Focussed Symposia

Arsenic trioxide (Trisenox®) in multiple myeloma: current clinical trials and future directions

CLINICAL EXPERIENCE WITH TRISENOX® (ARSENIC TRIOXIDE) AS A SINGLE-AGENT AND IN COMBINATION WITH ASCORBIC ACID (AA) AND DEXAMETHASONE (DEX)
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Arsenic trioxide is highly effective in the treatment of relapsed or refractory acute promyelocytic leukemia. In vitro data show that arsenic trioxide at concentrations between 2-5µmol is cytotoxic to myeloma cell lines and is not cross-resistant with dexamethasone or doxorubicin. Arsenic trioxide treatment also results in myeloma destruction through modulating integrins and caspase activation and through the over expression of CD38 and its ligand on plasma and LAK cells, respectively.

At the University of Arkansas, 14 patients with relapsed multiple myeloma (MM) or MM refractory to conventional salvage therapy were treated with Trisenox® using a dose and schedule shown to be effective in APL (0.15mg/kg/d for 60 days). The Trisenox® treatment regimen used in this study produced responses in 3 patients who failed previous high-dose chemotherapy and two different salvage regimens. A fourth patient experienced stable disease for over 6 months. Trisenox® was reasonably well tolerated in this heavily pre-treated group of patients. Based on these results, a multicenter study was designed to evaluate Trisenox® as a single agent at a higher dose and given on a shorter schedule. Twenty-four heavily pretreated patients with a median of 2.4 years from the time of diagnosis received treatment with Trisenox® (0.25 mg/kg, Mon-Fri, 2 weeks on/2 weeks off). Of the 24 patients, 15 were refractory to previous treatments and 9 relapsed. Thirteen of the 24 patients were evaluated for efficacy and 12 of those patients had an objective response or achieved stable disease. The response to Trisenox® in this study was durable with one patient maintaining stable disease for over 22 months after starting Trisenox® treatment. Neutropenia and leukopenia were the only common grade 4 toxicities noted. Overall, 70% of the treated patients achieved stable disease or > 25% decrease in the M-protein.

A number of investigators have shown that sensitivity to arsenic trioxide can be enhanced by depleting cellular levels of reduced glutathione (GSH) by buthionine sulfoximine (BSO) or ascorbic acid (AA). A phase 1 study was performed at the University of Miami to assess the toxicity of combined treatment with arsenic trioxide and AA and to determine if AA measurably depleted intracellular GSH. Six patients with stage IIIA relapsed/refractory MM were treated daily with Trisenox® (0.25 mg/kg) and AA (1000 mg) for 25 days. Two patients, both with thalidomide-refractory MM, achieved PR and 4 patients had stable disease. The combined treatment had acceptable toxicity and no effect on the pharmacokinetics of arsenic trioxide. Intravenous AA was able to reproducibly deplete intracellular GSH.

In-vitro data from the group at the Dana Farber show that the presence of arsenic trioxide in the growing media of myeloma cell lines sensitizes those cells to dexamethasone, resulting in a higher level of cytotoxicity at lower doses. These results, in addition to the new pharmacokinetic data, allowed us to initiate a phase 2 clinical trial to test the effect of Trisenox®-AA-dexamethasone (TAD). Data on the first 15 patients with active, progressive multiple myeloma, and who failed no more than 2 prior treatments, will be presented at the meeting. Preliminary data in the group of patients who completed the first cycle of therapy, showed that 6 patients (42%) achieved >50% reduction in M-protein and 1 patient had near CR after 1 cycle of therapy. Seven patients had stabilization of their disease process with 1 of these patients progressing during the second cycle. Preliminary results show that this drug combination of Trisenox®-AA-dexamethasone is active in the group of relapsed myeloma patients tested and that the regimen was well tolerated.

NEW ADVANCES IN THE BIOLOGY AND TREATMENT OF MULTIPLE MYELOMA
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Trisenox® (arsenic trioxide) injection is highly effective for the treatment of relapsed or refractory acute promyelocytic leukemia (APL). Trisenox® has unique, multifaceted mechanisms of action, offering a scientific rationale for investigation in diseases other than APL. At clinically relevant concentrations, it causes apoptosis in various tumor cell lines and has anti-angiogenic effects in vitro and in vivo. Human-myeloma-derived cell lines and freshly isolated myeloma cells are particularly sensitive to Trisenox®, and there is no apparent cross-resistance in myeloma cell lines that are resistant to other agents.

A number of clinical trials are investigating Trisenox® in multiple myeloma (MM), including an ongoing, single-center, phase 1/2 trial of Trisenox® in patients with advanced MM. The objectives of this study are to assess the safety and efficacy of Trisenox® given as maintenance therapy twice weekly or given with high-dose corticosteroids to patients whose disease progresses after Trisenox® single-agent therapy. Preliminary results show that Trisenox® given twice a week as a single agent or in combination with steroids is well tolerated and has signs of activity at two doses, 0.25 mg/kg or 0.35 mg/kg. These early results include a PR achieved by 1 out of the 7 patients treated at the low dose, and so far a minimal response in 1 out of 3 patients who received the higher dose of Trisenox®.

In preclinical studies designed to determine the effectiveness of Trisenox® combined with other agents, non-cytotoxic concentrations of Trisenox®, in combination with melphalan, were cytotoxic to myeloma cell lines. In another preclinical study, combined treatment with Trisenox® and ascorbic acid resulted in a marked sensitization of myeloma cell lines to the cytotoxic effects of Trisenox® alone. These results led to our early clinical work with melphalan-Trisenox®-ascorbic acid. In this clinical experience, three patients with relapsing MM failed other multiple therapies; 2 of the 3 patients received combined treatment of melphalan and a second agent as a prior therapy. The patients treated in this clinical experience also had significant secondary renal dysfunction (serum creatinine of 5.1, 5.1, and 6.1); however, even though these patients were seriously ill, all of them responded to a melphalan-Trisenox®-ascorbic acid regimen of melphalan 0.1 mg/kg daily for the first four days of a 4-6 week cycle, Trisenox® 0.25 mg/kg twice weekly, and ascorbic acid 1 g twice weekly. The responses to this therapy included a decrease in serum or urine M-proteins and a marked and sustained improvement in serum creatinine levels and creatinine clearance. These encouraging pre-clinical results and initial clinical
experiences indicate the clinical benefit of Trisenox® as a single agent or in combination with other agents for the treatment of relapsed or refractory MM.

TRISENOX (ARSENIC TRIOXIDE) AND BEYOND: OPPORTUNITIES TO IMPROVE PATIENT OUTCOME

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Arsenic trioxide has significant anti-myeloma activity through direct and indirect means. This agent induces apoptosis in drug-resistant cells from patients and in myeloma cell lines, through caspase-9 activation, and enhances apoptosis induced by dexamethasone. Arsenic trioxide reduces growth-promoting effects of IL-6 and blocks these same effects in the bone marrow microenvironment. Clinically, Trisenox® (arsenic trioxide) has been evaluated as a single-agent treatment in phase 2 studies and in combination with low dose melphalan. In a European trial, patients are given arsenic trioxide and if they do not respond to the drug, they are given a combined treatment of arsenic trioxide and dexamethasone.

Several other new treatment options for multiple myeloma are currently being evaluated in Europe. These treatments include thalidomide-dexamethasone compared to melphalan-prednisone as first line treatments, proteasome inhibitor PS-341(Velcade) in patients relapsing after first line induction treatment or after > 3 lines of previous treatments, ImIDs (Revimid) in patients failing after previous treatments, anti-IL6, farnesyl-transferase inhibitors, and others.

As we wait for the results from these studies, several simple and currently available measures, which might improve quality of life or possibly survival of myeloma patients, remain underused or not used at all. Examples of some of these measures are presented here. Studies have documented that physical exercise before and during autologous transplantation reduces nausea, vomiting, and neutropenic fever and enhances a sense of well-being. Female myeloma patients usually present with more severe osteoporosis than males; therefore, hormone replacement treatment, in addition to standard bisphosphonate therapy, might reduce progression of osteopenia. Interferon maintenance treatment has been shown in two meta-analyses to prolong remission by approximately 6 months and survival by 6-7 months. Erythropoietin clearly improves quality of life and reduces transfusion dependency in myeloma patients. In a preclinical study using a mouse myeloma model, erythropoietin treatment induced tumor regression and improved survival, possibly by enhancing T-cell activity. In humans, the effects of erythropoietin treatment are unknown.

In conclusion, preliminary data indicate important clinical activity of arsenic trioxide with acceptable toxicity, and tests are in progress in Europe for several other new promising drugs. A significant improvement in quality of life and survival, however, could be obtained with currently available drugs and concepts if they were offered to every eligible European multiple myeloma patient.

Thalidomide and IMiDs in multiple myeloma

THALIDOMIDE ALONE OR WITH DEXAMETHASONE FOR RESISTANT OR RELAPSING MULTIPLE MYELOMA.

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Since the surprising discovery by Dr. Barlogie and colleagues of activity of thalidomide against multiple myeloma, there have been many trials with this drug in various phases of disease. We treated 43 patients, with disease resistant or relapsing despite a high-dose dexamethasone-based regimen, with thalidomide in a daily evening dose of 200 mg increased with tolerance to a maximum of 800 mg and remissions were observed in 11 patients (26%). Criteria for response were based on >50% reduction of serum myeloma protein and/or >75% reduction of Bence Jones protein. The median remission time was 12 months, these results being similar to those of the Arkansas group. We then added intermittent high-dose dexamethasone (20 mg/m²/day for 4 days beginning on days 1, 9, 17) to those who had not responded and observed remissions in 40%. Thus, resistance of myeloma to serial trials with dexamethasone and thalidomide was overcome by concurrent use of both drugs. In vitro studies have also shown that addition of dexamethasone to thalidomide enhances anti-myeloma activity in a dose-responsive manner.

The combination was then given to 47 consecutive patients with resistant or relapsing disease to multiple prior treatments, including repeated high-dose dexamethasone and/or intensive treatment supported by autologous stem cells, but not thalidomide. We used criteria for response based on >75% reduction of serum myeloma protein production and/or >90% reduction of Bence Jones protein. Remission was observed in 22 patients (47%) including 5 with complete remission. Side effects were frequent, mild and usually reversible with more relation of increasing dose to toxicity than to clinical response. Thromboembolic complications occurred in 8% of these patients (and in 24% of other newly diagnosed patients despite prophylactic coumadin 1.0 mg p.o. daily), but has been largely prevented by therapeutic anticoagulation in recent patients. Survival and remission times were longer among patients with primary resistant, than with relapsing disease, similar to outcomes observed after prior rescue therapies (VAD, intensive therapy). These findings support the use of thalidomide-dexamethasone as soon as resistance to standard therapy is recognized, including patients who had received dexamethasone or transplant-supported therapies.

Thalidomide-dexamethasone was also given to 21 patients with persistent partial remission at least 6 months after intensive therapy supported by autologous blood stem cells. Thalidomide was given in a daily evening dose of 100 mg increased with acceptable tolerance to 300 mg; dexamethasone was prescribed as outlined previously. Further marked reduction of myeloma based on percentage change occurred in 57% of patients, including 17% converted to complete remission. However, the absolute magnitude of further reduction usually represented <5% of the initial tumor mass. This observation extends the previously established effect of TD against resistant or relapsing myeloma to subclones of plasma cells that persist at a low, stable level despite intensive therapy. Further controlled studies are necessary to
assess whether such a program will prolong remission and survival times.

**PIVOTAL TRIALS ON THALIDOMIDE OUTSIDE US**

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Singhal et al first reported that thalidomide, an oral agent with immunomodulatory and antiangiogenic properties, induced partial responses (ie at least 50% reduction of monoclonal protein concentration) in one-third of patients with refractory multiple myeloma most of whom have failed high-dose therapy. Several European studies have confirmed the activity of single agent thalidomide in patients with refractory or relapsing multiple myeloma (Table 1). It is now established that thalidomide can induce partial response in approximately one-third of patients and 10% to 20% of patients may achieve a minor response (≥25% reduction). The time to response is short with most patients responding within 2 months. Despite some evidence of a thalidomide dose-response relationship, responses may occur with doses as low as 50 mg and thus the optimal dose of thalidomide has yet to be defined. The most common side effects of thalidomide are constipation, tremor, headache, xerostomia, mood changes, edema and morning somnolence. The incidence and severity of these adverse effects may be dose-related and drug intolerance may be more prolonged in older patients. However, the most disabling complications have been deep vein thrombosis and peripheral neuropathy. Responding patients experience improvement of their performance status, reduction of pain and correction of their anemia. The median survival of thalidomide-treated patients is at least 1 year. Prognostic factors including low serum β2 microglobulin, absence of abnormalities of chromosome 13, younger age, normal platelet count, low plasma cell labeling index. Retrospective analysis suggested that after the addition of thalidomide to the therapeutic armamentarium, the three-year survival of the patients has improved by approximately 10%.

The activity of thalidomide as a single agent in advanced myeloma along with in vitro evidence of synergism with dexamethasone, provided the rational for investigation of the combination of thalidomide and dexamethasone (TD). Based on the clinical data of Weber et al who showed activity in approximately 50% of patients, several European studies confirmed the activity of this combination (Table 2). The median time to response with TD is less than one month. While it appears that the addition of dexamethasone to thalidomide increases the response rate by 20%, it is not clear that the combination improves event-free or overall survival compared to treatment with thalidomide alone. Recent retrospective analysis indicate that the administration of TD after failure of first line chemotherapy improves event-free and overall survival of the patients compared to the administration of conventional chemotherapy.

Thalidomide and dexamethasone have also been combined with chemotherapy for the treatment of patients with advanced myeloma (Table 3). Responses have been reported in approximately 60% of patients and the patients’ median survival is approaching 18 months. It appears that the incidence of deep vein thrombosis is increased when thalidomide is combined with chemotherapy (anthracyclines in particular). Several investigators recommend prophylactic anticoagulation with coumadin or low-molecular weight heparin since treatment with aspirin is not effective. It appears that thalidomide-based regimens not only lack a negative effect on blood stem cell collection but they may be used as mobilizing regimens. Prospective randomized trials are required in order to define the optimal thalidomide-based regimen for patients with advanced myeloma.

### Table 1: Thalidomide alone for refractory or relapsing myeloma

<table>
<thead>
<tr>
<th>Series</th>
<th>Thal Dose</th>
<th>Pt No</th>
<th>≥PR</th>
<th>EFS @1 year</th>
<th>OS @1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Juliusson 2000</td>
<td>200 to 800mg</td>
<td>23</td>
<td>43%</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Blade 2001</td>
<td>200 to 800mg</td>
<td>23</td>
<td>13%</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Oakervee 2001</td>
<td>Median 400mg</td>
<td>32</td>
<td>25%</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Prince 2002</td>
<td>Median 600mg</td>
<td>75</td>
<td>28%</td>
<td>50% @5.5months</td>
<td>50%@14.6</td>
</tr>
<tr>
<td>Yakoub-Agha 2002</td>
<td>Median 400 mg</td>
<td>83</td>
<td>48%</td>
<td>EFS 50% @1year</td>
<td>57%@1year</td>
</tr>
<tr>
<td>Neben 2002</td>
<td>Max 400mg</td>
<td>83</td>
<td>20%</td>
<td>EFS 45% @1year</td>
<td>86%@1year</td>
</tr>
<tr>
<td>Tossi 2002</td>
<td>Max 800mg</td>
<td>65</td>
<td>28%</td>
<td>50% &gt;8 months</td>
<td>NA</td>
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</table>

### Table 2: Thalidomide-dexamethasone for refractory or relapsing myeloma

<table>
<thead>
<tr>
<th>Series</th>
<th>Thal Dose</th>
<th>Pt No</th>
<th>≥PR</th>
<th>EFS @1 year</th>
<th>OS @1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimopoulos 2001</td>
<td>Thal 400 Dex pulses</td>
<td>44</td>
<td>55%</td>
<td>50% @10 months for responders</td>
<td>50%@12.6 months</td>
</tr>
<tr>
<td>Palumbo 2002</td>
<td>Thal 100 Dex pulse monthly</td>
<td>120</td>
<td>52%</td>
<td>50% @12 months</td>
<td>50%@27 months</td>
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</table>

### Table 3: Thalidomide and chemotherapy for refractory or relapsing myeloma

<table>
<thead>
<tr>
<th>Series</th>
<th>Regimen</th>
<th>Pt No</th>
<th>PR</th>
<th>EFS @1 year</th>
<th>OS @1 year</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moehler TM 2001</td>
<td>Thal CTX VP16 dex</td>
<td>56</td>
<td>68%</td>
<td>50% @16months</td>
<td>55%@16 months</td>
</tr>
<tr>
<td>Garcia-Sanz 2002</td>
<td>Thal CTX dex</td>
<td>22</td>
<td>53%</td>
<td>51% @1year</td>
<td>52%@1 year</td>
</tr>
<tr>
<td>Kropp 2002 ASH</td>
<td>Hyper CTD</td>
<td>60</td>
<td>72%</td>
<td>50% @11months</td>
<td>50%@19 months</td>
</tr>
<tr>
<td>Dimopoulos 2003</td>
<td>Pulsed CTD</td>
<td>43</td>
<td>67%</td>
<td>50% @12 months</td>
<td>50%@17.5 months</td>
</tr>
</tbody>
</table>

**THALIDOMIDE IN NEWLY DIAGNOSED PATIENTS: OVERVIEW OF SMOLDERING/INDOLENT DATA**

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Numerous trials have confirmed the activity of thalidomide in relapsed, refractory myeloma, with response rates averaging 30-35%. Based on these promising results, several trials with thalidomide alone or in combination with other active agents have been initiated in patients with newly diagnosed myeloma. A recent Mayo Clinic phase II trial studied 50 patients with newly diagnosed symptomatic myeloma with the combination of thalidomide plus dexamethasone. In this study, a confirmed
response (50% or greater reduction in M protein) was seen in 32 patients yielding a response rate of 64% (95% CI 49-77%). If minor responses are included (25-49% reduction in M protein), the overall response rate increased to 92%. Similar results were seen in an independent trial of thalidomide plus dexamethasone in newly diagnosed patients conducted at M. D. Anderson Cancer Center. Based on these results, a randomized controlled trial comparing dexamethasone versus thalidomide plus dexamethasone was initiated by the Eastern Cooperative Oncology Group, the results of which will define the role of thalidomide as initial therapy for early stage myeloma.

Given the incurability of myeloma and the leukemogenic potential of alkylating agents, the current standard of care is to delay therapy until symptomatic disease occurs. However, patients with asymptomatic myeloma are at high risk of progression to symptomatic disease, with a median time to progression of approximately 1-2 years. With the advent of effective non-cytotoxic biologic agents, the time is right to challenge this paradigm of myeloma therapy with carefully conducted clinical trials. We hypothesized that early therapy with thalidomide may be effective in delaying progression from asymptomatic to symptomatic multiple myeloma. Therefore, we conducted a phase II trial at the Mayo Clinic to determine the response rate and time to progression with thalidomide therapy in patients with smoldering and indolent (asymptomatic; early stage) multiple myeloma. Thirty-one patients with smoldering or indolent multiple myeloma were studied at the Mayo Clinic. Two patients were deemed ineligible because they were found to have received prior therapy for myeloma and were excluded from analyses except for toxicity. Thalidomide was initiated at a dose of 200 mg/day, and escalated as tolerated to a maximum of 800 mg/day. However, the dose of thalidomide was adjusted to as low as 50 mg per day, as needed, to minimize toxicity.

Of the twenty-nine eligible patients, ten (34%) had a partial response to therapy with at least 50% or greater reduction in serum and urine monoclonal (M) protein. When minor responses (25-49% decrease in M protein) were included, the response rate was 66%. Three patients had progressive disease while on therapy. Kaplan-Meier estimates of progression free survival are 80% at 1 year and 63% at 2 years. Major grade 3-4 toxicities included 2 patients with somnolence and 1 patient each with neuropathy, deep vein thrombosis, hearing loss, weakness, sinus bradycardia, and edema. Mild grade 1-2 neuropathy, sedation, and constipation were seen in 87%, 87% and 74% of patients, but these were generally amenable to appropriate dose reductions. Because of the lack of a control arm and the toxicities of early therapy, we presently do not recommend thalidomide for asymptomatic myeloma outside the setting of an approved clinical trial until randomized studies can be conducted. A phase III trial comparing zole dronic acid versus thalidomide plus zoledronic acid is due to open at the Mayo Clinic shortly, the results of which will shed light on the role of thalidomide as initial therapy for early stage myeloma.

DOXIL, VINCristine, DECAdron AND THALIDOMIDE (DVD-T) FOR NEWLY DiAGNosed, AND RELAPSED/REFRACTORY MULTIPLE MYELOMA; RESPONSE TO THERAPY, AND SUPPORTIVE CARE ISSUES

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DVd is an effective and well tolerated regimen in newly diagnosed MM patients resulting in an overall response rate of 90%, however only 10% of the patients achieve complete remission. In relapsed/refractory group of myeloma patients, only 22% and 5% achieve 50% & 90% reduction in the M-Protein respectively. Patients achieving >90% decrease in the M-Protein on DVD had a durable response. Thal/Dex in a similar group of patients results in 60% overall response with rare cases achieving 90% reduction in the M-Protein. Thalidomide modulates integrins thus interrupting the myeloma cell-stroma interaction results in the malignant cell becoming sensitized to therapy as well as a significant decrease in the supportive cytokine environment. We evaluated the role of Thalidomide in combination with DVD with the objectives is to enhance the quality of response in the newly diagnosed patients, i.e., complete remission, and near complete remission rate, and in the relapsed refractory group enhance the response rate as well as the quality of response, in addition to assessing the tolerability of the combination. In both groups the regimen was administered as follows. On day 1 Doxil was given at 40 mg/ m2 IVPB; Vincristine at 2 mg IPV & reduced dose decadron at 40 mg PO daily X 4 days. Thalidomide was started at 50 mg a day, to be increased by 50 mg a day q week to maximum tolerated dose and not to exceed 400 mg a day. DVD was repeated q4 W, for a minimum of 6 cycles & 2 cycles after best response. Patients achieving a plateau phase were maintained on prednisone 50 mg qod & the maximal tolerated dose of Thalidomide until disease progression. All patients were screened for vitamin B12 and folate deficiency, and were allowed to use erythropoietin and bisphosphonate therapy for anemia, and bony disease respectively. Response was assessed according to SWOG criteria. However, for (CR) we required in addition to the standard SWOG criteria, the BM to show polyclonal PC’s by immune staining. Following an increased incidence of neutropenia, infections, oral herpes simplex, increased incidence of neuropathy & Deep venous Thrombosis (DVT’s) in the first group of patients; the protocol was amended to initiate all patients on prophylactic amoxicillin 250mg BID, acyclovir 400 mg BID until completion of chemotherapy, GM-CSF or G-CSF if the total WBC was less than 5000/µL on day 1, & Aspirin 81mg daily. The vincristine dose reduction algorithm was further modified to be more aggressive in response to grades 1 and 2 neuropathy. 35 newly diagnosed, and 50 relapsed/refractory myeloma patients are currently enrolled. 70 patients (25 newly and 45 relapsed/refractory) will be reported for response, and 71 for toxicity. All patients enrolled had progressive disease, and none of the relapsed/refractory group was non-responder/non-progressor. Patients’ demographics and prognosticators are outlined in table 1.

Table 1

<table>
<thead>
<tr>
<th></th>
<th>Newly diagnosed</th>
<th>Relapsed/refractory</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age</td>
<td>60</td>
<td>63</td>
</tr>
<tr>
<td>PS</td>
<td>&lt;3</td>
<td>&lt;3</td>
</tr>
<tr>
<td>β2</td>
<td>2.9</td>
<td>6.6</td>
</tr>
<tr>
<td>Albumin</td>
<td>3.8</td>
<td>3.2</td>
</tr>
</tbody>
</table>
The overall response rate was noted in 22 (88%) and 34 (76%) patients; with CR (Disappearance of the M-protein by immune fixation, & the presence of polyclonal plasma cells in the bone marrow by immune staining) is achieved in 6 (24%) and 5(11%) patients who were newly diagnosed or relapsed/refractory correspondingly. The break down of the different responses by M-protein values is outlined in table 2.

<table>
<thead>
<tr>
<th></th>
<th>CR</th>
<th>NCR</th>
<th>&gt;75%-&lt;90%</th>
<th>50%-&lt;75%</th>
<th>SD</th>
<th>PD</th>
</tr>
</thead>
<tbody>
<tr>
<td>Newly</td>
<td>6 (24%)</td>
<td>4 (16%)</td>
<td>3 (12%)</td>
<td>9 (36%)</td>
<td>2 (8%)</td>
<td>1 (4%)</td>
</tr>
<tr>
<td>Relapsed</td>
<td>5 (11%)</td>
<td>15 (33%)</td>
<td>4 (9%)</td>
<td>10 (22%)</td>
<td>4 (9%)</td>
<td>7 (15%)</td>
</tr>
</tbody>
</table>

Toxicity prior to amending the protocol included 21 of 35 patients with Grade 3/4 neutropenia, 7 cases of pneumonia requiring IV antibiotic therapy, 1 septic arthritis, 2 GI bleeds. Following the amendments these cytopenia related complications has been reduced to only 1/19 grade 3 neutropenia & fevers. Grade 3 neuropathy was reduced from 18 of the first 31 patients to none after the amendments. Even though no excessive deep venous thrombosis was noted in our protocols that utilized thalidomide as a single agent or in combination with steroids or non anthracyclines combination regimens; in the current protocol deep venous thrombosis was significantly increased with the newly diagnosed patients more likely to be afflicted than relapsed/refractory (newly diagnosed 50% vs 10% & relapse/refractory 26% vs 10%). Activated protein C resistance (APCR), factor V Leiden (FVL), platelet aggregation activity (PA), & von Willebrand factor (vWF) were measured in 28 newly diagnosed & 51 relapsed/refractory MM patients before & after each cycle of D-Vd-T. 3 patients with prior DVT, 1 pt on Warfarin for mechanical heart valve & 1 pt on ASA prior to the study were excluded from the analysis. Patients were grouped in 2 categories: 39 patients received ASA & 35 patients did not at the start of therapy. None of the patients had homozygous Factor V Leiden. 14 treatment-related DVT occurred with a mean of 85.6 days post-therapy. 3 with post-therapy DVT in the ASA group stopped ASA (mean= 11 days) prior to their DVT. Excluding these 3 patients, the difference between DVT rates in non-ASA group (10/35) and ASA group (1/36) is statistically significant (p=0.003).With intent-to-treat, the ASA group continued to have a lower incidence of DVT (p=0.04) after the addition of ASA, the relapsed/refractory group of patients showed a trend towards less incidence of DVT (5/46) as compared to newly diagnosed patients (6/25) (p=0.144). Compared to pre-therapy levels, vWF (p=0.03) & PA to ristocetin 1500 mcg/ml (p=0.04) were significantly elevated at day 30 after start of DVD-T.

In summary DVD-T following supportive care modifications is well tolerated. The addition of low dose aspirin reduced the incidence of deep venous thrombosis to what is noted in historical data, and did not result in any increase incidence of bleeding. Compared to historical data in a similar pt population receiving Dvd or Thal/Dex, the quality of response in both groups of patients, and the response rate in the relapsed/refractory patients is significantly enhanced by combining both regimens, & reducing steroids.

ROLE OF IMMUNOMODULATORY DRUGS IN MULTIPLE MYELOMA

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We have carried our preclinical and clinical studies of the immunomodulatory drug (IMiD) Revimid. It induces growth arrest or apoptosis of drug resistant multiple myeloma (MM) cell lines and patient cells; abrogates binding of MM cells to bone marrow stromal cells (BMSCs) and extracellular matrix proteins; inhibits production cytokines (IL-6, IGF-1, VEGF) which confer growth, survival, and drug resistance in the BM; and stimulates patient anti-MM NK cell and ADCC. Revimid triggers activation of caspase 8, enhances MM cell sensitivity to Fas-induced apoptosis, and downregulates NF- B activity as well as expression of cellular inhibitor of apoptosis protein-2 and FLICE inhibitor protein. It potentiates the activity of TRAIL/Apo2L, dexamethasone, and proteasome inhibitor PS-341. Revimid activates PI3-K/PKC, and NF-AT2, with resultant nuclear translocation of NF-AT2 and upregulation of IL-2 transcription in T cells. It induces phosphorylation of CD28 on T cells, and increases proliferation of T cells following activation via CD3 or dendritic cells. When MM cells are injected subcutaneously into SCID mice in the context of matrigel, Revimid inhibits growth of human MM cells and associated angiogenesis, as well as prolongs survival. Phase I trial showed no constipation, neuropathy, or somnolence, and established the MTD of 25mg daily. Remarkably, >25% MM paraprotein decreases were observed in 63% patients with relapsed refractory MM, and stable paraprotein or better was achieved in 80% patients in this phase I trial. A phase II trial has examined 30mg once daily versus 15 mg twice a day in patients with relapsed refractory MM. Preliminary analysis reveals thrombocytopenia requiring dose reduction in 25% patients, more commonly in patients receiving the twice daily drug regimen. Importantly, 38 of 46 (85%) patients either stabilized their disease or responded, including complete responses. A multicenter phase III trial of Dexamethasone and placebo versus Dexamethasone and Revimid is ongoing in an attempt to achieve 50% prolongation of time to progression. Given its remarkable clinical activity in advanced relapsed and refractory MM, Revimid is being evaluated in treatment protocols for newly diagnosed MM, and as maintenance therapy to prolong progression free survival after high dose therapy and stem cell transplantation.

THALIDOMIDE (THAL), REVIDIM (REV) AND VELCADE (VEL) IN ADVANCED MULTIPLE MYELOMA

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The Myeloma Institute for Research and Therapy (MIRT), University of Arkansas for Medical Sciences, Little Rock, AR and Cancer Research And Biostatistics (CRAB), Fred Hutchinson Cancer Research Center, Seattle, WA.

Myeloma relapsing after high dose therapy (HDT) has a poor prognosis, especially if response duration is short and cytogenetic abnormalities (CA) are present at relapse. THAL represented the first-effective therapy for such post-HDT relapses, no longer responsive to standard DEX or chemotherapy such as DCEP. An update is provided of 169 patients receiving a dose-escalation schedule of THAL (200 mg with escalation of 200 mg q 2 weeks, according to tolerance, to a maximum of 800 mg). Seventy-six percent had relapsed from 1 and 53% from 2 cycles of HDT; 67% exhibited CA including 37% with del 13. By 8 months from

S5
initiation of treatment (intent-to-treat), 31% were estimated to have achieved partial response (> 50% myeloma protein reduction, MPR). Most responders could be identified within 2 months using > 25% MPR criteria (Figure 1). Survival was superior among the 52 patients presenting without CA and with low B2M (< 3 mg/ml) prior to therapy; survival shortened progressively in the presence of 1 (n=55) and especially 2 (n=46) adverse parameters; 16 patients lacked cytogenetic and B2M data (Figure 2). Significant myelosuppression was not noted but cumulative and acute dose-related peripheral neuropathy was dose-limiting.

REV is another immunomodulatory drug designed to be devoid of neurotoxicity. In an initial phase I/II study, dose escalation was performed, from a starting level of 5 mg q d (n=3), to 10 mg (n=3), 25 mg (n=3) and 50 mg (n=6). All patients had relapsed from prior HDT and most had been exposed to and were resistant to THAL. MPR by > 25%/> 50% occurred overall in 4/4 patients, mainly at >25mg (7 patients). These data formed the basis for a randomized phase II trial for post-HDT relapse patients, comparing REV 25 mg q d x 20 with REV 50 mg daily x 10, with cycles to be repeated in both arms q 28 days. DEX 40mg q d x 4 was added with cycle 3. After entry of 38 patients, the Revimid 50 mg arm appeared inferior in terms of response (21% vs. 42%, p=0.162), prompting modification to an alternating day schedule (50 mg qod x 10 doses). In addition, in both arms, bridging doses were introduced of 5 mg x 8 doses (REV 25 mg arm) and 10 mg qod x 4 doses (REV 50 mg arm). A total of 22 patients have been treated on the 25 mg arm and 25 on the 50 mg arm (19 prior to and 6 after revision). At 8 mos after start of REV, MPR >50% was observed in 25% on the 25mg and 9% on the 50mg arm (p=.16) (Figure 3). No significant sedative or neurotoxic effects were observed. Myelosuppression, especially thrombocytopenia, was dose-limiting.

VEL (PS 341) has recently been shown to exhibit marked anti-myeloma activity, even in the setting of post-transplant and post-THAL relapse. A phase I/II study was designed to evaluate the combination of PS-341 plus THAL in such high-risk patients (Table 1). Patients were enrolled at a starting level of PS-341 of 1.0 mg/m² administered on days 1, 4, 8 and 11 (q 21 days) with thalidomide to be added in a dose escalation fashion from 50 to 100 to 150 to 200 mg, whenever 7 patients had completed the second cycle without evidence of significant worsening of baseline neurotoxicity. DEX pulsing could be added for those patients not achieving at least PR status. Figure 4A displays a representative example of a responding patient and cumulative response rates using different levels of MPR (Figure 4B). It is apparent that 60% achieved PR status (> 50% MPR) at the end of cycle 3.

Collectively, these data indicate an important advance in the therapeutic armamentarium of myeloma management. Accompanying gene expression analysis at baseline has been used toward response prediction to PS-341 (Figure 5A). Follow-up examinations 48 hours after single drug treatment have revealed drug-unique gene expression changes which will aid in the understanding of their molecular mechanisms of action (Figure 5B).
Table 1: VEL + THAL for Refractory MM (N=30)

<table>
<thead>
<tr>
<th>Prior autotx (99%)</th>
<th>29</th>
</tr>
</thead>
<tbody>
<tr>
<td>Prior THAL (80%)</td>
<td>24</td>
</tr>
<tr>
<td>Prior Rx &gt; 5 lines (77%)</td>
<td>23</td>
</tr>
<tr>
<td>Abn. Cytogenetics (63%)</td>
<td>19</td>
</tr>
<tr>
<td>del 13 (43%)</td>
<td>13</td>
</tr>
<tr>
<td>B2M &gt; 4mg/L (53%)</td>
<td>16</td>
</tr>
</tbody>
</table>

Figure 4A Example of response

Figure 4B Cumulative % M-Protein response by cycle

Figure 5A

Figure 5B
Pathophysiology of myeloma bone disease and management of skeletal complications

MULTIPLE MYELOMA PATHOPHYSIOLOGY AND DISEASE OVERVIEW

Robert Vescio, MD

The great majority of patients with multiple myeloma develop pain and complications related to the destruction of bone at sites of disease. It is often the presentation of bone pain and fatigue that brings patients with multiple myeloma to medical attention. It has been estimated that 80% of patients with multiple myeloma will have lytic lesions present on radiographic skeletal survey. These lesions predominate in areas of particularly high concentrations of tumor cells within the bone marrow. However, even patients without discernible lytic lesions on radiographic assessment, often are noted to have significant osteoporosis on bone densitometry. These patients often have a more diffuse infiltration of tumor throughout their marrow and can present with features mistakenly attributed to complications of severe osteoporosis such as kyphosis of the spine due to vertebral fractures.

Unfortunately, even patients who respond to chemotherapy may have progression of skeletal disease and once a lytic bone lesion develops, recalcification and radiographic improvement are rare. Pathologic fractures within the spine are particularly problematic and can lead to chronic pain since these lesions rarely fully heal without surgical intervention. To appreciate the impact this bone destruction can induce without treatment, one can look at the results of the first trial demonstrating a benefit for the use of bisphosphonates in this disease. In this 370 patient trial, patients with multiple myeloma were randomized to receive monthly pamidronate or placebo. Within 9 months, 30% of patients receiving placebo infusions developed a fracture and 22% of similarly randomized patients required radiation treatment for symptoms of bone pain or impending fracture. Even more catastrophic was the 3% of patients receiving placebo who developed spinal cord compression. In this trial, patients with relapsed disease randomized to receive pamidronate had a seven month improvement in median survival as well. The explanations for this survival advantage vary. Certainly, some survival advantage may be attributable to the reduced pain, reduction in need for radiation and surgical procedures and the consequent complications when bisphosphonates were used. However, recent evidence suggests that these bisphosphonates may directly or indirectly impair tumor growth. Initially, Mundy and colleagues identified a number of proteins known as OAFs, (osteoclastic activating factors), which were thought to be the proteins responsible for enhanced bone loss in myeloma patients.(1) These factors including lymphotoxin (tumor necrosis factor (TNF) β), and interleukin-1 (IL-1) β were identified in the supernatants from cultures from myeloma cells lines and fresh myeloma bone marrow in these early studies. However, more recent studies have suggested that other factors are more important in the high rate of bone turnover in multiple myeloma. The role of lymphotoxin in myeloma bone disease has been downplayed by more recent studies failing to find significant differences in the amount of this cytokine in supernatants derived from myeloma patients compared to controls.(2) In addition, antibodies to lymphotoxin do not reduce the bone resorbing activity of fresh bone arrow plasma from myeloma patients.(3) Another tumor necrosis factor, TNF , is found at higher levels in supernatants from these patient’s bone marrow cultures, and is capable of stimulating osteoclast formation. Its effects are mediated by stimulation of the proteolytic breakdown of IκB that leads to the release of NF-κB. This enhancer translocates into the nucleus where it induces transcription of specific genes, some of which are involved in enhancing bone resorption. The importance of NF-κB in bone resorption is supported by studies showing that NF-κB knockout mice show osteopetrotic bones.(4) It is likely that the proteosome inhibitor PS-341 (Velcade) which inhibits NF-κB and is highly active in multiple myeloma may also be a potent osteoclast inhibitor.

The pathophysiology of osteoclast development has more recently been elucidated. Osteoclasts develop from monocytes which have been exposed to either M-CSF or VEGF. This process is insufficient for complete osteoclastic development, however. These immature osteoclasts only mature into active bone resorbing after direct contact with osteoblasts, stromal cells or by exposure to the ligand for the receptor for activation of NF-κB (RANK) called RANKL. The identification of RANK expressed on the surface of osteoclasts and RANKL on osteoblasts and stromal cells explains how this interaction leads to osteoclast development. TNF itself is capable of stimulating osteoclasts to increase the expression of RANKL. We have recently noted that malignant plasma cells from myeloma patients also express RANKL and can replace exogenous sRANKL as a promotor for complete osteoclast development. This may further explain how multiple myeloma cells promote local bone resorption. In fact, a vicious circle of osteoclast stimulation and subsequent myeloma cell proliferation is likely to exist in the bone marrow of patients with multiple myeloma. IL-6, which is mainly produced by nonmalignant cells in the bone marrow of myeloma patients, is a cytokine capable of stimulating growth and preventing apoptosis of the malignant cells in myeloma patients. Stromal cells, osteoclasts and osteoblasts are all major sources of IL-6 in the bone marrow microenvironment. Recent studies show that malignant cells from myeloma patients increase IL-6 production by osteoclasts both by direct cell-to-cell contact and release of soluble factors. Since some studies report that malignant cells from myeloma patients produce IL-1 and TNF and both of these cytokines stimulate IL-6 production by osteoclasts,(5, 6) either or both may be the soluble factor(s) involved in myeloma cell-induced release of IL-6 by osteoclasts. Thus, these studies suggest the role of bone cells not only in bone-related changes in these patients but also in the promotion of growth and prevention of apoptosis of the tumor cells themselves as mediated by IL-6.

Importantly, a soluble decoy receptor called osteoprotegerin (OPG) was discovered that binds RANKL and prevents the binding of the ligand to RANK. The importance of this natural inhibitor of bone resorption was shown in experiments using knockout mice whereby OPG deficient animals suffered from profound osteoporosis. It appears that the balance between soluble OPG and RANKL may significantly determine the degree of bone resorption in the local bone marrow microenvironment. Clinical trials using synthetic OPG analogues or blocking antibodies to RANKL have been initiated and show early promise in clinical trials. The first trial using AMGN-0007 showed that obtainable levels of this synthetic OPG analogue could suppress bone resorption markers to a similar degree as pamidronate.(7) The drug was also well tolerated. Unfortunately, one patient developed an antibody to the agent which led to temporary bone turnover greater than baseline by presumably interfering with
naturally occurring OPG. Present trials are focusing on RANK-Fc which also blocks the RANK-RANKL interaction.

Recently, macrophage inflammatory protein-1α (MIP-1α) has been identified as an important factor involved in myeloma bone disease.(8) Levels of this cytokine are elevated in the bone marrow of these patients. This chemokine is capable of inducing osteoclast formation in vitro, and antibodies to this protein block the induction of osteoclast formation by fresh bone marrow plasma from myeloma patients. In addition, this chemokine attracts and activates monocytes, and is a potent inhibitor of early hematopoiesis.

As noted earlier, VEGF is another cytokine important in promoting osteoclast development. It is now clear that VEGF is produced by malignant plasma cells, and the receptors that bind this factor are expressed on bone marrow stromal cells.(9) In fact, recent results show that VEGF increases IL-6 production by bone marrow stromal cells from myeloma patients. This may indirectly lead to enhanced bone loss in these individuals.

It is hoped that a combination of these newer agents and bisphosphonates may further reduce the devastating effects that myeloma has on bone. While further osteoclastic inhibition should improve the quality of life that multiple myeloma patients have, it may also promote improved survival by disrupting this vicious cycle of myeloma cell mediated growth by the bone marrow microenvironment.

characterized inhibitors of osteoclastic resorption are bisphosphonates. Bisphosphonates are analogues of inorganic pyrophosphate in which the central oxygen atom is replaced by a carbon atom to generate the P-C-P motif, which is responsible for the high affinity of these compounds for bone. Modifications to the side chains of this central motif influence the affinity of bisphosphonates for hydroxapatite and determine their anti-resorptive potency. One or the most potent bisphosphonates is zoledronic acid (1) and this bisphosphonate is now being examined for its ability to inhibit bone resorption and prevent the development of bone disease in multiple myeloma.

Zoledronic acid has now been studied in several murine models of myeloma. In the 5T2MM syngeneic model, treatment of mice, with zoledronic acid (twice weekly) from the time of tumor cell injection, or from the time the paraprotein was detected, prevented the formation of osteolytic bone lesions (2). Zoledronic acid also prevented the tumor-induced reduction in cancellous bone area and total bone mineral density and inhibited osteoclast formation. These effects were associated with 31-35% reduction in serum paraprotein concentration and a significant reduction in tumor burden in bone. Treatment of mice bearing 5T2MM cells with zoledronic acid also reduced microvessel density in areas of tumor cell invasion, suggesting that this bisphosphonate may be able to inhibit angiogenesis. Importantly, treatment of 5T2MM bearing mice, from the time of paraprotein detection, with zoledronic acid, was associated with a significant increase in time to first signs of morbidity (2). Subsequent studies have also demonstrated that a single dose of zoledronic acid, administered at the time of paraprotein detection, is still able to prevent the development of osteolytic bone disease and influence survival. Zoledronic acid has also been investigated in the SCID-hu system (3). As was observed in the 5T2MM model, zoledronic acid was also able to prevent the bone loss and decrease osteoclast numbers induced by the presence of primary human myeloma cells. Treatment of myeloma bearing animals reduced serum paraprotein concentration, whereas, pre-treatment prevented the appearance of a serum paraprotein altogether.

The mechanisms responsible for the anti-myeloma effect remain unclear. This could reflect an indirect effect on bone and by inhibiting bone resorption zoledronic acid may alter the local microenvironment providing a less favourably environment for myeloma cells to grow. Alternatively zoledronic acid may effect myeloma cells directly. Indeed, studies have demonstrated that bisphosphonates, including zoledronic acid are able to inhibit the growth of myeloma cells and to induce myeloma cell apoptosis in vitro (4-6). Furthermore, zoledronic acid is able to inhibit the adhesive properties and metastatic potential of tumor cells and may be able to alter features of the tumoral environment. However, there are currently little data demonstrating a direct anti-myeloma effect in vivo.

Taken together these studies demonstrate that zoledronic acid is effective in preventing the development of myeloma bone disease, in vivo, in pre-clinical models of myeloma. These studies have also shown that zoledronic acid treatment may be associated with an anti-myeloma activity and can promote survival. However, it remains to be established whether the anti-myeloma effect and the effects on survival observed in vivo are mediated by a direct effect on the tumor cells or indirectly via an effect on bone.


**Long-term Efficacy and Safety of Zoledronic Acid in the Treatment of Multiple Myeloma**

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Introduction: Patients with multiple myeloma are at risk for severe, painful complications from osteolytic bone destruction. Current American Society of Clinical Oncology Practice Guidelines recommend intravenous bisphosphonates to prevent skeletal complications in patients with multiple myeloma and evidence of osteolytic bone destruction (J Clin Oncol. 2002, 20:3719-3736). A previous report demonstrated that after 12 months of treatment, 4 mg zoledronic acid is at least as effective as 90 mg pamidronate in reducing skeletal complications in a large, randomized non-inferiority trial in 1,648 patients with either multiple myeloma or bone metastases from breast cancer (Cancer J. 2001, 7:377-87). Herein, the long-term follow-up for patients with multiple myeloma enrolled in this study is reported.

Patients and Methods: The trial was an international, multicenter, double-blind, double-dummy, randomized, parallel group trial designed to demonstrate the non-inferiority of zoledronic acid compared with pamidronate. Patients were eligible if they had a confirmed diagnosis of multiple myeloma (Durie-Salmon Stage III) and at least one osteolytic bone lesion on a conventional radiograph or a histologically confirmed diagnosis of breast cancer with at least one bone metastasis confirmed by a conventional radiograph (plain film). Prior to randomization patients were stratified into 3 groups as follows: multiple myeloma, breast cancer patients receiving first- or second-line hormonal therapy, or breast cancer patients receiving chemotherapy. In this trial, a total of 510 patients with Durie-Salmon Stage III multiple myeloma were randomly assigned to receive either pamidronate (90 mg via 2-hour infusion) or zoledronic acid (4 or 8 mg via 15-minute infusion) every 3 to 4 weeks for 1 year, and 194 patients continued to receive study medication for an additional year. The 8-mg dose of zoledronic acid was associated with an increase in serum creatinine levels, was subsequently reduced to 4 mg, and efficacy conclusions were not drawn from this treatment group. This report summarizes results from the 25-month follow up of the subset of patients with multiple myeloma. The primary efficacy endpoint was the percentage of patients who experienced at least 1 skeletal-related event (SRE), defined as pathologic fracture, spinal cord compression, surgery to bone, or radiation therapy to bone. Secondary efficacy endpoints included the time to first SRE, the annual incidence of SREs, and Andersen-Gill multiple event analysis of the overall risk of experiencing an SRE; these analyses included hypercalcemia of malignancy as an SRE.

Results: For the primary endpoint, 50% of patients treated with 4 mg zoledronic acid experienced at least 1 SRE versus 54% of patients treated with pamidronate (P = .499). The median time to first SRE was delayed by almost 100 days for patients treated with 4 mg zoledronic acid (median 380 days versus 286 days for pamidronate), but this difference did not achieve statistical significance (P = .539). The mean annual incidence of SREs was 1.32 for 4 mg zoledronic acid versus 0.97 for pamidronate (P = ...
Finally, the multiple event analysis hazard ratio for the 4 mg zoledronic acid treatment group versus pamidronate was 0.932 (95% CI = 0.719, 1.208), suggesting that the risk of developing an SRE was similar for both treatment groups. The incidence of adverse events reported over 25 months of treatment was similar between treatment groups. As expected, the most commonly reported adverse events were bone pain and transient, “flu-like” effects common after intravenous bisphosphonate treatment (nausea, fatigue, pyrexia, and vomiting). Importantly, 4 mg zoledronic acid (via 15-minute infusion) exhibited a renal safety profile similar to 90 mg pamidronate, based on Kaplan-Meier analysis of time to first elevated serum creatinine (hazard ratio = 0.764; 95% confidence interval 0.348, 1.677; P = .502).

Conclusions: This long-term analysis demonstrates that zoledronic acid (4 mg) is at least as effective as 90 mg pamidronate for the prevention of skeletal complications associated with multiple myeloma and exhibits a long-term safety profile similar to that of pamidronate after 2 years of treatment. In addition to the more convenient 15-minute infusion time for zoledronic acid versus the 2 hours required to infuse pamidronate, these results provide rationale for the use of zoledronic acid in patients with multiple myeloma.

**USE OF ZOLEDRONIC ACID IN EARLY STAGE DISEASE, MGUS AND INDOLENT MYELOMA**

*Philip R. Greipp, M.D., David Vesole, M.D., Ph.D., S. Vincent Rajkumar, M.D., Robert A. Kyle, M.D.*

Purpose. The purpose of this presentation is to provide background and rationale for the potential usefulness of zoledronic acid in asymptomatic (smoldering) myeloma, high risk monoclonal gammopathy of undetermined significance (MGUS), and ‘solitary’ plasmacytoma of bone, and to provide a strategy to discover whether or not zoledronic acid may be effective in delaying myeloma progression.

Background. Myeloma is preceded in most instances by an asymptomatic phase. Patients with MGUS develop myeloma at 1% per year. MGUS patients with higher M-protein levels progress at a higher rate. Patients with asymptomatic (smoldering) myeloma and ‘solitary’ plasmacytoma progress at even higher rates. The conversion to active myeloma requiring therapy is almost always accompanied by the development of bone disease. Effective prevention has not been demonstrated.

Rationale. Myeloma progression is most often heralded by the development of bone lesions. Osteoclastic activation and recruitment occur early in progression and forecasts the development of bone lesions. Bone matrix breakdown and cytokine production due to further progression of bone disease provides vital growth factors for myeloma. Bisphosphonates interrupt the vicious cycle of myeloma growth, bone destruction, and cytokine production by inhibiting osteoclast recruitment, activation, and function. In addition, zoledronic acid may have special anti-tumor activity against myeloma.

Methods. We are initiating a phase II trial, E1A98 in the Eastern Cooperative Oncology Group (ECOG) to explore the efficacy of zoledronic acid in high risk MGUS, asymptomatic (smoldering) myeloma, and ‘solitary’ plasmacytoma of bone. A controlled trial may be necessary to determine possible anti-tumor effects of zoledronic acid. In another trial, E1A00 we will have the opportunity to examine the potential effects of thalidomide and zoledronic acid on the recently described renal toxicity. In another strategy we examine the possible additive or synergistic effects of zoledronic acid with thalidomide in the early stages of myeloma. At the Mayo Clinic a National Cancer Institute grant (SVR and PRG) will support correlative laboratory analyses to study the beneficial or toxic effects of zoledronic acid versus the combination.

Summary and Conclusion. Patients with high risk MGUS, asymptomatic (smoldering) myeloma and ‘solitary’ plasmacytoma of bone are at increased risk of progression to myeloma. Progression is usually associated with the development of bone lesions. Since the altered bone microenvironment provides growth factors for myeloma it is reasonable to postulate that the use of bisphosphonates, which limit osteoclast resorption of bone might limit or delay progression of myeloma. While no study has yet proven the effectiveness of bisphosphonates in preventing or delaying myeloma progression it is reasonable to initiate clinical trials using these agents in early phase disease and to study clinical and biological endpoints of progression and the potential toxicity of these agents. Zoledronic acid is particularly attractive for such trials because of its safety profile, ease of administration relative to pamidronate, and potential unique anti-myeloma effects. However, in the absence of clinical evidence of efficacy and considering the costs and potential risks to this group of patients over potentially many years of follow-up, we cannot recommend the routine use of bisphosphonates for early stage disease as defined in this presentation.

References


Waldenström’s macroglobulinemia: update from the 2nd International Workshop on Waldenström’s macroglobulinemia

GENETIC BASIS AND PATHOGENESIS OF WALDENSTRÖM’S MACROGLOBULINEMIA

LM Pilarski, SS Sahota, R Fonseca, H Avet-Loiseau, N Mitsiades, ML McMaster, D Leitch, RG Owen, S Adamia & J Shaughnessy

The Second International Workshop on Waldenström’s Macroglobulinemia (WM) held in September 2002 brought together emerging data on the biology and genetic characteristics of WM, and these findings are summarized and updated here. WM, a slowly progressive clonal lymphoid disorder, involves a lymphoplasmacytic BM proliferation accompanied by a serum monoclonal IgM component, various autoimmune disorders, and organomegaly in some patients. Although WM includes both lymphocytic and plasmacytoid cells, a clonal relationship between the B cell subpopulations in WM is likely and direct evidence is now emerging to address this issue. The dynamic differentiation of the WM clone over time and in the face of treatment clearly has potentially profound implications for clinical management of the disease. In effect, the clinical therapeutic arsenal must hit a “moving target”. Although phenotypic characterization of WM suggests that the malignant cells express sIgM/CD19/CD20, the plasmacytic components expressing CD38/CD138 can vary. Interestingly, phenotypic markers normally associated with T-cells also appear to be expressed by some clonal cells. The analysis of Ig variable region (V) genes in WM has established a monoclonal tumor identity, and indicates origins from a cell which has undergone somatic mutation and transforms prior to isotype switch. This presentation will discuss new findings relating to the cell types that comprise WM, the clonal origins, cytogenetics and familial relationships in WM. It will also address novel gene profiling and proteomic analysis of WM, comparative analysis of IgH switch region translocations and the relationships between IgM myeloma and WM, dysregulation of apoptosis and potentially oncogenic overexpression of hyaluronan synthases in WM. The identification of molecular WM signatures will enable the extent of minimal disease to be readily monitored and early relapse detected. In addition, with the impending availability of sensitive molecular tests to detect the WM signature, the potential exists to identify novel therapies that may be better able to eradicate WM clonal populations.
WM is an uncommon lymphoproliferative disorder characterized primarily by bone marrow infiltration and IgM monoclonal gammopathy. It should be considered a distinct clinicopathological entity rather than a clinical syndrome secondary to IgM secretion. The underlying pathological diagnosis in WM is lymphoplasmacytic lymphoma as defined by the WHO and REAL classification criteria. The concentration of monoclonal IgM can vary widely in WM and it is not possible to define a concentration, which reliably distinguishes WM from MGUS and other lymphoproliferative disorders. A diagnosis of WM can therefore be made irrespective of IgM concentration if there is evidence on a bone marrow trephine biopsy of bone marrow infiltration by lymphoplasmacytic lymphoma with predominantly an intertrabecular pattern and this is supported by marrow infiltration by lymphoplasmacytic lymphoma with evidence on a bone marrow trephine biopsy of bone marrow infiltration also justifies treatment. The presence of anemia with a hemoglobin value of $\leq 10 \text{ g/dL}$ or a platelet count $<100 \times 10^9 / \text{L}$ due to marrow infiltration also justifies treatment. Certain complications such as hyperviscosity syndrome, symptomatic sensorimotor peripheral neuropathy, systemic amyloidosis, renal insufficiency, or symptomatic cryoglobulinemia may also be indications for therapy. Recommendations for follow-up of watch and wait patients are that patients with monoclonal gammopathy of undetermined significance (MGUS) should have serum protein electrophoresis repeated each year. Patients with asymptomatic (smoldering) macroglobulinemia should be followed every six months.

Regarding prognostic markers, hemoglobin and beta-2 microglobulin levels at diagnosis are important prognostic markers in WM: they influence the timing of treatment and survival. Age is consistently important prognostic factors for survival. However, the panel felt that current data are inadequate to support the use of any prognostic marker to select the timing and type of therapy, and called for studies on the application of prognostic markers in WM.

**TREATMENT RECOMMENDATIONS IN WALDENSTROM’S MACROGLOBULINEMIA:**

**CONSENSUS PANEL III RECOMMENDATIONS FROM THE SECOND INTERNATIONAL WORKSHOP ON WALDENSTROM’S MACROGLOBULINEMIA**

Morie A. Gertz$, Athanasios Anagnostopoulos$, Kenneth Anderson$, Andrew P. Branagan$, Morton Coleman$, Stan Frankel$, Sergio Giralt$, Todd Levine$, Nikhil Munshi$, Alan Pestrnak$, Vincent Rajkumar$, and Steven P. Treon$

Mayo Clinic, Rochester, MN, USA; Dana Farber Cancer Institute and Harvard Medical School, Boston, MA, USA; Mayo Clinic Rochester, MN, USA; National Cancer Institute, Bethesda, MD, USA; Niguarda Ca’Granda Hospital, Milan, ITALY; University of Salamanca, Salamanca, SPAIN; University of Athens, Athens, Greece.

The faculty adopted the following statements for the management of patients with Waldenstrom’s macroglobulinemia:

i) Alkylating agents, nucleoside analogues, and rituximab are reasonable choices for first line therapy of WM;

ii) Both cladribine and fludarabine are reasonable choices for the therapy of WM;

iii) Combinations of alkylating agents, nucleoside analogues, or rituximab should at this time be encouraged in the context of a clinical trial;

iv) In WM, rituximab can cause a sudden rise in serum IgM and viscosity levels in certain patients which may lead to complications, therefore close monitoring of these parameters and symptoms of hyperviscosity is recommended for WM patients undergoing rituximab therapy;

v) For relapsed disease, it is reasonable to use an alternate first line agent or re-use of the same agent; however, since autologous stem cell transplantation may have a role in treating patients with relapsed disease it is recommended that in patients who autologous transplantation is seriously being considered that these patients should have limited exposure to alkylator or nucleoside analogue drugs;

vi) Combination chemotherapy for patients who can tolerate myelotoxic therapy, thalidomide alone or with dexamethasone are reasonable choices for relapsed patients.

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**CLINICOPATHOLOGICAL DEFINITION OF WALDENSTROM’S MACROGLOBULINEMIA: CONSENSUS PANEL I RECOMMENDATIONS FROM THE SECOND INTERNATIONAL WORKSHOP ON WALDENSTROM’S MACROGLOBULINEMIA.**

Robert A. Kyle$, Steven P. Treon$, Raymond Alexanian$, Bart Barlogie$, Magnus Bjorkholm$, Madhav Dhodapkar, T. Andrew Lister$, Giampaolo Merlini$, Pierre Morel$, Marvin Stone$, Andrew R. Branagan$, Veronique Leblond$

Mayo Clinic, Rochester, MN, USA; Dana Farber Cancer Institute and Harvard Medical School, Boston, MA, USA; The University of Texas M.D. Anderson Cancer Center, Houston, TX, USA; Myeloma Institute for Research and Therapy, Little Rock, AR, USA; Department of Medicine, Karolinska Hospital and Institute, Stockholm, SWEDEN; Laboratory of Tumor Immunology and Immunotherapy, The Rockefeller University, New York, NY 10021, USA; Department of Oncology, St Bartholomew’s Hospital, London, UK; Scientific Biotechnology Research Laboratories, University Hospital IRCCS Policlinico San Matteo, Pavia, ITALY; Service d’Hematologie Clinique, Centre Hospitalier Schaffner, Lens, FRANCE; Baylor Charles A. Sammons Cancer Center, Dallas, TX, USA; and Departement d’Hematologie, Hopital Pitié-Salpêtriers, AP-HP, Paris, FRANCE.$

The panel recommended that initiation of therapy should not be based on the IgM level per sé since this may not correlate with the clinical manifestations of WM. The consensus panel agreed that initiation of therapy was appropriate for patients with constitutional symptoms such as recurrent fever, night sweats, fatigue due to anemia, or weight loss. The presence of progressive, symptomatic lymphadenopathy or splenomegaly provide additional reasons to begin therapy. The presence of anemia with a hemoglobin value of $\leq 10$ g/dL or a platelet count $<100 \times 10^9 / \text{L}$ due to marrow infiltration also justifies treatment. Certain complications such as hyperviscosity syndrome, symptomatic sensorimotor peripheral neuropathy, systemic amyloidosis, renal insufficiency, or symptomatic cryoglobulinemia may also be indications for therapy. Recommendations for follow-up of watch and wait patients are that patients with monoclonal gammopathy of undetermined significance (MGUS) should have serum protein electrophoresis repeated each year. Patients with asymptomatic (smoldering) macroglobulinemia should be followed every six months.
vii) Autologous stem cell transplantation may be considered for patients with refractory or relapsing disease.

viii) Allogeneic transplantation should only be undertaken in the context of a clinical trial.

ix) Plasmapheresis should be considered as interim therapy until definitive therapy can be initiated.

x) Rituximab should be considered for patients with IgM-related neuropathies.

xi) Corticosteroids may be useful in the treatment of symptomatic mixed cryoglobulinemia.

xii) Splenectomy is rarely indicated but has been used to manage painful splenomegaly and hypersplenism.

UNIFORM RESPONSE CRITERIA IN WALDENSTROM’S MACROGLOBULINEMIA: CONSENSUS PANEL IV RECOMMENDATIONS FROM THE SECOND INTERNATIONAL WORKSHOP ON WALDENSTROM’S MACROGLOBULINEMIA

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Although previous response criteria for Waldenstrom’s macroglobulinemia (WM) have generally incorporated parameters for monoclonal protein reduction and/or improvement of marrow/nodal involvement, specific and uniform response criteria are needed. This is of particular importance as new agents are developed and evaluated. During the Second International Workshop on Waldenstrom’s Macroglobulinemia, Consensus Panel IV proposed the following response criteria:

**Complete Response (CR)**
- Complete disappearance of serum and urine monoclonal IgM by immunofixation, resolution of adenopathy/organomegaly, and no signs or symptoms that are directly attributable to Waldenstrom’s macroglobulinemia (unexplained recurrent fever > 38.4°C, drenching night sweats, > 10% body weight loss, hyperviscosity, or symptomatic cryoglobulinemia. Absence of malignant cells by bone marrow histologic evaluation is required.
- Reconfirmation of the CR status is required at least 6 weeks later.

**Partial Response (PR)**
- A > 50% reduction of serum monoclonal IgM concentration on protein electrophoresis, and > 50% improvement in bulky adenopathy/organomegaly on CT scan. No new signs, symptoms, or other evidence of disease.

**Not Evaluable (NE)**
- Insufficient data/time for a determination of response to treatment.

**Progressive Disease (PD)**
- A greater than 25% increase in serum IgM monoclonal protein levels from the lowest attained response value as determined by serum electrophoresis, confirmed by at least one other investigation, or progression of clinically significant disease related symptom(s).

**Relapse from CR**
- Reappearance of serum IgM monoclonal protein levels as determined by immunofixation studies, confirmed by at least one other investigation, or progression of clinically significant signs or symptoms attributable to disease, or development of any other clinically significant disease related symptom(s).

The panel also recommended that evidence of PD or relapse from CR should not necessarily indicate that at this juncture therapy needs to be re-initiated, and that the criteria proposed by Consensus Panel Two with regard to criteria for initiation of therapy should apply in these circumstances.
Plenary Sessions

1. Development of normal and malignant plasma cell

P1.1 PRIMARY IGH TRANSLOCATIONS AND D-TYPE CYCLIN EXPRESSION PROVIDE A NOVEL MOLECULAR CLASSIFICATION OF MULTIPLE MYELOMA

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During the secondary immune response activated B cells undergo IgH somatic mutation and isotype switch recombination then home to the bone marrow and differentiate into long-lived plasma cells. Multiple myeloma is the malignant counterpart of these cells. We hypothesized that errors in these B cell specific DNA modification processes may be responsible for IgH translocations in MM, and developed a Southern blot assay to identify them. Identification and cloning of translocation breakpoints in MM has identified two types of IgH translocations. Primary translocations have breakpoints that cluster within the switch and JH regions (11q13, 6p21, 4p16, and 16q23), while secondary translocations have breakpoints outside of these regions (8q24, 6p25, 20q11, other non-recurrent partners). We postulate that these primary translocations, which occur in 40% of MM patients at diagnosis, are early, disease-defining events. Other unidentified IgH translocations (not 11q13, 6p21, 4p16, or 16q23) are identified in 20% of patients at diagnosis, although the frequency of primary and secondary translocations within this group is unknown. Finally, 40% of patients do not have IgH translocations at diagnosis, and the molecular pathogenesis of this subset remains enigmatic.

The primary translocations identify homogeneous groups of patients with similar phenotypic features and response to treatments. Seventy-five percent of patients with t(4;14) ectopically express FGFR3 and proliferate abnormally in response to FGF. Inhibition of FGFR3 signaling in these MM inhibits FGF induced growth, and in cell lines with activating mutations can induce growth arrest followed by differentiation, then apoptosis. Ectopic expression of c-maf, most pronounced in t(14;16) MM, results in expression of integrin beta 7, increased adhesion to E-cadherin and stroma and increased expression of cyclin D2, a direct transcriptional target of cyclin D2. Inhibition of c-maf inhibits growth and tumorigenicity in nude mice of c-maf-expressing MM cell lines. These results demonstrate the importance of these translocations to MM biology and validate the genes dysregulated by these translocations as attractive targets for drug development.

In addition these results highlight the importance of cyclin D dysregulation, either directly (Cyclin D1 - 11q13, Cyclin D3 - 6p21) or indirectly (Cyclin D2 – 16q23, 4p16), as a common pathway dysregulated by IgH translocation in MM. If we examine the expression of cyclin D in a large group of MM patients (200) it is evident that almost all patients (not only those with translocations) ectopically express a single cyclin D, a result incongruous with such a low proliferative tumor. Therefore dysregulation of the cyclin D pathway provides a unifying hypothesis for myelomagenesis. In this analysis, 60% (119/200) of patients do not have a primary translocations, with 35% (70/200) that express cyclin D1 (at a level below that seen with t(11;14)), 20% (40/200) that express cyclin D2, and 5% (9/200) without a predominant cyclin D. This large group of patients with a low level of cyclin D1 expression and lacking a t(11;14) (that we call D1 lo) have a distinct gene expression profile suggesting that they represent a homogeneous population of patients. We do not observe MM cell lines with this phenotype, and postulate that they may be particularly dependent on the bone marrow microenvironment for their growth and survival. We postulate that this group of patients represents an important new molecular subtype of MM that may include most of the patients lacking IgH translocations. In a recent cytogenetic analysis the prognostic importance of ploidy has been noted, with hypodiploid MM associated with a worse prognosis (median OS 12.6 mo) and more frequent IgH translocation (56%), while hyperdiploid MM had a better prognosis (median OS 33.8 mo), and less frequent IgH translocations (11%). This suggests that the D1 lo group, lacking primary IgH translocations, overlaps significantly with this hypodiploid group.

Based on this analysis of the data generated by Avet-Loiseau and the IFM3, Fonseca and the Mayo group4, and Shaughnessy and the Arkansas group5, and Smadja, Bastard and the Groupe Francais de Cytogenetique Hematologique6, we propose the following simple molecular classification of MM:

**Translocation and Cyclin D (TC) Molecular Classification of MM v1.0**

<table>
<thead>
<tr>
<th>Class</th>
<th>D1</th>
<th>D2</th>
<th>D3</th>
<th>Diploid</th>
<th>Incidence</th>
<th>Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>TC1</td>
<td>6p21</td>
<td>11q13</td>
<td>16q23</td>
<td>Non-hyper</td>
<td>3%</td>
<td>88%</td>
</tr>
<tr>
<td>TC2</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>Non-hyper</td>
<td>15%</td>
<td>75%</td>
</tr>
<tr>
<td>TC3</td>
<td>None</td>
<td>None</td>
<td>None</td>
<td>?Hyper</td>
<td>3%</td>
<td>74%</td>
</tr>
<tr>
<td>TC4</td>
<td>4p16</td>
<td>16q23</td>
<td>Non-hyper</td>
<td>Non-hyper</td>
<td>6%</td>
<td>23%</td>
</tr>
</tbody>
</table>

*Overall Survival at 80 months from Moreau et al, 2002.

This model has important implications for identifying genetically homogeneous groups of patients for treatment protocols, and defines two distinct groups (TC2 and TC3) in need of greater molecular dissection.


P1.2 IG TRANSLOCATIONS, CYCLIN D DYSREGULATION, AND OTHER GENETIC EVENTS IN MULTIPLE MYELOMA

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MM is post-germline center tumor of bone marrow plasmablasts/plasma cells. Germininal center B cells modify DNA by sequential rounds of somatic hypermutation and antigen selection, and IgH switch recombination. Post-germinal center B cells can generate plasmablasts (PB) that migrate to the bone marrow (BM), where stromal cells enable terminal differentiation into long-lived plasma cells (PC). MM, a low proliferative tumor that corresponds to long-lived PB/PC, often is preceded by a pre-malignant MGUS tumor, and more proliferative progresses to extramedullary MM. Virtually all human MM cell lines (HMCL) come from extramedullary MM, including primary PC leukemia that occurs without apparent intramedullary MM.

Ig translocations: frequent and often early events in the pathogenesis of MM. Ig translocations dysregulate oncogenes by juxtaposing them near one of the strong Ig enhancers. Translocations usually involve the IgH locus, whereas the IgL locus is involved infrequently, and the Igk locus rarely. The prevalence of IgH translocations appears to be related to the stage of the disease: about 50% in MGUS, 55-70% in intramedullary MM, >80% in extramedullary MM, and more than 90% in HMCL. Primary Ig translocations occur in germinal center B cells, usually a result of errors in IgH switch recombination, or less often somatic hypermutation.

Secondary (Ig) translocations occur during tumor progression. Secondary translocations are mediated by different mechanisms than most primary translocations, since B cell specific DNA modification processes are inactive in normal or malignant PB/PC. Features that often distinguish secondary translocations include: breakpoints not within or near IgH switch or V(D)J sequences, complex and unbalanced translocations or insertions, heterogeneity within a tumor, and sometimes lack of involvement of an Ig enhancer. The dysregulation of c-, N-, or L-MYC, which usually occurs as a very late progression event, provides a paradigm for secondary translocations. The increased prevalence of Ig translocations at later stages of disease and in HMCL probably is explained partly by secondary Ig translocations but also by selective progression of tumors with Ig translocations.

Different oncogenes for primary and secondary translocations. Four recurrent chromosomal loci (oncogenes) are involved in primary translocations: 11q13 (cyclin D1); 6p21 (cyclin D3); 4p16 (MMSET; FGFR3); 16q23 (c-maf); and these account for IgH translocations in about 40% of tumors. Loci involved in secondary translocations include: 8q24 (c-myc), 2p23 (N-myc), 20q12 (maf B), and 6p25 (MUM-1/IRF-4), but these probably account for IgH translocations in only 5% of tumors. Non-recurrent loci are involved in IgH translocations in nearly 20% of tumors, but the fraction of primary vs secondary translocations is unclear.

Dysregulation of cyclin D1, 2, or 3: a possible early, unifying event in MM. Despite the low proliferative index of MM tumors, microarray expression analyses indicate that most tumors express one of the cyclin D genes at a level that is similar to proliferating PB, and distinctly higher than quiescent PC. The four recurrent primary translocations appear to lead directly (11q13 - cyclin D1 or 6p21 - cyclin D3), or indirectly (4p16 and 16q23 - cyclin D2) to cyclin D dysregulation. Another 20% of MM tumors express cyclin D2, the major cyclin D gene expressed by normal PB. Remarkably, however, despite the lack of expression of cyclin D1 in normal hematopoietic cells, about one third of MM tumors express substantial levels of cyclin D1 in the absence of a t(11;14) translocation; the mechanism responsible for cyclin D1 expression is obscure, but it is notable that this group of tumors (that we call D1 lo) is represented rarely, if ever, by HMCL. Perhaps, cyclin D1 expression requires a continued interaction of the MM tumor cell and BM stromal cells.

Other oncogenic events in MM. Numeric (and possibly structural) karyotypic abnormalities, including monosomy of chromosome 13 or deletion of 13q14, are often present in MGUS, but the timing and molecular consequences of these abnormalities is unclear. Activating mutations of K- or N-RAS [or FGFR3 in tumors with (4;14)] are absent or rare in MGUS, but present in 40% of early MM, and perhaps 50% of advanced MM; this may represent a marker if not a cause of progression from MGUS to MM. Dysregulation of c-MYC, and mutations or mono-allelic deletion of p53 appear to occur as very late progression events. Presumably telomerase or the ALT pathway is activated during tumorigeneis, but it is uncertain how or when this occurs. Disruption of the Rb pathway occurs in most human tumors; but even though dysregulation of cyclin D appears to be a universal early event in MM, inactivation of RB or INK4 cyclin dependent kinase inhibitors (p16INK4a and p18INK4c) can occur during tumor progression and further disrupt the RB pathway.

P1.3 THE MYELOMA HIERARCHY: HETEROGENEITY WITHIN THE MYELOMA CLONE

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Early stage members of the multiple myeloma (MM) clone contribute to disease progression: A variety of evidence suggests that MM represents a hierarchy of monoclonal B lineage cells in the blood and BM that includes late stage B cells and plasma cells, pre-B-like cells and slgM+ pre-switch B cells. Others have described circulating cells expressing the MM idiotype, and circulating cells expressing the unique clonotypic IgH VDJ are readily detectable in blood. We have shown that MM includes circulating B cells that persist despite chemotherapy and may mediate relapse. Even though MM plasma cells are often severely depleted in response to therapy, residual disease and bone lesions usually persist, suggesting a failure of therapy to restore normal bone remodeling and the involvement of clinically cryptic components of the MM clone. The heterogeneity seen among ex-vivo clonotypic B and plasma cells suggests an in vivo hierarchy of sequentially related differentiation stages. These early stage members of the MM clone are morphologically cryptic in that their physical appearance and phenotypic profiles are largely normal, preventing their identification using as “morphologically abnormal” cells. Only their expression of the
clonotypic IgM VDJ signature confirms their relationship within the malignant clone. Our observations imply that circulating, early stage components of the MM clone are clinically important. 1) Circulating clonotypic MM B cells are able to produce and secrete IL-6 ex vivo (1), and strongly express CD31, the ligand for CD38, predicted to facilitate paracrine interactions with the osteoclast lineage and to exacerbate bone resorption in MM. Clonotypic MM B cells express surface IL-6 receptor a subunit, a prerequisite for autocrine and/or paracrine stimulatory loops. 2) Xenografted early stage MM B cells give rise to lytic bone disease, clonotypic progeny and are self-renewing in murine BM. 3) Hematopoietic progenitor fractions of G-CSF mobilized blood, comparable to those used for autotransplants and shown to include clonotypic MM components, and MM B cells, engraft the myeloma clone and generate bone lesions in xenografted mice. 4) Chemotherapy-resistant, pre-switch progenitors of the MM clone correlate with advanced disease at diagnosis and with significantly reduced survival (2). Direct evidence that circulating, pre-switch progenitors have a strong influence on MM progression comes from longitudinal analysis showing a strong correlation between persistent, and thus drug-resistant, pre-switch MM cells and reduced survival (p<0.0001). This reinforces the idea that evaluation of progenitor function in MM must take into account the parameters of time and maturation stage. 5) MM cells with the t(4;14) IgH switch translocation circulate in blood throughout the course of disease, and are present in mobilized autografts (3).

Despite phenotypic similarity to hematopoietic progenitors or immature B cells, all members of the MM clonotypic hierarchy are relatively late stage cells based on their IgH VDJ sequence. Even among phenotypically immature stages, including the MM CD34+ progenitor-like fractions and pre-switch MM B cells, the clonotypic IgH VDJ is maintained as a sequence that is identical to that expressed by end stage plasma cells. For all the MM B cell subpopulations detected, the IgH VDJ sequence is somatically mutated suggesting a post-germinal center, memory B cell phenotype. Further, even clonotypic IgM from preswitch progenitors is clonally homogeneous (4), indicating that MM B cells synthesizing clonotypic IgM have escaped from antigen-driven selective processes and from control by regulatory T cells, consistent with the ability of cells expressing clonotypic IgM to engraft mice and the strong clinical impact of drug resistant B cells expressing clonotypic IgM. The extensive body of evidence characterizing these persistent members of the malignant clone supports the contention that they are responsible for highly significant and potentially multifaceted contributions to the origins and progression of MM.

Genetic abnormalities among early stage MM cells: The RHAMM oncogene is overexpressed in all MM patients analyzed to date. RHAMM oncosgene in MM may result from dysregulated gene splicing as well as from increased levels of RHAMM. We have observed increased expression of RHAMM exon 4 splicing contributes to disease progression through alterations in protein function. Patients with increased levels of exon 4 splicing had significantly reduced survival compared to patients having low levels of exon 4 splicing (see abstract by Reiman et al.). Further, the extent of RHAMM exon 4 splicing in circulating MM cells predicts for disease activity (abstract by Reiman et al.). Although itinerant in its cellular localization, RHAMM is a centrosomal protein. Dysregulation of RHAMM results in highly abnormal centrosomes (5) and apparent chromosomal misseggregation. RHAMM may contribute to extensive chromosomal instability by transiently compromising separation of the mitotic spindles, consistent with the pervasive and complex chromosomal abnormalities, found in MM, perhaps resulting from the extensive centrosomal abnormalities detected in MM (see abstract by Reimall et al.). Centrosomal abnormalities may also impact DNA replication in MM. Consistent with this, circulating, clonotypic MM B cells are hyperdiploid, with 3-30% excess DNA. After culture in colchicine to arrest mitosis, hyperdiploid B cells were reduced and MM B cells accumulated in a diploid G2/M, suggesting that hyperdiploidy in MM may represent a transient S phase arrest (6), perhaps a result of RHAMM-mediated centrosomal abnormalities. Hyaluronan synthase produces hyaluronan, a ligand for RHAMM. We have identified a novel variant of HAS1 (HAS1Vb) that appears to be restricted to malignant B cells (see abstract by Adamia et al.). HAS1Vb in circulating B cells provides a significant indicator of poor survival that measures attributes of circulating malignant B cells, providing further evidence in support of a key role for early stage MM cells in malignant progression and suggesting potential mechanisms involving HA, RHAMM and mitotic abnormalities through which MM B cells may impact on disease progression.


P1.4 Comparison of gene expression profiling of normal plasmaBLASTIC cells and malignant plasma cells
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In order to improve the knowledge and treatment of multiple myeloma (MM), it would be useful to obtain normal cells with a phenotype as close as possible to that of malignant cells. These cells would allow the identification of genes that are deregulated in tumor plasma cells, and that may be involved in the emergence...
of the disease or may code for tumor antigens useful for immunotherapy strategies. In addition to the identification of myeloma-specific genes, obtaining plasma cells in vitro can be a helpful tool for studying the critical factors involved in the terminal step of B-cell differentiation. However, plasma cells are very rare cells in vivo, representing only 1% to 2% of tonsillar mononuclear cells and less than 0.5% of bone marrow cells in healthy individuals. Such polyclonal plasma cells cannot be routinely purified from normal donors and MM patients. Therefore, one way to get a normal counterpart of malignant plasma cells from MM patients would be to induce in vitro peripheral blood (PB) B-cell differentiation into plasma cells. We have shown that the in vitro differentiation of peripheral blood B lymphocytes, purified from healthy individuals or multiple myeloma patients, makes it possible to obtain a homogeneous population of normal plasmablasts. These cells were identified by their plasmablastic morphology, phenotype (CD20+, CD21+, CD37, CD22+, CD38+), production of polyclonal immunoglobulins, and expression of the major transcription factors involved in B-cell differentiation (Pax5-, bcl-6-, IFR4+, PRDI-BF1+, XBP-1+).

Using Affymetrix microarrays, we have compared the gene expression profiles of highly purified malignant plasma cells from nine patients with multiple myeloma (MM) and eight myeloma cell lines to those of highly purified nonmalignant plasma cells (eight samples) obtained by in-vitro differentiation of peripheral blood B cells. Two unsupervised clustering algorithms clustered these 25 samples into two distinct clusters: a malignant plasma cell cluster and a normal plasma cell cluster. Two hundred fifty genes were significantly up-regulated and 159 down-regulated in malignant plasma samples compared to normal plasma samples. For some of these genes, an overexpression or downregulation of the encoded protein was confirmed (cyclin D1, c-myc, BMI-1, cystatin c, SPARC, RB). Two genes overexpressed in myeloma cells (ABL and cystathionine beta synthase) code for enzymes that could be a therapeutic target with specific drugs. We also found several genes of the cancer testis tumor family overexpressed in myeloma cells (MAGE, SSX).

These data should help to disclose the molecular mechanisms of myeloma pathogenesis and to define new therapeutic targets in this still fatal malignancy. In addition, the comparison of gene expression between plasmablastic cells and B cells provides a new and powerful tool to identify genes specifically involved in normal plasma cell differentiation.

**P1.5 MOLECULAR INSIGHTS INTO THE MULTI-STEP PATHOGENESIS OF MYELOMA**

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A number of lines of evidence, including IgH mutation analysis, adoptive transfer of disease and molecular investigations suggest that the target for therapy in myeloma is the myeloma plasma cell. A full understanding of the molecular events underlying the pathogenesis of this cell should allow us to rationally target novel therapies. Until now the tools to look at pathogenic mechanisms have been limited but more recently a number of new approaches have become available, exponentially increasing the amount of data available to shape our understanding of this process. The classical model put forward to define the multi-step nature of the myeloma process has relied upon the transition of normal through MGUS to myeloma.

The evaluation of the results of Southern analysis of recurrent chromosomal translocations in myeloma cell lines has highlighted the role of aberrant class switch recombination early in the pathogenesis of myeloma. We have used a vectorette PCR approach to characterise examples of the t(4;14) and t(11;14) from patients, which may better reflect processes occurring in vitro. This data shows that breaks in the t(4;14) do occur in the switch region implying that the translocation arises during the process of physiological class switch recombination. However, a second mechanism not involving this physiological recombination has also been identified, where the breaks occur upstream of the switch region, and could be associated either with somatic hypermutation (SHM) or random double strand breaks (DSB). For fully cloned reciprocal translocations the structure of the break identified are compatible with staggered breaks and error prone DNA repair. Non-Homologous end joining (NHEJ) is the major repair mechanism for DSB, and in order to pursue the involvement of this pathway in the predisposition to the development of these translocations we have used a molecular epidemiological approach. We have characterised the effect of inherited variants affecting this pathway and the risk of developing multiple myeloma. In particular we have analysed a large case control series of over 200 patients looking at the distribution of novel lig IV variants and their impact on risk of developing a variety of lymphoid malignancies.

Expression microarrays have provided a new tool with which to analyse myeloma and we have used this technique to examine the patterns of genes altered during the transition of normal through MGUS to myeloma. Approximately 300 genes were differentially expressed between N and multiple myeloma or N and MGUS plasma cells. Whereas less than 100 genes were differentially expressed between MGUS and multiple myeloma. As well as confirming pathways already known to be involved in the pathogenesis of myeloma a number of new pathways including deregulation of developmental genes, transcription factors and changes in chromatin structure have been highlighted. In particular we have demonstrated that FRZB is up regulated strongly implicating this gene and signalling through the WNT and hedgehog pathways in myeloma.

**N 21 GENES**

Transcription - FOXG1A, RING1
Development - ARMET, RB
Cell proliferation

**MGUS 91 GENES**

Transcription - RING1
Signalling - MD2, MACS
Structural - ADD1, VCL

**MM 172 GENES**

Transcription - RING1
Development - FRZB
Survival - TNFSF7
Cell proliferation

**H 22 GENES**

Adhesion - LFA4, GPCR4
DNA repair

**PCL**

In keeping with the multi-step pathogenesis model of myeloma, recent data looking at MGUS suggests that it is not a uniform disease, a point of which is of some importance for the use of microarrays in a ‘class prediction’ fashion. We have performed flow cytometry and identified two distinct phenotypic subsets, which seem to have a different prognosis based on the presence or absence of normal plasma cells. This data is compatible with the work of Zojer et al who used IgH mutation analysis to show that MGUS can be characterised on the basis of ongoing exposure to somatic hypermutation, transition to multiple myeloma being associated with outgrowth of a single clone. Tentatively MGUS
can be considered as two entities, one with exposure to ongoing
mutation with a very long history and the other lacking this
intraclonal variation destined for the early progression and
development of clinical symptoms. Definition of subtypes of
myeloma using arrays need to incorporate these different types of
plasma cell from which myeloma may putatively arise.
References
Davies FE, Morgan GJ. Innovative techniques
Davies FE, Rawstron AC, Owen RG, Morgan GJ. Controversies
surrounding the clonogenic origin of multiple myeloma. Br J
Fenton JAL, Pratt G, Rawstron AC, Sibley K, Rothwell D, Yates
Z, Dring A, Richards SJ, Ashcroft AJ, Davies FE, Owen RG,
Child JA, Morgan GJ. Genomic characterization of the
chromosomal breakpoints of t(4;14) of multiple myeloma
suggests more than one possible aetiological mechanism.
Oncogene 2003, 1-11.
Proffitt J, Fenton J, Pratt G, Yates Z, Morgan G . Isolation and
characterisation of recombination events involving
immunoglobulin heavy chain switch regions in multiple myeloma
using long distance vectorette PCR (LDV-PCR). Leukemia
1999; 1100-1107.
Roddam PL, Rollinson S, O'Driscoll M, Jeggo PA, Jack A,
Morgan GJ (2002). Genetic variants of NHEJ DNA ligase IV can
affect the risk of developing multiple myeloma, a tumour
characterised by aberrant class switch recombination. J Med
Genet: 900-905.
Sibley K, Fenton JA, Dring AM, Ashcroft AJ, Rawstron AC,
Morgan GJ. A molecular analysis of the t(4;14) in multiple
Zojer N, Ludwig H, Fiegl M, Stevenson FK, Sahota SS. Patterns
of somatic mutations in VH genes reveal pathways of Clonal

2. Genetic heterogeneity in MM: impact
on diagnosis and therapy

P2.1 ELEVATED EXPRESSION OF WNT SIGNALING
ANTAGONISTS DKK1 AND FRZB BY MALIGNANT
PLASMA CELLS IS STRONGLY ASSOCIATED WITH
LYTIC BONE DISEASE IN MYELOMA

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Focal lytic bone lesions and systemic osteopenia are major causes
of morbidity and mortality in multiple myeloma (MM). Bone
destruction is linked to increased osteoclast activity, which can be
traced to perturbations in IL-6, MIP1 and RANK signaling.
Bisphosphonates have been shown to be highly effective agents
in reducing the progression of bone lesions, however, a lack of
repair to preexisting damage suggests that a defect in osteoblast
function is also an important component of the bone destruction
process. Indeed, studies have shown that whereas osteoblast
numbers are increased in MM, their functional capacity is
dramatically impaired.

Bone lesions are located adjacent to medullary plasmacytoma
suggesting that MM PC secrete factors that activate osteoclast
and/or induce osteoblast apoptosis. Thus, in an effort to identify
genes linked to bone disease we performed a genome wide scan
for altered gene expression patterns in a comparison of CD138-
enriched PC from newly diagnosed MM with no radiological
evidence of osteolytic bone lesions (n = 87) or ≥ 3 lytic lesions (n
= 83). Of a total of ~12,000 genes studied, 367 were identified as
being significantly differentially expressed (P < .001) using a
combination of 2, Wilcoxin Rank, and SAM statistical analysis.
Of these, 229 were higher and 138 lower in PC from MM with
lytic lesions. Elevated expression of genes associated with cell
proliferation, e.g. PCNA, TYMS, PRKDC, CENPA and TOP2A
was seen in MM with lytic lesions. In contrast ARHE, IL-6R,
WNT10B, and the B-cell receptor molecules SLAM, TACI, and
LNHR, were the most significantly under expressed genes in MM
with lytic lesions. Importantly, the WNT signaling antagonists,
FRZB3 and DKK14, represented the only genes coding for
secreted factors within the top 50 up-regulated genes. Moderately
high expression (Signal >1000) of DKK1 and/or FRZB was seen
in 74% of all MM cases with 46% of all cases expressing both
genes above 1000. Importantly, neither gene was detectable by
microarray in PC from 45 normal bone marrow donors or 10
Waldenstrom’s macroglobulinemia, a PC malignancy that lacks
bone disease. Although FRZB was expressed at similar levels in
MGUS and MM, DKK1 was rarely found in PC from this
condition. Immunohistochemistry of bone biopsies revealed an
inter- and intra-patient variability in expression in MM PC, yet a
strong correlation with DKK1 and FRZB gene expression data. In
patients with high gene expression, protein expression was
heterogeneous and tended to be highest in PC lining the bone.
Interestingly, in cases with low FRZB gene expression intense
FRZB staining was observed in microvessels. Simultaneous

S19
can be considered as two entities, one with exposure to ongoing mutation with a very long history and the other lacking this transformation from MGUS to multiple myeloma. Blood 2003. of somatic mutations in VH genes reveal pathways of Clonal characterisation of the chromosomal breakpoints of t(4;14) of multiple myeloma suggests more than one possible aetiological mechanism. Oncogene 2003, 1-11. Isolation and characterisation of recombination events involving immunoglobulin heavy chain switch regions in multiple myeloma using long distance vectorette PCR (LDV-PCR). Leukemia 1999; 1100-1107. Genetic variants of NHEJ DNA ligase IV can affect the risk of developing multiple myeloma, a tumour characterised by aberrant class switch recombination. J Med Genet: 900-905. A molecular analysis of the t(4;14) in multiple myeloma. Br J Haematol 2002, 514-520.

Proffitt J, Fenton J, Pratt G, Yates Z, Morgan GJ. Innovative techniques for altered gene expression patterns in a comparison of CD138-enriched PC from newly diagnosed MM with no radiological evidence of osteolytic bone lesions (n = 87) or ≥ 3 lytic lesions (n = 83). Of a total of ~12,000 genes studied, 367 were identified as being significantly differentially expressed (P < .001) using a combination of 2, Wilcoxon Rank, and SAM statistical analysis. Of these, 229 were higher and 138 lower in PC from MM with lytic lesions. Elevated expression of genes associated with cell proliferation, e.g. PCNA, TYMS, PRKDC, CENPA and TOP2A was seen in MM with lytic lesions. In contrast ARHE, IL-6R, WNT10B, and the B-cell receptor molecules SLAM, TACI, and LNHR, were the most significantly under expressed genes in MM with lytic lesions. Importantly, the WNT signaling antagonists, FRZB and DKK1, represented the only genes coding for secreted factors within the top 50 up-regulated genes. Moderately high expression (Signal >1000) of DKK1 and/or FRZB was seen in 74% of all MM cases with 46% of all cases expressing both genes above 1000. Importantly, neither gene was detectable by microarray in PC from 45 normal bone marrow donors or 10 Waldenstrom’s macroglobulinemia, a PC malignancy that lacks bone disease. Although FRZB was expressed at similar levels in MGUS and MM, DKK1 was rarely found in PC from MM with lytic lesions. Elevated expression of genes associated with cell proliferation, e.g. PCNA, TYMS, PRKDC, CENPA and TOP2A was seen in MM with lytic lesions. In contrast ARHE, IL-6R, WNT10B, and the B-cell receptor molecules SLAM, TACI, and LNHR, were the most significantly under expressed genes in MM with lytic lesions. Importantly, the WNT signaling antagonists, FRZB and DKK1, represented the only genes coding for secreted factors within the top 50 up-regulated genes. Moderately high expression (Signal >1000) of DKK1 and/or FRZB was seen in 74% of all MM cases with 46% of all cases expressing both genes above 1000. Importantly, neither gene was detectable by microarray in PC from 45 normal bone marrow donors or 10 Waldenstrom’s macroglobulinemia, a PC malignancy that lacks bone disease. Although FRZB was expressed at similar levels in MGUS and MM, DKK1 was rarely found in PC from this condition. Immunohistochemistry of bone biopsies revealed an inter- and intra-patient variability in expression in MM PC, yet a strong correlation with DKK1 and FRZB gene expression data. In patients with high gene expression, protein expression was heterogeneous and tended to be highest in PC lining the bone. Interestingly, in cases with low FRZB gene expression intense FRZB staining was observed in microvessels. Simultaneous

References Davies FE, Morgan GJ. Innovative techniques for altered gene expression patterns in a comparison of CD138-enriched PC from newly diagnosed MM with no radiological evidence of osteolytic bone lesions (n = 87) or ≥ 3 lytic lesions (n = 83). Of a total of ~12,000 genes studied, 367 were identified as being significantly differentially expressed (P < .001) using a combination of 2, Wilcoxon Rank, and SAM statistical analysis. Of these, 229 were higher and 138 lower in PC from MM with lytic lesions. Elevated expression of genes associated with cell proliferation, e.g. PCNA, TYMS, PRKDC, CENPA and TOP2A was seen in MM with lytic lesions. In contrast ARHE, IL-6R, WNT10B, and the B-cell receptor molecules SLAM, TACI, and LNHR, were the most significantly under expressed genes in MM with lytic lesions. Importantly, the WNT signaling antagonists, FRZB and DKK1, represented the only genes coding for secreted factors within the top 50 up-regulated genes. Moderately high expression (Signal >1000) of DKK1 and/or FRZB was seen in 74% of all MM cases with 46% of all cases expressing both genes above 1000. Importantly, neither gene was detectable by microarray in PC from 45 normal bone marrow donors or 10 Waldenstrom’s macroglobulinemia, a PC malignancy that lacks bone disease. Although FRZB was expressed at similar levels in MGUS and MM, DKK1 was rarely found in PC from this condition. Immunohistochemistry of bone biopsies revealed an inter- and intra-patient variability in expression in MM PC, yet a strong correlation with DKK1 and FRZB gene expression data. In patients with high gene expression, protein expression was heterogeneous and tended to be highest in PC lining the bone. Interestingly, in cases with low FRZB gene expression intense FRZB staining was observed in microvessels. Simultaneous
immunofluorescence staining for DKK1 and cytoplasmic immunoglobulin light chain (cIg) on cytospin preparations from MM bone marrow aspirates confirmed the heterogeneous expression pattern in clonotypic PC and also revealed DKK1 in a subset of cells with neutrophil nuclear morphology. A similar analysis on normal healthy donors revealed cytoplasmic DKK1 expression unique to 1/3 to 1/2 of cIg-positive plasma cells. A quantitative ELISA for DKK1 in sera from bone marrow and peripheral blood showed that normal bone marrow aspirates contained a mean of 10ng/ml with no case showing greater than 15ng/ml. One the other hand, MM cases could have as much as 125 ng/ml in both the bone marrow and peripheral blood. There was a strong correlation between plasma levels of DKK1 protein and DKK1 gene expression.

Since lytic bone lesions develop at sites of MRI-defined plasmacytoma (MPCT), MRI represents a highly sensitive surrogate for present and future osteolytic lesions. As MRI-defined MPCT can be observed in the absence of x-ray detectable lytic lesions, we hypothesized those cases lacking lytic lesions, yet having high DKK1 and/or FRZB gene expression levels may have underlying MRI detectable MPCT. To address this issue we combined x-ray and MRI data and applied the same analyses used above to analyze differences between those cases with ≥3 lytic lesions and MPCT (n = 65) and MM with no lytic lesions or MPCT (n = 45). A total of 107 genes differentiating the two groups (P < .001) were identified. Here many of the same genes, e.g. cell cycle genes, identified above remained significant and, importantly, the degree of significance increased. For example, whereas the ratio (≥3 lytic lesions/no lytic lesions) of the mean expression level for DKK1 in the first comparison was 2.45, the mean value increased to 6.25 in the latter comparison. This reflected the fact that virtually all cases with no lytic lesions and moderate to high DKK1, had MRI-defined focal lesions. The mean expression level of DKK1 in the no lytic lesion group was 1674 (range 40 to 10828) whereas the mean DKK1 level in the no lytic lesion & no MPCT group dropped to 625 (range 57 to 4183). It is important to note that DKK1 and FRZB expression, as determined from bone marrow aspirates of the iliac crest, although very powerful, can not account for the presence of bone lesions in all patients, as 10 of 83 (12%) of cases with >3 x-ray lesions did not express appreciable levels of DKK1 or FRZB. A quantitative trait locus (QTL) for low bone mass in the general population has been identified and may enhance bone loss, even in the presence of low levels of DKK1 and FRZB. Alternatively, Wnt signaling antagonism-independent mechanisms also certainly play a role in bone disease.

We have recently shown that expression of the cell cycle control and DNA metabolism genes TYMS, UBE2C, CCNB1, PCNA, TK1, BUB1, BUB1B, EZH2, and TOP2A is significantly higher in MM with metaphase cytogenetic abnormalities and that these features are linked to poor survival. These same genes were also over-expressed in MM with lytic lesions and MPCT and enhanced in cases with both, suggesting this type of MM is also likely to have a high proliferation index, thus providing a molecular explanation for the classification of MM with ≥3 lytic lesions as stage III disease in the Durie-Salmon system.

Although exhibiting highly variable and sometimes very high expression in MM PC, MPI (CCL3/SCYA3), a chemokine implicated in OCL development and MM bone disease, was not significantly differentially expressed in this analysis. In addition, RANKL, a known osteoclast differentiation factor with conflicting data over its expression on MM PC 3,4 was not detected in any MM PC or normal bone marrow PC sample tested with our microarray system.

The relevance of elevated DKK1 and FRZB expression in MM bone disease is derived from several recent studies that have shown that functional Wnt signaling is critical for osteoblast differentiation and function. Patients with loss-of-function mutations in the low-density lipoprotein receptor-related protein 5 (LRP5), a co-receptor for the Wnt ligand, have a condition known as osteoporosis-pseudoglioma (OPPG). Remarkably, separate and distinct mutations in LRP5 result in a high bone mass (HBM) phenotype. In contrast to the OPGP mutations, the HBM defects represent gain-of-function mutations that effectively block binding of the inhibitory protein DKK1. Elevated expression of FRZB results in a block in chondrocyte maturation and function. Thus, data presented here suggests that MM PC exert a powerful negative effect on bone growth through the secretion of two independent WNT signaling antagonists that likely interfere with osteoblast function.

Increasing evidence has demonstrated that genetic factors are involved in the pathogenesis of multiple myeloma. Genetic alterations or gene deregulation influences not only the initiation of disease but disease progression and therapeutic response. One of the difficulties in predicting disease progression and therapeutic response is the genetic heterogeneity in malignant plasma cells – both at the level of deregulated levels of expression and at the level of genetic variations that alter protein function. Moreover, reprogramming of the bone marrow microenvironment has been shown to play an important role in stimulating plasma cell growth, altering therapeutic response, and contributing to secondary complications. Among the factors induced in bone marrow stromal cells, IL-6 has been shown to play a prominent role in plasma cell proliferation. One of the most common genetic alterations in myeloma plasma cells (40-50% of patients) is the activating mutations in the \( ras \) family of oncogenes. Furthermore, cells with mutant \( ras \) show resistance to a variety of therapeutic agents. Recent evidence from gene expression profiles demonstrates myeloma plasma cells can be distinguished from their normal plasma cell counterpart. However, different proliferative signals might be expected to influence different sets of genes. In order to distinguish the contributions made by IL-6, stromal cell contact, or mutant \( ras \) activation, we have compared the gene expression profile of myeloma cell lines grown in IL-6, stromal co-culture and cells stably transfected with a mutant \( Nras \) gene. A simple expectation was that mutant \( Nras \) may induce a subset of genes seen in IL-6 response, and IL-6 response would provide a subset of the pattern derived from stromal interactions. However, our results show a more complex pattern of expression induced by the three conditions of growth induction.

With support from the Multiple Myeloma Research Foundation we developed gene expression profiles using the Affymetrix U95A GeneChip containing 12,626 known genes. Cell cycle analysis demonstrated that the myeloma derived ANBL-6 cell line could be significantly induced to proliferate by addition of IL-6, or by growth in co-culture with bone marrow stromal cells, or after stable expression of the mutant \( Nras61 \) gene. Six untreated controls, four IL-6 treated cell cultures, three mutant \( ras \) containing cultures, and five co-cultures with bone marrow stroma (3 normal; 2 patient derived) were analyzed. Hierarchical clustering was used to visualize groups of genes that showed common and distinct expression patterns under the four conditions. From this analysis we were able to identify signature gene expression patterns that defined each of the proliferative signals, including sets of genes that were up-regulated as well as down-regulated. 138 genes were identified that were significantly differentially expressed when comparing untreated cells with IL-6 treated cells. Not surprisingly, the highest percentage represented cell cycle genes (54%). 84 genes were differentially expressed in comparisons of IL-6 and stromal co-cultures; with a distinct set of genes differentially expressed from the stromal interactions, that were not identified in IL-6 responses. A high percentage of these were extracellular matrix associated genes and chemokines. Interestingly, there were 130 genes that distinguish IL-6 and mutant \( ras \) responses, with patterns suggesting that mutant \( ras \) does not simply induce a common subset of IL-6 response genes. Additional comparisons and specific gene patterns will be presented that demonstrate the similarities and differences in gene expression among these common myeloma cell responses.

**Hierarchical Clustering Analysis**

Notably, of the 30 most differentially expressed genes that distinguish MM1 and MM4 in the patient expression profiles, we could account for 24 of these derived from one or more of the conditions we assayed. Some genes showed induced expression in all treatments, others were induced specifically by only one of the conditions examined. RT-PCR confirms common or differential expression patterns. One gene of interest that was induced is the EZH2 gene, a polycomb group gene that is involved in transcriptional repression. EZH2 is not expressed in normal plasma cells, confers a proliferative phenotype in other cancer cells, is active in aggressive myeloma (MM4) cells, and is induced by the conditions we studied. Further analysis of EZH2 and its role in myeloma cell proliferation is underway.

While gene expression profiles can provide important clinical classifications, it is also important to consider not only the level of expression, but the functional variation of key genes in the patient population. Indeed, expression patterns may target further studies of functional genetic variants. And while genetic mutations in oncogens or tumor suppressor genes are associated with myeloma, genetic variants of cytokine or growth factor genes, drug response genes, and DNA repair genes may contribute to variability in both the growth and therapeutic response seen in patients, as well as secondary complications. We have chosen a set of candidate genes that meet the following criteria for analysis of single nucleotide polymorphisms (SNPs): 1) each gene has been shown to be involved in myeloma growth, drug response, or DNA repair; 2) each gene has polymorphic alleles that exist at frequencies in at least 5% of the general population; 3) each polymorphism has a known functional consequence on protein activity. Using this set of criteria we identified 16 candidate genes that are being analyzed in three ECOG phase III clinical trials, and correlated to survival, disease progression, bone disease, toxicity, response, and incidence of secondary malignancies. This study provides a systematic analysis of genetic polymorphisms and their effects on critical disease factors, and serves to complement the clinical correlations derived from gene expression profiling. The ultimate goal of genetic correlations to clinical outcome is the development of individualized approaches to therapy. This represents the beginning of international efforts to establish a large DNA bank and develop a population based study of genetic variants in myeloma (Bank On A Cure:\[BOACTM\]; supported by the International Myeloma Foundation).
The initiating molecular event in multiple myeloma is defined by several nonrandom chromosomal translocations leading to immortalization of malignant plasma cells. In contrast to B-cell lymphomas, in which a unique partner is involved, several partners have been identified in HMCL. Three cases present a translocation involving an Ig gene. However, more recent data obtained on human myeloma cell lines (HMCL) have enhanced the interest to the IgH gene in MM. Cytogenetic and molecular analyses of HMCL have shown that almost all the HMCL present an illegitimate IgH rearrangement. However, in contrast to B-cell lymphomas, in which a unique partner is involved, several partners have been identified in HMCL. Three partners are more frequently involved: FGFR3/MMSET at 4p16, CCND1 at 11q13, and c-maf at 16q23, observed in about 25% of the HMCL each. In the other quarter of HMCL, several chromosomal bands are translocated to 14q32. Do these results reflect the situation observed in patients? Actually, analyses using techniques that do not require metaphases (i.e., independent of the proliferative index), such as interphase fluorescence in situ
hybridization (FISH), have shown that the real incidence of illegitimate IgH rearrangements in MM patients was between 60% and 75%, as opposed to 30% in cytogenetic series. This discrepancy is especially related to the chromosomal location of the IgH and most partner genes, close to the telomers. Consequently, many of the translocations are cryptic, i.e., cytogenetically silent. FISH analyses with probes specific for the three main IgH translocations have shown that 2 of these partners are also frequent in patients, i.e., FGFR3/MMSET and CCND1, albeit with a lower incidence, 15% of the patients, respectively. In contrast, c-maf is rarely involved, in only 2% to 5% of the cases. In the 25% to 40% of patients with other illegitimate IgH rearrangements, very few other recurrent partners have been identified. It is actually plausible that many random chromosomal regions are involved in these patients. What is the role of these 14q32 translocations in MM? First of all, even though some differences in incidence are observed in the published series, they are not observed in 100% of the patients. Thus, they are probably not necessary for the malignant phenotype in all the cases. Second, these translocations are also present in at least half of the individuals with MGUS. Thus, not only the translocations are not necessary to confer a malignant phenotype, but they are also not sufficient. An attractive hypothesis could be that specific translocations may identify different MM subtypes. This situation would be reminiscent to that observed in B-cell lymphomas. In this hypothesis, the recurrent translocations would discriminate novel biological and clinical entities that could require specific therapeutic schemes. This hypothesis is very likely for the two main recurrent translocations, i.e., t(4;14) and t(11;14).

In t(4;14), two genes located at 4p16 are deregulated by the translocation: FGFR3, and MMSET. FGFR3 is telomeric to the translocation breakpoints, and is thus moved to the derivative chromosome 14. Its close association with the IgH enhancer leads to a strong activation of FGFR3 transcription. On the other side of the translocation breakpoints, another gene, MMSET, has been identified. All the breakpoints reported so far fall within the first MMSET introns, leading to its upregulation by the IgH enhancer. Recent data have suggested that MMSET would be the major target gene, since about 1/3 of the patients with t(4;14) do not overexpress FGFR3, probably because of the loss of the derivative chromosome 14. However, its role (in physiology or pathology) is still unknown, even though the presence of a SET domain suggests a role in chromatin remodeling. The translocation is associated with some biological features. It is more frequently associated with IgA isotype, and, overall, is almost constantly associated with del(13). These two correlations, plus a significantly higher β2-microglobulin level, may explain the short survival observed in these patients. In t(11;14), all the breakpoints are located on the centromeric side of CCND1, dispersed over at least 350 kb. However, despite this scattering, a constant upregulation of CCND1 is observed, probably related to the IgH enhancer activity. The activation of another gene (more centromeric), myeov, has been reported in a series, but not confirmed by other studies. Because of the presumed role of cyclin D1 in cell cycle control, proliferation activation would be expected in these patients. Surprisingly, opposite results have been observed in patients: t(11;14) is associated with a lower labeling index. Several hypotheses might be proposed, including the downstream activation of other genes, or the inhibition of cell cycle by so far unidentified proteins co-activated in these patients, or even the upregulation of other genes by the translocation. Apart a frequent lymphoplasmacytoid morphology, no other biological feature has been associated with t(11;14). However, a longer survival seems to be observed in these patients, especially in those receiving high-dose chemotherapy.

What about the other 70% of the patients? About half of them present an illegitimate IgH rearrangement, with non- (or low) recurrent partners. Except for patients with t(14;16) who present characteristics similar to those with t(4;14) (i.e., almost constant del(13), high β2-microglobulin level, short survival), other patients with 14q32 abnormalities do not share any typical feature, and may correspond to a very heterogeneous group. Recent data have proposed another classification of the patients, according to the ploidy status. The basis of this classification is essentially prognostic, since patients with hypodiploidy display a shorter survival than those with hyperdiploidy. Interestingly, these two categories present also apparent differences in the repartition of chromosomal abnormalities. The former group is associated with a high incidence of del(13) (about 75% versus 35% in the hyperdiploid group), and a higher incidence of structural abnormalities, including a significantly higher incidence of illegitimate IgH rearrangements (C Bastard and R Fonseca, personal communications, February 2003). However, we have to keep in mind that these results are obtained by cytogenetics, i.e., in proliferative patients. Whether these findings can be extended to all the patients is currently unknown. Nevertheless, it appears obvious that genetic features will define MM subgroups with different biological and survival characteristics.

Recently, a few studies based on gene expression profiling have been reported in the literature. Such approaches have been shown to be highly powerful in several malignancies, but especially in high-grade lymphomas. Gene profiling has shown that “diffuse large B-cell lymphomas” (DLBCL) represent in fact a heterogeneous compilation of several biological and clinical entities. In MM, data are less clear-cut, probably because of the difficulty in obtaining pure PC populations. Thus, a pre-step consisting in the positive selection of plasma cell is absolutely required. With the availability of cell separation technologies based on antibody-coated microbeads, this step is not anymore a brake for gene profiling approaches. However, purification processes are widely performed since only 2 to 3 years, preventing the generation of survival data for a couple more years. Furthermore, the amount of purified PCs is generally low, so far preventing the analysis of large cohorts of patients. Thus, these analyses have been limited to the comparison of gene expression profiles obtained in PCs purified from MM patients, HCL, normal bone marrow and tonsil, or generated in vitro. These studies have shown a huge heterogeneity within MM patients, but no clear-cut separated subgroups have been identified, except possibly a subgroup of MM resembling to HCL. In contrast, several genes are differentially expressed between normal bone marrow and tonsil PCs, as well as between these cells and MM. Interestingly, we have shown that the most prominent heterogeneity factor in MM, i.e., Ig chains expression, was associated with highly differentiated gene clusters. For instance, IgLκ and IgLλ were associated with many genes in different clusters, highlighting different biological features, especially regarding bone disease.

In conclusion, genetic and molecular studies do reveal a major heterogeneity in MM. The extension of these studies should help in the dissection of MM in several different entities, allowing improvements in our understanding of the oncogenetic pathways present in the disease, and thus in the therapeutic management of the patients. These studies should also improve our knowledge in the PC physiology, as well as in the first steps of clonal PC generation.
Molecular Cytogenetics of Myeloma: Biology, Clinical and Prognostic Implications

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1. INTRODUCTION: Cytogenetic and genetic abnormalities are thought to be critical for the pathogenesis of multiple myeloma (MM). We have embarked on the study of genetic abnormalities in MM to better understand their relationship to pathogenesis and clinical outcome. We have studied these abnormalities using a combination of classical molecular techniques, molecular cytogenetics (cIg-FISH), conventional karyotype analysis and novel genomic platforms (gene expression analysis). We have carefully considered the implications of specific chromosome abnormalities for a) the different stages of the disease, and b) evidence of ongoing genomic evolution and heterogeneity.

2. IgH TRANSLOCATIONS: The work done by several groups has now revealed that IgH translocations, mostly occurring at the time of isotype class switching, are present in the majority of patients with MM (~60%). They appear to be early genetic (clone initiating?) events since they are observed since MGUS. Additional evidence suggest that IgH translocations may represent the immortalizing event for the clone; their biologic plausibility, upregulation of oncogenes, presence in other B-cell neoplasms, initiated by B-cell specific recombination, clonally selected and most recently associated with dissimilar clinical outcomes. We have recently found that with serial evaluation IgH translocations are always conserved, and that with development of human MM cell lines and extramedullary MM, gaining extra copies of the derivative chromosomes seem to be advantageous for clonal expansion.

3. CLINICAL IMPLICATIONS FOR THE IgH TRANSLOCATIONS: Our group and others have now shown that the different IgH translocations have a significant impact on the ultimate outcome of patients; patients with the t(4;14)(p16.3;q32) and with t(14;16)(q32;q23) have very aggressive disease and have shorter survival. In contrast, patients with t(11;14)(q13;q32) seem to have a better prognosis. These effects on patients’ outcome seem to be influenced by treatment modality administered; for instance high-dose chemotherapies would not provide a significant benefit for patients with the t(11;14)(q13;q32). In contrast it appears that high dose therapy does not provide much benefit for patients with the more aggressive IgH translocations. These observations suggest that MM may be composed, much like AML, of subgroups of patients categorized by the specific IgH translocations. While the relative effect on overall prognosis of IgH translocations would seem to comparable to that of the most robust prognostic variables, determining their presence will be of even greater importance for the development of targeted therapies.

4. AНЕУПЛОИДИЯ: Aneuploidy as a category is the single most common abnormality in MM. Aneuploidy in MM at first glance appears to be random, but detailed studies of abnormal karyotypes have now shown that not to be the case. While numerical abnormalities are seen in the vast majority of patients, monosomies are seen in nearly all karyotypes while trisomies are only seen in 50% of patients. The chromosomes involved trisomies and monosomies are not random; trisomies predominantly involve chromosomes 3, 5, 7, 9, 11, 15, and 19, and monosomies involve chromosomes 8, 13, 14, 16 and 22.

5. HYPERDIPLOID AND NON-HYPERDIPLOID MM: Based on the presence of numerical abnormalities alone four sub-categories of MM are discernible; hypodiploid; pseudo-diploid, hyperdiploid and near tetraploid. Because of the extreme similarities between the hypo-, pseudo- and tetraploid karyotypes we have proposed the classification of MM into two major subtypes; hyperdiploid and non-hyperdiploid MM. This classification is highly relevant, as it has been recently shown that there is a marked difference in the prevalence of IgH translocations between the two groups; high in the non-hyperdiploid MM and low in the hyperdiploid MM. The striking association has suggested that two major pathways may be possible for MM pathogenesis; one that is initiated by IgH translocations and one that is initiated by a yet to be identified mechanism and whose end result is hyperdiploidy. It appears the same associations are present since MGUS but further work is needed to address these fundamental observations of disease pathogenesis. While the aforementioned studies were based on data derived form the study of abnormal karyotypes, the same observations are seen, and in the same degree when ones studies patients samples with FISH probes or via DNA content by flow. Hypodiploid MM is associated with a very poor prognosis, short survival and low response to treatment. Non-hyperdiploid MM seems to provide a proliferative advantage to the clone such that it emancipates the cells from the bone marrow and allows for the growth of cells in extramedullary sites. In fact, all human MM cell lines derive from the non-hyperdiploid MM and new models for the ex-vivo study of hyperdiploid MM are needed (e.g. mouse passage of hypodiploid MM cells).

6. CHROMOSOME 13 DELETION/MONOSOMY: Loss of chromosome 13, both deletion of 13q14 and monosomy, (∆13) have emerged over the last decade as a prominent prognostic cytogenetic factors. The negative prognostic associations are observed whether ∆13 are detected by karyotype analysis or by FISH. ∆13 detected by karyotype identify a group of patients with a grave prognosis, and more commonly hypodiploid. Observing ∆13 via karyotype is of course including the negative prognostic implications of obtaining any abnormal metaphase (proliferation) plus the negative biologic implications of having ∆13. In contrast ∆13 detected by FISH identifies a group of patients with the biologic abnormality. Several features suggest ∆13 are important (and not simply a marker) for pathogenesis; recurrent nature, clonally selected, effect on prognosis and the lack of trisomy 13. However, there is no specific gene identified as importantly associated with the loss yet. In addition it is not clear whether ∆13 is merely a surrogate marker of hypodiploid variant MM. Hypodiploid MM is associated with a high incidence of aggressive IgH translocations (e.g. t(4;14)(p16.3;q32)) and with an extremely poor prognosis. In fact we have recently shown that many other monosomies may also be associated with a significantly shorter survival. Much like IgH translocations we also observed ∆13 in a similar fraction of MGUS patients.

7. STAGES OF THE DISEASE: The current available information does not allow full elucidation of genomic aberrations that are seen in MM but not in MGUS. While differences still exist with regards to the prevalence of specific lesions (e.g. ∆13 or t(4;14)(p16.3;q32)), all cytogenetic features seen in MM have been described as well in MGUS by FISH. The only two abnormalities that are consistently reported as lower in prevalence in MGUS, and present in a sizable fraction of MM, are ras mutations and p16 gene silencing by methylation. In contrast other abnormalities seem to be increasingly common with advancing stages of the disease; c-myc abnormalities, p53 inactivation and secondary translocations.

8. GLOBAL INTEGRATION OF GENETICS, CYTOGENETICS AND GENE EXPRESSION PROFILING: As more information has emerged with regards to the genetic nature of MM, it has become increasingly
clear that the biggest challenge for the future will be to integrate this information, derived from the multiple techniques, multiple cohorts of patients and different stages of the disease. We need to integrate it all in a coherent model of disease pathogenesis. While this seems an ambitious task, the high power of high throughput technology coupled with the careful and detailed classical molecular studies should help elucidate some of these complex interactions. Furthermore confirmatory experimental studies are needed to validate observations emanating form the ongoing genetic studies of MM. For instance the presence of silencing of tumor suppressor genes (e.g. p16 methylation) will need to be evaluated in the context of its downstream signaling pathways, its relation to the baseline genetic abnormalities deregulating the CDK/CyclinD1 pathway, its impact on clinical outcome and its relationship to therapy responsiveness.

9. Conclusion: A genetic and molecular cytogenetic classification of MM is being formed by the work of multiple laboratories that soon will likely integrate a global model for the pathogenesis of the disease. Genetic abnormalities correlate closely with specific biologic and prognostic features. The available genetic modeling provides the best available evidence that MM is composed of subgroups of patients categorized according to their underlying genomic aberrations. More importantly, a comprehensive and accurate understanding of the genetic nature of MM will ultimately set the platform from which to develop true targeted therapies. Our efforts have been focused on the generation and validation of these targets as crucial for clonal expansion and maintenance. Those fulfilling the last category should make attractive therapeutic targets.

RF is a Clinical Investigator of the Damon Runyon Cancer Research Fund. This work was supported in part by Public Health Service grant no. R01 CA83724-01 (RF) and the Fund to Cure Myeloma. PRG is supported by the ECOG grant CA21115-25C from the National Cancer Institute

3. Immunobiology

P3.1
A CELLULAR MODEL FOR MYELOMA CELL GROWTH AND MATURATION BASED ON CD45 HIERARCHY.

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CD45 is a protein tyrosine phosphatase required for lymphocyte activation and development (1). As soon as its first description as a leucocyte common antigen, CD45 has been found to be expressed on B lymphocytes, its expression declining during plasma-cell differentiation (1, 2). More recently, the variations of CD45 expression during plasma-cell differentiation have been re-evaluated carefully in vivo by comparing CD45 expression with that of other antigens on human plasma cells (PC) of different origins: tonsils, peripheral blood and bone marrow (3). This important study has first confirmed the existence of a gradient of increasing maturity of the different human PC compartments from tonsils to bone marrow through peripheral blood. Second, with regard to CD45 specifically, a decreasing pattern of expression was confirmed, with a clear reduction only in bone marrow PC. This unique comparative study has confirmed previous works showing separately either a bright expression of CD45 on immature PC in tonsils and peripheral blood or a rather low expression on mature PC inside the bone marrow (4,5,6). Although these data strongly support the association between a CD45 bright expression and proliferation in immature PC as those in tonsils and peripheral blood, contrasting with the down-regulation of CD45 expression observed in the bone marrow during final maturation, and corresponding to proliferation arrest, this has never been previously shown directly in vivo.

As soon as 1988, Multiple Myeloma (MM) has been described as a tumor presenting with either a weak to intermediate expression of CD45 or even lacking CD45 expression (7). A major advance in the biology of CD45 in MM has been made by JOSHUA D et al (8) who demonstrated that CD45 expression was highly correlated with the proliferation rate of myeloma cells. In this study, the brightest expression of CD45 was associated with the highest proliferation rate (labeling index, LI) of myeloma cells. Furthermore, the proliferation of myeloma cells declined parallel to that of CD45 expression. Although the restriction of the highest proliferation to a CD45 bright compartment in MM has been confirmed by FUJII R et al (9), it was recently disproved by RAWSTRON A C et al (10) and thus this point remains pending.

In the current study, we have evaluated directly the expression of CD45 on normal and malignant PC of different origins in relation to their proliferation in vivo. More particularly, we have re-evaluated the expression of CD45 on human myeloma cells in order to better understand the meaning of CD45 bright and CD45 low myeloma cells and that of the annihilation of CD45 on myeloma cells.

CD45 expression was evaluated on normal malignant plasma cells (PC) in relation to their proliferation in vivo (labeling index, LI). In Tonsils (n=8) and peripheral blood (n=5), all PC were highly proliferating CD45 bright PC. All reactive plasmocytoses (n=12) turned out to be homogeneous expansions of this type of PC with unusually high LI (30%). CD45 bright expression declines with proliferation arrest and final maturation of PC in bone marrow only (n=11). In MM (n=37), CD45 expression is heterogeneous as in normal bone marrow. Proliferation is always restricted to a minor (12%) CD45 bright population of myeloma.
clear that the biggest challenge for the future will be to integrate this information, derived from the multiple techniques, multiple cohorts of patients and different stages of the disease. We need to integrate it all in a coherent model of disease pathogenesis. While this seems an ambitious task, the high power of high throughput technology coupled with the careful and detailed classical molecular studies should help elucidate some of these complex interactions. Furthermore confirmatory experimental studies are needed to validate observations emanating from the ongoing genetic studies of MM. For instance the presence of silencing of tumor suppressor genes (e.g. p16 methylation) will need to be evaluated in the context of its downstream signaling pathways, its relation to the baseline genetic abnormalities deregulating the CDK/CyclinD1 pathway, its impact on clinical outcome and its relationship to therapy responsiveness.

9. CONCLUSION: A genetic and molecular cytogentic classification of MM is being formed by the work of multiple laboratories that soon will likely integrate a global model for the pathogenesis of the disease. Genetic abnormalities correlate closely with specific biologic and prognostic features. The available genetic modeling provides the best available evidence that MM is composed of subgroups of patients categorized according to their underlying genomic aberrations. More importantly, a comprehensive and accurate understanding of the genetic nature of MM will ultimately set the platform from which to develop true targeted therapies. Our efforts have been focused on the generation and validation of these targets as crucial for clonal expansion and maintenance. Those fulfilling the last category should make attractive therapeutic targets.

RF is a Clinical Investigator of the Damon Runyon Cancer Research Fund. This work was supported in part by Public Health Service grant no. R01 CA83724-01 (RF) and the Fund to Cure Myeloma. PRG is supported by the ECOG grant CA21115-25C from the National Cancer Institute

3. Immunobiology

P3.1
A CELLULAR MODEL FOR MYELOMA CELL GROWTH AND MATURATION BASED ON CD45 HIERARCHY.

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CD45 is a protein tyrosine phosphatase required for lymphocyte activation and development (1). As soon as its first description as a leucocyte common antigen, CD45 has been found to be expressed on B lymphocytes, its expression declining during plasma-cell differentiation (1, 2). More recently, the variations of CD45 expression during plasma-cell differentiation have been re-evaluated carefully in vivo by comparing CD45 expression with that of other antigens on human plasma cells (PC) of different origins: tonsils, peripheral blood and bone marrow (3). This important study has first confirmed the existence of a gradient of increasing maturity of the different human PC compartments from tonsils to bone marrow through peripheral blood. Second, with regard to CD45 specifically, a decreasing pattern of expression was confirmed, with a clear reduction only in bone marrow PC. This unique comparative study has confirmed previous works showing separately either a bright expression of CD45 on immature PC in tonsils and peripheral blood or a rather low expression on mature PC inside the bone marrow (4,5,6). Although these data strongly support the association between a CD45 bright expression and proliferation in immature PC as those in tonsils and peripheral blood, contrasting with the down-regulation of CD45 expression observed in the bone marrow during final maturation, and corresponding to proliferation arrest, this has never been previously shown directly in vivo.

As soon as 1988, Multiple Myeloma (MM) has been described as a tumor presenting with either a weak to intermediate expression of CD45 or even lacking CD45 expression (7). A major advance in the biology of CD45 in MM has been made by JOSHUA D et al (8) who demonstrated that CD45 expression was highly correlated with the proliferation rate of myeloma cells. In this study, the brightest expression of CD45 was associated with the highest proliferation rate (labeling index, LI) of myeloma cells. Furthermore, the proliferation of myeloma cells declined parallel to that of CD45 expression. Although the restriction of the highest proliferation to a CD45 bright compartment in MM has been confirmed by FUJII R et al (9), it was recently disproved by RAWSTRON A C et al (10) and thus this point remains pending.

In the current study, we have evaluated directly the expression of CD45 on normal and malignant PC of different origins in relation to their proliferation in vivo. More particularly, we have re-evaluated the expression of CD45 on human myeloma cells in order to better understand the meaning of CD45 bright and CD45 low myeloma cells and that of the annihilation of CD45 on myeloma cells.

CD45 expression was evaluated on normal malignant plasma cells (PC) in relation to their proliferation in vivo (labeling index, LI). In Tonsils (n=8) and peripheral blood (n=5), all PC were highly proliferating CD45 bright PC. All reactive plasmocytoses (n=12) turned out to be homogeneous expansions of this type of PC with unusually high LI (30%). CD45 bright expression declines with proliferation arrest and final maturation of PC in bone marrow only (n=11). In MM (n=37), CD45 expression is heterogeneous as in normal bone marrow. Proliferation is always restricted to a minor (12%) CD45 bright population of myeloma.
THE ROLE OF T CELLS IN MYELOMA

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Although multiple myeloma is a neoplasm of mature B cells, there exists a range of numerical, phenotypic and functional abnormalities within the T cell compartment of patients with this disease. Interest in the immunoregulatory role of T cells and the possibility of enhancing the immune response against tumour cells through a tumour vaccination strategy has generated an increase in the number of studies involving T cells of patients with myeloma.

1. Expanded clones of T cells in myeloma

A number of studies have reported the presence of T cell clones in myeloma not found in other B cell malignancies. Expanded T-cell populations have been detected by Southern blot by an abnormal repertoire of expression of the T cell receptor (TCR) variable regions, TCR CDR3 fragment length analysis, determination of J beta gene usage and nucleotide sequencing. The most significant observation concerning expanded T cell clones is that their presence is associated with a prolonged overall survival suggesting that these T cells have some anti-tumour activity. Expanded T cell populations in patients with myeloma have been shown to have the phenotype of cytotoxic T cells, i.e. CD8+, CD45RA+, CD57+, CD28- and perforin positive.

Recently it has been demonstrated by TCR CDR3 fragment length analysis and nucleotide sequencing that it is the CD8+CD57+ cells within the expanded TCRVβ family that are clonal. While age-matched normal controls also contain expanded T cell populations, these are almost exclusively CD4+ T cells. The functional capacity of CD8+ T cell expansions in patients with myeloma and their specificity to malignant plasma cells is a key issue that requires further study.

2. T cells and idiotype reactivity in humans

When peripheral blood T cells were stimulated with idiotypic (F(ab')2) fragments, T cell responses of specific T cell subsets were observed in both proliferation and cytokine secretion assays. These are mainly Th1-type T cell response (IFN-γ and IL-2 secreting T helper cells) and are inhibited by an anti-HLA-DR antibody suggesting that the idiotype-induced T cell stimulation is MHC class II restricted. In addition, idiotype-induced T cell stimulation was shown to require the presence of antigen presenting cells (APC), such as B-cells or monocytes indicating that the idiotype alone is not sufficient to mount a T cell proliferative response. Malignant plasma cells are poor APC but the idiotype can be transferred from myeloma cells to other types of APC for MHC class II presentation to CD4+ T cells. However, when idiotype-induced reactivity was studied in patients with restricted TCRVβ expansions, idiotype recognition was not confined to the expanded populations. The exact nature of the idiotype protein induced effects on T cells is still rather obscure.

3. Immunodominant idiotype-derived peptides

The identification of immunodominant peptides is an important consideration if tumour specific peptides are to be used in idiotype vaccination strategies. The strength of the T cell response depends on the binding affinity of the peptide to the HLA molecule, the stability of the HLA-bound peptide and the avidity of the T cell receptor to the peptide-HLA complex. Bioinformatics can be used to predict which human immunoglobulin-derived peptides are capable of inducing a T cell response.

References:

9-Fujii R, Ishikawa H, Mahmoud MS, Asaoku H, Kawano MM. MPC-1CD49e immature myeloma cells include CD 45+ subpopulations that can proliferate in response to IL-6 in human myelomas. Br J Haematol, 1999, 105; 131-140
response and have demonstrated that CD8+ cells can recognise shared immunoglobulin-derived peptides bound to MHC class I molecules. Thus, it may be possible to develop a small set of shared peptides capable of inducing a T cell response in a range of patients. Certainly the ability to predict immunodominant peptides has significant implications for vaccination strategies in the treatment of all B-cell malignancies. We have recently used this method to predict immunodominant peptides from the sequence of the CDR3 region of the IgH gene of 16 patients with myeloma. (8) CDR3 peptides from most patients failed to achieve a BIMAS score of 100, suggesting that the poor affinity between the unique peptides and the patient's HLA would fail to generate a significant T cell response. As most immunodominant peptides in other B cell malignancies were found outside the CDR3 region and more often in framework regions, future studies in patients with myeloma should not expect that immunodominant peptides with the potential to stimulate anti-tumour T cell activity will only be found in the CDR3 region.

4. Identification of idiotype-specific T cells using MHC Class I Tetramers

MHC tetrameric complexes offer the possibility to identify and manipulate peptide specific T cells. We have sequenced the hypervariable regions of both the heavy and light chain hypervariable regions of 6 patients with expanded CD8+ clones, used bioinformatics to demonstrate that only 3 of the six patients had immunodominant idiotypic peptides and prepared tetrameric MHC class I complexes containing CDR-derived immunodominant peptides to search for idiotype-specific T cells in the blood of the 2 surviving patients. Initial studies which failed to detect tetramer positive cells in either patient suggested that idiotype-specific T cells were deleted. Modified staining techniques demonstrated that tetramer positive T cells comprised 1-10% of an IL-2 activated T cell population (<2% of the total T cells) in both patients. (8) CD80+ T cells CD80 is expressed at different concentrations on mature dendritic cells (DC) as well as B cells and monocye/macrophages. The expression of CD80 on APC may increase during maturation or may be upregulated by agents such as soluble CD40 ligand or lipopolysaccharide. CD80 has also been reported to be present on the T cells of some patients with HIV, lupus, RA and other autoimmune conditions. The CD80+ T cell population appears to represent a tolerized, post antigen presentation population of memory T cells and is likely to be common to a variety of situations characterised by chronic antigen stimulation. We have investigated the presence and significance of CD80+ and CD86+ T cells in patients with myeloma. Our studies show that CD80+ and CD86+ T cells are common in patients with myeloma, that these are memory T cells and may be either CD4 or CD8 but do not represent a single clonal expansion. The lack of CD80 mRNA in purified T cells expressing CD80 protein and the failure to upregulate CD80 on T cells with huCD40LT suggests that CD80 expression on the T cells of these patients is acquired rather than endogenously upregulated. CD80+ T cells may therefore offer a useful marker for prolonged T cell exposure to tumour antigen. 6. Dendritic cell – T cell interactions TGFβ and IL-10 have been shown to suppress T cell proliferation and inhibit T cell-DC signalling. In recent years both TGFβ and IL-10 have been reported to be expressed at high levels in patients with B cell lymphoma and are considered to be responsible for tumour-induced immunosuppression. TGFβ derived from malignant plasma cells has been shown to inhibit activation and IL-2 responses in T lymphocytes of patients with myeloma and the removal of TGFβ renders myeloma cells highly immunogenic. It is therefore likely that TGFβ and IL-10 are responsible for T cell unresponsiveness in myeloma. We have previously demonstrated that in patients with myeloma, T cells express increased levels of CTLA-4 and decreased levels of CD28. Our recent studies (9) have demonstrated that while huCD40LT can upregulate the expression of CD80 on all normal DCs, TGFβ and IL-10 inhibit the upregulation of CD80 on DC of 6/13 patients with myeloma. Identification of these cytokines as the inhibitors was determined by neutralisation studies with anti-TGFβ and anti-IL-10. In other studies we demonstrated that rTGFβ inhibited upregulation of CD80 on normal DC and the malignant plasma cells of the patients who fail to upregulate CD80 expression produce increased levels of TGFβ (10). Upregulation of CD80 on high potency DC may provide a more effective strategy for immunotherapy. More recent studies indicate that this can be achieved with IL-12.

7. Idiotype vaccination programs and the future

A variety of idiotype vaccination strategies have been used in clinical trials. Ruffini et al (2002) (10) have provided a recent review of the major trials reported. Most protocols include idiotype-pulsed autologous dendritic cells or idiotype-specific proteins conjugated to KLH as immunogens, followed by GM-CSF or IL-2 as immunoadjuvants.

To date, in vitro responses have been minimal and clinical responses have generally been very modest, if present at all. Vaccination protocols may require new therapeutic strategies with a more complex and multi-faceted approach to optimise antigen, antigen presenting cells and to overcome T cell tolerance. Factors relating to APC may include the generation of an increased number of high potency, functionally normal DC, enhanced recruitment of DC with Flt3L, affinity purification of DC and optimisation of the loading of DCs with antigen. If idiotype peptides are to be used as antigen, bioinformatics could be used to predict the most appropriate immunodominant epitopes. Upregulation of the expression of costimulatory molecules on the malignant cell population with a biological modifier such as CD40L may be necessary to induce plasma cells to function as APC and also to induce the differentiation of high potency DC. Finally it will be necessary to overcome T cell tolerance. This may require the addition of exogenous cytokines like IL-2 and IL-12. Thus T cells play a central role in tumour immunology. Restoration of T cell function by either active or passive immunotherapy continues to hold some promise in the future therapy of not only patients with multiple myeloma but also many other malignancies. Further studies need to be performed, however, to understand how T cells and other players of the immune system can be induced to reach their normal functional state.

5. Yi Q, Eriksson I, He W, Holm G, Mellstedt H, Osterborg Idiotype-specific T lymphocytes in monoclonal gammopathies:


9. Brown et al. Dendritic cells from patients with myeloma are numerically normal but functionally defective as they fail to upregulate CD80(B7-1) expression after huCD40LT stimulation due to inhibition by TGFβ and IL-10. Blood 2001;98: 2992-2998.


P3.3 PLASMA CELL/MICROENVIRONMENT INTERACTIONS IN MULTIPLE MYELOMA: A PARADIGM AND A CHALLENGE

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Multiple Myeloma (MM) is a paradigmatic example to illustrate the concept that host-tumor interactions contribute to cancer cell proliferation, survival and progression and to show the mutual importance of the "seed" and the "soil". BM microenvironmental proliferation, survival and progression and to show the mutual interactions constitute a minimal anti-tumour response. Blood 2001;98: 652a.

In conclusion, it is not unreasonable to postulate that the acquisition of the capacity to activate endogenous stromal cells is the real turning point in the natural history of MGUS. It likely gives a decisive impulse to the malignant clone growth and marks the progression of MGUS into overt MM. MM PC have also an extended life span due to defective apoptosis. Both external stimuli and intrinsic genetic defects of the neoplastic cell may concur to influence the ability of MM PC to avoid apoptosis. In the initial phases of the disease, when the malignant clone growth is highly dependent upon the supportive role of the BM stromal microenvironment, the stroma itself may be involved in the production of anti-apoptotic factors. Dissecting the multiple molecular mechanisms that underlie these interactions may spawn new treatment approaches.

P3.4 TUMOR CELL TARGET STRUCTURES AND T-CELL FUNCTIONS IN MYELOMA

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The myeloma clone consists of plasma cells and pre-plasma cells. Within the tumor clone, cells are expressing MHC class I as well as class II molecules. A hallmark of the myeloma clone is the idiotypic immunoglobulin, of which complete molecules are expressed on the surface of pre-plasma cells. As the idiotypic protein is also present in the cytosol peptides of the idiotypic protein should be presented in the groove of MHC class I and class II molecules. Peptide sequences of the idiotype presented by IL-6 in the culture supernatants.

Taken together, a number of observations suggest that MM plasma cells are able to "instruct" the local components of BM environment to help the expansion of the malignant clone. Despite the fact that the clonal founder cell of MM must have been involved in a T cell-dependent antigenic response, the evolution of MM malignant clone appears to be T-cell independent but strongly dependent on the BM stromal cell stimulating and nourishing activity, thereby explaining why MM is confined within the BM. It may be asked why several environmental cells, that normally would be quiescent, above all in a certain age range, are instead activated in the BM of MM. As normal BM stromal cells produce IL-6 after activation by inflammatory cytokines like IL-1, it is not unreasonable to consider the activated state of stromal cells in MM as a direct consequence of the influence of accessory cell-activating cytokines produced by the expanding monoclonal B cell population. In MGUS patients, the clonal population is below the threshold size that may produce enough cytokines to initiate the activation of BM stromal cells. Once the threshold size is reached, BM stromal cells become activated and trigger a self-perpetuating mechanism of mutual help and recruitment between malignant plasmacells and BM stromal cells that favours the progressive expansion of the B cell clone.
variable region of the idiotypic immunoglobulin heavy chain in multiple myeloma.

A large number of HLA (class I and II) peptides were identified in five studied patients. The frequency of predicted epitopes was dependent on the data base used: 245 in BIMAS and 601 in SYFPEITHI. Most of the peptides displayed a binding half-life or score in the low to intermediate affinity range. The majority of the predicted peptides were complementarity determining regions (CDR) rather than framework regions (FR) derived (52 – 60% vs. 40 – 48%).

Most of the predicted peptides were confined to the CDR2-FR3-CDR3 “geographical” region of the IgVH region (70%). Significantly fewer peptides were found within the flanking (FR1-CDR1-FR2 and FR4) regions (p<0.01). Eight to ten amino acid (aa) long peptides corresponding to the CDRs and fitting to the actual HLA-A/B haplotypes recognized spontaneously type I T cells (γ-IFN) indicating an ongoing MHC class I restricted T-cell response. Most of those peptides had a low binding half-life (BIMAS) and a low/intermediate score (SYFPEITHI).

Furthermore, 15-20 aa long CDR1-3 derived peptides recognized also spontaneously type I T cells indicating the presence of MHC class II restricted T cells as well. Thus, a large number of HLA-binding idiotypic peptides can be identified in patients with myeloma. Such peptides may spontaneously induce a type I MHC class I as well as class II restricted memory T-cell response.

As idiotypes may naturally induce a T-cell response, idiotypes might be utilized as an immunogen in a vaccination approach. However, as native idiotypic peptides seem to be of low to intermediate affinity peptides should be produced with a higher affinity but with preserved specificity, “heteroclitic” peptides, for the induction of an effective immune response. Such peptides might have the possibility to induce an effective lytic response. To be able to induce an effective T-cell response the functional capability T cells should be well preserved, otherwise the immunization procedure might be ineffective. It is known that patients with malignant diseases have a varying degree of T-cell dysfunction, which might hamper the capability of the patients to mount an effective tumor specific immune response.

T-cell immune dysfunction in patients with malignant tumors has been attributed to abnormal signal transduction, partly through altered expression of components of the TCR/CD3 complex and their associated intracellular protein tyrosine kinases. Four-color cytomtery was applied to study surface bound molecules TCRz-chain, CD28, CD152 and CD154 involved in T-cell signalling and the intracellular components of the TCR/CD3 complex CD3 z-chain, p56lck, p59fyn, ZAP-70 and PI3-k as well as the cytokines γ-IFN, IL-4 and IL-2 of blood T cells in myeloma patients at different stages of the disease. Multiple abnormalities were demonstrated of in vivo CD4 and CD8 populations as well as after stimulation with the TCR-binding molecule Staphylococcus Enterotoxin B (SEB), a superantigen activating T cells. There was a marked reduction, particularly in advanced stage in the number of T cells expressing CD28, CD152, CD3-z-chain, p56lck, p59fyn, ZAP-70 and PI3-k (p<0.01). The cytokine production of γ-IFN and IL-4 in resting T cells was significantly higher in patients than in controls (p<0.05). However, T cells of patients did not react normally to SEB stimulation.

Profound and multiple dysfunctions, especially in advanced stages, were found in patients with multiple myeloma. This should be taken into consideration when developing T-cell therapeutic approaches, e.g. vaccination. Addition of IL-2 to the vaccination procedure might restore T-cell dysfunction.

P3.5 The Regulation of fast induced Apoptosis in Multiple Myeloma
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Introduction: The Darwinistic selection of cellular variants with the capacity of resisting apoptosis is an important aspect of the tumour progression in human multiple myeloma (MM). If deregulated or overexpressed, anti-apoptotic signals will allow the tumour cell to escape apoptosis induced by e.g. Fast, DNA damage or glucocorticoids[Nilsson, 1998]. These alterations may also contribute to the development of resistance to cytotoxic drugs in transformed cells. We have previously shown that pretreatment with interferons (IFN) sensitize previously highly resistant MM cells to Fas-induced apoptosis [Specs, 1998]. This suggests that IFN may have a synergistic effect with other therapeutic agents and opens a window for new treatment strategies of Fas resistant MM tumors. The anti-tumor effects of IFNs in MM have been extensively studied in vivo and in vitro. However, it remains elusive how IFN-induced signaling pathways and gene expression are linked to susceptibility to apoptosis in MM.

Interestingly, both IFN and interleukin (IL)-6 important for maintaining survival in MM, activate STAT-Proteins, a family of transcription factors that have been associated with regulation of growth and survival of hematopoietic cells. State has been shown to be constructively activated in a number of human cancers, and has also been implicated as a crucial mediator of the pro-survival function of interleukin (IL)-6 in MM cell lines [Carlett-Falcone, 1999]. In contrast, Stat1, which is activated by IFNs has been proposed to play an important role in promoting apoptosis. Intriguingly, several reports suggest that Stat3 and Stat1 may counteract each other’s effects. In this study, we explore the mechanism of IFN-mediated sensitization to Fas-induced apoptosis in MM by investigating STAT activation and the effect of IFNs on the expression of candidate genes encoding different components of the apoptosis pathways in U266-1970 MM cells [Dimberg, submitted].

Results: We found that, in addition to inducing activation of Stat1, IFN treatment led to attenuation of IL-6 induced activation of Stat3. In a screen to identify IFN-regulated target genes involved in the apoptotic machinery, TRAIL and Fas were identified to be transcriptionally regulated by IFNs. Fas has been shown to be differentially regulated by Stat1 and Stat3. We found that IFN-induced TRAIL up-regulation was also dependent on Stat1 activation. Blocking TRAIL, using rhTRAILR1:Fc, consisting of the extracellular domain of the TRAIL-R1 receptor, had no apparent effect on IFN-mediated sensitization of Fas-induced apoptosis, suggesting that the TRAIL up-regulation may not be directly involved in this process (Fig 1). Interestingly, although U266-1970 cells express a considerable basal level of Fas, a higher level of Fas-expression was indeed associated with an increased sensitivity to Fas-induced apoptosis in these cells.

Conclusion: The work reported here show a candidate pathway for re-establishing the apoptosis program in MM by exposure to IFN-γ. We propose that IFN-induced activation of Stat1 and attenuation of IL-6-mediated Stat3-activation, followed by an up-regulation of Fas, is a plausible mechanism by which myeloma cells can be sensitized for Fas-induced apoptosis.
Fig.1 IFN-induced sensitization to Fas-induced apoptosis is independent of TRAIL. Agonistic anti-body CH11(FasL), recombinant soluble Apo2L/TRAIL (rTRAIL) or isotype specific control IgM in the presence (right panels) of blocking agent rhTRAIL-R1:Fc (Fc-DR4) was continually added in the experiments. The percentage of Annexin V-positive/PI-negative apoptotic cells was evaluated by flow cytometry and indicated.

References

In human myelomas, there is a heterogeneity of myeloma cells morphologically and phenotypically. Myeloma cells can be classified phenotypically into at least 5 subpopulations; MPC-1-CD45+CD49e-, MPC-1-CD45+CD49e- intermediate myeloma cells, MPC-1+CD45+CD49e- mature myeloma cells. Immature myeloma cells( MPC-1-CD49e-) have a capacity of proliferating in vitro and in vivo, but only CD45+ immature myeloma cells can respond directly to IL-6 to proliferate. In the U-266 cell lines, IL-6 can lead to the induction of CD45 expression and CD45+ U-266 cells can proliferate in response to IL-6. In both CD45- and CD45+ U-266 cells, STAT3 and MAPK(ERK1/2) can be activated in response to IL-6 equally between them, but src family kinases such as Lyn, Fyn can be activated only in CD45+ U-266 cells. Thus, the activation of the src family kinases associated with CD45 expression is a prerequisite for the proliferation of myeloma cells. Antisense oligonucleotides specific for Lyn, or PTK inhibitors(PP2 or herbimycin A) blocked enhancement of IL-6-induced proliferation of CD45+ U-266 cells. In addition, CD45+ U-266 cells were more sensitive to apoptotic stimuli such as serum-free condition, heat shock, UV irradiation, H2O2 treatment, or melphalan treatment than CD45- U-266. Therefore, we can speculate that in the bone marrow of human myelomas IL-6 can induce proliferation of CD45+ immature cells, but the amount of IL-6 is too low to support CD45+ myeloma cells and loss of CD45 results in no direct response to IL-6 to proliferate but confers resistance to stress condition leading to the longer survival at the limited amount of IL-6.

Fig.1 Heterogeneity of human myeloma cells in the bone marrow
MPC-1-CD45+CD49e- immature myeloma cells can proliferate in response to IL-6. In most cases of multiple myelomas, MPC-1-CD45+CD49e- proliferative immature cells are only 0.1 to 2 % of bone marrow mononuclear cells, and dominant subpopulations are MPC-1+CD45+CD49e- intermediate cells which are considered to be non-proliferative fractions.
4. From MGUS to symptomatic MM

**P4.1 CRITERIA FOR THE CLASSIFICATION OF MONOCLONAL GAMMOPATHIES, MULTIPLE MYELOMA, AND RELATED DISORDERS: A REPORT OF THE INTERNATIONAL MYELOMA WORKING GROUP**


The monoclonal gammopathies are a group of disorders associated with monoclonal proliferation of plasma cells. The characterisation of specific entities is an area of difficulty in clinical practice. The International Myeloma Working Group has reviewed the criteria for diagnosis and classification with the aim of producing simple, easily used definitions based on routinely available investigations. In monoclonal gammopathy of undetermined significance (MGUS) or monoclonal gammopathy, unattributed/unassociated (MGIu) the monoclonal protein is <30g/L and the bone marrow clonal cells <10% with no evidence of multiple myeloma, other B-cell proliferative disorders, or amyloidosis. In asymptomatic (smouldering) myeloma the M-protein is $\geq$30 g/L and/or bone marrow clonal cells $\geq$10% but no related organ or tissue impairment (ROTI) (end-organ damage); which is typically manifested by increased calcium, renal insufficiency, anaemia, or bone lesions (CRAB) attributed to the plasma cell proliferative process. Symptomatic myeloma requires evidence of ROTI. Nonsecretory myeloma is characterised by the absence of an M-protein in the serum and urine, bone marrow plasmacytosis, and ROTI. Solitary plasmacytoma of bone, extramedullary plasmacytoma, and multiple solitary plasmacytomas (± recurrent) are also defined as distinct entities. The use of these criteria will facilitate comparison of therapeutic trial data. Evaluation of currently available prognostic factors may allow better definition of prognosis in multiple myeloma.

**P4.2 RISK FACTORS FOR EARLY PROGRESSION OF ASYMPOTOMATIC MULTIPLE MYELOMA**


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Most patients with multiple myeloma require chemotherapy at diagnosis because of symptoms due to pathologic fracture, anemia, renal impairment or hypercalcemia. Recent trends include frequent laboratory screening of apparently normal subjects, so that approximately 20% of patients with multiple myeloma are now recognized by chance without significant symptoms. Various terms including “smoldering” myeloma, “indolent” myeloma, and “asymptomatic” myeloma have been used to denote disease which cannot be classified as MGUS, but which may remain stable for long periods without treatment. The term asymptomatic seems preferable since this term describes the clinical status at diagnosis and the subsequent course is unpredictable.

Various criteria have been proposed to distinguish between those asymptomatic patients who are likely to remain stable for a long period and those at high risk who may benefit from earlier treatment. These features have included the presence of a lytic bone lesion, high level of serum myeloma protein, Bence Jones protein, and an abnormal pattern on magnetic resonance imaging.
Fig. 7  Hypothetical model of myeloma cell proliferation in the bone marrow

REFERENCES


4. From MGUS to symptomatic MM

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Various criteria have been proposed to distinguish between those asymptomatic patients who are likely to remain stable for a long period and those at high risk who may benefit from earlier treatment. These features have included the presence of a lytic bone lesion, high level of serum myeloma protein, Bence Jones protein, and an abnormal pattern on magnetic resonance imaging.
some have claimed that levels of hemoglobin or marrow plasmacytosis were also useful.

In order to clarify the prognostic features of these patients we updated our previous experience which identified low, intermediate and high risk groups based on 3 variables (M protein > 3 g/dl, IgA protein type, and BJB > 50 mg/d) for time to progression. Patients with any lytic bone lesions by skeletal x-ray survey were excluded because this feature had been associated with early progression at multiple centers. The features evaluated included serum myeloma protein level (M protein), B2M, marrow plasmacytosis, levels of uninvolved immunoglobulins, myeloma protein type, level of Bence Jones protein, age, albumin, and hemoglobin. For those patients with an available MRI of the spine, the impact of this study was also assessed. Univariate analysis revealed abnormal MRI, myeloma (M) protein > 3 g/dl, B2M > 2.5 mg/L, marrow plasmacytosis >25% and suppression of uninvolved IgM to ≤ 30 mg/d/l to be indicators of a shortened time to progression and multivariate analysis was limited to 5 covariates after eliminating highly correlated independent variables (72 pts with complete information for all 5 variables). The risk categories were identified for time to disease progression as follows: low risk (33 pts) – normal MRI and serum M protein ≤ 3 g/dl (median TTP 79 mos), intermediate risk (28 pts) – abnormal MRI or M protein > 3 g/dl (median TTP 30 mos), and high risk (11 pts) – abnormal MRI and M protein > 3 g/dl (med TTP 17 mos.) (p<.01).

Because MRI was not available for all pts and our previous model had included BJB, we also performed multivariate analysis eliminating MRI and including BJB (109 pts with complete information for all 5 variables). In addition to serum M protein level, the only covariate that contributed significantly to the model was immunoglobulin type, with a higher risk to progression associated with IgA type. Again, 3 risk categories were identified as follows: low risk (50 pts) – serum M protein ≤ 3 g/dl and IgG type (median TTP 52 mos), intermediate risk (51 pts) – M protein > 3 g/dl or IgA type (median TTP 25 mos), and high risk (8 pts) – M protein > 3 g/dl and IgA type (med TTP 9 mos.) (p<.01). Thus, even without MRI our previous model was simplified to only 2 convenient laboratory variables.

In order to improve prognostic precision, we then assessed the impact of MRI on 41 patients with an available MRI and an intermediate risk of progression according to the latter model (M protein > 3 g/dl or IgA type). An abnormal pattern (n=38) distinguished patients more likely to have a shorter time to symptomatic progression (median 25 months) than 3 patients with a normal MRI (median 97 months). Thus, the addition of MRI for intermediate risk patients effectively identified a group of patients likely to have prolonged stability (no positive features or 1 risk factor and normal MRI, median TTP 57 months) in contrast to those with earlier progression (2 risk factors or 1 risk factor and abnormal MRI, median TTP 20 months). (Validation of this analysis in an independent data set is necessary because of the small numbers of patients evaluated). Because a serious complication (fracture, hypercalcemia, renal failure) occurred in approximately one quarter of patients with early disease progression, standard chemotherapy or a trial of new agents may be justified for such patients. The remaining patients are at such low risk for early progression that they may be followed at long intervals without treatment.

**P4.3 CYTOGENETIC CHANGES IN THE EVOLUTION OF MONOCLONAL GAMMOPATHY OF UNDETERMINED SIGNIFICANCE (MGUS) TO MULTIPLE MYELOMA (MM).**

Thierry Facon, Hervé Avet-Loiseau, Xavier Leleu, Régis Bataille

CHU Lille and Nantes, on behalf of the IFM group.

Monoclonal gammapathy of undetermined significance (MGUS) is a condition defined by the detection of a monoclonal protein in the serum without evidence of any causal disease, such as multiple myeloma (MM), Waldenström macroglobulinemia, primary amyloidosis or any related disorder. MGUS is not unusual, corresponding to 1% of the population over 50 years of age, and 3% of the population over 70 years of age. The origin of MGUS is largely unknown and the condition is heterogeneous. Some patients (pts) remain asymptomatic for decades, whereas others evolve to malignant diseases in less than 1 year. The overall incidence of MM transformation is estimated to be 1-2% per year, but little is known about what triggers the occasional progression from MGUS to MM.

As far as conventional cytogenetics is concerned, the evaluation of MGUS is unsuccessful. Some years ago, we and others reported the use of fluorescence in situ hybridization (FISH) on interphase cells for the investigation of numeric chromosomal abnormalities [1-3]. We demonstrated that numeric abnormalities were shared both by MGUS and MM pts and were thus not related to an overt disease. Using FISH at diagnosis and follow-up, we also showed that MGUS pts acquire slowly, gradually, but ineluctably numerical chromosome changes, distributed within several related subclones but not directly related to transformation into MM [3].

To extend the knowledge of MGUS to structural chromosomal abnormalities our group and others performed FISH experiments with probes directed to 14q32 (immunoglobulin H locus) and 13q14 chromosomal regions. Chromosomal translocations involving 14q32 are thought to be early events in the pathogenesis of many B-cell neoplasms, including MM. These translocations involve non random, recurrent, partner chromosomes, especially loci 4p16.3, 11q13 and 16q23. In a recent study, we evaluated such abnormalities in 855 pts with MGUS, smoldering MM (SMM) and overt MM. The results are presented in the Table [4]. The incidence of 14q32 rearrangements was almost 50% in MGUS and SMM, suggesting that they occur early in the clonal development, and the incidence of 11 (11;14) was similar in all conditions (approximately 15%). In contrast, t(4;14) was almost never encountered in MGUS/SMM, possibly because this translocation directly precipitates clonal plasma cells (PCs) into fully malignant PCs, bypassing a stable MGUS. Chromosome 13 deletion (del(13)) was observed in all stages. We found a lower incidence in MGUS compared to MM (21% versus 43%), but other authors found a similar 50% incidence [5]. Del(13) is an early event in MM oncogenesis but the issue whether del(13) is of importance in the progression from MGUS to MM is still debated. In our experience, the presence of a t(14;16)(q32;q23) was very rare, in MGUS as well as in MM. Overall, similar translocations are found in MGUS and MM. In MM, 14q32 and 13q abnormalities correlate with natural history, immunological features, and clinical presentation. In contrast, no obvious clinical or biological correlations have been found in MGUS. Very recently, it was thought that microarray analysis could provide insight into the mechanisms of disease progression [6,7]. It could identify genes or gene families important in the transition of MGUS to MM and these genes might be future therapeutic targets. In two concordant studies, the number of
genes separating MGUS and MM was found considerably less than those separating normal and MM PCs. So far, this approach has failed to explicitly distinguish MM and MGUS, probably because of a lack of sufficient differences between these two conditions.

<table>
<thead>
<tr>
<th>Chromosomal abnormalities</th>
<th>MGUS (n = 147)</th>
<th>SMM (n = 39)</th>
<th>MM (n=186)</th>
<th>MGUS/MM</th>
<th>MM (n = 669)</th>
</tr>
</thead>
<tbody>
<tr>
<td>t(11;14)</td>
<td>66/147 (44)</td>
<td>90/39 (23)</td>
<td>28/186 (15)</td>
<td>NS</td>
<td>68/669 (10)</td>
</tr>
<tr>
<td>t(1;14)</td>
<td>3/147 (2)</td>
<td>1/39 (3)</td>
<td>4/186 (2)</td>
<td>&lt;.001</td>
<td>1/183 (1)</td>
</tr>
<tr>
<td>t(14;16)</td>
<td>1/147 (7)</td>
<td>0/39 (1)</td>
<td>1/186 (&lt; 1)</td>
<td>NS</td>
<td>14/669 (2)</td>
</tr>
<tr>
<td>del(13)</td>
<td>31/147 (21)</td>
<td>11/39 (28)</td>
<td>42/186 (23)</td>
<td>&lt;10^-6</td>
<td>285/669 (43)</td>
</tr>
</tbody>
</table>

*for difference with MM


P4.4 CIRCULATING PLASMA CELLS, LABELING INDEX, AND ANGIOGENESIS IN MGUS AND SMOLDERING MULTIPLE MYELOMA

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Monoclonal gammopathy of undetermined significance (MGUS) affects nearly 2-3 percent of persons over 50 years of age. Patients with MGUS are at risk for progression to multiple myeloma (MM) or related malignancy at a rate of 1% per year, but do not require therapy. For the definition of MGUS, patients must have a serum monoclonal (M) protein <3g/dL, bone marrow plasma cells <10%, and no evidence of anemia, hypercalcemia, renal failure or bone lesions. Smoldering multiple myeloma (SMM) refers to patients who have a serum M protein ≥3g/dL and/or ≥10% plasma cells in the bone marrow, without anemia, bone lesions, hypercalcemia, or renal insufficiency. The risk of progression to MM is higher with SMM than MGUS, with a median time to progression of about 2 years.

Predictors of progression in MGUS: We have determined that the size of the M protein is the single best predictor of progression in MGUS. Another risk factor is the type of M protein: M proteins of the IgM type have a higher risk of progression than IgG and IgA subtypes. Bone marrow plasma cell percentage has been identified as a third risk factor. Conflicting reports are present regarding two other factors: Reduction of uninvolved immunoglobulins and the presence of urinary M proteins. Clearly, additional laboratory predictors of progression to MM are necessary if we are to develop interventions that can target patients with high risk MGUS/MM. Given that MM is incurable, prevention (or delay) of progression of high risk MGUS/MM assumes greater significance.

Circulating plasma cells and labeling index in SMM: Patients with abnormal peripheral blood monoclonal plasma cell studies defined as an increase in the number or proliferative rate of circulating plasma cells by immunofluorescent assays are at higher risk for earlier progression to MM. In a study of 57 patients, Witzig and colleagues found the median time to progression was 9 months for those with abnormal circulating plasma cell values versus 30 months for those with normal studies (P <.01). We have also found an elevated plasma cell labeling index to be an adverse prognostic factor for progression in SMM.

Circulating plasma cells in MGUS: We retrospectively evaluated the prognostic value of circulating plasma cells in 330 persons with MGUS, who had been evaluated at the Mayo Clinic between 1984 and 1997. Presence of plasma cells were determined by slide based immunofluorescence method. The primary endpoint was progression to another plasma-cell disorder. Thirty-eight patients (12%) progressed to MM (23), SMM (11), amyloidosis (3) or Waldenstrom’s macroglobulinemia (1) during the follow up period. Patients with circulating plasma cells were twice as likely (RR 2.2) to have progression to another plasma cell disorder, compared to the rest (P=0.02; 95% CI, 1.1, 4.3). When considering only those with > 0.5 X 10^9/L plasma cells, the relative risk of progression was 2.5 (P=0.016; 95% CI, 1.2, 5.3). The median overall survival was lower for those with circulating plasma cells compared to the rest of the group (P=0.03, logrank test).

Angiogenesis in MGUS and SMM: Similar to most malignancies, the transformation of MGUS to MM may involve induction of an angiogenic switch. BM angiogenesis is increased in MM, has prognostic importance in the disease and is correlated with disease activity and plasma cell proliferative capacity. We studied BM’s from 400 patients with plasma cell disorders seen at the Mayo Clinic: MGUS (76 pts), smoldering MM (SMM ) (112 pts), newly diagnosed, untreated MM (99 pts), relapsed MM (26 pts) and AL (87 pts). By grading, high grade angiogenesis was present in 0% of controls and AL, 1% of MGUS, 3% of SMM, 29% of MM, and 42% of relapsed MM, P<0.001. Studies are ongoing to determine if bone marrow angiogenesis and angiogenic ability of clonal plasma cells in a novel human in vitro angiogenesis assay are predictors of progression in MGUS and SMM.
P4.5 CYTOKINES IN MGUS: THERAPEUTIC INTERVENTIONS TO PREVENT PROGRESSION

John A. Lust, M.D., Ph.D. Kathleen A. Donovan, Ph.D. Mayo Clinic, Rochester, MN

In previously published work, we demonstrated that detection of IL-1β expression by RT/PCR or ISH was useful at differentiating active MM from MGUS. However, we were unable to differentiate the clinically benign condition, SMM from active MM using RNA based techniques. Therefore, we hypothesized that quantitative differences in IL-1β function may differentiate SMM from active MM. Measurement of IL-1β concentration by ELISA proved to be inadequate because of insufficient sensitivity. More importantly, ELISA measured only IL-1β concentration whereas IL-1β functional activity is modulated by several IL-1 family members such as IL-1 receptor antagonist and soluble IL-1 receptor. Therefore, we measured IL-1β functional activity with a bioassay using IL-6 production by bone marrow stromal cells as a highly sensitive surrogate marker. IL-1β bioactivity was determined by quantitating IL-6 production by cultured bone marrow stromal cells using an IL-6 ELISA. Ficoll purified bone marrow cells from patients with various plasma proliferative disorders were cultured at 2 x 10^5 cells/ml and incubated at 37°C for 48 hours. Supernatants were pooled, aliquoted and frozen at -80°C. Normal stromal cells (Clonetics, Walkersville, MD) were plated at 1 x 10^5 cells/ml and incubated at 37°C for 48 hours. After 48 hours, the stromal cells were washed and either IL-1β standards or patients’ supernatants were added with or without an IL-1β inhibitor (anti-IL-1β antibody or IL-1 receptor antagonist). Cultures were incubated at 37°C for another 48 hours. Supernatants are harvested and frozen at -80°C for analysis and subsequently analyzed for IL-6 using the Biosource ELISA kits according to the manufacturer’s specifications. Response of the stromal cells to IL-1β was calibrated with a standard curve using recombinant IL-1β. These cells respond to IL-1β in a sigmoid fashion with as little as 1 pg/ml of IL-1β inducing 20,000 pg/ml of IL-6.

Culture supernatants of unsorted bone marrow cells from 77 untreated patients (2 normal, 12 MGUS, 18 MM, 45 SMM/IMM) were tested in this assay. Results were expressed as fold increase = [patient IL-6 – media control IL-6]/media control IL-6. A fold increase was utilized to allow for inter assay comparison between different batches of stromal cells. IL-1β specificity was determined by inhibition of the IL-6 production with anti-IL-1β antibody. IL-1 specific IL-6 production showed that myeloma patients induced a 2.2 - 24.1 fold increase in IL-6 that was statistically different from the -0.25 - 1.9 fold increase by normal/MGUS patients (p<0.001). The SMM/IMM patients fell into two groups i.e. those patients that had stable disease (n=31) and those that progressed to active myeloma (n=14). The SMM/IMM patients with stable disease generated a -0.63 - 6.91 fold increase in IL-6 production that was statistically different from the 1.62 - 33.16 fold increase by the SMM/IMM patients that progressed to active MM (p=0.001). This stromal cell IL-6 production could be inhibited with an IL-1β inhibitor by >90% in 41/53 patients tested (100% in 32/53). A logistic regression analysis was performed on 62 of the 75 patients that had been followed for at least a year or progressed during that time. Variables examined were % bone marrow plasma cells, plasma cell labeling index, albumin, M-protein level, beta-2 microglobulin, creatinine, calcium, hemoglobin, and stromal cell IL-6 production. Only two factors, stromal cell IL-6 production and bone marrow plasma cells, were found to be significant (p < .01) in predicting progression of SMM to myeloma. Subsequently, a Kaplan-Meier estimate was performed on the 45 patients with SMM/IMM. The time to progression was defined as the time from the date of the bone marrow on which the IL-1β bioassay was performed to the date of progression to active MM. Seventeen patients with SMM/IMM had a fold increase less than 1.6 and, to date, none of these patients have progressed to active disease. In contrast, 28 patients with SMM/IMM demonstrated a FI ≥ 1.6 and 14 of these 28 patients have progressed to active MM with a median time to progression of approximately 2.5 years.

Bone marrow cells from a patient with MM were sorted into CD138+ plasma cells and CD138- populations and examined for IL-6 production by the IL-1β stromal cell bioassay. The IL-6 production was induced predominantly by the CD138+ plasma cells. These results correlate with our previously published data by ISH demonstrating that the monoclonal plasma cells from virtually all myeloma patients express IL-1β mRNA. In addition, the IL-6 production was significantly inhibited with IL-1 receptor antagonist. The above studies served as the preclinical data for a Phase II trial of IL-1 receptor antagonist (IL-1Ra) in patients with SMM/IMM. We have now accrued eight patients in the clinical study using IL-1Ra in patients with SMM/IMM and preliminary studies are available on four. Patients that have ≥10% bone marrow plasma cells and/or M-spike ≥ 5 g/dL and do not require immediate chemotherapy are eligible. Patients receive 100 mg of Anakinra SQ qd. Clinical results are available on four patients with 1-3 months of follow up. The underlying hypothesis is that IL-1β stimulates paracrine IL-6, the major growth factor for myeloma cells and that IL-1 receptor antagonist (IL-1Ra) will inhibit paracrine IL-6 production ultimately leading to apoptosis of the myeloma cells. Using CRP as a surrogate marker for IL-6 levels, all four patients demonstrated a reduction in CRP levels. For example, Patient 1 had a reduction in CRP (mg/dL) from 3.82 to 0.825 to 0.122 or a 96% reduction; patient 2 a 74% reduction in CRP after 2 months. In a similar fashion, urine NTX (nmole/L), which is a marker of osteoclast activity, was decreased in three patients (a followup urine was not available on one of the patients). Patient #1 also had an increase in her hemoglobin from 10.1 to 11.9. Monoclonal protein levels have remained stable in three of the patients so far. However, patient #2 demonstrated a gradual decline in the CRP with IL-1Ra that paralleled a reduction in the serum monoclonal protein (see Figure). Toxicity has been minimal with mild injection site reactions during the first month of therapy. One patient developed an asymptomatic Grade 4 neutropenia and the IL-1Ra was held. The patient’s counts returned to normal within 1 week and the IL-1Ra was restarted at 100mg qod. Although preliminary, these results demonstrate that IL-1Ra has biologic activity in patients with SMM/IMM and support the hypothesis that upregulation of IL-1β production is a critical event in the transition of SMM/IMM to active myeloma.

Donovan KA, Lacy MQ, Kline MP, Ahmann GJ, Heinimbach JA, Kyle RA, Lust JA:. Contrast in cytokine expression between patients with monoclonal gammopathy of undetermined significance or multiple myeloma. Leukemia 12:593-600, 1998


Donovan KA, Lacy MQ, Gertz MA, Lust JA. IL-1β Expression in IgM Monoclonal Gammopathy and Its Relationship to Multiple Myeloma. Leukemia, March 2002, 16:382-385.
5. Signal transduction pathways and cytokine networks

P5.1 OVERVIEW OF MULTIPLE MYELOMA SIGNAL TRANSDUCTION PATHWAYS

Anderson K

Cytokines in the BM microenvironment mediate growth of MM cells [interleukin-6 (IL-6), insulin-like growth factor-1 (IGF-1), vascular endothelial growth factor (VEGF), tumor necrosis factor-α (TNF-α); tumor cell survival or resistance to apoptosis (IL-6, IGF-1, IL-21); and migration (VEGF, stromal cell-derived factor-1 (SDF-1))]. Adhesion of tumor cells to BM stromal cells further upregulates transcription and secretion of cytokines in BM stromal cells and/or MM cells, thereby promoting autocrine and paracrine tumor cell growth and survival. Moreover, cytokines can modulate adhesion of MM cells in BM. For example, TNFα in the BM milieu induces NF-κB dependent upregulation of cell surface adhesion molecules [ICAM-1, vascular cell adhesion molecule-1 (VCAM-1)] on both MM cells and BM stromal cells, with related increased binding as well as induction of transcription and secretion of cytokines (IL-6, VEGF) in BM stromal cells. The delineation of signaling cascades mediating proliferation, survival, and migration of MM cells in the BM milieu both enhances understanding of pathogenesis and provides the framework for identification and validation of novel molecular targets. Proliferation of MM cells triggered by cytokines (IL-6, IGF-1, VEGF, TNFα, SDF-1α, IL-21) is mediated primarily via the Ras/mitogen activated protein kinase kinase (Mek)/p42/44 mitogen-activated protein kinase (MAPK) signaling cascade. Cytokine-induced survival or resistance to apoptosis in MM cells is mediated via Janus kinase (JAK)/signal transducers and activators of transcription 3 (STAT3) (IL-6), as well as the phosphatidylinositol 3-kinase (PI3-K)/Akt (IL-6, IGF-1, TNFα, SDF-1α) pathways. In contrast, MM cell migration induced by cytokines (VEGF) is mediated via a protein kinase C (PKC) dependent, p42/44MAPK-independent, pathway. Importantly, the BM microenvironment also confers drug resistance via two mechanisms. First, MM cell binding to fibronectin confers cell adhesion mediated drug resistance (CAM-DR) associated with induction of p27 and G1 growth arrest. Second, cytokines in the BM milieu induce JAK/STAT and PI3-K/Akt signaling which mediates resistance to conventional and novel therapies. Specifically, DNA damaging agents, irradiation (IR), Fas, TNF-related apoptosis-inducing ligand (TRAIL), as well as Thalidomide (Thal) and its immunomodulatory derivatives (IMiDs) activate caspase 8 and arsenic trioxide (As2O3) activate caspase 9; and proteasome inhibitor PS-341 activates both caspases 8 and 9. In all cases downstream death signaling is mediated via activation of caspase 3, poly ADP ribose polymerase (PARP) cleavage, and apoptosis. Apoptosis triggered by Dex, commonly used clinically to treat MM, is associated with activation of related adhesion focal tyrosine kinase (RAFTK) as well as release of second mitochondria activator of caspase (Smac), but not cytochrome c, from mitochondria. IL-6 confers drug resistance via activation of JAK/STAT signaling and upregulation of Bel-1L and Mcl-1 expression. In addition, IL-6 activates SHP2 phosphatase, which blocks Dex induced activation of RAFTK and apoptosis. Both IL-6 and IGF-1 inhibit drug-induced MM cell apoptosis via PI3K/Akt signaling and NF-κB activation, with downstream induction of intracellular inhibitors of apoptosis (IAPs) including FLICE inhibitory protein (FLIP), survivin, cIAP-2, A1/BFL-1 and XIAP. These studies therefore both define mechanisms of tumor cell adhesion and cytokine mediated anti-apoptotic sequelae in the BM milieu, and identify potential novel therapeutic targets.

P5.2 GENETIC DEREGULATION EFFECTS ON SIGNALING

Alan Lichtenstein, Joseph Gera, Yijiang Shi, Fuyuhiko Tamanoi and Brian Van Ness

Oncogenic K-ras or N-ras mutations are some of the most common genetic defects in myeloma cells, occurring in up to 40-50% of patients in selected series. The associations of these mutations with stage III disease, disease progression and plasma cell leukemia suggest they impart an aggressive phenotype to their myeloma clones. Constitutive activity of mutant ras proteins results in deregulated downstream signaling through several cascades. Use of the IL-6-dependent ANBL-6 myeloma cell line has allowed us to identify these cascades. When ANBL-6 cells are stably transfected with oncogenic N-ras or K-ras genes, they become IL-6-independent. In addition, expression of oncogenic ras in these cells results in the following constitutively upregulated signal pathways: 1) MEK/ERK; 2) PI3-kinase/AKT; 3) mTOR/p70S6kinase, and; 4) NF-kB. As these pathways are known to promote myeloma cell growth, they may be contributing to the ras-dependent proliferation and anti-apoptotic signals in the ANBL-6 model.

Although ras mutations certainly appear to provide a growth advantage and aggressive behavior in myeloma cells, they may also confer a sensitivity to therapy targeted to ras or the above described upregulated downstream pathways. For example, in model systems, hyperactive ras is known to induce an apoptosis-sensitive phenotype via its ability to upregulate expression of caspases and accelerate/stimulate release of cytochrome C from mitochondria. Thus, mutant ras-containing myeloma cells may be either specifically deficient in those apoptotic effects of mutant ras or additional ras-dependent downstream pathways may protect against this ras signature of enhanced apoptosis. Investigations in this arena with the goal of re-instituting the apoptosis signature would have therapeutic potential. A second strategy for targeting oncogenic ras is to prevent its farnesylation with newly developed farnesyl transferase inhibitors (FTIs). Ras proteins must be processed by farnesylation or geranylgeranylation in order to be properly localized to the cell membrane. Promising work on use of FTIs in patients will be presented later in this symposium. However, there are theoretical problems with this therapeutic concept. For example, although H-ras is very sensitive to inactivation by FTIs, K-ras and N-ras are much less so, owing to their greater avidity for farnesyl transferase itself, the target of FTIs, or their great facility for geranylgeranylation in the face of farnesyltransferase inhibition. In fact, using the same ANBL-6 model transfected with oncogenic ras or additional ras-dependent downstream pathways may protect against this ras signature of enhanced apoptosis. Investigations in this arena with the goal of re-instituting the apoptosis signature would have therapeutic potential. A second strategy for targeting oncogenic ras is to prevent its farnesylation with newly developed farnesyl transferase inhibitors (FTIs). Ras proteins must be processed by farnesylation or geranylgeranylation in order to be properly localized to the cell membrane. Promising work on use of FTIs in patients will be presented later in this symposium. However, there are theoretical problems with this therapeutic concept. For example, although H-ras is very sensitive to inactivation by FTIs, K-ras and N-ras are much less so, owing to their greater avidity for farnesyl transferase itself, the target of FTIs, or their great facility for geranylgeranylation in the face of farnesyltransferase inhibition. In fact, using the same ANBL-6 model transfected with oncogenic ras, we found a strong apoptotic effect of FTIs in the absence of any effect on ras processing, suggesting a mechanism of action independent of effects on ras itself. In a third approach, we have targeted the upregulated AKT activation in mutant ras-containing myeloma cells. Our prior work with myeloma cells containing heightened AKT activation due to PTEN mutations indicated that upregulated AKT activity was associated with hypersensitivity to mTOR inhibitors such as rapamycin and its newer analog, CCI-779. This influence of AKT on sensitivity to mTOR inhibitors has been seen in other tumor models as well. MTOR inhibitors induce cytostasis by...
WNT SIGNALING IN MULTIPLE MYELOMA

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Wnt comprises a family of secreted proteins that interact with receptors consisting of a Frizzled (Fz) family member alone or complexed with LDL receptor related proteins (LRP5/6). Wnt signaling plays a crucial role in both development and differentiation and activation of a ‘canonical’ Wnt pathway resulting in -catenin stabilization is associated with several types of human cancers the most well studied of which are colorectal tumors, but also include fibromatosis, gastric, and hepatocellular carcinoma. -catenin involvement in cancer is commonly associated with mutations in the amino terminal region that make the molecule resistant to processing and degradation. Mutations in other proteins in this pathway, most notably the APC gene in colon cancer, similarly lead to -catenin accumulation. A role for other Wnt activated (non-canonical) pathways in disease has yet to be determined. To date, little is known about potential Wnt signaling in mature lymphocytes or lymphoid neoplasia. Herein, we have analyzed Wnt signaling in mature B cells (lymphomas) and plasma cells (multiple myeloma). Both Fz and LRP5/6 mRNAs were expressed in myeloma lines, but LRP5/6 were not observed in lymphomas. In myelomas, a canonical Wnt signaling pathway was activated following treatment with Wnt-3a as assessed by accumulation of -catenin and transcriptional activation, but -catenin levels actually decreased in lymphoma cells. Wnt-3a treatment further led to striking morphological changes in myeloma cells accompanied by rearrangement of the actin cytoskeleton. Morphological changes resulted in cells developing filopodia-like processes and becoming attached to culture dishes. The alterations in morphology were associated with a second Wnt pathway dependent on Rho activation and could be blocked by an inhibitor of Rho-associated kinase. These results suggest that Wnt responsiveness is a stage specific phenomenon in B cell neoplasia and that the morphological changes associated with Wnt signaling may play a role in the motility and metastatic potential of myeloma cells.

P5.4
APOPTOTIC AND SURVIVAL SIGNALING: THERAPEUTIC IMPLICATIONS

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Diverse classes of chemotherapeutic agents induce apoptosis in multiple myeloma (MM) cells. In contrast, various growth factors and cytokines present within the bone marrow (BM) microenvironment trigger MM cell growth and block the apoptotic effects of drugs. For example, studies in MM cells have shown that BM-growth factors such as, interleukin-6 (IL-6), insulin growth factor (IGF) or vascular endothelial growth factor (VEGF) trigger growth and provide protection against Dexamethasone (Dex)-induced apoptosis in these cells. Novel agents that directly and simultaneously target the tumor cell and its BM microenvironment are required to both enhance drug anti-MM activity and prevent development of drug-resistance. Delineation of cellular growth and apoptotic signaling pathways identify molecule(s) that may serve as novel therapeutic targets. Our studies have shown that IL-6, IGF or VEGF induce proliferation of MM cells by activating MAP kinase, PKC and/or PI3K/Akt pathways. Pretreatment of cells with specific biochemical inhibitors of these pathways blocks MM cell growth. Various drugs either alone or in combination with biochemical inhibitors cause synergistic anti-MM effect via downregulation of growth pathways. Conversely, novel anti-MM agents such as Proteasome inhibitor (PS-341), 2-methoxyestradiol (2ME2), Thalidomide and its immunomodulatory derivatives (IMiDs) trigger apoptotic signaling that enables the protective effects of the BM microenvironment, as well as overcomes drug-resistance.

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in MM cells. The apoptotic mechanism(s) mediating the anti-MM effects of these novel agents is unclear. Our studies to date have shown that all these agents share some common signals. For example, a decrease in the mitochondrial transmembrane potential, caspase-3 activation and Poly (ADP ribose) polymerase (PARP) cleavage are universal events triggered in response to all agents. However, these agents also induce differential and/or additional upstream signaling cascades that lead to caspase-3 or PARP cleavage. As an example for this diversity, here, we have shown the delineation of 2ME2- or PS-341- versus Dex-induced mitochondrial apoptotic signaling in MM cells. Mitochondria harbor two key modulators of apoptosis, cytochrome-c (cyto-c) and Smac (Second mitochondria-derived activator of caspase) or DIABLO, which are released from mitochondria into the cytosol during apoptosis. Both these proteins activate caspase-9 via different mechanisms: cytosolic cyto-c binds to Apaf-1 > Apaf-1 oligomerization > Caspase-9 activation; cytosolic Smac binds to XIAP (an inhibitor of apoptosis protein) and thereby eliminates its inhibitory effects on caspase-9. In the context of MM, we showed that both 2ME2 and PS-341, but not Dex, triggers the release of cyto-c; all these agents activate caspase-9. IL-6, a growth factor for MM, blocks Dex-, but not 2ME2 or PS-341-, induced apoptosis by preventing the release of Smac. Irradiation and IMiDs also trigger the release of cyto-c, suggesting that the cyto-c is essential for most drug-induced apoptosis in MM. The lack of cyto-c signal in response to Dex, coupled with the finding that IL-6 protects MM cells against Dex-induced apoptosis, suggests that a combination of Dex with the novel agents that restores an additional cyto-c signal will enhance the anti-MM activity of Dex.

**Figure Schema showing drug-induced signaling via mitochondria.**

The mechanism(s) mediating the release of cyto-c or Smac is unclear. The c-Jun-NH2-terminal kinase (JNK) translocates to mitochondria after genotoxic stress and inhibits the anti-apoptotic function of proteins belonging to Bcl2 family members, thereby allowing the mitochondrial apoptotic proteins to cytosol and subsequent activation of caspase cascades. Since both 2ME2 and PS-341 induce cyto release, we next asked whether 2ME2 or PS-341 affects JNK. Our results demonstrate that 2ME2 or PS-341-induced apoptosis in MM cells is associated with activation of JNK, translocation of JNK from cytosol to mitochondria, and release of Smac from mitochondria to cytosol. Blocking JNK either by dominant-negative mutant (DN-JNK) or cotreatment with a specific JNK inhibitor SP600125, abrogates both stress-induced release of Smac and induction of apoptosis. These findings demonstrate that activation of JNK is an obligatory event for the release of cyto-c and Smac during 2ME2 or PS-341-induced apoptosis in MM cells. Importantly, our prior studies have also shown that Dex-induced apoptosis is not associated with activation of JNK. Collectively, the cellular signaling data in MM cell have important biologic and therapeutic implications. First, our results showing that anti-MM drugs induce apoptosis via release of mitochondrial Smac suggest that Smac antagonists or active Smac peptides may sensitize MM cells to various anti-MM agents. Second, the observation that Dex-induced signaling lacks cyto-c and JNK activation provides a rationale for combination of Dex with novel agents that trigger both cyto-c release and JNK, thereby allowing enhanced anti-MM activity.
transducing component of the interleukin 6 (IL-6) receptor, to cross-communicate with unrelated receptor systems (3-5).

The goal of this study, therefore, was to determine whether IL-6 signaling via gp130 interfaces with signals mediated through other receptors expressed by myeloma cells. In this regard, we have had a long-standing interest in interferon-alpha (IFN-α) and insulin-like growth factor-I (IGF-I). IFN-α is a cytokine that typically inhibits myeloma cell growth, and IGF-I is a growth factor that can directly stimulate myeloma cell growth and in some circumstances, synergistically enhance IL-6-stimulated myeloma cell growth. We have made a number of interesting observations which will be presented including novel evidence for receptor cross-talk between gp130 and the IFN-α receptor system in myeloma cells and the ability of IGF-IR levels to influence the magnitude of IL-6 stimulated myeloma cell proliferation. In conclusion, these studies suggest that atypical receptor expression levels and accompanying unexpected receptor interactions may be a common occurrence in multiple myeloma and may underlie variable regulation of malignant plasma cell growth and survival.

This work was supported by National Institutes of Health grants CA62242 and CA62228.

References:


6. Role of microenvironment

P6.1

THE INFLUENCE OF THE TUMOR MICROENVIRONMENT ON MYELOMA PROGRESSION AND SURVIVAL

William S. Dalton, Yulia Nefedova, Melissa Alsina, Terry Landowski, Kenneth Shain, and Lori Hazlehurst

H. Lee Moffitt Cancer Center and Research Institute At the University of South Florida Tampa, Florida

Classically, studies of drug resistance in cancer have focused on the molecular biology of single cancer cells. These types of studies have provided important information regarding certain drug resistance mechanisms, including mechanisms that reduce intracellular drug accumulation, alter or repair drug-induced damage, and reduce drug-induced apoptosis. While these cellular mechanisms undoubtedly contribute to the overall phenomenon of drug resistance, it is now evident that the tumor cell microenvironment also influences how a tumor cell behaves and responds to cytotoxic drugs or radiation. Two different forms of tumor cell-environmental interaction may explain how some myeloma cells survive initial drug exposure and eventually express classical mechanisms of drug resistance. The first form involves soluble mediators, such as interleukins, that are secreted by non-tumor, stromal cells. Interleukin-6 (IL-6) is a classical example of how a soluble mediator secreted by the tumor microenvironment is capable of enhancing myeloma cell survival and blocking apoptosis (Catlett-Falcone et al 1999). The second form of tumor cell-environment interaction requires direct cell contact and has been given the term cell adhesion mediated drug resistance (CAM-DR). In this case, binding extracellular matrix ligands in the tumor microenvironment may activate cell adhesion molecules, such as the integrins, and these interactions result in the activation of signal transduction pathways that block drug-induced apoptosis. Interrupting the tumor cell-environment interactions or the associated signal transduction pathways may represent a new approach for the treatment of myeloma.

Our laboratory has shown that adhesion of myeloma cells to fibronectin (FN) via β1 integrins contributes to a reversible de-novo drug resistance or CAM-DR (Damiano et al 1999). More recently, we have extended this observation to myeloma cells adhered to bone marrow stromal cells (Nefedova 2003). We have also observed that, in addition to inhibiting intrinsic pathways of apoptosis induced by cytotoxics, that myeloma cell adhesion to FN blocks extrinsic pathways of apoptosis induced by CD95 (Shain et al 2002). Most recently, we have compared de novo and acquired resistance to melphalan induced cell death in the human myeloma cell line RPMI 8226. Our findings show that acquired resistance to melphalan functionally correlates with reduced melphalan interstrand crosslinks and a complex array of gene expression changes involving DNA repair genes, transporters, detoxifying molecules, and antiapoptotic genes. By comparison, myeloma cells exhibiting a temporal melphalan resistance following adhesion to FN are resistant to melphalan induced mitochondrial perturbations and apoptosis compared to drug sensitive cells despite no changes in melphalan induced DNA damage. Changes in the transcriptome when 8226 myeloma cells were adhered to FN were less complex compared to cells with acquired drug resistance; however, similar changes in gene expression profiles were observed between cells with de novo and acquired melphalan resistance. We propose that CAM-DR induces reversible genomic changes that predispose cells to
transducing component of the interleukin 6 (IL-6) receptor, to cross-communicate with unrelated receptor systems (3-5).

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survive initial drug exposure and allows for the ultimate acquisition of resistance to drugs, including melphalan.

References:


P6.2
CELL ADHESION ALTERS GENE EXPRESSION AND ENHANCES CELL SURVIVAL IN MM

Terry H. Landowski, Nancy E. Olashaw, D. Agrawal, and William S. Dalton
H. Lee Moffitt Cancer Center and Research Institute, University of South Florida, Tampa, FL, and University of Arizona Cancer Center, University of Arizona, Tucson, AZ.

Resistance to chemotherapeutic drugs is the primary obstacle to the successful treatment of multiple myeloma. A large number of studies have demonstrated that mechanisms of resistance to cell death are frequently induced in response to drug exposure, however, more recent work has documented the contribution of the tumor microenvironment to the anti-apoptotic phenotype. The interaction between tumor cell and environment may explain how some tumor cells survive the initial drug exposure and acquire classical mechanisms of drug resistance. We have previously described the phenomenon of cell adhesion mediated resistance (CAM-DR) to programmed cell death in hematopoietic cell lines (1,2). This phenotype is characterized by increased resistance to physiological stimuli, such as CD95 ligation, and to a wide variety of chemotherapeutic drugs following adhesion to fibronectin through B1 integrins (3,4). While extensive research has identified a number of individual mechanisms contributing to CAM-DR, the paramount question remaining is how do malignant cells coordinate global changes in gene expression to alter the phenotypic state of the tumor from that of pro-apoptotic, to that of pro-survival?

To identify signal transduction pathways and gene products that may contribute to CAM-DR, we have utilized oligonucleotide microarray analysis of 8226 myeloma cells adhered to FN compared to cells maintained in suspension (5). Cells maintained in suspension were designated as the reference population, and genes with altered expression in cells adhered to FN ranked by fold increase. Of the 53 genes induced by FN, 11 are known to be regulated by NF-κB.

EMSA analysis demonstrated NF-κB binding activity significantly increased in cells adhered to fibronectin compared to cells in suspension. Supershift analysis with antibodies specific for NF-κB family members demonstrates the majority of the DNA binding activity in FN adhered cells is composed of RelB/p50 heterodimers with very low levels of p65. This activity was distinct from that seen following treatment with TNFα, which induces p65/p50 within 30 minutes of exposure.

Several of the NF-κB gene products identified by microarray analysis in our study are well-characterized survival factors in multiple myeloma, including interleukin 6, cIAP-2. Rnase protection and Western Blot analysis confirmed a 2 fold induction of the anti-apoptotic molecule cIAP-2 following B1 integrin-mediated adhesion to FN. We propose that activation of RelB/NF-κB in myeloma cells by adhesion to fibronectin in the bone marrow microenvironment may contribute to the CAM-DR phenotype.

References:

Landowski TH, Olashaw NE, Agrawal D., and Dalton WS Cell Adhesion Mediated Drug Resistance (CAM-DR) is Associated with Activation of NF-κB (RelB/p50) in Myeloma cells. Oncogene 2003, in press.

Table I. NF-κB regulated genes induced by adhesion to fibronectin

<table>
<thead>
<tr>
<th>Gene Accession</th>
<th>Description</th>
<th>Fold change</th>
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<tr>
<td>X66867</td>
<td>X-box binding protein</td>
<td>2.1</td>
</tr>
<tr>
<td>X66365</td>
<td>cyclin-dependent kinase 6</td>
<td>2.0</td>
</tr>
<tr>
<td>Y00081</td>
<td>interleukin 6 (interferon, beta 2)</td>
<td>4.9</td>
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<tr>
<td>Y00787</td>
<td>interleukin 8</td>
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<td>X56692</td>
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<tr>
<td>D79206</td>
<td>cIg protein, syndecan-4</td>
<td>3.9</td>
</tr>
<tr>
<td>M92357</td>
<td>tumor necrosis factor, alpha-induced protein 2</td>
<td>6.5</td>
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<tr>
<td>X53683</td>
<td>small inducible cytokine A4 (homologous to mouse Mip-1b)</td>
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<td>Y00787</td>
<td>interleukin 8</td>
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<tr>
<td>X66867</td>
<td>X-box binding protein</td>
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Table I. The GM-CSF expression profile of 8226 myeloma cells adhered to fibronectin for 8 hours was compared to cells in suspension and ranked by fold increase. Of the top 53 genes induced by FN, 11 are known to be regulated by NF-κB.
**P6.3 ENDOTHELIAL CELL-TUMOR CELL INTERACTIONS IN MULTIPLE MYELOMA.**

Ivan Van Riet¹, Isabelle Vande Broek¹, Kewal Asosingh¹, Els Van Valckenborgh¹, Liesbeth Hellebaut¹, Xavier Leleu², Thierry Facon², Ben Van Camp¹ and Karin Vanderkerken¹

¹Department of Hematology and Immunology, Vrije Universiteit Brussel (VUB), Brussels, Belgium; ²Department of Hematology, Hopital Huriez, Lille, France.

Endothelial cells (EC) represent, within the tumor-microenvironment, important interactive partners for multiple myeloma (MM) cells, since they are involved in different aspects of the paracrine network that underlies the pathogenesis of MM. Myeloma cells interact with bone marrow EC (BM-EC) during extravasation/homing and can directly activate EC resulting in neovascularization.

Using the in vivo 5T2MM-mouse model our group previously demonstrated that the specific localization of myeloma cells in the bone marrow (BM) is a result of the combination of both a selective entry/adhesion and a selective survival/growth of the tumor cells in the BM (1). In the same murine model the selective entry in BM could be associated with a selective adhesion to BM-EC, involving CD44v10 (1, 2). After adhesion to BM-EC, myeloma cells will receive chemotactic signals that will stimulate their migration to the extravascular (medullar) compartment. We could demonstrate that the migration of both human and murine MM cells could be triggered by laminin-1 and MCP-1 (both produced by BM stromal cells, including EC). These migratory responses are mediated by the 67kD laminin-receptor (LR) and the CCR-2 chemokine-receptor, respectively (3, 4, 5). It was also found that 67kD LR can be up-regulated in MM cells after contact with BM-EC and that this receptor is also involved in the bone marrow homing of 5T2MM cells in vivo (3). In order to cross bone marrow endothelium, MM cells also need to degrade the basement membrane. Using Matrigel invasion assays, we could demonstrate that human isolated (CD138 positive) MM cells are indeed invasive and that this invasive capacity could be enhanced by the presence of BM-EC. Moreover we found that the transendothelial invasion of MM cells involves MMP-9. In the 5T33MM model we could demonstrate an in vivo, bone marrow microenvironment-dependent, transcriptional upregulation of MMP-9 in the MM cells (6). In vitro experiments demonstrated that the production of this protease could be up-regulated in both murine (5T33MM) and human (CD138 positive) MM cells by interaction with BM-EC (6, 7). In human MM cells, this BM-EC-mediated up-regulation of MMP-9 seems to involve hepatocyte growth factor (7). After extravasation, MM cells continue to interact with BM-EC, directly contributing to the formation of new blood vessels. We could demonstrate/confirn that murine (5T33MM) and human MM cells have angiogenic-inducing potential and express different pro-angiogenic factors including VEGF-A, b-FGF and/or angiopoietin-1 (8, 9). Moreover we found that the expression of some of these factors (VEGF and bFGF) in human MM cells is regulated by the BM-stroma. Comparing the functional activity of human MM-cell derived VEGF-A and bFGF in the in vitro proliferation and migration of EC, we found that VEGF plays a major but not exclusive role (9). Most recently we found that both human and murine MM cells also express transcripts for platelet-derived growth factor PDGF (A, B, C and D). This factor induces angiogenic responses similar to VEGF, through interaction with receptor tyrosine kinases (RTK). We are currently investigating in the in vivo 5T33MM-mouse model the effect of a new potent RTK inhibitor, i.e. SU11657, that blocks the receptors for VEGF and PDGF.

In conclusion, EC are involved in different aspects of tumor-host communication in MM and may therefore also represent, as important component of the stromal cell population, an interesting target for new therapeutical approaches. Future efforts, including the use of microarray analysis, should further clarify the molecular background of these MM cell-EC interactions.

I. Vande Broek et al., Blood, 100, 209a, 2002.

**P6.4 ANGIOTIC ENDOThelial CELLS WITHIN THE BONE MARROW OF MULTIPLE MYELOMA. A PHENOTYPIC AND FUNCTIONAL ASSESSMENT**

Angelo Vacca, Roberto Ria, Domenico Ribatti, Fabrizio Semeraro, Francesca Merchionne, Franco Dammacco

Department of Biomedical Sciences and Human Oncology, Section of Internal Medicine and Clinical Oncology, and Department of Human Anatomy and Histology, University of Bari Medical School, I-70124 Bari, Italy.

Endothelial cells of tumor vessels greatly differ from those of quiescent normal vessels due to their rapid proliferation which reflects enhanced angiogenesis associated with tumor progression (growth, invasion, metastasis) (1). They also differ in the profile and level of cell adhesion molecules because attachment to one another and to extracellular matrix during sprouting (that implies cell proliferation and migration) is remarkably reduced. Their survival is largely dependent on growth factors secreted by the tumor and its microenvironment (cells and matrix), and on their expression of specific receptors for these factors. Moreover, they are abnormal in shape and highly permeable due to fenestrae, vesicles, transcellular holes, widened intercellular junctions, a discontinuous basement membrane and scarce accessory stabilizing cells such as pericytes. They share the lining of new vessels with tumor cells able to mimic vessels. The fast growth of endothelial and tumor cells coupled with structural and functional abnormalities of endothelial cells make tumor vessels tortuous and dilated, with uneven diameter, excessive branching and shunts. Thus, tumor blood flow is chaotic and variable, and leads to hypoxic and acidic regions in the tumor that stimulate further angiogenesis.

Bone marrow angiogenesis is mandatory for progression of multiple myeloma (MM) (2, 3), and is characterized by thin, tortuous and arborized vessels, and single or clustered endothelial cells (2). This process is sustained by vascular endothelial growth factor (VEGF) (4), basic fibroblast growth factor (bFGF), and matrix metalloproteinases (MMPs) (2) secreted by plasma cells. Besides their morphological picture, information on phenotype, function and structure of endothelial cells is circumstantial (2). In this presentation bone marrow endothelial cells of active MM are compared to normal quiescent endothelial cells in an attempt to add further information to the issue.

Endothelial cells were extracted from bone marrow of 57 patients, and compared for the antigenic and genetic phenotype, functions and ultrastructural morphology with their normal
quiescent counterpart, the human umbilical vein endothelial cells (HUVEC). MM endothelial cells express highly: a) vascular endothelial growth factor receptor-2 (VEGFR-2) and tyrosin kinase with Ig and EGF homology-2 (Tie2/Tek), suggesting their engagement in vessel sprouting, i.e. in angiogenesis; b) CD34 and CD133 (AC133), suggesting recruitment of endothelial progenitor cells into an ancillary vascular network, i.e. into embryonic vasculogenesis; c) basic fibroblast growth factor receptor-2 (bFGFR-2) and bFGFR-3, suggesting that they are prone to this growth factor secreted by plasma cells and stromal cells; d) endoglin, a marker of tumor vessels; d) E-selectin and β3 molecules, suggesting more opportunities of interactions with plasma cells; e) aquaporin 1, suggesting hyperpermeability. On the contrary, they poorly express vascular-endothelial (VE)-cadherin, as angiosarcoma cells. Fluorescent activated cell sorting (FACS) analysis of some antigens shows their heterogeneous expression, suggesting well defined subpopulations of cells. The main genetic markers are Tie-2/Tek, VEGFs, bFGFs and the corresponding receptors. MM endothelial cells rapidly form a close capillary network in vitro (matrigel assay), and generate on their turn numerous new vessels in vivo (chick embryo chorioallantoic membrane [CAM] assay). They secrete VEGF, bFGF, metalloproteinase-2 (MMP-2) and MMP-9, that are growth and invasive factors both for themselves and plasma cells. Ultrastructurally, they show vesicles, fenestrae, and hyperplasia of endoplasmic reticulum that are absent in HUVEC. Thalidomide interferes with their proliferative activity and capillarogenesis on matrigel. Our data suggest that both embryonic vasculogenesis and angiogenesis concur to the formation of vascular tree of MM bone marrow and disease progression. Because of the heterogeneous antigenic phenotype, a mixture (or sequence) of antiangiogenic agents coupled with thalidomide is envisaged as a possible biologic therapy of MM.


P6.5 GENOMIC AND PROTEOMIC CHANGES FOLLOWING MM CELL-MICROENVIRONMENTAL INTERACTION

Constantine S. Mitsiades1,2, Nicholas S. Mitsiades1,2, Ciaran McMullan1,2, Galinos Fanourakis1,2, Reshima Shringarpure1,2, Nikhil C. Munshi1,2, Towa Liberman3, Kenneth C. Anderson1,2.

1. Jerome Lipper Multiple Myeloma Center, Department of Medical Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts, USA; 2. Department of Medicine, Harvard Medical School, Boston, Massachusetts, USA; 3. Harvard Institutes of Medicine, Beth Israel Deaconess Medical Center, Boston, Massachusetts, USA.

The response of multiple myeloma (MM) patients to conventional therapies is significantly affected by interactions of MM tumor cells with their local bone marrow (BM) microenvironment, including biologic sequelae induced by BM-derived cytokines, and adhesion to extracellular matrix proteins and BM stromal cells (BMSCs). Indeed, these interactions can confer protection to MM cells against pro-apoptotic therapies (e.g. dexamethasone or cytotoxic chemotherapy), with adverse implications for patient outcome. The need to develop rational strategies to target and abrogate this microenvironment-derived drug-resistance of MM cells has provided the impetus for comprehensive profiling of the molecular sequelae triggered by exposure of MM cells to microenvironmental stimuli, such as BM-derived cytokines (such as IL-6 and insulin-like growth factors (IGFs)) or co-culture with BMSCs. IL-6 is known for its role as a growth/survival factor for MM cells and an important regulator of osteoclastogenesis, while the MM-BMSCs interaction is known to trigger NF-êB-mediated IL-6 secretion by BMSCs. The major emphasis on IGFs is warranted by our recent studies showing that IGFs not only stimulate MM cell proliferation, survival and attenuated response to apoptosis-inducing agents (e.g. Dex or Apo2L/TRAIL), but are also expressed at high levels in serum of MM patients (endocrine IGF), as well as locally in the BM microenvironment by autocrine (MM cells) and paracrine (including BMSCs and osteoblasts) sources (CS Mitsiades et al. Blood 2002; 100, 170a).

Importantly, we have recently shown that IGF-1 receptor (IGF-1R/CD221) is expressed on all MM cell lines and patients cells tested and that its inhibition by several different strategies (including neutralizing antibodies, inhibitory peptides or small molecule Tyr kinase inhibitors) significantly suppresses MM cell proliferation, survival and resistance to other drugs, both in vitro and in vivo (CS Mitsiades et al. Blood 2002; 100, 170a).

To characterize the molecular sequelae triggered by these microenvironmental interactions of MM cells with their BM milieu, we performed gene expression profiling, using U133A Affymetrix oligonucleotide microarrays, and proteomic analyses of the signaling state of MM cells, using multiplex immunoblotting arrays, as recently described (N. Mitsiades et al. Blood 2003;101(6):2377 and CS Mitsiades et al. Semin Oncol, in press). These studies involved ex vivo stimulation of MM cells with pathophysiologically-relevant concentrations of IGF-1, IGF-2 and IL-6, as well as incubation of MM cells in an ex vivo model of co-culture with BMSCs. In this model, MM-1S cells stably transfected with a construct for Green fluorescent protein (GFP) were co-cultured with BMSCs: the 2 cellular compartments were subsequently sorted by fluorescence activated cell sorting (FACS) on the basis of the GFP + status of MM cells vs GFP- of BMSCs (thereby minimizing any potential background signaling and transcriptional changes that may be induced during mAb-based positive selection and maximizing the post-sort yield of tumor cells).

Molecular profiles of co-cultured cells were compared with their respective profiles in isolated cultures, as well as with profiles generated by co-culture in the setting of treatment with novel anti-MM agents such as proteasome inhibitor PS-341, hsp90 inhibitor 17-AAG, histone deacetylase inhibitor SAHA, and anti-IGF-1R inhibitor. Analyses of these gene expression and proteomic data (using hierarchical clustering, functional clustering and relevance networks algorithms, as well as subsequent confirmatory and mechanistic assays) showed that the distinct molecular signatures of MM cells treated with cytokine or co-cultured with BMSCs also feature overlapping patterns of activation of proliferative /anti-apoptotic signaling events. Indeed, BM-derived cytokines and co-culture with BMSCs triggered activation of PI-3K/Akt and Raf/MAPK signaling pathways in MM cells; upregulated the
transcriptional activity of NF-κB; induced phosphorylation, cytoplasmic sequestration and functional neutralization of pro-apoptotic Forkhead transcription factors; and upregulated the expression of intracellular inhibitors of apoptosis (e.g. survivin). Furthermore, IGF-stimulation and co-culture with BMSCs triggered a wide constellation of previously unappreciated proliferative/anti-apoptotic molecular events, such as transcriptional activation of genes encoding 20S proteasome subunits; increase in activity of the proteasome (as evidenced by 20S proteasome chymotryptic activity assays); upregulation of molecules with key role in MM survival, including molecular chaperones hsp90, hsp70 and caspase inhibitors (including clAP-2 and FLIP); as well as increased expression of DNA synthesis and repair enzymes (e.g. DNA-PK and MSH2) and oncoproteins (e.g. myb, vav). The overlap in molecular sequelae of IGF stimulation and co-culture of MM cell with BMSCs may be attributed, at least in part, to the upregulation of IGF secretion by MM and BMSCs, triggered by their co-culture. Despite the overlapping patterns of signaling pathways triggered by BM-derived cytokines, important qualitative and quantitative differences were also noted. Of particular interest is the more pronounced and sustained biologic sequelae triggered by stimulation of MM cells with pathophysiologically relevant levels of IGFs vs. IL-6. Indeed, IGF-1 induced more pronounced and protracted effects on the activity of NF-κB, Akt, proteasome or Forkhead transcription factors; and triggered upregulation of a broader spectrum of intracellular anti-apoptotic molecules (e.g. IGF-1 upregulated FLIP, XIAP, cIAP2, survivin, while IL-6 upregulated only survivin). These findings may account for the fact that inhibition of IGF/IGF-1R signaling sensitizes MM cells against a broader spectrum of anti-MM agents (e.g. Dex, Doxorubicin, PS-341, Apo2L/TRAIL), in contrast to IL-6 that cannot protect MM cells from e.g. PS-341- or geldanamycin analogs which inhibit the ATPase activity and, abrogated at the level of hsp90 function (by 17-AAG or other geldanamycin analogs which inhibit the ATPase activity and, thus, the chaperoning function of hsp90) or at the level of hsp expression (by SAHA which reduced hsp90 transcription). These findings are of major pathophysiological and clinical interest, because they delineate novel roles of the proteasome and heat shock proteins in mediating survival of MM cells in the BM milieu, and because they provide mechanistic insight into why novel therapies targeting the proteasome, the heat shock proteins (e.g. hsp90) and the IGF/IGF-1R pathway can neutralize the protective effects of the BM milieu against pro-apoptotic therapies and yield objective anti-MM responses in vivo. Furthermore, our GFP-based model of MM-BMSC co-culture represents a useful tool, not only to delineate the role of the BM microenvironment in MM, but also to test novel therapies targeting the BM milieu of MM.

7. New prognostic criteria for classification and monitoring MM

P7.1 DEVELOPMENT OF AN INTERNATIONAL PROGNOSTIC INDEX (IPI) FOR MYELOMA: REPORT OF THE INTERNATIONAL MYELOMA WORKING GROUP


On behalf of the International Myeloma Working Group* **+(CRAB)

Cancer Research and Biostatistics, Seattle, WA *Supported by the International Myeloma Foundation - Los Angeles, CA

Background: Proper staging is important for accurate prognosis and for comparison of data from clinical trials from different institutions and groups. Investigators have recognized since the 1960’s that variables such as hemoglobin, creatinine and calcium forecast survival in multiple myeloma (MM) 1,2. In 1975, Durie and Salmon developed a staging system (DS) to identify patients with higher or lower tumor burden that includes measurement of the level of M-protein in the serum and urine, hemoglobin, calcium, bone lesions, and creatinine1. Attempts to improve on the widely accepted DS system have stimulated development of numerous prognostic systems. Acceptance of new systems has been limited because of inconsistent use of tests at the community level, lack of agreement on relative merits of the individual components, and difficulty reaching consensus on how best to combine variables into a single, easy to use staging system. We wished to develop an international consensus on a new myeloma staging system which could be adopted as the myeloma International Prognostic Index (IPI) for both standard and high dose therapy. We also wished to analyze the prognostic impact of karyotype analysis in the subset of patients in which conventional cytogenetics had been performed.

Methods: Meetings were held in St. Thomas in 2000 and 2002, and at the American Society of Hematology meetings in 2000, 2001, and 2002. Attendance included an international community from North and South America, Europe, Asia, and Africa. There was general acknowledgment of the need to update DS and to develop a simple, easy to use, reliable myeloma IPI. Solicitations of support were sent worldwide and data requests were sent to institutions and groups who agreed to participate. The data was received and closed to entry by March 1, 2003. CRAB developed data spread sheets, coordinated data collection, corrected/evaluated incomplete data and performed the subsequent data analysis. An executive committee, supervised the collection and analysis of data, and held a series of teleconferences.

CRAB and the Executive Committee adopted an overall strategy to identify 2 or 3 variables that would provide the best separation of survival and to identify a group at highest risk. Test availability, relative risk, independent prognostic significance, reproducibility, biologic relevance, and practicality were considered in choosing and combining candidate variables. Final choices for inclusion in the model were data driven and decided by consensus once the univariate assessment was complete and several models were tested. In addition to test variables consideration was given to performance status, age, gender, race and ethnicity.

Less commonly available data such as plasmablastic morphology, the plasma cell labeling index, and circulating myeloma cells, though prognostically very relevant, were not used for the final
transcriptional activity of NF-κB; induced phosphorylation, cytoplasmic sequestration and functional neutralization of pro-apoptotic Forkhead transcription factors; and upregulated the expression of intracellular inhibitors of apoptosis (e.g. survivin). Furthermore, IGF-stimulation and co-culture with BMSCs triggered a wide constellation of previously unappreciated proliferative/anti-apoptotic molecular events, such as transcriptional activation of genes encoding 20S proteasome subunits; increase in activity of the proteasome (as evidenced by 20S proteasome chymotryptic activity assays); upregulation of molecules with key role in MM survival, including molecular chaperones hsp90, hsp70 and caspase inhibitors (including clAP-2 and FLIP); as well as increased expression of DNA synthesis and repair enzymes (e.g DNA-PK and MSH2) and oncogenes (e.g. myb, vav). The overlap in molecular sequelae of IGF stimulation and co-culture of MM cell with BMSCs may be attributed, at least in part, to the upregulation of IGF secretion by MM and BMSCs, triggered by their co-culture. Despite the overlapping patterns of signaling pathways triggered by BM-derived cytokines, important qualitative and quantitative differences were noted. Of particular interest is the more pronounced and sustained biologic sequelae triggered by stimulation of MM cells with pathophysiologically relevant levels of IGFs vs. IL-6. Indeed, IGF-1 induced more pronounced and protracted effects on the activity of NF-κB, Akt, proteasome or Forkhead transcription factors; and triggered upregulation of a broader spectrum of intracellular anti-apoptotic molecules (e.g. IGF-1 upregulated FLIP, XIAP, clAP2, survivin, while IL-6 upregulated only survivin). These findings may account for the fact that inhibition of IGF/IGF-1R signaling sensitizes MM cells against a broader spectrum of anti-MM agents (e.g. Dex, Doxorubicin, PS-341, Apo2L/TRAILE), in contrast to IL-6 signaling (which cannot protect MM cells from e.g. PS-341- or Apo2L/TRAILE-induced apoptosis). Furthermore, these findings are also consistent with the observation that IGF-1R inhibitors had more potent effect in suppressing the drug-resistance (e.g. against Dex) conferred to MM cells by adhesion to BMSCs (CS Mitsiades et al. Blood 2002; 100, 170a).

Importantly, novel biologically-based therapies can counteract key anti-apoptotic molecular events triggered by MM cell adhesion to BMSCs; e.g. the proteasome inhibitor PS-341 abrogated the MM-BMSC adhesion-induced upregulation of survivin and clAP-2; the upregulation of 20S proteasome activity could be counteracted either by inhibition of its proteolytic active site by PS-341, or by agents (e.g. the HDAC inhibitor SAHA or IGF-1R inhibitors) who target the expression of proteasome subunits and ubiquitin pathway members. In addition, the molecular sequelae of adhesion-induced hsp90 upregulation were abrogated at the level of hsp90 function by 17-AAG or other geldanamycin analogs which inhibit the ATPase activity and, thus, the chaperoning function of hsp90. These findings are of major pathophysiologic and clinical interest, because they delineate novel roles of the proteasome and heat shock proteins in mediating survival of MM cells in the BM milieu, and because they provide mechanistic insight into why novel therapies targeting the proteasome, the heat shock proteins (e.g. hsp90) and the IGF/IGF-1R pathway can neutralize the protective effects of the BM milieu against pro-apoptotic therapies and yield objective anti-MM responses in vivo. Furthermore, our GFP-based model of MM-BMSC co-culture represents a useful tool, not only to delineate the role of the BM microenvironment in MM, but also to test novel therapies targeting the BM milieu of MM.

7. New prognostic criteria for classification and monitoring MM

P7.1 DEVELOPMENT OF AN INTERNATIONAL PROGNOSTIC INDEX (IPI) FOR MYELOMA: REPORT OF THE INTERNATIONAL MYELOMA WORKING GROUP

On behalf of the International Myeloma Working Group* **(CRAB) Cancer Research and Biostatistics, Seattle, WA *Supported by the International Myeloma Foundation - Los Angeles, CA

Background: Prognostic staging is important for accurate prognosis and for comparison of data from clinical trials from different institutions and groups. Investigators have recognized since the 1960’s that variables such as hemoglobin, creatinine and calcium forecast survival in multiple myeloma (MM) 1-5. In 1975, Durie and Salmon developed a staging system (DS) to identify patients with higher or lower tumor burden that includes measurement of the level of M-protein in the serum and urine, hemoglobin, calcium, bone lesions, and creatinine. Attempts to improve on the widely accepted DS system have stimulated development of numerous prognostic systems. Acceptance of new systems has been limited because of inconsistent use of tests at the community level, lack of agreement on relative merits of the individual components, and difficulty reaching consensus on how best to combine variables into a single, easy to use staging system. We wished to develop an international consensus on a new myeloma staging system which could be adopted as the myeloma International Prognostic Index (IPI) for both standard and high dose therapy. We also wished to analyze the prognostic impact of karyotype analysis in the subset of patients in which conventional cytogenetics had been performed.

Methods: Meetings were held in St. Thomas in 2000 and 2002, and at the American Society of Hematology meetings in 2000, 2001, and 2002. Attendance included an international community from North and South America, Europe, Asia, and Africa. There was general acknowledgment of the need to update DS and to develop a simple, easy to use, reliable myeloma IPI. Solicitations of support were sent worldwide and data requests were sent to institutions and groups who agreed to participate. The data was received and closed to entry by March 1, 2003. CRAB developed data spread sheets, coordinated data collection, corrected/evaluated incomplete data and performed the subsequent data analysis. An executive committee, supervised the collection and analysis of data, and held a series of teleconferences.

CRAB and the Executive Committee adopted an overall strategy to identify 2 or 3 variables that would provide the best separation of survival and to identify a group at highest risk. Test availability, relative risk, independent prognostic significance, reproducibility, biologic relevance, and practicality were considered in choosing and combining candidate variables. Final choices for inclusion in the model were data driven and decided by consensus once the univariate assessment was complete and several models were tested. In addition to test variables consideration was given to performance status, age, gender, race and ethnicity.

Less commonly available data such as plasmablastic morphology, the plasma cell labeling index, and circulating myeloma cells, though prognostically very relevant, were not used for the final
models. Cytogenetic results were analyzed on a subset of patients and analyzed for association with commonly available data. Following is a preliminary report of the univariate analysis and cytogenetic associations, as well as a preliminary report of combinations of variables that predict survival.

Results: The period of study was from 1981 to 2002. We accumulated data on 11,179 cases with initial chemotherapy and last contact date known. 7,323 patients have died (65.5%). Participation included 17 institutions or groups from Asia, Africa, Europe, North America, and South America. Of all the cases, 8,690 received standard treatment, and 2,489 received high dose therapy plus transplant as initial planned treatment, while 1,307 had a salvage transplant.

Using available data, median age was 60 years. 77% were white, 18.2% Asian, 3.6% Black, and 1.2% were other. 58% were IgG, 23.3% IgA, 3.4% IgM, 2.9% IgD, and 10.9% were light chain only, 0.1% were bicalonal and 2.1% were other. Regarding performance status (PS), 17.4% were PS0, 40.1% PS1, 24% PS2, 13.5% PS3, and 5% PS4. Median serum M-protein level was 3.9 g/dL, hemoglobin 10.5 g/dL, platelets 221 x 10^3/mcL, creatinine 1.1 mg/dL, B2M 3.8 mcg/mL, CRP 0.3 mg/dL, albumin 3.6 g/dL, LDH 257.5 U/L, bone marrow plasma cell percent (PC) 40.0%. Widespread bone disease was reported in 47.5%, 22.1% had no bone lesions, 42.4% had 3 or more bone lesions, 24.4% had pathologic fractures, and 33.2% had compression fractures. Median plasma cell labeling index (PCLI) was 0.5%, and flow cytometric S-phase was 1.6%. Plasmablastic morphology was present in 15.5% of cases.

Cytogenetic data by karyotype was available from Arkansas, Netherlands, ECOG, and Japan. Of 1143 patients, 36.5% had a clonal karyotypic abnormality; 12.9% of 529 reported analyses had del 13; 21.5% of 381 reported patients had a complex karyotype. Clonal karyotypic abnormalities were associated with low albumin, high B2M, calcium, creatinine, and low platelet count. Del 13 by karyotype was associated with a high B2M, BMPC%, high creatinine, low hemoglobin, high LDH and low platelet count. Complex karyotype was associated with age, higher BMPC%, calcium, creatinine, high LDH and low platelet count.

Distribution of characteristics was relatively uniform across centers, but occasional variation suggested there were some population differences. For instance, younger age and lower B2M reflected a transplant population in certain groups. To select cutoffs for the continuous variables we graphically examined the log rank and relative risk (RR) of survival of each factor over the range of values. RR at specific cutoffs was as follows: age 1.6 (>65), albumin 1.3 (<3.5), B2M 1.8 (>4), BMPC 1.3 (>33%), calcium 1.35 (>10), CRP 1.3 (>8), hemoglobin 1.5 (>10), LDH 1.6 (>100), platelet 1.7 (<130).

Univariate survival analysis. Survival was analyzed at cutoffs chosen for these variables and for nominal groups. Overall survival of the entire group was 44 months. There were no differences in survival by gender. There was a trend toward shorter survival among blacks, 36 months, versus 43 months for whites, and 46 months for Asians. Age >65 was associated with shorter survival, 33 versus 51 months. Performance status 3 and 4 had median survivals of 30 and 24 months compared to 63, 45, and 37 months for PS0, PS1, and PS2. There was some distribution difference by PS among certain centers. There was a major difference in survival by standard versus high dose therapy with transplant on intent to treat basis, because the risk factors for these populations differed significantly. For high versus low B2M median survivals were 33 and 56 months; for LDH, 29 and 50 months, for BMPC 39 and 51 months, for calcium 35 and 47 months, for creatinine 24 and 47 months, for CRP 40 and 51 months. There was a trend toward shorter survival in patients with bone lesions present 42 versus 48 months. Patients with low albumin had shorter survival, median 37 versus 48 months as did patients with low hemoglobin, 34 versus 51 months, and patients with low platelet count, 25 versus 45 months.

For an early analysis of combinations predicting for poor survival we looked at several existing systems. All systems separated groups of patients with better or worse survival, p<.0001. By DS stage patients with stage IIIb disease fared worse with a median survival of 24 months. Using the Intergroup/ECOG/Mayo staging system, patients with a B2M >4 and platelet count <150,000 had the worst survival, median 23 months. By SWOG stage patients with B2M >5.5 and albumin <3 had the worst survival, median 26 months. The longest survival was seen in those with early SWOG stage (B2M <2.5) median survival 63 months. Shortest survival for individual variables were PS4 at 24 months, high creatinine at 24 months, high LDH at 29 months, and low platelet count at 25 months.

Conclusion: In a group of 11,179 cases, univariate analysis of survival showed, a not unexpected significant prognostic importance for a wide range of variables. Arguably the most significant variables were age>65, PS=4, B2M >2.0, platelets <130,000, and high LDH. Albumin less than 3, and %BMPC >33% are factors to be considered. The final model will likely include some of these variables. A multivariate model using regression tree analysis may include parameters not identified as the strongest among the univariate comparisons. The final analysis will include a test set and validation set for the combinations selected. Missing data imputation will not used. Variables missing in a greater than optimal number of cases will not be included in the final model. We will perform validation among different groups, including age, race/ethnicity, country or institution of origin. Models for both standard therapy and transplant will be evaluated collectively and separately. Mention that the potential contribution of cytogenetics will be evaluated. An updated report and a recommended IPI for myeloma will be presented at the International Myeloma Workshop in Salamanca in May 2003.

** Contributing centers: ARGENTINA: FUNDALU, Buenos Aires, AUSTRIA: Wilhelminenspital Der Stat Wien *on behalf of the Central European Myeloma Study Program, CANADA: University of Toronto & Royal Victoria Hospital (Montreal), National Cancer Institute of Canada, FRANCE: Intergroupe Francais du Myelome, ITALY: Italian Multiple Myeloma Study Group including the Institute of Hematology Seragnoli & Cattedra Ematologia, Torino, JAPAN: Japan Myeloma Study Group, NETHERLANDS: HOVON Group, Rotterdam, NORWAY: Nordic Myeloma Study Group, SOUTH AFRICA: Constantiaberg Medi-Clinic, Cape Town (in association with the British Medical Research Council Myeloma 9 Trial), SPAIN: PETHMA Group & New Spanish Myeloma Group, Salamanca TURKEY: AUTF (Ankara University), Istanbul University, Eg University and Inonu University, UNITED KINGDOM: Royal Marsden Hospital, London & Northern Yorkshire, Clinical Trials Unit, Leeds, UNITED STATES: Dana Farber Cancer Institute, Boston, UNITED STATES: Eastern Oncology Collaborative Group, UNITED STATES: Mayo Clinic, Rochester, UNITED STATES: Southwest Oncology Group, UNITED STATES: Univeristy of Arkansas Medical Sciences , Little Rock

References 1. Carbone P, Kellerhouse L, Gehan E. Plasmacytoma myeloma a study of teh relationship of survival to various clinical

PT.2
THE MAJOR PROGNOSTIC VALUE OF CYTOGENETICS IN MYELOMA
Guido Tricot, MD, PhD
Cyogenetic information is limited in multiple myeloma because it is a malignancy composed mainly of almost terminally differentiated B-cells with low proliferative activity. Abnormal karyotypes are found in only 30 to 50% of cases. Typically, previously treated and relapsed patients have a higher frequency of chromosomal abnormalities compared with newly diagnosed patients. This is a reflection of the more proliferative nature of myeloma in its advanced stages. The presence of abnormal cytogenetics by conventional karyotyping has been associated with an inferior outcome. Flow cytometry-derived aneuploidy data and fluorescence in situ hybridization (FISH) analysis indicate the presence of cytogenetic abnormalities in at least 90% of myeloma patients. Therefore, the majority of normal karyotypes in myeloma are derived from normal hematopoietic cells and not from the myeloma clone. Even at the stage of monoclonal gammopathy of undetermined significance, flow cytometry and FISH analysis demonstrate aneuploidy or cytogenetic abnormalities in at least 50% of cases.

Of the myeloma patients with cytogenetic abnormalities, approximately 65% have a hyperdiploid karyotype. A pseudodiploid and hypodiploid karyotype are found in approximately 15 and 20% of patients, respectively. The most common abnormalities are gains of a whole chromosome 3, 5, 7, 9, 11, 15 and 19. Every single chromosome can potentially be involved in either deletions, gains, additions or translocations. The finding of a 13q abnormality with conventional karyotyping has been associated with poor outcome in patients treated with either conventional chemotherapy or with tandem transplants. By conventional cytogenetics, deletion of chromosome 13 has been detected in approximately 15 to 20% of patients. Using interface FISH, deletions of 13q is present in 50% of patients. The presence of 13q deletion as assessed by FISH studies has also been associated with poor outcome. To assess the impact of FISH-13 compared to abnormalities of chromosome 13 by conventional cytogenetics, we have evaluated event-free and overall survival of our Total Therapy II patients, all of whom had both of those tests performed. Patients without FISH-13 had an excellent outcome with a three year event-free survival of 75%. For patients with FISH-13, there was a major difference between those with cytogenetic abnormalities and those without any cytogenetic abnormalities as determined by conventional cytogenetics. Those with FISH 13 and no cytogenetic abnormalities had a three year event-free survival of 75%, while those with cytogenetic abnormalities had a three year event-free survival of only 30%. (p = < 0.0001)
The second cytogenetic abnormality associated with poor outcome is hypodiploidy. Hypodiploidy is often associated with deletion of chromosome 13. Analysis of our data on 1,475 myeloma patients scheduled to receive tandem transplants showed that 65% of patients with hypodiploid or hypotetraploid karyotypes had deletion of chromosome 13 compared with 29% of those with pseudodiploid karyotypes and 36% of those with hyperdiploid karyotypes. Median event-free survival for patients with hypodiploid/hypotetraploid karyotype was 10 months; median overall survival 19 months, compared with a median event-free survival of 28 months and an overall survival of 51 months for patients with normal karyotypes and 19 and 36 months, respectively, for patients with abnormal non-hypodiploid karyotypes. Event-free survival and overall survival were poor in patients with chromosome 13 abnormalities irrespective of their ploidy status, but survival was also poor in patients with hypodiploidy irrespective of deletion of chromosome 13.
Recently, we have also observed that patients who have a MDS signature (-5/-5q, -7/-7q, +8, t(1;7), del 20q) in an otherwise typical myeloma karyotype (MM-MDS) also have a poor outcome. The median event-free survival of previously treated and untreated patients with MM-MDS is 11 months and the overall survival 18 months. This compares to an event-free survival of 13 months and an overall survival of 24 months for patients with chromosome 13 abnormalities or hypodiploidy. Patients with other cytogenetic abnormalities not including chromosome 13 abnormalities or hypodiploidy or MM-MDS, had a median event-free survival of 20 months and a median overall survival of 41 months. Patients with no cytogenetics at all had a median event-free survival of 25 months and an overall survival of 56 months.
In summary, cytogenetic abnormalities are the strongest predictor of poor outcome after tandem transplants. Biologically, cytogenetic abnormalities as detected by conventional cytogenetics have a different meaning than those found by FISH analysis. To detect cytogenetic abnormalities by FISH, there is no requirement for proliferation. In contrast, finding cytogenetic abnormalities by conventional cytogenetics is an indication of stroma independence. When plasma cells are taken away from stroma support, they will quickly undergo apoptosis. If myeloma cells can be removed from the stroma and still grow and divide, they have become stroma-independent. As such it is an excellent marker, probably the best available at this point in time, to detect stroma-independent myeloma.

PT.3
THE IMPORTANCE OF IMAGING IN MYELOMA STAGING, PROGNOSTIC CLASSIFICATION AND MONITORING.
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Appropriate imaging is essential for myeloma management. Imaging establishes the (1) presence, (2) location, and (3) activity of myeloma lesions. This information allows prognostic classification and provides discrete information for monitoring purposes.
Considering each of the imaging techniques sequentially:
Standard Radiology – is an important baseline. Approximately 75% of patients with myeloma bone disease will manifest lytic lesions and/or osteopenia with or without fractures on x-ray (1). Other Imaging Techniques – such as MRI, CT-scanning, FDG/PET and 99m Tc-MIBI are additionally helpful, in a complementary fashion, both overall and especially in the 25% of patients with negative x-rays, to establish the presence, location and activity of myeloma lesions. Particular advantages are:

MRI – This is the most reliable technique to document the anatomic distribution of myeloma lesions (2-5). MRI is useful both if x-rays are negative and/or if specific clinical problems require delineation, e.g., neurologic compromise, pain. The practical problem is how to perform MRI of the whole body. Although we have developed a wide field screening technique for this, it remains cumbersome and expensive.

CT Scanning – This is very helpful for detailed evaluation of localized sites of disease, especially to evaluate bone destruction and/or in preparation for radiation therapy (6). We have therefore evaluated the additional benefit of whole body FDG/PET scanning. (7) The purpose of this evaluation was to assess the clinical utility of whole body positron emission tomography with [18F] fluorodeoxyglucose (FDG/PET) in patients with multiple myeloma and related monoclonal diseases, such as MGUS and solitary plasmacytoma.

Methods: Between July 1, 1996 and December 2001, 84 patients underwent 122 FDG/PET scans with 34 patients having 2 or more scans. Results were compared with routine clinical and staging information including MRI and CT scans, as indicated. Of the 84 patients: 21 had previously untreated active myeloma, 16 had monoclonal gammapathy of undetermined significance (MGUS), 13 were in remission and 34 had relapsing disease. Results: Negative whole body FDG/PET reliably predicted stable MGUS. Of the 16 MGUS patients with follow-up of 3-55+ months, only 1 (6%) has developed myeloma at 8 months. Conversely, the 21 previously untreated (PU) patients with active myeloma all had focal and/or diffusely positive scans. 5/21 (24%) PU patients with positive FDG/PET scans had negative full radiologic surveys. Another 5/21 (24%) patients had focal extra medullary disease. This was confirmed by biopsy and/or other imaging techniques. Extra medullary uptake also occurred in 8/34 (23%) relapse patients. This extra medullary uptake was a very poor prognostic factor both pretreatment and at relapse: e.g., median survival 7 months for relapsing patients. Persistent positive FDG/PET post induction therapy and/or stem cell transplantation predicted early relapse. 13/16 (81%) relapsing patients had new sites of disease identified. The FDG/PET scan results were especially helpful in identifying focal recurrent disease in patients with nonsecretory or hyposecretory disease amenable to local irradiation therapy used in 6 patients. Serum FreeJite testing is being cross correlated with serial FDG/PET imaging. Serum FreeJite levels > 200mg/L correlate with discrete changes on FDG/PET.

Conclusions: Whole body FDG/PET imaging provides important staging and prognostic information, which reliably identifies active myeloma versus MGUS. FDG/PET also identifies poor risk patients pretreatment and the potential for early relapse post induction and/or stem cell transplantation. Identification of focal relapse in nonsecretory patients is especially helpful. Formal cost effectiveness analysis is recommended.

Prior to our investigations of the role of 18F-FDG PET (2), we and others had evaluated the role of 99m Tc-MIBI (8-11). The new question is: what are the relative merits of 99m Tc-MIBI versus 18F-FDG PET? From an ongoing comparative analysis with serial 99m Tc-MIBI scanning, a few comments can be made. Both are positive in approximately 25% of patients with negative radiographs. Both can give helpful prognostic information. Since multiple drug resistant, p-glycoprotein positive myeloma is negative with 99m Tc-MIBI and FDG/PET is positive, there is differential utility in this setting (10). Both 99m Tc-MIBI and FDG/PET are usually negative in MGUS. However, slow growing, smoldering or asymptomatic myeloma can be positive with 99m Tc-MIBI, often in the form of a diffuse marrow “superscan” effect (11). FDG/PET is much better for detection and monitoring of discrete sites of more rapidly growing active myeloma both within bone and in extra medullary sites. The detection of lesions, especially “hot spot” foci, is enhanced by tomographic nuclear medicine techniques. PET offers the advantage of being a whole body, tomographic study and can often detect lesions not seen with planar (non tomographic) imaging. SPECT MIBI may be helpful in selected sites. The unpredictable GI activity as well as other abdominal organ uptake is a disadvantage for MIBI compared to FDG-PET. Our results have been much more favorable with FDG-PET in the lower spine and pelvis regions. Nonetheless, it is very helpful to have two nuclear imaging techniques capable of providing different types of clinical information and correlations. Overall, having several complementary imaging techniques with excellent results gives greater flexibility in evaluating patients with myeloma.

REFERENCES


chains in only 9 of the patients. Thus, serum assays could replace
26 patients compared with normalisation of serum free light
patients. 4 A further four patients had suppression of one or both
Bence Jones protein urine tests for patients with light chain
monitoring of 82 patients, changes in serum and urine free light
trials, all were correctly identified from serum samples. During
of either kappa or lambda free light chains (and abnormal
kidneys can metabolise 10-30gm of free light chains per day, so
that urine concentrations may not accurately reflect tumour
synthesis. Therefore, from a theoretical viewpoint, serum
measurements would be preferable, just as blood glucose
measurements are better than urine measurements for managing
patients with diabetes mellitus. Unfortunately, serum
measurements have been hampered by the lack of high affinity
antisera that are specific for free light chains. Recent
publications indicate that satisfactory serum immunoassays have
now been developed and are useful in a variety of clinical
situations. 1,2 In a study of patients with light chain multiple myeloma,
immunoassays for serum free light chains were compared with
traditional urine tests. 3 Of 224 patients tested at entry to clinical
trials, all were correctly identified from serum samples. During
monitoring of 82 patients, changes in serum and urine free light
correlated, but urine free light chains became negative in
26 patients compared with normalisation of serum free light
chains in only 9 of the patients. Thus, serum assays could replace
Bence Jones protein urine tests for patients with light chain
multiple myeloma.

In patients with nonsecretory multiple, increased concentrations
of either kappa or lambda free light chains (and abnormal
kappa/lambda ratios) were detected in the sera of 19 out of 28
patients. 4 A further four patients had suppression of one or both
light chains while the remaining five sera had normal or raised
free light chain concentrations with substantially normal
kappa/lambda ratios. Six of the patients with an elevated single
free light chain, who were studied during follow-up, had changes
in disease activity that mirrored changes in free light chain
concentrations.

Serum free light chain concentrations have also been assessed in
497 patients with intact immunoglobulin multiple myeloma at the
time of clinical presentation. These comprised 314 patients with
IgG, 142 with IgA, 36 with IgD and 5 patients with IgE multiple
myeloma. The results showed that overall 88% had elevated free
light chains with the following breakdown: IgG 84%, IgA 92%,
IgD 94% and IgE 100%. Some patients had normal or reduced
concentrations of free light chains but abnormal κ/λ ratios
indicating monoclonality in association with bone marrow
suppression. In total, 95% of patients had abnormal free light
chain concentrations or abnormal κ/λ ratios. This percentage is
higher than previously reported, reflecting the increased
sensitivity of the free light chain immunoassays. A comparison
was also made between the effect of treatment on the serum
concentrations of intact monoclonal immunoglobulins and free
light chains. Because the serum half-life of free light chains is
only 2-6 hours, compared with 21-25 days for IgG, short-term
responses to therapy could be identified. This might be useful for
identifying the most suitable treatment regimens in patients who
are refractory to conventional chemotherapy.

Other studies have shown that serum free light chains are
raised in nearly all patients with AL amyloidosis, 2,5 light chain
deposition disease 2 and Waldenström’s macroglobulinaemia.
In conclusion, serum free light chain measurements may obviate
the need for urine tests in most patients with monoclonal plasma
cell diseases. The serum assays may also find use in the early
assessment of responses to treatment because of their short half-
life compared with intact monoclonal immunoglobulins.
Bradwell AR, Carr-Smith HD, Mead GP, Tang LX, Showell PJ,
Drayson MT, Drew R. Highly sensitive automated immunoassay
for immunoglobulin free light chains in serum and urine. Clin

Katzmann JA, Clark RJ, Abraham RS, Bryant S, Lymp JF,
Bradwell AR, Kyle RA. Serum reference intervals and diagnostic
ranges for free κ and free λ immunoglobulin light chains: Relative sensitivity for detection of monoclonal light
Bradwell AR, Carr-Smith HD, Mead GP, Harvey TC, Drayson
MT. Serum test for assessment of patients with Bence Jones

Drayson MD, Tang LX, Drew R, Mead GP, Carr-Smith HD,
Bradwell AR. Serum free light-chain measurements for
identifying and monitoring patients with nonsecretory multiple

Abraham RS, Katzmann JA, Clark RJ, Bradwell AR, Kyle RA,
Gertz MA. Quantitative analysis of serum free light chains. A
new marker for the diagnostic evaluation of primary amyloidosis.
strategy for the detection of rearranged immunoglobulin heavy-chain genes (IgH). Nested PCR proved to be sensitive and specific, detecting up to $10^3$ /$10^6$ tumor cells in bone marrow samples (1).

Recently, molecular monitoring of minimal residual disease (MRD) after allo-HSCT has been proposed as a prognostic parameter potentially helpful to take clinical decisions regarding the immune-suppression tapering or the timing of donor lymphocyte infusions. However, its effective role in the clinical setting is still a matter of debate. Thus, we have recently taken advantage of our sensitive qualitative PCR based approach to address the issue of the prognostic value of MRD monitoring in myeloma patients receiving myeloablative allo-HSCT. In an EBMIT retrospective study, we have longitudinally analyzed MRD status in 48 myeloma patients in complete clinical remission for whom a specific molecular marker was identified. Sixteen of 48 patients (33%) remained persistently negative during the follow up (NEG group). Thirteen of 48 (27%) were persistently positive (POS group), while the remaining 19 subjects had a mixed PCR response (i.e. detection of alternatively positive or negative PCR results during the follow up, MIX group). The actuarial risk of relapse at 5 years in the NEG, MIX and POS groups was 0%, 33% and 100% respectively. Patients with persistent PCR-negativity or mixed PCR results had a better relapse free survival than those who had never reached PCR negativity ($p=0.0001$ and $p=0.002$ respectively). Although the retrospective selection of the patients has limited the possibility to correlate MRD results with clinical parameters, we could not find any clinical feature significantly associated with the molecular outcome.

Taken together, all these data suggest the existence of a graft versus myeloma effect and outline the potential role of such an effect in maintaining a long term remission in allografted patients. Thus, MRD monitoring could be used as an indirect parameter of malignant clone control by effector cells of the donor immune system. Furthermore, our results about patients experiencing mixed PCR results could reflect the ongoing balance between graft versus tumor and tumor immune escape. These findings prompted us to set up a more accurate technique to evaluate molecular disease in order to better understand a dynamic situation. Although extremely sensitive, qualitative monitoring of MRD by nested PCR, does not provide information about the amount of residual tumor load, and does not show the kinetics of its possible elimination by the immune system.

To address this issue, recently real-time quantitative PCR methods (TaqMan PCR) have been used for MRD monitoring (2, 3). We are thus evaluating quantitative MRD analysis of tumor specific IgH rearrangements in multiple myeloma patients by two different strategies: the first strategy relies on a panel of family specific consensus probes annealing to the framework region 2 or the framework region 3 of the rearranged IgH genes. In this case the specificity for the patient sequence is obtained using primers derived from complementarity-determining regions (4). The second strategy, which is more time consuming and expensive, relies on the design of patient specific probes; the major advantages of this approach are the sensitivity and the independence from the ratio of hypermutation of the IgH sequence. TaqMan protocols that we are currently using can detect up to 10 copies of patient specific IgH rearrangements diluted in 200 ng of polyclonal genomic DNA. However, since it has not been clarified yet, neither the reproducibility of quantitative data among different laboratories, nor the sensitivity of the technique, which can be influenced by the specific primer and probe combination, in this phase we are performing the experiments in duplicate with standard nested PCR to check reliability and sensitivity of the quantitative approach. Our preliminary data indicate that a TaqMan PCR based approach is feasible for monitoring patients with multiple myeloma after allogeneic HSCT. In figure 1, we report the quantitative MRD monitoring in a representative patient who reached the molecular remission after myeloablative allo-HSCT. In this case the progressive decrease of tumor specific DNAs was concomitant to the occurrence of chronic GVHD. The other two patients we have investigated by TaqMan PCR have never obtained a molecular response. Nevertheless in one case, quantitative MRD monitoring allowed the detection of a progressive increase in the number of tumor genomes 10 months prior to the clinical relapse.

In conclusion, our results prompt the use of quantitative methods to assess the kinetics of graft versus myeloma effect and suggest that these new techniques of molecular monitoring may be used to evaluate the tumor burden in order to develop an “individualized” post-transplant immunotherapy, reducing the GVHD risk and optimizing the chance of response.

References
were designed (i.e. CD38/CD56/CD19/CD45). A highly sensitive approach to the aberrant antigenic profile observed in the PC at diagnosis was the quadruple monoclonal antibody (MoAb) combinations adapted to "live-gate" drawn in the CD38+++ fraction –where PC are located– corresponding to the total BM cellularity was assessed, and at least 3,000 PC per test. Firstly, acquisition of 20,000 cells and, simultaneously, there was a higher recovery of the normal plasma cell (PC) compartment in patients with MM, since it discriminates between myelomatous (my) and normal PC (nPC), even when both populations coexist in the BM. This is based on the presence of phenotypic aberrations in the former PC population -which are absent in the nPC- and which could be considered as a "tumour-associated markers". The limit of detection of residual myPC by this technique ranges between 10⁻² and 10⁻⁴. For identification of residual myPC, patient-specific quadruple monoclonal antibody (MoAb) combinations adapted to the aberrant antigenic profile observed in the PC at diagnosis were designed (i.e. CD38/CD56/CD19/CD45). A highly sensitive two-step acquisition procedure was performed in order to screen at least 3,000 PC per test. Firstly, acquisition of 20,000 cells corresponding to the total BM cellularity was assessed, and secondly, phenotypic information of those events included in a “live-gate” drawn in the CD38+++ fraction –where PC are located– was recorded. In all cases, the percentage of myPC as well as nPC referred to the total cellularity, and the proportion of nPC within the total PC (Prn) were calculated.

Using this approach, we have previously shown that ASCT is more efficient than CC in reducing tumor load: ASCT produced a significantly higher reduction in the number of residual my-PC and, simultaneously, there was a higher recovery of the normal PC population. The level of recovery of non-involved immunoglobulins correlated with the number of nPC after treatment. Moreover, the proportion of patients that achieved an immunophenotypical remission after ASCT was also significantly higher than after CC. Regarding the influence of MRD in BM, the cut-off level of % nPC/total PC ≥30% showed the highest predictive value to discriminate among MM patients those who were at different risk of relapse. However, higher cut-off levels of %nPC/total PC might be more accurate for the specific assessment of patients undergoing ASCT. Rawstron et al have obtained similar results in a series of 45 transplanted patients showing that detectable neoplastic PC at three months post-transplant predicts early relapse against those with normal phenotypically normal PC.

At present, we are analysing the impact of MRD transplanted patients with MM (n=113) treated according to the current GEMM multi-centre protocol. All received 6 courses of alternating cycles of VBCMP/VBAD and subsequently underwent ASCT conditioned with melphalan 200 mg/m² or BUMEL (12 mg/kg Busulphan-140 mg/m² Melphalan); stem cell was collected after the fourth cycle of chemotherapy. Patients that achieved immunological CR after ASCT went into maintenance therapy while patients in partial response (PR) received a second transplant (either autologous or mini-allogeneic transplant). MRD was evaluated at 3 months after the first ASCT and only in those patients achieving CR (n=87). In 31 of these 87 cases, MRD was subsequently evaluated at two or more consecutive time-points post-ASCT (median: 3 studies/case; range: 2 to 8 studies). All these 87 patients showed less than 5% BMPC at all morphological examinations post-ASCT. CR was defined as absence of monoclonal component on electrophoresis. 75% of the patients also displayed negative immunofixation (IFE) –CR-, response- while the remaining 25% were electrophoresis negative but IFE positive –CR+ response-. The median follow-up from diagnosis was 22 months. Phenotypically aberrant PCs were detected at 3 months after ASCT in 37 out of 87 CR patients (42%) at a median level of 0.035% myPC (range: 0.002% to 3.18%). Comparing the level of MDR between IFE positive and IFE negative cases, we have observed a significantly lower level of myPC in IFE negative cases (myPCmedian: 0%, range: 0% to 3.18%) than in IFE positive cases (myPCmedian: 0.008%; range: 0% to 0.38%)(p=0.014) together with a higher recovery of nPC (nPCmedian: 0.18% vs 0.25%)(p=0.029) and a superior number of cases in immunophenotypical remission (77% cases vs 46%, p=0.028). Follow-up studies showed MRD negativization in six cases while it became positive in four other cases.

Finally, although the follow-up is still very short to reach firm conclusions, we have explored the impact on RFS of MRD in the BM obtained at 3 months after ASCT within 87 patients in eletrophoretic CR. Preliminary data showed that patients in whom ≥85% of the total BMPC displayed a normal phenotype presented a longer PFS as compared to that of patients with <85% Prn (32 months vs 20 months, p=0.03). In addition, follow-up studies indicate that patients that remained MRD positive or became positive displayed significantly worse outcome than the MRD negative cases. In summary, investigation of MRD by immunophenotyping may be a useful tool for disease monitoring in MM patients.

References
8. Mouse models for MM

P8.1 THE 5TMM MODEL: A USEFUL MODEL FOR THE STUDY OF MULTIPLE MYELOMA

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The 5TMM murine models of myeloma were initially developed by J. Radl (1). Multiple myeloma (MM) developed spontaneously in 0.5% of mice (of the inbred strain C57BL/KaLwRij) older than 2 years. The MM cells were localized in the BM of the mice and the serum paraprotein concentration correlated well with the development of the disease. The latter was associated with a decreased concentration of normal polyclonal immunoglobulins. The primary diseased BM was intravasally transplanted into young syngeneic animals and by doing so several in vivo growing cell lines were developed, each with its own characteristics. The 5TMM model hereby belongs to the de novo myelomas and its clinical characteristics resemble the human disease closely: the tumor cells are located in the bone marrow, the serum paraprotein concentration is a measure of disease development, neovascularization is increased (this was determined for 5T2MM and 5T33MM (2)) and in certain lines a clear osteolytic bone disease develops. All the original 5TMM models are maintained and propagated in vivo (3,4).

The 5T2MM model best represents human MM, with a moderate growth and the development of osteolytic bone lesions. These osteolytic lesions are associated with a decrease in cancellous bone volume, decreased bone mineral density and increased numbers of osteoclasts (5). The 5T33MM model has a more rapid tumor take and in addition to the bone marrow, also grows in the liver (6). For the 5T33MM model an in vitro, stroma independent growing cell line, clonally identical to the in vivo line (7), has been developed (8). Additional lines include the 5T7MM line which is a model for smouldering MM while the 5T14MM line is osteoblastic. The latter was associated with a decrease in cancellous bone and the development of osteolytic bone lesions. These osteolytic lesions are associated with a decrease in cancellous bone volume, decreased bone mineral density and increased numbers of osteoclasts (5).

The 5T2 and 5T33MM models have been extensively characterized. Specific monoclonal antibodies have been raised against the idiotypes of both 5T2 and 5T33MM allowing the detection, with great sensitivity, of the serum paraprotein by ELISA and the specific staining of the tumor cells both by FACS analysis and immunostaining of histological frozen sections (6). The sequence analysis of the VH gene enables the detection of cells by RT-PCR and Northern blot analysis (9).

The 5TMM models can be used for both in vitro and in vivo experiments. The specific antibodies allow the separation of MM cells by flow cytometry or with magnetic beads, generating pure MM cell populations for further in vitro investigation. The 5TMM models generate a typical MM disease and different methods are available to assess tumor load in the bone marrow, serum paraprotein concentrations, bone marrow angiogenesis (by measuring the microvessel density) and osteolytic bone lesions (by a combination of radiography, densitometry and histomorphometry). The investigation of these latter parameters allows the use of the 5TMM models in a preclinical setting and study the growth and biology of the myeloma cells in a complete syngeneic microenvironment. Both, molecules targeting the MM cells themselves and molecules targeting the bone marrow microenvironment, can be studied. While the 5T33MM model can be used to target both the microenvironment and the MM cells themselves, the 5T2MM model can also be used to study the myeloma associated bone disease.

The 5TMM models have now been used to unravel the mechanisms of homing to the bone marrow (10) and this in vivo setting, the evaluation of possible therapies including DNA vaccination (11), the use of biphosphonates (12,13) and the blocking of the RANKL/RANK interaction (5,14). Radl J et al. Idiopathic paraproteinemia. J Immunol. 1979; 122: 609-13.


P8.2 MYELOMA BIOLOGY REVEALED BY THE SCID-HU MODEL FOR PRIMARY HUMAN MYELOMA.

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The human bones of SCID-hu mice consistently support growth of freshly obtained myeloma cells. Growth of the myeloma cells is restricted to the human bones, and induces changes in the human bone marrow microenvironment, most notably increase in microvessel density, increased osteoclast activity and decrease in osteoblast numbers. Growth of myeloma cells is also associated with typical myeloma manifestations, the most dramatic of which is osteolysis of the human bone. By supporting the growth of primary human myeloma cells in a human bone marrow microenvironment, the SCID-hu model provides a platform for studying important aspects of myeloma biology and therapy previously beyond the power of our experimental models, among them whether myeloma plasma cells are proliferative and the role of the bone marrow microenvironment in the disease process. Experiments in which myeloma plasma cells, purified from bone marrow aspirates, were used demonstrated unequivocally that the recognizable tumor cells of plasma cell morphology produce myeloma in the SCID-hu model, with all its manifestations. Moreover, myeloma cells recovered from one mouse could be sequentially transferred from one SCID-hu mouse to another, clearly demonstrating that myeloma plasma cells are or contain a subpopulation of proliferative cells with self-renewal capacity. In contrast, the myeloma plasma cell-depleted bone marrow and blood specimens or purified B cells from myeloma patients did not produce myeloma in SCID-hu mice.

In some experiments, myeloma cells were injected only into one human bone of mice implanted with two, contralaterally placed bones. As myeloma developed, plasma cells disseminated,
presumably through the circulation, to the second bone, yet no myeloma cells were detected in any of the murine tissues, demonstrating the total dependence of the cells on the human bone marrow.

In most cases, myeloma cells grew only within the bone marrow of the human bone. However, cells from patients with extramedullary disease grew also along the outer surface of the human bones. This growth pattern indicates that extramedullary disease, while still dependent on a human microenvironment, no longer requires elements present only in the bone marrow, highlighting a biological difference between these and classical myeloma cells.

The absolute dependence of the myeloma cells on the human microenvironment offered an opportunity to study whether the changes in the microenvironment associated with their growth are merely consequences, or if these changes are important for disease subsistence. Anti-angiogenic or anti-osteoclastic agents were used to block myeloma-induced angiogenesis and osteoclastogenesis.

Thalidomide demonstrated anti-myeloma activity only in SCID-hu mice that contained also human liver implants, demonstrating that metabolism is important for the drug’s anti-myeloma efficacy. While anti-myeloma activity was associated with reduced microvessel density, cause and effect could not be determined. Treatment with endostatin elicited response in 50% of cases, suggesting that in some cases, angiogenesis may have a role in the disease process.

While treatment with inhibitors of osteoclast activity effectively halted destruction of the human bones, only Zoledronic acid and RANK-Fc reduced the number of osteoclasts, whereas pamidronate had no effect on osteoclast number. Still, all three agents in addition to preserving bone had profound anti-myeloma effects, indicating that myeloma depends on osteoclast activity.

In contrast to classical myeloma, while myeloma cells from patients with extramedullary disease were sensitive to thalidomide, they were completely resistant to all three anti-myeloma plasma cells and displayed similar changes in gene expression. These interactions induced changes in gene expression in both the myeloma cells and the osteoclasts. Osteoclasts from myeloma patients and from healthy donors were equally supportive of myeloma cells and displayed similar changes in gene expression.

In contrast to supporting extended survival and proliferation of myeloma plasma cells attracted committed osteoclast progenitors and, upon contact, induced their differentiation into morphologically mature and functionally active osteoclasts in a RANKL-mediated process. Osteoclasts, in turn, supported survival and proliferation of purified myeloma plasma cells for extended periods, a phenomenon that required physical contact between the myeloma cells and osteoclasts. These interactions induced changes in gene expression in both the myeloma cells and the osteoclasts. Osteoclasts from myeloma patients and from healthy donors were equally supportive of myeloma cells and displayed similar changes in gene expression.

Studies with the SCID-hu model have demonstrated that myeloma plasma cells are or contain a population of proliferative cells with self-renewal capacity; that osteoclast activity is essential for survival of tumor cells from classical myeloma; that cells from patients with extramedullary disease no longer depend on osteoclast activity; that metabolism of thalidomide is required for its activity in myeloma, and suggested that in some cases myeloma could be sensitive to anti-angiogenic therapy. These studies also indicate that the non-myelomatous human bone microenvironment in the SCID-hu mice, which is derived from fetal bones and hence is not inherently abnormal, can support the myeloma disease process.


Supported by grant CA-55819 from the National Cancer Institute.

P8.3 IN VIVO MOUSE MODELS FOR THE DEVELOPMENT OF NOVEL BIOLOGICALLY BASED THERAPIES FOR MM

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The rapid bench-to-bedside translation of novel anti-cancer therapies requires pre-clinical testing in animal models that accurately recapitulate the natural history of human cancers and their response to therapy. Yet, the overwhelming majority of conventional in vivo models for MM (and other tumors) do not optimally fulfill these requirements because they involve subcutaneous (s.c.) tumor cell xenografts, which a) do not reflect the diffuse, systemic nature of MM lesions; and b) place MM cells in a cutaneous microenvironment, which is radically different from the bone marrow (BM) milieu, which constitutes the predominant site for MM cell homing, by virtue of its role to promote MM cell proliferation, survival and drug resistance. Although intravenous (i.v.) injections of MM cells can lead to diffuse lesions, their exact anatomic location(s) cannot be readily detected with high sensitivity and specificity by conventional imaging modalities (due to low sensitivity of radiographic analyses or prohibitive high cost of CT or MRI imaging), while thorough whole-body histopathologic analyses are highly time-consuming and can be performed only after necropsy, and not serially during administration of an anti-tumor regimen.

To address the limitations of conventional MM models, we developed a series of novel in vivo MM models, which allow establishment of diffuse MM bone lesions and their reproducible quantitative real-time detection and spatio-temporal monitoring using the technologies of wholebody fluorescence and/or bioluminescence imaging. In these models, human MM cells, stably transfected/transduced with constructs for Green Fluorescent Protein (GFP), firefly luciferase (luc) or a fusion GFP/luciferase (GFP+/luc+) protein, are injected i.v. in SCID/NOD mice. The subsequently established diffuse MM lesions can be monitored in live anesthetized mice by: a) fluorescence imaging, where GFP+ tumors detected by illumination of mice with near infra-red light in a LT-9500 fluorescent light box; b) bioluminescence imaging, where luc+
MM cells digital camera detects bioluminescence emitted from luc+ MM cells when mice are injected i.v. with luciferin; c) combined use of both modalities (which is feasible due to lack of interference between signals emitted by GFP and luciferase). The marked visual contrast generated GFP+ and/or luc+ MM cells vs. non-fluorescent/non luminescent (GFP-/luc-) normal tissues, allowed us to characterize in detail the total number and size of MM lesions (even small lytic lesions which escape detection by X-rays) and to monitor, serially and non-invasively, their anatomic distribution in the skeleton, s.c. tissues, and visceral sites of potential tumor infiltration. The small size of Mmbearing mice results in limited attenuation of the GFP/luc signals, which are captured by digital cameras, for computerized quantification of MM tumor burden. Confirmatory studies in cohorts of >70 mice (for each of these imaging modalities) injected with MM cells (including RPMI-8226/S, MM-1S or MM-1R cell lines) expressing GFP+ and/or luc, which are functional (and thus detectable) only in live MM cells. Practically all mice (>98%) in these studies developed skeletal lesions, primarily in the axial skeleton, e.g. spine (>96% of mice), skull and pelvis. BM homing of GFP+ MM cells was confirmed by flow cytometry (detection of cells expressing GFP+ and human CD38/CD138 markers in BM aspirates from tumor sites) and histopathological analyses. Extra-skeletal lesions were also formed (e.g. s.c. plasmacytomas in >50% of mice), but their presence did not Abstract of Presentation (Scientific Session: Mouse models for MM) significantly impact upon MM survival and quality of life, while visceral MM lesions in lung, liver, spleen or kidney developed only rarely (~5% of mice). Comparison of histopathologic examination and imaging data showed no MM lesions lacking GFP and/or luc expression, and no GFP+/luc- signal from normal cells of the host, confirming the very high sensitivity and specificity of in vivo MM detection with these modalities. The most important determinant of overall survival was the development of spinal lesions (but not extraskeletal MM tumors), which were associated with hind limb paralysis (78.8% of mice), after a median of 29 (range 22-43) days, prompting sacrifice of mice per protocol. These models fulfill major prerequisites for a clinically-relevant in vivo model of MM: essentially all mice develop diffuse bone lesions, with anatomic distribution (primarily in the axial skeleton, e.g. spine (>96% of mice), skull and pelvis) and resulting manifestations (e.g. paralysis) consistent with the clinical picture of the disease in human patients. The clinical relevance of these models and the capability for noninvasive, sensitive and specific, real-time monitoring of precise tumor distribution has already allowed us to confirm the in vivo anti-MM activity of novel therapies such as hsp90 (heat shock protein-90) inhibitors (CS Mitsiades et al. Blood 2002; 100, 106a) or IGF-1R tyrosine kinase inhibitors (CS Mitsiades et al. Blood 2002; 100, 170a). GFP+ MM cells can also be rapidly purified by flow cytometry-based cell sorting (without need for additional positive selection steps) from non-fluorescent normal cells, and subsequently analyzed by conventional or high-throughput gene expression or proteomic profiling (CS Mitsiades et al. Blood 2002; 100, 106a), thus providing insight into in vivo mechanisms of action of novel agents. These use of these models has already provided important pathophysiological and clinical insight: the overall survival and quality of life in mice with diffuse MM lesions is not affected by the total tumor burden in a strictly proportional manner, because (in the overwhelming majority of mice) vertebral bone lesions (even the size of only a small fraction of the total tumor burden) cause paralysis and necessitate sacrifice in the overwhelming majority of mice, while lesions in other less critical skeletal or extraskeletal (e.g. s.c. tissues) areas with greater local tumor involvement, did not have the same impact on the course of the disease. These findings suggest that systemic markers of total tumor burden (e.g. serum or urine monoclonal Ig levels), while informative of changes in total tumor burden, may not reflect how individual MM lesions at different site can differentially affect survival and quality of life of mice, or even how tumor cells homing in different organs may differentially respond to therapy, a concept that warrants further study both from a preclinical and clinical standpoint (Blade et al. Br J Haematol 2001;113, 422-4). These in vivo models also highlight the marked osteotropism of MM cells, as well as their heterogeneous, but not random, skeletal distribution, which is analogous to the clinical observation of variable % of BM infiltration by MM cells in different skeletal sites of the same patients, thus raising the hypothesis of molecular heterogeneity of the BM microenvironment in various skeletal areas, with potential implications in the pathogenesis of MM and selective therapeutic targeting of its local milieu. Importantly, even in cases of MM cell lines derived from plasma cell leukemic patients (e.g. RPMI-8226/S), bone lesions developed in all mice and were the predominant cause of death, suggesting that the genetic abnormalities in advanced MM/PCL cells may allow their survival even in the absence of interaction with the BM microenvironment, yet these cells can apparently still be responsive to BM microenvironmental stimuli (e.g. cytokines), to such an extent that they still preferentially home to the BM milieu, indicating its important role as a therapeutic target even in very aggressive cases of MM. The application of whole-body fluorescence and/or bioluminescence imaging has led to development of clinically-relevant in vivo models of diffuse MM lesions which not only provide insight into the pathophysiology of MM, but have also allowed for more accurate quantitative preclinical evaluations of novel anti-MM therapies.
9. Chemotherapy, maintenance treatment and supportive care

P9.1
OPTIMAL CHEMOTHERAPY FOR INDUCTION AND MAINTENANCE

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Before therapy of a myeloma patient is started two important questions have to be answered. First, is the patient really in need of chemotherapy? If the answer is no, i.e. the patient has smoldering or stage I myeloma, he/she should be carefully watched but no active therapy given. There are no established means to delay the onset of symptomatic disease, but the results of ongoing trials using thalidomide and other drugs are awaited. Second, if the patient needs immediate therapy, next question to be raised is if he/she is a candidate for intensive therapy (usually high-dose melphalan with autologous stem cell support, given once or twice; or in a small minority of patients allogeneic bone marrow transplantation). This will today constitute the standard therapy for patients below the age of 60 (at least), 65 (in most places) or 70 years or more (at certain institutions).

If the patient will undergo high-dose therapy with stem cell support up-front this decision influences the choice of initial chemotherapy. Melphalan should be avoided, since it may damage the hematopoietic stem cells and reduce the yield at the stem cell harvest. The standard pretransplant induction therapy has for many years been VAD (vincristine, adriamycin and dexamethasone), usually given as a continuous 4-day infusion. This regimen is, however, both toxic and technically complex (indwelling catheter, pump, frequent hospital visits). Furthermore, neither vincristine nor adriamycin are potent anti-myeloma drugs, showing minimal activity when given as single drugs, and dexamethasone seems to be the most potent component of the drug combination. This has led to several ongoing trials, in which dexamethasone alone, dexamethasone plus thalidomide (e.g. Mayo Clinic, ECOG) or dexamethasone plus cyclophosphamide (NMSG) is compared to VAD as induction therapy before stem cell harvest and high-dose melphalan. A further advantage of a mainly dexamethasone-based induction regimen might be a shortening of the time from diagnosis/start of therapy to the high-dose melphalan (and hopefully subsequent good response). Negative might be that fewer patients would achieve a partial response on the induction therapy, but this fact does not preclude a good response to high-dose melphalan.

For the patients not considered for intensive therapy (and with a median age of 70 years in an unselected patient population this will still be the majority of cases) intermittent melphalan and prednisone (MP) has since more than 40 years been the therapy of choice. Since the absorption of melphalan is variable the dose should be stepwise escalated to achieve a moderate leuko- and thrombocytopenia between the courses (nadir reached 14-21days). In patients with renal failure the dose should be reduced. High fluid intake is important. For patients with initial cytopenias, especially thrombocytopenia cyclophosphamide may be an alternative instead of melphalan. With MP a partial response can be demonstrated in 50-60 % of patients (but CR in less than 5 %), and the median duration of response is about two years.

After a patient has entered a plateau phase continued MP therapy is generally considered of no value. Inspired by the dramatic effect multidrug cytostatic regimens was shown to exert in other B-cell neoplasms a very large number of clinical trials has been performed also in multiple myeloma, from the early 60-ies onwards, evaluating combination chemotherapy vs traditional MP therapy. A number of drug combinations have been investigated, most of them comprising vincristine, an anthracycline, one or two alkylating agents and corticosteroids. This led to many years of discussion regarding the advantages of different regimens, at least in certain situations, over MP. However, in the overview, performed by the Myeloma Trialists' Collaborative Group (1998), that included individual data on 6623 patients from all known trials worldwide (n = 27), it was not possible to demonstrate that combination chemotherapy has an advantage in comparison to MP. It could neither be shown that multiagent chemotherapy conferred a survival benefit to poor-risk patients.

Interferon alone was in the early 80-ies shown to induce responses in a certain fraction of newly diagnosed myeloma patients, but the addition of this drug to MP or combination chemotherapy did not convincingly increase the response rate and not the overall survival. Today several large clinical trials are ongoing, both in Europe and in the US, exploring the value of adding thalidomide and/or other drugs to the induction therapy, but no results are yet available.

Patients responding to initial chemotherapy inevitably relapse after a period of varying length, months to years. Great interest has therefore been focused, and is focused today, on methods to prevent or delay relapse. Alfa-interferon has since the early 80-ies been used for this purpose in several trials, recently summarized in an overview performed by the Myeloma Trialists' Collaborative Group (2001), comprising 12 maintenance studies. Even if a significant advantage was demonstrated for interferon-treated patients with regard to both time to progression and total survival (c.a 6 months), this moderate gain is by most physicians (and many patients) considered too small to outweigh the negative side-effects and the cost of the therapy. Today much interest is focused on a number of new drugs with activity in myeloma (immunomodulators, proteasome-inhibitors, arsenic compounds), and several of them are in different stages of clinical trial evaluation. Almost every clinical trial group is in one or other way including thalidomide maintenance in their running protocols. Some results from these studies may be available at this meeting. Other trials, in which prolonging the time in "remission" for myeloma patients with the help of chemotherapy has been explored, have not been successful. A number of options are still not fully examined, e.g. repeated post-remission chemotherapy, late intensification therapy, early treatment of "subclinical" relapses with the help of minimal residual disease status. However, it seems more realistic to believe that immunotherapy (in some form) rather than chemotherapy will be a practicable way to prolong remission or perhaps even cure myeloma patients with a heavily reduced tumor burden or a stable plateau phase.

References

P9.2 CURRENT MANAGEMENT OF MYELOMA PATIENTS WITH RENAL FAILURE

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Introduction: Renal failure is seen in up to 50% of patients with myeloma at one time or another over the course of the disease, and is secondary to cast nephropathy (myeloma kidney), amyloidosis, light chain deposition disease, other metabolic abnormalities and nephrotoxic therapy.

While the management is dependent upon the underlying cause, the general principles of management are outlined below.

Supportive therapy: This is critical at all stages of therapy, and includes the following:
- Adequate hydration
- Correction of hypercalcemia
- Correction of other metabolic abnormalities
- Plasmapheresis if hyperviscosity syndrome present
- Consideration of limited plasmapheresis during induction therapy in patients with acute renal failure even in the absence of hyperviscosity syndrome
- Avoidance of nephrotoxic drugs
- Use of newer low-osmolar, non-ionic, monomeric contrast agents such as iohexol if radiographic studies with contrast administration are essential
- Use of n-acetylcysteine with the use of unavoidable nephrotoxic agents
- Slow bisphosphonate administration (2-3 hours for pamidronate and 30 minutes for zoledronate)

Induction therapy: Prompt initiation of therapy is important in patients presenting with renal failure because, unless the renal failure is purely of metabolic etiology, reduction of tumor burden is essential for improvement of renal function. The reversibility of renal failure decreases with passing time.

While standard induction chemotherapeutic regimens such as VAD can be employed safely in patients with renal failure, toxicity (particularly from vincristine) may be more significant. High-dose dexamethasone is safe and effective, and is usually the treatment of choice in patients with compromised renal function. Thalidomide is another reasonable option; either by itself or in combination with corticosteroids.

High-dose therapy and transplantation: High-dose therapy and autotransplantation, which is effective in patients without renal failure, is feasible in patients with renal failure too since melphalan pharmacokinetics are not significantly affected by renal failure.

Adequate data exist to show that autografting is reasonably safe in patients with renal failure. However, overall toxicity is higher than in patients with normal renal function. High-dose therapy therefore ought to be used judiciously in patients with renal failure; particularly older individuals, those with concomitant medical problems, and those who are already in complete remission. The place of tandem transplantation, already debatable in myeloma, is questionable in patients with renal failure.

Non-myeloablative allogeneic hematopoietic stem cell transplantation is feasible in myeloma patients with renal failure. However, in view of a 20% risk of treatment-related mortality, it should only be undertaken in patients with high-risk disease.

Role of renal transplantation: Because myeloma is considered incurable, myeloma patients can almost never qualify for a cadaveric renal allograft. However, a renal allograft from a living (usually related) donor is feasible. This should only be undertaken only in patients who are in complete remission and have disease that is expected to remain under control for a reasonable period of time based upon its biological features. An attractive possibility is a hematopoietic stem cell and a renal allograft from the same donor (an HLA-identical sibling) which could cure myeloma while inducing specific transplantation tolerance.

P9.3 NEW ADVANCES IN THE USE OF BISPHONATONES IN MYELOMA.

James R. Berenson, MD
Professor of the Myeloma and Bone Metastasis Programs at Cedars-Sinai Medical Center

Recent large placebo-controlled clinical trials have shown the efficacy of bisphosphonates in reducing skeletal complications in myeloma patients, and suggested that these agents may also alter the overall course of the disease. Large randomized trials of long-term bisphosphonate use have now been published, and involved evaluation of oral administration of daily etidronate, clodronate, or pamidronate or intravenously administered pamidronate, ibandronate or zoledronic acid.

In the Canadian study involving daily oral etidronate compared to placebo for newly diagnosed myeloma patients who also received oral melphalan and prednisone, there was no difference was found between the two arms. Similarly, the other outcome measures (new fractures, hypercalcemic episodes, and bone pain) showed no differences between the two arms.

Two large randomized, double-blind trials have been published using oral clodronate in myeloma patients. In the Finnish trial, 350 previously untreated patients were entered, and 336 randomized to receive either clodronate (2.4 g) or placebo daily for 2 years. Although the proportion of patients with progression of lytic lesions was less in the clodronate treated group (12%) than in the placebo group (24%), the progression of overall pathological fractures, as well as both vertebral and non-vertebral fractures, was not different between the arms. The Medical Research Council has published the results of a large randomized trial involving 536 recently diagnosed myeloma patients randomized to receive either oral clodronate 1.6 g or placebo daily in addition to alkylator-based chemotherapy. After combining the proportion of patients developing either non-vertebral fractures or severe hypercalcemia including those leaving the trial due to severe hypercalcemia, there were less clodronate-treated patients experiencing these combined events than placebo patients. However, the number of patients developing hypercalcemia was similar between the two arms. The number of patients experiencing non-vertebral and vertebral fractures was lower in the clodronate group. The proportion of patients requiring radiotherapy was similar between the two arms. There was no difference in time to first skeletal event or overall survival.

In a double blind randomized trial, a Danish-Swedish cooperative group evaluated daily oral pamidronate (300 mg/day) compared to placebo in 300 newly diagnosed myeloma patients also receiving intermittent melphalan and prednisone. After a median duration of 18 months, there was no significant reduction in the primary endpoint defined as skeletal-related morbidity (bone fracture, surgery for impending fracture, vertebral collapse, or increase in number and/or size of lytic lesions), hypercalcemic episodes, or survival between the arms. A large randomized, double-blind study was conducted to determine whether monthly 90 mg infusions of pamidronate compared to placebo for 21 months reduced skeletal events in patients with multiple myeloma who were receiving chemotherapy. Patients were
stratified according to their amyloidoma therapy at trial entry: stratum 1, first-line chemotherapy; stratum 2, second-line or greater chemotherapy. The primary endpoint, skeletal events (pathologic fractures, spinal cord compression associated with vertebral compression fracture, surgery to treat or prevent pathologic fracture or spinal cord compression associated with vertebral compression fracture, or radiation to bone) and secondary endpoints (hypercalcemia, bone pain, analgesic drug use, performance status and quality of life) were assessed monthly. The proportions of amyloidoma patients having any skeletal event was 41% in patients receiving placebo but only 24% in pamidronate-treated patients. In addition, the number of skeletal events/year was half in the patients treated with pamidronate. Although overall survival in all patients was not significantly different between the two treatment groups, in stratum 2 the median survival time was 21 months for pamidronate patients compared to 14 months for placebo patients.

Ibandronate is a nitrogen-containing bisphosphonate that in pre-clinical models shows more anti-bone resorative potency than pamidronate and the other non-nitrogen-containing bisphosphonates. The results of a phase III placebo-controlled trial of 214 stage II or III amyloidoma patients with osteolytic bone disease were recently published. Patients either received monthly injections of 2 mg of ibandronate or placebo in addition to their antineoplastic therapy. Ninety-nine patients were evaluable in each arm for efficacy. The mean number of events per patient year on treatment was similar in both groups. In addition, there was no difference in pain, analgesic usage or quality of life between the arms. However, among patients treated with ibandronate who showed a sustained and marked reduction in bone resorption markers, fewer skeletal complications occurred. There was no difference in overall survival.

Zoledronic acid is an imidazole-containing bisphosphonate that shows more potency in pre-clinical studies than any other bisphosphonate currently available. Two small Phase I studies and one large randomized Phase II trial established the safety and marked sustained reduction in bone resorption markers for patients with amyloidoma and other cancers associated with metastatic bone disease with monthly infusions of small doses given over several minutes. Thus, a larger Phase III trial evaluated two doses of zoledronic acid (4 and 8 mg) compared to pamidronate (90 mg) infused every 3-4 weeks for treatment of amyloidoma or breast cancer patients with metastatic bone disease. Importantly, the primary efficacy endpoint of this trial was designed to show the noninferiority of zoledronic acid compared to pamidronate in reducing skeletal complications for patients with amyloidoma or breast cancer metastatic to bone. The trial involved 1643 patients who were stratified among individuals with amyloidoma (n=513) or breast cancer on either hormonal therapy or chemotherapy (n=1130). Importantly, during the clinical trial, rises in creatinine were more frequently observed in the zoledronic acid arms, and the infusion time was increased to 15 minutes. Despite this increase in infusion time, patients receiving the 8 mg dose continued to be at a higher risk of developing rises in serum creatinine, and these patients were subsequently changed to the 4 mg dose for the remainder of the trial and were excluded from efficacy conclusions. Recently, the final results of the trial with an additional year of randomized follow-up (25 months overall) have become available. The primary efficacy endpoint was the percentage of patients who experienced a skeletal event. Secondary efficacy endpoints included the time to first skeletal events, the annual incidence of skeletal events, and Andersen-Gill multiple event analysis of the overall risk of experiencing a skeletal event; these analyses included hypercalcemia of malignancy as skeletal event. For the primary endpoint, 50% of patients treated with 4 mg zoledronic acid experienced a skeletal event versus 54% of patients treated with pamidronate (P = .499). The median time to first skeletal event was delayed by almost 100 days for patients treated with 4 mg zoledronic acid (median 380 days versus 286 days for pamidronate), but this difference did not achieve statistical significance (P = .652). The mean annual incidence of skeletal events was 1.32 for 4 mg zoledronic acid versus 0.97 for pamidronate (P = .505). Finally, the multiple event analysis hazard ratio for the 4 mg zoledronic acid treatment group versus pamidronate was 0.932 (95% CI = 0.719, 1.208). Importantly, 4 mg zoledronic acid (via 15-minute infusion) exhibited a renal safety profile similar to 90 mg pamidronate.

Recently, the American Society of Clinical Oncology published guidelines based on the recommendations of the ASCO Bisphosphonates Expert Panel. The panel recommended that for multiple amyloidoma patients who have on plain radiographs evidence of lytic bone disease either intravenous zoledronic acid 4 mg infused over 15 minutes or pamidronate 90 mg delivered over 120 minutes every 3 to 4 weeks. The panel also believes it is reasonable to start these agents for patients with osteopenia but without evidence of lytic bone disease. Once initiated, the Panel recommended that the intravenous bisphosphonate be continued until there was a substantial decline in the patient’s bone scan or imaging. The Panel also recommended intermittent monitoring of renal function as well as urinary protein evaluation to assess possible renal dysfunction from these agents. However, for patients with either solitary plasmacytoma or indolent amyloidoma, no data exists to suggest their efficacy. In addition, although clinical studies would be interesting to conduct for patients with monoclonal gammopathy of undetermined significance, the panel did not recommend treatment of these patients with bisphosphonates. Importantly, the role of bisphosphonates for amyloidoma patients may go beyond simply inhibiting bone resorption and the resulting skeletal complications. Some studies suggest that these drugs may have antitumor effects both directly and indirectly. Using the murine 5T2 multiple amyloidoma model, Radl and colleagues suggested that pamidronate might reduce tumor burden in treated mice. In vitro studies also suggest pamidronate may possess anti-amyloidoma properties as demonstrated by its ability to induce apoptosis of amyloidoma cells and suppress the production of IL-6, an important amyloidoma growth factor, by bone marrow stromal cells from amyloidoma patients. A recent in vitro study may help explain the induction of apoptosis by these compounds. These drugs inhibit the mevalonate pathway; and, as a result, decrease the isoprenylation of proteins such as ras and other GTPases. The antitumor effects of these agents appear to be synergistic with glucocorticoids. Several recent studies show that bisphosphonates are markedly anti-angiogenic, and the recent demonstration of the marked anti-amyloidoma clinical effects of the anti-angiogenic agent thalidomide in amyloidoma patients suggests another putative mechanism by which bisphosphonates may possess anti-amyloidoma effects. In addition to the effects on the tumor cells and the tumoral microenvironment, recent studies suggest that nitrogen-containing bisphosphonates may stimulate T lymphocytes and induce antiplasma cell activity in amyloidoma patients. Several recent murine models of human amyloidoma show that the administration of pamidronate or zoledronic acid both reduces the development of lytic bone disease and tumor burden. Because of the survival advantage observed in relapsing patients in the large randomized placebo-controlled pamidronate trial, attempts were made to increase the dose of pamidronate to more clearly show the anti-amyloidoma effect of this agent but were accompanied by the development of albuminuria and azotemia. However, since 4 mg of zoledronic acid may be administered safely over 15 minutes, it may be possible to increase the dose of this newer agent with longer infusion times and clearly show the hoped for anti-amyloidoma effects clinically that have been suggested from the pre-clinical studies mentioned above. As a result, a Phase I trial has been initiated to...
explore higher doses of zoledronic acid for patients with myeloma. These studies suggest the possibility that in the near future that bisphosphonates may not only be used to reduce skeletal complications but also treat the underlying myeloma itself.

P9.4
OPTIMIZING TREATMENT FOR ANEMIA
Heinz Ludwig
Department of Medicine and Medical Oncology, Wilhelminenspital, Vienna, Austria

Anemia is a frequent complication of myeloma and a negative prognostic factor. In several studies up to 60% of patients presented with hemoglobin <12g/dl and about 20% with moderate to severe anemia (hemoglobin <10g/dl).

The mechanism of anemia in myeloma usually is multi-factorial with inadequate endogenous erythropoietin production as one of the leading causes. Other factors responsible or contributing to anemia are infections, side effects of cytotoxic treatment or radiotherapy and direct destruction of erythroid precursors via Fas mediated apoptosis of erythroid precursors by myeloma cells. Dilutional anemia due to hyperviscosity is rare, but also a myeloma specific cause of anemia. Other causes are hemolysis, vitamin deficiency, bleeding and rarely disseminated intravascular coagulation.

Anemia is associated with impaired quality of life (QOL) and with poor prognosis. Erythropoietin, the most important stimulator of erythropoiesis, has soon after its introduction been used for treatment of anemia in myeloma. After initial successful phase II studies, prospective randomized trials elaborated the optimal dose for treatment in myeloma which has been defined as 10,000 Units, 3x/ week or 40,000 Units once weekly subcutaneous. The advent of the novel erythropoiesis stimulating substance (Aranesp) will probably allow even longer treatment intervals.

Studies on the incremental gain in quality of life indicate that the highest gain in subjective wellbeing is obtained when hemoglobin is increased from 10g/dl to 12g/dl; hence 12g of hemoglobin/dl have been defined as target level for treatment. Increasing hemoglobin above that level will lead to further improvement in QOL but at lower incremental gain. In order to prevent overshooting of hemoglobin levels, the dose of erythropoietin needs to be tapered in several patients, either by lowering the dose or by increasing the interval between dosing.

Iron supplementation is recommended in patients with iron deficiency indicated by low transferrin saturation (<20%). Oral iron supplementation is usually recommended although parenteral iron will result in faster restoration of iron stores and faster increase in erythropoiesis.

It is unclear at present whether erythropoietin treatment will impact also on outcome of cancer treatment and survival. An animal model indicated that unphysiologically high doses of erythropoietin induce tumor regression in plasmacytoma bearing mice. In conclusion, erythropoietin treatment in myeloma has been shown to be safe and effective in terms of reduction of transfusion need and improvement in quality of life, physical activity and enhanced sense of well-being and therefore is recommended in patients afflicted with symptoms of anemia.

10. Has autologous stem cell transplantation (ASCT) become the gold-standard treatment in multiple myeloma?

Roundtable 1: ASCT VERSUS CONVENTIONAL CHEMOTHERAPY Randomised studies

P10.1.1
THE IFM-90 TRIAL.
Jean-Luc HAROUSSEAU, Michel ATTAL
On behalf of the IFM Group

The Intergroupe Francais du Myelome was the first to conduct a randomized trial showing the superiority of high dose therapy (HDT) with autologous bone marrow transplantation as compared to conventional chemotherapy (CC) (N Engl J Med 1996;335:91-97). In this trial, at the time of diagnosis, 200 patients less than 65 years of age with Durie –Salmon stage II or III were randomly assigned to receive either CC or HDT. CC consisted of alternating cycles VMCP/BVAP administered at 3-week intervals for 12 months for a total of 18 cycles. HDT was administered after four to six cycles of VMCP/BVAP to all patients with WHO performance status of 2 or less, a serum creatinine level less than 150 mol/L and more than 2 x 10^8 nucleated cells/kg in the marrow collected and unpurged. Patients were prepared with HDm (140 mg/m²) and total-body-irradiation (TBI) (8 Gy). Interferon alfa (IFNα) was administered at a dose of 3 x 10^6 U/m² 3 times a week until relapse in both arms. Comparison of the two therapeutic modalities was made on an intention-to-treat basis, with all patients studied in their assigned treatment groups. None of the initial characteristics differed significantly between treatment groups. The response to initial chemotherapy and the compliance with interferon treatment were also comparable. In this IFM 90 trial, HDT significantly improved the response rate since 38% of patients enrolled in the HDT arm achieved a complete remission or very good partial remission (>90% reduction of the M-component) versus 14% of patients enrolled in the CC arm (p<0.001). An updated analysis of this study confirms that, with a median follow-up of 7 years, HDT significantly improves event free survival (EFS) (median 28 months versus 18 months, 7-year EFS 16% versus 8%, p<0.01) and overall survival (OS) (median 57 months versus 44 months, 7-year OS 43% versus 25%, p=0.03).

P10.1.2
HIGH DOSE CHEMOTHERAPY WITH AUTOLOGOUS STEM CELL TRANSPLANTATION VERSUS CONVENTIONAL CHEMOTHERAPY: THE BRITISH EXPERIENCE.

Department of Clinical Haematology, The General Infirmary, Leeds LS1 3EX; Northern and Yorkshire Clinical Trials and Research Unit (NYCTRU) Department of Immunology, University of Birmingham.
On behalf of the Medical Research Council (MRC) Adult Leukaemia Working Party

The approaches introduced at the Royal Marsden Hospital in the early 1980s with escalation of melphalan to 140mg/m² without stem cell support and related studies under the aegis of the MRC were the basis for the introduction of high dose therapy (HDT) with supporting autologous bone marrow and, subsequently,
explore higher doses of zoledronic acid for patients with myeloma. These studies suggest the possibility that in the near future that bisphosphonates may not only be used to reduce skeletal complications but also treat the underlying myeloma itself.

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Peripheral blood stem cell transplantation in the UK. In previously untreated patients the complete response (CR) rate as assessed at that time approached 50%. The strategy which emerged was initial conventional dose anthracycline-containing infusional chemotherapy followed by high dose melphalan (HDM) at 200mg/m² or HDM at 140mg/m² with the addition of total body irradiation (TBI). Although the depth of remission was evidently greater than with purely conventional dose therapy the remissions were not enduring. The concept of maintaining longer remissions was introduced with the use of interferon-alpha (IFN-α) in a maintenance role. At the time of the initiation of MRC Myeloma VII in 1993 there was a clear need for outcome data on HDT in comparison with conventional dose chemotherapy in the large randomised trial setting. In the series of MRC trials leading up to Myeloma VII, the most effective conventional dose regimen was ABCM² and this was chosen as the standard treatment: doxorubicin 30mg/m² i.v., carmustine 30mg/m² i.v. short infusion d1; cyclophosphamide 100mg/m² orally and melphalan 6mg/m² orally d22-25; cycle repeating every 6 weeks to maximal response (plateau) maximum 12 cycles. Patients could switch to cyclophosphamide 300mg/m² i.v. weekly in the event of undue myelosuppression. Planned maintenance was IFN-α (Roferon A) initially at 3 megunits 3x/week. The intensive regimen was C-VAMP: doxorubicin 9mg/m²/d and vincristine 0.4mg/d continuous infusion d1-4; methylprednisolone 1mg/m² i.v. or orally (max 1.5g) d1-5; cyclophosphamide 500mg i.v. d1, 8 and 15; cycle repeating every 21 days to maximal response with minimum of 3 cycles before stem cell harvesting. Adjustments for cytopenias/renal dysfunction were applied. Stem cells were mobilised using cyclophosphamide 2-4g/m² and G-CSF d5-12. The HDT comprised HDM at 200mg/m² followed by the infusion of PBSCs at 24hrs. Bone marrow autograft and TBI + melphalan 140mg/m² were permissible options. Methylprednisolone 1.5g/d was given i.v. for the first 4 days following HDM. The melphalan dose was modified according to creatinine clearance. The planned maintenance in this treatment arm was also IFN-α. The EMBT/IBMTR response criteria were used whereby CR required paraprotein negative by immunofixation. A total of 407 previously untreated patients under 65 years of age were randomised to receive “Standard” or “Intensive” treatments. Only 30 (15 percent) patients in the Standard group went on to receive an autograft and 4 (2 percent) an allograft, outside protocol. In the 401 evaluable patients, response rates for the Intensive group were higher than in the Standard group: CR 44.3% v 8.5% (p<0.001); PR 42.3% v 40.5%. In an intention to treat (ITT) analysis better overall survival and progression-free survival was seen in the Intensive group than the Standard group, (log rank p=0.04 and p <0.001 respectively). Overall there was an improvement in median survival of approximately one year in the Intensive group: 54.1 (95% confidence interval 44.9 to 65.2) months compared with 42.3 months (95% confidence interval 33.1 to 51.6) in the Standard group. Other analyses showed a trend to improved survival from minimal response (MR) 25.6 months through partial response (PR) 39.8 months to CR, 88.6 months. A significant interaction between treatment group and pretreatment serum beta 2 microglobulin (β2m) level was seen (p=0.003, Cox model). Stratified log rank analysis was carried with the β2m strata <4.0, 4-8.0 and >8.0mg/l (as defined in previous MRC studies). Within each stratum the Intensive group had a longer median survival than the Standard group but this difference was greatest in those with baseline serum β2m >8mg/l – median survival 41.9 months in the Intensive group compared with 13.1 months in the Standard group. In cohort studies to further investigate the impact of attaining minimal residual disease (MRD) after HDT, immunophenotypic characterisation of plasma cells by flow cytometry suggested that patients who were immunofixation negative and consistently produced plasma cells with a normal phenotype post-HDT had an improved survival compared to patients who were immunofixation negative but with plasma cells of malignant phenotype³⁻⁵. Although the worldwide data on the straight non-selected randomised comparison of conventional-dose treatment versus treatment incorporating HDT are limited, an overview including published data from the comparable IFM and MAG studies suggest that there is a significant survival benefit from HDT. It is unlikely that further trials of this nature will be pursued. In the forthcoming MRC Myeloma IX Trial, all younger/fitter patients (not defined strictly by age) will receive HDT as a component of their first line treatment.

References

P10.1.3 HIGH-DOSE THERAPY AUTOTRANSPANTATION/ INTENSIFICATION VERSUS CONTINUED CONVENTIONAL CHEMOTHERAPY IN MULTIPLE MYELOMA PATIENTS RESPONDING TO INITIAL CHEMOTHERAPY. RESULTS OF A PROSPECTIVE RANDOMIZED TRIAL FROM THE SPANISH COOPERATIVE GROUP PETHEMA.
On behalf of PETHEMA. Hospital Clinic. Barcelona. Spain.
Background: Treatment of patients with multiple myeloma (MM) is disappointing. In this regard, several large phase III trials from the SWOG and PETHEMA showed that increased doses of conventional chemotherapy did not result in a significant survival prolongation (1,2). Furthermore, the Nordic Myeloma Study Group reported no survival improvement in conventionally treated younger myeloma patients during the last two decades (3). These limitations led to the current tendency of offering high-dose therapy/stem cell rescue (HDT/SCT) to most younger patients with MM as part of the
initial therapy. However, the role of HDT/SCT in the management of patients with MM is still a matter of controversy. A randomized trial by the French Intergroup showed that HDT significantly increased the complete remission (CR) rate, progression-free survival (PFS) and overall survival (OS) (4). More recently, the MRC VII trial showed a significant benefit for HDT/SCT versus conventional chemotherapy in both PFS and OS (5). In contrast with these results, Fermand et al (6) showed, in a prospective randomized trial, that HDT/SCT was not superior to conventional chemotherapy in patients aged 55 to 65 years.

Aim: The objective of the present study was to compare HDT/SCT and continued conventional chemotherapy in MM patients responding to the initial treatment.

Patients and Methods: From May 1994 to October 1999, 216 patients (122M/94F), median age 56 yrs, stage II or III, ECOG < 3) were registered. The diagnosis was made according to the criteria of the Chronic Leukemia Myeloma Task Force. The initial chemotherapy consisted of 4 courses of alternating BVMCP/VBAD. Responding patients received either 8 additional courses of BVMCP/VBAD (arm A) or intensification with HDT/SCT - melphalan 140 mg/m²/TBI 12 Gys or melphalan 200 mg/m² (arm B). Maintenance treatment consisted of alpha-interferon and dexamethasone in both arms.

Results: One-hundred and eighty five patients responded to initial chemotherapy (CR: 15%, PR: 68%, and MR: 17%). Twenty-one of these responding patients were not randomized due to different reasons. Among the 164 randomized patients 83 were allocated to continued chemotherapy and 81 to HDT/SCT. The degree of initial response as well as prognostic features did not differ in both groups. The results were updated as of November 15, 2002 after a median follow-up of 44 months from the initiation of treatment and analyzed on an intention-to-treat basis. CR (negative electrophoresis) was significantly higher in the HDT/SCT arm (30 vs. 11%, p=0.002). However, PFS was not significantly different between HDT/SCT and conventional chemotherapy (median, 43 vs. 34 mos; p=NS) and the OS was similar in both groups (median, 62 vs. 56 mos.; p=NS).

Conclusions: This study shows that, as delivered in this trial, HDT/SCT intensification significantly increases complete remission rate but has no significant impact on PFS and OS in myeloma patients responding to initial chemotherapy.

References

P10.1.4
HIGH DOSE THERAPY VERSUS CONVENTIONAL CHEMOTHERAPY FOR NEWLY DIAGNOSED MULTIPLE MYELOMA: HISTORICAL COMPARISON OF TOTAL THERAPY I VERSUS STANDARD SWOG TRIALS AND US INTERGROUP TRIAL SWOG 9321.

Bart Barlogie, Joth Jacobson, Kenneth Anderson, Philip Greipp, Robert Kyle and John Crowley
The Myeloma Institute for Research and Therapy (MIRT), University of Arkansas for Medical Sciences, Little Rock, AR, USA; Cancer Research And Biostatistics (CRAB), Fred Hutchinson Cancer Center, Seattle, WA, USA; Mayo Clinic, Rochester, MN; Dana Farber Cancer Institute, Harvard Medical Center, Boston, MA.

Improved long-term survival of patients with myeloma is directly linked to the widespread use of high-dose melphalan usually at 200mg/m² (MEL 200) with peripheral blood stem cell support (PBSC). Since mucositis is dose-limiting, tandem transplants were evaluated in TT I to further augment cell kill toward increasing CR and extending event-free and overall survival. Of 231 patients enrolled between 1989 and 1994, 152 were previously entirely untreated and could be closely matched with 152 contemporaneous patients receiving standard dose therapies (SDT) as part of SWOG trials (matching criteria included age, B2M, albumin). No significant differences existed in the proportions of patients with IgA isotype and creatinine ≥2mg/dL (Table 1). With a median follow-up of 10 years for all patients, 10-year survival and event-free survival rates were markedly higher with TT I than with SWOG SDT (33 vs 15%, p=0.001; 16 vs 5%, p=0.001) (Figure 1). Indeed, according to multivariate analysis of baseline prognostic variables together with treatment modality, TT I emerged as the single most important variable associated both with superior overall and event-free survival (Table 2). Thus, regardless of standard risk factors (cytogenetics were unavailable in SWOG trials), TT I extended survival significantly. Total Therapy II builds on the success of TT I and employs more intensive remission induction, consolidation chemotherapy after tandem transplants with MEL 200mg/m², and up-front randomization to +/- thalidomide.

Unfortunately, follow-up was deemed still too short to present the results of US Intergroup trial S9321 that randomized patients, after VAD induction, to MEL 140 + TBI 12 Gy vs VBMCP SDT. According to a recursive partitioning model, platelet count and LDH distinguished 3 risk groups with superior median survival of 68 months among 437 patients with good risk, 44 months among 211 patients with intermediate risk and 27 months among 170 patients with poor risk (p<0.001) (Figure 2).

Table 1. Patient Characteristics

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<th>TT I (N=152)</th>
<th>SWOG (N=152)</th>
<th>P-value</th>
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<tr>
<td>Age ≥60 years</td>
<td>26%</td>
<td>26%</td>
<td>0.90</td>
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<td>Albumin &lt;3.5</td>
<td>24%</td>
<td>22%</td>
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<td>B2M ≥3</td>
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<td>Creatinine ≥2</td>
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<td>HGB &lt;10</td>
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<td>28%</td>
<td>0.45</td>
</tr>
<tr>
<td>Platelets &lt;150</td>
<td>10%</td>
<td>12%</td>
<td>0.47</td>
</tr>
<tr>
<td>IgA isotype</td>
<td>17%</td>
<td>18%</td>
<td>0.88</td>
</tr>
</tbody>
</table>
## Roundtable 2: SINGLE VS TANDEM AUTOLOGOUS TRANSPLANTS

**P10.2.1**

**DOUBLE AUTOLOGOUS TRANSPLANTATION IMPROVES SURVIVAL OF MULTIPLE MYELOMA PATIENTS: FINAL ANALYSIS OF A PROSPECTIVE RANDOMIZED STUDY OF THE "INTERGROUP FRANCOPHONE DU MYELOME" (IFM 94).**

Michel Attal, Jean-Luc Harousseau, Thierry Facon, François Guilhot, Chantal Doyen, Jean-Gabriel Fuzibet, Mathieu Monconduit, Cyril Hullen, Denis caillot, Reda Bouabdallah, Laurent Voillat, Jean-Jacques Sotto, Bernard Grosbois and Regis Bataille

For the IFM.

High dose therapy supported with autologous stem cell transplantation has been introduced in the management of aggressive myeloma and promising survivals from single institutions, case-controlled and randomized studies have been reported. However after a single transplant (ST), almost all patients ultimately relapse. In order to improve these results the role of double transplantation (DT) has been evaluated in uncontrolled studies. However, direct comparison of these results with those observed after ST is unsatisfactory as patients undergoing high dose therapy are subject to considerable selection bias (including young age, good performans status, and normal renal function). Thus, multicentric and prospective randomized trials designed to avoid these sources of bias were required to compare ST and DT. The "Intergroupe Francophone du Myélome" (IFM) initiated such a trial. From October 1994 to March 1997, 399 untreated myeloma patients under the age of 60 years were randomized at diagnosis to receive ST prepared with melphalan (140 mg/m2) and TBI (8 Gy) or DT : the first one prepared with melphalan (140 mg/m2) and the second one prepared with melphalan (140 mg/m2) and TBI (8 Gy). Patients were initially treated with 3-4 cycles of the VAD regimen.

The response rate was not significantly different between the 2 groups. Indeed, 42% of patients enrolled in the ST arm achieved a complete response or a very good partial response (more than 90% of reduction of the M component on electrophoresis) versus 50% of patients enrolled in the DT group (p=0.15).

DT was found to improve event-free-survival (EFS). Indeed, the 7-year post-diagnosis probability of EFS was 20% (95% CI=14-26) in the DT arm versus 10% (95% CI=5-15) in the ST arm (p<0.03).

DT was found to improve overall survival. Indeed, the 7-year post-diagnosis probability of survival was 42% (95% CI=34-49) in the DT arm versus 20% (95% CI=13-29) in the ST arm (p<0.01).

In this trial 4 factors were found to be associated with a longer survival : low beta-2-microglobulin at diagnosis (p<0.01), young age (p<0.05), low LDH at diagnosis (p<0.01) and treatment arm (p<0.05).

In conclusion, the final analysis of the IFM94 trial demonstrates that DT improves the overall survival in MM and could be recommended for patients aged under 60 years.
P10.2.2
SINGLE VERSUS TANDEM HIGH DOSE THERAPY (HDT) SUPPORTED WITH AUTOLOGOUS BLOOD STEM CELL (ABSC) TRANSPLANTATION USING UNSELECTED OR CD34-ENRICHED ABSC: RESULTS OF A TWO BY TWO DESIGNED RANDOMIZED TRIAL IN 230 YOUNG PATIENTS WITH MULTIPLE MYELOMA (MM).

Jean-Paul Fermand, Corinne Alberti and Jean-Pierre Marolleau
For the group “Myélome-Autogreffe”, Caen, Créteil, Limoges, Paris, Strasbourg, France.

In 1996, we initiated a multicenter prospective trial where patients aged under 56 with newly diagnosed symptomatic MM were randomly assigned up-front to receive either a single HDT (HDT1) or two sequential HDT (HDT2). In addition, all patients were independently randomized to be transplanted with unselected ABSC (unselected arm) or CD34-enriched ABSC (CD34 arm).

In all cases, patients first received one or 2 courses of high dose steroid containing regimens and ABSC were thereafter mobilized by cytoxan (CTX) (4 g/m²) and lenograstim (10 mg/kg/d). When appropriate (CD34 arm), part of collected ABSC were selected using the Isolx®300i system. The selection procedure resulted in a median purity of 95% (65-100) and in a more than two log tumor cell depletion. In the HDT1 arm, HDT was preceded by 3 monthly courses of a VAD-like regimen and combined a multi-drug regimen (carmustine, etoposide, melphalan 140 mg/m² (MLP 140) and CTX 60 mg/kg) with a TBI (12 grays in 6 fractions). Patients treated in the HDT2 arm received MLP 140 alone (always supported by unselected ABSC) followed 2 to 3 months later by a second MLP 140 combined with etoposide (30 mg/kg) and 12-gray TBI. In both arms, TBI including HDT were supported with unselected or CD34 enriched ABSC.

Two hundred and thirty patients were included in the study. At the reference data of 01/12/2002, median follow-up was 52 months since randomization. Baseline characteristics of HDT1 (n=94) and HDT2 (n=99) groups were close. Similarly, there was no significant difference between the unselected (n=94) and CD34 (n=99) arms.

All analysis were performed in an intent to treat basis. There was no evidence for benefit of CD34 selection as compared to the use of unselected ABSC. Post transplant hematological recovery was similar but immunological recovery was delayed in the CD34 selected group in which the incidence of serious infections was abnormal in 37% (45% del 13/13q-, 50% abnormal 1p/q, 32% β2-M were not significantly different between the two groups (45 and 51 deaths in each arm and the 2 OS curves were nearly identical (p= 0.55).

In conclusion, present analysis of the study did not show any significant benefit of single HDT versus tandem HDT and of CD34+ selection of autografts which appeared to increase the incidence of serious infections.

P10.2.3
INTENSIVE VERSUS DOUBLE INTENSIVE THERAPY IN UNTREATED MULTIPLE MYELOMA: UPDATED ANALYSIS OF THE PROSPECTIVE PHASE III STUDY HOVON 24 MM*

For the Dutch-Belgian Haematology-Oncology Cooperative Group (HOVON), The Netherlands

The benefit of high-dose therapy with hemapoietic stem cell rescue in the treatment of multiple myeloma has been demonstrated in phase II/III studies. One randomized trial demonstrated a superior long-term clinical outcome of double as compared to single high-dose therapy. In 1995 HOVON started a prospective randomized multicenter trial to compare the efficacy of intensified treatment followed by myelo ablative therapy and peripheral stem cell transplantation with intensified treatment alone in newly diagnosed patients. We now report the results of a second analysis in 441 eligible patients with stage II (22%) and stage III (78%) disease. The median age was 55 years (range 31-65 years). Remission induction treatment consisted of 3-4 courses of VAD by rapid infusion. 63 patients up to 55 years who had an HLA identical sibling were candidates for an allogeneic transplantation and will be presented separately. After VAD, patients without a sibling were randomized to receive melphalan 140 mg/m² divided in 2 doses of 70 mg/m² (IDM) without stem cell rescue (arm A) or the same regimen followed by myelo ablative treatment with cyclophosphamide (120 mg/kg) and TBI with peripheral stem cell transplantation (arm B). Peripheral stem cells were mobilized by cyclophosphamide (4 g/m²) and G-CSF after VAD. Interferon-α 2-a was given as maintenance therapy in both arms.

Patient characteristics with regard to sex, age, stage of disease, Ig isotype, and β2-M were not significantly different between the two arms. The median follow-up from randomization was 40 months. 81% of patients received both cycles of IDM (79% in arm A and 83% in arm B) and 82% of patients actually received autologous peripheral stem cell transplantation in arm B. CR and PR rate were 14% and 72% in arm A versus 28% and 62% in arm B (P=0.004). The event-free survival (EFS) at 48 months from randomization was 15% (arm A) vs 29% (arm B) (logrank P=0.03). Progression free survival (PFS) at 48 months was not different between both treatment arms (18% vs 31%, logrank P=0.08). Overall survival (OS) was equal between both treatments (55% vs. 50% at 48 months, logrank P=0.31). Time to Progression (TTP) was significantly worse in arm A (80% vs 61% at 48 months, logrank P=0.003). Multivariate analysis showed that treatment arm A, hemoglobin ≤ 6.21 mmol/l, serum β2-M > 3 mg/l and elevated serum LDH were adverse prognostic factors for EFS. Cyto genetic analysis in 150 registered patients was abnormal in 37%(45 del 13/13q, 50% abnormal 1p/q, 32% disease) rates were 39% and 37%, respectively. During follow-up, there were 53 deaths in each arm and the 2 OS curves were not statistically different (p= 0.14 by the log rank test). The EFS curves were nearly identical (p= 0.55).
disappointingly low, suggesting that the procedure was not the 5-year projected probability of event-free survival was with conventional chemotherapy. However, in both these studies outcome with autologous stem cell transplantation in comparison by the Nordic Myeloma Study Group (2) demonstrated superior prospective randomized study by the Intergroupe Francais du the management of multiple myeloma (MM). Results of a hematopoietic stem cell support has enjoyed wider application for

In conclusion, in this trial myeloablative treatment with cyclophosphamide/TBI when added to intensified chemotherapy (VAD/IDM) resulted in a superior EFS and TTP, but not PFS nor OS. *C.M. Segeren, P.Sonneveld, B. van der Holt et al, Blood, March 15, 2003 in press.

**P10.2.4**

**SINGLE VS. TANDEM AUTOLOGOUS TRANSPLANTS IN MULTIPLE MYELOMA: ITALIAN EXPERIENCE.**

Michele Cavo, Elena Zamagni, Claudia Cellini, Patrizia Tosi, Sonia Ronconi, Delia Cangini, Paola Tacchetti, Antonio De Vivo, Roberto Massimo Lemoli, Monica Benni, Francesca Bonifazi, Mauro Fiacchini, Maria Rosa Motta, Simona Rizzi, Michele Baccarani and Sante Tura, writing committee of the "Bologna 96" clinical trail. Institute of Hematology and Medical Oncology "Seràgnoli", University of Bologna, Italy.

Over the last decade, high-dose therapy (HDT) with autologous hematopoetic stem cell support has enjoyed wider application for the management of multiple myeloma (MM). Results of a prospective randomized study by the Intergroupe Francais du Myélome (IFM) (1) and of a retrospective population-based study by the Nordic Myeloma Study Group (2) demonstrated superior outcome with autologous stem cell transplantation in comparison with conventional chemotherapy. However, in both these studies the 5-year projected probability of event-free survival was disappointingly low, suggesting that the procedure was not curative. In an attempt to improve the results by furtherly increasing the dose intensity, administration of two sequential courses of HDT with double, or tandem, autotransplants was proposed by several groups, initially in patients with advanced and refractory disease and subsequently as first-line therapy (3, 4). Significantly longer overall survival and event-free survival reported with double autotransplants in comparison with historical controls receiving either conventional chemotherapy (5) or a single line of HDT (6) supported the notion that "more is better" in MM. However, this issue warrants confirmation in controlled clinical studies. An Italian multicenter (a list of participating centres appears in the Appendix) phase III study designed to prospectively compare a single autotransplant (arm A) versus double autotransplants (arm B) as primary therapy for MM was started in January 1996 and was closed in December 2001. In both arms of the study, treatment plan consisted of the following phases: I) conventional remission induction chemotherapy with vincristine, doxorubicin and dexamethasone (VAD) administered at 4-week intervals, for a total of 4 courses; II) mobilization and collection of peripheral blood stem cells (PBSC) with high-dose cyclophosphamide (HD-CTX) (7 g/m²) and granulocyte-colony stimulating factor (G-CSF); III) PBSC-supported HDT (Tx-1) with high-dose melphalan (MEL) (200 mg/m²); IV) maintenance therapy with recombinant alpha interferon (IFN) following the completion of autotransplant(s). In patients randomized to arm A, a second autotransplant (Tx-2) with the combination of melphalan and busulfan (Mel-Bu) (120 mg/m² and 12 mg/kg, respectively) was planned within 90 to 180 days after Tx-1. Primary endpoints were response rate, as evaluated according to previously reported criteria (7), time to relapse/progression (TTR), overall survival (OS) and event-free survival (EFS). Curves for OS, EFS and TTR were plotted according to the method of Kaplan and Meier starting from the time of initiation of VAD chemotherapy and were compared by the log-rank test. An event included disease progression and death from any cause. An analysis of the first 220 patients who entered the study from January 1996 to December 1999 was performed on July 2001 and results are herein reported. Comparison of the presenting clinical and hematological characteristics between the two groups of patients (arm A: 110 patients) (arm B: 110 patients) revealed that they were well balanced with respect to the most common variables presumed to have prognostic relevance. The probability of receiving VAD x 4, HD-CTX and Tx-1 for patients randomized to arm A of the study was 87%, 83% and 80%, respectively. The corresponding figures for patients randomized to arm B were 96%, 86% and 85%, respectively. Thirty-six % of patients who were assigned to receive double autotransplants failed to complete their assigned treatment program (15 % due to non medical reasons); among patients who actually received Tx-2 the median interval between Tx-1 and Tx-2 was 4 months. The median number of PBSC collected following HD-CTX was 10.4x10⁶ CD 34+/kg; 16% of patients failed to collect the minimum target cell dose (≥ 4x10⁶ CD 34+/kg). The most frequent nonhematologic toxicity associated with HDT was mucositis which was graded III-IV (WHO criteria) in 14% of patients receiving MEL and in 13% of those treated with Mel-Bu. On an intention-to-treat analysis, the probability of attaining stringent defined complete remission (CR) increased with the progression through VAD, HD-CTX and HDT up to a final CR rate of 21% in arm A and of 24% in arm B (p not significant). Overall response (≥ partial response) rates for patients who actually received a single autotransplant or double autotransplants were 90% and 87%, respectively, including 25% CRs in group A and 30% CRs in group B (p, not significant).
With a median follow-up of 38 months from the start of VAD therapy, no statistically significant difference in OS was observed between the two groups (median, 56 months for Tx-1 vs. 60 months for Tx-2). Patients who died due to treatment-related causes were 3% in group A and 4.9% in group B. Compared to group A, patients assigned to the the double transplant arm of the study had a significantly longer duration of remission (median TTR, 27 vs. 44 months, respectively; p = .005) and extended EFS (median, 34 months for patients randomized to group B vs.25 months for those assigned to group A; p = .05).

Results of the present analysis confirmed that double autotransplants as primary therapy for MM could be timely performed in slightly less than two thirds of patients aged below 60 with a risk of treatment-related mortality that did not exceed 5%. Nonhematological toxicity of sequential chemotherapy consisting of MEL and Bu-Mel was minimal and no cumulative toxicity was observed in the Tx-2 arm of the study compared to Tx-1. Double autotransplants, albeit not correlated with significantly higher CR rates in comparison with Tx-1, effected responses of “better quality”. This finding ultimately resulted in extended duration of remission and longer EFS for patients assigned to receive double autotransplants in comparison with those randomized to a single autotransplant. With a median follow-up of approximately 3 years no difference in OS was disclosed between the two treatment groups. It is worthy of note that data similar to those herein described were reported by the French Myeloma Intergroup at a preliminary analysis of the IFM-94 trial of single versus double autologous transplants, both prepared with TBI and MEL, 140 mg/m², preceded by MEL in case of double transplants (8). More recently, the same group updated the results of the trial with a longer follow-up and found that double autotransplants significantly extended the 5-year projected probability of OS and EFS in comparison with a single transplant (9). Mature data derived from the final analysis of our study must be awaited before definite conclusions can be given concerning the impact of double autotransplants on the ultimate outcome of MM.

Supported in part by Università di Bologna, Progetti di Ricerca ex-60% (M.C.)

Appendix


REFERENCES


P10.2.5 SINGLE VS. TANDEM AUTOLOGOUS TRANSPLANTATION IN MULTIPLE MYELOMA: THE GMMG EXPERIENCE

Goldschmidt H
On behalf of the German-Speaking Myeloma Multicenter Group, GMMG

High-dose (HD) therapy followed by autologous stem cell transplantation (ASCT) has improved event-free (EFS) and overall survival (OS) in multiple myeloma (MM), but nonetheless virtually all patients eventually relapse. Single center experience of the Arkansas group has indicated that total therapy including tandem ASCT improves clinical outcome further, and results of the French IFM-group have shown in a randomized trial that MM patients benefit from tandem ASCT. No significant benefit of double ASCT could be demonstrated in the studies performed by the HOVON-group, the Bologna-group and in the MAG95-study. Melphalan without total body irradiation is currently considered as the best high-dose therapy regimen.

Between 1996 and 2000, patients with MM who had received no more than 6 previous cycles of conventional chemotherapy were included in the GMMG-HD2 trial. In the study two randomizations were possible. The first randomization was optional and compared standard VAD vs. vincristine, oral idarubicine and dexamethasone (VID) as induction treatment. Results showed no difference in response and number of stem cells mobilized. Hematological toxicity was higher after VID compared to VAD. The second randomization was obligatory and compared a single cycle of HD-melphalan vs. two sequential cycles of HD-melphalan, each followed by peripheral blood ASCT. Interferon-alpha was started as maintenance treatment for all patients at a dose of 13.5 million units per week. The median follow up of the patients in the study is 36 month. The GMMG will present results of the first trial comparing a single vs. two sequential cycles of HD-melphalan (200 mg/m²) followed by ASCT.
An alternative to tandem autotransplantation is wait until relapse before giving the second transplant. We have previously shown that results with a single autotransplant conditioned with HD-M200 are comparable to those with tandem transplants (Sirohi et al, Proc Am Soc Clin Oncol 2002; 21:269a). We have analysed a group of 96 myeloma patients (34-77 years, median 55) relapsing after one autograft who underwent a second autograft with HD-M200. The median interval between the two autografts was 3.2 years (range, 6 weeks to 11.8 years). At relapse, of these 96 patients 87 received reinduction with infusional chemotherapy (n=86) or other (n=1); 9 received HD-M200 with the cells harvested while in first CR. At the time of salvage autograft 6 patients were in CR, 43 partial remission (PR) and 47 were non-responders (NR) or had progressive disease (PD). 32% of patients attained or continued in CR after salvage autograft and the overall response rate was 76%. 9 patients died of treatment-related mortality and 6 died of PD. 56 patients experienced disease progression at a median of 10.5 months. Overall, 33 patients are alive at the last follow-up 2 weeks to 10.9 years post salvage autotransplant. The actuarial 3-year probabilities of relapse, disease-free survival and overall survival from salvage autotransplant were 84%, 13%, and 35% respectively. The variables analysed for prognostic significance in multivariate analysis at the time of salvage autotransplant were age, sex, immunologic subtype of myeloma, interval between two autografts, disease stage at salvage autograft, calcium, creatinine, beta-2-microglobulin (B2M), albumin, performance status and source of cells. The results of this analysis are shown in the Table.

<table>
<thead>
<tr>
<th>Event</th>
<th>Favorable co-variates</th>
<th>RR</th>
<th>P</th>
</tr>
</thead>
<tbody>
<tr>
<td>OS</td>
<td>Interval between two autografts &gt; 2 years</td>
<td>0.25</td>
<td>0.0004</td>
</tr>
<tr>
<td>EFS</td>
<td>Interval between two autografts &gt; 2 years B2M &lt;= 4 mg/L Creatinine &lt; 85 micromol/L</td>
<td>0.44</td>
<td>0.007</td>
</tr>
<tr>
<td></td>
<td></td>
<td>2.43</td>
<td>0.048</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.58</td>
<td>0.036</td>
</tr>
<tr>
<td>Relapse</td>
<td>B2M &lt;= 4 mg/L Creatinine &lt; 85 micromol/L</td>
<td>3.26</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.5</td>
<td>0.02</td>
</tr>
<tr>
<td>Probability of CR2</td>
<td>Being in CR1 after first autograft</td>
<td>5.1</td>
<td>0.0005</td>
</tr>
<tr>
<td></td>
<td>Being in CR/PR at salvage autograft B2M &lt;= 4 mg/L</td>
<td>2.2</td>
<td>0.02</td>
</tr>
<tr>
<td></td>
<td></td>
<td>0.38</td>
<td>0.048</td>
</tr>
</tbody>
</table>

These data suggest that all eligible patients should be candidates for a salvage autotransplant. For high-risk patients with a shorter interval between the two transplants, high B2M and creatinine are not in CR1 with the first autograft, salvage autografts should be offered with the addition of newer agents given as consolidation/maintenance therapy. From the time of first autograft, the actuarial survival of this group is 61% at 5 years and the median survival is 6.4 years which is equivalent to that of tandem autotransplantation. Autograft at relapse policy saves a high proportion of patients from receiving an unnecessary second autograft as some patients become long-term survivors and some patients may not benefit due to refractory disease. In a study of 220 patients, we have also shown that response to induction chemotherapy is not essential to obtain survival benefit from autotransplantation in myeloma (Singhal S et al, Bone Marrow Transplant, 2002;30:673-9).
cells could be harvested following chemotherapy and growth factor, or growth factor alone. Mobilization with filgrastim alone avoids the morbidity associated with chemotherapy-induced neutropenia, and is more conducive for planning ahead for dialysis.

<table>
<thead>
<tr>
<th>Study</th>
<th>Regimen</th>
<th>No. of Pts</th>
<th>No. on HD</th>
<th>ED</th>
<th>EFS</th>
<th>OS</th>
</tr>
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<tbody>
<tr>
<td>Badros</td>
<td>TX1: Mel 200</td>
<td>81</td>
<td>60</td>
<td>38</td>
<td>6%</td>
<td>48% at 3 yrs</td>
</tr>
<tr>
<td></td>
<td>Mel 140</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>TX2: Mel 200</td>
<td>31</td>
<td>24</td>
<td>13</td>
<td>13%</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mel 140</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ballester</td>
<td>Bu 16 Cy 120</td>
<td>6</td>
<td>3</td>
<td>1</td>
<td></td>
<td>5 alive in</td>
</tr>
<tr>
<td></td>
<td>Mel 80</td>
<td></td>
<td></td>
<td></td>
<td>remission</td>
<td>6+ 39+</td>
</tr>
<tr>
<td>Tosi</td>
<td>Bu 12 Mel 140</td>
<td>6</td>
<td>1</td>
<td>0</td>
<td>2+, 15+, 16+</td>
<td></td>
</tr>
<tr>
<td></td>
<td>Mel 80</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>5 alive 2+</td>
</tr>
<tr>
<td></td>
<td>Mel 80</td>
<td></td>
<td></td>
<td></td>
<td>26+</td>
<td></td>
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</tbody>
</table>

High-dose therapy: Melphalan is administered over 30 minutes; the total dose could be administered in one or two days. Thus dialysis can be avoided during the day of melphalan administration. The stem cell could be reinfused 24 hours after melphalan. The dialysis could be done prior to stem cell administration. And, for patients not dialysis, attention to the hematocrit of the stem cell products and administering stem cells in divided doses may avoid further worsening of renal function.

The hematopoietic recovery has been shown to be prompt, and comparable to patients without renal impairment.

The toxicity during the neutropenic phase is generally higher, and is dose dependent. Mucositis incidence and severity is higher among patients receiving melphalan at 200 mg/M² as compared to patients receiving a lower dose of melphalan. Pulmonary complications, often requiring ventilatory support, are more common with the higher dose of melphalan and in patients requiring hemodialysis. Transplant-related mortality is less when the dose of melphalan is lower and tandem transplant is avoided.

As in the case of standard therapy, once the patient achieves a remission, the outcome of high-dose therapy is comparable between patients with or without renal failure. Only a small proportion of patients on dialysis at the time transplantation become dialysis independent post transplant. With proper planning and dose modification, it should be possible to keep transplant-related mortality to fewer than 5%.

References:
We have transplanted 125 patients with AL. 118 had a monoclonal light chain in serum or urine. The remaining 7 all had clonal plasma cells in the bone marrow with an overall kappa to lambda ratio of 1:2.6. At the time of diagnosis signs of amyloid were seen in the kidney in 83 (66%), the heart in 60 (48%), peripheral nerves in 17 (14%) and the liver in 21 (17%). The characteristics of the patient are given in the table.

Characteristics of Patients with AL (n=125) before ASCT

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Number (%) or Median (Range)</th>
<th>Number (%)</th>
<th>Abnormal Value</th>
</tr>
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<tbody>
<tr>
<td>Male gender</td>
<td>68 (54)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Age</td>
<td>55 (31-71)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin</td>
<td>2.8 (1.0-4.4)</td>
<td>54 (43)</td>
<td>&lt;2.5 g/dl</td>
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<tr>
<td>Creatinine</td>
<td>1.1 (0.6-3.9)</td>
<td>13 (10)</td>
<td>≥2.0 mg/dl</td>
</tr>
<tr>
<td>Alkaline phosphatase</td>
<td>177 (67-1350)</td>
<td>19 (15)</td>
<td>&gt;375 (1.5 x normal)</td>
</tr>
<tr>
<td>Serum M protein (N=92)</td>
<td>0.15 (0.1-2.6)</td>
<td>24 (19)</td>
<td>≥1.0 g/dl</td>
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<tr>
<td>24-hour urine total protein</td>
<td>3.9 (0.02-2.85)</td>
<td>71 (57)</td>
<td>&gt;3 g/dl</td>
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<tr>
<td>Urine M protein (N=114)</td>
<td>0.19 (0.02-2.85)</td>
<td>48 (38)</td>
<td>&gt;0.25 g/d</td>
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<tr>
<td>% BM plasma cells</td>
<td>8 (0.4-49)</td>
<td>22 (18)</td>
<td></td>
</tr>
<tr>
<td>Echo IVS (mm)</td>
<td>12 (7-25)</td>
<td>29 (23)</td>
<td>≥15</td>
</tr>
<tr>
<td>EF%</td>
<td>65 (28-84)</td>
<td>25 (20)</td>
<td>&lt;60</td>
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<tr>
<td>β2M</td>
<td>2.21 (1-9.7)</td>
<td>31 (25)</td>
<td>≥3</td>
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<table>
<thead>
<tr>
<th>Number of organs involved</th>
<th>Number (%)</th>
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<td>1</td>
<td>64 (51)</td>
</tr>
<tr>
<td>2</td>
<td>42 (34)</td>
</tr>
<tr>
<td>&gt;2</td>
<td>19 (15)</td>
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</tbody>
</table>

Eight of the 125 patients died prior to day 30 and were not evaluable for neutrophil engraftment. Of the remaining 117 patients all achieved a neutrophil count of 500/μL ranging from 7 to 116 days (median 14) following transplantation. There were 15 patients who died without having achieved a platelet count of 20,000/μL. The time to 20,000 platelets for the remaining 110 patients ranged from 7 to 406 days (median 17). There was one mobilization-related death and one patient died on day 0 during stem cell infusion. Treatment related mortality for the entire group was 15% with death due to a wide variety of complications including intractable intestinal bleeding, acute treatment-related renal failure, cardiac arrhythmias, disseminated fungus infection, etc. There are two major predictors of survival which include the serum creatinine at the time of transplantation and the number of organs involved at the time of transplantation (p=0.001). Median survival has only been reached for those patients who had greater than two organs involved, 16.5 months. We have 4 patients with single organ involvement that now have been followed for greater than 5 years and 2 patients with two organ involvement that have been followed in excess of 5 years. The presence of long-term survival should not be equated with a proven advantage of stem cell transplant since these patients are highly selected. However, the overall response of 64% exceeds anything that we have previously achieved with conventional chemotherapy. This is a technique worth pursuing, although a phase III trial is necessary.

POEMS syndrome:

We have transplanted six patients with POEMS syndrome, all of whom had lambda light chains, three Gλ, two Aλ, one free λ. The age range of the six was from 20 to 62. Two of the patients were previously treated with radiation therapy and progressed. One patient died of graft failure and was invaluable for response. Of the remaining five patients four had complete eradication of the monoclonal protein and one had a greater than 50% reduction in the monoclonal protein. Four of the five had definite improvement in their neuropathy and neuropathic symptomatology, all of whom had both sensory and motor neuropathy. The use of high dose therapy for patients with POEMS syndrome can produce both hematologic and symptomatic responses. Five patients are alive with survivals ranging from 8 to 48 months.
11. What is the role of allogeneic transplantation in MM?

Roundtable 1: CONVENTIONAL CONDITIONING

P11.1.1 PROGRESS IN ALLOGENEIC TRANSPLANTATION WITH ABLATIVE CONDITIONING. USE OF PROGNOSTIC FACTORS.


Department of Medicine, Karolinska Institutet at Huddinge University Hospital, SE-141 81 Stockholm, Sweden

Allogeneic transplantation has been performed in patients with multiple myeloma since the early 1980s. During the first ten year period of transplantation the overall median survival was short and transplant related mortality high. However, relapse rate after complete hematologic remission was shown to be lower after allogeneic transplantation than after autologous transplantation, although autologous transplantation was associated with better overall survival due to lower transplant related mortality. Molecular studies of minimal residual disease have shown that about half of the 50 % of patients that enter a complete hematologic response after allogeneic transplantation have no sign of persistent disease, while only occasional patients are free of disease following autologous transplantation. Thus the prospects for cure appear better following allogeneic than autologous transplantation.

A recent comparison of allogeneic transplantation performed during the two time periods 1983 – 1993 and 1994 – 1998 showed a significant improvement in survival during the later time period. The improvement was due to decreased transplant related mortality. This in turn seemed to be due to many factors, i.e. earlier transplantation and more effective treatment of bacterial, fungal and viral infections. No change in relapse rate was seen. A change to an increasing number of peripheral blood stem cell (PBSC) transplants was not the reason for the improvement. On the contrary a later update indicates that there may be a small, but borderline significant disadvantage of using PBSC, which may be related to a higher risk of chronic GVHD. Despite these improvements in allogeneic transplantation the transplant related mortality remains high. Selection of patients based on prognostic parameters could be one way to decrease overall mortality. Good prognosis parameters for allogeneic transplantation are female gender, low age, low beta2-microglobulin, stage I at diagnosis, responsiveness to previous treatment and only one treatment regimen before transplantation. However most of these parameters also indicate good prognosis with other treatment modalities such as autologous transplantation.

Selection of the best donor is important. Matched sibling donor transplants are associated with better prognosis than unrelated matched transplants. Also a gender-matched sibling donor is better than a gender mismatched one. The worst combination appears to be a female to male transplant.

Procedural factors probably play a role, but documentation is poor. It has not been possible to show an advantage of any of the most commonly used high dose conditioning regimens. Until now the most frequently used regimen has been a combination of cyclophosphamide 60 mg/kg x 2 plus 10 Gy total body irradiation with lung shielding to 9 Gy. However, the dose and the way the total body irradiation is given varies, i.e. fractionated or non-fractionated etc. Other regimens include melphalan, usually 140 – 200 mg/m². These regimens are considered to be ablative for the bone marrow. Until now it has not been possible to specify an ablative regimen that is superior to the most commonly used ones.

The most common regimen for prophylaxis of GVHD, methotrexate plus cyclosporine, is standard and other regimens have not proven to be superior in terms of improving survival. However, attempts to manipulate the GVHD preventive regimen have been successful in diminishing GVHD by using various T-cell depletion regimens. One promising method has been to use T-cell depletion with later CD4 cell add back in an attempt to reduce GVHD and still obtain a graft versus myeloma effect. The use of low intensive (or non-myeloablative ) conditioning methods has increased dramatically. They are usually combined with later donor lymphocyte infusions. Preliminary results are encouraging. The rationale for this approach is that the transplant related mortality can be reduced by the less intensive pretransplant conditioning while preserving the graft versus myeloma effect, which may be more important than the conditioning regimen in preventing relapse. Later donor lymphocyte infusions directed against the myeloma may counteract a potentially increased risk of relapse particularly when T-cell depletion is used.

Many non-myeloablative conditioning regimens have been tried with initial success. Very promising results have been obtained with fludarabine 30 mg/m² days -4,-3,-2, followed by total body irradiation 200 cGy on day 0. The initial transplant related mortality is low and some patients may even be treated as out-patients. The EBMT is presently running a study comparing this regimen after a previous autologous transplantation compared to autologous transplantation alone based on the availability of an HLA matched sibling donor. Post-transplant DLI is included for all patients except those in complete remission with full donor chimerism.

In summary allogeneic ablative conditioning regimens may be an option for patients with stage IIIA disease, preferentially for younger women that were diagnosed when they had stage I disease and that have an HLA matched female sibling donor. Patients that have been responsive to previous conventional treatment and have received only one treatment regimen before the transplant are the best candidates. However some patients that have not responded and are poor candidates for conventional treatment or autologous transplantation may be offered an allogeneic transplant if their general condition is otherwise good. The best offer for a younger myeloma patient may be to enter a trial of non-myeloablative transplantation following an autologous transplant and be prepared to later receive donor lymphocyte transfusions.


transplantation was improved in more recent years, we investigate whether the outcome of allogeneic stem cell transplantation for MM patients have been for a long time the these observations, indications for the use of allogeneic stem cell change appreciably over the time period analyzed (6). Based on allografted group (41% vs 13% for autotransplants) and did not between 1983 and 1994, TRM was significantly higher in the Group for Blood and Marrow Transplantation (EBMT) (2, 3). Moreover, additional studies on single-centre experiences were also published by other groups (4, 5). From the analysis of all transplants performed in Europe was opened; since then, the use of allotransplants has grown over the years and more than The first patient who received an allotransplant at our centre for even in patients who failed on prior conventional chemotherapy. The first patient who received an allotransplant at our centre for relapsing MM is still in serological and molecular CR 19 years after transplant. Following initial encouraging reports, interest in the use of allotransplants has grown over the years and more than 2000 patients have been performed worldwide. In 1983, a registry of allotransplants performed in Europe was opened; since then, several updates of registry data were reported by the European Group for Blood and Marrow Transplantation (EBMT) (2, 3). Moreover, additional studies on single-centre experiences were also published by other groups (4, 5). From the analysis of all these studies it appears evident that the most crucial issue related to the use of allogeneic stem cell transplantation for MM was the high treatment related mortality (TRM) that exceeded 40% in most series (4). In a retrospective case-matched study performed by the EBMT group and aimed at comparing autologous and allogeneic bone marrow transplantation reported to the registry between 1983 and 1994, TRM was significantly higher in the allografted group (41% vs 13% for autotransplants) and did not change appreciably over the time period analyzed (6). Based on these observations, indications for the use of allogeneic stem cell transplantation for MM patients have been for a long time the matter of debate and controversies still exist. In an attempt to investigate whether the outcome of allogeneic stem cell transplantation was improved in more recent years, we retrospectively analyzed a series of 84 consecutive patients (median age, 43 years; stage III, 64%; refractory or progressive, 67%) who received allotransplants from HLA-identical sibling donors (n=79) or HLA-matched unrelated donors (n=5) at our centre between 1990 and 2002. Patients were divided into 3 groups according to the period in whom allotransplants were performed (group 1, from 1990 to 1993; group 2, from 1994 to 1995; group 3, from 1996 to 2002). Notably, conditioning regimens and treatments used to prevent graft-versus-host disease (GVHD) were different over the periods of the study. More specifically, patients in group 1 (n=20) received a combination of busulfan (total dose, 16 mg/kg) and cyclophosphamide (CTX) (total dose, 200 mg/kg), whereas patients in groups 2 (n=14) and 3 (n=30) were mostly treated with combined total body irradiation (TBI), CTX and melphalan (MEL) (total dose, 100-120 mg/m2). Cyclosporine + methotrexate (CsA + MTX) were used to prevent GVHD in patients included in groups 1 and 3, whereas patients in group 2 received a T-cell depleted marrow. Bone marrow was the only source of repopulating hematopoietic stem cells used from 1990 to 1995 (groups 1 and 2), while the bone marrow donors in groups performed after 1995 (group 3) were performed with peripheral blood (PB) stem cells. TRM rate at 1 year was significantly lower among patients included in group 3 (16%), as compared with patients in group 2 (43%) and in group 1 (30%) (P=0.0002). Notably, the decreased TRM observed in recent years was not related to a more favorable selection of the patients (stage III, 70%; refractory/progressive, 66%). At the opposite, comparison between the 3 groups showed that patients in group 3 were transplanted earlier than the others (median time interval from diagnosis to transplant: 13 months vs. 18 and 20 months for patients in groups 2 and 1, respectively). Posttransplant mortality due to infections was 8% among patients in group 3, as opposed to 36% for patients who received TBI-including regimens and a T-cell depleted marrow (group 2). As a result of the lower TRM rate, overall survival for patients in group 3 was significantly longer than that for the other groups (66% projected at 4 years, as opposed to 35% for group 2 and 20% for group 1) (P=0.001). Similarly, the progression-free survival for patients in group 3 was significantly longer than that for the other groups (41% projected at 4 years, as opposed to 21% and 15% for groups 2 and 1, respectively) (P=0.008). These results compare favorably with those of a recent study carried out by the EBMT registry demonstrating that TRM during the period 1994-1998 was significantly reduced in comparison with earlier time periods (21% as opposed to 38% observed before 1994), mainly as a result of a significant decrease in the frequency of deaths due to interstitial pneumonia or bacterial and fungal infections (7). As in the EBMT study, also in the present analysis we were unable to demonstrate any improvement over time in the response to transplant. On an intent-to-treat basis, the CR rate was 38% in group 1, 43% in group 2 and 44% in group 3. Overall, the 4-year and 7-year projected probabilities of relapse were 33% and 46%, respectively. Due to several relapses occurring as late as 9 and 10 years following transplantation, there was no apparent plateau in the relapse-free survival curve. This finding raises the issue of whether allotransplant has the potential ability to cure MM. To address this poorly investigated issue, we recently used a polymerase chain reaction-based (PCR) strategy to retrospectively analyze the presence of residual myeloma cells in serial posttransplant bone marrow samples obtained from a series of patients in sustained CR following allogeneic stem cell transplantation (8). For this purpose, patient-specific primers were generated from complementarity determining regions 2 and 3 of the rearranged IgH gene. It was

**P11.1.2 WHAT IS THE ROLE OF ALLOGENEIC TRANSPLANTATION IN MM? BOLOGNA EXPERIENCE**

*Michele Cavo, Claudia Cellini, Francesca Bonifazi, Paola Tacchetti, Elena Zamagni, Delia Cangini, Patrizia Tosi, Giuseppe Bandini, Sante Tura, Michele Baccarani*

*Institute of Hematology and Medical Oncology "Seràgnoli; University of Bologna, Italy*

Clinical studies aimed at investigating the feasibility and toxicity of allogeneic stem cell transplantation for the treatment of MM were pioneered by our group (1) and other groups in the early 1980s. Initial studies mostly included heavily pretreated patients with refractory disease and provided demonstration that chemo(radio)therapy administered at myeloablative doses overcame chemoresistance and induced complete remission (CR), even in patients who failed on prior conventional chemotherapy. The first patient who received an allotransplant at our centre for relapsing MM is still in serological and molecular CR 19 years after transplant. Following initial encouraging reports, interest in the use of allotransplants has grown over the years and more than 2000 patients have been performed worldwide. In 1983, a registry of allotransplants performed in Europe was opened; since then, several updates of registry data were reported by the European Group for Blood and Marrow Transplantation (EBMT) (2, 3). Moreover, additional studies on single-centre experiences were also published by other groups (4, 5). From the analysis of all these studies it appears evident that the most crucial issue related to the use of allogeneic stem cell transplantation for MM was the high treatment related mortality (TRM) that exceeded 40% in most series (4). In a retrospective case-matched study performed by the EBMT group and aimed at comparing autologous and allogeneic bone marrow transplantation reported to the registry between 1983 and 1994, TRM was significantly higher in the allografted group (41% vs 13% for autotransplants) and did not change appreciably over the time period analyzed (6). Based on these observations, indications for the use of allogeneic stem cell transplantation for MM patients have been for a long time the matter of debate and controversies still exist. In an attempt to investigate whether the outcome of allogeneic stem cell transplantation was improved in more recent years, we
found that 75% of patients who were analyzed remained persistently PCR negative for a median of 3 years. In some of these patients PCR negative results were documented up to 4-10 years after allotransplants. It is concluded that allogeneic stem cell transplantation is associated with a graft-versus-myeloma (GVM) effect which results in more frequent molecular CR and decreased probability of relapse as compared with autotransplant(s). Mortality due to treatment-related complications, mainly infective, has been significantly reduced over the last years, as a result of earlier timing of the procedure and better supportive care. The challenge for clinical investigators will be to furtherly reduce the mortality associated with allotransplants and to increase the rate of sustained CR. Transplants with low-intensity, nonablative regimens aimed at decreasing early toxic complications, even in heavily pretreated patients, while retaining a GVM effect to induce CR are currently under investigation. Moreover, molecular profiling may help identify high-risk patients who do not benefit from autotransplant(s) and for whom allotransplants deserve further investigation.

REFERENCES


P11.1.3

THE ROLE OF DONOR T CELLS IN THE TREATMENT OF MYELOMA


Upfront Myeloablative Allo-SCT.

The necessity of performing Allo-SCT in MM is disputed. Median OS in different reports varies from 18 to 28 months from transplantation. A survival advantage for patients receiving an Allo-SCT compared to patients with matched characteristics who were treated with Auto-SCT and no SCT at all has not been shown.1

We have determined in a prospective study the efficacy, toxicity and long term outcome of upfront myeloablative Allo-SCT.2 In the prospective phase III study HOVON 24 MM , 53 patients with an HLA-identical sibling (median age at Tx 48 yrs, range 31-56) were allocated to a partial T-cell depleted Allo-SCT after induction therapy. The overall response after Allo-SCT was 89% (47/53) including 19% (10/53) of patients with a complete remission (CR). Five patients achieved a CR only after Allo-SCT.

Five of 7 (71%) primary refractory patients obtained a response to Allo-SCT, all of which had a PR. With a median follow-up of 38 months (range 25-61), 20 patients are alive since Allo-SCT, 33 patients have died, of whom 14 from progressive disease, 18 (34%) from Treatment Related Mortality (TRM) and one from another cause. Occurrence of acute Graft versus Host Disease grades II-IV predicted for higher TRM in a time-dependent analysis. Median progression-free survival after Allo-SCT was 18 months. Median overall survival after Allo-SCT was 25 months or 29 months since start of therapy (figure 1). The outcome of Allo-SCT patients was inferior as compared to a comparable group (age) of patients who received autologous stem cell transplantation or α-Interferon maintenance as part of the HOVON 24 trial. These patients had a comparable PFS but their overall survival was significantly longer (47 months vs 25 months) due to a much lower TRM (5%).
patients with sensitive disease to reinduction have a high probability of response to DLI and prolonged remission.

Graft versus Myeloma and deletion of chromosome 13: We retrospectively evaluated the impact of deletion 13, as determined by double colour interphase FISH, on the outcome of myeloablative Allogeneic Stem Cell Transplantation (Allo-SCT) in 51 patients treated at the department of Haematology, University Medical Center Utrecht. A del(13) was found in 16 patients (31%). No significant influence of del(13) was found on post transplant progression free survival (median 32 months versus 39 months, p=0.36). Overall survival tended to be shorter in the patients with del(13) (median 16 versus 59 months, p=0.12), but this difference was partly due to a higher TRM. The 2 longest surviving patients (>10 years), who had a del(13) at diagnosis, enjoy an extended molecular remission and are probably cured.4

Conclusion:
The results of this first prospective evaluation of Upfront Allo-SCT in MM in comparison with intensive treatment show that there seems to be no indication for standard Allo-SCT as part of first line therapy due to a high TRM which is not compensated for by a GVM effect. In order to make a better use of the GVM effect of DLI, especially in high risk myeloma like patients with a deletion 13, this procedure should probably best be applied as pre-emptive therapy after less toxic transplantation strategies.

Literature:

Roundtable 2: DOSE REDUCED INTENSITY REGIMENS

P11.2.1 Non-Myeloablative Allogeneic Transplant Strategies for the Treatment of Multiple Myeloma

WI Bensinger, DM Maloney, B Sandmaier, and R Storb
Fred Hutchinson Cancer Research Center (FHRCRC), University of Washington, Seattle, WA, USA

Although high-dose chemoradiation therapy followed by allogeneic stem cell transplantation (SCT) is capable of producing remissions and long-term survival for patients with multiple myeloma, the transplant-related mortality (TRM) of 25-50%, even in “good-risk” patients, limits the application of this approach. Furthermore, since the majority of patients who develop multiple myeloma a
patients with responsive disease 3 died of transplant complications, 2 have relapsed, 16 remain in CR, 5 in PR and 2 with stable disease. Among 26 patients with relapsed or refractory disease, 5 died of transplant complications, 11 remain in CR, and 4 in PR. Patients with relapsed or refractory disease had a poorer survival than patients responding to initial chemotherapy prior to transplant.

Non-ablative or reduced intensity regimens prior to allogeneic SCT for MM have been reported from other centers. One promising report utilized melphalan 100 mg/m² to prepare 31 SCT for MM have been reported from other centers. One Non-ablative or reduced intensity regimens prior to allogeneic chemotherapy prior to transplant.

had a poorer survival than patients responding to initial refractory disease, 5 died of transplant complications, 11 remain with responsive disease 3 died of transplant complications, 2 have relapsed, 16 remain in CR, 5 in PR and 2 with stable disease. Among 26 patients with relapsed or refractory disease, 5 died of transplant complications, 11 remain in CR, and 4 in PR. Patients with relapsed or refractory disease had a poorer survival than patients responding to initial chemotherapy prior to transplant.

Thus, non-ablative allogeneic transplant regimens can result in reliable donor engraftment with relatively low transplant related mortality compared to high dose regimens. It appears, however, that substantial cytoreduction pre-allografting is necessary due to a limited GVM effect. Preliminary results suggest the tandem auto/min-allogeneic strategy can result in CR’s in at least 50% of patients with multiple myeloma; similar to what can be achieved with a single high dose conditioning regimen, yet avoiding the high early mortality. It will be important, however, to have longer follow-up of patients transplanted used non-ablative regimens in order to document the durability of these remissions and to document the rates and severity of chronic GVHD.

The studies using low intensity, non-ablative regimens appear to effectively reduce the early complications and mortality of allogeneic transplants, while retaining GVM effects sufficient to induce remissions. Such treatments could be combined with infusions of allogeneic donor lymphocytes or subsets of lymphocytes in the form of “engineered grafts”; for example CD4 lymphocytes, which may have a GVM effect without increasing myeloma effect has been well documented, harnessing this effect versus tumor effect mediated by donor lymphocytes independent of chemotherapy has led to the exploration of less intense strategies aimed at reducing the toxicity of allografting in myeloma patients are not well understood. Older age is likely to contribute in part to the higher mortality rates, since age is a major risk factor for both regimen related toxicity and graft versus host disease (GVHD). (6-8) The existence of a graft-versus-tumor effect mediated by donor lymphocytes independent of chemotherapy has led to the exploration of less intense preparative regimens for multiple myeloma. (9-12)

Experience with non-ablative preparative regimens in multiple myeloma: The first report from MDACC was presented at ASCO in 1998.(13) In this report, 13 patients with a median age of 50 years (range, 46-55), 10 of which had refractory disease and 5 had failed prior autologous transplant. With a combination of fludarabine/melphalan 12
patients had 100% engraftment of donor cells (including 4 recipients of unrelated donor grafts). One patient died before day 30 from tumor lysis and toxicity. Seven patients achieved complete remission, and 5 failed to respond. At the time of the report 6 patients were alive between 3 and 24 months after transplant (median, 18 months), two in continued complete remission. The major causes of treatment failure in this group of refractory and heavily pretreated patients was GVHD (n=4) and disease (n=2). Thus engraftment of allogeneic progenitor cells after non myeloablative therapies is feasible in patients with myeloma, and further exploration of this strategy in patients with less advanced disease is warranted.

The Seattle group has pioneered an innovative approach to non-ablative transplantation using low dose TBI at a dose of 200 cGy in a single fraction in conjunction with post transplant immune suppression with cyclosporine and mycophenolate mofetil. (14) In the initial experience patients with myeloma and CML who had not received prior intensive therapy had a high risk of graft rejection and autologous reconstitution of over 20%. (15) To improve upon these outcomes, the Seattle group explored the safety and efficacy of high dose melphalan with autologous stem cell support followed 90-120 days by a non ablative preparative regimen of 200 cGy of TBI followed by an allogeneic peripheral blood stem cell infusion from an HLA identical sibling for myeloma patients who had never received a prior autograft. Twelve patients were treated with a median age 49 years (42-63) with a median time to transplant of 12 months (range, 4-57 months). Toxicity from the allografting was minimal with a median of 0 days of neutropenia (range, 0-10) and a median nadir neutrophil count of 760 neutrophils/ l (range, 150-1270 neutrophils/l). All patients had donor cell engraftment with a median percentage of donor cells of 82%. Six patients developed grade III acute GVHD and 2 developed grade IV GVHD. Seven patients achieved a CR and 3 patients have died, 9 patients were alive with a median follow up of 7 months at the time of the report. (16)

Further evidence for the role of non-ablative preparative regimens for multiple myeloma is provided by the number of papers presented in the 42nd Annual Meeting of the American Society of Hematology (Table 1).

Table 1: Other Non Ablative Stem Cell Transplant Trials with >10 Myeloma Patients Reported at the 42nd Annual Meeting of the American Society of Hematology.

<table>
<thead>
<tr>
<th>Ref</th>
<th>N</th>
<th>Age</th>
<th>Regimen</th>
<th>Graft Failure</th>
<th>NRM</th>
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<tr>
<td>16</td>
<td>50</td>
<td>48</td>
<td>FM:25</td>
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<td>27%</td>
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<tr>
<td>17</td>
<td>23</td>
<td>47</td>
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<td>22</td>
<td>54</td>
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<tr>
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<td>16</td>
<td>57</td>
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<td>1</td>
<td>3/16</td>
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Of interest among the papers presented are the results reported to the EBMT in which patients with good risk disease (chemosensitive relapse or remission consolidation) had a 10% non relapse mortality and a progression free survival of 80%. (17) In all reported series to date GVHD is an important cause of treatment failure. The use of pretransplant CAMPATH 1H has been reported to decrease the incidence of this complication, according to the experience reported by Peggs et al. (18) Melphalan at a lower dose of 100 mg/m^2 has also been used as a preparative regimen for non-ablative or reduced intensity conditioning. This dose as reported by the University of Arkansas group results in high rates of engraftment and low incidence of toxicity in patients who relapsed after either one or two autologous transplants. (19,20) All studies to date have demonstrated that chemosensitivity as well as extent of prior therapy are important prognostic factors for outcomes with few patients with refractory disease obtaining long term disease control.

SUMMARY: The goals of therapy for myeloma have changed substantially from the initial days of melphalan and prednisone. (21) The advent of autologous transplant, and the recognition of the graft versus myeloma effect make it possible to search for complete remission and long term disease control in a substantial proportion of myeloma patients. However, the ability to exploit the graft versus myeloma effect has been hampered by the high incidence of transplant related mortality seen in the myeloma population. Reducing the intensity of the preparative regimen has been explored as a strategy to improve the outcomes of allogeneic transplant in myeloma. The results of the limited experience to date demonstrate that non-ablative and reduced intensity conditioning regimens are feasible in myeloma. This procedure results in donor cell engraftment, acceptable toxicity, and long term disease control in a fraction of patients. However, graft versus host disease and disease recurrence particularly in patients with relapsed or refractory disease continue to be the major obstacles to overcome. Further studies will be needed to define the role of reduced intensity and non-ablative conditioning in the treatment of multiple myeloma.

REFERENCES


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**P11.2.3 HIGH DOSE THERAPY VERSUS CONVENTIONAL CHEMOTHERAPY FOR NEWLY DIAGNOSED MULTIPLE MYELOMA: HISTORICAL COMPARISON OF TOTAL THERAPY I VERSUS STANDARD SWOG TRIALS AND US INTERGROUP TRIAL SWOG 9321.**

Bart Barlogie, Joth Jacobson, Kenneth Anderson, Philip Greipp, Robert Kyle and John Crowley. The Myeloma Institute for Research and Therapy (MIRT), University of Arkansas for Medical Sciences, Little Rock, AR; Dana Farber Cancer Institute, Harvard Medical Center, Boston, MA.

Improved long-term survival of patients with myeloma is directly linked to the widespread use of high-dose melphalan usually at 200mg/m² (MEI) 200) with peripheral blood stem cell support (PBSC). Since mucositis is dose-limiting, tandem transplants were evaluated in TT I to further augment cell kill toward increasing CR and extending event-free and overall survival. Of 231 patients enrolled between 1989 and 1994, 152 were previously entirely untreated and could be closely matched with 152 contemporaneous patients receiving standard dose therapies (SDT) as part of SWOG trials (matching criteria included age, B2M, albumin). No significant differences existed in the proportions of patients with IgA isotype and creatinine ≥2mg/dl (Table 1). With a median follow-up of 10 years for all patients, 10-year survival and event-free survival rates were markedly higher with TT I than with SWOG SDT (33 vs 15%, p<.0001; 16 vs 5%, p<.0001) (Figure 1).

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**Figure 1: Total Therapy I vs Standard SWOG Therapy (pairwise analysis according to age, B2M and albumin)**

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Indeed, according to multivariate analysis of baseline prognostic variables together with treatment modality, TT I emerged as the single most important variable associated both with superior overall and event-free survival (Table 2). Thus, regardless of standard risk factors (cytogenetics were unavailable in SWOG trials), TT I extended survival significantly. Total Therapy II builds on the success of TT I and employs more intensive remission induction, consolidation chemotherapy after tandem transplants with MEL 200mg/m², and up-front randomization to +/- thalidomide. Unfortunately, follow-up was deemed still too short to present the results of US Intergroup trial S9321 that randomized patients, after VAD induction, to MEL 140 + TBI 12 Gy vs VBMCP SDT. According to a recursive partitioning model, platelet count and LDH distinguished 3 risk groups with superior median survival of 68 months among 437 patients with good risk, 44 months among...
211 patients with intermediate risk and 27 months among 170 patients with poor risk (p<0.0001) (Figure 2).

Table 1. Patient Characteristics

<table>
<thead>
<tr>
<th>TTI (N=152)</th>
<th>SWOG (N=152)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≤60 years</td>
<td>26%</td>
<td>26%</td>
</tr>
<tr>
<td>Albumin &lt;3.5</td>
<td>24%</td>
<td>22%</td>
</tr>
<tr>
<td>B2M ≥3</td>
<td>52%</td>
<td>48%</td>
</tr>
<tr>
<td>Creatinine ≥2</td>
<td>9%</td>
<td>11%</td>
</tr>
<tr>
<td>HGB &lt;10</td>
<td>32%</td>
<td>28%</td>
</tr>
<tr>
<td>Platelets &lt;150</td>
<td>10%</td>
<td>12%</td>
</tr>
<tr>
<td>IgA Isotype</td>
<td>17%</td>
<td>18%</td>
</tr>
</tbody>
</table>

Table 2. Multivariate Analysis

<table>
<thead>
<tr>
<th>Overall Survival</th>
<th>Event-Free Survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>TTI</td>
<td>HR P</td>
</tr>
<tr>
<td>Age ≤60 years</td>
<td>1.9 &lt;0.001 2.0 &lt;0.001</td>
</tr>
<tr>
<td>Albumin &lt;3.5</td>
<td>1.3 0.125 1.2 0.178</td>
</tr>
<tr>
<td>B2M ≥3</td>
<td>1.4 0.228 1.0 0.752</td>
</tr>
<tr>
<td>HGB &lt;10</td>
<td>1.3 0.094 1.4 0.051</td>
</tr>
<tr>
<td>Platelets &lt;150</td>
<td>1.4 0.111 1.5 0.066</td>
</tr>
<tr>
<td>IgA Isotype</td>
<td>1.1 0.760 1.1 0.612</td>
</tr>
</tbody>
</table>

Figure 2. Survival on US Intergroup S9321

P11.2.4

STEM CELL TRANSPLANTATION FROM RELATED AND UNRELATED DONORS IN MULTIPLE MYELOMA AFTER A REDUCED INTENSITY FLUDARABINE/MELPHALAN CONDITIONING

Nicolaus Kröger1, Avichai Shimoni2, Arnon Nagler2, Herbert Gottfried Sayer2, Rainer Schwerdtfeger2, Michael Kiehl3, Helmut Renges1, Tatjana Zabelina1, Boris Fehse1, Francis Ayuk1, Axel Rolf Zander1.

1Bone Marrow Transplantation, University Hospital Hamburg, 2Dept of Bone Marrow Transplantation Chaim Sheba Medical Center, Tel Hashomer, Israel, 3Dept of Oncology and Hematology University Jena, 4Dept of Bone Marrow Transplantation Wiesbaden and 4Sider-Oberten, and 5Dept of Hematology A.K. St. Georg, Hamburg, Germany.

This work was supported by a grant of the Roggenbuck- Stiftung. Dose-reduced regimens based on fludarabine and melphalan demonstrated stable engraftment of allogeneic stem cells from related and unrelated donors in patients with hematological diseases including multiple myeloma (1,2). We report the results of two multicenter phase I/II study investigating the feasibility of a fludarabine-melphalan dose-reduced intensity regimen followed by stem cell transplantation in patients with advanced multiple myeloma. Our program is focussing on four issues: Auto-allo Tandem approach in newly diagnosed or less advanced patients (melphalan 200mg/m² plus auto PBSC followed after 3 months by melphalan 100 mg/m² and allo PBSC) (3)

Allo-Transplant in patients who had relapsed to a prior autograft (Melphalan 140 mg/m²)

Effect of donor lymphocyte infusion for persistent or relapsed disease

The reduced intensity conditioning regimen consisting of fludarabine (150 mg/m²), melphalan (100-140 mg/m²) and anti-thymocyte globulin (ATG: 3 x 10-20mg/kg).

So far 64 patients with a median age of 52 years (range 31-64) are included in both protocols.

The median number of prior chemotherapies was 5 (r:2-26). A prior autograft was performed in 63 patients: 34 of 63 had experienced relapse to an autograft while in 29 patients autograft was part of the auto-allo tandem approach. No graft failure was observed and the median time to ANC 1 x 10⁹/L and platelet 50 x 10⁹/L was 16 days (r: 11-23) and 43 days (r: 12-22), respectively. Acute GvHD II-IV was noted in 38% (MUD 55% vs Related 31%; p=0.05). Severe grade II/IV aGvHD was seen in 15%. Chronic GvHD was observed in 37%, while only 10% experienced extended cGvHD.

The 1 year TRM was 22% (MUD: 25% vs Related: 17%, n.s.). After allografting 45% of the patients achieved a complete remission with negative immunofixation. The estimated overall and progression free survival at two years was better in patients treated with the auto-allo approach than in patients who had already relapsed to an prior autograft: 65% vs 38% (p=0.03) and 48% vs 25% (p=0.06), respectively. In a multivariate analysis bone marrow as stem cell source (HR 1.98: 95% CI: 1.08 – 3.64; p=0.03) is the most important factor for TRM, while female sex of the donor and relapse to autograft are significant for OS (HR 2.17; 95%CI: 1.36-3.45; p=0.001) and HR 2.38: 95% CI: 1.43-3.96; p=0.008) and for PFS (HR 1.69: 95%CI:1.18-2.44; p=0.005 and HR 2.02; 95% CI: 1.35-3.03; p=0.0007), respectively.Twenty-one patients received donor lymphocyte infusion because of persistent disease (n=6) or progressive disease (n=15). The median CD3+ cell dose for MUD (n=10) was 1 x 10⁶/kg and for related donors (n=11) 5 x 10⁶/kg. Acute GvHD II-IV was seen in 25%, but one patients experienced a fatal grade IV GvHD. Despite the lower cell dose the probability of developing aGvHD was higher after MUD-DLI than after related DLI (p=0.01). The response rate was 42% (3 CR and 4 PR).

Melphalan/fludarabine reduced conditioning with pretransplant ATG followed by related or unrelated stem cell transplantation provides rapid and sustained engraftment with durable complete donor chimermism, and low one year reatment related mortality. Because of the better outcome in patients without prior failure to autograft, allogeneic stem cell transplantation should be performed at an earlier phase of the disease. Randomized studies comparing dose-reduced allograft after a autograft with a second autograft in high risk patients are ongoing in Germany and within the EBMT.


Concluding remarks on high dose therapy/stem cell transplantation

P11.3.1 FREQUENCY AND IMPACT OF COMPLETE REMISSION WITH INTENSIVE THERAPY FOR MULTIPLE MYELOMA.

University of Texas M. D. Anderson Cancer Center, Houston, Texas, U.S.A.

Approximately 10% of patients with multiple myeloma who received primary therapy that included high-dose dexamethasone achieved complete remission (CR) based on negative immunofixation; an additional 30% of remaining patients achieved CR with intensive therapy supported by autologous stem cells. Most centers have reported similar findings, with variations attributed to failure to require negative immunofixation in defining CR, inclusion of patients with only Bence Jones protein or “nonsecretory” disease where CR is difficult to define, and inclusion of patients intensified after one year when there is declining sensitivity of myeloma. Consequently, our analyses focused on symptomatic patients ≤60 years old, with serum myeloma protein, treated initially with a high-dose dexamethasone-based regimen, intensified within the first year, with results compared with matched controls denied intensive therapy for socioeconomic reasons. Among 234 patients with these features treated between 1986-2001, treatment-related mortality was 5%.

Among 153 patients responsive to dexamethasone-based therapies (>75% reduction of serum myeloma protein production and >95% reduction of Bence Jones protein), conversion of partial to complete remission occurred in 36%. For patients with rapid reduction of serum myeloma protein (<0.5 month) and to ≤1.0 gm/dl after dexamethasone, complete remission occurred in 74% in contrast to 11% of patients without either feature (p<.01). Markedly improved survival was observed only among those in CR (median 9.9 years), survival of the remaining patients with persistent PR being similar to that of control patients.

Outcomes following intensive therapy for responsive or unresponsive patients

<table>
<thead>
<tr>
<th>Control*</th>
<th>Intensive Treatment</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>PR</td>
</tr>
<tr>
<td>Prior Partial Remission</td>
<td></td>
</tr>
<tr>
<td>Percent of total</td>
<td>—</td>
</tr>
<tr>
<td>Median survival (yrs)</td>
<td>5.9</td>
</tr>
<tr>
<td>Prior Resistance</td>
<td></td>
</tr>
<tr>
<td>Percent of total</td>
<td>NR</td>
</tr>
<tr>
<td>Median survival (yrs)</td>
<td>1.8</td>
</tr>
</tbody>
</table>

151 control patients had guaranteed survival of 3 months, the minimum survival for transplanted patients. NR: No response

PR: Partial remission
CR: Complete remission

Among 81 patients with disease unresponsive to primary therapy, 68% achieved remission including 19% with complete remission. The higher frequency of benefit for patients with resistant disease explains the longer lifespan with intensification vs standard therapy among all patients evaluated, in comparison with analyses limited to responsive patients. Because occurrence of PR was not predictable from any prognostic factor, all patients with primary resistant disease are candidates for intensive therapy. Resistant disease persisted in one-third of patients who remain at high risk for complications and with limited survival for whom effective new agents are indicated. Outcomes of 27 separate patients with primary resistant disease and only Bence Jones protein were similar in terms of longer survival for responding patients. Thus, approximately one-half of all patients intensified showed meaningful reduction of myeloma that translated into significantly longer survival.

P11.3.2 POST-TRANSPLANT CONSOLIDATION THERAPY TOWARD IMPROVING LONG-TERM DISEASE-FREE SURVIVAL: PRELIMINARY RESULTS OF TOTAL THERAPY II (TTII)

Bart Barlogie, Joth Jacobson, Elias Anaissie, Athanasios Fassas, Choon-Kee Lee, Raymond Thertulien, Guido Tricot, Fritz van Rhee, Maurizio Zangari, Giampaolo Talamo, Jason McCoy, John Crowley
The Myeloma Institute for Research and Therapy (MIRT), University of Arkansas for Medical Sciences, Little Rock, AR, USA; Cancer Research And Biostatistics (CRAB), Fred Hutchinson Cancer Research Center, Seattle, WA, USA.

Total Therapy II (TT II) was designed to improve upon results of Total Therapy I (TT I). Upon up-front randomization to +/- thalidomide (THAL), patients proceeded through 4 phases of treatment: 1) induction with VAD, DCEP, CAD (→ PBSC), DCEP; 2) tandem autotransplants with MEL 200 mg/m² 3 mos apart; 3) consolidation chemotherapy with DCEP q3mos x 4 vs DCEP alternating with CAD q6 wks x 8 or DEX in case of persistent thrombocytopenia; 4) interferon maintenance (with DEX pulsing during the first year). As of February 24, 2003, 541 patients have been enrolled with an accrual goal of 660 patients. Data are blinded as to THAL randomization. Myeloma protein reduction (MPR) by at least 50% was observed in 90% of patients, >75% MPR in 83% and CR/nCR (only IFE +) in 71%. The 4-yr estimate of overall survival (OS) is 69% (CI:61%,76%) and of event-free survival (EFS) 62% (CI:55%,69%) (Figure1).
Cytogenetic abnormalities (CA), present in 35%, imparted poor prognosis. Thus, OS and EFS were superior in the absence of CA (4yr OS 83% and 4yr EFS 82%); however, in the presence of CA13 or hypodiploidy, 4yr OS was only 41% and 4yr EFS 37%; an intermediate survival was observed in patients with “other CA” (4yr OS 59%, 4yr EFS 57%) (all p<.0001) (Figure 2).

When examined from the onset of consolidation chemotherapy, 3yr estimates of OS/EFS were 96% /84% in the absence of CA; 47%/52% with CA13 & hypodiploidy and 76%/59% with “other CA” (p<.0001). In the “no CA” group, no difference is yet apparent whether patients received chemotherapy or DEX pulsing for consolidation; however, among those with CA, chemotherapy was superior to DEX (3yr OS 69% vs 40% and 3yr EFS 64% vs 47%).

Comparing the first 231 TT II patients and all 231 TT I patients (exhibiting similar baseline features except older age in TT II, p=<.05) (Table 1), TT II patients fared significantly better in terms of CR/nCR (80% vs 46%, p=<.0001) (Table 2), 4-yr OS (70% vs 62%, p=0.19) and 4-yr EFS (63% vs 34%, p=<.0001) (Figure 3). According to multivariate analysis, TT II was an independent favorable feature (p=0.05) for both OS and EFS in addition to no CA, low LDH, low CRP and age <65 years. In summary, TT II appears feasible, safe and effective. Information on the contribution of THAL to clinical outcome is anxiously being awaited.

**Table 1. Characteristics**

<table>
<thead>
<tr>
<th></th>
<th>TTI</th>
<th>TTII</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age ≥65 years</td>
<td>24%</td>
<td>39%</td>
</tr>
<tr>
<td>B2M ≥4 mg/L</td>
<td>30%</td>
<td>28%</td>
</tr>
<tr>
<td>CRP ≥4 mg/L</td>
<td>47%</td>
<td>55%</td>
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<td>Creatinine ≥2 mg/dL</td>
<td>10%</td>
<td>9%</td>
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<td>LDH ≥190 U/l</td>
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<td>22%</td>
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<tr>
<td>Any CA</td>
<td>27%</td>
<td>28%</td>
</tr>
<tr>
<td>CA 13</td>
<td>12%</td>
<td>14%</td>
</tr>
<tr>
<td>* P&lt;.05</td>
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**Table 2. Protocol Completion, TRM & CR**

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<td>Completed</td>
<td>TTI</td>
<td>TTII</td>
<td>TTI</td>
</tr>
<tr>
<td>Protocol</td>
<td>TTII</td>
<td>TTII</td>
<td>TTII</td>
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<tr>
<td>&lt;65 years</td>
<td>92%</td>
<td>92%</td>
<td>87%</td>
</tr>
<tr>
<td>≥65 years</td>
<td>67%</td>
<td>89%</td>
<td>62%</td>
</tr>
<tr>
<td>TRM (cumulative)</td>
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</tr>
<tr>
<td>&lt;65 years</td>
<td>2%</td>
<td>2%</td>
<td>3%</td>
</tr>
<tr>
<td>≥65 years</td>
<td>9%</td>
<td>6%</td>
<td>11%</td>
</tr>
<tr>
<td>CR and</td>
<td></td>
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</tr>
<tr>
<td>Near-CR</td>
<td></td>
<td></td>
<td></td>
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<tr>
<td>&lt;65 years</td>
<td>21%</td>
<td>44%</td>
<td>36%</td>
</tr>
<tr>
<td>≥65 years</td>
<td>15%</td>
<td>29%</td>
<td>34%</td>
</tr>
</tbody>
</table>

Shaded = P<.05
12. Role of novel therapies targeting the myeloma cell and its marrow microenvironment

Roundtable I

P12.1.1 ROLE OF NOVEL THERAPIES TARGETING THE MYELOMA CELL AND ITS MICROENVIRONMENT

Anderson KC

Novel agents targeting MM cells in the BM, including Thalidomide (Thal) and Immunomodulatory Analogs Thal/IMiDs: induce G1 growth arrest/apoptosis of drug resistant MM cells via inhibiting NF-κB and activating caspase 8; inhibit adhesion of MM cells to BM stromal cells (SCs); inhibit bioactivity and/or secretion in MM cells and/or BMSCs of cytokines; inhibit angiogenesis; induce T cell and NK cell anti-MM immunity; and decrease human MM cell growth in a SCID mouse model. IMiD (Revamid) is more potent in preclinical studies and achieved stable disease or response in 79% patients in phase I study of relapsed refractory MM without somnolence, constipation, or neuropathy; it achieved responses, including CRs, in phase II trials. Phase III trial is comparing Dex/placebo versus Revimid/placebo in relapsed MM. The proteasome inhibitor PS-341 (Velcade): inhibits 26S proteasome activity; induces apoptosis via caspase-8, 9, and 3 in drug resistant MM cells; downregulates adhesion molecules and binding of MM cells and BMSCs; blocks constitutive and adhesion-induced NF-κB dependent cytokine secretion in BMSCs; and inhibits angiogenesis. PS-341 downregulates growth and survival gene transcription; and induces apoptotic, ubiquitin/proteasome, and stress response gene transcripts. Proteomics shows that PS-341 inhibits DNA repair kinases, and it can overcome resistance to DNA damaging agents. PS-341 induced cleavage of p130 may account for MM sensitivity. PS-341 inhibits human MM cell growth, decreases angiogenesis, and prolongs survival in SCID mice. Anti-MM activity was observed in phase I trials, and a phase II trial of PS-341 in refractory relapsed MM achieved 35% responses (10% CR, near CR). Responses were durable (12 months) and associated with clinical benefit: improved quality of life; increased hemoglobin and decreased transfusions; stable renal function; and increase in normal immunoglobulin levels. PS-341 is being compared with Dex in phase III trial for relapsed MM. Other agents include Arsenic Trioxide, 2-Methoxyestradiol, and Lysophosphatidic Acid Acyltransferase β-Inhibitors. Novel agents targeting MM cell signaling cascades include VEGFR tyrosine kinase inhibitor PTK787/ZK222584; Farnesyltransferase Inhibitors; Histone Deacetylase Inhibitors suberoylanilide hydroxamic acid and cinnamyl hydroxamic acid LAQ824; and Heat Shock Protein-90 Inhibitors alone or to enhance response to PS-341. Novel agents targeting BM include IκB Kinase Inhibitor PS-1145 and p38 MAPK inhibitor which do not inhibit growth of isolated MM cells, but do block tumor growth and cytokine secretion in BM. Novel agents targeting cell surface receptors include Tumor Necrosis Factor -Related Apoptosis-Inducing Ligand/Apo2 Ligand; Insulin-like growth factor -1 receptor inhibitors to overcome cytokined-induced growth, survival, and drug resistance; Statins to disrupt upstream lipid raft and downstream growth signaling; and anti-CD 20 against CD20 + MM cells. These novel agents, used alone or in combination with conventional or other novel agents, offer great promise to
Figure 3b. Event-free Survival

<table>
<thead>
<tr>
<th>Events / N</th>
<th>4-Year Estimate</th>
</tr>
</thead>
<tbody>
<tr>
<td>TII</td>
<td>74 / 231 63% (55,70)</td>
</tr>
<tr>
<td>TTI</td>
<td>196 / 231 34% (29,41)</td>
</tr>
</tbody>
</table>

Logrank P-value < .0001

12. Role of novel therapies targeting the myeloma cell and its marrow microenvironment

Roundtable I

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improve patient outcome in MM. Importantly, gene array and proteomic evaluation samples from patients treated with these novel agents on will define molecular mechanisms of tumor cell sensitivity versus resistance, thereby providing the framework for developing next generation more potent, selective, and less toxic, targeted MM therapies.

P12.1.2
THE ROLE OF ARSENIC TRIOXIDE IN MULTIPLE MYELOMA
Mohamad Hussein, MD
Multiple Myeloma Research Program, Cleveland Clinic Cancer Center, Cleveland, OH, USA

Introduction
In recent years the delineation of the different cytokines and cellular interactions influencing plasma cells has provided the drug industry with a rationale to develop target specific molecules. Multiple Myeloma is incurable as a result of the complex, redundant and effective mechanisms maintaining the plasma cell’s survival. Effectively influencing the malignant plasma cell microenvironment to modulate and/or reset to a normal level of activity could change the disease into a chronic process. Molecules that act on different levels of the immune system, or a combination of such agents will be needed to overcome the redundancy and positive feedback loops in the myeloma cell support system. These molecules have diverse effects and activities; therefore toxicity tends to be a global byproduct of treatment. Clinical trials that are well designed and conducted are critical in the development of therapy for myeloma.

Trisenox® (arsenic trioxide) is a novel anticancer agent with unique, multifaceted mechanisms of action. At clinically relevant concentrations, it causes apoptosis in various tumor cell lines and has anti-angiogenic effects in vitro and in vivo. Human myeloma cell lines and freshly isolated cells are particularly sensitive to Trisenox® and there is no apparent cross-resistance to the drug in myeloma cell lines that are resistant to other agents such as dexamethasone or doxorubicin.

Preclinical effects of arsenic trioxide when used as a single agent
Results from preclinical studies show that arsenic trioxide, when used as a single agent, appears to act directly on mitochondria to induce apoptosis. This activity is unlike conventional cytotoxics, which trigger pro-apoptotic signal transduction pathways upstream of mitochondria. The induction of apoptosis by arsenic trioxide is thought to occur by several mechanisms that involve generation of reactive oxygen species (ROS) and inhibition of the glutathione (GSH) cellular redox system. These activities appear to directly damage the mitochondria leading to apoptosis of the myeloma cells.

Other preclinical work indicates that the effects of arsenic trioxide treatment also occur at the cell surface. The results from this work show myeloma cell destruction through modulating integrins and caspase activation and through the over expression of CD38 and its ligand on plasma and LAK cells, respectively.

Preclinical effects of arsenic trioxide when used as a combined agent
Depletion of cellular levels of GSH with agents such as ascorbic acid (AA) or buthionine sulfoximine (BSO) can enhance apoptosis of human myeloma cells by arsenic trioxide. Through different mechanisms, AA and BSO reduce GSH levels and accentuate mitochondrial damage and apoptosis of myeloma cells. Investigators observed that AA could potentiate arsenic trioxide-mediated increases in the production of superoxide and increase disruption of mitochondrial membrane potential in myeloma cell lines. The investigators also found that when myeloma cells from patients are treated with AA and arsenic trioxide, the cells are more sensitive to the apoptotic effects of arsenic trioxide. The combination of arsenic trioxide with AA or BSO also affected drug-resistant myeloma cell lines known to express various mechanisms of drug resistance.

Clinical effects of Trisenox® when used as a single agent
PHASE 1 STUDY
In a phase 2, multicenter study, 24 heavily pretreated myeloma patients received treatment with Trisenox® (0.25 mg/kg, Mon-Fri, 2 weeks on/2 weeks off). Of the 24 patients, 15 were refractory to previous treatments and 9 relapsed. Thirty of the 24 patients were evaluated for efficacy and 12 of those patients had an objective response or achieved stable disease. The response to Trisenox® in this study was durable with one patient maintaining stable disease at 22+ months after starting Trisenox® therapy. Neutropenia and leukopenia were the only common grade 4 toxicities in this study.

Clinical effects of arsenic trioxide when used as a combined agent
PHASE 2 STUDY
A phase 2 clinical trial was initiated at the Cleveland Clinic Cancer Center to test the effect of Trisenox®-AA-dexamethasone (TAD) in the treatment of 15 patients with active, progressive multiple myeloma who failed no more than 2 prior treatments. This study and its treatment regimen are based on the results others have reported from previous clinical studies with Trisenox® as a single agent and in vitro work. The in vitro studies showed that Trisenox® sensitizes myeloma cells to dexamethasone (Dex) or that AA enhances the effect of Trisenox® on plasma and human cell lines.

Treatment regimen for phase 2 TAD study Cycle 1: Week 1, load with Trisenox® 0.25mg/kg IV days 1-5, AA 1000mg IV within 30 minutes after each Trisenox® infusion, and Dex 40 mg orally days 1-4. Weeks 2-12, Trisenox® 0.25 mg/kg IV twice weekly, AA 1000 mg IV within 30 minutes after each Trisenox® infusion, and Dex 40 mg orally days 11-14, 29-32, 39-42, 57-60, and 67-70. Weeks 13-15, rest period. Trisenox® and AA regimens are the same during cycles 2 and 3, but the frequency of Dex is reduced to Dex 40 mg orally days 1-4, 29-32, 57-60, and 67-70.

The most current results from this phase 2 TAD study show that 6 patients (42%) achieved >50% reduction in M-protein and 1 patient had near CR after 1 cycle of therapy. Seven patients had stabilization of their disease process with 1 of these patients progressing during the second cycle. Preliminary results show that this combination of drugs is active in the group of relapsed myeloma patients tested and that the regimen was well tolerated.
Longer follow-up of these patients will help to determine the role of Trisenox® in sensitizing myeloma cells to dexamethasone.

CLINICAL EXPERIENCE
The encouraging results from preclinical studies done to test the cytotoxic effect of drug combinations, such as Trisenox® with melphalan or Trisenox® and AA on myeloma cells, led to the early clinical work with melphalan-Trisenox®-ascorbic acid. In this clinical experience, three patients with relapsing myeloma failed multiple therapies; 2 of the 3 patients received combined treatment of melphalan and a second agent as a prior therapy. The patients treated in this clinical experience also had significant secondary renal dysfunction (serum creatinine of 5.1, 5.1, and 6.1); however, even though these patients were seriously ill, all of them responded to a melphalan-Trisenox®-ascorbic acid regimen (melphalan 0.1 mg/kg daily for the first four days of a 4-6 week cycle, Trisenox® 0.25 mg/kg twice weekly, and ascorbic acid 1 g twice weekly). The responses to this therapy included a decrease in serum or urine M-proteins and a marked and sustained improvement in serum creatinine levels and creatinine clearance. Understanding the unique mechanism of action of arsenic trioxide and its interaction with other agents provides an opportunity to develop new drug regimens that are well tolerated and that act differently from the traditional cytotoxic agents currently used as multiple myeloma therapies. These new regimens may include using arsenic trioxide alone or combined with other agents to enhance the cytotoxic effects of arsenic trioxide. In addition to the studies summarized here, other clinical trials with Trisenox® are in progress or are being designed to learn more about the clinically beneficial effects of Trisenox® in the treatment of multiple myeloma.

P12.1.3
THE PROTEASOME INHIBITOR BORTEZOMIB IN MULTIPLE MYELOMA (MM)
Paul G. Richardson, MD, Toru Hideshima, MD, and Kenneth C. Anderson MD
In MM cell lines and patient (pt) MM cells in vitro, bortezomib (VELCADE™, formerly PS-341) inhibited proliferation, prevented binding to bone marrow stromal cells, induced apoptosis, and produced additive cytotoxicity with conventional treatment, including dexamethasone (Dex). Additionally, bortezomib inhibited tumor growth, induced apoptosis, and reduced angiogenesis in a murine MM xenograft model in vivo. As part of a phase I study, pts with MM (n=8) were treated with bortezomib (0.4–1.38mg/m², 2x/w x 4 q6w) and significant antitumor activity was seen, including 1 CR. In a phase II trial of bortezomib of heavily pre-treated relapsed and refractory MM pts (n=202, median number of prior regimens=6), bortezomib (1.3mg/m² 2x/w x2 q3w) induced a 10% CR rate (4% CR using Blådè criteria, and 6% with residual positive immunofixation only); an overall response rate of 35% (MR+PR+CR), and 59% of pts achieved SD or better in the evaluable population (n=193). The response was independent of prior therapy. Overall (n=202) median survival was 16 months and median time to progression was 7 months (vs. 3 months on last prior therapy). In CR+PR pts, parameters of clinical benefit, including hemoglobin, platelet count, and KPS, improved with therapy. PD/SD pts could receive combination bortezomib-Dex (20mg on day of and day after bortezomib). Of these pts (n=74), 24% showed improved response. The most common attributable AE’s included nausea, diarrhea, fatigue, thrombocytopenia and peripheral neuropathy (PN). Bortezomib-related AE’s led to discontinuation in 18% of pts (with no specific event accounting for >5%). Genomic profiles associated with response (vs. nonresponse) were identified, and these will be further characterized in future trials. In another phase II trial of pts with earlier relapsed or refractory MM and less prior therapy (n=54), bortezomib (1.3 or 1.0mg/m² 2x/w x2 q3w) induced 1 CR at 1.3mg/m² and 1 CR at 1.0mg/m² by Blådè and 2 additional CRs (1.0mg/m²) with residual positive immunofixation: MR+PR+CR was achieved in 50% (13/26 evaluable pts) and 33% (9/27), respectively. PD/SD pts could have Dex (20mg 4x/w x 2 w) added; 5/12 (25%) and 3/16 (19%) pts treated achieved MR or better in each dose group, respectively. The most common attributable AE’s to bortezomib alone were fatigue, nausea, diarrhea and PN, with toxicities more frequent at 1.3mg/m² and 8 pts discontinuing due to drug-related AE’s. Preliminary results in pts with relapsed MM treated with bortezomib (0.9–1.20mg/m² 2x/w x2 q3w) and pegylated liposomal doxorubicin (30mg/m², d4) demonstrated the combination’s feasibility. DLT’s included G3 diarrhea, hypotension, confusion, and syncope in a pt with underlying Crohn’s. Bortezomib (1.0mg/m²) with thalidomide (50 and 100mg at cycle 2) is being tested in pts with MM resistant to or relapsed from auto-SCT or salvage therapies. Anti-MM activity (MR or better) was seen, with toxicities including G4 neutropenia and G3 hyponatremia, but PN has not been reported. Studies to further assess the clinical utility of bortezomib (alone and in combination), including a pivotal, comparative phase III trial in MM, are ongoing.


P12.1.4
Results of Thalidomide and IMIDs in multiple myeloma
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The prognosis of patients with symptomatic myeloma has improved significantly over the last 40 years due to the administration of alkylating agents in standard doses, of high-dose steroids, of high dose therapy with autologous stem cell support and more recently of thalidomide. This oral agent and its immunomodulatory derivatives (IMIDs) represent new treatments which target the myeloma cell-host interaction and the bone marrow microenvironment. Whatever its exact mechanism of action, thalidomide has remarkable activity in myeloma, as first reported by Singhal et al. These investigators demonstrated that at least 50% reduction of monoclonal protein concentration occurred in one-third of patients with refractory multiple myeloma. [1] Multiple other studies have also indicated that thalidomide can induce partial responses in 30 to 40% of patients with refractory or relapsing myeloma (Table I). With patients with normal cytogenetics, low PCLI and low levels of serum beta2 microglobulin respond better to thalidomide. Some of the responses are remarkably durable with 9% of patients remaining free of progression at 4 years. Despite some evidence of a thalidomide dose-response relationship, responses occur frequently with doses varying from 50 to 200 mg/day and thus the optimal dose of thalidomide has yet to be defined. Thalidomide has been used alone in newly diagnosed asymptomatic patients and one third of patients achieved at least 50% reduction of monoclonal protein. Approximately 60% of responding patients remain free of progression at 2 years [2]. Because intermittent high-dose dexamethasone alone has been effective in approximately one-fourth of patients with refractory...
myeloma and in 40% of previously untreated patients, its combination with thalidomide (TD) was assessed. Approximately 50% of previously treated patients responded. [3] Despite the higher response rate of TD it is not clear whether this combination is associated with longer survival than that achieved with single agent thalidomide; however many patients responded after resistance to sequential administration of thalidomide and pulses dexamethasone separately, which suggests synergy of the two agents. The thalidomide-dexamethasone combination induced objective responses in 72% and 64% of previously untreated patients in 2 series respectively. [2, 4] With this non-myelosuppressive regimen 86% of responsive patients were in remission within 2 months. Blood stem collection was rapid and efficient with the use of G-CSF alone in most instances. [2] While this active oral regimen obviates the need for a long-term central venous catheter, it is associated with increased risk for thrombosis. This risk is even higher when chemotherapy and particularly doxorubicin is administered with TD. Despite several preliminary studies the mechanism of deep vein thrombosis is not clearly understood and firm recommendations regarding antithrombotic prophylaxis are lacking.

There have been several studies concerning the efficacy of thalidomide combined with dexamethasone and chemotherapeutic agents such as cyclophosphamide, melphalan, doxorubicin, etoposide and cisplatin. While responses are being reported in approximately 60% of previously treated patients, the impact of these combinations on patients’ outcome remains unclear. Ongoing studies will establish the role of these combinations as primary treatment for multiple myeloma.

Certain of the thalidomide analogues demonstrate enhanced antitumor activity with an improved toxicity profile. An immunomodulatory derivative of thalidomide is well tolerated in patients with relapsed multiple myeloma and is used in clinical trials, showing remarkable antimyeloma activity against the combination treatment are still a field of research. Immunomodulatory derivatives of thalidomide are already used in clinical trials, showing remarkable antimyeloma activity with an improved toxicity profile.

References

Table I. Outcomes after thalidomide alone, with dexamethasone +/- other agents in previously treated multiple myeloma

<table>
<thead>
<tr>
<th>Series</th>
<th>Regimen</th>
<th>PR %</th>
<th>CR %</th>
<th>EFS</th>
<th>OS</th>
</tr>
</thead>
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<tr>
<td>Barlogie 2002 Ash</td>
<td>Thal 200 to 800mg</td>
<td>169</td>
<td>33%</td>
<td>20% @2y</td>
<td>48% @2y</td>
</tr>
<tr>
<td>Takahash-Aqua 2002</td>
<td>Median 400 mg</td>
<td>83</td>
<td>48%</td>
<td>50% @1y</td>
<td>57% @1y</td>
</tr>
<tr>
<td>Neben 2002</td>
<td>Thal 400mg</td>
<td>83</td>
<td>20%</td>
<td>45% @1y</td>
<td>86% @1y</td>
</tr>
<tr>
<td>Richardson 2001 Ash</td>
<td>Thal 200-600</td>
<td>30</td>
<td>42%</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Julussow 2000</td>
<td>Thal 200 to 800mg</td>
<td>23</td>
<td>43%</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Biades 2001</td>
<td>Thal 200 to 800mg</td>
<td>23</td>
<td>13%</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Prince 2002</td>
<td>Thal 600mg</td>
<td>75</td>
<td>28%</td>
<td>50% @5.5m</td>
<td>50% @14.6</td>
</tr>
<tr>
<td>Dimopoulos 2001</td>
<td>Thal 400mg Dex pulses</td>
<td>44</td>
<td>55%</td>
<td>50% @10m for responders</td>
<td>50% @12m</td>
</tr>
<tr>
<td>Paumbo 2001</td>
<td>Thal 100 Pul Dex monthly</td>
<td>120</td>
<td>52%</td>
<td>50% @12m</td>
<td>50% @27m</td>
</tr>
<tr>
<td>Anagoss-E 2003</td>
<td>Thal 200 to 800 Dex pulses</td>
<td>47</td>
<td>57%</td>
<td>50% @16m</td>
<td>50% @38m</td>
</tr>
<tr>
<td>Coleman 2002</td>
<td>Thal max200 clar 5002 dex 40/w</td>
<td>50</td>
<td>74%</td>
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<td>NA</td>
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<tr>
<td>Mohtler F M 2002</td>
<td>Thal CTX VP16 dex</td>
<td>58</td>
<td>68%</td>
<td>50% @16m</td>
<td>55% @16m</td>
</tr>
<tr>
<td>Garcia-Sanz 2002</td>
<td>Thal CTX</td>
<td>22</td>
<td>53%</td>
<td>51% @1y</td>
<td>52% @1y</td>
</tr>
<tr>
<td>Chiook-Kee Lee 2003 Ash</td>
<td>DTPACE</td>
<td>156</td>
<td>43% (&gt;75%)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Tkarnov 2002</td>
<td>Thal myel dex</td>
<td>21</td>
<td>70%</td>
<td>50% @6m</td>
<td>50% @13m</td>
</tr>
<tr>
<td>Zipfel 2002 Ash</td>
<td>Hyper CTX</td>
<td>60</td>
<td>72%</td>
<td>50% @11m</td>
<td>50% @19m</td>
</tr>
<tr>
<td>Hussein 2002 Ash</td>
<td>Thal-doxil VCR dex</td>
<td>35</td>
<td>74%</td>
<td>NA</td>
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</tbody>
</table>

Roundtable II

P12.2.1 FARNESYLTRANSFERASE INHIBITORS IN MULTIPLE MYELOMA.

Melissa Alisa, M.D.
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Multiple myeloma (MM) patients with mutated RAS are less likely to respond to chemotherapy and have a shortened median survival. Therefore, targeting RAS farnesylatation, which is required for its function, may be a novel approach to treatment of MM. In a recently published article by Bolick et al., the prenylation inhibitors, FTI-277 and GGTI-2166, a farnesyl transferase inhibitor and geranylgeranyl transferase inhibitor, respectively, were shown to induce apoptosis in myeloma cell lines selected for resistance to classic cytotoxics including doxorubicin and melphalan. Similarly, we and others have shown that FTI-R115777 (Zarnestra) induces a dose and time dependent growth inhibition and apoptosis in myeloma cell lines. We have evaluated in a phase II trial the activity and tolerability of the farnesyltransferase (Fase) inhibitor Zarnestra and correlated these to inhibition of protein farnesylatation and oncogenic/tumor survival pathways in patients with advanced multiple myeloma. Eligibility criteria included patients with relapsed or refractory myeloma, ECOG performance status ≤ 3, normal renal function and

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This work was supported by CTEP/NIH grant, Multiple Myeloma agent in combination with other cytotoxics in patients with myeloma. Stabilization of disease in 62% of patients with advanced myeloma. In patients, the farnesyltransferase inhibitor FTI-R1155777 induced a new class of agents with significant antimyeloma activity in vitro. Apoptosis in myeloma. In summary, farnesyl transferase inhibitors are survival pathway plays an important role in FTI-R115777 induced when treated with FTI. Our data suggests that the AKT tumor survival pathway. FTI-R115777 inhibited proliferation in all tumor lines examined. The levels of phosphorylated Akt and STAT3 but not Erk1/2 in bone marrow from patients where these oncogenic tumor survival pathways were constitutively activated. We conclude that Zarnestra is tolerable, can induce disease stabilization in multiple myeloma patients, and that 300 mg BID is sufficient to inhibit FTase activity, protein farnesylation and oncogenic/tumor survival pathway. From the clinical trial we learned that protein farnesylation was inhibited in all patients, this did not correlate with clinical activity. Clinical results did indicate that FTI-R115777 reduced the levels of phosphorylated Akt and STAT3 in bone marrow from patients where these tumor survival pathways were constitutively active, and the former correlated with disease stabilization in the limited number of patients examined. The PI3 kinase/AKT2 pathway have been shown to be a critical target for FTI induced apoptosis in ovarian cancer cell lines. Therefore, we examined the mechanisms of cytotoxicity of FTI-R115777 on myeloma cell lines and its correlation to the AKT tumor survival pathway. FTI- R115777 inhibited proliferation in all cell lines except MM1.s at concentrations <5uM, with RPMI 8226 showing the most sensitivity, IC 50 2x10^{-8} M, and U266 and H929 a more moderate 2x10^{-6} and 4x10^{-7} respectively. Propidium iodide cell cycle analysis data indicated 8226 and H929 cells accumulate in G2-M phase and G1-G0 phase respectively, in a dose dependent manner. Annexin V-PI analysis indicated a dose dependent increase in the number of apoptotic cells in all cell lines except MM1.s. One uM FTI-R115777 induced pro-caspase 3 cleavage in 8226 cells, but not in MM1.s cells, between 12 and 24 hours after treatment. FTI- R115777 induced a dose dependent pro-caspase 3 cleavage in 8226, U266, and H929 cells within 72 hours. MM1.s cells under the same conditions failed to exhibit a similar response through 72 hours. FTI inhibited AKT phosphorylation in a dose dependent manner in all MM cell lines examined. The levels of phospho AKT expression correlated with resistance to FTI, with the more resistant cell lines showing higher levels of phospho AKT and incomplete inhibition when treated with FTI. Our data suggests that the AKT tumor survival pathway plays an important role in FTI-R115777 induced apoptosis in myeloma. In summary, farnesyl transference inhibitors are a new class of agents with significant antimonyeloma activity in vitro. In patients, the farnesyltransference inhibitor FTI-R1155777 induced stabilization of disease in 62% of patients with advanced myeloma. Further clinical studies will examined the clinical activity of this agent in combination with other cytotoxics in patients with myeloma. This work was supported by CTEP/NIH grant, Multiple Myeloma Research Foundation Senior Research Award and the Myeloma Research Foundation.

References:

P12.2.2 THE ROLE OF OSTEOPROTEGERIN (OPG) IN THE BONE DISEASE OF MULTIPLE MYELOMA.

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Osteolytic bone destruction is responsible for much of the morbidity associated with multiple myeloma. The presence of myeloma cells within the bone marrow affects the fine-tuned balance between bone-forming osteoblasts and bone-resorbing osteoclasts. The activity of osteoclasts appears to be mainly regulated by the osteoblasts. In osteoblasts, the molecules maintaining this regulation are the protein RANKL (Receptor Activator of NF-kB Ligand), and osteoprotegerin(OPG), which is a soluble decoy receptor for RANKL. The RANKL binds the cell surface receptor RANK on osteoclasts, thereby promoting both osteoclastogenesis and osteoclast activity. The balance between osteoblast expression of RANKL and OPG thus act as the main molecular switch determining the degree of bone resorption. OPG naturally occurs as a dimer, and the protein contains a domain capable of binding heparin and heparan sulphate proteoglycans. The presence of myeloma cells within the bone marrow affects the balance between bone-forming osteoblasts and bone-resorbing osteoclasts. We have shown that serum as well as bone marrow plasma OPG levels are lower in myeloma patients with bone disease than in patients without detectable bone lesions. Moreover, there is a correlation between OPG levels in bone marrow and serum, both in multiple myeloma patients and in non-myeloma patients. OPG binds to cell surface heparan sulfates on myeloma cells and the binding may result in internalization and subsequent degradation of the protein in lysosomal compartments. Syndecan-1 is the main heparan sulfate proteoglycan on myeloma cells. ARH-77 lymphoblastoid cells transfected with syndecan-1 also bind, internalise and

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degrade OPG in the same way as myeloma cells do. Plasma cells in bone marrow biopsies from myeloma patients stained positive for OPG. The binding, internalisation and degradation of OPG by myeloma cells may contribute to the reduced OPG levels observed in the bone marrow of multiple myeloma patients. Furthermore, OPG may be removed from both osteoblast surfaces as well as the bone marrow microenvironment by the presence of myeloma derived soluble heparan sulphates.

Alternatively, myeloma cells may interfere with osteoblast expression of OPG, either by affecting OPG synthesis and secretion, or by affecting osteoblast differentiation and maturation. The opportunities in manipulating functional bone marrow OPG levels in patient treatment will be discussed.

References:
NFkB. A NEW THERAPEUTIC TARGET FOR OVERCOMING DRUG RESISTANCE.

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Director of Multiple Myeloma and Bone Metastasis Programs at Cedars-Sinai Medical Center, Los Angeles, CA

Activation of apoptosis in cancer cells resulting from NF-kB inhibition suggests that NF-kB inhibition could be used as a mechanism to treat cancers. To inhibit the activity of NF-kB, several genetic studies were carried out by homologous recombination to either directly destroy NF-kB/p65 function or indirectly suppress NF-kB activity through destroying IkB (IkB kinase) function and thereby upregulating IkB activity. To establish that inhibition of NF-kB activity induces apoptosis in MM cells, we recently carried out viral transduction experiments in which dominant negative IkBα was introduced into both melphalan-sensitive and -resistant MM cells. The cellular apoptosis was noted to greatly increase in melphalan-sensitive and resistant MM cells compared to viral vector alone. These studies confirm the notion that inhibition of NF-kB activity can precipitate cell death in MM cells through induction of apoptosis. The proteasome inhibitor, PS-341 (Bortezomib or Velcade), is a novel drug that was designed to specifically block the signal transduction pathways mediated by NF-kB. The destruction of IkBα proteins following their phosphorylation by IKK and subsequent ubiquitination is primarily mediated by the proteasome degradation that can be inhibited by PS-341. Blocking the proteasome degradation of IkBα by PS-341 significantly inhibits NF-kB activity, resulting in the stimulation of apoptosis of myeloma cells.

PS-341 has been shown to be quite effective in inhibiting human myeloma cell growth both in vitro and in vivo. Specifically, the growth of both chemo-sensitive and -resistant MM cell-lines was substantially inhibited by PS-341 treatment. Interestingly, there is a "right shift" in the dose-response curves for chemoresistant cell lines, suggesting that the chemoresistant cell-lines appear to be more sensitive to the treatment of PS-341 than the chemosensitive lines. The alteration in NF-kB activity appears to be one of the major mechanisms of anti-myeloma activity of PS-341.

In support of this, the nuclear translocation of NF-kB and its subsequent DNA binding are decreased in MM cell lines that were treated with PS-341. Many tumor cells especially MM tumor cells display constitutively high levels of NF-kB activity. In response to chemotherapy, the activity of NF-kB is further enhanced resulting in chemoresistance. Thus, the inhibition of its activity can be used to reverse the chemoresistant phenotype of a variety of cancer cells. Using NF-kB inhibition together with cytotoxic agents has also been tried in MM treatment, and this combination strategy has been evaluated using in vitro studies since the availability of PS-341. We demonstrated a marked synergistic effect exists between PS-341 and various chemotherapeutic agents in inhibiting MM cell growth. We treated several chemo-sensitive MM cell-lines along with chemo-resistant lines with several chemotherapeutic agents, including doxorubicin, mitoxantrone and melphalan, that were used either alone or in combination with a low, non-cytotoxic dose of PS-341 (5 ng/ml). We saw no significant growth inhibition of chemo-resistant lines when they are treated with chemotherapeutic agents alone until high concentrations of chemotherapy were applied. However, when the cells were treated with PS-341 together with chemotherapeutic agents, these chemo-resistant cell-lines became extremely sensitive to chemotherapeutic agents. For example, the cytotoxic dose of melphalan when used together with PS-341 was 1,000,000-fold lower than the concentration necessary for melphalan alone to induce cytotoxicity in a highly melphalan-resistant MM cell lines. Similar effects were observed between PS-341 and doxorubicin or mitoxantrone as the combination markedly increased the sensitivity of both doxorubicin-resistant and mitoxantrone-resistant MM cell-lines by approximately 100,000-fold. Parallel with the increase in chemosensitivity, there also was a marked increase in apoptosis of chemoresistant MM cell lines induced by this combined approach.

The synergy observed between PS-341 and chemotherapy agents appear to be cell-type specific. Synergistic effects between PS-341 and chemotherapeutic agents were not found when they were used together to treat other types of tumor cell lines. Similar experiments were also performed on normal unstimulated and mitogen stimulated peripheral blood mononuclear cells (PBMCs) and CD34-selected BMMCs obtained from healthy individuals. Suppression of proliferation in these non-MM cell-lines or normal hematopoietic cells was not found with PS-341 treatment except at higher concentrations (IC50 50 - 75 ng/ml). Moreover, the addition of PS-341 to chemotherapy had minimal synergistic inhibitory effects on cell growth in these same samples. This observation is interesting because the extent of synergy between PS-341 and cytotoxic agents also correlates with the baseline levels of NF-kB activity identified in each cell type evaluated. This finding is also important and clinically relevant since the difference in cell response to the combined treatments between myeloma cells and normal cells could provide an excellent therapeutic/toxicity ratio for this approach for treating MM patients. As a result of these encouraging in vitro results, we began a Phase I clinical trial to study the efficacy lower doses of both PS-341 (using 40% of the dose/month in the previous large Phase II SUMMIT trial) and oral melphalan as combination therapy in treating refractory and relapsed MM patients. Even among all three patients receiving the lowest melphalan dose (only 0.025 mg/kg daily X 4), dramatic decreases in paraprotein levels were observed, and, in fact, responses have been observed in all five 3-patient cohorts (see H Yang et al, IXth International Workshop on Multiple Myeloma for details). Importantly, this combination has been associated with minimal neurotoxicity. In addition to the proteasome inhibitors, other pharmacotherapeutic agents also can block NF-kB signaling. One example is arsenic trioxide. Arsenic trioxide has been shown to be a potent NF-kB inhibitor. It binds to the cysteine residue 179 in the activation loop of IKK catalytic subunits and thereby blocks the IKK activity. This results in a lack of IkB phosphorylation and inability for the IkB to be ubiquitinated and proteasome degraded. Indeed, exposure of MM cells to arsenic results in accumulation of IkB and reduced nuclear accumulation of NF-kB and DNA-binding of this transcription factor. Similar to PS-341, arsenic trioxide also sensitizes myeloma cells to chemotherapy in vitro and in vivo. Based on these preclinical findings, we treated eight relapsing myeloma patients with a combination of low-dose oral melphalan, arsenic trioxide and ascorbic acid.

The latter agent was used because previous studies showed that this vitamin was able to reduce glutathione levels and increase the anti-myeloma effects of arsenic trioxide. Seven of the eight patients showed reduction in paraprotein (25-58%), and four of the patients with renal failure showed marked improvement in renal function on this regimen which was well tolerated. These studies suggest that inhibition of NF-kB activity may allow use of reduced doses of both chemotherapy and the NF-kB blocking agents resulting in enhanced anti-myeloma effects with reduced toxicity.
Monoclonal antibody therapy, particularly with rituximab (Rituxan, MabThera) has been successfully used in the treatment of B-cell malignancies. In an effort to extend the activity of rituximab, we conducted studies using rituximab alone and in combination therapy in Waldenström’s macroglobulinemia (WM) and multiple myeloma (MM). In studies examining extended dose (i.e. 8 infusions) rituximab, we observed an overall response rate (ORR) of 73% (69% PR; 4% MR) and an estimated median time to treatment failure of 20+ months, which compared favorably to those reported by us and others (6-9 months) using standard dose (i.e. 4 infusions) rituximab. Significant improvements in hematological function were also observed in patients receiving extended dose rituximab. Pre-rituximab therapy, 35% of patients were anemic and 28% were thrombocytopenic which decreased to 5% and 9%, respectively following therapy. Interestingly, response to extended dose rituximab was correlated with pre-therapy IgM levels, but not BM tumor cell burden. Eighteen of 20 patients (90%) with an IgM level of <6,000 mg/dL responded, whereas only 1/6 (16%) patients with an IgM level of >6,000 mg/dL responded (p=0.002). The reason for this finding remains under active investigation. In an effort to further extend the activity of rituximab in WM patients, we also examined in a multicenter trial combination therapy with extended dose rituximab and 6 cycles of fludarabine. Interim analysis of the first 23 patients on this study showed that 90% (15% CR; 61% PR; 14% MR) of patients attained a response. Unlike in WM, a role for rituximab in MM appears particularly limited to those patients whose BM plasma cells (BMPC) express CD20. In a multicenter study, we treated 19 MM patients with extended dose rituximab, regardless of their CD20 status. Six of 19 (32%) patients demonstrated either a PR (n=1) or SD (n=5), with a median time to treatment failure of 5.5 months (range 3-21+ months). 5/6 of these patients had CD20+ BMPC, while CD20 status could not be determined in one patient. In an effort to advance the use of monoclonal antibody therapy in WM and MM, we have sought to define mechanism(s) by which rituximab facilitates tumor cell killing. As part of these efforts, we conducted studies to determine a role for antibody dependent cell mediated cytotoxicity (ADCC) by evaluating polymorphisms in position 158 of the Fc gammaRIIa (CD16) receptor. The Fc gammaRIIa receptor has previously been reported to modulate human immunoglobulin G1 binding, and antibody dependent cell mediated cytotoxicity (ADCC). Genetic dimorphisms at position 158 result in expression of either the valine (V), or phenylalanine (F) amino acids which may be expressed either in a homozygous (V/V; F/F) or heterozygous (V/F) phenotype. In these studies, we used allele specific PCR analysis to determine position 158 polymorphisms for 58 patients with Waldenström’s macroglobulinemia for whom clinical responses to rituximab therapy were known. PCR amplifications of exon 4, intron 4 and most of intron 5 were performed using primers which specifically amplified Fc gamma RIla but not Fc gamma RIIb. Each end of the PCR product was then sequenced, and sequence information from exon 4 was used to provide genotype information for codon 158, while the sequence information from exon 5 end was used to confirm that the PCR product was specifically from the Fc gamma RIla gene. Of the 58 WM patients examined, 10/58 (17%) were homozygous for V (Fc gammaRlla-V/V); 26/58 (45%) were heterozygous (Fc gamma RIIla-V/F); and 22/58 (38%) were homozygous for F (Fc gamma RIIla-F/F). No significant differences in sex, age, baseline IgM levels, number of prior therapies, and number of rituximab infusions received was seen among the three allelotype groups. The overall response rate (major, i.e. >50% decline in IgM and minor, i.e. >25% decline in IgM responses) for the three allelotype groups were as follows: 6/10 (60%) Fc gamma RIIla V/V; 13/26 (50%) Fc gamma RIlla V/F; and 8/22 (36%) Fc gamma RIIla F/F (V/V and V/F vs. F/F p=0.28). Comparison of major RR among the V carriers vs. F/F revealed even greater differences: 4/10 (40%) Fc gamma RIIla V/V; 9/26 (36%) Fc gamma RIIla V/F (13/36 for V carriers combined), and 2/22 (9.0%) for Fc gamma RIIla F/F (V/V and V/F vs. F/F p=0.03). The results of these studies support an association between V carrier status and higher response rates (particularly for major responses) to rituximab, which analogue Revamid were previously shown by us to enhance myeloma cell lysis by natural killer cells. As a follow-up to these studies, we have performed studies using immunomodulators to enhance the ADCC function of rituximab. Thalidomide and its analogue Revamid significantly enhanced rituximab mediated ADCC of CD20+ ARH-77 lymphoblastocytic cells. In view of these results, a clinical trial combining Thalidomide and Rituximab is contemplated. Lastly, ongoing pre-clinical and clinical studies are underway to develop monoclonal antibody therapy targeting CD20 using yttrium90 conjugated Zevalin, as well as unconjugated monoclonal antibodies targeting CD22, CD40, and CD52. These studies were funded through generous grants or gifts from the Research Fund for Waldenström’s at the Dana Farber Cancer Institute, the International Waldenström’s Macroglobulinemia Foundation, the International Myeloma Foundation, the Multiple Myeloma Research Foundation, the American Society of Clinical Oncology Young Investigator Award, a National Institutes of Health Career Development Award, the Peter and Helen Bing Fund, the Bailey Family Fund, Genentech Bio-Oncology Inc., IDEC Pharmaceuticals Inc., Celgene Corporation, and Berlex Oncology Inc. References 1. Treon SP, Shima Y, Preffer FI, Doss DS, Eilman L, Schlossman RL, Grossbard ML, Belch AR, Pilarski LM, Anderson KC. Treatment of plasma cell dyscrasias by antibody immunotherapy. Sem Oncol 1999; 26: 97-106. 2. Treon SP, Shima Y, Grossbard ML, Preffer FI, Belch AR, Pilarski LM, Anderson KC. Treatment of multiple myeloma by antibody mediated immunotherapy and induction of myeloma selective antigens. Ann Oncol 2000; 11:107-111. 3. Treon SP, Anderson KC. The use of rituximab in the treatment of malignant and non-malignant plasma cell disorders. Sem Oncol 2000; 27:79-85. 4. Treon SP, Raje N, Anderson KC: Immunotherapeutic strategies for the treatment of plasma cell malignancies. Sem Oncol 2000; 27:598-613. 5. Davies FE, Raje N, Hideshima T, Lentzsc S, Young G, Tai YT, Lin B, Podar K, Gupta D, Chauhan D, Preffer F, Richardson PG, Schlossman RD, Morgan GJ, Muller GW, Stirling DI, Anderson KC: Thalidomide and immunomodulatory derivatives augment natural killer cell cytotoxicity in multiple
12. Treon SP, Hayashi T, Anderson KC, Treon SP. Rituximab induced antibody dependent cell mediated cytoxicity (ADCC) is enhanced by thalidomide and its analogue Revimid. Blood 2002; 100:314b.
13. Vaccination strategies in multiple myeloma

P13.1
DNA VACCINATION: FROM THE LABORATORY TO THE CLINIC

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Introduction: DNA vaccines deliver encoded protein to the immune system and can activate antibody and cellular responses. Many new DNA vaccines against infectious diseases are in clinical trial in normal adults. For cancer, the concept of placing safe tumor-derived gene sequences into a vaccine format, and inducing specific anti-tumor immunity, has obvious attractions. However, for myeloma, there are two major problems. The first is that tumor antigens are only weakly immunogenic, and the second is that patients tend to be immunosuppressed, due either to disease or to treatment. Both these problems have been addressed in our study.

To strengthen immunogenicity, we have fused a sequence from tetanus toxin to the tumor-derived sequence. This strategy dramatically amplifies the response to the tumor protein and in pre-clinical models leads to protective immunity. Our first target tumor antigen for myeloma is the idiotypic immunoglobulin secreted by the neoplastic plasma cells. This is coded by the variable region genes, VH and VL which can be readily identified and isolated from patients’ tumor cells. We assemble these as single chain Fv (scFv) a convenient way of making a single gene able to produce idiotypic protein. The tetanus toxin sequence codes the non-toxic C-terminal fragment (Fragment C (FrC)), which is highly immunogenic and includes a “promiscuous” helper determinant able to activate high levels of T-cell help. The DNA scFv-FrC fusion gene induces specific immunity against a model myeloma (5T33) with protection mediated by anti-idiotypic CD4+ T cells. This vaccine design is in a trial for patients with lymphoma which is almost complete. No significant side-effects of the vaccine have been observed, and immune responses against both components of the fusion gene are evident in most patients.

To circumvent the problem of immunosuppression in patients with myeloma, we are currently vaccinating donors of allogeneic stem cell transplants. The safety of the DNA vaccines allows this procedure, and ensures that a maximal response will be obtained. Immune cells are being transferred to the recipient patients at the time of donor lymphocyte infusion (DLI). Clearly, to extend the approach to more patients, we need information on the capacity of the immune response following conventional chemotherapy with or without autologous transplantation.

Idiotypic antigen is a useful and safe tumor target, but we aim also to vaccinate myeloma patients against other antigens. One class of antigens which is attractive is the cancer-testis antigens, known to be expressed only by cells of the testis, and by cells of a range of cancers, including myeloma. To attack these intracellular antigens, it is necessary to induce cytotoxic T cells (CTL) which can recognize processed peptides presented by the MHC Class I molecules. For this, we have designed a novel vaccine incorporating a gene encoding an engineered domain of the FrC sequence (p.DOM) fused to an epitope from a cancer
12. Treon SP, Hayashi T, Anderson KC. Combination therapy with rituximab and fludarabine is highly active in Waldenstrom’s macroglobulinemia. Blood 2002; 100:211a.

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antigen. This induces high levels of epitope-specific CTL able to produce IFN and to kill tumor cells. To test operation of this design in human subjects, we have used an epitope derived from cytomegalovirus (CMV). The chosen epitope is a focus of the HLA-A*0201-restricted immune response during a natural infection with CMV. We have shown that the pDOM-epitope design induces high levels of responding CTL in HLA-A*0201 transgenic mice, and we are currently vaccinating normal donors of transplants to raise immunity prior to transfer into immunosuppressed patients, vulnerable to reactivation or infection with CMV. For myeloma, we have assessed the pre-clinical performance of a vaccine design incorporating an epitope from a known epitope from a cancer-testis antigen.

Results:
Clinical testing:
So far we have vaccinated the first donor with DNA scFv-FrC with the scFv derived from the recipient’s tumor. A specific T-cell proliferative response against both FrC and against the patient’s idiotypic protein was observed to develop at the 16 week time point and to persist for several weeks. The response to FrC was a memory response with apparent fluctuation of levels of responding T cells during the infection period, likely due to the movement of cells to the site of injection. The response to idiotypic protein rose steadily from week 12 onward with no significant response to control Ig. The immune cells have been transferred to the patient during DLI and the patient appears to be doing well.

Analysis of immune status of patients with myeloma:
To consider extending the approach to a larger number of patients, we need information on the immune status of myeloma patients undergoing more conventional chemotherapy. We have first assessed patients who have undergone autologous stem cell transplant following high dose melphalan. We have used conventional tetanus toxoid as a test vaccine to facilitate comparison of immune responses with those of patients being vaccinated with the DNA constructs containing the gene encoding the FrC portion of tetanus toxin. Patients were assessed from 2-15 months post transplant for their ability to produce antibody or T cell responses to tetanus toxoid. Most patients responded with antibody and proliferative responses, and all antibody or T cell responses to tetanus toxoid. Most patients responded with antibody and proliferative responses, and all


P13.2
Abstract withdrawn

P13.3
DENDRITIC CELL-BASED IMMUNOTHERAPY IN MULTIPLE MYELOMA.
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Myeloablative therapies requiring hematopoietic stem cell support can induce complete remissions in up to 40% of multiple myeloma (MM) patients. However, ultimately patients experience disease progression and there is no cure. Novel therapeutic interventions for this disease are therefore needed to specifically target the myeloma cell and its microenvironment. The potential susceptibility of multiple myeloma to immune based therapy has been demonstrated in the allogeneic transplantation through graft versus myeloma effect. A major focus of investigation therefore has been the use of immune-based therapies in a minimal disease setting to decrease the risk of relapse and potentially achieve curative outcomes.

Multiple Myeloma (MM) is associated with a number of dysfunctions in both humoral and cellular immunity (1). Abnormalities in immune cell numbers have been associated with inferior disease prognosis. However, both cellular and humoral immune responses have been observed against both viral and tumor antigens. We have also confirmed improvement in the immune responsiveness in MM patients following effective therapy especially high-dose chemotherapy followed by autologous stem cell transplantation by recovery of uninvolved immunoglobulins and by significantly higher frequencies of viral antigen-specific T cells in the peripheral blood (2). These results have confirmed optimal time for immunotherapy of MM.

We have investigated various immunotherapeutic approaches using idiotype as a myeloma-specific antigen. In a clinical protocol involving 49 patients with minimal disease status following tandem autologous transplantation, we utilized patient-specific Id protein coupled with KLH, as a vaccine. The development of an anti-KLH response confirmed immune competence of the myeloma patients. Moreover, induction of Id-specific immune responses including generation of CTL specifically able to lyse MM cells, and a preliminary evidence of a survival benefit was observed.

To improve on these results we have investigated the role of dendritic cells (DCs), the most potent antigen-presenting cells (APCs) equipped with the necessary co-stimulatory, adhesion and
Multiple myeloma (MM) is still a fatal disease. Despite advances in high-dose chemotherapy and autologous stem-cell support, relapses of the underlying disease remain the primary cause of treatment failure. Novel therapeutic approaches that have a mode of action different from and non-cross-resistant with cytotoxic chemotherapy are required to eradicate tumor cells that have become multidrug-resistant. To this end, immunotherapy aimed at inducing or enhancing myeloma-specific immunity in tumor-bearing patients may be desirable. Indeed, in the post-allograft relapse setting of MM (in which patients are chemotherapy refractory), long-lasting disease remission has been achieved after infusion of donor lymphocytes, suggesting that chemotherapy and T cell-mediated cytotoxicity kill myeloma cells by different modes of action that are non-cross-resistant. Nevertheless, the development of severe graft-versus-host disease in this setting calls for more specific immunotherapeutic strategies.

Plasma cells represent the major tumor burden and constitute 10% to 100% of the total bone marrow cell count. Myeloma plasma cells secrete a monoclonal M-protein and express cytoplasmic, but not surface, Ig that carry idiotype (Id) determinants. We and others have shown that myeloma plasma cells may express MHC class I, adhesion molecules CD44, CD56, CD54, and costimulatory molecules CD40 and CD28. These cells were able to activate alloreactive T cells and present recalled antigens to autologous T cells. Thus, it is a consensus that T cell-mediated immunity may play a role in controlling the growth of myeloma cells. Ideally, a tumor-specific immunotherapy should induce or expand only the beneficial immune responses mediated by cytotoxic T lymphocytes (CTLs) including CD4+ Th1 and CD8+ Tc1 subsets that have sufficient cytotoxic effects towards tumor cells.

Id proteins are tumor-specific antigens and active immunization against Id determinants on malignant B cells has produced resistance to tumor growth in transplantable murine B-cell lymphoma and plasmacytoma. Various approaches have been used to visualize the existence of Id-specific immune response in human disease. By using the enzyme-linked immunospot (ELISPOT) assay, we were able to detect a low frequency of Id-specific T cells in most of myeloma patients with an early disease. We have also shown that Id-specific Th1 cells were significantly higher in patients with indolent disease than those with advanced MM. In contrast, cells secreting Th2-subtype cytokine (IL-4) were seen more frequently in advanced patients. These findings provide indirect evidence that Id-specific T cells may have regulatory effects on human myeloma cells.

To examine whether Id-specific T cells can recognize and kill myeloma cells, we generated Id-specific CTL lines from myeloma patients. The results showed that Id-specific CTLs not only recognized and lysed autologous Id-pulsed dendritic cells (DCs) but also significantly killed autologous primary myeloma cells. The cytotoxicity was MHC class I- and, to a lesser extent, class II-restricted, suggesting that myeloma cells could process Id protein and present Id peptides in the context of their surface MHC molecules. No cytolytic activity against K562 was noted, indicating that the cytotoxicity was not attributed to natural killer cells. The CTLs lysed the target cells mainly through the perforin-mediated pathway.
To explore the possibility of using myeloma cells, which may contain a multitude of tumor antigens that can stimulate an increased repertoire of anti-tumor T cells, as the source of tumor antigens for immunotherapy, myeloma-specific CTLs were generated from patients by culturing T cells with autologous DCs pulsed with myeloma freeze-and-thaw cell lysate. These CTL lines proliferated in response to autologous primary myeloma cells and DCs pulsed with autologous, but not allogeneic, tumor lysate and secreted predominantly IFN-γ and TNF-α. TheCTLs had strong cytotoxic activity against autologous tumor lysate-pulsed DCs and primary myeloma cells. Interestingly, some of the CTLs killed, to a lesser degree, autologous Id-pulsed DCs and allogeneic myeloma cells, suggesting that Id was sometimes a part of tumor antigens present in the tumor lysate and that there were shared tumor antigens between patients. No killing of autologous peripheral blood mononuclear cells, purified B cells, or Epstein-Barr virus-transformed B-cell lines was observed. These data demonstrate that CTLs induced by tumor lysate-pulsed DCs specifically kill autologous tumor cells, but not normal blood cells, and provide a rationale for vaccination with tumor cell-pulsed DCs in myeloma patients.

To evaluate the anti-myeloma effects of specific CTLs in vivo, a myeloma SCID-hu host was established by inoculation of a myeloma cell line, ARK-RS, into the implanted human bones in the mice. After myeloma was established, defined by the appearance in mouse serum of human Ig secreted by the tumor cells, an ARK-RS-specific CTL line was injected into the tumor sites. Adoptive transfer of specific T cells, but not control CTLs, strongly suppressed tumor growth in vivo. Eight weeks after tumor inoculation, control mice and mice received control CTLs had large tumors (5-10 g) around the implanted human bones, while specific CTL-treated mice had no visible tumor mass or circulating human Ig. Two weeks after the final injection, circulating human T cells were still detected in the circulation, indicating that human T cells survived in the host. These findings indicate that myeloma-specific CTLs can control or even eradicate tumor cells in vivo.

In conclusion, our studies demonstrate that Id- and tumor lysate-specific CTLs can be generated from myeloma patients. These CTLs killed specifically primary myeloma cells but not normal blood cells in vitro. Adoptive transfer of myeloma-specific CTLs could eradicate tumor cells in SCID-hu host. Thus, our studies have laid the basis for adoptive immunotherapy in myeloma patients using these CTLs, which may be able to eradicate myeloma cells without causing tissue damage.

References


Supported by grants from the National Cancer Institute (RO1-CA96569) and Multiple Myeloma Research Foundation and McCarty Cancer Foundation, and by Translational Research Grants from the Leukemia and Lymphoma Society (6548-00 and 6041-03).

P13.5 VACCIBODIES: A NOVEL APPROACH FOR ID VACCINATION

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Immunoglobulins (Ig) have highly diversified variable (V) regions that contain unique antigenic determinants called idiotopes (Id). Idiotype vaccination is promising in treatment of B cell lymphomas and multiple myelomas, and both anti-idiotypic antibodies and Id-specific T cells may be of importance (1). However, Id is a weak self-antigen in its original context (as part of Ig). Therefore, for vaccine purposes, it is important to enhance the immunogenicity of Id. To this end, we have now designed a novel type of recombinant Ig-like molecules called Vaccibodies, which hopefully induce both strong Id-specific Ab and T cell responses. The Vaccibodies consist of two scFv’s derived from the myeloma protein M315 connected through Cy3 domains and a hinge to two scFv’s with specificity for MHC class II molecules expressed on APC (Fig 1).

Legend Fig 1. The structure of the Vaccibody. The two scFvs (top) target the Vaccibody to surface molecules on APC. The two patient-derived M-component scFvs are at the bottom. The two types of Fv are connected by a hinge and Cγ3 domains. The hinge contributes to flexibility of the two NH2-terminal scFvs relative orientation and offers disulfide bridges between the monomers. The Cγ3 domains act as a spacer between the NH2 and COOH terminal scFvs and participate in the dimerization through hydrophobic interactions.

Vaccibodies have been prepared for use in the MOPC315 mouse myeloma model in which Id-specific CD4+ T cells protect mice against myeloma development (2-5). The Vaccibodies have been genetically constructed, and transfected NSO cells produce and secrete the recombinant molecules. The Vaccibodies appear to have a correct structure. In particular, the scFv’s at each end of the Vaccibodies exhibit binding to MHC class II and DNP (specificity of M315), respectively. Thus, the domains retain the same folding pattern as in their original context. The Vaccibodies have been constructed to induce strong T cell responses. The produced Vaccibodies are designed to bind MHC class II’ APC and, subsequently to endocytosis and antigen processing, be presented as Id-peptides on class II molecules to Id-specific CD4+ T cells. Furthermore, since they have intact Fv of the M-component, they should bind anti-id B cells and, with the help of Id-specific T cells, induce differentiation of such B cells into plasma cells that produce anti-id antibodies. Moreover, the Vaccibodies lack the Cγ2 domains and hence the FcyR binding sites, and should therefore exclusively be taken up by MHC class II and not by FcRs on APC. We have compared class II-specific Vaccibodies with hapten (NIP)-specific Vaccibodies as a negative (nontargeted) control. Initial experiments in BALB/c
mice demonstrate that class II-specific Vaccibodies are much more potent at inducing anti-Id\textsuperscript{315} antibodies than are NIP-specific Vaccibodies. Moreover, class II-specific Vaccibodies are 100-1000 fold more potent on a per molecule basis at inducing proliferation of Id\textsuperscript{315}-specific CD4\textsuperscript{+} T cells in vitro compared to NIP-specific Vaccibodies. Tumor challenge experiments with MOPC315 are yet to be performed. The plasmids encoding Vaccibodies allow for easy exchange of targeting and patient Fv. Thus, the strategy should be easy to extend to new targeting specificities and new patient Fvs.

Refs.
Scientific sessions

1. Development of normal and malignant plasma cell

001 The Essential Role of Oncogenic FGFR3 in Maintenance of t(4;14) Myeloma.


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Chromosomal translocations to the immunoglobulin heavy-chain locus on chromosome 14q32 are present in the majority of multiple myeloma (MM) patients and may represent the first and defining genetic event that leads to the development of MM. The t(4;14) translocation which occurs in approximately 15% of patients results in the dysregulated expression of fibroblast growth factor receptor 3 (FGFR3) and MMSET. Wild-type FGFR3 appears to be weakly transforming in a hematopoietic murine model. The subsequent acquisition of FGFR3 activating mutations is associated with disease progression and is strongly transforming in experimental models. These findings suggest a pathogenic correlation between FGFR3 expression and myeloma. However, it remains to be proven how dysregulation of FGFR3 mediates an early oncogenic process in MM and whether FGFR3 is required for tumor maintenance. We have used pharmacological inactivation of FGFR3 to address this question directly in human MM.

We have developed 3 screening assays for identification of FGFR3 inhibitors and have used these to establish PD173074 as a selective inhibitor of FGFR3. Using this inhibitor we confirmed that inactivation of FGFR3 blocks its oncogenic potential. We have previously shown that activated forms of FGFR3 induce transformation of NIH 3T3. Using this same assay we tested the ability of PD173074 to inhibit the Y373C-FGFR3 induced transformation of NIH 3T3 cells. Although it had no effect on Ras-induced transformation, it completely inhibited FcRi formation induced by activated FGFR3. Similarly, PD173074 prevented in vivo growth of Y373C-FGFR3 transfected NIH 3T3 cells in nude mice but had no inhibitory effect on growth of Ras V12 expressing cells.

To establish that FGFR3 activation provides a critical and non-redundant pro-proliferative and anti-apoptotic signal in MM we exposed FGFR3 expressing myeloma cell lines to PD173074. PD173074 inhibited cell proliferation of FGFR3 expressing KMS11 and KMS18 cells with an IC50 of 12.5 nM and 20 nM, respectively. 8226 cells, which lack FGFR3 expression, displayed no growth inhibition demonstrating that PD173074 exhibits minimal nonspecific cytotoxicity. Further characterization of this finding demonstrated that inhibition of cell growth is related to G0/G1 cell cycle arrest. PD173074 also induced delayed, dose-responsive apoptosis of these cells. Immunohistochemical analysis demonstrated an increase in cleaved caspase 3 positivity, suggesting that FGFR3 activation protects MM cells from caspase-dependent cell death. To explain the marked delay in apoptosis we speculated that inhibition of FGFR3 in these cells induces cell cycle arrest and differentiation. Inhibition of FGFR3 resulted in the differentiation of KMS11 and KMS18 cells from a plasmablast-like phenotype to a more mature plasma cell characterized morphologically and by the induction of CD31 expression and increase in light chain secretion. In addition, FGFR3 inactivation had similar effects in vivo inducing growth arrest, apoptosis and differentiation of KMS11 tumors in a xenograft mouse model. Most importantly the reversion of the malignant phenotype was associated with delayed tumor progression and enhanced overall survival of PD173074 treated mice. These results provide evidence that FGFR3 is important for genesis and maintenance of myeloma. Further, they validate FGFR3 as a therapeutic target for a subset of MM patients.

002 The role of MMSET in t(4;14) myeloma

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The t(4;14)(p16;q32) translocation, that occurs in about 15-20% of multiple myeloma (MM), causes the concomitant dysregulation of two genes by their juxtaposition to the two immunoglobulin enhancers. FGFR3, is brought on the der(14) under the control of the IgH 3' enhancer and the intrinsic enhancer, Emu, is translocated on der(4) where it dysregulates MMSET expression. The breakpoints are clustered in two groups: one falls in the 5' UTR of MMSET, outside the coding sequence; the other falls into the 5' coding exons, resulting into a N-term truncation of MMSET protein. Although the acquisition by the tumor cells of FGFR3 activating mutations indicates a role for FGFR3 in tumor progression in the few informative cases, we and other investigators reported the loss of der(14) or FGFR3 expression in about 20% of t(4;14) myelomas. On the contrary, there is only one example of a t(4;14) MM that has lost der(4) and does not contain Ig/MMSET hybrid transcripts. Therefore MMSET seems to be the crucial gene in t(4;14) myeloma. MMSET belongs to the trithorax family of nuclear proteins characterized by the presence of a SET domain and several PHD-type zinc fingers and involved in chromatin remodeling. One of them, MLL, is located on 11q23 and translocated in acute leukemia. MMSET, also known as Nsd2, Trx5 and WHSC1, is the gene deleted in Wolf Hirsch Syndrome. Highly related genes, Nsd1 and Nsd3 have also been implicated in neoplastic transformation and found translocated in AML. There are two classes of MMSET mRNA transcripts, based on alternative splicing: MMSET type I encodes for a 674 aa protein, MMSET type II extends at the 3' end of the type I and encodes for a 1365 aa protein. Although we have found that the type I protein can block transformation of NIH3T3 fibroblasts by a variety of oncogenes, there is no direct evidence that MMSET can function as an oncogene. To study the oncogenic contribution of MMSET dysregulation in myeloma we followed two approaches 1) We generated retroviral vectors carrying EGFP, MMSET1 and MMSETII fused to an IRES-neomycin cassette, and infected human myeloma cell lines (HMCL) that do not have a t(4;14) translocation and do not express MMSET; 2) we generated transgenic mice in which MMSETI and –II are under the control of the lex minimal promoter and Emu – a strategy that had previously enabled us and others to generate mice expressing transgenes in B and T cell lineages. Although we obtained HMCL neomycin resistant clones expressing MMSET mRNA, we were unable to detect MMSET protein expression. Under the same condition, however, MMSET exogenous protein was
detected in 293 and NIH3T3 cells. Similarly, MMSET transgenic protein was detected in T cells from thymus, lymph nodes and spleen of the transgenic mice, but not in any B cells, whose number and phenotype were otherwise normal, excluding an MMSET inhibitory effect on B cell development. We conclude that MMSET protein expression must be tightly regulated in B cells and that it shares more features in common with a tumor suppressor gene than with an oncogene.

003 Cancer-Related Potential Target Genes of MMSET in Multiple Myeloma

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In Multiple Myeloma (MM) the frequently detected translocation t(4;14)(p16.3;q32) results in a dysregulation of two potential oncogenes, the recently discovered MM SET domain (MMSET) and of four plant homeo domain (PHD) zinc modulation of chromatin remodelling based on the presence of a putative transcription factor expressed as two mRNA isoforms, and the fibroblast growth factor receptor 3 (FGFR3). MMSET is an oncogene, the recently discovered MM SET domain (MMSET) t(4;14)(p16.3;q32) results in a dysregulation of two potential oncogenes.

In Multiple Myeloma (MM) the frequently detected translocation t(4;14)(p16.3;q32) results in a dysregulation of two potential oncogenes, the recently discovered MM SET domain (MMSET) and of four plant homeo domain (PHD) zinc fingers. In this study the oncogenic role of MMSET in MM has been examined by defining potential target genes of MMSET by a cDNA array analysis.

To investigate the role of MMSET in MM, MMSET type I and II were cloned into a pCMS-EGFP mammalian expression vector. The vectors containing MMSET type I or II were transfected into the human erythroleukemia cell line, K562, expressing MMSET at a low level. Successful transfection was documented by flow cytometry detection of the enhanced green fluorescent protein (EGFP) and subsequent MMSET quantitation by real-time RT-PCR. The EGFP+ K562 cells transfected with the vectors containing MMSET type I or II and with the empty vector were FACs sorted after 6, 12 and 24 h and RNA was extracted. After verifying a high level of MMSET type I or II expression, gene expression profiling was performed by cDNA arrays (U133A, Affymetrix). So far, we have identified 25 genes with a significant upregulation caused by MMSET type I. 3/25 genes (CLI/Clusterin, FOSB and JUN) were selected for further analysis because of their described association with cancer. To examine whether these genes had a relation to MMSET dysregulation in primary MM tumors a quantitative detection by real-time RT-PCR was performed on FACS purified plasma cells from MM patients with an upregulation of either MMSET (n=4) or cyclin D1 (n=4) as detected by real-time RT-PCR. The presence of the t(4;14) translocation in the MMSET+ patients was verified by detection of the IgH-MMSET hybrid transcript. The results documented that an upregulation of CLI/Clusterin, FOSB and JUN was detected exclusively in the patient samples with a MMSET dysregulation. The downstream upregulation of cancer-related genes supports the hypothesis that MMSET is an oncogene.

004 Secondary Translocations Dysregulate c-, N-, or L-MYC in Multiple Myeloma

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Simple, reciprocal chromosomal translocations juxtaposing c-MYC to an Ig locus are an invariant event in murine plasmacytoma tumors. These primary Ig translocations occur early in tumorigenesis as a result of errors in one of two B cell specific DNA modification processes (IgH switch recombination or somatic hypermutation). Expression of the translocated c-MYC allele is dysregulated, whereas the normal c-MYC allele is silent, corresponding to the absence of c-MYC expression in terminally differentiated plasma cells. Although primary Ig translocations are found in a majority of multiple myeloma (MM) tumors, these translocations rarely – if ever - involve c-MYC. Instead, dysregulation of c-, N-, or L-MYC is mediated by secondary (Ig) translocations as a late progression event in MM. Cloned translocation breakpoints infrequently occur within or near V(D)J or switch sequences, consistent with a lack of involvement of B cell specific DNA modification processes. Three color FISH analyses of metaphase chromosomes showed complex translocations and insertions of c-MYC, L-MYC (cell line), or N-MYC (one tumor) in 25 of 29 (86%) human MM cell lines (HMCL) and 18 of 38 (47%) MM tumors examined. These karyotypic abnormalities often are non-reciprocal and frequently involve 3 chromosomes, sometimes with associated inversions, deletions, or duplications. Surprisingly, only half of the karyotypically abnormal MYC loci include associated Ig sequences. The MYC karyotypic abnormalities are associated with dysregulation of a single allele, since all 13 informative MM cell lines express either L-MYC or one of two genetically distinguishable c-MYC alleles; and 2/82 primary tumors express N-MYC but not c- or L-MYC. Corroborative studies by Avet-Loiseau et al have shown that similar karyotypic abnormalities of c-MYC are detected by interphase FISH in 5% of MGUS tumors and 15% of MM tumors, often with heterogeneity within a single tumor specimen. Together, these studies indicate that the dysregulation of one MYC allele is mediated by a complex translocation that is associated with increased proliferation and autonomy from the influence of bone marrow stromal cells. We have used a combination of FISH mapping, and molecular cloning to better understand the structures of complex translocations that do or do not involve an Ig enhancer. Translocations can have breakpoints that occur up to 1 Mb telomeric or centromeric to c-MYC. Also, as little as 100 kb from the c-MYC locus can be inserted at other chromosomal locations, including translocation breakpoints that involve two other chromosomes. For correlations of structure and expression, we have also used a combination of DNA and RNA FISH on HMCL, and also constructed HMCL X mouse plasmacytoma hybrids to correlate abnormal chromosome structures with human MYC expression. A summary of our results will be presented, including the Karpas 620 HMCL that apparently underwent sequential t(11;14) and t(8;11;14) translocations involving the same IgH locus. Large regions of chromosomes 11 and B were duplicated during the second translocation: der14 t(14;11;8) contains and expresses c-MYC; whereas der(8) t(8;14;11) contains c-MYC and CYCLIN D1 but expresses only CYCLIN D1 and not c-MYC. The implications of these various results will be discussed.
HETEROGENEOUS PATTERN OF THE CHROMOSOMAL BREAKPOINTS INVOLVING THE MYC LOCUS IN MULTIPLE MYELOMA

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Chromosomal translocations juxtaposing the MYC locus with one of the immunoglobulin (IG) loci represent an invariant oncogenetic event in Burkitt’s lymphomas and murine plasmacytomatas. In human multiple myeloma (MM), a malignant tumor of somatically mutated, isotype-switched plasma cells, the involvement of the MYC locus has been thought to be a rare event. Conventional cytogenetic studies have identified 8q24 translocations involving the IG loci in less than 5%, and Southern blotting has revealed rearrangements of the MYC locus in only a few cases, generally not involving the IG loci. Over the last few years, novel molecular approaches such as fluorescence in situ hybridization (FISH) have demonstrated that IG translocations, mainly affecting the IGH switch regions, occur in virtually all MM cell lines and most primary tumors, and may involve a large number of target loci. Using this approach, recent studies have shown that MYC locus abnormalities, often characterized by complex translocations and insertions not involving the IG loci, are very common in MM cell lines and represent a recurrent event in MM patients with aggressive disease and a poor outcome. In our study, we used dual-colour FISH to characterize the breakpoint locations of chromosomal translocations/rearrangements involving the MYC locus at 8q24 found in a panel of 14 MM cell lines and 70 primary tumors (66 MM and 4 PCL). MYC locus alterations were observed in 21 cases: MYC/IGH (mainly IGH) fusions in 11 cell lines and three patients (2 MM and 1 PCL), and extra signals and/or abnormal MYC localizations in seven patients (5 MM and 2 PCL). Fourteen of these cases were investigated by FISH analyses using a panel of BAC clones covering about 6 Mb encompassing the MYC locus. The breakpoints were localized in a region 100-250 kb centromeric to MYC in four cases, a region 500-800 kb telomeric to the gene in four cases, and regions ≥ 2 Mb centromeric or telomeric to MYC in five cases. Two different breakpoints were detected in KMS-18 cell line, while the insertion of a MYC allele was found in a complex t(16;22) chromosomal translocation in RPMI8226 cell line. Our data document a relatively high dispersion of 8q24 breakpoints in MM.

S. Fabris and T. Storlazzi contributed equally to this work

Supported by: the Associazione Italiana Ricerca sul Cancro (AIRC) and the Italian Ministry of Health.

Distinct developmental pathways of the multiple myeloma identified by mRNA expression analysis of the chromosomal translocation-associated protooncogenes

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[Purpose] Accumulation of chromosomal translocations involving immunoglobulin heavy chain gene locus is responsible for the development of multiple myeloma (MM). Among them, deregulation of CCND1 and FGFR3 expression is often found in MGUS, indicating that t(11;14) and t(4;14) are the primary translocations, which result in the development of MGUS. However, t(6;14), t(8;14), t(14;16) and t(14;20), by which respectively deregulate MUM1, c-MYC, c-MAF and MAFB genes are rarely found in MGUS, although they are occasionally encountered in MM cases, suggesting that they belong to the secondary translocations, which are relevant to the disease progression of the MM. To pursue the developmental pathways and to classify MM based on the genetic alterations, we established RQ/RT-PCR system to quantify their mRNA expression and applied it to investigate primary MM samples. [Method] mRNA expression of the abovementioned six genes was quantified by RQ/RT-PCR with an aid of Light Cycler. After establishing the optimal conditions, we studied the gene expression in 19 MM cell lines, 30 MM, 4 MGUS and 5 reactive plasmacytosis patients. In patients’ specimens, 1ug of total RNA extracted from purified plasma cells using CD138 bead selection was reverse transcribed for cDNA, diluted and used for RQ-PCR. Standard curves of the gene expression were generated using diluted plasmids. Expression level was corrected using housekeeping gene expression. [Result] In 19 MM cell lines, mRNA expression of the CCND1, FGFR3, c-MAF and MAFB genes was extremely high when chromosomal translocations involving these gene loci exist. In contrast, expression level of the MUM1 and c-MYC mRNA was exclusively higher in 19 MM cell lines than in fresh MM samples irrespective of the status of the chromosomal rearrangements involving these gene loci, suggesting that high expression of these two genes are associated with highly proliferative ability. Of the six genes, 10 out of 19 MM cell lines harbored high expression of more than two genes. Of the 30 MM samples, eight(26%), six(20%) and five(18%) showed ectopic expression of the CCND1, FGFR3 and c-MAF genes. Analogous to the data obtained from cell lines, two and one CCND1+ cases coexpressed high levels of c-MYC and MUM1 genes, respectively. Three out of the six FGFR3+ cases showed ectopic expression of the c-MAF. One c-MAF+FGFR3+c-MYC+ case and one c-MAF+c-MYC+MUM1+ case were diagnosed as having primary plasma cell leukemia and highly aggressive MM, respectively. Interestingly, CCND1+ MM cases tended to carry c-MYC or MUM1 overexpression and never carried c-MAF mRNA expression, and FGFR3+ cases tended to carry c-MAF overexpression (P<.05), vice versa. Moreover, c-MYC overexpression was significantly associated with low levels of hemoglobin concentration, serum albumin and platelet count and high levels of 2-microglobulin and narrow plasmacytosis (P<.05). [Conclusions] Our findings support a
hypothesis that (1) CCND1 and FGFR3 contribute to the early development of MM, followed by (2) c-MYC/MUM1 and c-MAF play crucial roles in the progression of CCND1+ and FGFR3+ MM, respectively. Our diagnostic system will provide a new insight into understanding genetics-based MM classification and into its application for the appropriate molecular targeting therapies.

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IGH REARRANGEMENTS PATTERNS IN MULTIPLE MYELOMA.

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An increased rate of complete responses in myeloma multiple (MM) patients through high dose chemotherapy and transplantation of cell progenitors, has lead to the design of strategies for the analysis of minimal residual disease (MRD). One example is the use of PCR for amplification of immunoglobulin (Ig) gene rearrangements that are specific for each malignancy. Recent studies have shown that “real time quantitative PCR” (RQ-PCR) analysis of the Ig heavy chain gene (IgH) rearrangements can be used for MRD detection in acute lymphoblastic leukemias. This approach is more complicated when applied to B-cell malignancies with somatically mutated lymphoblastic leukaemias. This approach is more complicated when applied to B-cell malignancies with somatically mutated IgH genes like MM. We analyzed the presence of incomplete DH-JH and complete VH-JH rearrangements in a series of 84 MM with DH and VH family primers in combination with a consensus JH primer. Genescanning was used to evaluate the clonality of the rearrangements. After purification in polyacrylamide gels, clonal VH-JH and DH-JH rearrangements were sequenced to identify the VH, DH and JH gene segments. Germline VH, DH and JH segments were identified by comparison to the V, IGMT and BLAST databases. These analyses also allowed the assessment of somatic mutations in both VH-JH and DH-JH rearrangements. The overall detection rate of clonality was 94% (79/84). In particular, VH-JH rearrangements were detected in 73/84 patients (84%). Moreover, more than 50% of patients displayed incomplete DH-JH rearrangements (50 out of the 84 patients, 60%). Of the 11 patients in whom no VH-JH product could be obtained, the DH-JH PCR showed a monoclonal band in 6 of them. All VH segments carried >2% deviation from the germline sequence compared to molecular databases (median 7.2%; range: 2-22). By contrast, 88% of the incomplete DH-JH rearrangements possesses >98% homology with its closest germline gene segments. We also observed a biased VH, DH and JH gene segment usage. In VH-JH rearrangements we found an over-representation of VH3-30, VH1-69, and VH3-9, DH3-22 and DH2-21 as well as JH4. In DH-JH rearrangements we found an over-representation of DH1-7 and DH4-4, and JH4 gene segments. In conclusion, incomplete DH-JH rearrangements are present in 60% of MM patients with the special feature that the vast majority of them are unmaturated which make them the preferential target for MRD studies in MM patients by RQ-PCR using consensus JH probes. Furthermore, these findings offer a new insight into the regulatory development model of IgH rearrangements.

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DNA mismatch repair defects in the pathogenesis and evolution of myeloma

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Genetic instability is a prominent feature in multiple myeloma (MM) and progression of this disease from MGUS and smouldering MM is associated with increasing molecular and chromosomal abnormalities. The mismatch repair (MMR) pathway is a post replicational DNA repair system that maintains genetic stability by repairing mismatched bases and insertion deletion loops mistakenly incorporated during DNA replication. Deficiencies in proteins pivotal to this pathway result in a higher mutation rate, particularly at microsatellites. We have investigated the proficiency of the MMR pathway in clinical samples and myeloma cell lines. Microsatellite analyses showed instability at one or more loci of 9 examined in 15/92 patients: 7.7% of MGUS/MM, 19.3% of MM/plasma cell leukaemia and 25% relapsed MM/PCL. An in vitro heteroduplex G/T repair assay found reduced repair in 2 cell lines, JIM1 and JIM3 and in 2 from 4 PCL samples. Thus we show that microsatellite instability occurs in plasma cell dyscrasias and the increased frequency during more active stages of disease may suggest defects in the MMR pathway have a contributory role in disease progression.

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Cell Cycle Control of Plasma Cell Differentiation and Tumorigenesis

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Cell cycle control and apoptosis are major determinants of homeostasis during B cell differentiation and tumorigenesis. Although multiple myeloma cells rarely cycle and normal plasma cells are cell cycle arrested, virtually nothing is known about cell cycle control of B cell differentiation. Frequent translocation of D type cyclins in MM cells implies that cell cycle dysregulation may contribute to MM pathogenesis. Cell cycle progression, however, is controlled not by the absolute levels of D type cyclins, but by the balance between positive regulators, cylins together with cyclin-dependent kinases (CDK), and negative regulators, the CDK inhibitors. (CDKI) This suggests a critical role for CDKIs in plasma cell generation and tumorigenesis. To test this hypothesis, we have dissected the roles of CDKIs in normal plasma cell differentiation. We demonstrate that one specific CDKI, p18INK4c, is elevated by IL-6 to cause G1 cell cycle arrest through inhibition of Rb phosphorylation by CDK6 during differentiation of human lymphoblastoid cells to plasma cells (1). Targeted disruption of p18INK4c in mouse leads to B and T cell hyperproliferation (2). Most significantly, plasma cell differentiation in vivo and in vitro is defective in the absence of p18INK4c, but not other CDKIs (3). The requirement for p18INK4c is temporal specific, because there is no impairment in the formation of germinal centers and memory cells, class switch recombination or bone homing in a T-cell dependent antibody response. Syndecan-1- positive plasmacytoid cells containing high levels of secreted-form of immunoglobulin are generated in the absence of p18INK4c. However, they fail to efficiently
differentiate to antibody-secreting plasma cells and are eliminated by apoptosis. Thus, p18INK4c is specifically required for cell cycle arrest and differentiation of functional plasma cells, and it modulates plasmacytoid cell survival. Given that MM cells may represent inappropriate intermediates of plasma cell differentiation that survive in the bone microenvironment, work is in progress to address the expression of CDKI in MM cells. A model for coordinated cell cycle and apoptosis control in MM pathogenesis will be discussed.

Supported by NIH grants (CA 80204, AR49436) and a Specialized Center of Research for Myeloma grant by the Leukemia and Lymphoma Society of America.


Molecular Analysis of the Mitotic Checkpoint Genes BUB1, BUBR1 MAD1L1, MAD2, and MAD2B in Multiple Myeloma

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Introduction: Chromosomal instability (CIN) occurs in the context of defective mitotic checkpoints, as in colorectal cancer, with the end result being aneuploidy. Multiple myeloma (MM) is characterized by ubiquitous aneuploidy, which is an early event detectable in MGUS. Hyperdiploidy, characterized by gains of chromosomes 3, 5, 7, 9, 11, and 15, is seen in 50% of cases, predominantly those without IgH translocations. Key mitotic checkpoint genes include BUB1, BUBR1, MAD1L1, MAD2 and MAD2B. We therefore assayed for abnormalities of these genes in MM.

Samples and Methods: Our analysis included FISH, Southern-blot, Northern-blot, molecular screening and sequencing of these genes. We studied 5 human MM cell lines; JNJ3, OCI-MYS, MM1, KAS 6/1, ANBL-6 (all harbor IgH translocations) and 10 patients with MM and no IgH translocations (by FISH). Northern and Southern blot analysis were done on the cell lines. Both patient samples and MM cell lines were screened for mutations using conformation sensitive gel electrophoresis followed by manual screening in abnormal cases. BAC clones including the genomic loci of these genes were used as FISH probes. Interphase FISH combined with the cytoplasmic light-chain and cytomorphology were used to analyze the MM patients. Metaphase and interphase FISH was used for the analysis of the cell lines.

Results: No abnormal qualitative RNA production was detected by Northern blot analysis of the cell lines. The genomic loci appeared intact as Southern blot, using EcoRI digested fragments, did not reveal large deletions, insertions or inversions with the exception of a point mutation in MAD2B IVS(4) which appeared to be polymorphic. RNA sequencing of the BUBR1 gene revealed three single base alterations causing the following transitions: 161C>T (T40M), 1088A>G(Q349R) and 1895T>C(V619A). Population studies from 100 normal individuals revealed these transitions are polymorphic. RNA sequencing of the BUBR1 gene did not reveal large deletions, insertions or inversions with the exception of a point mutation in MAD2B IVS(4) which appeared intact as Southern blot, using EcoRI digested fragments.

Concurrent with the genomic profiling, cells were analyzed by genomic profiling and interphase fluorescent in situ hybridization (FISH) analysis from 10 MM patients revealed no predominant deletion pattern for any of the 5 checkpoint genes. FISH analysis on the cell lines displayed abnormal deletion patterns in 3 of the checkpoint genes. Kas 6/1 showed deletion of BUB1 in 40% of metaphases while OCI-MYS showed deletions of MAD2 and MAD1L1 in each metaphase observed.

Conclusion: Our preliminary findings indicate that mutational inactivation of BUBR1, MAD1L1 and MAD2 could result in defective checkpoint allowing the generation of aneuploidy in some cases (30%) of MM. However none of these mutations were constant. Further investigations into the role of mitotic checkpoint genes and their relationship to aneuploidy is warranted.

Establishment of the JMW Myeloma Cell Line: In vitro Analysis of Multiple Myeloma Clonal Evolution

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Human multiple myeloma (MM) cell lines have proven to be useful tools in understanding this progressive disease. However, establishment of human myeloma cell lines is a difficult task and a further complication is the uncertain relationship between in vivo tumor cells and tumor cells that survive in vitro giving rise to cell lines. Thus, the selection pressures in vitro may differ markedly from in vivo selection pressures. We wished to study this more closely and have done so by genomic profiling and interphase fluorescent in situ hybridization (FISH) analysis from the initial stage of procurement of patient tumor cells to permanence as a cell line (designated as JMW).

The IgA- expressing JMW cell line was derived from the blood of a 67-year-old female presenting with aggressive MM and many circulating plasma cells. Purified myeloma cells were cultured in RPMI 1640 media with MCS, IL-6, and IGF-1. The JMW cell line is CD2-, CD5-, CD19-, CD38+, CD40+, CD44+, CD28+, and EBV negative. The IgVH sequence of the cell line is identical to the sequence of the primary tumor cells (VH 4-39 with 6.8% somatic mutations). This cell line is IL-6 dependent, but also displays a smaller proliferative response to IGF-1. Interferons alpha and gamma inhibited proliferation of the cell line whereas IL-1, IL-2, IL-3, IL-4, IL-10, IL-11, IL-12, GM-CSF and TGF-beta were without effect on proliferation. DNA from both the initial cell population and the established line was used for cDNA array analysis using the Affymetrix U95Av2 biochip. A significant number of genes were differentially expressed between the two time points and these results will be presented. Genes of interest include HSP 70, IAP-1, IGFBP-4 and several human ribosomal proteins.

Concurrent with the genomic profiling, cells were analyzed by FISH for genetic abnormalities. The (t;14)(p16.3;q32) was detected since the time of diagnosis in nearly all cells and was conserved throughout disease evolution, and in the stable cell line. As expected monosomy 13 (94-97% of the cells) was present in all samples. Patient tumor cells did have a complex karyotype that was shown both by karyotype analysis and by FISH to include an unbalanced complex translocation resulting in LOH of 13q and 17p (der13 t(13;17)(q14;p11)), in the context of a hypodiploid karyotype. FISH analysis shows divergence in the chromosome complexity between the cell line and subsequent
differentiate into both a memory B-cell and a PC. In this study, germinal center (GC) or post GC B-cell. The GC B-cell can

Introduction. It is believed that myeloma cells are derived from a lymph nodes recirculate through bone marrow, peripheral blood and

One of the striking features that emerged from immunoglobulin variable (V) region analysis in multiple myeloma (MM) is that a specific population of B-cells expressing the IgH V4-34 gene is excluded from the myelomagenic pathway. Other features have established a post-follicular stage of neoplastic arrest. This asymmetry in VH gene use in MM contrasts markedly with usage in the normal B-cell repertoire of ~7%, and derivation of a range of other B-cell tumors from such cells. Recently, it was reported that there may be a developmental censoring of normal B-cells to end-differentiated plasma cells, using a MoAb (9G4) specific for V4-34. Such censoring could also explain the fact that V4-34 is not found in malignant plasma cells. To address this further, we have examined V4-34 at the gene level in purified normal plasma cells from bone marrow, and in plasma cells generated in-vitro from normal circulating CD19+ B cells. We also examined in-vitro matured plasma cells from circulating B-cells from a MM patient. In both the normal and tumor setting, we detected isotype-switched somatically mutated V4-34 functional sequences, indicating no block in maturation. Interestingly, in >50% of cases, somatic mutation had occurred in sequences involved in binding-site-associated idiotope expression. Our data suggest that normal B cells expressing V4-34-encoded Ig can mature to plasma cells in-vivo and in-vitro, but emerging cells may lose reactivity to 9G4 by somatic mutation. Failure to detect V4-34 in plasma cell tumors is therefore not due to a maturational defect, but is likely to be a tumor-specific feature.

In multiple myeloma clonotypic memory B-cells recirculate through bone marrow, peripheral blood and lymph nodes

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Introduction. It is believed that myeloma cells are derived from a germinal center (GC) or post GC B-cell. The GC B-cell can differentiate into both a memory B-cell and a PC. In this study, we investigated the recirculating potential of memory B-cells clonally related to the myeloma plasma cell (termed clonotypic).

Materials and methods. From 10 multiple myeloma (MM) patients bone marrow (BM) aspirates obtained at time of diagnosis and peripheral blood (PB) samples were collected. From 7 out of the 10 MM patients a single peripheral lymph node (PLN) was aspirated. The VHDH immunoglobulin gene rearrangement that represents the MM clone was identified for the 10 MM patients and allele-specific oligonucleotides (ASO) IgH RT-PCR assays were designed for each patient. BM mononuclear cells (BMMC) and PBMC were stained with the monoclonal antibodies CD19, CD27, CD38, CD62L, CCR6, CXC4, CCR5, CCR7 and different memory B-cell subsets were flow-sorted as single cells directly to PCR tubes followed by ASO RT-PCR analysis.

Results. Clonotypic memory B-cells were identified in 7/10 patients and both CD62L positive and negative clonotypic memory B-cells were identified suggesting the presence of clonotypic memory B-cells with different migration/homing potential. Further, clonotypic memory and later stage B-cells (CD38+) were identified in CXC4+/− subsets, whereas all clonotypic memory and later stage B-cells were CCR5 positive. Comparable frequencies of clonotypic cells were found in the CCR6+/− memory B-cell subsets, but only few clonotypic CCR7+ memory B-cells were observed in a single patient. Different clonotypic memory B-cell subsets were identified in both PB and BM.

To extend these studies we investigated whether clonotypic cells were present in PLNs obtained from 7 myeloma patients. In 2 out of 7 patients we were able to identify clonotypic cells in the PLN illustrating that a subset of clonotypic cells enters the PLNs.

Discussion. We identified a CD19+/CD27+/CD38− subset of clonotypic cells in the majority of MM patients and as all clonotypic cells have accumulated somatic mutations, these cells meet all the characteristics of memory B-cells. The heterogeneous expression of the CD62L, CXC4, CCR5 and CCR6 molecules on clonotypic memory B-cells probably reflects their diverse homing/recirculating possibilities including a potential to extravasate secondary lymphoid organs. In accordance with their immunophenotype, clonotypic memory B-cells were identified in PLNs. Clonotypic memory B-cells seem to have the same diverse recirculating/homing capacity as normal memory B-cells. Although the clonotypic memory B-cells showed a normal immunophenotype and recirculating potential, these cells comprised up to 4% of the memory B-cell pool. The malignant potential of clonotypic memory B-cells is currently under investigation.

Identification of a new human plasma cell subset from which myeloma and ‘progressive’ MGUS are derived


Plasma cells in MGUS patients are heterogeneous with respect to phenotype, karyotype, and degree of intraclonal variation. A correlation between plasma cell characteristics and clinical outcome has not yet been demonstrated, partly because progression to myeloma is a rare event. However, many patients do show progression, with gradually increasing paraprotein,
plasma cell levels, or degree of immuneparesis. In order to determine whether plasma cell biology can predict progressive disease characteristics, we assessed the CD19 expression of marrow plasma cells in 88 MGUS patients followed for a median of 34 months (range 12 – 55).

Patients with predominantly CD19- plasma cells (the pattern seen in myeloma) had a high probability of disease progression: 10/39 (26%) showed evidence of increasing tumour burden at a median of 31 months. In contrast, those with a mixture of CD19+ (seen in normal bone marrow) and CD19- plasma cells had an extremely low probability of disease progression: only 1/49 patients (2%) had a raising paraprotein at 47 months (Log Rank P<0.0001).

Significantly, 6/49 (12%) patients in this group showed a decreasing paraprotein level with time, raising the possibility that the paraprotein resulted from a reactive process.

This was supported by assessment of intracranial light-chain expression in 6 MGUS patients with both CD19+ and CD19- plasma cells: CD19+ plasma cells were always polyclonal, whilst the CD19- plasma cells were monoclonal in 4/6 but polyclonal in 2/6 cases. Detailed analysis of normal bone marrow from healthy donors with no paraprotein (n=20) confirmed that CD19- plasma cells with a normal kappa:lambda ratio are frequently present, representing a median 14% (range 0 – 54%) of total plasma cells. It is therefore likely that some cases of MGUS represent an expansion of ‘monotypic’ but reactive CD19- plasma cells.

A ramification of this finding is that some genes/proteins reported to differentiate “neoplastic” and “normal” plasma cells actually represent differences that occur normally between the predominant CD19+ and the rare CD19- subset from which myeloma is derived. To assess this, we have performed detailed phenotypic analysis on a series of normal (n=10) and neoplastic (MGUS n=20, myeloma n=20) plasma cells using a panel of 66 antigens identifying stage of differentiation, chemokine/cytokine receptors, and markers identified from microarray analyses.

Antigens such as CD9/CD28/CD39/CD63/CD56/CD81 discriminate normal plasma cell subsets, rather than discriminating normal from neoplastic. However, antigens such as CD27(TNF7)/CD40/CD117/MPC-1 do discriminate neoplastic cells from their normal CD19- counterparts. CD19 downregulation is not linked to class-switching, as similar proportions of both subsets secrete IgM. However, the high bcl-2 (median 2.0, range 1.2 – 4.4 fold increased) and lack of fas (CD95) expression by CD19- plasma cells compared to their CD19+ counterparts suggests that the cells may represent long- and short-lived plasma cells respectively.

In conclusion, our results from normal donors raise the possibility that CD19- plasma cells in those patients with non-progressive MGUS may represent an expanded non-neoplastic population. Understanding the normal function and differentiation pathway of this CD19- plasma cell population will be critical to further understanding of the pathogenesis of myeloma.

015 Expression profiles of transcriptional factors associated with B lymphopoiesis in multiple myeloma

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Background: There is a growing body of literature indicating that mutations in certain transcriptional factors such as AML1, C/EBPα, and PU.1 are linked to leukemogenesis of acute leukemia. Multiple myeloma (MM) is a neoplasm of plasma cells that is constituted terminal stage of B-cell development. In this study, we asked whether genetic alterations in transcriptional factors associated with B cell maturation play a role in the oncogenesis of MM. We examined four genes, B lymphocyte induced maturation protein (Blimp-1), nuclear factor (p65) in human B cells (NF-κB), PU.1, and NF-κB. Blimp-1 and XBP-1 are transcriptional factors specifically required for the terminal differentiation of B-lymphocytes to plasma cells. Transfection of Blimp-1 into murine B-cell lymphoma cells results in differentiation to plasma cells (Turner, et al, Cell, 1994; 77: 297). XBP-1 knockout mice show deficiency of plasma cells (Reimold et al. Nature, 2001; 412: 300). PU.1 gene controls differentiation of B cells and macrophages. In PU.1 knockout mice, the development of myeloid and lymphoid cells is impaired (Scott et al, Science, 1994; 265: 1573). NF-κB is up-regulated in MM cells and has been an experimental target for therapy of MM.

Materials and Methods: Myeloma cells were purified from bone marrow (n=35), plasmacytoma (n=2), peripheral blood (n=2), pleural effusion (n=1) samples by negative selection method using immunomagnetic beads. Plasma cells from MGUS (n=3) and reactive plasmacytosis (n=1) were also purified. Expressions of Blimp-1, XBP-1, p65 (a component of NF-κB), and PU.1 were examined using RT-PCR and the PCR products were subjected to direct sequencing. In search for activation of XBP-1, PCR products of XBP-1 was digested by ApaLI since ApaLI site exists in 26 bp intron of XBP-1 mRNA which is spliced out by ER stress. We performed same analysis against 8 MM cell lines and 7 non-MM lines.

Results: Blimp-1, XBP-1 and p65 mRNA were found to be expressed in all myeloma cell lines but also in other non-MM cell lines. All 36 freshly isolated MM samples showed the expression of Blimp-1 and XBP-1 mRNA, while no mutations were identified. XBP-1 was active in 25 of 34 MM cases, suggesting the presence of ER stress in those cases. PU.1 mRNA was detected in 3 of 8 MM cell lines, and 22 of 40 MM cases while no mutations were seen in these samples.

Conclusion: The present data showing that Blimp-1, XBP-1, and p65 were expressed in all MM cell lines and fresh MM cells, while there was heterogeneity of expression of PU.1 in these samples. Accompanying no mutations in such factors suggest the oncogenic events in MM might differ from the leukemogenesis of acute leukemia, although other B-cell/plasma cell-associated transcriptional factors have to be examined. Additionally, it might be important to know the significance of heterogeneity in expression of PU.1 and activation of XBP-1 gene in MM cells.
016 NEUTRAL ENDOPEPTIDASE (CD10) KNOCKOUT MICE DEVELOP B CELL LYMPHOMAS AND PLASMACYTOSIS
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BACKGROUND: Neutral endopeptidase (CD10) is a metalloprotease that reduces the cellular response to certain peptide hormones. CD10 has been found to play a role in B cell maturation. However, its precise function and mechanism of action are unknown. Moreover, the role of CD10 in lymphomagenesis has not been previously investigated.

DESIGN: CD10 knockout mice (CD10KO) were sacrificed and autopsied at 6, 10, and 16 months and compared to wild type (WT) controls for changes in lymphoid compartments and for the development of tumors. Spleen, bone marrow, thymus, lymph node and tumor sections were analyzed for morphologic changes as well as for expression of B220 (CD45R), CD3, CD138, IgG, IgM, Igκ, and Igλ. Relative numbers of B, T, and plasma cell subsets (PCs) were compared using image analysis.

RESULTS: At 6 months of age, there were no gross abnormalities in the mice (3KO and 3 WT). At 10 months, 3 WT mice were phenotypically normal, but 7 of 10 (70%) CD10KO had large mesenteric masses that showed follicular hyperplasia and plasmacytosis, usually marked. At 16 months, 5 WT mice were phenotypically normal but all 5 CD10KO had large mesenteric masses; 3 large B cell lymphomas and 2 nodular lesions, possibly follicular lymphomas. In addition, all the 16 month old CD10KO had marked marrow IgM plasmacytosis. The percentage of IgG PCs was markedly reduced.

CONCLUSION: This study shows that CD10 is required for normal B cell maturation and plasmacytogenesis. It appears that in the absence of CD10, there is a buildup of IgM PCs with failure to undergo normal immunoglobulin heavy chain class switching. In addition, these results suggest that CD10 may function as a tumor suppressor.

017 Hereditary Multiple Myeloma (MM): An International Consortium for MM Family Studies
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Inherited genetic factors in the development of multiple myeloma (MM) have received limited attention; the rarity of extended MM families is an obstacle to progress. There have been several reports describing familial clustering of myeloma within families however a systematic approaches to delineate a genetic predisposition is lacking.

We have identified 15 MM families with one or more members affected with MM. Many of the relatives in these families present with pancreatic cancer or malignant melanoma. Some of the families also have members with chronic lymphocytic leukemia or lymphoma, as well as solid tumors of the colon and breast. In two of the families, individuals have been diagnosed with monoclonal gammopathy of unknown significance (MGUS) in addition to the MM cases. We have collected biological specimens from 53 individuals in these families. Of the 53 samples, 27 have been tested for CDKN2A (p16) mutations. No alterations were detected in 26 of these, while one showed an alteration of unknown significance. We have plans to test all of the samples and any additional samples from new families for CDKN2A mutations, as well as, other candidate genes. We also plan to carry out general linkage studies to localize potential susceptibility genes.

The occurrence of familial MM is an important phenomenon and discovery of its genetic basis may illuminate biological pathways of MM development and contribute to pharmacotherapeutic advances. However, a sufficient number of informative families are needed to accomplish these goals. Therefore we propose an international consortium to study familial MM and invite interested colleagues to participate. A web site is available, http://medicine.creighton.edu/HGCC for rapid communication between members in the consortium.

018 Risk Factors For Multiple Myeloma: A Possible Role For Breast Implants
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Although autoimmune like symptoms have been attributed to silicone gel-filled breast implant (SGBI) exposure, few studies have documented this link. Dr. Potter’s group discovered that silicone gel removed from commercial mammary implants and injected intraperitoneally into BALB/c mice induced multiple myeloma (MM) development at high rates. We subsequently noted a high incidence of breast implant exposure in our clinical practice. As a pilot study, serum samples were obtained from a cohort of 630 women with SGBIs followed in a rheumatology practice. Elevated quantitative immunoglobulin levels were found in 23% of the samples and a monoclonal gammapathy was present in 1.7% of a smaller 284 patient cohort. Consequently, we initiated a case-control trial to determine the incidence of SGBI exposure in women diagnosed with MM. California Cancer Registry data was used to contact women who developed MM between the years 1991 and 1997. It had been estimated that 91% of women with SGBIs are < 60 years of age so only such women were interviewed to reduce study cost. A spouse proxy was interviewed if available for deceased cases. Cases were asked about occupational, medical and social history. In order to match socioeconomic status, controls of similar age and ethnicity were ascertained by random digit phone dialing using the area code and prefix of the corresponding case. Up to 5 controls were interviewed for each of the 208 Caucasian cases. Control women were required to be within 5 years of age of the case. Spouse proxies were also interviewed and served as the control for cases in which a proxy was used. All exposures occurring in the cases and controls after the date of MM diagnosis were excluded in the analysis. Breast implant usage was associated with an unadjusted odds ratio of 2.31 on univariate analysis (95% C.L. 1.01-5.26, p=0.048). Prior work on a farm, in an airplane, around microwaves, or use of a computer were also risk factors for MM development. A higher incidence of complications such as pain and contracture was noted in the cases with breast implants vs. controls with breast implants after chi-squared analysis. The adjusted odds ratio for breast implants with contracture was 4.74 (95% C.L. 1.44-15.68, p=0.01). These results suggest that breast implant exposure may contribute to the development of MM. A larger national registry study or prospective analysis of women currently receiving SGBIs may be required to increase statistical power and conclusively link or refute this association. The higher incidence of MM in farm workers has been noted by others and
may be related to pesticide exposure. The higher incidence of computer use and lower educational background of the cases is difficult to explain although electromagnetic radiation could be implicated in the former.

**019 COEXISTENCE OF PRODUCTIVE AND NON-PRODUCTIVE IGH GENE REARRANGEMENTS IN MULTIPLE MYELOMA**

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Multiple Myeloma (MM) is a B-cell neoplastic disease characterized by clonal expansion of plasma cells in the bone marrow. Analysis of the VDJ rearrangement of the immunoglobulin heavy chain (Igh) gene provides a unique clonotypic marker to monitor the residual tumor cells and to verify the efficacy of chemotherapy. The VDJ rearrangement is considered to remain constant in the patient thorough the course of the disease; furthermore, the presence of independent clones in the same MM patient is rarely reported, mostly in association with a second hematological malignancy.

The aim of this study was to assess the recurrence of specific clones in a panel of consecutive MM patients included in a high-dose treatment program enrolled at our Department from January 1999 to December 2002. Ninety consecutive MM patients were first screened at the genomic DNA level; mRNA analysis was subsequently performed in those patients showing multiple rearrangements to verify the real coexistence of multiple productive clones evolved independently or the presence of rearrangements involving both alleles of a single cell. VDJ rearrangement was determined using different set of VH family-specific primers for FR1. PCR products were subjected to capillary electrophoresis and analyzed by gene scanning in an automated DNA sequencer ABI Prism 310. A monoclonal population was detected in 64/90 (71%); three patients (3%) showed double rearrangements. These rearrangements used different VH, D and JH genes, as confirmed by sequencing. Total RNA from samples collected prior to high-dose therapy was reverse transcribed using random primers and cDNA was separately amplified with the same VH family-specific primers which gave positivity at the DNA level. PCR products were analyzed by gene scanning. The absence of contaminating DNA was assessed using primers for the beta-actin gene, designed to discriminate between DNA and RNA.

In two of the three patients a monoclonal peak was detected on cDNA only with one VH-family. This finding confirms the hypothesis of rearrangements involving both alleles, one of which was a non-productive rearrangement. In the third patient, two monoclonal population were identified, for family VH.1 and VH.3 respectively. In this case two monoclonal population are really detectable, although the presence of an associated, clinically silent disease, can not be excluded. Biclonality or oligoclonality are widely documented for ALL and lymphomas; this study strongly suggests that multiple rearrangements could be identified even in a small number of MM patients (~3%), although the presence of more than one functional clone remains a rare event (~1%). In these cases mRNA analysis is mandatory to distinguish between real biclonality and productive or non-productive rearrangements involving the same cell and to monitor residual tumor plasma cells. Clinical and molecular follow-up using tumor-specific primers of the patients with double clonality is currently under way.

**020 Isolation and culture of human multiple myeloma medullary plasmocytes**

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 Aim: Studies of freshly isolated multiple myeloma patient bone marrow samples are often limited because of massive and rapid cell death. So far, no human myeloma cell line has been obtained from patients with medullary involvement alone. We are developing a plasma cell culture, which keeps some of them alive after a week. Materials and methods: Bone marrow from five multiple myeloma patients (stage III) at diagnosis were collected into heparin with the patient’s informed consent. Mononuclear cells were isolated by Ficoll Hypaque sedimentation, then plasma cells were purified using the anti-CD138 plasma cell isolation system (Miltenyi-Biotech). Purity of isolated plasma cells was determined by incubation with PE anti-CD138 monoclonal antibody and analyzed on a FACScalibur flow cytometry system (Becton Dickinson). Purified plasma cells were used immediately or frozen in fetal calf serum containing 10 % DMSO and stored in liquid nitrogen before use. Fresh or thawed plasma cells were maintained in liquid culture in ISCOVE medium with IL-6, GM-CSF, IL-3 and SCF for 8 days. On day 0, 4 and 8, the plasmocyte culture was examined for viability (using trypan blue dye exclusion), morphology (air-dried cytopsin stained with Wright-Giemsa) and cell surface phenotype (CD38 and CD138 antibodies). Results: After immunomagnetic bead separation, purity was 76% (55%-98%) and yield was 54% (11%-100%). On day 0, the cell count was started with a cell range from 0.6 to 4.6 X 10^6 plasmocytes. After a 4-day culture, 41% of myeloma cells (15%-69%) were viable as assayed by trypan blue and checked by morphological examination. On day 8, the mean plasmocyte number decreased to 15% (4%-24%). In terms of viability, we didn’t observe any difference starting the culture with fresh plasma cells or after thawing. FACSc analysis was very homogeneous, CD138 decreasing steadily during the cell culture as cells were dying and failed to express Syndecan-1. CD38 had a higher expression compared to CD138 because of a more non-specific staining.

Conclusion: In this preliminary study, we showed that the combined use of IL-6, GM-CSF, IL-3 and SCF enabled some viability of medullary plasma cells during at least an 8-day culture and as such avoided the common early death of freshly uprooted myeloma cells. This culture method could be used for short-term functional or cytogenetic studies.
2. Genetic heterogeneity in MM: impact on diagnosis and therapy

2.1 IGH rearrangement

Low frequency of IGH rearrangement in UK plasma cell dyscrasias.

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Interphase FISH has been carried out on CD138-purified plasma cells from 251 cases of plasma cell dyscrasia (2 PCL, 219 MM, 2 MGUS, 3 primary amyloidosis) from 32 UK cases who 75% cases were studied at diagnosis. 95% were abnormal when tested for IGH rearrangement, 13q14 loss, p53 loss, or numbers of centromeres of chromosomes 3, 6, 7, 9, 10, 11 and 17. Clear associations between these abnormalities were observed. Only 43% overall showed an IGH translocation (44% MM, 100% PCL, 26% MGUS, 66% PA), much less than expected from the literature. However, the overall rates of t(11;14) (16%), t(4;14) (7%) and t(14;16) (3%) were not significantly different from expected. Part of the explanation for the low IGH abnormality rate may have been due to a higher proportion of older patients in our series, as we saw a clear trend towards decreasing IGH rearrangement with increasing age (53% in the 40s to 32% aged 80+).

IGH rearrangement was slightly increased in IgA cases (53%) relative to IgG cases (37%) and t(4;14) was significantly over-represented in IgA cases (18%) vs 3% in IgG cases. The t(11;14) was seen in 2/3 non-secretory MM. MGUS cases did not show t(4;14) or t(14;16). 29% of t(4;14) cases had lost the signal for the derived 14 in a high proportion of cells, confirming that the FGFR3/IGH fusion is less important in maintenance of myeloma than the MMSET/IGH fusion on the der(t4). 37% cases overall showed deletion of Rb and/or D13S319 from chromosome 13. The rate was higher in MM than MGUS (40% vs 15%). The 6 cases of MM known to have had a preceding MGUS had a 66% del(13) rate lending support to the theory that acquisition of del(13) may be involved with the development of MM from MGUS.

64% cases appeared to be substantially hyperdiploid. As expected, these had a lower frequency of del(13) (25%) than those cases that were probably pseudodiploid or hypodiploid (51%). 88% of cases with no visible numerical abnormalities (counting del(13) as a numerical change) had t(11;14). The t(11;14) was rarely associated with del(13) (18%) or hypodiploidy (15%). The t(4;14) and t(14;16) cases showed del(13) in 76% and 71% respectively. However, conventional cytogenetic results available for 3 of these cases with apparently normal 13s indicated that loss of 13 relative to a near-triploid or near-tetraploid karyotype had been masked. The t(4;14) cases also showed a reduced rate of significant hyperdiploidy (18%) but had evidence of many more abnormalities of single chromosomes than t(11;14) cases.

Only 8 cases (3%) had significant deletion of p53. However 78% of 14 cases with an additional 17 centromere did not show an extra p53. It is unclear whether this relative loss of p53 is significant. Only one of 50 cases tested for ATM deletion was abnormal indicating this is not an important cause of chromosome instability in myeloma. Thus FISH studies are continuing to demonstrate and refine patterns of chromosome abnormalities and their association with other features of myeloma.

14q32 translocations in multiple myeloma patients

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Translocations involving the immunoglobulin heavy chain (IGH) genes are frequent in multiple myeloma (MM) patients. Using conventional cytogenetics, MM can be separated into two groups according to the chromosome number pattern, and 14q32 translocations are more frequently associated with hypodiploid than with hyperdiploid karyotypes (Smadja et al, Blood, 2001 : total IGH rearrangements 31%, hyperdiploid group 11%, hypodiploid group 56%). However, conventional cytogenetics misses cryptic translocations, especially t(4;14)(p16;q32). Furthermore, recent interphase fluorescent in situ hybridization (FISH) studies found 14q32 translocations in as much as 75% MM patients. In order to identify in which CC group we failed to detect these translocations, we design a study using FISH with a dual color break apart IGH probe on previously R-banded metaphases, allowing to detect both 14q32 translocations and overall chromosomal abnormalities in a new series of 55 patients with abnormal karyotypes. Upon conventional cytogenetics analysis, 2/29 hyperdiploid (7%) and 9/26 hypodiploid patients (35%) had a 14q32 translocation. Using the FISH assay, twelve t(4;14) were identified, in 2 hyperdiploid (7%) and 10 hypodiploid (38.5%) cases, respectively. This abnormality was always associated with a chromosome 13 monosomy. We therefore confirm that 14q32 translocations are much more frequent in hypodiploid (73.5%) than in hyperdiploid patients (14%), (p< .0001), and that cryptic t(4;14)(p16;q32) is strongly associated with hypodiploid karyotypes (p< .01). This study also confirms the heterogeneity of the hypodiploid group, in which several subgroups can be recognized depending on IGH genes translocations. Using this reliable assay, only 42% of patients had a 14q32 translocation.

Detection of chromosomal breakpoints affecting the IGH, IGL and IGK loci by interphase FISH in MM and MGUS with normal karyotype.

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The routine application of conventional cytogenetic techniques for the diagnosis of plasma cell disorders (PCD) is often unsuccessful. The percentage of patients with non-informative karyotypes is around 60-70% in multiple myeloma (MM) and
cases were less informative. Only 4 cases (8%) presented signal detected at least one structural or numerical chromosomal alteration in the IGL and IGK loci (Martín-Subero et al., Int J Cancer 2002) by FISH in 50 MM and 50 MGUS with normal karyotype. From all these patients, cytogenetic suspensions from bone marrow samples were studied by interphase cytogenetics. A triple-color assay for the IGH locus and double-color assays for the IGL and IGK loci (Martin-Subero et al., Int J Cancer 2002) were applied. In sixteen out of 50 MM patients (32%), FISH with these probes detected at least one structural or numerical chromosomal alteration. Ten cases (20%) carried translocations affecting the IGH locus whereas 5 (10%) displayed IGL breakpoints. In contrast, no IGK translocation was detected. Four (8%) cases and 1 (2%) case displayed three copies of the intact IGK and IGH locus, respectively. No gain of IGL was detected. The 50 MGUS cases were less informative. Only 4 cases (8%) presented signal patterns indicative for a translocation affecting the IGH locus. Neither evidence for illegitimate IGL or IGK rearrangements nor for numerical aberrations was found.

Our results support the hypothesis that interphase FISH in bone marrow specimens should be an important adjunct to conventional cytogenetics in MM with normal karyotype, but probably not in MGUS. Because of the frequent low bone marrow infiltration with malignant plasma cells particularity in MGUS, combined immunophenotyping and FISH (FICTION technique) or FISH after MACS (Magnetic Cell Sorting) might be more suited than conventional interphase FISH for the diagnosis of PCD with normal karyotype.

Here, we present new multicolor interphase FISH (MI-FISH) assays for the rapid and simultaneous detection of IGH translocations in MM. Probes flanking the chromosomal breakpoints of the most common translocation partners were designed and labeled in a dual-color fashion. These probes were validated in healthy donors and the cut-off for false-positive results was calculated. Their suitability to detect their respective chromosomal breakpoints was proven by using cytogenetically-positive controls. After this thorough validation process, the probes for each gene were pooled and differentially labeled with DEAC, Spectrum Orange, Texas Red or Cyanine 5. The first multicolor assay was made of a probe for the IGH locus labeled in Spectrum Green together with probes for the most frequent gene partners: CCND1, FGFR3/MMSET, MAF and CCND3. By means of this assay, the t(11;14)(q13;q32), t(4;14)(p16;q32), t(14;16)(q32;q23) and t(6;14)(p21;q32) translocations can be detected in a single experiment. The second multicolor assay included a probe for the IGH locus together with the new probes for the less frequent IG gene partners: IRF4, MAFB and IRTA1/2 to detect the t(6;14)(p25;q32), t(14;20)(q32;q11) and t(1;14)(q21;q32) translocations. These MI-FISH assays were tested in negative and positive controls and hybridized in a small series of MM cases with normal karyotypes where IGH breakpoints were previously detected. Thus, we could validate the capacity of the new probe sets to simultaneously detect the most common translocations in MM. Furthermore, the modular probe design allows easy combination with probes for IGL or IGK instead of IGH to detect variant translocations.

In the near future, in order to increase the sensitivity of the MI-FISH approach, multicolor combined immunophenotyping and FISH assays will be established. This technique will pave the way for the accurate study of low infiltration with chromosomally altered plasma cells, as it occurs in monoclonal gammopathy of unknown significance (MGUS).

024 Multicolor interphase FISH for the detection of IGH translocations in multiple myeloma

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Translocations involving the immunoglobulin heavy chain (IGH) locus at 14q32, or its variants, are among the most common chromosomal abnormalities in multiple myeloma (MM). These translocations juxtapose IG regulatory sequences next to various oncogenes, whose expression is subsequently altered. IGH partners in MM targeted by such translocations are CCND1 (11q13), FGFR3/MMSET (4p16), MAF (16q23), CCND3 (6p21), IRF4 (6p25), MAFB (20q11) and IRTA1/2 (1q21). Some of these translocations affecting IGH in MM are difficult to detect by conventional chromosome analyses because they involve subtelomeric regions on both affected chromosomes, e.g. t(4;14)(p16q32), t(14;16)(q23;q23) or t(6;14)(p25;q32). This limitation can be overcome by fluorescence in situ hybridization (FISH) using suitable locus-specific probes. The second multicolor assay included a probe for the IGH locus together with the new probes for the less frequent IG gene partners: IRF4, MAFB and IRTA1/2 to detect the t(6;14)(p25;q32), t(14;20)(q32;q11) and t(1;14)(q21;q32) translocations. These MI-FISH assays were tested in negative and positive controls and hybridized in a small series of MM cases with normal karyotypes where IGH breakpoints were previously detected. Thus, we could validate the capacity of the new probe sets to simultaneously detect the most common translocations in MM. Furthermore, the modular probe design allows easy combination with probes for IGL or IGK instead of IGH to detect variant translocations.

In the near future, in order to increase the sensitivity of the MI-FISH approach, multicolor combined immunophenotyping and FISH assays will be established. This technique will pave the way for the accurate study of low infiltration with chromosomally altered plasma cells, as it occurs in monoclonal gammopathy of unknown significance (MGUS).

025 Genomic characterisation of the chromosomal breakpoints of t(4;14) and t(11;14) Multiple Myeloma suggest distinct mechanisms of recombination.

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Translocations involving the IgH locus are the commonest genetic lesion in multiple myeloma (MM), with recent reports suggesting they are a virtually universal event. Studying the nature of the breakpoints will help us to understand the molecular mechanism leading to MM. We have characterised the genomic breakpoints of nine MM patients, seven t(4;14) translocations, and two t(11;14), using inverse and long range PCR techniques. By convention chromosome 14q32 breakpoints in MM are believed to be located in the IgH S switch region on der(4) or der(11) and a further downstream switch (S) region on der(14), with deletion of intervening 14q32 DNA occurring as a result of aberrant class switch recombination (CSR). Our analysis showed such breakpoints did occur, but there was also evidence of more complex recombination events in four of the eight patients examined. In two t(4;14) patients it was possible to demonstrate that rearranged hybrid switch region sequence was joined to DNA from chromosome 4p16. For the first patient it was found, on der(4), that S sequence had recombined with S 3 sequence before a further recombination site with 4p16 was observed. Similarly for the second case S had apparently recombined with S before being joined to 4p16 sequence. We also provide...
To access the importance of flanking genes we have updated our cohort, which now includes 272 MM and 77 MGUS patients. Using an RT-PCR screening assay we have found 38 (14.0%) t(4;14) positive MM patients and 1 (1.3%) positive MGUS patient. This strategy can identify three major breakpoint clusters, termed MB4-1, 2, and 3. The 38 MM patients segregate into 26 MB4-1, and 6 each of MB4-2 and MB4-3, while the MGUS patient is an MB4-1. FGFR3 is expressed in 27/37 (73.0%) positive MM patients and in the one MGUS patient. All FGFR3 non-expressers lacked a detectable der(14) RT-PCR product. Furthermore, for 54 negative and 11 t(4;14) positive patients we have tested multiple BM samples, primarily diagnosis and relapse, and have yet to find any differences in t(4;14) status or FGFR3 expression. To define the level of gene expression we have chosen to use quantitative RT-PCR. We are particularly interested in genes that are not interrupted by any of the breakpoints or genes that may promote the oncogenic process by either overexpression or haplo-insufficiency. One gene that falls into the latter category is the Response Element-II Binding Protein (RE-IIBP). Transcription of RE-IIBP initiates downstream of all known t(4;14) breakpoints and thus, unlike MMSET type I & II, RE-IIBP could be overexpressed in its native form by the mu enhancer within all t(4;14) patients. To date we have observed an 8-15 fold increase in the expression of RE-IIBP in t(4;14) positive cell lines compared to other human myeloma cell lines. Work is ongoing to identify other genes located on chromosome 4 that are deregulated by t(4;14).
In conclusion, we identified a novel recurrent primary translocation involving 14q32, i.e. t(14;20)(q32;q12). Aberrant expression of MafB and/or TIMAP may be involved in the oncogenic transformation of myeloma cells, harboring this t(14;20).

028 Studies on Cyclin D1 Gene Amplification and the t(11;14) Chromosome Translocation in Multiple Myeloma
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Malignant diseases are characterized by abnormal regulation of the cell cycle and by uncontrolled cell division. CYCLIN D1 is a protein derived from the CCND1 gene and is responsible for the transition from G1 (resting) phase of the cell cycle to the S phase (DNA synthesis) via phosphorylating the product of the retinoblastoma gene (pRB). CYCLIN D1 is expressed in 50% of human mammary carcinoma; in parathyroid adenoma and mantle cell lymphoma CYCLIN D1 is activated by translocation. In many cancers, including colon, breast, lung, head and neck, CYCLIN D1 is overexpressed when detected by immunohistoc hemical techniques, and around 30% of MM express CYCLIN D1.

In Multiple Myeloma (MM), the role of CYCLIN D1 is less established. Approximately 30% of MM express CYCLIN D1 when detected by immunohistochemical techniques, and around 50% of untreated MM patients are characterized by a CYCLIN D1 overexpression when studied by RT-PCR. Only one previous study analyzes amplification of CCND1 gene by FISH and detected the amplification in 38% of cases. The aim of this study is to evaluate overexpression of CCND1 gene in MM at diagnosis. Furthermore we investigate if CCND1 overexpression derives from the t(11;14) translocation or it is activated by gene amplification.

Plasma cell were purified from bone marrow samples with CD138 magnetic beads. Two sets of probes were used for interphase FISH investigations: LSI CCND1/CEP11 (Vysis, UK) dual color probe for the detection of CCND1 amplification and LSI IGH/CCND1 (Vysis, UK) dual color probe for the detection of the t(11;14) translocation. FISH analysis was applied to a series of 25 patients at diagnosis.

Results. Cyclin D1 amplification was found in 9 out of 25 samples analyzed (36%). Trisomy 11 occurred in 7 patients (28%). 4 patients were positive for the t(11;14) (16%). The cut-off level for CYCLIN D1 amplification was defined at 20%. Combined analysis shows CYCLIN D1 overexpression in 3 of the 4 cases bearing the t(11;14), in 2 of the 7 cases with trisomy 11 and in 5 cases without the t(11;14) (55% of the amplified cases, 16% of all cases).

Of note, the amplification of CCND1 gene was not detected in a sample despite the finding of the t(11;14). In this case breakpoint on chromosome 11 may occur at least 400 Kb upstream the CCND1 gene.

Conclusion. A CCND1 overexpression is frequently observed in MM patients (36%). Amplification of CCND1 gene was not correlated with the t(11;14) translocation in 5 out 9 patients (55%). In a subset of MM alternative mechanism of CCND1 amplification occurs. The same mechanism has been observed in the majority of solid tumors. This subgroup of myeloma may have a different biological and clinical behavior.

029 The t(9;14)(p13;q32)/IGH-PAX5 rearrangement is not a hallmark of low grade lymphoplasmacytic lymphoma
The t(9;14)(p13;q32) is a rare chromosomal translocation presumably associated with low grade lymphomas showing plasmacytid differentiation. On a molecular level this aberration results in a deregulated expression of the PAX5 gene (9p13) due to the proximity of IGH transcriptional enhancers/promoters. PAX5 encodes the BSAP (B-cell specific activator protein) transcription factor that normally is downregulated at the transition between the mature B-cell and plasma cell. Thus, the plasmacytid phenotype of t(9;14)-associated lymphomas possibly reflects an inappropriate expression of PAX5 that should be turned off at terminal differentiation to plasma cells. The t(9;14)/IGH-PAX5 translocation detected in approximately 50% of lymphoplasmacytic lymphoma (LPL) by Iida et al (1996) has been occasionally reported in other lymphoma subtypes including FL, MZL and DLBCL (with or without a preceding phase of a low-grade lymphoma). These latter observations suggest that the t(9;14) either reflects the underlying low grade malignancy, or that its association with LPL is not specific. In order to clearly define the diagnostic and prognostic significance of a t(9;14) we performed a retrospective multi-center study of patients with a suspected or proven t(9;14)(p13;q32).

Interestingly, in 3 of these cases this translocation was hidden by three-way rearrangements detected by MFISH analysis. In order to facilitate the screening for IGH-PAX5, we designed two and three color interphase FISH assays with probes spanning the IGH and PAX5 loci and validated their application in the KIS-1 cell-line carrying the t(9;14)(p13;q32). Using that approach, a coinciding rearrangement of the PAX5 and IGH loci was demonstrated in all patients. It is worth to note that two additionally analyzed DLBCL cases with a postulated variant t(3;9)(q27;p13) (Iida et al, 1996) showed the 9p13 breakpoint outside PAX5 (400kb proximal to the gene) pointing other recurrent 9p13 targets in NHL. Particularly interesting is that only 2 of 8 cases with the identified t(9;14) were diagnosed as low grade NHL (Waldenström’s macroglobulinemia and MZL), and only one of them showed features of plasmacytid differentiation. All the remaining patients (6/8) suffered from de novo DLBCL. Predominant occurrence of this high grade lymphoma in the present series of IGH-PAX5 positive tumors indicates that translocation t(9;14)(p13;q32) has a much wider clinical spectrum as previously assumed, and therefore may not be regarded as a genetic hallmark of low grade lymphomas with features of plasmacytid differentiation.

2.2 Chromosome instability

030 Centrosomal disorganization in myeloma: Role of the receptor for hyaluronan mediated motility (RHAMM)
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Multiple myeloma (MM) is characterized by infiltration of monoclonal plasma cells in the bone marrow, chromosomal instability (CIN) and translocations involving the
immunoglobulin heavy chain (IgH) locus. Overexpression of receptor for hyaluronan mediated motility (RHAMM), and its splice variants (RHAMM-exon4 and RHAMM-exon13), also characterizes the myeloma clone. Recently, we reported that RHAMM, an itinerant protein that functions outside and within the cell, is a centrosomal/spindle pole protein that maintains mitotic stability. GFP-RHAMMF and GFP-RHAMM-exon13, but not GFP-RHAMM-exon4, interact with microtubules. RHAMM, like nuclear-mitotic apparatus protein (NuMA), depends upon direct microtubule contact, mediated by exon 4, and indirect microtubule contact through the dynein motor complex to crosslink spindle microtubules. As RHAMM overexpression characterizes MM, we investigated centrosomal structure within CD138+ plasma cells from archived bone marrow cores taken from MM (n=41), MGUS (n=8) and control (n=4) patients. Centrosomal number and qualitative structural abnormalities were analysed visually. Immunofluorescence analysis, in combination with confocal microscopy and 3-dimensional reconstruction, allowed quantitative assessment of structural abnormalities. While visual inspection of centrosomal numbers outlined a difference between MM and control (p=0.03) but not MGUS (p=0.09), quantitation of centrosomal structure demonstrated significant differences between MM and control (p=0.002) as well as MGUS (p=0.01) samples. These data illustrate the necessity for quantitative analysis of centrosomal structure and the pervasive centrosomal abnormalities in MM. Other centrosomal/spindle pole gene products that are intimately associated with RHAMM mitotic function(s) (TACC3, NuMA, dynein light chain 2B) map telomeric to recurrent IgH translocation sites (4p16.3, 14q13, 16q23.3) and may also be disregulated in MM. IgH translocations may elevate the expression of gene products by positioning them next to strong enhancer elements; moreover, the occurrence of unbalanced IgH translocations, and occasional loss of derivative chromosomes, may induce haploinsufficiency of translocated gene products. Using RHAMM as a model of spindle pole proteins, we show that both overexpression and inhibition of function affects mitotic integrity. Overexpression of GFP-RHAMMF induces ectopic nucleation of microtubules and, in the absence of centrosomal replication defects, multipolar spindles. Inhibition of RHAMM function, through the microinjection of purified RHAMM antibodies, disrupts mitotic integrity and induces tripolar (11+/- 3.4% of injected cells) and tetrapolar (14+/- 4.3%) spindles. Isoform balance may be another important determinant of RHAMM function. Fluorescent recovery after photobleaching examination of GFP-RHAMMF and GFP-RHAMM-exon4 dynamics illustrates differences in isoform mobility within the cytoplasm and at the centrosome. Within the cytoplasm, GFP alone was highly mobile (99.24% mobility) which translated as an extremely low time for 50% recovery (t1/2=0.218s). The dynamics of GFP-RHAMMF(t1/2=8.42s, 70.6% mobile) differed from GFP-RHAMM-exon4(t1/2=2.96s, 88.9% mobile) within the cytoplasm but not at the centrosome. Although not yet examined at spindle poles, relative overexpression of RHAMM-exon4, shown by us to significantly correlate with poor survival, may inhibit microtubule crosslinking, adversely affect the ability of RHAMM complexes to maintain spindle poles and lead to CIN. Thus, MM is characterised by centrosomal disregulation that can result from elevated, inhibited and/or differential isoform expression of RHAMM, perhaps in concert with other centrosomal proteins that may be disregulated by recurrent translocations in MM.

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Altered RHAMM splicing is an adverse prognostic factor, and upregulated RHAMM correlates with lytic bone disease.

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RHAMM is a centrosomal protein which is overexpressed in myeloma bone marrow plasma cells (BMPC) relative to normal B cells. Aberrant RHAMM expression in vitro leads to mitotic errors. In myeloma we have observed variable deletion of RHAMM exon 4, a region critical to binding of microtubules. These observations imply a model in which aberrant RHAMM expression and/or splicing leads to compromised mitotic spindle integrity, contributing to the chromosome instability that drives myeloma progression. Our model is supported by microarray-based global gene expression profiles (GEP) of BMPC from 112 myeloma patients with lytic bone disease, in comparison to 43 patients lacking bone lesions. RHAMM was found to be more highly expressed in patients with lytic bone lesions (p<0.001), suggesting a relationship between RHAMM expression and disease activity. Interestingly, although RHAMM was upregulated in GEP from 20 of 21 human myeloma cell lines examined, the absolute levels of RHAMM expression in myeloma patient BMPC were not substantially elevated relative to normal BMPC, suggesting that upregulation of RHAMM in myeloma PC is not the sole mechanism of RHAMM dysfunction and consistent with the idea that RHAMM splicing patterns may have clinical relevance. To dissect the relevance of RHAMM mRNA splicing, we performed RT-PCR and capillary fragment analysis to measure the extent of RHAMM exon 4 deletion in the BM and peripheral blood of myeloma patients. This measure is designated R, and is the ratio of fluorescence intensity of spliced : unspliced RHAMM in a single RT-PCR reaction using fluorescent PCR primers flanking exon 4. R varied significantly among 101 BM aspirates from the time of diagnosis (range, 0.37-2.67). Patients were divided into three groups based on R. Overall survival (OS) strongly correlated with RHAMM splicing. Those patients with relatively high exon 4 splicing had significantly reduced OS when compared to those with patients having low exon 4 splicing patterns (p=0.002). There was no significant correlation observed between RHAMM splicing and %BMPC, lytic bone disease, other baseline prognostic factors or response to therapy, suggesting a contribution to clinical outcome that is independent of disease burden. RHAMM splicing in the blood of myeloma patients was followed over time in 5 patients, and was found to be low post transplant and to be prominent at relapse, suggesting that exon 4 deletion characterizes circulating MM cells as disease progresses.

Conclusions: In MM, survival strongly correlates with the extent of RHAMM splicing, with significantly reduced survival when exon 4 splicing is high and significantly improved survival when exon 4 splicing is low. Further confirming that RHAMM is clinically important in MM, MM patients with bone disease have been found to have significantly greater RHAMM expression than those lacking bone disease, as measured by microarray analysis. RHAMM expression and splicing are intimately linked to myeloma progression, likely through aberrancies in mitosis and the subsequent development of chromosomal instability. Therapeutic targeting of RHAMM may halt the genetic evolution of the myeloma clone.
Hyaluronan (HA) regulates MM cell behavior through the interactions with the HA receptor RHAMM, and may also mediate intracellular interactions. Both HA and its receptor RHAMM appear to be clinically important in the biology of MM. HA is synthesized by hyaluronan synthases (HASs)- HAS1, HAS2, HAS3. We characterized the expression of HASs in MM peripheral blood (PB) and BM using rigorously controlled single stage RT-PCR, capillary electrophoresis and GeneScan analysis software. We examined 142 BM and 70 PB samples from MM patients taken at the time of diagnosis. Differential expression of HAS1 or HAS2 was detected in MM PB and BM samples respectively. For 82/142 MM patients BM cells expressed HAS1, while 112/142 patients expressed HAS2 transcripts, suggesting preferential expression of HAS1 in MM PB. We identified two novel splice variants of HAS1 (HAS1Vα and HAS1Vβ) in MM patients. We find that HAS1Vα is overexpressed in MM PB and BM, as well as in human thymocytes. HAS1Vβ is a result of abnormal intronic splicing, and is detectable only in malignant cells. Although HAS1Vα is found in both PB and BM, expression of HAS1Vβ which is detectable only in the PB, is found in MM B cells, but is absent from non-B cells in MM PB. Statistical analysis of 41 PBMC and 117 BM samples from MM patients showed that expression of HAS1Vβ in PBMC either alone or in combination with HAS1Vα, HAS1 or HAS2, was strongly correlated with poor survival (P=0.002). Furthermore, all 6 MM patients expressing HAS1Vβ in BM cells had inferior survival when compared to 117 MM patients lacking BM expression of HAS1Vβ. HAS1Vβ is detected in PB MM B cells, but is undetectable in purified BM PC from MM, raising the possibility that BM HAS1Vβ expression may derive from BM B cells rather than PC. The remarkable association between PB HAS1Vβ and poor survival, together with the relative lack of this variant in the BM, suggests that HAS1Vβ may be preferentially upregulated in circulating malignant B cells, providing further evidence in support of a central role for early stage MM cells in malignant progression and suggesting potential mechanisms through which MM B cells may impact on disease progression. We speculate that HASs, particularly the newly identified HAS1Vβ, play important roles in disease progression in MM. Based on the clinical significance of HAS1Vβ expression in early stage components of the MM hierarchy, HASs may also participate in the initial oncogenic events giving rise to MM. In addition to a role in promoting malignant dissemination, we speculate that novel variants of HAS1 may synthesize intracellular HA, a ligand for intracellular RHAMM. This may modulate intracellular associations of RHAMM with the mitotic apparatus and thus promote survival of malignant cells with altered chromosomal complements, increasing genetic instability of the MM clone and facilitating the emergence of aggressive clonal variants. Funded by CIHR and the National Cancer Institute (USA).
Instability of Pericentromeric Heterochromatin and Associated Gene Duplication in Multiple Myeloma (MM).


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Chromosome instability is a hallmark of cytogenetic progression in MM. One of the largest subsets of structural chromosome anomalies in the progression of MM involves the duplication of all or part of chromosome 1q. We investigated chromosome 1q associated duplications in 35 patients which showed 1q aberrations by G-banding. Spectral karyotyping (SKY) and locus-specific fluorescence in situ hybridization (FISH) were used to help further define these aberrations. FISH probes for the pericentromeric heterochromatin (sat II/III sequences) at 1q12 were used in conjunction with locus specific probes for BCL9 and IL6Ra, which map to 1q21. G-banding analysis identified the most frequent aberrations as whole-arm or “jumping” translocations of 1q (21 cases). The second most frequent aberrations were partial duplications of the 1q12–32 region (16 cases). FISH analysis of the partial duplications revealed both direct and inverted sequential duplications of the 1q12–32 region, which were associated with up to three copies of the satII/III sequences on a single chromosome arm. Inverted duplications (five cases) resulted in larger numbers of duplicated genes with up to five copies of BCL9 and/or IL6Ra per chromosome arm. Subsequent whole chromosome duplications of both normal and abnormal chromosomes 1 in hyperdiploid cells resulted in up to 12 copies of BCL9 and IL6Ra in a metaphase spread. SKY detected cryptic insertions of non-homologous chromosome segments next to the pericentric 1q12 regions in three cases. In two of these cases duplications of BCL9 and IL6Ra were found. In one case, however, the insertion/translocation of satII/III next to the c-myc gene region resulted in the duplication of multiple copies of the c-myc gene. A surprising finding in this study was that, not only does the 1q12–24 region undergo sequential duplication at the original chromosome 1q locus, but also undergoes sequential duplication after being translocated to non-homologous chromosomes. In four cases duplications of multiple copies of satII/III were found translocated, and in two cases the evolution of subclones showed the sequential duplications of satII/III and extra copies of BCL9 subsequent to the translocation. No evidence of duplication of BCL9 or IL6Ra on non-homologous chromosomes was found without the presence of satII/III sequences. We have found an association of duplication of sat II/III sequences and the subsequent duplication of BCL9, IL6Ra, and c-myc in MM. The behavior of the tandemly repeated satellite DNA sequences and transposon-derived repeats in the pericentromeric region may be affected by the methylation status of the cell. The characteristic pattern of decondensation of the pericentromeric heterochromatin at 1q12 found in many of these cases suggests hypomethylation of the satII/III sequences could be one factor which facilitates instability and duplication of this region.

Hypomethylation of Chromosome 1 Satellite 2 Sequences and Hypermethylation of p16 Gene in Multiple Myeloma and Amyloidosis

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Introduction: Epigenetic regulation of gene expression and its interrelated effects on chromatin organization seem to play critical roles in the genesis of human neoplasias. Hypomethylation of pericentromeric satellite sequences of chromosome 1 (Chr1) predisposes to Chr1 rearrangements in the vicinity of centromere. This has been observed in the ICF syndrome (immunodeficiency, centromere instability, and facial anomaly) and in a human pro-B cell line treated with 5-azadeoxycytidine. In multiple myeloma (MM), Chr1 aberrations in the pericentromeric region are found in 40% of patients, and Chr1 rearrangements confer negative prognostic implications. In contrast, hypermethylation of the p16 promoter, which is linked to silencing of this tumor suppressor gene on 9p21, has been found in 30-40% of examined human MM cases. Hypermethylation of p16 gene is associated with increased cell proliferation and shorter patient survival. We thereby hypothesized that altered DNA methylation may contribute to the pathogenesis of MM.

Patients and methods: By Southern blot analysis, we detected satellite hypomethylation using a CpG methylation-sensitive enzyme to digest DNA (Bst BI) followed by hybridization with a Chr1-specific satellite 2 probe. Human sperm DNA served as the hypomethylation standard, and human lung and brain DNA as hypermethylation standards. We also looked for p16 methylation using a methylation sensitive PCR after sodium bisulfite modification. Among the 12 patients with primary plasma cell neoplasms studied, 8 had MM, 3 light chain associated amyloidosis and 1 smoldering MM. DNA was purified from CD138+ plasma cells. We also studied 5 human MM cell lines; SK-MM-1, OPM-2, MM-1, JJN3, and KP16.

Results: Overt hypomethylation was detected in all five human MM cell lines. Cytogenetic data were available for four of the five cell lines, all of which showed chr1 pericentromeric rearrangement. All 12 patient samples showed Chr1 satellite 2 hypomethylation. The degrees of hypomethylation in both cell lines and patients’ specimen were equivalent to that in human sperm DNA, in which many tandem repeats are hypomethylated. In contrast, 3 out of 12 patients had hypermethylation of p16 gene (25%), including one with amyloidosis and two with MM.

Discussion: Our study suggests that plasma cells may be epigenetically prone to Chr1 pericentromeric rearrangements mediated through DNA hypomethylation of Chr1 satellite sequences. However, satellite hypomethylation and p16 hypermethylation gene silencing can co-exist, both of which could contribute to carcinogenesis independently.
2.3 Molecular cytogenetic and prognostic impact

CYTOGENETICS AND MULTIPLE MYELOMA: A SPANISH MULTICENTER STUDY OF 497 PATIENTS


On behalf of GEM group.

Introduction: The information about cytogenetics in Multiple Myeloma (MM) is opened to identify and define the role of chromosomal abnormalities on prognosis of MM. We have initiated a National cytogenetics studio with newly diagnosed MM Spanish patients, in order to permit a more accurate description of cytogenetics anomalies in MM.

Methods and Patients: 497 untreated MM patients were studied, recruited from 68 centres in Spain. Cytogenetics analysis was performed on bone-marrow processed by 72 and 96h unstimulated cultures. Chromosomes were identified by G-banding. Aberrant chromosomes were examined by FISH, using painting region-specific, and/or centromeric probes. Furthermore, we performed FISH technique with (RB/LSID13S319/LSID13S25) and LSI 14q32/11q13 loci probes. Karyotypes were designated according to the ISCN criteria.

Results: We present cytogenetics results on 318 patients. An abnormal karyotype was detected in 143 patients (45% of evaluable patients): 17% of karyotypes were Pseudodiploid, 15% Hyperdiploid and 11% Hypodiploid. Numerical and structural abnormalities were found at the same time in the 45% of cases. Monosomy of chromosome 13 was the most common numerical abnormalities (22%), following of trisomy of chromosomes 5, 9 and 7 (17, 18 and 10% of cases). The most frequent recurrent chromosomal abnormalities were del(13)(q14) detected in 53 patients (37%), and 14q32 abnormalities detected in 22 patients (15%), 15 of them as t(11;14)(q13;q32). The 45% of del(13)(q14) and the 53% of t(11;14)(q13;q32) showed additional abnormalities on karyotypes. FISH analysis showed del(13)(q14) and t(11;14)(q13;q32) in a 42% (n=22) and in a 40% (n=6) of cases that have been no detected by conventional cytogenetics. The 43% (n=62) of clonal karyotypes were complex. Chromosome 1 abnormalities represent the most frequent aberration, observed in 31% of cases (n=45), implicated in translocations with others chromosomes, detecting trisomies 1q and monosomies 1p near with many chromosomal abnormalities. FISH technique allowed more complete description of karyotypes.

Conclusions: 1) The simultaneous analysis of conventional and molecular cytogenetics allowed the detection of 45% clonal karyotypes. 2) We detected del(13)(q14) and t(11;14)(q13;q32) with many others chromosomics abnormalities in a high frequency of cases (98%), 3) FISH analysis were more perceptible for detection of del(13)(q14) and t(11;14)(q13;q32). 4) Chromosome 1 abnormalities represented the most frequent cytogenetics anomalies. They could be secondary aberrations associated to complex karyotypes, suggesting that chromosome 1 could be implicated in a more aggressivity of tumour. 5) The great heterogeneity and complexity of newly diagnosed MM karyotypes suggest that when the tumour is diagnosed it is already in an advanced state of disease.

Our experience showed the necessity to carry out not only specific FISH study but conventional cytogenetics in order to permit: a) looking for cytogenetics abnormalities specifics of MM, and b) establish the true role of recurrent abnormalities: del(13)(q14) and t(11;14), and will arrive to define which of them or others, are primary or secondary abnormalities. (Supported by Grant from Spanish FIS G03/136)

Hierarchical clustering for the analysis of chromosome aberration patterns in Multiple Myeloma.

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The knowledge of chromosome aberration patterns in Multiple Myeloma have been hampered by the low proportion of clonal abnormalities found by conventional cytogenetics, and by the high complexity of those cases with altered karyotypes. Nevertheless, karyotypes of around 1000 cases have been reported, and the main chromosomal features may be summarized as follow: (1) abnormal clones appear to be divided into those with modal chromosome numbers in the hypodiploid, pseudodiploid and hyperdiploid range, being hyperdiploid more common (approximately 46-68%) than pseudo- or hypodiploid. The main numerical imbalances reported by conventional cytogenetics are gains of chromosomes 3, 5, 7, 9, 11, 15 and 19, and losses of 13, X (females), 14, 6q, 8, 16, and Y. (2) The chromosome bands most commonly affected by structural abnormalities are 13q14, mainly as deletions and 14q32, which is involved in various translocations like t(11;14)(q23;q32) or complex rearrangements with unidentified chromosomal partners. Moreover, a prognostic implication has been postulated for some of these aberrations, e.g. 13q deletions have been described as an adverse prognostic factor in MM. In order to better understand the biological relevance of abnormal karyotypes in myeloma cells, a systematic characterization of the karyotypic patterns is required. Thus, the aim of our study was to identify distinct subgroups of cytogenetic patterns among myeloma patients using appropriate statistical approaches. A total of 276 myeloma cases, all of them with abnormal karyotypes, were retrieved from our own database and from previously published series. The presence or absence of chromosomal aberrations by G-banding of all cases were recorded, and those abnormalities present in more than 5% of the cases entered the statistical analysis. A total of 43 variables were included for the first approach. Chromosomal abnormalities are asymmetrical binary data, thus, hierarchical clustering analysis was performed. For these kind of variables the most adequate method for computing distances is the Jaccard coefficient or any of its variants like Dice or Sokal. For genetic data usually two methods of hierarchical clustering are used, average linkage or CAST (Cluster Affinity Search Technique). As the last one is still experimental we have choosen average linkage using three measures of distance, i.e. Jaccard’s, Dice’s and Sokal’s coefficients. The three cluster trees resulting from these analysis were analysed using the Stata and Clustan software. Our preliminary results are in good concordance with those recently published by Fonseca et al.(2003). Two major
clusters were found, the first was composed of gains of chromosomes 3, 5, 7, and 9, as well as hyperdiploidy, and the second of losses of chromosome 1, 4, 8, 13, 14, 16, 17, 20, 21, 22 and hypodiploid karyotypes. Moreover, a third group including those cases with “more than triploid karyotype” was observed, possibly conforming an independent cytogenetic subgroup. Further studies will focus in the relationship between these chromosomal abnormality patterns, clinical parameters and outcome.

038 AML/MDS-associated cytogenetic abnormalities in multiple myeloma and monoclonal gamopathy of undetermined significance: evidence for de novo occurrence and stem cell involvement of del(20q) as a sole change

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The plasma cell dyscrasias multiple myeloma (MM) and monoclonal gamopathy of undetermined significance (MGUS) are cytogenetically characterized by various translocations involving 14q32, -13 or del(13q), and trisomies of chromosomes 3, 7, 9, 11, and 15. However, karyotypic patterns characteristic for acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS), eg hypodiploidy with total or partial losses of chromosomes 5 and 7, may occasionally also occur in the setting of plasma cell dyscrasias. In most instances, such AML/MDS-associated cytogenetic features signify the development of therapy-related malignancies, in particular t-AML/t-MDS in MM patients previously treated with alkylating agents. In a few instances, however, “myeloid” chromosomal changes are detected in untreated plasma cell disorders without any morphologic features of AML/MDS. Whether these aberrations have arisen in myeloid precursor cells, heralding an ensuing myeloid malignancy, or whether they exist in the MM/MGUS cells is unknown. In order to characterize the “myeloid” abnormality patterns in MM and MGUS patients, we have ascertained and reviewed all 123 MM and 25 MGUS cases as well as all 19 t-AML/t-MDS occurring after previous chemotherapy for MM cytogenetically analyzed in our department. Among these, 67 (54%) MM, 7 (28%) MGUS, and 16 (84%) t-AML/t-MDS were karyotypically abnormal, with 7 (10%) MM and 2 (29%) MGUS displaying “myeloid” abnormalities, ie +8 (one case) and 20q- (eight cases), without any evidence for AML/MDS. In the MM cases, interphase fluorescence in situ hybridization analyses with a probe for 20q12 revealed the presence of the del(20q) in all cell populations investigated (CD34+, CD34+CD38−, CD34+CD38+, CD34−, CD19+, CD15+, and CD3+). The present data indicate that del(20q) as a sole anomaly occurs as a de novo aberration in approximately 10% of karyotypically abnormal MM/MGUS cases and also strongly suggest that it arises at the stem cell level.

039 Cytogenetics, Interphase and Multiplex FISH of Multiple Myeloma.

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Translocations involving the immunoglobulin heavy chain gene (IGH) at 14q32 have been proposed to be primary abnormalities in myeloma. Monosomy or deletions of chromosome 13 have been associated with a poor prognosis. Interphase fluorescence in situ hybridisation (FISH) has now become the method of choice for the detection of these abnormalities due to the difficulties of conventional cytogenetic analysis. The limitation of interphase FISH is that interpretation of karyotypes is restricted to the specific chromosomal sites under investigation and important abnormalities of these regions may be masked within complex karyotypes and those with very high chromosome numbers. However, even when conventional cytogenetics is successful, the complexity of the karyotypes often precludes accurate characterisation. The complementary application of 24 colour FISH (M-FISH), cytogenetics and FISH with specific probes can resolve many of the problems of interpretation.

We have applied these techniques to a series of 19 myeloma patients with abnormal karyotypes. In agreement with other studies, cytogenetics and M-FISH revealed that chromosomes 3, 5, 7, 9, 11, 15, 19 and 21 were most frequently gained in hyperdiploid karyotypes and chromosomes 13 and 22 were often lost. Although no new recurring structural chromosomal changes were identified in this small series, a high incidence of complex abnormalities were observed in all cases. Multiple rearrangements involving chromosome 1 were observed in 17/19 cases. Other frequently occurring structural changes involved chromosomes 5, 6, 7, 8, 14 and 16, many of which were only visible by M-FISH. Cryptic abnormalities of chromosomes X and Y were also shown by M-FISH only. Metaphases from cases with apparent discrepancies between the cytogenetics/M-FISH and interphase FISH were re-hybridised with the IGH or locus-specific chromosome 13 probes. This revealed cryptic and complex involvement of chromosome 14 with a range of partners, including chromosomes 1, 2, 7, 8 and 12 and a cryptic chromosome 13 deletion. Our findings have confirmed that accurate interpretation of karyotypes benefits from the complementary application of these techniques. This approach facilitates the search for new chromosomal abnormalities with clinical or prognostic significance and helps to identify significant patterns of genetic change, which will further our understanding of the role of genetics in the development and progression of myeloma.

040 IN SEARCH FOR NEW RECURRENT CHROMOSOME BREAKPOINTS IN MULTIPLE MYELOMA

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The use of advanced molecular cytogenetic techniques, as Spectral Karyotyping (SKY), on multiple myeloma (MM) has
lead to the identification of recurrent chromosome rearrangements which are specific for the disease and in some instances of clinical and biologic significance. It is now well established that up to 50% of the MM cases show rearrangements involving the IGH locus at 14q32. This proportion might be even higher if rearrangements of the IG lambda (IGL) locus at 22q11.2 are included. Three specific translocations involving the IGH locus at 14q32, i.e. t(4;14), t(1;14) and t(14;16), account for nearly half of the 14q32 translocations in MM. The remaining IGH and IGL translocations are obviously of much lower frequency but nevertheless recurrent, like t(6;14), t(14;20) or t(8;22). Nowadays, two groups of cases are of major interest for the identification of genetic markers in MM: those with immunoglobulin breakpoints involving novel partner genes and those with recurrent breakpoints not involving IG.

We have selected from our files 12 cases of de novo MM with complex karyotype which by G-banding lacked the most common translocations t(4;14), t(1;14), and t(14;16). These cases were analysed by SKY as well as by FISH using probes flanking the IGH and IGL genes.

IGH breakpoints at 14q32: Four cases that showed 14q32 rearrangement were re-classified by SKY as t(11;14)(q13;q32) (3 cases) and as t(4;14)(q32;q13.1) (1 case). FISH analysis detected the involvement of the MAFB gene in the latter case. IGL breakpoints at 22q11.2: Eight cases showed a rearrangement at 22q11.2 by SKY. FISH analyses detected involvement of the IGL locus in four of these cases. All four cases showed the t(8;22)(q24;q11.2) variant Burkitt translocation. These translocations were always balanced. Non-IGL-breakpoints at 22q11.2: The remaining four MM lacked evidence for IGL involvement by FISH. An unbalanced translocation der(1)(12;22)(p13;q11.2) was present in two cases. In addition, two other translocations with a non-IGL breakpoint in 22q11.2 were noted: a t(10;22)(q25;q11.2) and a t(11;22)(p11.2;q11.2), each one being present in one case. Other new recurrent non-IG-breakpoints: The most frequent breakpoint location was in band 1p13 (9/12 cases). Except for the two cases with the recurrent translocation t(1;22) and two cases with deletions the 1p13 breakpoints were due to non-recurrent rearrangements. Breakpoints at 13q14-q21, Xp11.2, and Xq21 were detected in each three MM but different partners were involved.

The described cytogenetic findings warrant further molecular evaluation but clearly provide evidence for the existence of genes yet to be discovered that play an important role in the pathogenesis of MM.

**041 In multiple myeloma, five common genomic aberrations (+1q, +9q, +11q, 13q-, t14q) display a non-random distribution and suggest novel pathogenetic pathways**

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Introduction and Aims: Genomic aberrations play a key role in the pathogenesis of multiple myeloma (MM) and provide crucial prognostic information in this disease. Translocations involving the immunoglobulin heavy chain (IGH) locus on chromosome 14q32 (t14q) lead to the dysregulation of various oncogenes and are early genetic rearrangements in the development of plasma cell tumors. Deletion of chromosome arm 13q (13q-) is a powerful and independent predictor of an unfavorable outcome in MM. The biological implications and the prognostic significance of other recurring chromosomal losses and gains are unknown. Using FISH and a comprehensive, disease-specific DNA probe set, a high incidence of chromosome 1q, 9q, and 11q trisomies (+1q, +9q, +11q) were recently demonstrated in a large prospective series of myeloma tumors. To determine the significance of these frequent chromosomal gains, we evaluated the incidences of 13q- and t14q depending on the presence or absence of +1q, +9q, and +11q.

Material and Methods: Bone marrow aspirates from 182 patients with MM were obtained during routine diagnostic procedures. The following DNA probes were used for tri-color FISH: RP11-71L20 (mapping to chromosome band 1q21), RP11-40A07 (9q34), RP11-17M17 (1q25 – all from the RPCI human BAC library 11, National Center for Biotechnology Information, NCBI; http://www.ncbi.nlm.nih.gov/), PAC clone 272/3 (13q14, locus D13S272), and two DNA clones covering the IGH constant and variable region (BAC CH and cosmid VH). According to published data, t14q was diagnosed in tumors that exhibited segregating CH/VH signals in >25% of plasma cells.

Results: In a series of 182 cases, 13q- was significantly less common in tumors with +1q or +9q compared to those lacking these gains (31% vs. 70% and 39% vs. 65%, respectively; p<.001 – Fisher’s exact test). In contrast, tumors with +1q exhibited a significantly higher incidence of 13q- than those without +1q (64% vs. 43%; p=.006). In a subset of 84 pts., the overall incidence of t14q was 58%. t14q were significantly less common in tumors with +1q or +9q compared to tumors lacking these gains (45% vs. 77% [p=.004] and 44% vs. 79% [p=.001], respectively), while tumors with +1q exhibited a significantly higher incidence of t14q than those without this chromosomal gain (79% vs. 47%; p=.005).

Conclusions: Our data suggest novel, possibly t14q-independent and +9q/+11q-mediated pathways in the pathogenesis of MM and point to +1q as a secondary genetic change emerging during clonal evolution in this disease. The prognostic significance of +1q, +9q, and +11q is evaluated in ongoing phase III multicenter treatment trials.

Supported by a grant from the Deutsche Krebshilfe (70-2899-Li I) and the Wilhelm Sander-Stiftung (No. 2002.098.1) to P.L.

**042 Detection of trisomies 1q, 9q, and 11q in Monoclonal Gammopathy of Undetermined Significance (MGUS) using tri-color FISH**

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Introduction: The biological mechanisms responsible for the progression of Monoclonal Gammopathy of Undetermined Significance (MGUS) to overt multiple myeloma (MM) are still unknown. Genomic changes are most likely key events in this process, but no chromosomal aberration has been proven to account for the MGUS-MM transition to date. High incidences of chromosome 13q deletions (13q-) as well as translocations involving the IGH locus (14q32) have been found in both conditions. MGUS and MM, which suggested that these aberrations are early genetic events essential for the formation of
MGUS but most likely not crucial for the transition of MGUS to MM.

Recently, we have demonstrated that trisomies of chromosome arms 1q, 9q, and 11q (+1q, +9q, +11q) are among the most common chromosomal aberrations in MM. However, the incidence of these frequent trisomies in MGUS and their significance in the pathogenesis of MM remain to be explored.

Aims: To determine the incidences of chromosomal gains and losses involving chromosome bands 1q21, 9q34, 11q25, and 13q14 in individuals with MGUS and to distinguish primary aberrations (present in MGUS) from secondary genetic changes (not or infrequently detectable in MGUS, but common in MM) by comparing these incidences with that previously identified in a large series of myeloma tumors.

Material and Methods: Bone marrow aspirates from all individuals diagnosed with MGUS were obtained during routine diagnostic procedures. In 13 out of 21 cases, a positive selection of plasma cells using immunomagnetic beads (CD 138) was performed. The following DNA probes were used for tri-color FISH: RP11-71L20 (mapping to chromosome band 1q21), RP11-40A07 (9q34), RP11-17M17 (11q25 – all from the RPCI human BAC library 11, National Center for Biotechnology Information, NCBI; http://www.ncbi.nlm.nih.gov/), and PAC clone 272/3 (13q14, locus D13S272).

Results: The most frequent chromosomal imbalances in this preliminary study were (in order of decreasing prevalence): +9q (5/14 – 36%), 13q- (5/21 – 24%), +11q (2/14 – 14%), 11q- (2/14 – 14%), +1q (2/20 – 10%), and tetrasomy 1q (2/20 – 10%).

Conclusions: Our data obtained from a small series of cases show that +1q, +9q, and +11q are detectable in MGUS. While the incidence of +9q appears to be in the range of that found in MM, +1q and +11q, as well as 13q- seem to occur less frequently in MGUS. The precise incidences of all chromosomal imbalances have to be determined in a larger series of individuals with MGUS. This work is under way in our laboratory.

Supported by grants from the Deutsche Krebshilfe (70-2899-Li 1) and the Wilhelm Sander-Stiftung (No. 2002.098.1) to P.L.

**043 Cyto genetic s, fl ourescence in situ hybridization (FISH) and comparative genomic hybridization (CGH) studies of 11q in multiple myeloma.**

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Background: A large number of chromosomal and genetic abnormalities have been detected in multiple myeloma (MM), the most frequently are translocations affecting immunoglobulin heavy chain (IGH) and chromosome 13q deletions. Both of them have been associated with an adverse outcome. By contrast, abnormalities on 11q, other than t(11;14), have been less study. Purpose: To analyse 11q by conventional cytogenetics (CC), fluorescence “in situ” hybridization (FISH) and Comparative Genomic Hybridization (CGH).

Patients & Methods: A total of 53 patients were included. Three different groups were defined according to the chromosomal data: 1. Patients with both normal CC and CGH (n=20). 2. Cases with cytogenetics and/or CGH aberrations with no 11q involvement (n=19). 3. Patients with abnormalities in 11q assessed by cytogenetics and/or CGH (n=14). FISH analyses were performed with three different specific probes for the regions containing the genes BCL1 (11q13), ATM (11q22) and MLL (11q23).

Conventional cytogenetics and CGH were carried out according to standard methodologies.

Results: Overall 23 out of the 53 patients analysed by FISH (43%) showed abnormalities on 11q: gains on 11q were present in 20 cases (38%) while rearrangements on 11q were present in 4 cases (7%) (one case had both t(11;14) and gain on 11q). All but one gains involved the three different regions (BCL1, ATM and MLL genes) analysed. Only rearrangements of BCL1 were observed. In the group of patients with normal cytogenetics and CGH, 7 out 20 cases analysed (35%) showed abnormalities on 11q: 2 patients had ins(11;14), one patient a t(11;14) and the remaining 4 cases gains of 11q. Seven out the 19 (37%) patients displaying other cytogenetics abnormalities had also 11q gains. No translocations involving 11q were present in this group. In the third group FISH studies confirmed in all but one case the presence of gains on 11q detected by conventional cytogenetics or CGH. The negative case had a gain on 11q11-12 ascertained only by CGH.

Conclusions: Chromosomal abnormalities on 11q are frequent in MM. In these cases FISH refined cytogenetics and CGH studies and showed more sensitivity. Therefore FISH studies for abnormalities on 11q it should be use in the routine evaluation of MM.

(Partially supported by Grants of the Spanish FIS 01/1161 & G03/136)

**044 GENETIC CHANGES BY COMPARATIVE GENOMIC HYBRIDIZATION IN SMOLDERING MULTIPLE MYELOMA (SMM). DIFFERENT PATTERN ACCORDING TO THE SMM TYPE (EVOLVING vs NON-EVOLVING).**


Introduction: Patients with SMM met the diagnostic criteria of multiple myeloma (MM) but have no anemia, hypercalcemia, renal failure or extramedullary plasmacytomas. After a period of stability (median 3.5 yrs) most of these patients develop symptomatic MM. We identified two subsets of SMM: 1) evolving SMM, characterized by a progressive increase in the M-protein, a previous MGUS in most patients and a shorter time to progression into overt MM (median 1.6 yrs), and 2) non-evolving SMM, characterized by a stable M-protein that abruptly increases when symptomatic MM develops and a longer time to progression (median 4.3 yrs). Previous studies with comparative genomic hybridization (CGH) in patients with symptomatic MM have shown multiple abnormalities, including gains in 9q, 11q and15q and losses in 13q, 16q and 22q. However, there are no reports on CGH studies in patients with SMM.

Objective: To study the cytogenetics abnormalities by CGH in patients with both types of SMM.

Patients and methods: Seventeen patients with SMM (7 evolving, 8 non-evolving) were studied by CGH. Previous enrichment of plasma cell was performed in all bone marrow samples with monoclonal antibody BB4 conjugated with immunomagnetic beads. CGH was performed as described in the supplier’s protocol (Vysis®).

Results: Thirteen of the 15 patients (87%) showed chromosomal imbalances by CGH analysis. Twelve of the 13 patients (92%) had chromosomal gains whereas in only 6 patients (46%),
chromosomal losses were found. The most frequent abnormalities were gains of chromosomes 9 and 15, which were observed in 7 of the 13 patients (54%). Chromosomal gains were detected in all evolving and non-evolving patients with CGH imbalances. In contrast, chromosomal losses were observed in 5 of the 7 patients (71%) with evolving SMM and in only one of the 8 patients (16%) with non-evolving SMM. The most recurrent changes in evolving SMM were gains of 1q (57%) and deletion of 8p, 13q, 14q and 16q (43%). Patients with non-evolving SMM showed recurrent gains on 3p, 7, 11q and 18 (33%).

Conclusions: The most recurrent changes in SMM consist of gains of chromosomes 9 and 15. Evolving SMM displays multiple chromosomal imbalances with gains and losses (including deletion of chromosome 13), findings consistent with the characteristic chromosomal abnormalities commonly seen in patients with symptomatic MM. In contrast, in non-evolving SMM chromosomal losses are uncommon. Therefore, although the number of patients studied is low, the two subsets of SMM seem to have different cytogenetic patterns, which are associated with a different natural history.

045 Gene expression profiling of multiple myeloma reveals molecular portraits in relation to the pathogenesis of the disease

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Although multiple myeloma (MM) is a unique entity, a marked heterogeneity is actually observed among the patients, which has been first related to immunoglobulin (Ig) types and light chain subtypes, and more recently to chromosomal abnormalities. In order to further investigate this genetic heterogeneity, we analyzed gene expression profiles of 92 primary tumors according to their Ig types and light chain subtypes with DNA microarrays. Several clusters of genes involved in various biological functions such as immune response, cell cycle control, signaling, apoptosis, cell adhesion and structure significantly discriminated IgA- from IgG-MM. Genes associated with inhibition of differentiation and apoptosis induction were up-regulated while genes associated with immune response, cell cycle control and apoptosis were down-regulated in IgA-MM. According to the expression of the 61 most discriminating genes, BJ-MM represented a separate subgroup that did not express either the genes characteristic of IgG-MM, or those of IgA-MM at a high level. This suggests that transcriptional programs associated to the switch could be maintained up to plasma cell differentiation. Several genes whose products are known to stimulate bone remodeling could discriminate between κ and λ MM. One of these genes, Mip-1q was overexpressed in the κ subgroup. In addition we established a strong association (P<.0001) between κ subgroup expressing high levels of Mip-1q and active myeloma bone disease. This study shows that DNA microarrays enable to perform a molecular dissection of the bioclinical diversity of MM and provides new molecular tools to investigate the pathogenesis of malignant plasma cells.

046 Cytogenetic Analysis For Chromosome 13 Abnormality In Multiple Myeloma: Experience From A Cancer Center In India

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Introduction: Chromosomal abnormalities are rapidly emerging as one of the important prognostic factor in Multiple Myeloma (MM). The most common numerical abnormality reported is in relation to chromosome 13 (13q- or deletion of chromosome 13). It has been demonstrated to carry independent poor prognosis. However, there are no Indian data as regard to incidence of chromosome 13 abnormality in Indian patients. Material and Method: From March 2000 to May 2002, 22 newly diagnosed patients were enrolled onto this study to find out incidence and significance of chromosomal 13 abnormality in MM patients. Cytogenetic analysis on bone marrow aspiration was done using Trujillo’s method with some modification. Patients characteristics: Median age- 53.3 years (32-75 years), Sex- males 15, Stage distribution- IIA- 16, and IIIB- 6 patients. Seventeen patients had positive serum “M” band (median level 2.45gm/dl). Five patients had pure light chain myeloma. Median bone marrow plasma cells were 60%. Results: Of the 22 patients 16 had adequate analyzable metaphases, 2 had few and overlapped metaphases, and in 4 patients no metaphases were found. Median number of metaphases analysed were 9.5. Chromosome 13 abnormality in the form of deletion was seen in only one patient. This patient achieved PR to melphalan based therapy and is alive after follow up of for 2.5. Since, only one was detected to have chromosomal abnormality no meaningful correlation with M band level, plasma cell percentage, stage at diagnosis, and its prognostic significance was possible. As per literature 30-40% of patients will have chromosomal 13 abnormality. The possible reasons for low detection rate could be: only 16 patients had analyzable metaphases, median number of metaphases analyzed were low (<reflecting low mitotic index), and in many samples G banding resolution was poor. Conclusion- Conventional cytogenetics is a useful investigation modality for MM patients. However, we feel that further refinement of the technique is required in developing centers like ours so as to increase the yield of metaphases. This will than reflect the true incidence of chromosomal abnormality and its prognostic significance. Alternatively synchronization techniques or FISH should be employed.

047 COMPARISON OF THE PROGNOSTIC IMPACT OF CHROMOSOME 13 DELETION DETECTED BY CONVENTIONAL CYTOGENETICS vs FISH ANALYSIS IN MULTIPLE MYELOMA PATIENTS: AN SPANISH GEM GROUP STUDY.

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On behalf of GEM group.

Introduction: Abnormalities on chromosome 13 (13q- and monosomy) have been associated with unfavorable prognosis in patients with multiple myeloma (MM). The different prognosis significance of 13 abnormalities depending on the method of detection, conventional karyotype or FISH analysis, remains a matter of controversy.
Objective: To compare the impact on disease outcome of abnormalities on chromosome 13 detected by FISH vs karyotype detection in a series of MM patients treated with intensive chemotherapy.

Material and Methods: Cytogenetics (CG) and FISH analysis (LSI 13/RB1 probe, Vysis Inc.) were performed simultaneously in 318 consecutive patients with newly diagnosed MM, registered in the Spanish GEM trial since January 2000. So far, a preliminary prognosis study have been carried out in the first 140 enrolled patients which had clinical, biological and follow-up data available.

Results: An abnormal karyotype was detected in 48/120 (40%) successful cultures, of whom 13 (11%) showed chromosome 13 monosomy. A del(13q) by FISH was present in 35 of the 140 patients (25%): all patients with -13 by CG had del(13q) by FISH (group 1: CG+/FISH+) and in addition, FISH detected del(13q) in 16/92 (17%) patients with normal or non informative karyotype (group 2: CG-FISH+). 13q- by FISH was significantly associated with hypodiploidy (12 patients, p<0.005), and high serum 2-microglobulin (p=0.007), calcium (p=0.02) and % of bone marrow plasma cells (p=0.009). Patients with 13q- detected either by CG or FISH showed very poor prognosis. Upon comparing the outcome of group 1 vs group 2 patients no significant differences were observed. The estimated median EFS times were 17.4 vs 93.7 months for patients with and without del(13q), respectively. Abnormal karyotype affected EFS but not OS and the median EFS time (23.2 months) was longer than that observed in del(13q) group (17.4 months). When impact of del(13q) was analysed in the normal karyotype group, the adverse outcome of this abnormality remained invariant. On multivariate analysis, the most important negative factor for both EFS and OS was del(13q) (p=0.001).

Conclusion: The preliminary data confirmed that deletions on chromosome 13 detected either by FISH or conventional cytogenetics are associated with adverse outcome. Interestingly, our study shows that adverse impact of 13q- is particularly evident soon after diagnosis. FISH analysis of 13q is mandatory in all MM patients at time of diagnosis since normal karyotypes by CG with del 13q detected by FISH have a short survival. (Supported by Grants from Spanish FIS 01/1161 & G03/136)

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14q32 Translocations and 13q Deletions Determine The Course of Patients with Multiple Myeloma (MM)


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MM is a monoclonal malignant plasma cell disorder that shows a biological and clinical heterogeneity with genetic base. Chromosomal translocations to the immunoglobulin heavy-chain (IgH) locus on chromosome 14q32 have been identified in MM (50-80%). They represent the mechanism of activation of several proto-oncogenes. The loci most frequently involved are 11q13, 16q23, and 4p16, with the consequent dysregulation of cyclin D1, c-maf, and FGFR3 respectively.

IgH abnormalities are important early events in myeloma pathogenesis so, the main goals of this study were to determine their incidence and clinical significance. Thus, we analyzed bone marrow samples from 40 newly diagnosed MM patients using fluorescent in situ hybridization (FISH) with the probes specific for the following chromosomal changes: illegitimate rearrangements of the IgH gene (LSI IgH dual color, break apart), t(11;14)(LSI IGH/CCND1), and 13q14 deletions (LSI D13S25, D13S319). Interphase signals were evaluated in a minimum of 200 nuclei per slide.

Rearrangements of the 14q32 region were observed in 18 of 40 patients (45%), with the following distribution: t(11;14) in 5 (12.5%), and IgH translocations with other chromosomal partner (Tr-x) in 13 patients (32.5%). 13q14 deletions were found in 11 of 40 patients (27.5%). Patients with t(14q32) displayed more frequent del(13q) than patients lacking any t(14q32) (33.3% vs 22.7%). Furthermore, del(13q) were more common among cases with Tr-x than among cases with t(11;14) (38.4% vs 20%). Regarding clinical parameters, patients with IgH translocations were more likely to have a higher percentage of bone marrow plasma cells (69.5% vs 50%, p=0.019). Presence of both chromosomal abnormalities (IgH translocations and 13q deletions) was associated significantly with serum β2-microglobulin (median 8.8 mg/dl, p=0.034), and lactate dehydrogenase (median 457.5 U/l, p=0.049) levels. We confirmed a positive association between t(11;14) and IgH translocations and 13q deletions) displayed the worst OS (7 months, p=0.04), and progression free survival (6 months, p=0.001).

In conclusion, IgH translocations different from t(11;14) and 13q deletions are strongly associated with a subgroup of adverse outcome in MM.

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LOSSES ON 13q AND GAINS ON 11q AND 3q EVALUATED BY COMPARATIVE GENOMIC HYBRIDIZATION ANALYSIS ARE ASSOCIATED WITH A POOR OUTCOME IN MULTIPLE MYELOMA

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Aim: To analyze the prognostic value of comparative genomic hybridization (CGH) in patients with multiple myeloma.

Patients: Bone marrow samples from 90 untreated patients with MM were evaluated. Median age was 62 years (range: 45-90). All patients showed a bone marrow infiltration >35%. 74% of the patients were in stage III. All patients were homogeneously treated according to Spanish protocols (PETHMA 94 and 96). Survival analysis was carried out on the group of 73 patients treated with conventional chemotherapy without autotransplant.

Methods: Comparative genomic hybridization was carried out in all patients. Ratio values above 1.25 and below 0.75 were considered to represent chromosomal gains and losses respectively. A high-level amplification was considered when the fluorescence ratio values exceeded 1.5. Abnormalities on chromosomes 13 and 11 were confirmed by FISH. In all cases the
most relevant clinical and biological characteristics as well as the S-phase assessed by flow cytometry were also analysed. Results: Chromosomal imbalances were identified by CGH analysis in 60 out of the 90 cases (67%). A total of 320 changes were identified by CGH with a median of 4 changes per case (range: 1-28). Gains of chromosomal material were more frequent than losses (224 gains vs 96 losses). The most frequent aberrations among the cases with genomic changes were gains on chromosome regions 1q (44%), 9q (27%), 15q (25%), 11q (24%), 5q (24%), and 3q (18%), while losses mainly involved chromosomes 13 (44%), 16q (18%), 8p (11%) and 6q (9%). Interestingly the most frequent losses (13q-, 16q- and 8p-) were associated each other (p=0.008). In a similar way, gains on different chromosomes, leaving out 1q, tended to be present together. Thus, cases with gains on 1q displayed a significantly higher incidence of losses on 13q, 6q and 16q (p=0.03), but not of gains of other chromosomes. The clinical variables influencing negatively survival were: age > 70 (p=0.002), 2-microglobulin > 6 mg/L (p=0.02), calcium > 11.5 mg/dL (p=0.001), creatinine > 2 mg/dL (p=0.004), ECOG > 2 (p=0.01), and number of plasma cells in S-phase > 1.5% (p=0.001). Patients with losses on 13q and gains on 3q and 11q assessed by CGH had a significantly worse overall survival compared with patients without these abnormalities (p=0.03, p=0.04 and p=0.02 respectively). The rest of genomic changes did not confer an adverse prognosis. Conclusions: CGH detects a high incidence of chromosomal gains and losses in MM. Gains on 1q, 9q, 15q and 16q as well as losses on chromosomes 13 and 16q were the most frequent genomic changes. Losses on 13q and gains on 3q and 11q determined by CGH were associated with a shorter survival. (Supported by Grants from Spanish FIS 01/1161 & G03/136)

050 Cyclin D1 overexpression has no effect on survival in myeloma while upregulation of MM-SET may confer a favourable prognostic effect.
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Translocations involving chromosome 14q32 are amongst the most common genetic abnormalities in myeloma, occurring in up to 80% of cases. Despite the frequency of occurrence, their pathogenic significance is not yet clear. The most frequent translocations involve chromosomes 11q13, 4p16 and 16q32. We examined the expression of 2 candidate oncogenes on chromosomes 11q13 and 4p, Cyclin D1 (CND1) and MM-SET respectively, in purified myeloma plasma cell (MPC) populations, using real-time RT-PCR with β-actin as internal control, Sybr Green I as fluorophore and a series of standards containing 10^{-1} to 10^{7} cDNA copies. PCs were purified on the basis of CD38hi and CD138 expression by flow sorting. CND1 was quantitated in 18 MPC populations, with CND1: β-actin cDNA ratios ranging from 0.01 to 314. CND1 is not expressed in normal PCs (CND1: β-actin ≤ 0). In 11/18 samples, CND1: β-actin was 0.01-1.4, while 7 samples demonstrated increased ratios of 6.2 to 314, indicating CND1 overexpression. There was no significant difference between the 2 groups in survival, β2 microglobulin (4.2 and 60 mg/L respectively, normal range 0–2.4mg/L) or mean PC labelling index (PCLI) (5.0% and 5.8% respectively, normal range 0–4%) measured by flow cytometry. The correlation between CND1 expression and FISH detection of t(11;14) has been confirmed in 9 samples. The t(4;14) translocation was detected by nested RT-PCR for “hybrid” cDNA containing IgH and the candidate oncogene MM-SET, using myeloma cell lines carrying t(4;14) as internal controls. Of 32 samples examined, 7 were positive. Survival of the 2 groups also showed no significant difference. In a separate group of 12 patients, MPCs were examined for MM-SET expression using real-time RT-PCR. MM-SET: β-actin cDNA ratios ranged from 0.1 to 22. MM-SET expression has also been reported to be low or undetectable in normal PCs. Eight of the 12 MPCs examined showed MMSET: β-actin ratios of 0 to 1.6. The other 4 samples demonstrated higher ratios of 9 to 22, indicating increased MM-SET expression. Survival of these 4 patients appeared to be superior compared with the other 8 patients, although not significant due to small sample size. The mean PCLI of the 2 groups with low and high MMSET expression were 2.4% and 7.4% respectively (normal range 0–4%). In summary, expression analysis of candidate genes in chromosome 14q32 translocations in purified MPCs has shown no effect of CND1 overexpression on survival, while a possible favourable prognostic effect of MM-SET upregulation will be further examined in a larger cohort.

051 Frequency and prognostic relevance of t(4;14) in previously untreated Multiple Myeloma (MM) patients receiving either single or double autotransplants.
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In patients with MM a number of recurrent translocations involving chromosome 14 at band q32 have been recently identified, the most common being the t(11;14) and the t(4;14). Both these chromosomal abnormalities are closely associated with particular presenting features of the disease and may help to identify patients at different risk of death. In particular, the t(11;14) predicts for good prognosis, whereas the t(4;14) has been reported to be an unfavorable prognostic feature. The t(4;14) has been described almost exclusively in MM patients, although its exact role in the pathogenesis of the disease has not fully elucidated. The translocation affects at least two potential oncogenes, MMSET on der(4) and Fibroblast Growth Factor Receptor 3 (FGFR3) on der (14); the role of two additional genes (TACC3 and LETM1) located near the breakpoint region on chromosome 4 has not yet been evaluated. In the present study we investigated the frequency and the prognostic relevance of the t(4;14) in a series of 63 patients with de novo MM, who were randomized to receive either a single (Tx-1) or a double (Tx-2) autotransplant as primary therapy for their disease. For this purpose we analyzed (1) the presence of t(4;14) by RT-PCR of the hybrid transcript between MMSET and the IgH locus; (2) the overexpression of FGFR3 by Real-time RT-PCR; (3) the frequency of potentially activating point mutations in the FGFR3 translocated coding region, by direct sequencing of RT-PCR products; (4) the relationship between t(4;14), response to high-dose therapy and outcome of autotransplant(s).

Overall, the t(4;14) was detected in 17/63 patients (27%), a value slightly higher than that reported by others. 13/17 patients had both MMSET/IgH fusion gene and FGFR3 overexpression, while
4 patients had MMSET/IgH but did not overexpress FGFR3. This finding further confirms the possible discrepancy between MMSET/IgH positivity and FGFR3 overexpression. Comparison between t(4;14)+ and t(4;14)- patients revealed that both groups were well balanced with respect to the most common presenting features of MM. In 36 patients, for whom material was available, FISH analysis for the detection of 13q deletion and/or monosomy was performed. Results showed that t(4;14)+ patients were more likely to carry also del(13) than t(4;14)- patients (46% vs. 29%, respectively). Among patients who attained stringently defined complete remission following either Tx-1 or Tx-2, t(4;14)+-patients were 35%, as opposed to 6% of t(4;14)- patients (p = 0.05, intention to treat). With a median follow-up of 26 months, no difference in overall (OS) and event free survival (EFS) was disclosed between t(4;14)- and t(4;14)+ patients.

In summary, 27% of our MM patients carried the t(4;14). In this cohort of homogeneously treated patients, the t(4;14) predicted for lower response to high-dose therapy. Longer follow-up is required to assess the influence of these abnormalities on OS and EFS. In a subgroup of six patients carrying t(4;14), point mutations were detected in the FGFR3 coding region, thus suggesting a possible constitutive FGFR3 activation.

Supported by MIUR, Firb project RBAU012E9A_001 (M.Cavo), University of Bologna, Progetti di Ricerca ex-60% (M.Cavo) and Fondazione Carisbo.

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Expression of SSX Cancer Testis Antigen Genes Correlates with Reduced Survival in Multiple Myeloma
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The identification of genes selectively expressed by malignant cells has major implications for diagnosis, treatment, and understanding the biology of cancer. For many different types of cancer, the best treatment may be a combination of chemotherapeutic and immunotherapeutic regimens, the latter involving a vaccine consisting of tumor-specific antigens. Cancer testis antigens (CTA) are good tumor vaccine candidates because they are expressed in tumor and testis cells, but not normal cells. In this study, we analyzed SSX (Synovial sarcoma, X chromosome) CTA gene expression in Multiple Myeloma (MM) and its corresponding pre-malignant condition, monoclonal gammopathy of undetermined significance (MGUS). The SSX genes comprise a family of nine members on the X chromosome. SSX1, 2, 4, and 5 expression has been detected in many cancers, and SYT-SSX fusion via t(X;18) is considered a central transforming event in synovial sarcoma. The SSX genes encode as important new candidates for further consideration in the study and treatment of multiple myeloma.

independent of the frequency of plasma cells in the BM. Three groups consisted of singly expressed SSX genes: SSX1, 15/70 SSX+ samples; SSX4, 11/70; SSX5, 8/70. Only SSX4 expressed alone had any impact on survival, and in this case SSX4 expression was associated with increased survival. The remaining group (13/70 SSX+ samples) consisted of different combinations of two or three SSX genes expressed together and did not correlate with survival. SSX expression was also detected in 11/40 MGUS BM samples: 7/11 SSX+ samples were SSX4+; 2/11, SSX1+; and 2/11, SSX1 and 4+. No SSX expression was detected in uninvolved BM samples. Ongoing studies have localized SSX expression to the CD138+/CD38+ plasma cell population and are now focused on SSX protein detection in these cells and anti-SSX immune responses in MM patients. This study demonstrates that SSX expression is detectable in a significant fraction of MM and MGUS patient BM samples, and that in MM, expression of SSX4 alone is associated with increased survival and co-expression of the four SSX genes correlates with reduced survival. The relationship between SSX expression in MGUS and progression to MM remains unclear. This work identifies SSX genes and the proteins they encode as important new candidates for further consideration in the study and treatment of multiple myeloma.

052
Expression of SSX Cancer Testis Antigen Genes Correlates with Reduced Survival in Multiple Myeloma
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The identification of genes selectively expressed by malignant cells has major implications for diagnosis, treatment, and understanding the biology of cancer. For many different types of cancer, the best treatment may be a combination of chemotherapeutic and immunotherapeutic regimens, the latter involving a vaccine consisting of tumor-specific antigens. Cancer testis antigens (CTA) are good tumor vaccine candidates because they are expressed in tumor and testis cells, but not normal cells. In this study, we analyzed SSX (Synovial sarcoma, X chromosome) CTA gene expression in Multiple Myeloma (MM) and its corresponding pre-malignant condition, monoclonal gammopathy of undetermined significance (MGUS). The SSX genes comprise a family of nine members on the X chromosome. SSX1, 2, 4, and 5 expression has been detected in many cancers, and SYT-SSX fusion via t(X;18) is considered a central transforming event in synovial sarcoma. The SSX genes encode as important new candidates for further consideration in the study and treatment of multiple myeloma.
3. Immunobiology

053 IMMUNOPHENOTYPE PROFILE IN MONOCLONAL GAMMOPATHY UNDETERMINED SIGNIFICANCE AND MULTIPLE MYELOMA

Lemes A, Galvano E, de la Iglesias S, Santana A*, Gómez MT, Jiménez S. and Molero T. 
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Monoclonal gammopathy of undetermined significance (MGUS) is the most common plasma cell dyscrasia occurring in up to 10% of the population over age 75. Differential diagnosis between myeloma multiple (MM) and MGUS is sometimes uncertain especially in early phases of MM, and the distinct immunophenotype of plasma cells in each one can be helpful in order to reach a correct diagnosis.

The aim of this study was to describe the immunophenotype of bone marrow plasma cells (BMPC) in MGUS and MM.

Methods: Bone marrow samples from 57 patients with MM and 28 patients with MGUS were evaluated by flow cytometry (FC) after incubation with the following monoclonal antibodies: CD138FITC/CD56PE/CD19TC/CD38APC. The plasma cells were identified as CD38++/CD138 positive cells and a live gate acquisition strategy was performed.

Results: Four plasma cells subpopulations could be identified in the bone marrow (Table 1): CD19+/CD56-; CD19-/CD56+; CD19+/CD56+ and CD19-/CD56-. The number of BMPC displaying this phenotype in MGUS was 53% of MGUS displayed a CD19-/CD56- origin al immunophenotype in the 100% of the population over age 75. Differential diagnosis between myeloma multiple (MM) and MGUS is sometimes uncertain especially in early phases of MM, and the distinct immunophenotype of plasma cells in each one can be helpful in order to reach a correct diagnosis.

Discussion. Plasma cells in MGUS and MM showed a heterogeneous immunophenotype. Unlike other studies we observed a normal plasma cell population in MM. Nevertheless, 32% of MGUS cases displayed only an aberrant plasma cell phenotype. In summary, our data showed that flow cytometry analysis can be helpful in the differential diagnosis of MGUS and MM but we did not find a single parameter for this purpose. The CD56-/CD19- population in MGUS patients and their prognostic significance warrant further investigation.

054 Immunophenotypic differences between clonal plasma cells from MGUS (monoclonal gammopathy of undetermined significance), MM (multiple myeloma) and PCL (plasma cell leukemia)*

M Pérez de Andrés1, 2, M Martín-Ayuso1, 2, J Almeida1, 2, MA García-Marcos3, MI González Fraile4, MJ Moro4, J Galende5, MJ Rodríguez6, JF San Miguel2, 3, A Orfao1, 2

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Previous studies have reported on the existence of important phenotypic differences between normal and clonal plasma cells (PC). In contrast, few markers have been shown to be differentially expressed in clonal PC from patients with MGUS (monoclonal gammopathies of undetermined significance) as compared to MM (multiple myeloma) and PCL (plasma cell leukemia).

In the present study, we have comparative analyzed the expression of surface markers on clonal PC from MGUS, MM and PCL patients as well as normal PC from the same individuals and an age-matched healthy group of donors. Our major interest focused on the study of phenotypic markers of clonal PC that are involved in their interactions with the bone marrow microenvironment, these including costimulatory (CD40, CD80, CD86) and adhesion molecules (CD38, CD56, CD138, CD106), cytokine receptors (CD126, CD130), HLA molecules (HLA-Iα and β2-microglobulin) and the Fas receptor (CD95).

Bone marrow PC from total of 30 MGUS, 27 MM, 4 PCL (all newly diagnosed untreated patients) and 5 normal individuals were analyzed by flow cytometry specifically gating on CD38high and CD138+ cells. In all cases, antigen expression was evaluated as the mean fluorescence intensity (MFI).

Our results confirm that PC from normal BM display identical phenotypic features to those of normal PC from cases with MGUS. They were constantly positive for CD138, CD38, CD40, HLA-Iα and β2-microglobulin, while they lacked on the expression of the IL-6Rα-chain (CD126) and CD80 and displayed variable reactivity for CD130, CD86 and CD56 (Table 1).

Upon comparing this phenotype of normal PC with that of PC from patients with monoclonal gammopathies (MG), significant differences were found for all markers tested, except for CD138, CD130, CD80 and CD106. A detailed analysis of these differences shows that clonal PC from patients with MG constantly displayed abnormally higher levels of CD56, CD86 and CD126, together with low amounts of CD38, independently of the diagnostic subgroup. In addition, HLA-Iα and β2-microglobulin were expressed at abnormally high levels in MGUS and to a lower extent in MM but decreased or normal in PCL; in turn CD40, expression was abnormally decreased in MM and PCL but not in MGUS, whereas reactivity for CD95 was only detected in PC from PCL cases. The exact phenotypes of normal
and clonal PC from MGUS, MM and PCL patients are summarized in table 1.

Table 1: Immunophenotypic features of normal and clonal BM plasma cells.

<table>
<thead>
<tr>
<th></th>
<th>Normal BM PC</th>
<th>MGUS</th>
<th>Clonal PC</th>
<th>MM</th>
<th>PCL</th>
</tr>
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<tbody>
<tr>
<td>% of CD56+ cells</td>
<td>0.0±12.1</td>
<td>9.0±6.9</td>
<td>59.1±44.8</td>
<td>78.5±38.6</td>
<td>50.0±57.7</td>
</tr>
<tr>
<td>% of CD126+ cells</td>
<td>0.0±0</td>
<td>7.0±48.3</td>
<td>7.9±41.9</td>
<td>50.0±70.7</td>
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<tr>
<td>% of CD95+ cells</td>
<td>0.0±7.1</td>
<td>7.1±26.7</td>
<td>5.0±22.4</td>
<td>3.8±19.6</td>
<td>50.0±57.7</td>
</tr>
<tr>
<td>% of CD86+ cells</td>
<td>54.7±39.9</td>
<td>26.8±20.7</td>
<td>100±0</td>
<td>84.2±36.2</td>
<td>100±0</td>
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<tr>
<td>CD40 MFI</td>
<td>837.5±80.4</td>
<td>1164.4±75.3</td>
<td>952.0±84.4</td>
<td>540.8±70.6</td>
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<td>HLA-1k MFI</td>
<td>4567.9±200</td>
<td>1826.8±167.0</td>
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<tr>
<td>β2-microglobulin MFI</td>
<td>398.4±275.4</td>
<td>475.1±420.3</td>
<td>1168.8±986</td>
<td>597.0±744</td>
<td>209.1±161.1</td>
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<tr>
<td>CD38 MFI</td>
<td>8442.2±1566.9</td>
<td>7439.0±2399.0</td>
<td>5884.4±2051.1</td>
<td>4063.0±1856.4</td>
<td>2387.4±1119.1</td>
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</table>

Results expressed as mean±standard deviation; MFI: mean fluorescence intensity.

In summary, our results confirm and extend previous observations on the phenotypic differences existing between normal and clonal PC from patients with MG. In addition, we demonstrate that clonal PC from MGUS and from both MM and PCL patients display clearly different phenotype which might translate into different interactions between clonal PC and the BM microenvironment in the distinct diagnostic conditions.

056 Expression of Chemokine Receptors in Multiple Myeloma and Its Correlation with Clinical Status: Preliminary Results

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Hematology Department(1) and Immunology Department(2), Hospital Universitario de la Princesa, Madrid- Spain

Introduction: The mechanisms that contribute to the multiple myeloma cell recruitment to the bone marrow environment are not well known. Some recent findings suggest that chemokines could play a relevant role for this compartmentalization of MM cells in the bone marrow (1,2,3). Chemokines are molecules related with cellular adhesion and migration. These molecules are implicated in lymphocyte trafficking and homing. In this study we evaluate the expression of chemokine receptors in a group of MM patients and its relation with disease type and clinical status. Patients and methods. Bone marrow aspirates from 12 patients with multiple myeloma were studied for the expression of chemokine receptors: CCR7, CXCR5 and CXCR4. Such analysis was performed by using standard flow cytometry with mAb directed against CCR7, CXCR3 and CXCR4 on electronically gated myelomatous cells, previously identified by staining with anti-CD38, anti-CD45, anti-CD19 and anti-CD56 mAbs.

Results. Expression of chemokine receptors in the mentioned patients are shown in the next table:
Correlation of chemokine receptor levels and clinical status after treatment will be presented.

Conclusions. The expression of CXCR4, CXCR5 and CCR7 were demonstrated in bone marrow samples of patients with multiple myeloma. The different levels of expression could be related to the heterogenous MM clinical patterns and to previous treatment. More studies are need to evaluate the exact significance of this expression and to elucidate this possible correlation with clinical MM status and with novel therapies that could influence plasmocyte adhesion mechanism as thalidomide.

References:
1.-Moller C et al. Expression and function of chemokine receptors in multiple myeloma. Leukemia 2003, 1:203-210

057

pHENOTYPIC AND FUNCTIONAL CHARACTERIZATION OF CHEMOKINE RECEPTORS ON MALIGNANT plasma cells IN PATIENTS WITH MULTIPLE MYELOMA

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Chemokine receptors and their ligands play a relevant role in the organogenesis of lymphoid tissues and in the trafficking of B lymphocytes. Several data reported in the literature indicate the involvement of chemokines and their receptors in the pathogenesis of lymphoid neoplasias of B-cell lineage have been reported in the literature, whilst few data are available on plasma cells obtained from patients with multiple myeloma.

In this study freshly isolated malignant plasma cells obtained from the bone marrow of 30 patients with multiple myeloma, 3 myeloma cell lines and 5 normal PC suspensions were recovered from peripheral blood and pleural effusions.

Flow cytometry analysis showed that all the samples studied were positive for CD38++, CD138+ and CD117, while CD56+ was expressed on PC obtained from MM patients and MM cell lines. Other receptors, i.e. CCR1, CCR5, CXC1, CXC2, CXC3, CCR5 and CXCR5 are commonly absent on myeloma cells obtained from patients. CCR1 and CCR3 are found to be expressed in RPMI-8226 and OPM-2 cell lines. Functional in vitro evaluation showed a consistent migration of malignant plasma cells in the absence of exogenous chemotactic stimuli. In others, chemotaxis might be induced by several chemokines, mainly SDF and Mip3. Furthermore the analysis of chemokine production from malignant plasma cells revealed a heterogeneous pattern.

These observations indicate that myeloma cells have variable pattern of expression of chemokine receptors and displayed a marked capability to migrate in vitro, this property being more evident in patients with progressive disease or in patients with the leukemic form of the disease.

058

Expression of receptor activator of NF-κB ligand (RANKL) on bone marrow plasma cells from patients with multiple myeloma correlates with osteolytic bone disease

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Increased bone resorption is a hallmark of multiple myeloma and is due to excessive osteoclast activation. The recently characterized receptor activator of NF-κB ligand (RANKL) is a key mediator of osteoclastogenesis and plays a crucial role in bone destruction in malignant bone disease. Myeloma cells induce RANKL expression in bone marrow stromal cells. Recently, we detected RANKL protein on human myeloma cells using flow cytometry and immunocytochemistry. In this study, we analyzed the association of RANKL expression on plasma cells and osteolytic bone disease in patients with multiple myeloma. Flow cytometry was performed on bone marrow samples derived from controls and 50 multiple myeloma patients with (n=35) or without (n=15) osteolytic bone lesions on radiography. Plasma cells were identified as CD38++/CD138+ cells. The mean fluorescence index for RANKL on the surface of bone marrow plasma cells was correlated with the bone status of the patients. Bone marrow plasma cells from controls showed no or only a very weak expression of RANKL, the median mean fluorescence index (MFI) was 6. In contrary, RANKL could be detected on bone marrow plasma cells from all patients with multiple myeloma, median MFI was 47. The difference in MFI for RANKL expression on bone marrow plasma cells from controls and myeloma patients was highly significant (P < 0.0005). Myeloma patients with osteolytic bone lesions showed a significantly higher surface expression of RANKL (median MFI=60, range 16-2494) compared to patients without osteolysis (median MFI=16, range 6-229) (P < 0.0005). These findings show that the level of expression of RANKL on bone marrow plasma cells correlates with the bone status in multiple myeloma and underscores the clinical relevance of the RANKL expression by myeloma cells.

<table>
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<tr>
<th>UPN</th>
<th>MM Type</th>
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VEGF expression is very common, particularly at disease
in the biology of Multiple Myeloma.

Results: We investigated thirteen patients in different disease’s
stages. Six cases were performed in marrow clots, seven in biopsy
clinders, and in a single occasion an aspirate’s smear was
conducted, resulting in a negative report.

Their relationship to Thalidomide therapy could not be pursued,
the two patients received it, one was not evaluable because
early drug intolerance, and the other, who had a long lasting
response, could not be evaluated since her baseline marrow smear
was not evaluable.

The greatest possibility was found at diagnosis, being markedly
intense in 4 out of 7 cases, but all of them displaying expression.
On the other hand no receptors were detectable in 2 out of three
responding cases.

Conclusions: Cytokine induction is playing a more relevant role
in the biology of Multiple Myeloma.

VEGF expression is very common, particularly at disease
presentation, and consequently early phase therapy with VEGF
inhibitors seems more then appropriate.

From the technical standpoint, the marrow clot is as good as the
cylinder, and possibly more feasible.

Multiple Myeloma (MM) is characterized by a malignant
proliferation of monoclonal plasma cells in the bone marrow that
are highly chemotherapy resistant suggesting the involvement
of anti-apoptotic proteins. Subsequently we studied the expression
of Bcl-x, a downstream target of the transcription factor STAT3
that is controlled by IL-6. Forty MM patients were studied
upfront treatment and 25 MM patients at time of relapse. The
results were compared with the Bcl-xl expression in normal bone
marrow plasma cells. In addition the results were correlated with
the level of CRP, the microvessel density (MVD) and clinical
outcome. Upfront treatment 75% of the patients demonstrated a
normal Bcl-x expression compared to normal plasma cells, whereas
22% of the patients demonstrated an elevated expression. In
addition 20% of the patients demonstrated two populations of
plasma cells with normal and elevated Bcl-xl expression. At time
of relapse no change in Bcl-xl expression was observed compared
to upfront treatment. No distinct correlation was observed
between the level of CRP and the Bcl-xl expression suggesting an
IL-6 independent effect. In addition no significant correlation was
observed between Bcl-xl expression and clinical outcome. In
summary these data indicate that an elevated Bcl-xl expression can be observed in a subgroup
of MM patients which is likely IL-6 independent and will
contribute to chemotherapy resistance of the malignant plasma
cells.

BCL-xl expression in Multiple Myeloma
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Department of Hematology and Pathology, University Hospital
Groningen, Hanzeplein 1, 9700 RB Groningen, The Netherlands

β2-Microglobulin (2M) is the invariant chain of the major
histocompatibility complex (MHC) class-I molecules and free
B2M (usually <2 mg/L) is found in body fluids under
physiological conditions. Increased synthesis and release of B2M,
as indicated by an elevated serum B2M concentration, occurs in
autoimmune and infectious diseases and in hematological
malignancies including multiple myeloma (MM), all of which
involve activation or inhibition of the immune system. In MM,
the level of B2M is one of the most important independent
predictors of survival, attesting to an important yet unidentified
role of B2M in the disease. We hypothesized that B2M is not only
a surrogate for tumor burden but, at high concentrations, may
have a negative impact on the immune system. Indeed, our
preliminary study showed that addition of more than 10 µg/ml of
B2M to T-cell cultures significantly inhibited antigen-induced T-
cell activation in a dose-dependent manner (p < 0.01). Further
tests revealed that B2M mainly affected the function of antigen-
presenting cells (APCs). As dendritic cells (DCs) are the most
professional APCs and play a pivotal role in initiating primary
immune responses, we examined the effects of B2M on
monocyte-derived DCs. Our results show that addition of high
concentrations (~10 µg/ml) of B2M to the culture impaired in
vitro generation of DCs; B2M reduced the yield of DCs, inhibited
the upregulation of their surface expression of HLA-ABC, CD1a,
CD80 and CD86, diminished their ability to activate allospecific T cells and present recalled antigen (PPD) to autologous T cells (p < 0.05 to p < 0.01). The generation of type-1 T-cell response induced in allogeneic mixed lymphocyte reaction was also compromised when β2M-treated DCs were used as APCs. Compared with control cells, β2M-treated DCs produced much more IL-6, IL-8 and IL-10 (p < 0.01). After additional 48-hour culture in the presence of TNF-α and IL-1β (to induce DC maturation), 2M-treated (in the first 7 days) DCs expressed significantly fewer surface CD83, HLA-ABC, costimulatory and adhesion molecules, and were less potent at stimulating allospecific T cells (p < 0.05). During cell culture, β2M downregulated the expression of phosphorylated MAP kinases ERK and MEK, inhibited NF-κB and activated STAT3 (p < 0.01) in treated cells, all of which are involved in cell differentiation and proliferation. In conclusion, we demonstrate, for the first time, that β2M at high concentrations retards the generation of DCs, which may involve downregulation of the MHC class-I molecules, inactivation of Raf/MEK/ERK cascade and NF-κB, and activation of STAT3. Thus, our study identifies β2M as a negative regulator of the immune system, which is compatible with the clinical arena in MM and merits further study to examine its role on other components of the immune system.

063 PATIENTS WITH MONOCLONAL GAMMOPATHIES DISPLAY ABNORMAL NUMBERS OF FUNCTIONALLY ALTERED CIRCULATING DENDRITIC CELLS AND MONOCYTES.

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In the development and progression of monoclonal gammopathies (MG) the plasma cell microenvironment including inflammatory cells is believed to play a crucial role. The aim of the present study was to quantitatively analyze the distribution and function of circulating monocytes and dendritic cells (DC) in patients with MG in order to gain insight into their potential role in the clinical behaviour of the disease. PB samples corresponding to 34 untreated newly diagnosed patients with MG of undetermined significance (MGUS), 27 multiple myelomas (MM) and 4 plasma cell leukemias (PCL) were analyzed. PB samples from 12 healthy individuals aged >40 years, were studied in parallel.

As compared to normal PB, the absolute numbers of circulating DC were decreased in MGUS, normal in MM, and increased in PCL: overall number of circulating DC of normal individuals of 65±18 versus 54±32, 64±34 and 237±91 cells/µl respectively. However, absolute monocyte levels were increased in all MG, the highest values being found in PCL patients (509±523 monocytes/µl in MGUS, 482±345 in MM and 616±392 in PCL) as compared to healthy individuals (308±114 monocytes/µl). From the functional point of view, PB monocytes and DC from healthy and MGUS individuals showed no spontaneous production of inflammatory cytokines. In contrast, increased spontaneous ex vivo IL6 and TNFα production was observed in a variable proportion of MM and PCL, the percentage of cytokine-secreting cells being higher in PCL, where 50% showed spontaneous production. Overall, after in vitro stimulation with LPS+IFNγ, production of inflammatory cytokines by PB monocytes and DC was lower in MG than in healthy subjects. Interestingly, monocytes from MGUS cases had a lower response to LPS+IFNγ than patients with MM and PCL, as regards production of IL1, IL6, IL8, IL12 and TNFα. In contrast, no major differences were observed by the different subsets of PB DC in the three disease groups, except for TNFα (table1). Accordingly, a higher production of TNFα was observed in PCL and MM as compared to MGUS.

Table 1: Production of TNFα from stimulated DC in different MG

<table>
<thead>
<tr>
<th>Myeloid DC</th>
<th>Lymphoplasmoeytoid DC</th>
<th>CD16+ DC</th>
</tr>
</thead>
<tbody>
<tr>
<td>CONTROL</td>
<td>316 (38-613)</td>
<td>18 (7-35)</td>
</tr>
<tr>
<td>MGUS</td>
<td>107 (7-834)</td>
<td>16 (5-227)</td>
</tr>
<tr>
<td>MM</td>
<td>157 (12-1212)</td>
<td>15 (5-438)</td>
</tr>
<tr>
<td>PCL</td>
<td>194 (30-732)</td>
<td>30 (13-153)</td>
</tr>
</tbody>
</table>

- Results expressed as median and range (in brackets) of the mean fluorescence intensity/cell.

In summary, our results show that in MGUS and MM circulating DC are decreased in numbers, but increased in PCL, as compared to healthy individuals. In contrast, the absolute number of circulating monocytes is increased in all groups of MG. From the functional point of view circulating DC and monocytes from MM and PCL but not MGUS showed increased spontaneous production of inflammatory cytokines; this is associated in both MM and PCL as well as in MGUS with a lower in vitro response to LPS+IFNγ. The distinct subsets of DC and monocytes have an impaired function in the different MG.

064 Clonally expanded T cells in myeloma with a “late” memory/effector phenotype are associated with improved survival.

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We have previously demonstrated that the presence of an expanded CD8+CD57+ T cell clone in patients with myeloma is a significantly prolonged overall survival. Recently, it has been shown that a pair of differentially expressed co-stimulatory receptors on CD8+ T cells, CD28 and CD27, can be used to differentiate three distinct memory/effector phenotypes: CD28+CD27+, CD28-CD27+ and CD28-CD27- which correspond to “early”, “intermediate”, and “late” subsets respectively on a putative linear differentiation pathway (1). We have performed 5-color flow analysis on 22 patients with MM with a total of 29 expanded T cell receptor Vβ (TCRVβ) clones and found that the majority (73%) of patients had clonally expanded CD8+CD57+TCRVβ+ T cells of the “late” subset; while the remaining 27% were of the “intermediate” subset. These results demonstrate that the phenotype of the clonal T cells in myeloma is similar to the phenotype of the memory CD8 cells seen in patients with CMV infection and dissimilar to the phenotypic pattern seen in patients with hepatitis C, HIV and EBV. Using a CMVPP65 (NLVPMVATV) HLA-A2-tetramer on
two HLA-A2+ myeloma patients with CD28-CD27- TCRβ expansions, it was demonstrated that only 2-3% of total CD8 T cells were CMV-tetramer positive. As the expanded Vβ clones contained 10 times as many cells as the tetramer positive population and several patients with T cell clones had negative CMV serology it was considered that the T cell clones were not CMV specific. Patients whose expanded T cells expressed a “late” T cell phenotype had a significantly improved survival over those with an “intermediate” phenotype (p=0.013). The survival of patients with an “intermediate” T cell phenotype was not significantly better than those patients who had no detectable T cell clones. Thus it is the expanded CD8+CD57+ cell clones with a late memory/effector phenotype which are associated with a good prognosis in patients with myeloma. Although this “late” memory/effector phenotype is found in patients with chronic CMV infection, the T cell clones in patients with myeloma were not CMV specific but indicate a similar pattern of chronic antigen presentation and T cell recognition.


065

Idiotype specific T cells detected with MHC Class I Tetramers

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The extent to which idiotype-specific T cells have been either deleted or tolerised in patients with multiple myeloma is unknown. Previous attempts to detect these cells have traditionally used a range of surrogate or functional markers of the T cell response. As direct detection of antigen-specific T cells can be demonstrated with MHC tetramers we sequenced the hypervariable regions of both the heavy and light chain hypervariable regions of 6 patients (HLA-A0201) with expanded CD8+ clones and used bioinformatic programs (BIMAS http://bimas.dct.nih.gov/molbio/hla_bind and SYFPEITHI http://syfpeithi.bmi-heidelberg.com/Scripts/MHCServer.dll/EpPredict.htm) to demonstrate the presence of immunodominant peptides in only 3 of the 6 patients. We then prepared tetrameric MHC class I complexes containing CDR-derived immunodominant peptides to search for idiotype-specific T cells in the blood of the 2 surviving patients. CMV P65 HLA-A*0201 tetramer and the CMV-derived peptide NVLPVMVATV were used to optimise the novel staining strategies. The standard staining technique failed to detect tetramer positive cells in either patient and suggested that idiotype-specific T cells were deleted. Modified staining techniques, using different staining temperatures, crossover controls and preincubation with free peptide, demonstrated that low avidity tetramer positive T cells comprised 1-10% of an IL-2 activated T cell population (<5% of the total T cells) in both patients. Preincubation with free peptide caused downregulation of the TCR:CD3 complex on both CMV and idiotype-specific T cells, most likely due to inhibition of TCR recycling after peptide ligation. These studies identify a strategy for the selection of idiotype-specific peptides, monitoring the presence of peptide-specific T cells during idiotype vaccination and offer a potential means to isolate and expand tumour-specific T cells for adoptive immunotherapy.

066

T-cell epitopes within the CDRs and FRs of the tumor derived immunoglobulin heavy chain in multiple myeloma

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The idiotypic structure of the monoclonal immunoglobulin (Ig) in multiple myeloma (MM) might be regarded a tumor-specific antigen. The present study was designed to identify T-cell epitopes of the variable region of the Ig heavy chain in MM (n=5) using bioinformatics and analyze the presence of naturally occurring T cells against idiotype derived peptides. A large number of HLA-binding (class I and II) peptides were identified. The frequency of predicted epitopes was depended on the database used: 245 in BIMAS and 601 in SYFPEITHI. Most of the peptides displayed a binding-half-life or score in the low or intermediate affinity range. The majority of the predicted peptides were complementarity-determining region (CDR) rather than framework region (FR) derived (52-60% vs. 40-48%). Most of the predicted peptides were confined to the CDR2-FR3-CDR3 “geographical” region of the Ig-VH region (70%) and significantly fewer peptides were found within the flanking (FR1-CDR1-FR2 and FR4) regions (p=0.01). Eight to ten amino acid (aa) long peptides corresponding to the CDRs and fitting to the actual HLA-A/B haplo-types recognized spontaneously albeit with a low magnitude type I T cells (IFN-γ) indicating an ongoing MHC class I restricted T-cell response. Most of those peptides had a low binding half-life (BIMAS) and a low/intermediate score (SYFPEITHI). Furthermore, 15-20 aa long CDR 1-3 derived peptides recognized also spontaneously type I T cells indicating the presence of MHC class II restricted T cells as well. This study demonstrates that a large number of HLA-binding idiotypic epitopes can be identified in patients with MM. Such peptides may spontaneously induce a type I MHC class I as well as class II restricted memory T cell response.

067

THE EFFECTS OF PAMIDRONATE IN T γδ CELLS OF MULTIPLE MYELOMA AND PAGET'S DISEASE.

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Introduction: Several studies have assumed an antitumor activity for bisphosphonates, which could be mediated by the stimulation of γδT cells. We have analyzed in vivo activation of γδT cells in Multiple Myeloma (MM) and Paget’s disease (PD). Design and methods: 23 patients with MM and 5 with PD received pamidronate. Samples were taken before pamidronate and after 72-96h for flow cytometry measurements of γδ TCR, CD69+ and CD25+. Results: we have founded no differences between both diseases in γδT cells, but CD69+ γδT cells and CD25+ before infusion ranked higher in MM (CD69+ of 0.18% MM, 0.07% PD; CD25+ of 0.047% MM, 0.022% PD). In PD the increase in CD69+ γδT cells was greater, although the activation did not equal MM levels. The CD25+ γδT cells only increased in MM (0.13%). After 72h, the stimulation CD69+ was greater in PD (pre:0.07%, post:0.14%). The CD25+ γδT cells only increased in MM (pre:0.017%, post:0.031%). In MM, the CD69+ γδT cells increased after 72h (pre:0.20%, post:0.24%), but
decreased after 96h (pre:0.12%,post:0.07%), when CD25+ γδT cells levels were higher. We did not find significant differences in this study between the different groups, although this could be due to the small size of the subgroups. Conclusions: MM and PD have the same percentage of γδ T cells, but the activation was higher in MM. After pamidronate, the induction was rapid in MM: after 72h, MM did not show any increase in CD69+, but higher levels of CD25+. Stimulation was delayed in PD, with higher levels of CD69+ but no increase in CD25+. These results show that pamidronate stimulate γδT cells in MM and PD, but further studies are needed to assess this antitumor role.

068  
T-cells rendered to express chimeric immunoreceptors targeting B-cell antigens lyse multiple myeloma cells  
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There is increasing evidence that multiple myeloma patients may also harbour circulating multiple myeloma progenitors, which express early/intermediated B-cell antigens such as CD19 and CD20 but lack expression of the plasma cell antigen CD138. Transplantation of purified CD19+ PBMC from multiple myeloma patients into irradiated NOD/SCID mice results in multiple myeloma engraftment (Pilarski et al; Exp Hematol. 2002.; Matsui et al, ASH 2002). Furthermore multiple myeloma progenitors cells are also characterized by over-expression of the multi drug resistance gene and may therefore be resistance to standard high dose chemotherapy followed autologous stem cell transplantation.

Thus novel treatment strategies targeting such multiple myeloma cells effectively are highly warranted.

An attractive approach of combing the dynamic properties of cytolytic potential of T-cells with the development of high affinity specific for B-cell antigens is the construction and expression of chimeric immunoreceptors in T-cells. This was accomplished by the genetic modification of CTL to express a chimeric immunoreceptor composed of either the CD19 or the CD20-specific single-chain immunoglobulin extracellular targeting domain fused to a CD3ζ intracellular signalling domain. CD19- and CD20-specific CD8+ T-cells clones were established through limiting dilution and the surfaces expression of chimeric immunoreceptors determined by flow-cytometry with an anti-Fc polyclonal antibody. T-cell clones with the highest expression were then used as effectors in standard Cr-release assays with the CD19+ multiple myeloma cell lines CD19+ ARH-77 and HS-Sultan and as a control the CML cell line CD19+ K562. Both the CD19 and CD20-specific CD8+ T-cell clones could specifically lyse the MM cell lines ARH-77 and HS-Sultan (up to 65% at E/T of 1:1) whereas the CD19- targets where not recognized. Lysis was abrogated by preincubation of targets with anti-MHC-I antibody, demonstrating the MHC-I independent recognition of the targets. Similarly T-cells stimulated with the either MM cell line ARH-77 or HS-Sultan readily produced IFN-γ (ELISA) whereas incubation with the CD19- cell lines K562 no IFN-γ production was detected in collected supernatants.

In summary, construction and expression of either CD19 or CD20-specific chimeric immunoreceptors in CD8+ T-cells results in specific recognition of CD19+ multiple myeloma cells. These pre-clinical observation helps justify further investigation into the potential of this novel concept in preventing the engraftment of multiple myeloma in NOD/SCID mice.

069  
Myeloma Infiltrating Lymphocytes (MILS) as a Strategy for Enhancing Adoptive Immunotherapy  
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Sidney Kimmel Comprehensive Cancer Center at Johns Hopkins, Baltimore, MD 21231

T cell mediated immune responses can mediate a tumor-specific response in the appropriate setting. Unfortunately, the severe defects underlying immune responses in cancer-bearing hosts limit the efficacy of adoptive T cell transfer from such hosts. Ex-vivo activation of T cells with beads conjugated to CD3 and CD28 antibodies has been capable of activating and augmenting peripheral T cells in a non-specific polyclonal manner from cancer-bearing hosts. However, a major concern is the absence of tumor-specificity of this approach. T cells can be obtained from the tumor micro-environment with a heightened tumor specificity as compared to peripheral blood. In comparing T cells obtained from these two different compartments, we have demonstrated oligoclonal restriction of myeloma infiltrating lymphocytes (MILS) obtained from marrow aspirates. Utilizing the anti-CD3/CD28 conjugated beads, the MILS showed a greater expansion and an enhanced response to or plasma cells compared to peripheral blood lymphocytes, in several assays, suggestive of the activation of a memory/effector response with heightened tumor specificity.

This strategy provides the ability to enhance the efficacy of adoptive immunotherapy through the selective isolation and expansion of tumor-specific cell populations with a greater antigenic specificity compared to tumor antigen stimulated cells. The ability to infuse these polyclonal myeloma specific T cells and ultimately integrate the adoptive transfer of these cells with myeloma-specific vaccinations are the goals of the clinical implementation of such an approach.

070  
Ex Vivo Activation and Expansion of T Cells from the Peripheral Blood of Multiple Myeloma Patients Using the XcellerateTM Process  
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1) Cedars Sinai Medical Center, Los Angeles, CA; 2) Washington University, St. Louis, MO; 3) Johns Hopkins University, Baltimore, MD; 4) Hackensack University, Hackensack, NJ; 5) University of California, San Francisco, CA; 6) Xcye Therapies, Inc., Seattle, WA

Previous studies in multiple myeloma have demonstrated a significant association between survival and baseline levels of T cells prior to chemotherapy (Kay et al., Blood 2001) as well as lymphocyte recovery to >500/mm3 fifteen days following autologous transplantation (Porrata et al., Blood 2001). Therefore, infusion of large numbers of T cells post transplant may improve clinical results. We have developed the XcellerateTM Process for the ex vivo activation and expansion of T cells using immobilized anti-CD3 and anti-CD28 antibodies covalently linked to magnetic beads (Dynabeads® M-450 CD3/CD28 T). Adding the beads to peripheral blood mononuclear cell cultures results in the rapid co- ligation of the T cell receptor/CD3 complexes and CD28
molecules expressed on the surface of T cells. This, in turn, leads to the rapid activation of T cells and cell division.

Twenty-two expansions have been conducted to date using samples from multiple myeloma patients who have received a variety of prior therapies. The number of T cells increased a mean of 404 fold following an 8-14 day culture process. Purity and viability of T cells post-Xcellerate averaged 97% and 96%, respectively. The CD4:CD8 ratio increased from 1.3 to 2.1 during the course of culture. Significant upregulation of key surface/effector molecules, including CD25 (IL-2 receptor) and CD154 (CD40 ligand) occurred.

We have also developed a flow cytometric assay for evaluating residual myeloma cells in the final Xcellerated T cell product. Using analysis of surface marker expression, including CD38, CD138, CD45 and CD56, we have been unable to detect any myeloma cells in the final product within the limits of detection of the assay (~0.01%).

Based upon these data, we have initiated a clinical trial to evaluate the activity of Xcellerated T Cells in patients with multiple myeloma, who are undergoing an autologous stem cell transplantation. Following induction chemotherapy, patients undergo a leukapheresis to collect peripheral blood mononuclear cells for the Xcellerate Process. Patients then undergo stem cell mobilization and collection, followed by high dose chemotherapy with Melphalan (200 mg/m2). Patients receive a stem cell infusion, followed three days later by an infusion of 5-10 x 1010 autologous Xcellerated T Cells. Three patients have been treated to date. In the two evaluable patients, lymphocyte recovery to > 1,000/mm3 occurred within three days following T cell infusion (Day 6 post transplant). In contrast, lymphocyte recovery usually does not occur for > 3-4 weeks post transplant in myeloma patients treated with this regimen.

071 Comparison of single nucleotide cytokine polymorphisms between patients with myeloma and their siblings

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Aim: Multiple Myeloma (MM) is a monoclonal B cell disorder in which a network of multiple cytokines is highly active. Tumor Necrosis Factor-alpha (TNFα), Transforming Growth Factor-beta(TGF-β1), Interleukin-6 (IL-6), Interleukin-10 (IL-10) and Interferon-γamma (INF-γ) are among those studied the most. The role of these cytokine genes during the evolution to multiple myeloma is still under investigation. Polymorphisms of cytokines are associated with high or low secretion patterns. Although the frequency of some alleles have been analysed and compared to healthy controls there is no published study which has evaluated cytokine polymorphism patterns between patients and their siblings. Patients and Methods: 35 patients (M/F: 21/14) aged 28-73 (median: 51) admitted to Ankara University Ibn-i Sina Hospital were included in the study. Patients were Stage I (n:3), II A (n:3), III B (n:17), IIIB (n:6); presented with IgG (n:19), IgA (n:4), light chain (n:4), IgM (n:1), PCL (n:1) and plasmacytoma(n:4). Treatment was radiotherapy (n:8), MP (n:18) or VAD (n:23). Ten patients have been transplanted (8 auto and two tandem). Following a period of 3-81 months (median: 25) from diagnosis seven of the patients responded, 21 relapsed or became refractory following at least one protocol, six patients aren't evaluable yet. Patients had a median of two siblings (1-5).

Peripheral blood sample DNA was used for detection of TNFα -308, TGF-β110,25, Interleukin-10-1082,-819,-592 Interleukin-6-174 and Interferon γ+874genotyping. Gene polymorphism was detected using the CYTGEN kit (One-Lamba, USA).

Results: Frequency of all cytokines among patients compared to the frequency among siblings were similar:

<table>
<thead>
<tr>
<th>Cytokines</th>
<th>Frequency %</th>
<th>OS Disparities</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Patient</td>
<td>Sibling</td>
</tr>
<tr>
<td>TNF-α high low</td>
<td>11.8</td>
<td>8.9</td>
</tr>
<tr>
<td>TGF high intern low</td>
<td>76.5</td>
<td>23.5</td>
</tr>
<tr>
<td>IL-10 high intern low</td>
<td>11.4</td>
<td>28.6</td>
</tr>
<tr>
<td>IL-6 high Low</td>
<td>97.1</td>
<td>2.9</td>
</tr>
<tr>
<td>IFN-γ high intern low</td>
<td>20</td>
<td>51.4</td>
</tr>
</tbody>
</table>

However individual comparisons between patients and siblings revealed similar (TNF) or different expression patterns (TGF, IL-6, IL-10, INF). The greatest disparity was observed with IL-10. Khi-Square analysis between the the frequency of disparities in TNF(13.8%) and IL-10(81.3%) was not significant. Among the patients with single nucleotide polymorphism disparities, differences were detected in all(n:1), four(n:4), three(n:5), two(n:11) or one(n:8) of the cytokines. Patients carrying high TNFα, low IL-10, high IL-6 high IFN γ alleles had an insignificant survival advantage. Age at presentation and cytokine high/low patterns werenot associated with survival.

Conclusion: Increase in the duration of followup and sample size are required for better evaluation of prognostic-etiological effects of these cytokine profiles.

072 Elevated plasma OPN in multiple myeloma patients

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A characteristic feature of multiple myeloma is the development of osteolytic bone lesions. Osteopontin (OPN) is a noncollagenous matrix protein produced by various cell types including osteoblasts, osteoclasts and several types of tumor cells. OPN is essential for the migration, attachment, and resorptive activity of osteoclasts. Furthermore, it has been shown that OPN inhibits hydroxyapatite formation during bone mineralization. An association between elevated plasma OPN, increased tumor burden and decreased survival has been reported in women with metastatic breast cancer and men with prostate carcinoma. Here, we report that plasma levels of OPN are elevated in myeloma patients as compared to healthy individuals. However, levels of OPN are not associated with survival in the myeloma patients. We further show that myeloma cell-lines and primary myeloma cells produce OPN. After inculating OPN-producing ANBL-6 human myeloma cells in irradiated SCID-mice we were not able to detect human OPN in mouse plasma-samples. Interestingly, levels of circulating rodent OPN in these mice were elevated as compared to control mice. This suggests that
myeloma cells are able to induce OPN-secretion from host cells. The fact that myeloma-derived hepatocyte growth factor (HGF) induced OPN-secretion in a dose-dependent manner from the osteoblastic cell line Saos-2 supports this finding.

073

A novel recombinant bispecific single-chain antibody, Wue-1xCD3, induces T-cell mediated cytotoxicity towards human myeloma cells

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The development of antibody-based strategies for the treatment of Multiple Myeloma (MM) has been hampered by the fact that suitable plasmacell-specific surface antigens have been missing so far. Although normal and malignant plasmacells express a number of well characterized surface markers they all have turned out to be not plasmacell-specific. However, recently a novel monoclonal antibody, designated Wue-1, has been generated, which specifically binds to the cell surface of normal and malignant human plasma cells. Therefore, Wue-1 is an interesting and promising candidate to develop novel immunotherapeutic strategies for the treatment of multiple myeloma. One variant for an antibody-based strategy is the bispecific antibody approach. In particular, recombinant bispecific single-chain antibodies are interesting candidates because they show exceptional biological properties discriminating these molecules from conventional antibodies. We have generated a novel MM directed recombinant bispecific single-chain antibody, Wue-1xCD3 (bscWue-1xCD3) and analyzed the biological properties of this antibody using the MM cell line NCI-H929 and primary cells from the bone marrow of patients with multiple myeloma and autologous or allogeneic effector T-cells. We were able to show that the bscWue-1xCD3 induces efficient T-cell mediated cell death of NCI-H929 cells and primary myeloma cells in 10/11 cases analyzed so far. In contrast to conventional bispecific antibodies described so far, the bscWue-1xCD3, is efficacious at low E:T ratios and without T-cell pre-stimulation. Target cell lysis was specific for Wue-1 antigen positive cells and could be blocked by the Wue-1 monoclonal antibody. Wue-1 antigen negative NALM-6 cells were not lysed by bscWue-1xCD3 Ab. To our knowledge this is the first plasma cell directed bispecific antibody described so far, showing promising results, and might therefore be a new potential agent for the treatment of malignant plasma cell disorders.

074

A recombinant HLA class I-specific single-chain Fv diabody induces cell death in human myeloma cells

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Cross-linking of HLA class I molecules has been shown to induce programmed death of human neoplastic lymphoid cells as well as activated lymphocytes. We have generated a novel anti-HLA class I monoclonal antibody (2D7) to examine the possible role of this molecule expressed on multiple myeloma (MM) cells. Moreover, a recombinant single-chain Fv diabody was constructed from the parent mouse IgG antibody. This diabody (2D7-DB) effectively causes cross-linking of HLA class I molecules and induces cell death of target cells. In this study, we compared the levels of HLA class I expression in MM cells and normal bone marrow cells, and evaluated the in vitro effects of 2D7-DB to induce cytotoxicity and/or apoptosis in MM cells. Cell surface expression of HLA class I on bone marrow cells was analyzed by flow cytometry using fluorescein-labeled 2D7 and phycoerythrin-labeled anti-CD38 antibody for MM cell gating. Cells were cultured for 48 hours with 2D7-DB or 2D7 in the presence or absence of F(ab′)2 goat anti-mouse IgG (GAM). Cell viability was determined by trypan blue staining. Induction of apoptosis was assessed by Annexin V/propidium iodide staining. HLA class I molecules were more strongly expressed in 13/15 of patient MM cells than in normal myeloid cells or lymphocytes. Cross-linking of HLA class I with 2D7 and GAM induced cytotoxicity of patient MM cells. More importantly, 2D7-DB alone mediated cytotoxicity in MM cells at 100 ng/mL, but not in normal myeloid cells or lymphocytes. MM cells treated with 2D7-DB showed cytoplasmic vacuolization and positive staining for Annexin V. These findings indicate a functional role for HLA class I molecules in MM cell death and also provide new insights into the possible therapeutic strategies for targeting this molecule in MM.

075

Decitabine increases expression of cancer antigens MAGE-3 and NY-ESO-1 in multiple myeloma cell lines

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NY-ESO-1 and MAGE-3 both belong to the group of Testis Cancer Antigens (TCA’s). TCA’s are present in testis and various malignancies, but not in normal tissues. NY-ESO-1 and MAGE-3 are highly immunogenic, eliciting B-cell and HLA-class I/class II restricted T-lymphocyte responses. About 20% of newly diagnosed multiple myeloma (MM) patients express these antigens, to reach more than double in relapse.
Recognition of these antigens appears to be limited in cancer patients. This is caused by different mechanisms: deficiencies of antigen processing and recognition, or an antigen expression below the threshold for immune recognition.

Aim: By upregulating the expression of CTA’s under conditions potentially reachable in the clinical setting, a higher immunogenicity could be reached, which is a major advantage in the setting of immunotherapy.

It has recently been shown that the demethylating agent 5-aza-2’-deoxycytidine or decitabine (DAC) can enhance MAGE-3 and NY-ESO-1 expression in different cell lines.

We tried to determine if this is also true in MM cell lines.

Methods: Seven different cell lines originating from myeloma, lymphoid malignancy and lung cancer (U266, RPMI8226, Calu-6, U937, EJM, JJN3 and MMS1) were exposed to DAC at varying concentrations (0, 0.1, 1 or 10 M) and during different exposure times (0, 24, 48 and 72 Hrs.). The cultures were performed in RPMI 1640 media with 10% FBS, 1% penicilline/streptomycine, 1% L-glutamine and 1% Hepes buffer. Both MAGE-3 and NY-ESO-1 were evaluated by flowcytometry (FCM), PCR and quantitative PCR (TaqMan).

Results: 1) We observed an increase of expression of TCA’s in cell lines U937, HMS1 and U266 after exposure to the demethylating agent DAC. We’re the first group to report this finding.
2) NY-ESO-1 and MAGE-3 not only appear to be excellent candidate proteins for immunotherapy in MM, but their expression can be significantly upregulated by DAC.

4. From MGUS to symptomatic MM

4.1 Diagnostic guidelines

076 Nordic Myeloma Study Group Guidelines on the Diagnosis and Management of Multiple Myeloma

E Hippe1, M Hjorth2, I Turesson3, J Westin4, F Wisløff5 for the Nordic Myeloma Study Group

University Hospital of Copenhagen, Herlev1, Lidköping Hospital, Lidköping2, University Hospital Malmö3, University Hospital Lund4 and Ullevål University Hospital, Oslo5 (E-mail: hippe@dadlnet.dk)

The Nordic Myeloma Study Group (NMSG) is a network of clinicians and scientists working with myeloma patients and myeloma research projects within the Nordic countries. The group was founded in 1987 and today comprises 17 university clinics and 90 county hospital clinics in Denmark, Norway and Sweden, covering a population of about 12 million inhabitants. The aims of the group are: 1) to perform population based clinical studies to evaluate new therapeutic modalities in patients with multiple myeloma, with special regard to and their influences on the quality of life, 2) to study the mechanisms behind and the manifestations of the disease and 3) to inform and educate patients and their relatives about the disease and the therapeutic options available.

In 1995 NMSG first prepared a Nordic health care program covering all aspects of the diagnosis and care of multiple myeloma patients. The program was written in the Nordic languages and widely distributed among the participating clinics. The NMSG guidelines comprise: 1) Investigation, diagnosis and indications for treatment, 2) Initial therapy, 3) Treatment of complications, 4) Supportive care, 5) Management of relapsed/refractory disease, 6) Aspects of quality of life and economic evaluation of treatment modalities, and 7) Patient information. Where possible the guidelines end up in recommendations based on literature review and consensus of the NMSG expert opinion.

The guidelines have recently been updated and are from 2002 available on the NMSG homepage (http://www.nordic-myeloma.org). Also the web-based edition is written in the Nordic languages. Our recommendations are very similar to the guidelines later published by the UK Myeloma Forum. In the near future it would be possible to coordinate the Nordic/UK guidelines, and also to involve other interested European countries in the project.

077 Guidelines on the diagnosis and management of solitary plasmacytoma of bone (SBP) and solitary extramedullary plasmacytoma (SEP)

R. Soutar, H. Lucraft, A. Reece, J. Bird, G. Jackson, E. Low and Diana Samson

On behalf of the UK Myeloma Forum (UKMF)

SBP and SEP are rare tumours and most haematologists have little experience of treating such patients. Guidelines on the diagnosis and management of SBP and SEP would therefore be helpful. A working group of the UKMF, including a clinical oncologist (radiotherapist) and an orthopaedic surgeon, has drafted evidence-based guidelines. The main recommendations are as follows:
Recognition of these antigens appears to be limited in cancer patients. This is caused by different mechanisms: deficiencies of antigen processing and recognition, or an antigen expression below the threshold for immune recognition.

Aim: By upregulating the expression of CTA’s under conditions potentially reachable in the clinical setting, a higher immunogenicity could be reached, which is a major advantage in the setting of immunotherapy.

It has recently been shown that the demethylating agent 5-Aza-2'-deoxycytidine or decitabine (DAC) can enhance MAGE-3 and NY-ESO-1 expression in different cell lines.

We tried to determine if this is also true in MM cell lines.

Methods: Seven different cell lines originating from myeloma, lymphoid malignancy and lung cancer (U266, RPMI8226, Calu-6, U937, EJM, JNN3 and MMS1) were exposed to DAC at varying concentrations (0, 0.1, 1 or 10 M) and during different exposure times (0, 24, 48 and 72 Hrs.). The cultures were performed in RPMI 1640 media with 10% FBS, 1% penicillin/streptomycin, 1% L-glutamine and 1% Heps buffer. Both MAGE-3 and NY-ESO-1 were evaluated by flowcytometry (FCM), PCR and quantitative PCR (TaqMan).

Results: 1) We observed an increase of expression of TCA’s in cell lines U937, HSM1 and U266 after exposure to the demethylating agent DAC. We’re the first group to report this finding.

2) NY-ESO-1 and MAGE-3 not only appear to be excellent candidate proteins for immunotherapy in MM, but their expression can be significantly upregulated by DAC.

4. From MGUS to symptomatic MM

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Nordic Myeloma Study Group Guidelines on the Diagnosis and Management of Multiple Myeloma

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University Hospital of Copenhagen, Herlev1, Lidköping Hospital, Lidköping2, University Hospital Malmö3, University Hospital Lund4 and Ullevål University Hospital, Oslo5 (E-mail: hippe@dadlnet.dk)

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Guidelines on the diagnosis and management of solitary plasmacytoma of bone (SBP) and solitary extramedullary plasmacytoma (SEP)

R. Soutar, H. Lucraft, A. Reece, J. Bird, G. Jackson, E. Low and Diana Samson

On behalf of the UK Myeloma Forum (UKMF)

SBP and SEP are rare tumours and most haematologists have little experience of treating such patients. Guidelines on the diagnosis and management of SBP and SEP would therefore be helpful. A working group of the UKMF, including a clinical oncologist (radiotherapist) and an orthopaedic surgeon, has drafted evidence-based guidelines. The main recommendations are as follows:
Diagnosis requires a single area of bone destruction or an extramedullary mass of clonal plasma cells, with a normal BM aspirate and trephine, normal skeletal survey, no anaemia, hypercalcemia or renal impairment, and absent or low serum or urinary paraprotein. In addition, diagnosis of SBP requires an MRI of the spine showing no additional lesions. Over 50% of patients with apparent SBP progress to multiple myeloma (MM) and patients with an abnormal marrow appearance on MRI of the spine have a higher rate of progression to overt MM. Such patients should be considered to have MM at presentation. Further investigations which may distinguish between SBP and MM include immunophenotyping of bone marrow to look for an excess of kappa or lambda staining plasma cells, MRI examination of other areas to look for other plasmacytomas or abnormal BM appearances, and FDG-PET scanning to look for other foci of disease. In contrast to SBP, SEP rarely progresses to myeloma and there is no data on the use of MRI of uninvolved areas; the working group therefore considered that MRI of the spine is not required for the investigation and diagnosis of SEP.

Management: SBP should be treated with radical radiotherapy with a dose of 40 Gy in 20 fractions. A higher dose is recommended for tumours >5 cm. Surgery is reserved for patients with vertebral instability or neurological compromise; such patients should also receive radiotherapy. The timing of radiotherapy in relation to surgery should be determined for individual patients; while it may be preferable to carry out surgery before radiotherapy, the placing of metal supports can compromise the efficacy of radiotherapy. Reconstructive orthopaedic surgery may be required in patients with anterior column damage. Patients should be followed up carefully with regular paraprotein measurements, as over 50% will progress to myeloma. There is as yet no data on the use of the free light chain assay in monitoring patients with SBP.

SEP of the head and neck is also best treated with radiotherapy and radical surgery in this area should be avoided. A radiation dose of 45 Gy in 25 fractions is recommended. For SEP in other areas surgery should be considered as an alternative to radiotherapy. If surgical margins are involved then adjuvant radiotherapy should be given.

There are few data on adjuvant chemotherapy for either SBP or SEP, but it may be considered in patients with bulky tumours or histologically high-grade SEP.

078 Guidelines for the Diagnosis and Management of AL Amyloidosis
JM Bird, H Lachmann, J Cavenagh, A Mehta, PN Hawkins and Diana Samson
For the UK Myeloma Forum

Systemic AL amyloidosis is a protein conformation disorder in which monoclonal immunoglobulin light chains are deposited as AL amyloid fibrils, which progressively disrupt normal organ structure and function. It is often fatal within 2 years. AL amyloidosis poses special problems for patient care; diagnosis can be difficult and requires specialist expertise, while the optimum management remains unclear. The UK Myeloma Forum has been working with the UK National Amyloidosis Centre to formulate evidence-based guidelines for diagnosis and management. Some of the main recommendations are as follows:

Diagnosis requires histological confirmation of amyloidosis followed by immunohistochemical staining to characterize the fibril protein, although this technique is often not definitive in AL type. Evidence should be sought of an underlying B-cell disorder and serum and urine immunofixation should be performed in all cases even if routine electrophoresis is negative. DNA analysis may be required to exclude hereditary amyloidosis, since a monoclonal gammapathy may incidentally co-exist. Assessment of organ function requires assessment of renal, hepatic and cardiac function in all cases and neurological investigations in some patients. SAP scintigraphy allows determination of the extent and distribution of visceral amyloid deposits.

Monitoring Disease status should be monitored in terms of both amyloid deposition and the underlying B-cell disorder, including frequent monitoring of serum free light chains, serial SAP scintigraphy and assessment of associated organ dysfunction. Regression of amyloid and clinical improvement following chemotherapy in AL amyloidosis is always delayed for many months following adequate suppression of the underlying clonal disease, and treatment strategies in individual patients are presently best guided by their early effect on quantitative measurements of serum free light chains.

Treatment Chemotherapy regimens in AL amyloidosis are derived from those used in myeloma, but there have been few randomised, controlled trials in AL amyloidosis. Colchicine is ineffective. MP has been shown in phase III trials to be superior to colchicine in terms of response and survival, although response is slow and median survival still only 18-24 months. Alkylator based combination chemotherapy regimens have not been shown to be superior to MP. -Interferon has not been shown to be of benefit. An important objective is rapid suppression of amyloidogenic light chains, which infusional (VAD-type), monthly iv melphalan 25 mg/m2 ± dexamethasone and high dose regimens are able to achieve with broadly similar effect. However, high dose therapy with PBSCT has a TRM of 15-40% in AL amyloid. Its use should be restricted to selected patients (those with no cardiac involvement, 1-2 organs involved and GFR >50 ml/min), and particularly those who have not responded to less intensive regimens. In patients who are sufficiently fit, VAD is rational initial therapy, whereas monthly iv melphalan ± dexamethasone is an alternative when VAD is contra-indicated or ineffective. Other treatment options include oral MP, dexamethasone, thalidomide, novel therapies and palliative care. Supportive care is vital in all patients.

4.2 Characteristics of MGUS

079 MONOCLONAL GAMMAPATHY OF UNDETERMINED SIGNIFICANCE (MGUS): CLINICAL PREDICTORS OF MALIGNANT TRANSFORMATION IN 434 PATIENTS FROM A SINGLE INSTITUTION WITH A LONG FOLLOW-UP
Institute of Hematology and Oncology, Department of Hematology and Hematopathology Unit*, IDIBAPS, Hospital Clinic, Barcelona, Spain.

Background: MGUS is a frequent disorder characterized by the presence of a small serum M-protein in individuals with no evidence of multiple myeloma (MM), Waldenström’s macroglobulinemia (WM) or primary amyloidosis (AL). Although about one fourth of these individuals will evolve into a malignant disease, there are not well-established predictors of outcome.
Conclusions: In these series of patients with MGUS, the type (i.e. (p=0.009), the amount of M-protein (<15 vs. >15 g/L, p=0.005) transformation were IgA-type (p=0.003), kappa light chain respectively. The variables associated with a higher risk of CI: 10.5-20.3) and 34 % (95% CI: 22.6-45.3) at 10 and 20 years, (range: 1.4-16.9). The risk of transformation was 15.4 % (95%

After a median follow-up of 5.2 yrs, 50 patients (11.5 %) have After a median follow-up of 5.2 years, 50 patients (11.5 %) have

Results: The type of M-protein was IgG in 67.2 % of the cases, IgA in 18. %, IgM in 11.9 %, light chain in 1 % and biclonal in 1 %. The light chain was of kappa type in 56.4 % of the patients. The median M-protein size was 15.6 g/L (<10 g/L in 10.8 %, 10-20 g/L in 61.7 %, and >20 g/L in 27.4 %). The median percentage of BMPC in 305 reviewed samples was 4.6 % (range: 0.4-25). After a median follow-up of 5.2 years, 50 patients (11.5 %) have evolved into a malignant monoclonal gammopathy (44 MM, 5 WM and 1 AL). The median time to progression was 5.4 yrs (range: 1.4-16.9). The risk of transformation was 15.4 % (95% CI: 10.5-20.3) and 34 % (95% CI: 22.6-45.3) at 10 and 20 years, respectively. The variables associated with a higher risk of transformation were IgA-type (p=0.003), kappa light chain (p=0.009), the amount of M-protein (<15 vs. > 15 g/L, p=0.005) and the percentage of BMPC(<5 % vs. > 5 %, p=0.007).

Conclusions: In these series of patients with MGUS, the type (i.e. IgA or kappa) and size of M-protein (i.e. > 15 g/L) as well as the percentage of bone marrow plasma cells (i.e. > 5 %) predicted malignant transformation.

080 CLINICAL EVOLUTION AND PROGNOSTIC FACTORS IN 300 PATIENTS WITH ASYMPTOMATIC IgM MONOCLONAL GAMMOPATHY

M.Goldaniga, P.Gobbi, S.Cortelazzo, C.Brogli, A.Guffanti, E.Oldani, C.Stelitano, B.Bronzino, R.Calori, E.Pogliani, F.Merli and L.Baldini 1) GISL (Gruppo Italiano Studio Linfomi) 2) Divisione di Ematologia, Ospedali Riuniti , Bergamo, Italy

Asymptomatic clonal macroglobulinemia (ACM) is currently classified as an IgM MGUS or indolent Waldenström’s macroglobulinemia (WM), but its clinical relevance and propensity to evolve into lymphoid neoplasms is not well defined. We retrospectively evaluated 300 patients with ACM in order to identify the clinico– pathological features relating to its evolution into a symptomatic lymphoid neoplasm requiring treatment and create a prognostic score capable of distinguishing patient subgroups with different prognoses. The exclusion criteria were: 1) treatment-requiring conditions (high or rapidly increasing serum IgM levels, hyperviscosity or systemic symptoms, organomegaly and/or cytopenia); 2) the presence of an autoimmune disorder, HCV-related cryoglobulinemia, amyloidosis or other complications due to tissue deposits of IgM or clonal IgM leading to MM or low grade non-lymphoplasmacytic lymphoma; or 3) cases with an uncertain follow-up or unclear disease evolution or treatment criteria. The main characteristics at diagnosis are shown in the table.

After a median follow-up of 55 months (6-221), 43/300 patients (14.7%) required chemotherapy for symptomatic WM (70%), NHL (18.5%), amyloidosis (7%) or peripheral neuropathy (4.5%). Five- and 10-year overall survival was 98% and 90%, and evolution-free-survival 91% and 78%.

The features correlating with evolution to overt lymphoproliferative disease were: serum clonal IgM concentration (P<0.0001), serum nephelometric IgG concentration (P<0.0001), Bence Jones proteinuria (P<0.0001), hemoglobin (P<0.0001), nephelometric IgG concentration (P=0.0259) and albumin (P=0.0257) and male gender (P=0.04). The evolution-related variables at multivariate analysis were: serum IgM concentration, hemoglobin and male sex. The relative increased risk of evolution in males (vs females) was 4.095; that of a unit increase in serum IgM or decrease in hemoglobin value was respectively 2.68 and 0.787. By attributing arbitrary scores to the three prognostic variables (male=2, female=0; Hb <12g/dL=2, 12-15=1, >15=0; s-clonal IgM <0.7g/dL=0, 0.7-1.5=1; >1.5=2), we developed a simple prognostic index that separated the patients into those at low (score 1-3; 173 patients ) and high (score 4-6; 127 patients) risk of malignant evolution (P< 0.0001). We think that our prognostic index may have a practical diagnostic value and avoid the need to introduce new disease entities (such as indolent or asymptomatic WM) defined on the basis of strict but un reproducible clinico-hematological criteria.

<table>
<thead>
<tr>
<th>Variable</th>
<th>No. of evaluated pts</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age in years (mean ± SD)</td>
<td>300</td>
<td>63 ± 11</td>
</tr>
<tr>
<td>M/F (ratio)</td>
<td>300</td>
<td>186/114</td>
</tr>
<tr>
<td>Serum MC g/dL (median, min-max)</td>
<td>300</td>
<td>1.1 (0.11- 3.0)</td>
</tr>
<tr>
<td>Hb g/dL. (median, min-max)</td>
<td>300</td>
<td>13.8 (11.0-17.9)</td>
</tr>
<tr>
<td>Peripheral lymphocytes x 109/L (median, min-max)</td>
<td>287</td>
<td>2 (0.4-3.6)</td>
</tr>
<tr>
<td>PLT x 109/L (median, min-max)</td>
<td>298</td>
<td>238 (100-627)</td>
</tr>
<tr>
<td>Serum LDH UI (mean ± SD, min-max)</td>
<td>187</td>
<td>319 ± 104 (134-986)</td>
</tr>
<tr>
<td>Serum β2 microglobulin μg/ml (mean ± SD, min-max)</td>
<td>132</td>
<td>2.504 ± 1.052 (195-7.962)</td>
</tr>
<tr>
<td>No.of pts. with detectable Bence Jones proteinuria (%)</td>
<td>296</td>
<td>21/296 ( 7 )</td>
</tr>
<tr>
<td>No.of pts. with one serum polyclonal Ig reduction (%)</td>
<td>258</td>
<td>34/258 ( 13.2 )</td>
</tr>
</tbody>
</table>
| Bone marrow lymphoplasmocytes % (median, min-max) | 237 | 11 ( 3 - 92 )
**Incidence of solid tumors in cohort of patients with Monoclonal Gammopathies of Undetermined Significance along twenty years.**

Montañés MA, Franco-García E1, Martos C2, García-Carpintero G2, Recasens V, Gómez-López L2, Giralt M, Rubio-Félix D, Giraldo P.

Miguel Servet University Hospital, Military Hospital1, Aragon Health Service2. Zaragoza, Spain.

Background: Monoclonal gammopathy of undetermined significance (MGUS) is a disorder frequently related to immunological disfunctions of B-cell lines and aging, as well as several types of cancer, with an estimated incidence of 3% in people over 70s. Purpose of study: to determine the incidence of neoplastic disease in MGUS patients and to compare with cancer incidence in general population during a period of 20 years.

Patients & Methods: Since 1967 a cohort of 1,906 patients diagnosed of MGUS in the Hematology Department of Miguel Servet University Hospital was followed-up to 2002 (population at risk: 533,946 inh). A longitudinal, retrospective and descriptive study has been performed. Data source: clinical reports and population Cancer Registry of Zaragoza. Variables: demographic data (age, gender), date of MGUS diagnosis, immunoochemical subtype, date of cancer diagnosis, subtype and location of cancer. Cohort was stratified according to age and sex. Cancer diagnosis was considered as previous, concomitant or after MGUS diagnosis. Results: The incidence rate of MGUS in our population is 13.4 SD 1.6 cases/105 inh/y, as previously reported; estimated cancer incidence in our area was 255.2 SD 32.3 cases/105 inh/y for the period 1978-1998. During this period 639 MGUS developed cancer (33.1%), 684 neoplastic disorders were detected (range 1-3), in 35 cases (5.5%) two different neoplastic disorders were diagnosis and in 4 cases (0.5%) three different tumors were. In 25 cases a non-hematological and a hematological neoplasia were observed. In 180 cases (27.3%) both MGUS and neoplasia were simultaneous, in 293 cases (42.8%) cancer diagnosis preceded MGUS detection, and in the remaining 211 (30.9%) neoplasia appears along the follow-up.

The risk of cancer in patients with MGUS was 4.87 and 1.34 when only non hematological tumor were considered. Mean age: 65.5 SD 13.6 years. Gender: 384 M/255 F. MGUS subtype: IgG 433 (67.7%), IgA 58 (9.1%), IgM 142 (22.3%) and light chain disease 6 (0.9%); of these 30 were bicalon (4.7%) and 3 were tricalon (0.5%). Median time from MGUS to cancer was 60.1 SD 23.2 months (range 0-152). Cancer types associated to MGUS were: 12 MM, 294 lymphoproliferative disorders (NHL, CLL, HD, LAL), 5 LANL, 39 CMPD (PV, CML, MF, ET), 21 MDS and 294 non hematological cancers (skin 14.9%, digestive tract 12.6%, larynx and area 8.2%, urinary tract 7.8%, lung 7.1%, genital tract 4.7%, breast 3.4%, prostate 3.1%, thyroid gland 2.4%, CNS 1.4%, liver or gall bladder 1.1% and others 9.2%).

Incidence rate x 105 inh/year | gastric M/F | colorectal M/F | lung M/F | breast M/F | bladder M/F | prostate M |
--- | --- | --- | --- | --- | --- | --- |
General population | 28.8 / 19.1 | 58.0 / 45.6 | 92.6 / - | - / 92.0 | 53.2 / - | 76.5 |
MGUS population | 78.8 / 55.4 | 167.5 / 110.6 | 187.2 / 13.8 | - / 124.6 | 147.8 / 55.4 | 51.8 |

Comments: a higher incidence of cancer was observed among population having MGUS, compared to general population. This fact must be considered as not incidental, suggesting a strong relationship between MGUS and neoplasia.

**SYSTEMIC MANIFESTATIONS ASSOCIATED WITH MONOCLONAL GAMMOPATHY**

B. Grosbois, E. Laurat, M. Sebillot, C. Cazalets, J. Bracq, B. Cador, P. Jego

Department of Internal Medicine, CHU Hopital Sud, Rennes FRANCE

In a retrospective cohort of 580 monoclonal gammopathy (MG) from a single institution we studied the frequency and type of systemic manifestations non-fortuitously associated with MG. After exclusion of amyloidosis and cryoglobulinemia we found 30 MG patients (5.2%) presenting with systemic manifestations (26 with one single and 2 with double). The distribution according to the type of manifestations was 14 neurologic (12 peripheral neuropathy, 2 motoneurone disease), 8 dermatologic (2 urticaria, 2 facial oedema, 1 vascular purpura, 1 necrobiotic xanthogranuloma, 1 pyodema gangrenosum and 1 buccal aphthous associated with splenic aseptic abscesses), 6 rheumatologic (sero-negative polyarthritis), 4 hematologic (1 auto immune hemolytic anemia, 1 erythoblastopenia, 1 acquired factor V deficiency, 1 acquired factor VIII deficiency). In 26 patients systemic manifestations revealed MG. Immunoochemical type MG were IgM(20), IGG(9) and 1 IgA (1). MG were classified as 17 Waldenström’s Macroglobulinemia, 9 MGUS and 4 Multiple Myeloma. Sixteen patients received specific treatment of MG (polychemotherapy, chlorambucil ) with improvement in 13 cases. Seven patients received steroid therapy with immunosuppressive agents (cyclophosphamide, azathioprine) and improvement was observed in 6 cases.

We conclude that associated sytemic manifestations, although rare (5 %), often reveal MG. These manifestations are related to auto-antibody activity of MG. The most frequent type of MG is IgM related to Waldenström’s Macroglobulinemia. Specific treatment can frequently improve these manifestations.

**THE INCIDENCE OF SUBCLINICAL BONE DISEASE IN PATIENTS WITH MGUS OR SMOULDERING MYELOMA.**


Institute of Haematology and Department of Endocrinology, Royal Prince Alfred Hospital, Sydney, Australia

This two-part study was designed to assess the incidence of subclinical bone disease in patients with monoclonal gammopathy of unknown significance (MGUS) or untreated smouldering myeloma using markers of bone turnover, resorption and bone density measurements. In patients in whom abnormalities are identified we plan to assess the effects of a single dose of zoledronic acid (Zometar®). Patients attending our myeloma clinic were screened using the following criteria.

Age between 50 and 80 years, premenopausal women excluded.

Diagnosis of MGUS or smouldering myeloma and the demonstration of a stable paraprotein, IgA, IgG or light chain disease without evidence of lytic lesions.

No previous chemotherapy or treatment for myeloma (including bisphosphonates).

No use of glucocorticoids, calcitriol, estrogen or androgen hormone therapy, calcium or vitamin D supplementation in the 3 months prior to screening.

Normal liver and kidney function.

Assessment involved baseline measurement of:

- Serum bone density specific alkaline phosphatase (serum ostase)
Serum osteocalcin
Urinary aminoterminal telopeptide of bone collagen (NTx)
Urinary deoxypyridinoline (DPD)
Skeletal survey
Patients with elevated levels of serum or urinary markers of bone turnover went on to have a bone mineral density (BMD) test. The study was discussed with 15 potentially eligible patients. Of these, 7 declined to participate. Therefore, 8 patients underwent assessment between July 2002 and February 2003. Of these 8 patients:
5 had neither abnormal bone markers nor osteopenia.
1 had both elevated bone markers and bony lytic lesions that indicated that her disease had progressed to multiple myeloma.
1 had elevated deoxypyridinoline (DPD), suggesting bone resorption, but no evidence of osteopenia on bone mineral density tests.
1 had elevated deoxypyridinoline (DPD), suggesting bone resorption, and at the time of writing, we are waiting on the results of a BMD test.
The treatment phase of the study requires patients to have abnormalities suggesting bone resorption (raised urinary NTx or DPD or serum ostase or osteocalcin) and at least two sites of osteopenia. No patients have been identified who meet these criteria.
From this limited study (to date) we can conclude that the incidence of bone disease in MGUS and smouldering myeloma is low. It is unclear if the introduction of zoledronic acid will continue to be accrued and reassessed annually.

4.3 New criteria for differential diagnosis between MGUS and MM.

Jessica L Haug, Shaji Kumar, Thomas E Witzig, Michael A Thompson, Linda Weillik, Michael M Timm, Philip R Greipp, and S. Vincent Rajkumar
Division of Hematology, Mayo Clinic, Rochester, MN, 55905
Background: Bone marrow (BM) angiogenesis is a striking feature of multiple myeloma that correlates with plasma cell proliferation and overall survival. There is a progressive increase in the BM angiogenesis along the spectrum of plasma cell proliferative disorders from MGUS to smouldering myeloma (SMM) and newly diagnosed symptomatic myeloma (NMM). We studied the expression of VEGF, bFGF and their receptors on plasma cells from patients with MGUS, SMM, and NMM using various methods to address this question.
Methods and Materials: BM angiogenesis was studied using immunohistochemical staining for CD34 and graded as previously described. Expression of VEGF, bFGF, flt1, KDR (flk-1), FGFR-2 and FGFR-3 were studied by immunohistochemical staining of BM biopsy sections from patients with MGUS, SMM and NMM; results were expressed as the percentage of total plasma cells with cytoplasmic staining for the cytokine. Plasma levels of VEGF, bFGF and IL-6 were estimated using ELISA. Expression of VEGF, bFGF, flt-1 and KDR were studied using quantitative RT-PCR on CD138 sorted plasma cells from patients with MGUS, SMM and NMM. Results: Angiogenesis and angiogenic cytokine expression were studied by immunohistochemistry on 57 bone marrow biopsy samples (15 MGUS, 19 MM, 23 SMM). Plasma cells in MGUS, SMM, and NMM expressed VEGF, bFGF and their receptors (Table1), however there was no statistically significant difference between the three groups. When the expression pattern was correlated with grade of angiogenesis, patients with low grade of angiogenesis had significantly lower % of PCs expressing VEGF, bFGF and flt1. There was no difference in VEGF and bFGF plasma levels between SMM and NMM, P=0.79 and 0.62, respectively. Plasma levels of VEGF and bFGF did not correlate significantly with BM microvessel density (MVD) except in patients with SMM where there was correlation between MVD and bFGF. There was no significant difference in the mRNA expression of VEGF, bFGF, flt1 and KDR between the three groups by quantitative RTPCR.
Conclusion: Plasma cells express the angiogenic cytokines VEGF, bFGF and their receptors. We found trends correlating degree of BM angiogenesis with the expression of these cytokines/receptors. However, there was no significant difference in expression of VEGF, bFGF, and their receptors by plasma cells between MGUS, SMM and NMM. Increased angiogenesis along the spectrum of plasma cell disorders may therefore be a function of increased levels of VEGF and bFGF that occur as a result of increasing BM plasma cell % from MGUS to SMM to NMM.

<table>
<thead>
<tr>
<th></th>
<th>VEGF</th>
<th>flt1</th>
<th>KDR</th>
<th>bFGF</th>
<th>FGFR-2</th>
<th>FGFR-3</th>
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<tbody>
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<td>20</td>
<td>33</td>
<td>19</td>
<td>50</td>
<td>40</td>
</tr>
<tr>
<td>SMM</td>
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<td>33</td>
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<tr>
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<tr>
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</tbody>
</table>

All values expressed as median (range); pg/mL

085 Utility of Serum Cytokines in the Study of Monoclonal Gammopathies and Bone Disease of Multiple Myeloma (MM)
*Hospital General de Segovia, **Hospital Clínico de Salamanca, ***R.S. "Virgen de la Concha"-Zamora, ****Hospital del Bierzo-Ponferrada, *****Universidad Autónoma de Madrid, Cooperate Group of Castilla-León for study of Monoclonal Gammopathies

The narrow microenvironment plays a fundamental role in the etiopathogenesis of bone disease of Multiple Myeloma (MM), either through direct cell-to-cell stimulus or through the interaction of a network of cytokines produced by stromal, tumor or bone marrow cells. These cytokines are detectable in the patient’s serum.

We investigate the value of a series of serum cytokines for the study of bone disease of Multiple Myeloma and the distinction between MM and Monoclonal Gammapathy of Unknown Significance (MGUS).

MATERIALS AND METHODS: 176 newly diagnosed patients were included: 107 MM and 69 MGUS. As control groups we selected 25 patients with benign Osteoporosis (BO) and 32 healthy individuals (HI). In all patients, the serum levels of the following cytokines were measured by ELISA (Quantikine®-R&D System): IL-6, soluble IL-6 receptor (sIL-6R), Oncostatin-M (OSM), IL-1β, TNF-α and TNF-β, Vascular Endothelial
Growth Factor (VEGF), Hepatocyte Growth Factor (HGF) and IL-11. All values were expressed as pg/ml., except the OC which was analysed qualitatively (detectable vs. undetectable).

We determined three bone resorption markers (BRM) in urine: Pyridinoline total (Pyrt), Deoxypyridinoline total (Dpyrt) and Deoxypyridinoline free (DPDi), and two bone formation markers (BFM) in serum: Osteocalcin (OC) and Bone alkaline phosphatase (bAP).

RESULTS: IL-6, sIL-6R, TNF-α and HGF presented values which were significantly higher in the MM group than they were in the MGUS group (4.2 vs. 3.2 (p<0.01); 1015.3 vs. 825.2 (p<0.01); 5.5 vs.3 (p<0.001); 1764 vs.1168 (p<0.001), respectively). The same cytokines showed significant differences when MM patients were compared to each of the control groups (BO and HI). VEGF was significantly higher in the MGUS group than it was in the MM group (312.9 vs. 213.1 (p<0.01).

The serum concentration of IL-6 was the only significant difference between MM patients with lytic lesion and those without (6 vs. 3.2 (p<0.01). Nevertheless, the cytokines which distinguished MM patients without lysis and those patients with MGUS were sIL6R, TNF-α and HGF (1053.9 vs.825.2 (p<0.01); 5.2 vs.3 (p<0.001); 2337.5 vs. 1168 (p<0.01, respectively).

We analyzed the relationship between the cytokines studied and the BRM or BFM in the different diagnostic groups (Table 1). Thus, in MM, IL-6, TNF-α and OSM directly correlated with BRM, with a relationship also observed between IL-6 and bAP.

In the MGUS group, no significant correlation was observed. In conclusion, bone resorption and formation markers, especially the ratios, are useful in the evaluation of bone lesions in monoclonal gammapathies.

Supported by the Spanish FIS Grant 98/0206, Grant from CajaSegovia and FIS G03/136)

### Table 1: Distribution of Bone Remodeling Markers by Disease

<table>
<thead>
<tr>
<th></th>
<th>IL-6</th>
<th>sIL-6R</th>
<th>IL-1β</th>
<th>TNF-α</th>
<th>OSMc</th>
<th>HG F</th>
<th>VEG F</th>
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<tr>
<td>Pyrt</td>
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<td>Dpyrt</td>
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<td>OC</td>
<td>bAP</td>
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<tr>
<td>PyrtOC</td>
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<td>PyrtbAP</td>
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<td>DpyrtbAP</td>
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<td>DPDbAP</td>
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</table>

+ MM ; # BO ; & HI ( + , $&$, & p<0.05; ++, $&&$, $&&$ p<0.001)

CONCLUSIONS: The serum concentrations IL-6, sIL-6R, HGF y TNF-α are useful for discriminating patients with MM from those with MGUS, including those in early stages of bone disease.

The evaluation of bone disease in MM is usually carried out by conventional radiology. However this method has low reproducibility. Over the last decade, several serum and urine biochemical parameters, for evaluation of bone turnover, have become available. The present study was designed to explore the value of five bone remodeling markers in a series of 176 newly diagnosed patients with monoclonal gammopathies (107 MM and 69 MGUS). As control groups, we used 25 patients with benign osteoporosis (BO) and 32 healthy individuals (HI). The bone markers analyzed included: three bone resorption markers (BRM) (total Pyridinoline, total Deoxypyridinoline and free Deoxypyridinoline) and two bone formation markers (BFM) (bone Alkaline Phosphatase and Osteocalcin).

The present study shows that the serum and/or urinary levels of bone resorption markers are significantly higher in MM patients than they are in MGUS patients (Table I) (p<0.001, respectively). BO patients or HI. Interestingly, in our study free DPD were significantly higher in BO than in MGUS. Pyrt and DPDi values were higher in MM patients with lytic lesions (p<0.05, respectively). However, BRM did not discriminate MM patients without bone lesions from MGUS patients.

BFM didn’t show significant differences in the aforementioned comparisons (Table I), although a trend was observed towards higher values of OC and lower values of bAP in patients with early bone affection.

In order to explore the simultaneous impact of BRM and BFM, we established ratios for marker pairs. Ratios that contained bAP were those which exhibited the most significant differences between the MM group and other entities, as well as between the different MM subgroups. In fact, the ratios of BRM/bAP represented the parameters most able to discriminate the MM subgroup without lyse from the MGUS group (p<0.01).

In conclusion, bone resorption and formation markers, especially the ratios, are useful in the evaluation of bone lesions in monoclonal gammapathies.

Supported by the Spanish FIS Grant 98/0206, Grant from CajaSegovia and FIS G03/136)
Serum levels of carboxy-terminal telopeptide of type-I collagen (ICTP) are elevated in patients with multiple myeloma with skeletal abnormalities in MRI, who lack osteolyses in conventional radiography

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Introduction: Osteoclastic bone destruction is a major clinical problem in multiple myeloma. Increased bone resorption activity can even be present before osteolytic lesions can be discovered by conventional radiography. Magnetic resonance imaging (MRI) of the spine was established as a diagnostic tool to depict bone abnormalities with greater sensitivity than conventional radiography especially in early myeloma. In addition to common imaging techniques, type-I collagen degradation products such as the carboxy-terminal telopeptide of type-I collagen (ICTP) were introduced as novel biochemical parameters reflecting bone resorption activity in multiple myeloma. In the present study we investigated whether increased serum levels of ICTP can predict abnormal MRI patterns in multiple myeloma patients. Furthermore the prognostic relevance of elevated ICTP and abnormal MRI was evaluated. Magnetic resonance images of the spine were performed in 32 untreated patients with multiple myeloma in stages I-III (Durie and Salmon), who had no osteolytic lesions in conventional radiography. Simultaneously serum levels of ICTP were measured by a competitive radioimmunoassay (Orion Diagnostics, Espoo, Finland).

Results: Serum levels of ICTP were significantly (P = 0.002) elevated in patients with abnormal bone MRI compared to those patients with normal MRI findings. The positive and negative predictive value of serum ICTP for predicting bone abnormalities in MRI was 85% and 84%, respectively. Both serum ICTP and MRI were identified as prognostic factors for event-free survival (P < 0.001 and P = 0.003, respectively).

Conclusions: Our results demonstrate for the first time that abnormal skeletal MRI findings are accompanied by an increase in serum ICTP levels in myeloma patients who lack osteolyses in conventional radiography. We conclude that ICTP can be used as a surrogate parameter to identify multiple myeloma patients with normal skeletal surveys who have a high probability of myeloma bone disease and should be evaluated by sensitive diagnostic procedures such as MRI.

Conception of the inverse relationship between the proliferation and apoptosis activity in plasma cell compartments of patients with MGUS and multiple myeloma

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3rd Department of Internal Medicine, Faculty Hospital, Palacky University, Olomouc, Czech Republic

Background. Multiple myeloma (MM) is a clonal lymphoproliferative disease characterized by slow proliferative activity and different resistance to apoptosis with latent accumulation of myeloma cells in the bone marrow. Aim. The aim of this study was comparison of contemporaneously measured plasma cell proliferative and apoptotic indices in MGUS and in various phases of MM e.g. smoldering (SMM), stable/plateau (PMM) and the active (progressive/relapsing) phases of multiple myeloma (AMM). Methods. Analyzed group consists of 30 MGUS, 21 SMM, 82 patients examined at the time of MM diagnosis (DMM) and 64 patients analyzed during various phases of the disease. Plasma cell proliferative activity was measured using propidium iodide/CD138 index (PC-PI/CD138) while rate of apoptosis with help of annexin – V FITC/CD138 index (PC-AI/CD138). To estimate the statistical significance t-test and ANOVA test were used. Results. The MGUS individuals had overall low PC-PI index (M-1.8%) and relatively high levels of PC-AI index (M-9.1%), the relation was statistically significant (p-0.000). The patients with SMM had also low levels of PC-PI (M-1.8%) and high values of PC-AI (M-10.8%), the relation was also significant (p-0.000). The symptomatic DMM patients had PC-PI median level 2.5% and PC-AI median value 6.2%, the relation of these both indices was statistically significant (p-0.000). In the group of patients evaluated during various phases of MM after previous conventional or HD-therapy with ASCT support PC-PI median was 2.6% and PC-AI median 7.2 %, the relation was statistically significant (p-0.000). Statistical comparison of PC-PI and also
PC-AI levels of MGUS and SMM patients with patients in PMM phase did not bring any significant differences, the medians of their values were very similar (PC-PI: M -1.8, 1.7 and 2.1%; PC-AI: M-9.1, 10.8 and 9.0 %). The statistically significant differences of PC-PI and PC-AI were found, if comparing MGUS and SMM patients with group of AMM patients marked by a higher level of the PC-PI (M-1.8 and 1.7 vs. 3.2%, p<0.000) eventually by lower values of the PC-AI (M-9.1 and 10.8 vs. 4.8 %). In contrast to AMM patients, in the PMM phase significantly lower PC-PI levels (M-2.1 vs. 3.2%, p<0.000) and significantly higher PC-AI values (M-9.1 vs. 4.8%, p<0.000) were found. Conclusion. Our results supported the conception of usually inverse relationship between the proliferative and apoptotic activity of the plasma cell compartments of patients with MGUS, smoldering and overt/symptomatic forms of MM. The patients with MGUS, SMM and PMM phases have usually low proliferative and high apoptotic level, whereas patients in the AMM phase have usually high proliferative and low apoptotic level. These results suggest that not only proliferative but also apoptotic properties of myeloma cells are important from the point of view of clinical and laboratory manifestation of MM.

089
Aberrant methylation of tumor suppressor genes in patients with Multiple Myeloma and Monoclonal Gammopathy of Undetermined significance
Sonja Seidl, Jutta Ackermann, Hannes Kaufmann, Christoph C. Zielinski, Johannes Drach and Sabine Zöchbauer-Müller
Clinical Division of Oncology, University Hospital, Vienna, Austria

Aberrant methylation (referred to as methylation) of normally unmethylated CpG islands in the promotor region of tumor suppressor genes (TSGs) has been associated with transcriptional inactivation of these genes in human cancer. So far, several genes have been identified which are frequently methylated in malignant diseases. However, only little is known about methylation of these genes in patients with multiple myeloma (MM), plasma cell leukemia (PCL) or monoclonal gammopathy of undetermined significance (MGUS). Thus, we investigated the frequency of methylation of the genes p16, TIMP-3, p15, CDH1, DAPK, p73, RASSF1A, p14, MGMT and RARbeta in bone marrow aspirates from patients with MM (N = 109), PCL (N=7) and from patients with MGUS (N = 29) by methylation specific PCR. Methylation of the genes p16, TIMP-3, p15, CDH1, DAPK, p73, RASSF1A, p14, MGMT and RARbeta was detected in 34%, 27%, 26%, 24%, 20%, 17%, 16%, 7%, 5%, and 0% in MM patients, respectively. Aberrant methylation of at least one of the genes was detected in 77% of MM patients. The frequency of methylation in PCL was similar to MM patients in the case of TIMP-3, p15, DAPK, p73, RASSF1A, p14, MGMT and RARbeta but was higher in the case of p16 (57%) and CDH1 (57%). In MGUS patients, the frequency of methylation was similar to MM patients for most of the genes. However, the frequency of p15 methylation was lower. Interestingly, methylation of CDH1 was not found in any of the MGUS patients (p = 0.004). We also analysed bone marrow specimens from healthy bone marrow donors and patients with localized non-Hodgkin’s lymphomas. However, we did not observe methylation of any of these genes in control specimens. The methylation results will be compared with clinical characteristics as well as specific chromosomal alterations from these patients. In conclusion, our data demonstrate that methylation of several genes is a frequent event in patients with MM. Methylation of CDH1 was not detected in MGUS patients, but in MM patients and in an even higher percentage in patients with PCL suggesting that CDH1 methylation is an indicator of disease progression in this hematologic malignancy.

090
Bcl-2 and poly-ADP-ribosyl polymerase expression in plasma cells of MGUS and multiple myeloma patients: a comparative study
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3rd Department of Internal Medicine and Department of Pathology, Faculty Hospital, Palacky University, Olomouc, Czech Republic

Background. Multiple myeloma (MM) is a plasma cell malignancy, in which different proliferative activity and resistance to apoptosis of plasma cells play an important role in pathophysiology and clinical manifestation of this disease. Bcl-2 oncoprotein plays a very important role in hemopoietic cells by preventing apoptosis. Disregulation of this process may be important for oncogenesis due to illegitimate cell survival and increase in the chances for cells to acquire additional gene defects that promote aberrant growth and proliferation. The real clinical impact of bcl-2 or PARP (poly-ADP-ribosyl polymerase) expression in monoclonal gammopathies is not adequately known in this time. Aims. The aim of this study was the contemporaneous measurement of the bcl-2 (i), bcl-2 (m), PARP and Ki-67 expression intensity in plasma cells and correlations of these markers in the patients with MGUS (n=10), smoldering multiple myeloma (S-MM, n=11), patients evaluated at the time of MM diagnosis (D-MM, n=45) and in the various phases of the disease (R-MM, n=25). Methods. Immunoenzymatic methods were applied using McAb against bcl-2 protein (i and m), PARP and Ki-67 while the intensity of protein expression was assessed by the semiquantitative histomorphometry method (LUCIA M system, Prague) in the bone marrow biopsy samples. Data were analyzed and the groups compared using Pearson’s and ANOVA tests. Results. The intensity of bcl-2 (i) expression in plasma cells was weak and statistically insignificant in all four analyzed groups. The intensity expression of the bcl-2 (m) was also weak in patients with MGUS, S-MM and R-MM but high in D-MM patients, with statistical significance in comparison of MGUS, S-MM and R-MM vs D-MM (p<0.011, 0.008 and 0.001). The intensity of PARP expression was significantly different in comparison of MGUS, S-MM and R-MM vs D-MM patients (p<0.018, 0.000 and 0.000). There was observed a statistically significant correlation of the bcl-2(i) vs bcl-2(m) plasma cells activity in the situation of MGUS (p<0.05), S-MM (p<0.001) and R-MM (p<0.001) but no significant difference in D-MM. The significant relationship was also observed in comparison of bcl-2(m) vs PARP myeloma cells expression in the R-MM but not in the case of MGUS, S-MM and D-MM. There was no correlation of bcl-2(i), bcl-2(m) and PARP expression in comparison of Ki-67 proliferative rate of plasma cells in all four evaluated groups. Conclusion. There was demonstrated that the expression activity of bcl-2 (m) and PARP in plasma cells in overt form of MM (D-MM and R-MM) is statistic significantly higher than in MGUS or S-MM. Bcl-2(m) may play a significant role in the pathogenesis of malignant gammopathies, extending the survival of myeloma cells by protecting them from apoptosis and increasing the chance for cells to acquire new additional gene defect.

Supported by grant of IGA MH CR.
4.4 Evolving from MGUS/ SMOLDERING MM to symptomatic MM.

091 Characterisation of the Transition of MGUS to Multiple Myeloma using Expression Microarrays.


MGUS can be considered a premalignant phase of myeloma (MM) and MGUS plasma cells (PCs) although distinct in their clinical behavior are clearly related to MM PCs. Understanding the molecular basis of the transition from MGUS to MM can provide considerable insight into the multi-step pathogenesis of MM. This transition has been studied using cytogenetics and mutational analysis however consistent changes suitable for the further investigation have not been identified. Global expression based analysis can highlight genes and gene families important in this transition which may be potential future therapeutic targets. We have analyzed PCs from 5 normals (N), 5 MGUS and 31 MM following CD138+ selection and SMART PCR-based amplification using the Affymetrix U95Av2 gene chip comparing 12,000 known expressed sequences (10,000 genes).

Unsupervised analyses were performed to identify genes which had most variation across all samples and supervised analyses, using the ‘compare samples’ function in DCHIP, looked for genes which varied significantly between specific sample groups. Hierarchical clustering was then used to study the results. 380 genes separated N PCs from MM PCs, whereas 263 genes separated N PCs from MGUS PCs. Interestingly the majority of genes were downregulated (252 downregulated, 128 upregulated). The transition of MGUS to MM was more closely examined and using the same strict analysis criteria the number of genes separating MGUS and MM was considerably fewer (74 genes), than those separating N and malignant PCs suggesting that MGUS and MM PCs are more similar to each other than to PCs from normal donors. The underlying basis of these changes may be understood more clearly by looking at the functional classes of genes that are altered. Important genes in the N vs Malignant comparison include: oncogenes and tumor suppressor genes (c-myb, LAF4, DOC1, Rb1); cell signaling genes (CD163, small inducible cytokine subfamily C and CDK2 associated protein 2); death genes (MAD3 and beclin 1); DNA binding and transcription factors (XBP1, YY1, CBF and seven in absentia); and developmental genes (WNT and sonic pathways). In comparison the genes differentiating MGUS from MM are more limited but include a number of potentially important genes affecting cell growth and maintenance, signal transduction, structural proteins, and developmental processes. Interestingly no genes involved in apoptosis were highlighted as being differentially expressed between MGUS and MM. The data sets were validated by comparing the expression of genes differentiating N and malignant PCs using flow cytometry and RT-PCR. Using these techniques there was good correlation with gene expression data identified on the arrays, and also good correlation with previously published data. In conclusion gene array analysis highlights the differences in gene expression levels between N, MGUS, and MM PCs and supports the multi-step pathogenesis of MM. Genes involved in the control of transcription and developmental pathways are important in this transition, with the critical differences relating to the transition from normal to MGUS. To fully define this transition further analysis of different sub-populations of CD138+ PCs in MGUS in comparison with N PCs populations is required. The amplification technique used in this study will be critical for this analysis.

092 ROLE OF ANTI-TUMOR IMMUNE EFFECTORS IN THE CONTROL OF “MALIGNANT TRANSFORMATION” IN GAMMOPATHIES.

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Clonal expansion of a transformed cell is essential, but not sufficient for the development of clinical cancer. This is particularly puzzling in the case of monoclonal gammopathies, because many of the cytogenetic and genomic changes initially described in myeloma have now also been detected in pre-malignant monoclonal gammopathy of undetermined significance (MGUS). Immune system has been postulated to play a role in surveillance of tumors in mice. The role of immune effectors in the control of transformed cells in patients with gammopathies remains to be fully defined. We have studied tumor reactive immune effectors in the blood and tumor bed of patients with MGUS and myeloma. We have recently shown that freshly isolated T cells from blood or tumor bed of patients with progressive myeloma lack tumor reactive rapid effector function. Targeting tumor antigens to Fc receptors of dendritic cells (DCs) leads to enhancement of cross presentation and generation of tumor reactive effector T cells (Dhodapkar et al, J Exp Med 2002). Using this approach, T cells from the tumor bed of even patients with progressive tumors can be activated to yield tumor reactive killer T cells (Dhodapkar et al, PNAS 2002). Anti-tumor reactivity in these cultures was specific for autologous tumor, and mostly not directed against Ig derived determinants. Natural killer T cells are distinct lymphocytes that recognize glycolipid antigens in the context of CD1 family of antigen presenting molecules. We find that clinical progression in myeloma is associated with a loss of ligand reactive rapid effector function in NKT cells, which can be restored ex vivo using dendritic cells. Direct analysis of tumor specific effector T cell function in MGUS now indicates the presence of an active anti-tumor effector T cell response. This response is enriched in the marrow and is specific for antigens expressed by tumor cells in each patient. These data, to our knowledge, provide the first direct evidence for active and tumor specific immune recognition in the bed of a human non-viral “preneoplastic state”, and strongly suggest that the development of “clinical myeloma” is regulated at least in part at the level of the host immune response consisting of both innate and adaptive immune effectors. The finding that reversible host factors might determine clinical malignancy may also have important implications for therapeutic goals in these diseases.
The predominant pathway of transformation to multiple myeloma is associated with clonal homogeneity in VH genes at the MGUS stage

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The transition of MGUS to multiple myeloma (MM) is now being evaluated at the molecular level to identify important features of disease progression. These will impact on clinical evaluation and treatment. In B-cell tumors, immunoglobulin variable (V) region gene analysis identifies salient characteristics of the cell of origin of disease, and it's clonal history following neoplastic arrest to be identified. In MM, V gene analysis has firmly established features of extensive somatic mutation at this stage. Heterogeneity clearly does not correlate with a benign course. In each case, the same tumor-clone evolves to malignancy. In 1 case, clonal stability of V gene sequences is still apparent in this putative cell. In contrast, we showed previously that in some MGUS cases, there is marked intraclonal variation in tumor-derived V gene sequences, suggesting at least in these cases an origin from a less differentiated cell which is able to continually engage the somatic mutation mechanism. This cell is likely to be slg+ve to maintain on-going somatic mutation, which is generally thought to occur in the germinal center (GC).

We recently reported our findings in tracking progression from MGUS to MM longitudinally using VH gene analysis in 2 cases. In each case, the same tumor-clone evolves to malignancy. In 1 case, stability of sequence was observed at both stages of disease. In the other case, intraclonal heterogeneity in MGUS confirmed previous findings and at the MM stage on-going somatic mutation was apparently silenced. However, at this stage some residual MGUS clones showing heterogeneity could still be identified, most likely as time to transformation was notably short. These findings suggested transformation in a cell in which somatic mutation has ceased, with clonal outgrowth at the MM stage. Heterogeneity clearly does not correlate with a benign outcome. We have now expanded this study in a further 3 paired MGUS/MM cases. In these 3/3 cases, homogeneity of V gene sequences was observed at both stages of disease. Therefore, in our analysis of the transition of MGUS to MM, VH gene reveal that stability of sequences is already evident at the MGUS stage (4/5 cases), and that this is likely to be the predominant pathway of progression. Transformation to myeloma here retains homogeneity. Our data is consistent with clonal outgrowth of a cell which has transformed, in which cessation of somatic mutation suggests that slg may no longer be present. This also identifies a site in which mutational activity has ceased, such as the bone marrow. It raises the possibility of transformation at the level of a late stage B-cell, such as a plasmablast or MGUS plasma cell, which acquires the necessary complement of oncogenic events.
SMOLDERING MULTIPLE MYELOMA: PATTERN OF PROGRESSION AND SUBSEQUENT OUTCOME IN 53 PATIENTS FROM A SINGLE INSTITUTION


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Background: The majority of patients with multiple myeloma (MM) have symptomatic disease and require therapy. Smoldering multiple myeloma (SMM) was first recognized in six patients who fulfilled the diagnostic criteria of MM with no symptoms and who remained stable with no therapy for five or more years. However, there are no further reports on patients with SMM diagnosed according to the stringent criteria originally reported.

Objective: To describe the pattern of progression and the outcome after transformation (response to therapy and survival) in 53 patients fulfilling SMM criteria.

Patients and methods: From May 1978 to July 2001, 53 patients (22 M/31F) with a median age of 61 yrs (range, 41-88) were diagnosed with SMM (serum M-protein ≥ 30 g/L and > 10% bone marrow plasma cells -BMPC -). The mean serum M-protein at diagnosis were 35 ±7.3 g/L and 29 ±18%, respectively. The M-protein type was IgG in 40 cases, IgA in 11 cases, and light-chain and biclonal one case each.

Results: The overall survival from diagnosis was 8.3 yrs. Two subsets of SMM were identified: 1) patients with “evolving” SMM (n=23), with a progressive increase in serum M-protein and a previously recognized monoclonal gammopathy of undetermined significance (MGUS) in most cases and 2) patients with “non-evolving” SMM (n=25), with long lasting stable serum M-protein that abruptly increases when symptomatic MM develops. Patients with “evolving” disease had an earlier transformation than those with “non-evolving” SMM (60% & 90% vs 16% & 55% at 2 & 5 yrs respectively, p=0.007). However, no significant differences were observed in survival between both groups (6.4 vs 8.9 yrs, p=0.25). Thirty-four of the 53 patients developed symptomatic MM. The pattern of progression consisted of anemia (11 cases), bone lytic lesions (6), anemia and bone lytic lesions (12) and bone pain due to osteoporosis (5). No patient developed renal failure, hypercalcemia or extramedullary plasmacytomas at progression. Thirty-one patients were treated: 16 with single alkylating agents plus prednisone and 15 with combination chemotherapy. The partial response rate was 39%. The median survival from transformation was 3.5 yrs with no significant differences between both groups.

Conclusions: Patients with SMM have a prolonged survival. Patients with “evolving” disease usually have a previously recognized MGUS and a significantly shorter time to progression that those with “non-evolving” SMM. In both groups the pattern of progression consists of anemia and/or lytic lesions with no renal failure, hypercalcemia or extramedullary plasmacytomas. Although the response rate to therapy is poor, the survival from transformation to symptomatic MM is 3.5 yrs.

Deep-vein thrombosis in multiple myeloma after first-line chemotherapy without thalidomide.

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Deep venous thrombosis (DVT) is frequently observed in cancer patients with a small proportion of these subjects experiencing this event as the first manifestation of their neoplastic disease. In multiple myeloma (MM) VTE has recently emerged as the most single important complication of thalidomide therapy. Acquired resistance to activated protein C (APC) in the absence of factor V Leiden mutation has also reported as a significant risk factor for VTE in MM patients.

We performed coagulation studies included prothrombin time (PT), activated partial thromboplastin time (aPTT), fibrinogen and APC resistance in patients with MM referred to our hematological unit (untreated or with preceding chemotherapy). Twenty patients (15 males and 5 females; median age 64 years, range 38-85 years), without history of previous VTE preceding the diagnosis of MM and not receiving antiocoagulation were enrolled in this study.

None of the tested patients demonstrated abnormalities of these coagulation tests. Moreover 2 patients experienced VTE during the course of their disease (one and three months after diagnosis and after string chemotherapy not including thalidomide. No identifiable prothrombotic laboratory abnormalities were found in these subjects.

The trombogenicity in MM patients could finally be related to other abnormalities, i.e. elevated levels of the von Willebrand factor antigen observed with the increase of angiogenesis.
was associated with I.V. line, while 4 (30.8 %) were identified postoperatively. We identified several univariable correlates in MGUS patients, including family (HR=13.79, P= .014) and personal (HR=8.95, P=. 002) history of DVT, low serum albumin (HR=4.21, P=. 018) and increased white blood cells count (HR=3.41, P=. 032). IgG immunoglobulin type was protective in our analysis (HR=0.19, P=. 017). None of the patients received any type of active treatment for their disease. In conclusion, risk for thromboembolic diseases in patients with MGUS is increased as compared to general population, and is similar to incidence in MM patients (10%). Further studies are necessary to define the mechanisms involved.

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Amyloidosis and Deep Venous Thrombosis (DVT)

Gordan Srkalovic, Marte A. Cameron, Lisa Rybicki and Mohamad A. Hussein

Cleveland Clinic Foundation Multiple Myeloma Research Program

Coagulation problems in Amyloidosis are historically associated with bleeding tendencies (mostly F X abnormalities). Increased clotting was observed in isolated cases diagnosed with low grade DIC. Problem of DVT in Amyloidosis was not systematically investigated. We evaluated frequency of DVT and risk factors for DVT in 56 consecutive Amyloidosis patients with a documented disease evaluated and followed up at our Center from 1991-2001. Data were collected in 5 categories: (a) demographics, (b) disease and treatment, (c) thrombosis case information, (d) major risk factors for thrombosis and (e) baseline laboratory data. Uni- and multivariable correlates of DVT were assessed using Kaplan-Meier analysis and Cox proportional hazards analysis. Mean age of the patients was 61.8 (range 21 – 83). Male female percentage ratio was 70/30. 30 % of the patients had high creatinine level (> 1.4 mg/dl). Personal or family history of DVT was recorded in 1.8 and 0 % of patients, respectively. Known hypercoagulable state was present in 1 patient (1.8%). 7.6 % of patients were smokers. Of 56 patients 6 developed DVT (10.7%). Median time from diagnosis to DVT was 12.5 month (range 1-107). Treatment was given within 1.4 months (range 0-4) from the development of thrombosis. Only sites of DVT were lower extremities. No cases were associated with I.V. line. 1 (16.7 %) was identified postoperatively. We identified several univariable correlates of DVT in Amyloid patients, including age at diagnosis (HR=2.99, P=. 041), personal history of DVT (HR=47.7, P=.006), immobility (HR=11.78, P=.006). Paradoxically, presence of circulating serum M-protein had protective role in our analysis (HR-.08, 95% confidence interval .01-. 80, P=. 031). There was no correlation with the type of treatment patients was receiving. Family and personal history of DVT were also identified as risk factors in multivariable analysis of whole group (668) of patients with plasma cell dyscrasias. In conclusion, risk for thromboembolic diseases in patients with Amyloidosis is identical to one previously described for MM patients (10%). Further studies are necessary to define pathophysiological processes involved.

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Monoclonal gammopathies (MG) may be associated with unique M-protein induced disturbances of either primary hemostasis or plasma coagulation. We have investigated the possible interference of M-protein with antithrombotic systems. Decrease of antithrombin III, protein C, protein S and plasminogen levels or defect of APC resistance was found in 26.5% of patients. However, higher tissue-type plasminogen activator (t-PA) activity was the most frequent abnormality. The relationship between M-protein type and concentration and frequency of antithrombotic factor abnormalities was not found. The risk of venous thrombosis was higher in patients with the defect in comparison with the unaffected group (46% vs. 22%) but the difference was not statistically significant. Bleeding complications were markedly less frequent in the group of patients with the defect of anticoagulation mechanisms (0% vs. 17%). In conclusion, we have found the defects of anticoagulation and/or fibrinolytic system, analogous to well-known disturbances in hemostatic mechanisms, in more than a quarter of patients with MG. The interference of M-protein with coagulations inhibitors and/or fibrinolytic systems could contribute to the development of thromboembolic events.

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Occupation, Pesticide Exposure and Risk of Multiple Myeloma


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In this population-based case-control study, we examined the relationship between occupation, farm history, pesticide exposure and risk of multiple myeloma among blacks and whites in the U.S. The study included 573 cases (206 blacks and 367 whites) newly diagnosed with myeloma between 1986 and 1989 and 2,131 controls (967 blacks and 1,164 whites) from three U.S. geographic areas. Detailed information was obtained on socio-demographic factors, occupational and farm history, dietary factors, smoking, and medical history. Information on usual occupation and industry were coded according to standardized classification systems. A job/industry exposure matrix (JEM) was developed to estimate the level of occupational exposure to pesticides. Odds ratios (ORs) and 95% confidence intervals (CIs) for the analyses of occupational and farm history and pesticide exposure were estimated by unconditional logistic regression. Farmers and farm workers had ORs of 1.9 (95% CI=0.8-4.6) and 1.4 (95% CI=0.8-2.3), respectively. An OR of 1.7 (95% CI=1.0-2.7) was observed among those who lived or worked on a farm.
where sheep were raised, whereas no increased risks were found for those who lived or worked on a farm where cattle, beef, pigs, or chickens were raised. We observed a modestly increased risk for pesticides overall (OR=1.3, 95% CI=0.9-1.8), which was mainly due to exposure to herbicides (OR=1.5, 95% CI=0.7-3.0) and fungicides (OR=2.3, 95% CI=0.7-7.9). Significantly increased risks were also observed among pharmacists, dietitians and therapists (OR=6.1, 95% CI=1.7-22.5), roofers (OR=3.3, 95% CI=1.1-9.8), heating equipment operators (OR=4.7, 95% CI=1.4-15.8) and hand molders and casters (OR=3.0, 95% CI=1.0-8.4). In conclusion, our study suggests a modest increased risk of multiple myeloma for occupational exposure to pesticides. The observed increased risk among subjects who lived or worked on a farm where sheep were raised suggests that certain animal viruses may be involved in myeloma risk.

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Acquired fanconi’s syndrome is an indolent disorder in the absence of overt multiple myeloma.


Adult acquired Fanconi’s syndrome (FS) is a rare complication of plasma cell proliferative disorders. It is characterized by an absorptive defect of the proximal renal tubules. This results in aminoaciduria, glycosuria, hypophosphatemia, hypokalemia, hypouricemia, and metabolic acidosis. Methods: We retrospectively reviewed 32 patients who were diagnosed with monoclonal gammapathy associated adult acquired FS between April 1968 and June 2002. The median follow up of survivors was 56 months (ranges 2 to 238 months). Results: Various forms of plasma cell disorders, including Multiple Myeloma (MM), Smoldering Multiple Myeloma, Waldenström’s Macroglobulinemia (WM), and Monoclonal Gammapathy of Undetermined Significance (MGUS), were identified in these patients. 91% of these patients were found to have monoclonal . The remaining 9% of patients have monoclonal light chains in the urine. The median creatinine at diagnosis was 1.9 mg/dl (ranges 0.9-3.7). Currently, five patients developed ESRD. The median time to ESRD was 153 months (ranges 90-238 months). Pathologically, 47% of the available kidney biopsies demonstrated cytoplasmic crystals in the proximal tubular epithelial cells. One patient developed cytoplasmic crystals in the transplanted kidney 1 year after the transplant for ESRD secondary to FS. Only one out of the fourteen MGUS patients subsequently transformed to overt MM. 44% of the patients died during the period of follow up. The median survival was significantly different between patients who had overt MM at diagnosis (43 months) and MGUS (120 months). Only one patient died from renal failure. Chemotherapy was given to twenty-one patients for symptomatic MM or progressive renal failure. Improvement of renal function was found in only one minority of patients. However, four patients who were treated with chemotherapy developed secondary leukemia or myelodysplastic syndrome. The majority of the patients had elevated alkaline phosphatase levels, reflecting secondary osteomalacia. Renal osteodystrophy was found in only one patient who also had ESRD.

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IgM Myeloma: a rare subtype. Description of 4 cases


The distinction between multiple myeloma (MM) and Waldenström’s macroglobulinemia (WM) usually poses no diagnostic dilemma. Consistent with a diagnosis of MM is the presence non IgM monoclonal gammapathy associated to multiple osteolytic lesions and plasma cell infiltration of the bone marrow. On the other hand, characteristic of WM is the presence of an IgM monoclonal gammapathy associated to lymphadenopathy, hepatosplenomegaly, anemia, and hyperviscosity syndrome in conjunction with a monoclonal lymphoplasmacytoid proliferation in both the bone marrow and peripheral blood is characteristic . Despite that, few cases of IgM myeloma have been reported , with clinicopathologic features intermediate to those of MM and WM.. We present 4 patients with an IgM monoclonal gammapathy in whom morphologic and clinical features were consistent with the diagnosis of IgM myeloma.

From July 1973 to April 2002, we observed 3,176 monoclonal gammapathies of which 316 (9.9%) were of IgM type. At diagnosis, 187 (59.1%) were MGUS, 93 (29.4%) were WM, 10 (3.1%) were non Hodgkin Lymphoma (NHL) and only 4 (1.3%) were IgM Myeloma. Of the 186 MGUS, 21 (11%) evolved to WM and 1 to NHL during the follow-up.

The following Table reports the clinical characteristics of the 4 IgM myeloma:

<table>
<thead>
<tr>
<th>Case</th>
<th>Sex</th>
<th>Age</th>
<th>Light Chain</th>
<th>Stage</th>
<th>IgM grids</th>
<th>% of WM</th>
<th>Therapy</th>
<th>Response</th>
<th>Months of survival</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>M</td>
<td>79</td>
<td>κ</td>
<td>II</td>
<td>1.7</td>
<td>31</td>
<td>MP</td>
<td>SD</td>
<td>107</td>
</tr>
<tr>
<td>2</td>
<td>F</td>
<td>79</td>
<td>κ</td>
<td>II</td>
<td>2.2</td>
<td>20</td>
<td>RT</td>
<td>PD</td>
<td>31</td>
</tr>
<tr>
<td>3</td>
<td>M</td>
<td>65</td>
<td>κ</td>
<td>Smoldering</td>
<td>1</td>
<td>40</td>
<td>none</td>
<td>SD</td>
<td>172</td>
</tr>
<tr>
<td>4</td>
<td>M</td>
<td>66</td>
<td>λ</td>
<td>III</td>
<td>2</td>
<td>60</td>
<td>MP</td>
<td>SD</td>
<td>15</td>
</tr>
</tbody>
</table>

SD: stable disease, PD: Progressive Disease. Survival after primary diagnosis, MP: melphalan- prednisone; RT: radiotherapy. None of the 4 patients had diffuse osteolytic lesions. However, in case 2, who complained lumbal pain and paresthesia, Magnetic Resonance demonstrated the presence of L2-L5 fractures with associated pathologic tissue causing spine compression. The histologic analysis of this tissue showed a diffuse plasma cells infiltration. After laminectomy, the patient received local radiotherapy obtaining a reduction of symptoms but few months later he died of progressive disease. Of the remaining 3 patients only case 3 who had a smoldering IgM myeloma is still alive after 172 months from diagnosis.

In conclusion, IgM myeloma is a rare disease, accounting for about 1% of all monoclonal IgM and less then 0.5% of MM. The distinction between the WM and MM rests on the histologic finding of a lymphoplasmacytoid proliferation in WM as opposed to the predominantly plasma cell rich infiltrate in myeloma. Since MM and WM differ in prognosis and treatment strategies, the two disease entities should be distinguished based on clinical criteria and bone marrow morphology.
A definite diagnosis was made by tissue biopsy and there was no gastrointestinal hemorrhage. Patient characteristics: In each case, institution during the last 3 years, presenting amyloidosis-induced cases are rare. We report 5 patients with MG, from a single the physician should look for a MG.
diagnostic procedure for amyloidosis. More generally, gastrointestinal bleeding in a patient with MG should induce a Amyloid hemorrhage is most often due to amyloid infiltration of another of sudden cardiac arrest. In one multiple myeloma after massive hematemesis, one of kidney amyloidosis and patient had melena and rectooalgy with acute colitis. For two in four patients and two needed intensive care management. One patient had melena and rectorragy with acute colitis. For two patients, endoscopic findings were: severe hemorrhagic gastritis with white ulcerated nodules and prepyloric ulcerations in one case, severe diffuse hemorrhagic ulcerations of the bulbe and atrophic gastritis with microulcerations and a mucosal friability which bled after biopsy in the other case. One patient had both prepyloric ulcerations and diffuse ulcerative colitis. For another, necropy showed a massive gastric ulceration centred by a punctured artery. A diagnosis of amyloidosis was done twice by gastric, once rectal, once colic biopsy and once after necropy. All patients had amyloid deposition in the vessel walls with one involving the whole body middle size arteries. Muscularis mucosae was twice involved. Four of them died: two immediately after massive hematemesis, one of kidney amyloidosis and another of sudden cardiac arrest. In one multiple myeloma patient, bleeding never resumed after chemotheraphy with a double autologous transplantation. Following treatment, that patient had a normal colonoscopy but with persistent amyloidosis in the colon vessel walls. Our patients are most likely the first set occurring during evolution in three. Hematemesis was the major symptom in four patients and two needed intensive care management. One patient had melena and rectorragy with acute colitis. For two patients, endoscopic findings were: severe hemorrhagic gastritis with white ulcerated nodules and prepyloric ulcerations in one case, severe diffuse hemorrhagic ulcerations of the bulbe and atrophic gastritis with microulcerations and a mucosal friability which bled after biopsy in the other case. One patient had both prepyloric ulcerations and diffuse ulcerative colitis. For another, necropy showed a massive gastric ulceration centred by a punctured artery. A diagnosis of amyloidosis was done twice by gastric, once rectal, once colic biopsy and once after necropy.

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An exceptional association of diffuse anaplastic myeloma and microangiopathic anemia: a case report.
Erasmus hospital Université Libre de Bruxelles

A 70-year old man was admitted to the Haematology Department because of fatigue and acute pain in the right forearm for three weeks. Past medical history revealed orchitis four years before actual presentation. Four months before admission, he presented progressive bilateral paralysis of both inferior limbs and was admitted with suspicion of Guillan-Barré syndrome. Complete work-up showed demyelinating neuropathy, IgG-kappa monoclonal band on plasma protein electrophoresis and moderate light-chain proteinuria. Bone marrow biopsy revealed 10% of plasmaocytes, and the caryotype was normal. Diagnosis of peripheral polyneuropathy associated with monoclonal gammopathy of undetermined significance (MGUS) was made and the patient was successfully treated with high-dose methylprednisolone and discharged to start a rehabilitation program. He had progressive amelioration and began walking without support. Blood values were normal at that time. At home, treatment consisted of paroxetin, zolpidem and cholecalciferol. At admission he complained of progressive asthenia with mild exertional dyspnea. He could not sleep because of right arm pain which developed next to the wrist two weeks before. He had tried nonsteroidal anti-inflammatory drugs with no improvement of symptoms. He noticed progressive weakness of both legs. Vital signs were within the normal limits. Oxygen saturation was 94% while the patient was breathing room air.

Physical examination revealed left basal pulmonary hypoventilation associated with dullness, peripheral bilateral leg swelling with diffuse purpura, bilateral inferior limbs weakness (4/5) with ankle and patellar hyporeflexy (1/4).

Blood tests showed anaemia, thrombocytopenia and renal failure. There was proteinuria (1g/24h) consisting exclusively of kappa-light chain. Chest radiography showed left pleural effusion. Bone marrow biopsy revealed massive infiltration by plasmaocytes (58%); 5% of them stained positive for a kappa-light chain. Caryotype analysis showed partial deletion of chromosome 13.

CT-scan of the arm was normal. Complete bone radiographic study showed no lytic lesion. Diagnosis of multiple myeloma was made and VAD-type chemotherapy was started. Because of polynpea and light hypoxia, diagnostic thoracocentesis was made and numerous monoclonal plasmocytic cells were found in the pleural fluid.

On the second day of chemotherapy the patient complained of diplopia with left sixth cranial nerve palsy. Cerebral CT-scan and MRI showed no specific periventricular lesions. Nevertheless, lumbar puncture showed plasmocytic cells and intrathecal methotrexate was administered.

During chemotherapy, the patient developed Enterooccus Faecalis septicaemia treated by intravenous ampicilline and gentamycine. On the sixth day, haemolytic anaemia with aggravation of renal failure and coma were noticed.

Blood tests showed the following: haemoglobin 7.3 g/dL (13-17 g/dL), platelets 20000/mm3 (150000-400000/mm3), LDH 4637 IU/dL (NR: 60-310 IU/dL), schistocytes > 8/1000 , creatinin 1.9 g/dL (NR: 0.8-1.5 g/dL), haptoglobin <6 and plasmoblasts were assessed to 18%. Direct Coombs test and agglutinins research were negative, all these results suggest microangiopathic anaemia.

The patient died of massive haemoptysis with diffuse intravascular coagulation. Autopsy disclosed thoracic adenopathies and infiltrative masses in the left cardiac ventricle and the left kidney parenchyma. All were infiltrated by anaplastic plasma cells.

To our knowledge, this is the first case in the medical literature describing association of multiple myeloma and microangiopathic anaemia. All the biological findings suggesting microangiopathic anaemia were absent at the beginning of hospitalisation and they appeared at the same time than plasmoblasts in peripheral blood, therefore suggesting a link between spread of plasma cells and microangiopathic haemolytic anaemia.
105 Development of ATLL during follow up of Multiple Myeloma with previous positive sorology for Human T Cell Leukemia virus.

Martinez, G.A.; Kondo, A.; Fonseca, G.; Pereira, J.; Beillett, B.; Douthie-Llacuer, P.E.; Chamone, D.
Department of Haematology University of Sao Paulo, Hospital das Clinicas, Sao Paulo, Brazil. Fundacao Pr-Sangue Hemocentro de Sao Paulo.

We report a case of ATLL 23 months after the diagnosis of Multiple Myeloma. Case Report: A 73 years old black woman with bronchopneumonia and anemia was admitted for investigation. Laboratory data showed: Hb: 6.5g/dl; Serum electrophoresis: monoclonal globulin: 5.43g/dl. Immunoeléctrophoresis: IgA kappa. Renal function and serum calcium were both normal. The bone marrow showed increased number (40%) of pathological plasma cells (PC). A bone survey revealed collapse of T7 and L3 and osteopenia. A diagnosis of MM, stage IIIA, was made. At this time the sorology for HTLV was positive: WESTERN BLOT GD21(+) P19 (+), P24 (+). She was begun on melphalan 10 mg/m2 plus dexamethasone (DXM) 20mg vo (D1-D4). The controls 18 months after diagnosis showed: Hb10.7 g/dl; Leukocytes 4100/mm3; neutrophils: 2100/mm3; lymphocytes: 1300/mm3; Platelets: 276000/mm3, stable paraprotein 2.68g/dl and bone marrow aspirate showing 29.5% PC and 3% lymphocytes. One month after the last evaluation, she was admitted to the hospital in coma. Investigations showed anemia (Hb: 9.2 g/dl), leukocytosis: 199.400/mm3 with lymphocytosis (76%) and hypercalcemia: 7.6mg/dl (normal: 4.8-5.28mg/dl), monoclonal protein 2.18g/dl and high serum lactate dehydrogenase level: 2.174 (normal <225ui/L). Immunophenotype of peripheral blood: CD3: 97%; CD4:93%; CD2 96%, CD5 27%; CD1a 2%; CD19 3%; CD38 12%. Cy IgA, IgM, IgG negative. Diagnosis of T-Cell chronic lymphoproliferative disorder, ATLL, was made. The patient died 3 days after admission. To our knowledge only two descriptions of association of this two diseases have been reported. One case a patient with ATL who developed IgA kappa MM and another case of multiple myeloma superimposed on adult T cell lymphoma.

106 INTRACRANIAL PLASMACYTOMA. A RARE EVENT IN THE COURSE OF MULTIPLE MYELOMA

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Institut Catalá d’Oncologia, Institut de Diagnòstic per la Imatge*. Ciutat Sanitària i Universitària de Bellvitge. Barcelona, Spain.

Intracranial plasmacytoma (IP) is infrequent in multiple myeloma (MM) with an incidence <4%. IP can appear at diagnosis or in the setting of progressive systemic disease. In the other hand, less than 40 cases of solitary IP have been described and some can evolve to overt MM.

IP arises from the skull, dura or cranial base and may present as an external tumour or cause central nervous system (CNS) compression symptoms. The usually recommended treatment of IP is radiotherapy (RT) together or not with systemic chemotherapy, depending on the chemosensitivity status of the disease.

We present 3 cases of IP in a series of 279 consecutive MM patients from 1993 to 2001 (1.07%). Two patients (cases 1 and 3) had IP at diagnosis of MM, and in the third patient (case 2) IP occurred as a terminal event in the course of MM. All had more than 1 lytic bone lesions. All patients had CNS compression symptoms which ultimately were the cause of death in one of them (case 2). The remaining two patients responded to radiotherapy and subsequently received chemotherapy, which included high-dose melphalan followed by autologous stem cell rescue in patient 3 who is alive and in very good objective response 31 months after diagnosis.

<table>
<thead>
<tr>
<th>Case</th>
<th>Age (yr)</th>
<th>Sex</th>
<th>CNS symptoms</th>
<th>IP Dx of MM</th>
<th>CNS fete</th>
<th>MM type</th>
<th>Tx type</th>
<th>IP response</th>
<th>high-dose Tx</th>
<th>Survival (Mo)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>72F</td>
<td></td>
<td>Headache</td>
<td>at Dx of MM</td>
<td>Bone</td>
<td></td>
<td>RT</td>
<td>Complete</td>
<td></td>
<td>69</td>
</tr>
<tr>
<td>2</td>
<td>72F</td>
<td></td>
<td>Panaces IX-XII</td>
<td>at Dx of MM</td>
<td>Bone</td>
<td></td>
<td>RT</td>
<td>Complete</td>
<td></td>
<td>47</td>
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<tr>
<td>3</td>
<td>42M</td>
<td></td>
<td>Ataxia, nystagmus, headache</td>
<td>at Dx of MM</td>
<td>Bone</td>
<td></td>
<td>RT</td>
<td>Complete yes</td>
<td>31*</td>
<td></td>
</tr>
</tbody>
</table>

Dx: diagnosis; Mo: months; Bj: Bence Jones proteinuria; Tx: treatment; MP: melphalan- prednisone; VAD: viscristine-adriamicin-dexamethasone.

We present a rare case of pericardial myeloma representing one year after diagnosis of myeloma. This 73-year-old woman developed a chylos pericardial effusion with tamponade. The effusion contained numerous plasma cells of the IgG subtype. She had a pericardioctesis and was treated with high dose oral steroids. She survived a further nine months and died from hyperviscosity syndrome. This case raises questions regarding the optimal management of extramedullary pericardial myeloma. A literature review was undertaken to ascertain characteristics of presentation, trends in survival and optimal management of patients with pericardial myelomatous involvement. We excluded cases associated with amyloidosis and plasma cell leukaemia. Sixteen cases in the world literature were identified. We present a summary of these and our present case. The median age at diagnosis of myeloma was 67. The male:female (M: F) ratio was 9:8 (M=52%, F=48%). 3/17 (17%) patients had pleural and pericardial involvement while 14/17 (82%) had pericardial involvement only. 6/17 (35%) patients had pericardial involvement within 3 months of diagnosis and the rest presented either at disease relapse or progression. Pericardial tamponade was a feature in 10/15 (67%) patients. The median survival from diagnosis of pericardial involvement to death is 9.5 weeks. IgG isotypes accounted for 7/17 (41%) of cases and IgA for 6/17 (35%) of cases. All patients treated with systemic or intrapericardial myelosuppressive chemotherapy (4/4) died of sepsis as did one other case who received radiotherapy only.

Conclusions: Pericardial myeloma is rare and is usually a preterminal event with median survival <3 months. Pericardial tamponade previously reported as rare is seen in the majority of cases. There is little evidence of benefit from myelosuppressive chemotherapy as chemotherapy associated septic death follows all reported cases.

107 A Case of Pericardial Myeloma and Review of the Literature

W Abelman1, A Virchis1 and K. Yong2
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We present a rare case of pericardial myeloma representing one year after diagnosis of myeloma. This 73-year-old woman developed a chylos pericardial effusion with tamponade. The effusion contained numerous plasma cells of the IgG subtype. She had a pericardioctesis and was treated with high dose oral steroids. She survived a further nine months and died from hyperviscosity syndrome. This case raises questions regarding the optimal management of extramedullary pericardial myeloma. A literature review was undertaken to ascertain characteristics of presentation, trends in survival and optimal management of patients with pericardial myelomatous involvement. We excluded cases associated with amyloidosis and plasma cell leukaemia. Sixteen cases in the world literature were identified. We present a summary of these and our present case. The median age at diagnosis of myeloma was 67. The male:female (M: F) ratio was 9:8 (M=52%, F=48%). 3/17 (17%) patients had pleural and pericardial involvement while 14/17 (82%) had pericardial involvement only. 6/17 (35%) patients had pericardial involvement within 3 months of diagnosis and the rest presented either at disease relapse or progression. Pericardial tamponade was a feature in 10/15 (67%) patients. The median survival from diagnosis of pericardial involvement to death is 9.5 weeks. IgG isotypes accounted for 7/17 (41%) of cases and IgA for 6/17 (35%) of cases. All patients treated with systemic or intrapericardial myelosuppressive chemotherapy (4/4) died of sepsis as did one other case who received radiotherapy only.
5. Signal transduction pathways and cytokine networks

5.1 Surface reception.

108 ATYPICAL RECEPTOR INTERACTIONS IN MYELOMA CELLS: USE OF RNA INTERFERENCE (RNAi) TO INVESTIGATE FUNCTIONAL SIGNIFICANCE

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Our laboratory has had a long-standing interest in the growth regulation of myeloma cells. Furthermore, we have had a particular interest in multiple myeloma (MM) cell responsiveness to interferon-alpha (IFN-α) because of its ability to modulate myeloma cell growth in a variable manner. Thus, IFN-α typically results in growth inhibition of myeloma cells, however some MM cells, such as the KAS-6/1 MM cell line, display an atypical growth response. Moreover, during the course of other studies, we serendipitously and unexpectedly discovered that the IFN-α growth responsive KAS-6/1 MM cell line expresses ErbB3, whereas MM cell lines that are growth arrested by IFN-α do not. In this regard, there is a growing awareness of the complications introduced by unexpected receptor interactions that result in synergistic down-stream cellular effects, e.g., enhanced growth or resistance to apoptosis of cancer cells. We hypothesized, therefore, that ErbB3 expression may play a role in the atypical IFN-α, KAS-6/1 response. Indeed, we were initially able to demonstrate that IFN-α stimulation resulted not only in the activation of the IFN-α receptor, but also in the transactivation of ErbB3, as revealed by tyrosine phosphorylation. To address the significance of these observations, we employed two strategies. First, we transfected ErbB3 negative DP-6 MM cells with ErbB3 and analyzed whether IFN-α stimulation could transactivate ectopically expressed ErbB3. Although IFN-α transactivated ErbB3 in the DP-6 transfectants, it did not confer growth responsiveness to IFN-α. Second, we wished to use RNA inhibition (RNAi) to assess the functional significance of atypical expression of ErbB3 in the KAS-6/1 cells. RNA interference (RNAi) is a recently discovered natural response to double-stranded RNA (dsRNA) that can result in sequence specific gene silencing. In ancillary studies aimed at adapting this methodology to myeloma cells, we demonstrated the striking dependence of dsRNA-mediated gene silencing in some cells on the methods of dsRNA transfection. Thus, we were able to optimize the inhibitory effects of ErbB3 siRNA in the KAS-6/1 cells by utilizing electroporation, rather than conventional transfection agents, to transfect the cells. Using Western blot analysis, we were able to demonstrate that ErbB3 siRNA treatment resulted in the loss of this receptor from the KAS-6/1 cells. Of great interest, silencing ErbB3 expression in KAS-6/1 cells decreased the overall growth response to both IFN-α and IL-6. Taken together these two lines of investigation suggest that ErbB3 expression alone does not uniquely confer IFN-α growth responsiveness but instead, may amplify proliferation rates in MM cells that have acquired atypical expression of this receptor. In conclusion, we believe that novel receptor interactions have the potential to allow dysregulated tumor cell function. In this regard, atypical expression of ErbB3 in multiple myeloma may provide a mechanism whereby the tumor cell is afforded with either an ability to grow or to survive. We are currently investigating this possibility, along with analyzing primary myeloma cells for ErbB3 expression.

109 DOWN-MODULATION OF ERK PROTEIN KINASE ACTIVITY SUPPRESSES HUMAN MYELOMA CELL PROLIFERATION AND MYELOMA-INDUCED ANGIogenesis BY VEGF INHIBITION

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The mitogen-activated protein (MAP) cascade leading to the activation of ERK (Extracellular signal-regulated kinase) is critical for regulating myeloma cell growth, however the relationships of ERK1/2 protein kinases activity with vascular endothelial growth factor (VEGF) production and the effects of their down-modulation in myeloma cells are not completely investigated. In order to elucidate this issue we first studied the expression and the activation of ERK1/2 protein kinases, in RPMI-8226, XG6, U266 and OPM-2 human myeloma cell lines (HMCL) (5x106) growing in 5ml of complete RPMI medium with or without IL-6 (20ng/ml). By immunoenzymatic MAP kinase assay using Elk1 as substrate (Elk1-p) or dual-phosphorylation-ERK1/2 specific antisera (p-ERK1/2) we found that steady-state levels of phosphorylated ERK 1/2 (p-ERK) were significantly higher. Basal VEGF secretion, detected by ELISA was similar in OPM-2 (Mean±SD: 835±10 pg/ml), XG-6 (668±15 pg/ml), U266 (739±12 pg/ml) and higher in RPMI-8226 (1381.6±290 pg/ml). IL-6 stimulation significantly increased ERK1/2 activity and VEGF secretion in XG-6, U266, RPMI-8226. Therefore, we used the specific inhibitor of MEK, PD98059, in order to down-modulate activated ERK1/2 (p-ERK1/2). The treatment for 2 hours of myeloma cells with 40mM of MEK-1 inhibitor PD98059 produced a reduction of p-ERK1/2 levels of more than 80% and prevented the increase of p-ERK1/2 induced by IL-6 treatment. PD98059 induced a significant inhibition of HMCL proliferation (-33±5%) and blunted the stimulatory effect induced by IL-6. A more potent inhibition on HMCL proliferation was observed with the ERK inhibitor PD184352 at 2mM (-70%±4%). PD184352 but not PD98059 induced HMCL apoptosis in combination with anti CD95 mAb or Arsenic Trioxide 2m . A significant dose and time dependent inhibition on basal VEGF secretion by HMCL was induced by PD98059 treatment, more evident in RPMI-8226 (771±12 vs 1350±18 pg/ml, -43%; p<0.01, after 24 hours). In addition, PD98059 treatment suppressed the stimulatory effect of IL-6 on VEGF secretion by HMCL (RPMI-8226: 887±18 vs 1694±17 pg/ml, -47%; p<0.01). Consistently, in an "in vitro" model of angiogenesis (ANGIOkit TCS, UK), we found that PD98059 inhibits vessel formation induced by HMCL. In conclusion, our data indicate that a constitutive activation of ERK is present in HMCL and that the down modulation of ERK1/2 activity induced an inhibition of cell proliferation and myeloma-induced angiogenesis.
In conclusion, our data indicate that human myeloma cells increased in MM patients in comparison with normal subjects in the pathophysiology of MM.

We found that RPMI-8226, U266, OPM-2, XG-6 and fresh MM cells obtained from 12 patients were positive for IL-7 mRNA. On the other hand, IL-7R mRNA was not expressed in any HMCL tested while the EBV positive cell line ARH-77 was positive for IL-7R. Using an ELISA assay IL-7 was detected in conditioned medium of mononuclear cells or normal CD19+ B positive for IL-7R. Using an ELISA assay IL-7 was detected in any HMCL tested while the EBV positive cell line ARH-77 was positive for IL-7R. Using an ELISA assay IL-7 was detected in the supernatant of HMCL, in contrast IL-7 was undetectable in conditioned medium of mononuclear cells or normal CD19+ B cells or B leukemia cell line REH. IL-7 levels were significantly up-regulated when RPMI-8226, U266, XG-6 were cultured in presence of IL-6 (20-50 ng/ml) while IL-6 did not induce IL-7 production in normal B cells and REH as well as in EBV positive cells and in B cells obtained from patients with acute lymphoblastic leukemia, previously evaluated for CD126 expression by flow cytometry.

A stimulatory effect of IL-7 on IL-7R positive cells ARH-77 proliferation was found (+12%±1;p<0.05). In addition IL-7 production induced the critical osteoclastogenic factor RANKL by T lymphocytes and blocking anti IL-7 Ab inhibited the stimulatory effect of HMCL on RANKL production by T lymphocytes. Following we tested IL-7 levels in MM patients. We found that IL-7 serum levels were significantly higher in MM patients in comparison to healthy subjects (median: 12.15 pg/ml; range: 2.41-29.5 pg/ml vs. 1.91 pg/ml; range: 0-3.43 pg/ml; p<0.05). Similarly, IL-7 levels in BM plasma were significantly increased in MM patients in comparison with normal subjects (median: 8.67 pg/ml; range: 2.68-36.8 pg/ml vs. 40 pg/ml; range 0-46 pg/ml; p<0.05).

In conclusion, our data indicate that human myeloma cells produce IL-7 in presence of IL-6 and that IL-7 could be involved in the pathobiology of MM.

The role of the IL-6/gp130/STAT3 pathway for survival and drug resistance of multiple myeloma cells in the presence of bone marrow stromal cells

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Introduction: The interleukin 6/glycoprotein 130/signal transducer and activator of transcription 3 (IL-6/gp130/STAT3) pathway has been reported to play an important role in the pathogenesis of multiple myeloma (MM) and for the survival of MM cells. However, most data concerning the role of IL-6 and IL-6-triggered signaling pathways were obtained from MM cell lines and without considering the influence of the bone marrow microenvironment. Thus, the precise role of IL-6 and its intracellular signaling pathways for the survival and proliferation of human MM cells is still unclear.

Experimental model: Human MM cells (the IL-6-dependent MM cell line INA-6 and primary MM cells) were cultured in the presence or absence of bone marrow stromal cells (BMSCs), a major cellular constituent of the bone marrow microenvironment, and IL-6 receptor function was blocked either with the antagonist Sant7 or with an anti-gp130 monoclonal antibody. Within this setting, MM cells were additionally treated with dexamethasone and all-trans-retinoic acid (ATRA), drugs that are routinely used for the treatment of patients with hematological malignancies. These drugs have been shown to induce apoptosis in MM cells, presumably by altering the expression of IL-6 or its receptor.

Results: BMSCs protect MM cells from apoptosis induced by Sant7 or by an anti-gp130 mAb. The analysis of intracellular pathways in INA-6 cells revealed that Sant7 and the anti-gp130 mAb effectively precluded phosphorylation of gp130 and phosphorylation and DNA-binding activity of STAT3. These effects were observed in the absence and in the presence of BMSCs. Conversely, phosphorylation of ERK1,2 and Akt was only slightly affected. Furthermore, we found that while MM cells co-cultured with BMSCs were protected from apoptosis induced by a combined treatment with Dexamethasone and ATRA, blockade of the IL-6 receptor with Sant7 sensitized them for drug-induced apoptosis.

Conclusions: MM cells become independent of the IL-6/gp130/STAT3 pathway in the presence of BMSCs. These findings support the idea that the IL-6/gp130/STAT3 pathway is not essential for the survival of MM cells in their microenvironment, where additional factors that activate different pathways and substitute for the antiapoptotic function of the IL-6/gp130/STAT3 pathway, might be provided. Nevertheless, Sant7 and other compounds that abrogate signaling through the IL-6/IL-6R complex might be of potential therapeutic use, because blocking the IL-6/gp130/STAT3 pathway can overcome drug resistance of MM cells.

Insulin-like growth factor-1 induces adhesion and migration in human multiple myeloma cells via activation of β1-integrin and phosphatidylinositol 3-kinase/AKT signaling

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Insulin-like growth factor-1 (IGF-1) is a growth and survival factor in human multiple myeloma (MM). In the present study, we examine the effect of IGF-1 on MM cell adhesion and define the role of β1 integrin in this process. IGF-1 increases adhesion of two MM cell lines MM.1S and OPM6 to fibronectin (FN, 20 µg/ml) in a dose- and time-dependent manner. An IGF-1 receptor blocking antibody (αIR3) prevents IGF-1-stimulated adhesion, whereas an isotype control antibody MOPC21 has no effect. Since an integrin mediates MM cell adhesion to FN and bone marrow (BM) stroma in MM, we next investigated the role of β1
integrin in IGF-1-triggered MM cell adhesion and migration. A functional blocking antibody against β1 integrin and a RGD peptide, as well as cytochalasin D (cyt D: a cytoskeleton inhibitory agent), significantly reduced IGF-1-induced cell adhesion. Immunoprecipitation studies demonstrated that IGF-1 rapidly and transiently induces association of IGF-1R and β1 integrin, which is associated with phosphorylation of IGF-1R, IRS-1, and p85/P13K. IGF-1 also triggers phosphorylation of AKT and ERK. We next demonstrated that IGF-1R and β1 integrin comigrate to cholesterol-rich microdomains on plasma membrane (membrane rafts) following IGF-1 stimulation, using western blotting for the Triton-insoluble and Triton-soluble fraction of the cells and dual immunofluorescence staining. Using methyl-β-cyclodextrin to disrupt membrane raft structure, we further show that β1 integrin-mediated IGF-1-stimulated adhesion requires intact lipid rafts. In addition, IGF-1 (100 ng/ml and 40 ng/ml for MM.1S and OPM6, respectively) triggers polymerization of F-actin, indicating focal adhesion formation, and immunoprecipitation experiments confirm that IGF-1 induces interaction of β1 integrin with cytoskeletal and signaling proteins localized at focal adhesions, including focal adhesion kinase (p125FAK), α-actinin, and paxillin. Pretreatment of cyt D abrogates activation of p125FAK and paxillin induced by IGF-1. Importantly, IGF-1-induced MM cell adhesion to FN is achieved only when β1 integrin and P13K/AKT are activated. In a 96-well transmigration assay, IGF-1 induces a 2-3 (MM.1S) and 3-4-fold (OPM6) increase in migration. Conversely, IR3 and a blocking anti-β1 integrin mAb, as well as Cyt D, abrogate MM cell transmigration. Interestingly, IGF-1 induces MM cell migration independent of de novo protein synthesis. Finally, primary CD138+ MM cells isolated from 3 patients were also responsive to IGF-1-induced adhesion on FN-coated plates and BM stromal cells. Together, these studies demonstrate that IGF-1 induces MM cell adhesion and migration, suggesting a role of IGF-1 in the trafficking and localization of these cells in the BM microenvironment. Moreover, these results identify a functional cooperation between IGF-1R and β1 integrin in MM homing, supporting blockade of IGF-1/IGF1R system as a novel treatment strategy of MM.

113 HHV-8 viral IL-6 homologue is as active as human IL-6 on human myeloma cells

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Kaposi-Sarcoma-associated Virus/ Human-Herpesvirus-8 (KSHV/HHV-8) is associated with at least three human lymphoproliferative disorders: primary effusion lymphomas (PEL), Castleman’s disease (CD) and plasmablastic lymphoma. However, the role of HHV-8 in multiple myeloma (MM) is still controversial. Some groups detected HHV-8-DNA in bone marrow samples of MM patients whereas other groups failed to confirm these data. An important issue is the potential functional role of HHV-8 which encodes for several viral cytokines. Most interest was given to the viral homologue of IL-6 (vIL-6) since human IL-6 (hull-6) is not only an essential growth and survival factor for malignant plasma cells but plays an important role in PEL, CD and plasmablastic lymphoma. We could show that prokaryotically expressed vIL-6 (pro-v-IL-6) has biological activity on human myeloma cells in vitro (Burger et al., Blood 1998). However, the vIL-6 concentrations needed for a maximum stimulatory growth effect on the strictly IL-6 dependent human myeloma cell line INA-6 were dramatically higher (4000 ng/ml) than those needed for recombinant huIL-6 (1.25 ng/ml) This requirement for very high amounts of vIL-6 argued against a causative role of vIL-6 in human diseases. However, this study was performed with prokaryotically expressed recombinant vIL-6 which could be less active than vIL-6 expressed in human cells. Now, we could overcome these limitations. First we used conditioned medium (CM) of the HHV-8 infected PEL cell line BCBL-1, containing only small amounts of hu IL-6 (45 pg/ml). The addition of 50% CM was able to induce a proliferative response of the INA-6 plasma cell line which would have required 1 ng/ml of recombinant hulIL-6. Anti-gp130 antibody (Ab) or the combination of anti-IL-6 receptor and anti-gp130 Abs, but not anti-IL-6R Ab alone, were effective in inhibiting BCBL-1 CM activity on INA-6. These results are similar to those obtained with vIL-6 and indicate that the growth induction of BCBL-1 CM is mainly caused by vIL-6. In a second approach we used a baculovirus vector expression system in eukaryotic cells. Eukaryotically expressed vIL-6 (eu-v-IL-6) had a significantly higher activity than pro-v-IL-6 and induced a maximum stimulatory growth response in INA-6 cells at the same concentration as hulIL-6 (1.25 ng/ml). Similar to the results published previously for pro-vIL-6 and obtained for BCBL-1 CM. The growth effects induced by eu-v-IL-6 could be blocked by anti-gp 130 Ab or a combination of anti-IL-6R and anti-gp130 Abs, but not anti-IL-6R Ab alone. Thus, our data indicate that the HHV-8 viral homologue of IL-6 is as active on human cells as hu IL-6. HHV-8 vIL-6 may therefore act as a substitute for the human cytokine in multiple myeloma and HHV-8 associated diseases and could play a causative role in their development.

114 Macrophage inflammatory protein 1-alpha (MIP-1α) triggers migration and signaling cascades mediating survival and proliferation in multiple myeloma cells

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Recently, several studies have suggested that MIP-1 contributes to the pathophysiology of multiple myeloma (MM). Thus, it has been demonstrated that MIP-1α is an osteoclast stimulatory factor and that MIP-1α neutralizing antibody (ab) or antisense inhibit bone destruction and reduce tumor load in a SCID-mice model of MM. Furthermore, MM patients have significantly higher bone marrow plasma levels of MIP-1α than healthy controls.

The current study was designed to determine the direct effects of MIP-1α on MM cells. We found expression of very high levels of MIP-1α and its receptor CCR5 in MM cell lines and patient-derived primary cells. Furthermore, we were able to demonstrate that MIP-1α significantly increased proliferation of different MM cell lines (MM.1S, H929, OPM-2, INA-6) and patient-derived primary MM cells. MIP-1α specific migration could be observed in a dose-dependent fashion up to 21 fold compared to control. In MM cell line and patient MM cells, MIP-1α induces phosphorylation of p44/42 mitogen-activated protein kinase (MAPK) as well as AKT and its downstream target FKHR. STX13 was not activated by MIP-1α. In addition, inhibition of AKT activation by the PI3-Kinase (PI3-K) inhibitors wortmannin.
or LY 294002 did not influence MAPK activation, suggesting that there is no cross talk between MIP-1α-dependent activation of the PI3-K/AKT and MAPK pathway. Our data suggest that besides the role of development of osteolytic bone destruction, MIP-1α also directly affects cell signaling pathways mediating growth, survival and migration in MM cells and provide evidence that MIP-1α might play a pivotal role in the pathogenesis of MM.

115 Regulation of Hypoxia-Inducible Factor-1-Mediated VEGF Expression in Human Multiple Myeloma Cells

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Constitutive expression of the transcription factor, hypoxia-inducible factor-1 (HIF-1), has been associated with tumor angiogenesis and higher mortality in solid tumors such as breast, ovarian, prostate and colon. HIF-1 is composed of a HIF-1α subunit, which is degraded under normoxic conditions but stabilized under hypoxic conditions, and a constitutive HIF-1β subunit. To date, very little is known about the role of HIF-1 activity in hematological malignancies. Our studies demonstrate that multiple myeloma (MM) cells express constitutive HIF-1α protein under normoxic conditions. HIF-1 has recently emerged as a critical determinant in the pathophysiological response to hypoxia and regulates angiogenic factors such as vascular endothelial growth factor (VEGF). Hypoxia further increases HIF-1α protein levels in several MM cell lines as well as HIF-1 dependent transcriptional activity in ARP-1 cells. Furthermore, treatment of MM cells with insulin-like growth factor-1 (IGF-1) and interleukin-6 (IL-6), two major growth and survival factors for MM cells, induced HIF-1 DNA binding and HIF-1α protein levels in several MM cell lines. Induction of HIF-1α protein levels by IGF-1 and IL-6 correlated with VEGF transcription. Pharmacological inhibitors of phosphatidylinositol-3-kinase/Akt kinase (PI3-K/Akt), mammalian target of rapamycin (mTOR), and MAP kinase (MAPK) signaling pathways blocked IGF-1 and IL-6-induced HIF-1α protein and VEGF mRNA levels in ARP-1 cells. This study suggests an important role for HIF-1 in IL-6 and IL-6-induced MM cell growth and survival as well as a central role in the regulation of VEGF expression. The elucidation of the HIF-1 signaling pathway may therefore identify novel therapeutic targets.

116 Essential Role of Caveolae in IL-6- and IGF-I-Triggered Akt-1- Mediated Survival of Multiple Myeloma Cells

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Caveolae, specialized flask-shaped lipid rafts on the cell surface, are composed of cholesterol, sphingolipids and structural proteins termed caveolins; functionally, these plasma membrane microdomains have been implicated in signal transduction and transmembrane transport. In the present study, we examined the role of caveolin-1 in multiple myeloma cells. We show for the first time that caveolin-1, which is usually absent in blood cells, is expressed in multiple myeloma cells. Interestingly, Cav-1 was up-regulated in MM (158+/-36) versus MGUS (49+/-24) samples, suggesting a possible role for caveolae in the transition of MGUS to MM. Analysis of myeloma cell-derived plasma membrane fractions shows that caveolin-1 is co-localized with interleukin-6 receptor signal transducing chain gp130 and with insulin-like growth factor-1 receptor, but not with ERK. Cholesterol depletion by β-cyclodextrin results in the loss of caveolae structure in myeloma cells, as shown by transmission electron microscopy, and loss of caveolin-1 function. Interleukin-6 and insulin-like growth factor-1, growth and survival factors in multiple myeloma, induce caveolin-1 phosphorylation, which is abrogated by pre-treatment with β-cyclodextrin. Importantly, inhibition of caveolin-1 phosphorylation blocks both interleukin-6- induced protein complex-formation with caveolin-1 and downstream activation of the phosphatidylinositol 3-kinase/Akt-1 pathway. β-cyclodextrin also blocks insulin-like growth factor-1- induced tyrosine phosphorylation of insulin responsive substrate-1 and downstream activation of the phosphatidylinositol 3-kinase/ Akt-1 pathway. Therefore cholesterol depletion by β- cyclodextrin abrogates both interleukin-6- and insulin-like growth factor-1- triggered multiple myeloma cell survival via negative regulation of caveolin-1. Taken together, this study identifies caveolin-1 and other structural membrane components as potential new therapeutic targets in multiple myeloma.

117 Multiple Bone Marrow Derived Cytokines Stimulate Signaling Cascades and Mediate Survival and Proliferation in Multiple Myeloma (MM)

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Interleukin-6 (IL-6) has been reported to play a central role in malignant growth and survival of Multiple Myeloma (MM) cells. Several studies have demonstrated IL-6-dependent activation of STAT-3 signaling pathways regulating proliferation and survival of MM cells. However, recently we have shown that in the presence of bone marrow stromal cells survival of MM cells becomes independent of the IL-6-gp130-STAT3 pathway and IL-6 antibody therapies have failed to induce remissions in patients with MM questioning the singular role of IL-6 in MM. Therefore, it was the aim of this study to identify additional factors and their corresponding signaling pathways contributing to the growth of MM cells. We found that besides IL-6 a number of various bone marrow derived cytokines such as LIF, VEGF, βFGF, MIP-1α, SDF-1α, IL-1β, SCF, and IL-3 redundantly activates the PI3K/Akt pathway and its downstream target FKHR and the MAPK pathway. Inhibition of these pathways by specific small compound inhibitors induces apoptosis in both MM cell lines and primary MM cells. Furthermore LIF, VEGF, βFGF, MIP-1α, SDF-1α, IL-1β, SCF, and IL-3 induces proliferation of MM cell lines and mediates survival of primary human MM cells. Thus, we provide evidence that in addition to IL-6 a number of different factors secreted by the bone marrow microenvironment might redundantly trigger a few important growth promoting pathways thereby supporting proliferation and survival of MM cells. Therefore, blocking of such pathways rather than blocking a single factor might be a promising approach to develop novel treatment strategies in MM.
IL-6-Induced Phosphorylation of Gab-Family Proteins in MM Cells is Src-family tyrosine kinase dependent
Podar-K1, Mostoslavsky-G2, Tai-YT1, Satller-M1, Catley-LP1, Hideshima-T1, Chauhan-D1, Mulligan-RC2, Anderson-KC1
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Interleukin-6 is a potent growth and survival factor in multiple myeloma (MM), which initiates signaling pathways via binding to the interleukin-6 receptor. Early IL-6 mediating and modulating signaling events, which ultimately lead to the activation of extracellular signal-regulated kinase (ERK) and phosphatidylinositol 3-kinase, are poorly understood. Our studies show for the first time that Gab-family adaptor proteins Gab1 and Gab2 are expressed by multiple myeloma cells and that IL-6 mediates their phosphorylation and association with several signaling proteins. In addition, we demonstrate that IL-6-induced phosphorylation of both Gab1 and Gab2 and their association with several downstream signaling proteins are Src-family tyrosine kinase-dependent. Consequently, inhibition of Src-family tyrosine kinase by PP2 significantly reduced the activation of the downstream signaling molecules ERK and Akt-1, leading to significant reduction of MM cell proliferation and survival. These studies identify Src-family tyrosine kinases and downstream adaptor proteins as potential new therapeutic targets in multiple myeloma.

Activation of the Erk5 route by IL-6 in multiple myeloma
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Several signalling pathways have been implicated in the transduction of proliferation/survival responses. An important group of pathways is represented by the MAPK routes. Three classical MAPK pathways have been described in mammals: the extracellular signal-regulated kinase 1 and 2 (Erk1 and Erk2), the p38 and the Jun N-terminal kinases (JNK) routes. A novel MAPK pathway, the Big MAPK-1/Erk5 pathway, has recently been implicated in proliferative responses. The role of Erk5 in MM biology is still unknown. Studies on the expression of Erk5 in different MM cell lines indicated that the protein was expressed in all the cell lines investigated. The subcellular distribution of Erk5 was analysed by immunofluorescence microscopy, and in the different cell lines studied the antibody stained the cytoplasm of the cells. Some Erk5 staining was also localized in the nuclear compartment. Staining was prevented by preincubation with the peptide antigen against which the antibody had been raised, indicating that staining was specific. To analyze whether IL-6, a major survival and proliferation factor for MM cells, regulated the Erk5 pathway we treated MM cells with this factor and analysed Erk5 activation by Western blotting. Addition of 10 nM IL-6 to MM1S, MM1R and MM144 cells caused Erk5 to migrate as a more retarded band. The action of IL-6 on Erk5 activation was found to be time- and dose-dependent. Maximal effect on Erk5 activation was observed around 20 minutes of treatment with IL-6, and 5 nM of IL-6. At present we are evaluating the importance of the Erk5 route on MM proliferation by the use of a form of Erk5 mutated in the TEY microdomain.

GENE EXPRESSION PROFILING AS A MEANS TO UNDERSTAND MYELOMA CELL GROWTH CONTROL: IDENTIFICATION OF MEK-DEPENDENT IL-6 AND IGF-I INDUCED GENES
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Multiple myeloma (MM) is an invariably fatal disease that accounts for approximately 1-2% of all human cancers. Although progress has been made in better understanding growth control of MM cells, further investigation is required. We have been interested in using a genomic profiling approach to facilitate an understanding of the mechanism by which interleukin 6 (IL-6), a known growth factor for MM cells, stimulates tumor cell growth. We, and others, have also shown that insulin-like growth factor I (IGF-I) can also directly stimulate myeloma cell growth, particularly when IL-6 is present. The ability of IL-6 and IGF-I to stimulate myeloma cell growth is of interest because the receptors for these two growth factors are strikingly different. Thus, the IL-6 receptor requires non-receptor tyrosine kinases, e.g., Jaks, to initiate signaling downstream of gp130, whereas the IGF-I receptor is a receptor which itself has tyrosine kinase activity. Of interest, the proliferative responses stimulated by both IL-6 and IGF-I are substantially inhibited when cells are cultured in the presence of the highly specific MEK (MAPKK) inhibitor, U0126. Our goal in this study was to use gene expression profiling to identify genes induced by these two growth factors alone or in combination, and to use the U0126 MEK inhibitor as a tool to specifically focus on genes downstream of the Raf-MEK-ERK pathway.

Three MM cell lines were cultured with and without IL-6 and IGF-I and the MEK inhibitor for 24 hours before measuring DNA synthesis and isolating total RNA. IL-6 stimulation induced 17-, 46-, and 2-fold increases in DNA synthesis in the DP-6, KAS-6/1, and KP-6 cell lines, respectively and IGF-I stimulation resulted in 3-, 16-, and 4.5-fold increases, respectively. In each of the three cell lines, U0126 inhibited IL-6-, IGF-I, and IL-6+IGF-I stimulated responses by an average of 80%. For gene expression analysis, cRNA was prepared from each sample and hybridized to Affymetrix HG-U95Av2 gene biochips. Expression data were analyzed using recently developed algorithms that combine data normalization with a holistic-model analysis of the probe data. We have also employed permutation test methods to calibrate significance values. Finally, to better understand the interrelationships of groups of genes, we have also applied the pattern recognition software, Genes@Work. Of interest, we have identified a significant number of genes that are induced in both a MEK-dependent and MEK-independent manner and these results will be presented. Notable examples of MEK-dependent IL-6 induced genes included several currently unknown genes, bFGF, Itilotin-1, HSP-70, c-fos, CTP-synthetase, hasRNP, CSE-1, Ran/Tc4, LAS-1, and the Cdc45-like PORC-PL gene. Many of these genes have been associated with other human cancers and play roles in cell cycle control. However, our studies also link many of these genes for the first time with the Raf-MEK-ERK pathway. Because of the role that the Raf-MEK-ERK pathway plays in myeloma cell proliferation, this approach has the potential to specifically identify genetic targets important in tumor cell growth.
The role of membrane raft in initiating CD40 signaling and ERK activation in modulating CD40-induced cytokine production in human multiple myeloma cells

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CD40 activation of human multiple myeloma (MM) induces PI3K/AKT/NIK-NF-kB activation, which mediates MM cell transmigration. CD40 activation in human MM also activates the MAP kinase (MEK) pathway, evidenced by phosphorylation of ERK, but not JNK or p38. In the present study, we examined the role of membrane raft in initiating CD40 signaling and the role of ERK activation in CD40-dependent regulation of cytokine/chemokine production in MM.1S cells, as well as patient plasma cell leukemia cells. Following cross-linking by sCD40L or anti-CD40 mAb G28.5, CD40 receptors on human MM cells move to membrane rafts, cholesterol-rich plasma membrane microdomains, as illustrated by dual immunofluorescence staining with FITC-labeled cholera toxin B against membrane raft ganglioside GM1 and Alexa 568-conjugated anti-CD40 mAb. Since Src is a membrane raft associated-signaling protein, we determined whether Src and intact membrane rafts modulate tyrosine phosphorylation (pTyr) following CD40 activation. Using methyl-β-cyclodextrin to disrupt cholesterol structure on cell membrane or the Src-family specific inhibitor PP2, we demonstrated that CD40-induced pTyr events are initiated in membrane rafts and are dependent on Src family kinase activation. Since CD40 activation of MM.1S and plasma cell leukemia cells, even at concentrations as high as 20 µg/ml, did not significantly alter DNA synthesis (p = 0.15 and 0.19, respectively), we next studied effects of CD40-induced ERK phosphorylation on cytokine/chemokine mRNA expression in MM.1S and plasma cell leukemia cells. Following CD40 activation, ERK activation was induced and persisted for an hour following treatment of sCD40L (2 µg/ml) and phosphorylation of c-Myc was also observed. We found that chemokine RANTES and IL-8 mRNAs were up-regulated in MM.1S and plasma cell leukemia cells by CD40 activation for 6-24 hr. Macrophage chemoattractant protein-1 (MCP-1) mRNA was also significantly induced. These results are in contrast to published reports on CD40-induced MCP-1 expression in U1026, we further demonstrated that secretion of RANTES, IL-8, and MCP-1 is largely dependent upon ERK activation. IL-1β was transiently induced following CD40 activation at 24 hr and the induction is dependent on ERK activation. In addition, all mRNAs for TNFR-associated factor (TRAF) family members, intracellular adapter molecules coupling TNFRs to effector molecules, were expressed constitutively although CD40 ligation up-regulates TRAF1 and TRAF5 mRNA. The ERK1/2 pathway was not involved. These results identify a crucial role for membrane rafts in initiation of CD40 signaling in human MM cells, and delineate sequence of CD40-mediated pathways regulating cytokine production. Furthermore, our results demonstrate differential cytokine/chemokine production following CD40 activation in MM vs normal tonsillar B cells.

IGF-1 plays an important multifunctional role in MM: Involvement of the PI3K and the MEK-ERK pathway

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Insulin-like growth factor-1 (IGF-1), produced by the bone marrow stromal cells, has previously been described as an important factor in proliferation, apoptosis and migration of MM cells. The latter process is involved in the homing of MM cells. The occurrence of angiogenesis and its correlation with both development and prognosis of the MM disease has been described by several groups. VEGF (vascular endothelial growth factor) is one of the prominent factors involved in this process. Here we sought to determine the different functions of IGF-1 in the 5TMM mouse model with special emphasis on proliferation, migration and VEGF secretion and the signalling pathways which execute them. This mouse model is representative for the human disease and can combine in vitro and in vivo studies. Western Blot analysis revealed that the MAP kinases ERK1/2 were activated 5 min after stimulation with IGF-1. The activation was abolished by PD98059, a MEK inhibitor, and reduced by the PI3K inhibitor Wortmannin. This implies that the PI3K pathway is involved in the activation of ERK1/2. We also found that Akt (PKB), a downstream target in the PI3K pathway, became phosphorylated 1 min after stimulation. While Wortmannin inhibited this activation, PD98059 had no effect. We next determined the role of these two signalling pathways in the functions of IGF-1. Thymidine incorporation assays revealed that IGF-1 induced a two-fold increase in DNA synthesis in MM cells, which was reduced by 30% with Wortmannin and by 45% with PD98059. These results indicate that both pathways are involved in the proliferation of MM cells. However, our finding that Wortmannin reduces the phosphorylation of ERK1/2 suggest that PI3K and Akt may operate through activation of the MEK-ERK pathway, especially since when the 2 inhibitors were added together, no additive effect was seen. We have previously reported that F-actin assembly correlates with MM cell migration. F-actin measurements demonstrated that IGF-1 enhances F-actin assembly and that this assembly could be reduced by Wortmannin but not by PD98059 suggesting that the PI3K pathway is functionally involved in the migration of MM cells towards IGF-1. Real-time PCR and ELISA showed that IGF-1 was able to enhance the production of VEGF in the MM cells. In real-time PCR after 24h, the mRNA of the VEGF isoform 164 was increased 2.8-fold and that of VEGF120 1.8-fold, while in ELISA after 24h the VEGF production was doubled. Stimulation of VEGF secretion was reduced by PD98059 but not by Wortmannin indicating that the MEK-ERK pathway is involved in the IGF-1 stimulated VEGF production.

In summary, IGF-1 mediates its effects through different signal transduction pathways: PI3K is mainly involved in the migratory response of the 5TMM cells while the MEK-ERK pathway is involved in stimulation of both DNA synthesis and VEGF secretion. The latter may be of particular importance since we previously demonstrated that contact of MM cells with bone marrow endothelial cells upregulates the expression of the IGF-1 receptor, which in turn could enhance the effect of IGF-1 on the VEGF secretion in MM cells.
THE IGF/IGF-1R SYSTEM IS A MAJOR THERAPEUTIC TARGET FOR MULTIPLE MYELOMA AND OTHER MALIGNANCIES

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We and others have shown that insulin-like growth factors (IGFs) stimulate proliferation of multiple myeloma (MM) cells and protect them from Dexamethasone (Dex)- or Apo2L/TRAIL-induced apoptosis. We now show that the IGF/IGF-1 receptor (IGF-1R/CD221) pathway represents a major therapeutic target for MM cells and other neoplasias. We studied a panel of 25 MM cell lines, including cells resistant to Dex, melphalan, anthracyclines, Apo2L/TRAIL, thalidomide and its novel immunomodulatory derivatives; 30 tumor samples from MM patients, including patients resistant to IMiDs or PS-341; as well as 30 cell lines from several hematologic malignancies, including B- and T-ALL, AML, CML, various non-Hodgkin's lymphomas (NHL) subtypes, and solid tumors (e.g. breast, prostate, lung, colon, thyroid, ovarian, renal Ca, retinoblastoma). Cell surface IGF-1R expression was confirmed by flow cytometry in all cell lines and 30/30 MM patient samples tested. No correlation was noted between the degree of IGF-1R expression and the drug sensitivity pattern of MM cells. Because IGFs are present in high levels in peripheral blood serum (fetal bovine, from healthy donors or autologous MM patients' sera) and can stimulate proliferation/survival of tumor cells throughout the body, we specifically evaluated the impact of inhibiting the IGF/IGF-1R pathway in serum-cultured tumor cells, using anti-IGF-1R neutralizing monoclonal antibodies (mAb); an IGF-1-like peptide that binds IGF-1R but competitively inhibits activation of its Tyr kinase; and a specific IGF-1R Tyr kinase small molecule inhibitor (Novartis AG, Basel, Switzerland). All 3 IGF-1R-inhibitory molecules had comparable effect in profoundly suppressing serum-induced proliferation/survival of all MM cells (median % inhibition of >70%) and cell lines from both solid tumors and hematologic malignancies (with less activity, though, against some NHL cells), while specific anti-IL-6R neutralizing mAb's had minimal, if any, effect on serum-cultured MM cells. We also found that IGFs are not only present in circulation, but are also produced in the BM microenvironment by autocrine (MM cells) and paracrine IGFs (present in serum or from autocrine/paracrine sources) and can stimulate proliferation/survival of mice and enhances the anti-MM effect of cytotoxic chemotherapy, without major toxicities. Our studies indicate that IGFs (present in serum or from autocrine/paracrine sources) and IGF-1R signaling in tumor cells play critical roles in stimulation of proliferation, survival and drug-resistance of MM, as well as other malignancies. Most importantly, our studies provide the first proof-of-principle that inhibition of IGF/IGF-1R pathway can be achieved with favorable therapeutic window and that small molecule IGF-1R kinase inhibitors constitute promising therapeutic agents for MM, and other neoplasias.

5.2 Apoptosis and survival signalling

In myeloma cells apoptosis induced by inhibition of Janus kinase 2 is independent of IL-6- or BMSC-mediated STAT3 activation

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Introduction: It has recently been reported that constitutive activation of STAT3 signaling confers resistance to apoptosis in human multiple myeloma (MM) cell lines. Accordingly, it has been demonstrated that the Janus kinase 2 (Jak2) inhibitor tyrphostin AG490 inhibits STAT3 phosphorylation and induces apoptosis in certain MM cell lines. Therefore, tyrphostins are thought to be useful drugs for the treatment of MM. However, data concerning the role of tyrphostins in human MM are limited to cell lines and do not generally consider contributions of the bone marrow microenvironment, which has been found to confer resistance to drug treatment in MM. Data on primary MM cells are missing and data describing the specificity and potential side effects of tyrphostins are scant.

Experimental model: To determine the role of the STAT3 activation status for the induction of apoptosis by AG490, we analyzed two different human MM cell lines: INA-6, which is strictly dependent on IL-6 and consequently maintains an activated STAT3 pathway, and MM.1S which is independent on IL-6 and lacks constitutive activation of STAT3. We also employed a co-culture model with MM cells (INA-6 or primary MM cells) and bone marrow stromal cells (BMSCs) to analyze AG490-mediated effects. In order to assess effects of Jak2 inhibition on hematopoietic stem cells CD34+ enriched cells were treated with AG490.

Results: Here we show that treatment with AG490 induces apoptosis of human MM cells in the absence or presence of BMSCs to a similar extent. Interestingly, this effect was observed not only with INA-6 cells, where strict dependence on IL-6 keeps the STAT3 pathway activated, but also with MM.1S cells, where STAT3 activity is absent. Primary MM cells were susceptible to AG490-induced apoptosis as well. Of interest, these experiments revealed a strong inhibitory effect of AG490 on the growth of hematopoietic stem cells in vitro. Signaling analysis revealed that treatment with AG490 led to a significant inhibition of phosphorylation of STAT3 only in the absence of BMSCs and only at very high concentrations. In the presence of BMSCs and at lower concentrations, that were nonetheless sufficient to induce apoptosis, no inhibition of STAT3 could be observed. Additionally, treatment with AG490 did not result in significant
inhibition of phosphorylation of MAPK/ERK1,2, or Akt, in either the presence or absence of BMSCs.

Conclusions: These experiments show that the apoptosis-inducing effect of AG490 is not mediated through inhibition of the STAT3 pathway or other pathways commonly associated with the malignant growth of MM. Thus, the precise mechanism of action of AG490 in MM cells remains elusive. Although our data indicates that tyrphostins are of potential interest for the treatment of MM, adverse side effects - in particular on the hematopoietic system - may be expected.

125 Bone morphogenetic protein (BMP) family members BMP-4, -5, -6 and 7 induce apoptosis in human myeloma cells.

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BMPs are members of the TGFβ superfamily, originally identified as molecules capable of inducing bone and cartilage formation. More recent knowledge shows that BMPs regulate a broad spectrum of biological responses like proliferation, differentiation and apoptosis in a wide variety of cell types, including hematopoietic cells. Recently, we have discovered that several members of the bone morphogenetic protein (BMP) family, BMP-4,-5,-6 and -7, are able to induce apoptosis in myeloma cell lines as well as in primary myeloma cells. We found a dose-dependent induction of apoptosis in the majority of cell lines and primary myeloma cell samples examined. BMPs reduced viability in more than 70% of purified primary myeloma cell samples. Myeloma cells resistant to one type of BMP (e.g. BMP-4) were still sensitive to other BMPs (e.g. BMP-6). In a panel of 5 different myeloma cell lines this diversity was explained by different expression patterns of BMP receptors. The induction of apoptosis seemed dependent on synthesis of new proteins, as the signs of apoptosis appeared relatively late compared to FAS- and TRAIL- receptor-induced apoptosis (24-48 vs. 1-6 hours), and as the induction of apoptosis was inhibited by cycloheximide. Myeloma cells were protected from apoptosis by a general caspase inhibitor, indicating involvement of caspases in the development of apoptosis. The intracellular mechanisms of BMP- induced apoptosis in myeloma cells are under further investigation.

BMP-induced apoptosis was influenced by interaction with other factors. The BMP antagonist noggin was able to abolish BMP-4-induced apoptosis, but it did not influence BMP-6 or -7-induced apoptosis. Whereas growth induced by IL-6, IL-21, TNF or IGF-1 were almost completely inhibited by BMPs, IL-15 was able to partially counteract the growth-inhibiting effect of BMPs. Heparin in doses of 10-100 μg/ml was able to fully abolish BMP-5 and BMP-6-induced apoptosis, and partially inhibit BMP-4 and BMP-7-induced apoptosis. Thus, interaction between BMPs and heparan sulfate chains of syndecan-1 may be of importance in vivo.

BMPs have intriguing anti-tumor effects in vitro. BMP-7 is currently in use as local treatment against fracture non-unions. Intravenously, BMP-7 has shown promising results as an agent reducing ischemic damage following stroke in rat brains. It is possible that therapeutic use of BMP or BMP analogues could have impact on both myeloma bone disease and myeloma cell growth.

126 Both mitochondrial (Type 2) and non-mitochondrial (Type 1) apoptotic pathways are activated in authentic multiple myeloma cells by Apo2L/TRAIL.

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TNF-alpha related apoptosis inducing ligand (TRAIL/Apo2L) is a member of the TNF family and has unique potential as an anti-tumour agent. We have demonstrated previously that TRAIL induces apoptosis of primary multiple myeloma (MM) tumour cells from MM patients but does not appear to be harmful to normal haemopoietic cells. Furthermore, we have demonstrated that one or both of the effector receptors for TRAIL (R1 and R2) is present on 90% of primary MM tumour populations and that TRAIL resistance is not due to expression of TRAIL decoy receptors (R3 and R4). To develop a rational approach to overcoming TRAIL resistance and the development of synergistic drug combinations with anti-MM potential we have endeavoured to define the mechanism(s) by which TRAIL induces apoptosis. Five authentic MM cell lines expressing both R1 and R2 were used as targets for TRAIL evaluation. At 1 hour post-TRAIL treatment 3 lines OPM-2, RPMI 8226 and LP-1 demonstrated sensitivity to TRAIL with 39%, 37% and 34% apoptosis, respectively, whereas NCI H929 and U266 were resistant with only 8% and 5% apoptosis, respectively. The 3 sensitive lines all demonstrated efficient cleavage of Pro-caspase 8 within 15 minutes of TRAIL treatment but this was delayed and reduced in the resistant lines. All 5 lines exhibited similar cytosolic accumulation of both Cytochrome C and SMAC confirming activation of the Type 2 pathway. To clarify the relative contributions of the Type 2 pathway it was inhibited by pre-incubation with a Caspase 9 inhibitor and the effect on TRAIL-induced apoptosis determined. This revealed a negative correlation between uninhibited TRAIL-induced apoptosis and the effect of Type 2 pathway inhibition with a reduction in apoptosis of 7.1%, 20.3%, 48.8% and 64.3% for the lines RPMI8226, LP-1, NCI H929 and U266, respectively. The latter is consistent with a greater reliance on the Type 2 pathway in MM cells with low TRAIL-induced Procaspase 8 activation efficiency. In contrast, there is significant redundancy in cells with efficient TRAIL-induced Procaspase 8 activation. Based on this we hypothesised that enhancing Type 2 pathway activation in cells relatively resistant to TRAIL may enhance TRAIL-induced apoptosis. Preliminary experiments with NCI H929 have demonstrated significant synergy between TRAIL and dexamethasone consistent with this hypothesis. We conclude that both mitochondrial and non-mitochondrial apoptotic pathways are activated in MM cells by TRAIL and this observation provides a basis for a rational approach for the evaluation of synergistic anti-MM drug combinations with TRAIL.

127 Both CD45 and PTEN phosphatase expression are critical for Akt/PI-3Kinase signaling in Multiple myeloma

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In Multiple Myeloma, the Akt/PI-3 Kinase pathway is involved in the proliferation of myeloma cells. In the current study, we have investigated the mechanisms that control Akt/PI 3-kinase activation. We show that this activation is restricted to IGF-1 but not to IL-6 stimulation. Its level in response to IGF-1 is highly variable from one
human myeloma cell line (HMCL) to another. Actually, Akt activation is highly dependent on either PTEN or CD45 phosphatase expression. PTEN is a recently identified tumor suppressor gene that is an important negative regulator controlling the Akt activation. Indeed, out of 26 HMCL only 2 of them do not express PTEN and have the strongest Akt activation in response to IGF-1. Among the 15 myeloma cell lines which has been analyzed for Akt activation, 3 expressed CD45 on 100% of cells (XG-1, XG2, MDN), 2 expressed both CD45− and CD45+ subpopulations (U266 and JIN-3) and 9 are completely negative for CD45 (LP-1, NCI-H926, OPM-2, ANBL-6, BCN, NAN-1, RPMI-8226, L363, JIM-3 and Delta47). The Akt response was significantly weaker for CD45+ or CD45+/− HMCL. On the other hand, the highest Akt activation was restricted to CD45− HMCL. Furthermore, the duration of Akt phosphorylation in response to IGF-1 is more durable in CD45− myeloma cell lines (>3hours) whereas a return to baseline level is reached as soon as 30 min in CD45+ HMCL. Finally, the growth of CD45− HMCL is mainly or even totally controlled by the PI-3 Kinase pathway whereas that of CD45+ HMCL is modestly controlled by it. Altogether, the results suggest that CD45 as PTEN negatively regulate IGF-1-dependent activation of PI-3 Kinase. Since we recently demonstrated that patients lacking CD45 at diagnosis had a bad prognosis, strategies that block IGF-1R signaling and consequently Akt/PI-3Kinase pathway could be a priority in the treatment of CD45− MM patients.

128 Two NF-κB activation pathways mediate CD40 signals to promote primary myeloma cell survival

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Multiple myeloma cells represent plasma cell precursors which, as a result of prior oncogenic transformation, fail to fully differentiate to plasma cells and turn over by apoptosis. On this basis, we hypothesize that the survival of MM cells in the bone marrow is at least in part due to inappropriate retention of B cell surface receptors. One candidate is CD40, which we have recently demonstrated to be progressively reduced during normal B cell terminal differentiation. CD40 signaling in MM cells has been shown to correlate with IL-6 secretion, but its role in myeloma clonogenic colony formation remains controversial.

To test our hypothesis, we have characterized the functional consequence and the pathway of CD40 signaling in freshly isolated bone marrow MM cells. Nearly all freshly isolated CD138+ MM cells (20/21) expressed CD40 at varying levels, and in 7 of them CD40 was expressed in the majority of cells (65−100%). Stimulation with CD40L protected MM cells from apoptosis ex vivo in 75% of the samples, and the degree of protection correlated with CD40 expression. These results support our hypothesis that most MM cells retain CD40 expression and that the primary consequence of CD40 signaling is inhibition of apoptosis.

Next, we determined the signaling pathway(s) that mediate CD40 signals for MM cell survival. CD40 signaling in normal B cells leads activation of various NF-κB transcription complexes and the downstream cell cycle and survival genes via the “classical” pathway, which requires degradation of the inhibitor of NF-κB (IκB) by the ubiquitin-proteasome pathway. This “classical” pathway, notably the transcriptional activating NF-κB1 p50/p65 NF-κB complex, was activated in CD40 signaling in MM cells. Importantly, CD40 signaling also activated NF-κB p52 in MM cells through the newly discovered “alternative” pathway, which does not involve IκB degradation. The two NF-κB signaling pathways are crucial for the survival of MM cells, because inhibition by proteasome inhibitors dramatically accelerated MM cell apoptosis.

This study provides the first direct evidence that CD40 promotes MM cell survival through activation of both classical and alternative NF-κB signaling pathways. Our findings have important implications for the mechanism of MM cell survival, the timing of oncogenic transformation in MM pathogenesis, and the use of proteasome inhibitors and possibly CD40 antagonists for MM treatment. These will be discussed.

Supported by NIH grants (CA 80204, AR49436) and a Specialized Center of Research for Myeloma grant by the Leukemia and Lymphoma Society of America.

5.3 Drugs and signalling pathways

129 Selective receptor tyrosine kinase (RTK) inhibition downregulates paracrine IL-6 in myeloma - stroma crosstalk and induces apoptosis in t(4;14) myeloma cells: therapeutic implications.

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We have previously shown that basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF) derived from MM cells stimulate bone marrow stromal cells (BMSC) to produce and secrete interleukin-6 (IL-6), an important growth and survival factor for human MM. In turn, IL-6 stimulates MM cells to secrete bFGF and VEGF (Blood, 2002, Nov 27, epub ahead of print, Blood,95:2630-2636; 2000).

In this study we investigated effects of BIBF1000 (125nM - 1µM), a selective inhibitor of VEGF and FGF RTKs, on paracrine IL-6 secretion in non-contact MM-BMSC cocultures. IL-6 concentrations in supernatants of serum-free 72hr-cocultures were significantly decreased by BIBF1000. In detail, a 2.5−7.0-fold increase of stromal cell-derived IL-6 by non-contact coincubation with KMS-11, U-266, RPMI-8226, or MM patient cells, was abrogated by BIBF1000 to baseline levels of unstimulated BMSC monocultures. BIBF1000 decreased IL-6 more potently than anti-bFGF-, anti-VEGF-, or a combination of anti-bFGF- and anti-VEGF-antibodies. In summary, paracrine MM-BMSC circuits can be completely blocked by BIBF1000 suggesting that selective VEGF/FGF RTK inhibitors may be active in the treatment of MM, not only through their anti-angiogenic activity, but also by downregulation of paracrine IL-6 release.

Furthermore, we studied direct effects of BIBF1000 on proliferation and apoptosis in MM cells. In KMS-11 and OPM-2, but not in RPMI-8226, a significant inhibition of proliferation occurred by addition of BIBF1000 (250nM - 1µM) as analyzed by 3H-thymidine-uptake. In parallel, we observed a consistent 10−20% increase in apoptosis by Annexin V / PI staining in OPM-2, KMS-18, and KMS-11 cell lines, all of which were shown to carry the t(4;14) translocation and overexpress FGF receptor 3. The proapoptotic activity was partially antagonized by exogenous bFGF or VEGF. In summary, paracrine MM-BMSC circuits can be completely blocked by BIBF1000 suggesting that selective VEGF/FGF RTK inhibitors may be active in the treatment of MM, not only through their anti-angiogenic activity, but also by downregulation of paracrine IL-6 release.
mTOR, a novel therapeutic target for multiple myeloma?
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Recently, it was reported that the phosphatidylinositol 3-kinase (PI3-K) signaling pathway may play an important role for the growth and survival of malignant plasma cells in multiple myeloma (MM). The PI3-K and its downstream effector Akt, activated by interleukin(IL)-6 or by insulin-like growth factor(IGF)-1, were shown to mediate proliferative signals and to inhibit dexamethasone induced apoptosis. In addition, it could be shown that multiple downstream targets are phosphorylated in MM cells upon activation of the PI-3K. Rapamycin is a potent and highly selective inhibitor of the mammalian target of rapamycin (mTOR), a downstream effector of PI3-K which mediates activation of p70S6K and 4E-BP1 by Akt. Rapamycin is a naturally occurring product isolated from Streptomyces hygroscopicus, which has strong immunosuppressive properties. In addition to its immunosuppressive properties, rapamycin was found to inhibit the growth of normal and malignant B- and T-lineage cells. In myeloma cells, rapamycin was shown to be able to revert IL-6 and IGF-1 induced phosphorylation of p70S6K and 4E-BP1 as well as IL-6 and IGF-1 induced cell growth under serum-free conditions. However, the overall effect of rapamycin on myeloma cell growth was relatively modest in these experiments. From other systems, it is known that serum is a potent activator of the PI3-K signaling pathway. For example, serum albumin was recently identified as the major plasma component responsible for Akt activation in B-CLL cells (...). Therefore, experiments conducted under serum-free conditions may not reveal the relevance of PI3-K signaling for myeloma cell growth and the potential role of PI3-K signaling pathway inhibitors in the treatment of multiple myeloma. By Western Blot analysis, we were able to show that fetal calf serum (FCS) is a far more potent activator of the p70S6K than either IL-6 or IGF-1 in all five human MM cell lines tested. Subsequently, we studied the effect of rapamycin treatment on the growth of these MM cell lines in the presence of FCS. Rapamycin was highly effective at inhibiting the growth of all five MM cell lines tested, as determined by [3H]-Thymidine incorporation (IC50 ranging from 0.24 to 1.8 ng/ml, growth inhibition at 100 ng/ml ranging from 74 to 97 %). Furthermore, we could show that this growth inhibition is mainly mediated by a G1 cell-cycle arrest. Next, the in vivo efficacy of rapamycin was evaluated in a SCID mouse xenograft model for human plasmacytoma, based on the IL-6 dependent human myeloma cell line INA-6. Starting on day 2 after tumor inoculation, a total of 14 mice received an oral dose of 2 mg/kg rapamycin 3 times per week for a total of two weeks. This dose was comparable to the maximum tolerated dose determined in studies with patients with advanced solid tumors. Treatment with rapamycin significantly delayed tumor development and prolonged survival of mice compared to untreated controls (median survival: 108 days vs. 58 days). The in vitro and preclinical in vivo efficacy of rapamycin indicates that inhibition of mTOR may provide an interesting treatment option for patients with malignant plasma cell tumors.

Inhibition of Protein Geranylgeranylation Induces Apoptosis in Myeloma Cells by Reducing Mcl-1 Protein Levels
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Prenylation is a class of lipid modification involving covalent attachment of hydrophobic isoprenoid molecules to target proteins including small GTP-binding proteins such as Ras and members of the Rac and Rho families. Farnesyl transferase (FTase) and geranylgeranyl transferase (GGTase) catalyze the transfer and binding of farnesyl and geranylgeranyl moieties from farnesyl pyrophosphate (FP) and geranylgeranyl pyrophosphate (GGPP), respectively. Prenylation is essential for membrane attachment and the subsequent participation of prenylated proteins in diverse signaling pathways regulating growth and survival. Lovastatin inhibits 3-hydroxy-3-methylglutaryl coenzyme A (HMG-CoA) reductase, which is the rate-limiting enzyme of the mevalonate pathway. This pathway leads to formation of cholesterol and isoprenoids like FP and GGPP. Treatment of plasma cell lines and ex vivo purified tumor cells from myeloma patients with lovastatin resulted in the induction of apoptosis. Co-treatment of cells with lovastatin and mevalonate or GGPP, but not FP, abrogated lovastatin-induced apoptosis. Furthermore, induction of apoptosis and reduction of cell viability by inhibition of FTase were less pronounced when compared to inhibition of GGTase I. This implies that geranylgeranylation of proteins is critical for the regulation of survival of myeloma cells. Apoptosis triggered by inhibition of geranylgeranylation was associated with reduction of Mcl-1 protein expression, which, in turn, resulted in the collapse of the mitochondrial transmembrane potential, cytochrome c release from mitochondria into the cytosol, and stimulation of caspase-3 activity. Lovastatin enhanced the cytotoxic effects of dexamethasone and doxorubicin in a synergistic fashion. This synergism may be due to the lovastatin-mediated Mcl-1 downregulation. These results show that geranylgeranylation of proteins is a key event in the regulation of myeloma tumor cell survival, probably through the regulation of Mcl-1 expression. Based on these findings, we started a Phase I/II trial to evaluate the combination of dose-escalating lovastatin and CHOP or VAD chemotherapy in refractory non-Hodgkin’s lymphoma and multiple myeloma patients. At the moment, 5 patients have been included in this trial.

Targeting the IGF-I Receptor Survival Pathways in Multiple Myeloma Using Selective IGF-I Receptor Tyrosine Kinase Inhibitors
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The insulin-like growth factor I receptor (IGF-IR) is strongly suggested to play a key role in malignant transformation. In tumor cells from multiple myeloma (MM), IGF-I has been established as an important survival factor, and we and others have previously shown that a blocking anti-IGF-IR antibody (IR3) augments apoptosis induced by serum-starvation, Fas-
ligation or dexamethasone (Georgii-Hemming et al 1996, Nilsson et al 1998). However, the biological significance of individual anti-apoptotic elements downstream the IGF-IR is not fully elucidated.

Upon ligand interaction with the IGF-R -subunit, tyrosine residues in the intracellular, membrane-bound β-subunit are autophosphorylated. This enables phosphorylation and docking of the insulin receptor substrates (IRS), thereby activating two important pathways mediating proliferation and survival, i.e. the phosphatidylinositol 3-kinase (PI 3-K)/Akt and the mitogen-activated protein kinase (MAPK) pathways. Thus, interfering with signaling at the level of the receptor tyrosine kinase (RTK) might represent an attractive strategy to sensitize MM cells to apoptosis.

To characterize the effects of the IGF-I RTK inhibitors in MM, we used a panel of authentic MM cell lines as well as freshly purified CD138+ tumor cells from MM patients. The IGF-I RTK inhibitors proved to be effective in the MM cell lines and in the primary MM cells providing 50-90% cell death within 48 h incubation during standard, serum-containing culture conditions. The two drug resistant subclones of the MM cell line RPMI8226, RPMI8226/Dox 40 (doxorubicin) and RPMI8226/LR5 (melphalan), were also sensitive to the RTK inhibitors showing similar IC50s as the parental cell line. Additionally, the IGF-I RTK inhibitors were studied in combination with conventional cytotoxic drugs, e.g. dexamethasone.

Analysis of the IGF-I RTK activity using immunoprecipitation of the IGF-IR -subunit and Western blotting shows that the IGF-I receptor tyrosine kinase activity is downregulated both basal and ligand-induced RTK activity. Furthermore, the downstream consequences of the perturbed IGF-IR signaling were investigated using phosphorylation site-specific antibodies directed against signaling molecules and substrates of the PI 3-kinase/Akt and the MAPK pathways. To identify target genes crucial for the IGF-IR mediated survival, gene expression of candidate regulators of apoptosis, e.g. the Bcl-2 family of genes, were analysed by ribonuclease-protection assay and Western blotting.

133 Inhibition of Protein Kinase C delta negatively regulates the Akt signaling pathway and induces apoptosis of myeloma cells

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Multiple myeloma (MM) is an invariably fatal disease of terminally differentiated B-lineage or plasma cells. Several growth factors (IL-6, IGF-I, VEGF) provide proliferative and oncogenic signals to these cells, contributing to their resistant phenotype. These signals are often transduced by a family of phospholipid-regulated, serine / threonine kinases known as Protein Kinase C. The expression of these PKC isoenzymes is cell specific and each isoenzyme is reported to regulate a specific cellular function. We first determined that several myeloma cell lines (8226s, U266, MM1S and MM1R) expressed classical PKC isoforms (α,β,γ) as well as the novel isoform, PKC δ. Treatment of myeloma cells with the classical and novel PKC inhibitor, N-benzoyl-stauroporine (PKC412, Novartis) (500-1000nM) induced apoptosis in dexamethasone sensitive (MM1S) as well as dexamethasone (MM1R) and chemo-resistant (U266) cells. Co-treatment with IL-6 (100ng/ml) did not inhibit PKC412 induced cell death while the caspase inhibitor Z-VAD-fmk (100μM) significantly abrogated its effect. PKC412 induced activation of caspase 9, caspase 3 and cleavage of PARP. Most importantly PKC412 reduced ser 473 phosphorylation of Akt and its downstream phosphorylated substrates GSK3-β, FKHR and Bad. Similarly to PKC412, the specific PKC δ inhibitor, Rottlerin (3μM), reduced Akt phosphorylation, induced caspase 3 and PARP cleavage and apoptosis of treated myeloma cells as well. However the classical PKC (α, β, γ) inhibitor GÖ6976 did not significantly affect their viability. Finally PKC412 (1μM) and Rottlerin (3μM) induced apoptosis in primary myeloma cells (CD138 high) while they had no effect on CD138 low bone marrow mononuclear cells.

Our studies demonstrate that inhibition of PKCδ negatively regulates Akt in myeloma cells and activates the downstream apoptotic machinery providing a rationale for targeting PKC isoforms, especially PKCδ in multiple myeloma.

134 MOLECULAR PROFILE OF THE ANTI-MYELOMA ACTIVITY OF HISTONE DEACETYLASE (HDAC) INHIBITORS: BIOLOGICAL AND THERAPEUTIC IMPLICATIONATIONS.

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Histone deacetylases (HDACs) affect cell differentiation and survival at the transcriptional level, by regulating the acetylation status of nucleosomal histones and the function of transcription factor complexes. HDAC inhibition induces differentiation and/or apoptosis in transformed cells. We recently showed (Blood, in press) that HDAC inhibitors, such as the prototypic hydroxamic acid-based HDAC inhibitor suberoylanilide hydroxamic acid (SAHA), potently induce cell death (through caspase-independent/calcium-dependent mechanism) of human multiple myeloma (MM) cells, including cell lines and MM patient-derived tumor cells, either sensitive or resistant to conventional or novel anti-tumor agents. SAHA also sensitized MM cells to death receptor (e.g. Fas or TRAIL receptor)-mediated apoptosis and inhibited IL-6 secretion in cocultures of bone marrow stromal cells (BMSCs) with MM cells. These comprehensive effects of SAHA, both on MM cells directly and on their microenvironmental interactions, prompted further investigation of the molecular sequelae of this class of agents, with particular focus on the transcriptional profile of SAHA-treatment, since HDAC inhibition exerts its anti-tumor activity by targeting predominantly the regulation of gene expression. HDAC inhibition was originally pursued with intent to induce differentiation of malignant (e.g. leukemic) cells, by de-repressing transcriptional programs of cellular differentiation. Interestingly, however, our gene expression profiling (using U133A Affymetrix oligonucleotide microarrays) and subsequent confirmatory mechanistic and functional assays, indicate that
HDAC inhibition in MM triggers a distinct transcriptional signature hallmark by suppression of pathways critical for tumor cell proliferation, survival and drug resistance, including downregulation of insulin-like growth factor (IGF)/IGF-1 receptor (IGF-1R) and interleukin-6 receptor (IL-6R) signaling cascades; suppression of anti-apoptotic molecules (e.g. caspase inhibitors); oncopogenes (e.g. myb, maf, pim-1, Axl, Polo and Aurora kinases, abl, vav, PKA-1, ASK); DNA synthesis or repair enzymes; transcription factors (e.g. XBP-1, E2F-1); nucleocytoplasmic transport regulators; and adhesion molecules (e.g. RHAMM, integrins) implicated in MM pathophysiology. SAHA treatment upregulates p53 transcriptional activity, represses the activity of HIF-1α and NF-κB, suppresses 26S proteasome subunits and proteasome activity, but does not trigger major heat shock protein upregulation, in contrast to pronounced stress responses generated by treatment of MM cells with other anti-tumor agents, e.g. proteasome inhibitors. Importantly, SAHA enhances MM cell sensitivity to other anti-MM agents, including dexamethasone, cytotoxic chemotherapy, as well as thalidomide analogs, proteasome inhibitors or hsp90 inhibitors. SAHA treatment does not indiscriminately suppress or activate gene transcription: it modulates expression of a wide constellation of molecular targets, which correspond, however, to highly specific functional clusters, with known direct or indirect involvement in tumorigenesis and/or proliferation, survival and drug-resistance of MM cells, specifically, or malignant cells, in general. Our studies indicate that HDAC function is critical for MM cells by actively maintaining a transcriptional program indispensable for their uncontrolled proliferation and/or inappropriate resistance to proapoptotic stimuli. The pleiotropic anti-tumor effects of SAHA, its ability to enhance the anti-MM activity of multiple conventional or novel agents and, importantly, the fact that it was bioavailable, well-tolerated and achieved objective responses after oral administration in phase 1 clinical trials, provide the framework for future clinical applications of SAHA to improve patient outcome in MM.

135 GENE EXPRESSION AND PROTEOMIC PROFILING OF DRUG-TREATED MULTIPLE MYELOMA (MM) CELLS: MECHANISMS OF DRUG RESPONSIVENESS VS. RESISTANCE AND RATIONALE FOR DESIGN OF NOVEL COMBINATION THERAPIES FOR MM

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The ongoing expansion of the therapeutic armamentarium for multiple myeloma (MM) with an extensive series of novel biologically-based treatment strategies poses the translational dilemma of how such agents should be optimally combined clinically with other conventional or novel therapies for MM. Indiscriminate testing of all possible treatment combinations is not feasible at either clinical or pre-clinical level, highlighting the need for rational design of anti-MM combination therapies based on comprehensive molecular profiling (at the gene expression and proteomic level) of individual agents. We have performed such transcriptional and proteomic profiling of MM cells treated ex vivo with conventional therapies (Dex, Doxo); as well as novel agents with pre-clinical and/or clinical evidence of anti-MM activity, including proteasome inhibitor (PS-341), immunomodulatory thalidomide derivatives (IMiDs), inhibitors of the hsp90 chaperone (17-AAG), histone deacetylase inhibitors (SAHA), thiazolidinedione (TZD) PPAR-α agonists (cigitazone), and inhibitors of IGF-1 receptor (IGF-1R) activity, and HMGCoA inhibitors (lovastatin). The molecular profiling was coupled with bio-informatic analyses, including hierarchical clustering, functional clustering and relevance network analyses, as well as with confirmatory mechanistic studies. These profiling studies documented overlapping molecular features of such novel classes of agents, including effect of PS-341, hsp90 inhibitors and IGF-1R inhibition on the NF-κB pathway and its activity; and decreased expression of inhibitors of apoptosis (IAPs) conferred by treatment with PS-341, 17-AAG, IGF-1R inhibitors, SAHA or TZDs. On the other hand, distinct molecular sequelae were induced by certain agents e.g. hsp90 inhibitors induced depletion of intracellular levels of several kinases implicated in growth/survival cascades (IGF-1R, Akt, Raf, IKK-α), while SAHA suppressed expression of key heat shock proteins (hsp) and regulators of translation. These studies provide a framework for combination treatments to enhance anti-MM activity. For example, PS-341 induced pronounced upregulation of heat shock protein (hsp) transcription and protein expression, in an apparent cellular response to counteract the stress of accumulating intracellular undegraded proteins, by promoting their enhanced chaperoning. This suggests that upregulation of hsp's may modulate sensitivity to PS-341, and that agents which abrogate the chaperoning function of key hsp's, such as the hsp90 inhibitor geldanamycin and its analogs, may increase MM cell sensitivity and/or overcome resistance to PS-341 or other agents (e.g. Doxo, Dex) which also upregulate hsp expression, in our studies. These studies on drug-induced molecular profiling therefore provide a framework for rational design of anti-MM combination therapies including therapies which independently target distinct pro-apoptotic pathways, inhibit key proliferative/anti-apoptotic pathways at distinct molecular levels, affording more effective overall blockade of the targeted pathway, while capitalizing on the use of certain agents to abrogate anti-apoptotic mechanisms attenuating response to other drugs.
6. Role of microenvironment

6.1 Cell adhesion

136 Regulation of SDF-1-CXCR4 interactions by SOCS3 in Myeloma and MGUS bone marrow.

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Multiple myeloma (MM), among the hematological malignancies is second only to lymphoma in incidence and mortality statistics. From published data, it is evident that interactions between the bone marrow microenvironment and myeloma cell play an important role in the pathogenesis of disease. Our data show that the stromal cell-derived chemokine, SDF-1, transcripts are significantly upregulated in the bone marrow of myeloma (p = 0.04) and MGUS (p = 0.04) patients compared to normal controls. SDF-1 protein is also increased in the bone marrow plasma of myeloma and MGUS patients. Overexpression of SDF-1 and its interactions with its receptor, CXCR4, has been implicated in preventing apoptosis and stimulating growth of tumor cells in a number of neoplastic diseases, through activation of Erk1/2, PI3-K/Akt and members of the JAK-STAT family. Myeloma and MGUS plasma cells from patients as well as several myeloma cell lines tested showed expression of CXCR4. Short exposure to SDF-1 induces internalization of the CXCR4 receptor through the GRK2/arrestin pathway. Jurkat, a T cell line with constitutive expression of CXCR4 (positive control) showed internalization of more than half of the CXCR4 receptor molecules on the cell surface after exposure to 50ng/ml SDF-1 for 30 minutes. The IL-6-dependent myeloma cell line, KAS-6/1 also showed a similar reduction in CXCR4 expression while DP-6 showed only about 20% reduction. This may indicate dysregulation of the GRK2 pathway in some myeloma cell lines. Prolonged exposure to SDF-1 functionally inactivates CXCR4, without altering surface expression, through the suppressor of cytokine signaling –3 protein (SOCS3) and is mediated by the JAK-STAT pathway. We hypothesize that the difference between the ‘benign-pre-myeloma’ state of MGUS and myeloma lies not so much in the plasma cell but in the bone marrow microenvironment and its regulation through SOCS3 and possibly other proteins. SOCS3 transcripts are expressed constitutively in bone marrow stromal cells (BMSCs) cultured from patients with myeloma, MGUS or normals. SOCS3 mRNA is also seen in the IL-6-dependent myeloma cell lines, ANBL-6, KAS-6/1 and DP-6. SDF-1 transcripts are present in BMSCs from normals, MM and MGUS as well as in the 3 IL-6 MM cell lines. Interestingly, very preliminary observations indicate that the SOCS3 protein, as detected by Western blot, is seen only in BMSCs derived from MGUS and normal bone marrow but not in MM BM. We are in the process of validating and verifying this finding. However, if this is true, it would suggest that while levels of SDF-1 are comparable in MM and MGUS BM, the interaction of SDF-1-CXCR4 is differentially modulated by SOCS3. In other words, in the MGUS BM, the interaction of SDF-1 with CXCR4 is appropriately controlled by SOCS3, which prevents continual activation of the receptor. However, the absence of SOCS3 regulation in the MM BM would allow SDF-1-CXCR4 interactions to go unchecked, leaving the downstream signaling pathways constantly activated, triggering proliferation and block of apoptosis of the transformed plasma cell. Thus, SOCS3 may play a crucial role in determining the fate of the clonal plasma cell through SDF-1-CXCR4 interactions.

137 Myeloma cell transendothelial migration and invasion in response to chemokine stromal cell-derived factor-1

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Multiple myeloma is a B cell neoplasm characterized by accumulation of myeloma cells in the bone marrow (BM) in contact with stromal cells. Myeloma cells express the integrin α4β1 which mediates their attachment to VCAM-1 and fibronectin displayed on BM stroma. Using purified recombinant forms of these α4β1 ligands we previously reported that SDF-1α is a chemokine present in the BM microenvironment, modulated α4β1-dependent myeloma cell adhesion. In the present work we show that SDF-1α triggers transendothelial migration of MM-C3D8, and modulated α4β1-dependent myeloma cell adhesion. In addition, a key role for the small GTPase RhoA in the increase of this adhesion by SDF-1α is suggested by the inhibition exerted by C3 exoyyme, which interferes with RhoA activation. We also report here that SDF-1α promotes myeloma cell in vitro invasion across Matrigel, which is mediated by the metalloproteinase MT1-MMP, whereas MMP-9 played minor roles. These data indicate that SDF-1α/CXCR4 axis in MM might contribute to myeloma cell transendothelial migration and lodgement in the BM microenvironment involving modulation of α4β1 adhesive activity. Additionally, MT1-MMP and MMP-9 activities expressed by myeloma cells could mediate their invasion across basement membranes after their transendothelial migration in response to SDF-1α.

138 Endoplasmic reticulum stress induces apoptosis in myeloma cells

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Introduction: The endoplasmic reticulum (ER) is the primary organelle in which proteins are synthesized and modified for appropriate folding. Accumulation of unfolded proteins in the ER lumen, which is caused by various environmental stresses, leads to unfolded protein response (UPR) which results in activation of XBP–1(X–box binding protein–1), a transcriptional factor required for the expression of ER chaperons. When stresses are present in excess, cells undergo apoptosis through the caspase–12 pathway. Multiple myeloma (MM) cells bear abundant ER, prompting us to ask whether MM cells are under ER stress and whether ER stress in MM cells can be controlled.

Materials and Methods: Plasma cells from MM patients were purified using negative selection utilizing magnetic beads. The cDNA coding XBP-1 (a marker for ER stress) was obtained through RT-PCR of mRNA purified from MM cells of 34 cases and subjected to digestion with Apa–LI. Apoptosis was detected either
morphologically or using Annexin V/PI staining. For induction of ER stress, Thapsigargin (Tg) was utilized. Activation of XBP–1 and cleavage of caspase–12 in two MM cell lines, U266 and KMS–12–PE, were also analyzed using western blot.

Results: Activation of XBP–1 was present in 25 fresh MM cases, suggesting the existence of ER stress in vivo. Tg stimulation caused significant apoptosis in purified MM cells but not in normal peripheral blood mononuclear cells. The Tg-induced apoptosis in MM cell lines accompanied XBP–1 activation and caspase–12 cleavage. When MM cells were placed under an overgrowth state, apoptosis also occurred with caspase–12 cleavage, suggesting that ER stress is inducible by either exogenous stimulation or nutrition deficiency.

Conclusion: The present results suggest that MM cells undergo apoptosis through ER stress. Induction of apoptosis via ER stress could open a new avenue for the development of therapy of MM.

### 140

**Possible role of LeuCAM, VLA-6 and VCAM-1 adhesion molecules in multiple myeloma clinical progression**

**Raya Sanchez JM, Hernández Nieto L, Brito Barroso ML, Fernández Martin R, González Brito G.**

**BACKGROUND:** Normal plasma cells express a wide repertory of cell-adhesion molecules (CAM) on their membranes, which anchor them to the tissues where they usually stay. The loss or underexpression of some of these CAM and the acquisition of others, could be related with migration processes to bone marrow or other extramedullary tissues. In myeloma patients, these phenotypic changes could contribute to tumour spreading and clinical progression of the disease. Our aim was to analyze the differential expression of some CAM on myeloma plasma cells, in a group of myeloma patients distributed by Durie & Salmon clinical staging system. **PATIENTS AND METHODS:** We have studied 18 myeloma patients distributed according to Durie & Salmon staging classification. Expression of sixteen different CAM (VLA-1 to VLA-6, LFA-1, CR3, CR4, LeuCAM beta-2 subunit, HCAM, ICAM-1, NCAM, L-selectin, VCAM-1, and PECAM-1) was analyzed by flow cytometry (double-color protocols) in bone marrow myeloma plasma cells. Identification and analysis of plasma cells was performed by gating CD138 (syndecan-1) positive cells. Investigated parameters were percentage of CAM-positive cells and mean fluorescence intensity (MFI) for each monoclonal antibody, which explores the antigenic density of CAM on plasma cell membrane.

**RESULTS:** The percentage of CR3 (CD11b) positive plasma cells was significantly low in stage III myeloma patients, when compared with stages I and II (see Table). There were no differences in percentages of positivity between the three stages for the remain analyzed CAM. The value of MFI for LeuCAM beta-2 subunit (CD18), VLA-6 (CD49f) and VCAM-1 (CD106) decreased significantly from stage I to stage III, while the other studied CAM did not show differences.

<table>
<thead>
<tr>
<th>MoAb (CAM)</th>
<th>Stage I (n=6)</th>
<th>Stage II (n=7)</th>
<th>Stage III (n=5)</th>
<th>p value</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD11b (%)</td>
<td>50.7 +/- 37.7</td>
<td>52.5 +/- 39.9</td>
<td>20.6 +/- 19.3</td>
<td>0.038</td>
</tr>
<tr>
<td>CD18 (MFI)</td>
<td>4.06 +/- 3.61</td>
<td>1.78 +/- 0.64</td>
<td>1.44 +/- 0.41</td>
<td>0.044</td>
</tr>
<tr>
<td>CD49f (MFI)</td>
<td>2.39 +/- 1.29</td>
<td>1.35 +/- 0.55</td>
<td>1.20 +/- 0.65</td>
<td>0.013</td>
</tr>
<tr>
<td>CD106 (MFI)</td>
<td>1.69 +/- 0.44</td>
<td>1.15 +/- 0.17</td>
<td>0.88 +/- 0.35</td>
<td>0.035</td>
</tr>
</tbody>
</table>

**CONCLUSIONS:** Our findings suggest that clinical progression in myeloma patients could be related with the loss of certain membrane CAM in myeloma plasma cells. Underexpression of beta-2 integrins, VLA-6 and VCAM-1 may play a role in tumor spreading and progression of the disease. Immunophenotypic changes include a decrease in antigenic density for these molecules on myeloma plasma cells membranes. If confirmed by further studies, these preliminary results could contribute to design new therapeutic strategies.
Migration and adhesion are important events in the interaction between myeloma cells and their surroundings. This cell behavior is dependent on adhesion molecules and is regulated by stimuli from the microenvironment in which the cell is located.

We examined adhesion of myeloma cell lines to several matrix components (fibronectin (FN), VCAM-1, collagen I and IV, laminin and vitronectin), after stimulation by various cytokines as well as by Mn2+. The cytokines used were BMP-7, EGF, FGF2, VEGF, HGF, IFN-α, IGF-1, IL-1β, IL-6, IL-15, IL-21, TNF, SDF-1α, and MIP-1α. We found that INA-6 and ANBL-6 cells adhered to FN and VCAM-1 but not to collagen, laminin or vitronectin. Adhesion to FN increased upon stimulation of cells by HGF (INA-6 only), IGF-1 or SDF-1α (7–9 fold), whereas other cytokines gave little or no increase in adhesion.

Migration of myeloma cells was studied by use of transwell chambers. HGF and SDF-1α increased migration of INA-6 and ANBL-6 cells 3 – 4 fold compared to control, while IGF-1 increased migration 2 fold.

Both adhesion and migration may involve integrin activation. We screened eight myeloma cell lines for surface integrins (β1, β2, β3, α5, αv, αl, αx) by flow cytometry, and found that α4β1 (VLA-4) was by far the most abundant integrin. Cytokine-stimulated adhesion was inhibited completely by use of blocking monoclonal antibodies towards α4 or towards β1, whereas migration decreased 50% by the same mAbs. The amount of α4β1 varied widely between cell lines (more than 10 fold variation in mean fluorescence intensity). Adhesion properties did not only reflect surface level of α4β1, as INA-6 cells, with less than half the amount of α4β1 integrin on their surface compared to IH-1 cells, adhered much better to FN and VCAM-1 than IH-1 cells.

By use of blocking agents, we studied the intracellular pathways for HGF, IGF-1 and SDF-1α signaling leading to activation of αβ1 integrin. Cytokine-stimulated adhesion was blocked by PI-3 kinase-inhibitors (LY-294002 and Wortmannin), but not by MAP kinase inhibitors (U-0126 and PD-98059). A tyrosine kinase inhibitor specific for the HGF receptor c-Met inhibited HGF-mediated adhesion only, whereas pertussis toxin (an inhibitor of Gi-protein-linked receptors including chemokine receptors) inhibited only SDF-1α-mediated adhesion. This shows that IGF-1- and HGF-induced αβ1 integrin activation does not act via the receptor for SDF-1α or via other Gi-protein-linked receptors. Similarly, IGF-1 or SDF-1α are not upstream of the signal from the HGF receptor.

In conclusion, of many tested cytokines HGF, IGF-1 and SDF-1α were unique in their ability to strongly activate αβ1-integrin-dependent adhesion and migration of myeloma cells.
Evaluation of Bone Marrow Angiogenesis in newly diagnosed myeloma patients before and after treatment with the combination VAD containing liposomal doxorubicin plus Thalidomide.

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Background Bone marrow angiogenesis (BMA) is increased in Multiple Myeloma (MM) and its extent is considered as an important prognostic factor. However, the impact of treatment in BMA is still controversial. Aim of study. The purpose of this study was to evaluate in newly diagnosed myeloma patients the changes in BMA after treatment with combination VAD containing liposomal doxorubicin plus Thalidomide. Patients Nineteen consecutive myeloma patients, (12 females, 7 males) with median age 67 years (range 47-76) were included into the study. The patients were treated with liposomal doxorubicin (40mg/m2) day 1, vincristine (2mg) day 1, dexamethasone (40 mg d 1-4) orally, every 4 weeks. Thalidomide was given daily at the dose of 200mg. Patients were reevaluated for response after four cycles of treatment. Methods. We studied BMA in bone marrow biopsy specimens at diagnosis and after the fourth cycle of treatment. Angiogenesis was estimated by microvessel density (MVD) using standard immunohistochecmical staining for CD 34 (mean±SE: 6.23±0.2 vs. 2.94±0.1, median: 6.21 vs. 2.79; p<0.001) and the micrvascular density was significantly increased (32.98±1.7 vs. 14.55±1.3, median: 34.69 vs. 13.04; p<0.01; capillaries: 26.73±1.3 vs. 10.42±0.8, median: 24.06 vs. 9.04; p<0.01, small venules: 9.56 ±0.5 vs. 4.14±0.5, median: 10.60 vs. 3.65; p<0.01). Furthermore a significant positive correlation between Ang-1 expression and MVD was found in our cohort of patients (Pearson Chi-square: p=0.036, Cochran’s Linear Trend: p=0.01). In conclusion our data indicate that myeloma cells produced Ang-1 but not Ang-2 mRNA and protein expression in purified MM cells obtained from about 47% of 23 patients analysed either immediately after purification or after co-culture with BM stromal cells and endothelial cells. In a transwell co-culture system, we observed that myeloma cells up-regulated the ang-1 receptor Tie2 in human BM endothelial cells. Moreover, in an experimental model of angiogenesis the conditioned medium of HMCLs significantly stimulated vessel formation as compared to control or to VEGF treatment. The presence of anti-Tie2 blocking Ab completely blunted the pro-angiogenic effect of XG-6. Our in vitro results were supported by the in vivo finding of Ang-1 but not Ang-2 mRNA and protein expression in purified MM cells obtained from about 47% of 23 patients analysed either immediately after purification or after co-culture with BM stromal cells and endothelial cells. Angiogenesis was evaluated in bone biopsies obtained from 15 out of 23 patients. The number of microvessels per field was higher in Ang-1 positive patients in comparison with Ang-1 negative ones (mean±SE: 6.23±0.2 vs. 2.94±0.1, median: 6.21 vs. 2.79; p<0.001) and the micrvascular density was significantly increased (32.98±1.7 vs. 14.55±1.3, median: 34.69 vs. 13.04; p<0.01; capillaries: 26.73±1.3 vs. 10.42±0.8, median: 24.06 vs. 9.04; p<0.01, small venules: 9.56 ±0.5 vs. 4.14±0.5, median: 10.60 vs. 3.65; p<0.01). Furthermore a significant positive correlation between Ang-1 expression and MVD was found in our cohort of patients (Pearson Chi-square: p=0.036, Cochran’s Linear Trend: p=0.01). In conclusion our data indicate that myeloma cells produced Ang-1 that it is involved in MM-induced angiogenesis.
STUDY OF BONE MARROW ANGIogenesis IN WALDENSTROM’S MACROGLOBULINEMIA. PRELIMINARY RESULTS.


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Background: Waldenstrom's Macroglobulinemia (WM) is a rare clinical syndrome, which is usually associated with an underlying lymphoplasmacytic lymphoma. It is characterized by infiltration of bone marrow (BM) with lymphoplasmacytid cells and increased number of mast cells. The latter are considered strong mediators of angiogenesis. It has been reported that in non-Hodgkin's lymphomas, mast cell density is correlated with the extent of BM angiogenesis. However, there are limiting data regarding the presence, extent, and the prognostic significance of angiogenesis in WM.

Aim of the study. In this study we evaluated bone marrow angiogenesis (BMA) in conjunction with the presence of mast cells and the percentage of infiltration by lymphoplasmacytid cells in patients with WM at diagnosis and after treatment with Rituximab (monoclonal Anti-CD20 Ab).

Methods: Eight patients (5 males, 3 females) with median age 66 years (range 47-80) with symptomatic WM were treated with Rituximab (monoclonal Anti-CD20 Ab).

Results: Five of eight patients responded, according to the usual criteria. Bone marrow biopsies were obtained at diagnosis and after the completion of treatment. Bone marrow angiogenesis was estimated by microvessel density (MVD). Microvessels were identified using the standard immunohistochemical staining for CD34 moAb and counted in whole cellular bone marrow at 400x magnification. MVD was expressed as number of vessels per mm2. Angiogenesis was also studied in a control group of 10 normal bone marrow biopsies. Mast cells were detected by positive immunostaining of BM specimens for tryptase and c-kit (CD 117 moAb). Bone marrow specimens were also evaluated for % of lymphoplasmacytid infiltration and the expression of CD 20 by malignant cells.

Conclusions. Although the number of patients studied is too small, we observed that BMA is increased in patients with WM at diagnosis. This seems to be related to the degree of BM infiltration by lymphoplasmacytid cells as well as with the number of mast cells. This observation is of considerable biological interest and further studies are warranted to elucidate the role of angiogenesis in WM.

THE PROTEASOME INHIBITOR PS-341 MODULATES VEGF SECRETION AND ACTIVITY IN MULTIPLE MYELOMA.

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The proteasome inhibitor PS-341 inhibits IeB degradation, thereby preventing activation of NF-κB, and induces apoptosis in several types of cancer cells, including chemoresistant multiple myeloma (MM) cells. Importantly, it has marked clinical activity even in the setting of relapsed refractory MM. We have recently reported that PS-341 in a dose-dependent fashion inhibits tumor growth, inhibits associated angiogenesis, and prolongs host survival in a murine plasmacytoma model. We now investigated the mechanism of PS341-induced inhibition of angiogenesis, by evaluating its effect on i) human microvascular endothelial cell (HMVEC) viability and ii) the production of VEGF by the MM cell line MM.1S in vitro. We found that PS-341 decreased the viability of human microvascular endothelial cells in a dose-dependent manner. PS-341 also lowered their proliferative response to stimulation with VEGF and bFGF. Surprisingly, PS-341 stimulated VEGF mRNA expression and protein release by MM.1S cells in vitro. To characterize the mechanism of PS341-induced VEGF upregulation, we transiently transfected MM.1S cells with a reporter plasmid carrying the 1.7 kb sequence of the VEGF promoter (VEGFpr), as well as deletion mutants with respectively 1.0-kb, 0.8-kb, 0.5-kb and 0.1-kb from the VEGFpr, upstream of a luciferase gene. A promoterless vector served as control. We found that PS-341 stimulated reporter gene expression in cells transfected with the full-length (1.7kb) VEGFpr, but not in cells transfected with the 1.0 kb, 0.8 kb, 0.5 kb, and 0.1 kb promoter fragments, or the control vector. This step-by-step deletional mapping identified a 0.7 kb region that (between 1.0 kb to 1.7kb upstream of the initiation site) to be responsible for the PS-341-induced effect on VEGF expression.
of genes, one of them being the gene for transmembrane protein carbonic anhydrase isoenzyme IX (CA IX) which is involved in maintaining the acid-base balance of the cell. Since the degree of intratumoral hypoxia is positively correlated with HIF 1, the expression of CA IX has been proven an indirect parameter for the degree of tissue hypoxia.

Aim of the study: Determining the expression of CA IX, the endothelial cell proliferation fraction (ECP) and mean vessel density (MVD) of bone marrow (BM) biopsies in non-neoplastic diseases, haematological malignancies and metastatic carcinomas.

Materials and methods: BM biopsies were included at the time of diagnosis before any treatment was administered from 12 patients without malignancies (NON-NEO), 17 patients with MGUS, 23 myeloma patients (MM), 44 patients with a chronic myeloproliferative syndrome (CMPs), 13 patients with a CD34- negative AML, 15 patients with a CLL, 25 patients with a bone marrow metastasis of an epithelial tumour (META).

The BM biopsies were immunostained with anti-CA IX. The number of vessels per 0.22 mm² was determined on sections immunostained for CD34. The mean vessel density (MVD), the endothelial cell proliferation was determined by a double staining for CD34 and Ki-67. The mean vessel density (MVD), the endothelial cell proliferation (r=0.60, p=0.009) and the MVD (r=0.72, =0.0003) had a significant correlation between the tumorload and the MVD.

Results: 7 of 25 META had at least a focal membranous expression of CA IX whereas no expression was observed in all haematological neoplasms nor in MGUS and bone marrows of NON-NEO patients. There was a significantly (Kruskal Wallis: p < 0.0001) increased MVD in MM (19.1(11.3), META (24.0(7.7), CMPs (19.2(7.6), AML (20.8(6.8) compared to the NON-NEO (8.6(6.5) cases. There was no significant difference for MGUS (7.3(3.5) and CLL (9.2(4.6) compared to NON-NEO (p=0.05).

The MM (1.9(2.0), CMPs (0.9(0.8), AML(1.2(1.1) and the META (2.5(1.4) had a significantly increased ECP compared to the the NON-NEO(0.1(0.3) cases. For the CLL (0.1(1.3) and the MGUS (0.0(0.0) no significant difference could be demonstrated.

A significant difference in ECP was observed between MM and MGUS. The intensity of CD34 staining was graded: grade 1 corresponded to a weak to partially negative staining of the majority of the (sinusoidal) vessels. Grade 2 was attributed to the cases in which part of the vessels had a weak to partially negative staining and part of the vessels had a strong CD34 staining and grade 3 if the majority were strongly staining for CD34 (small sprouting vessels). MM with a diffuse growth pattern (7 cases) had grade 3 staining vessels; in the interstitial-nodular growth pattern (5 cases), 2 were grade 1, 2 were grade 2 and 1 was grade 1 and in the pure interstitial pattern (9), eight had grade 1 and 1 had grade 3 (chi-squared p-value= 0.0003).

In MM there was a significant correlation between the tumorload and the MVD (r=0.72, =0.0001), between the tumorload and the endothelial cell proliferation (r=0.60, p=0.03) and between the MVD and the endothelial cell proliferation (r=0.60, p=0.009)

6.3 Bone disease

148 Role of myeloma-induced osteoclastogenesis in the disease.

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We have studied the role of myeloma-induced osteoclastogenesis in the disease process. Initially, we determined whether myeloma plasma cells (MM PC) could induce osteoclastogenesis in a stromal cell-free environment. Next, we examined the effect of osteoclasts (OC) on the growth and survival of myeloma and healthy donor PC, and the molecular consequences of PC interactions with OC.

Cultures of osteoclast precursors (pOC) and mature active multinucleated OC were prepared from PBSCs and MNC from 5 healthy donors and PBSCs from 30 myeloma patients. CD138-enriched PC were purified from BM aspirates from 50 patients and from 6 healthy donors (NPC).

PM PC conditioned media from 9 patients increased migration of pOC across a 5 µm pore size membrane 1.6-4.2 fold from 127±37 (mean±SEM) in controls (p=0.01). In co-cultures with pOC, PM PC from 22 patients induced formation of multinucleated, bone-resorbing OC by 6 fold from 50±8 in controls (p<0.0002). PM PC co-cultured with pOC expressed RANKL on their surface, demonstrated immunohistochromically. Addition of RANK-Fc to 13 cultures inhibited MM PC-induced OC formation by 16-100% (p<0.008).

To investigate if, as suggested by in vivo experiments, OC affect survival and growth of MM PC, 0.5x103 MM PC from 25 patients were cultured alone or together with OC. After 14 days, although fewer (302±22 vs. 448±26 X103 in controls, p<0.0001), the PM PC in co-cultures had higher viability (95±1% vs. 38±3%, p<0.0001) resulting in more viable cells (287±21 X103 vs. 166±18 X103, p<0.0001), fewer annexin V positive cells (22±1% vs. 70±3%, p<0.0001), higher BrdU labeling indices (LI, 1.3±0.2 vs. 0.3±0.1, p<0.0005), and increased [3H]TdR incorporation (4547±1518 vs. 1579±723 cpm, p<0.0001) than PC cultured alone. OC removed dying PC by phagocytosis, explaining the higher total cell counts in controls cultures. These observations were not dependent on the source of osteoclasts, whether from healthy donors or myeloma patients. In contrast to MM PC, NPC did not survive in long term co-cultures with OC.

Physical contact between MM PC and OC was essential; the number of viable PC recovered from non-contact co-cultures was lower by 33% (p<0.003) from 308±31X103 in co-cultures. Blocking IL-6 activity, while having no effect on the LI, resulted in 30% reduction in the number of viable PC (p<0.0001), 22% in [3H]TdR incorporation, and a 83% increase in apoptotic cells (p<0.005), demonstrating that IL-6 is a myeloma survival rather than growth factor.

Microarray analyses were performed on 20 MM PC before and after 4 days co-culture and in 4 NPC after co-culture. 111 probe sets changed 2- to 110-fold (103 up- and 8 down-regulated) in at least 16 of the 20 MM PC after co-culture. Their role in myeloma pathogenesis will be discussed. 41 genes were differentially expressed between MM PC and NPC after co-culture, some of which are involved in cell cycle (NAP1L1, PTPRK), oncogenesis (ARHB, CAMP-GEFII, PVT1, DSIPI) and adhesion (STIM1).

We conclude that myeloma cells directly induce formation of OC, these, in turn, support the growth of MM PC through modulation of gene expression through a cell contact-mediated interaction.

149 Role and fate of osteoblasts in myeloma

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Progression of myeloma (MM) is associated with reduction in osteoblast numbers along the surface of involved bones. As in
patients, the growth of primary myeloma plasma cells (MM PC) in SCID-hu mice is associated with a reduced number of osteoblasts. The aim of the study was to shed light on the fate of osteogenic cells in MM and to test the effect of osteoblasts on myeloma cell growth and survival. Human bone sections from myelomatous SCID-hu mice double stained for TUNEL and for osteocalcin showed increased number of apoptotic osteoblasts and osteocytes as compared with non-myelomatous bone sections.

To investigate the effects of mesenchymal cells from myeloma patients, 0.5-1x106 EGFP-transduced mesenchymal stem cells (MSCs), established from bone marrow aspirates of MM patients, were injected directly into the human bones of myelomatous SCID-hu mice, resulting in marked inhibition of tumor growth in 3 of 9 experiments and stable disease in 2 additional mice. The levels of hlg in responding mice was reduced from 492±206 to 205±68 µg/ml (p<0.04), whereas in the non-responders they increased from 310±114 to 909±271 µg/ml. Immunohistochemical staining for EGFP revealed positive staining mostly in osteoblasts and less frequently in adipocytes and fibroblasts, indicating that the MSCs differentiated mostly into osteogenic cells. To investigate the effects of bone cells on myeloma cell growth and survival, a novel co-culture system was developed using 0.45-1 µM pore-size Transwell inserts. Multinucleated, TRAP-and vitronectin receptor-positive, bone-resorbing osteoclasts, prepared by culturing patients’ PBSCs with RANKL, M-CSF and dexamethasone for 1-2 weeks, were sutured in the upper chamber prepared by culturing patients’ PBSCs with RANKL, M-CSF and dexamethasone for 2-3 weeks and characterized by increase expression of alkaline phosphatase followed by calcium deposition and high expression of osteocalcin and BMP-2, were cultured on the backside of the inserts’ membranes. The cytoplasmic villi of stromal cells pass through the membrane pores, allowing contact with tumor cells. CD138-enriched MM PC were cultured alone or co-cultured with osteoclasts, osteoblasts and osteoclasts+osteoblasts for 7 days. MM PC (n=8) in co-cultures with osteoclasts had more viable cells (313±62 vs. 189±44, p<0.02), fewer annexin V positive cells (22±3 vs. 70±6, p<0.0001) and higher BrdU labeling indices (LI, 11±5 vs. 3±1, p<0.02) than in co-cultures with osteoblasts. The presence of osteoclasts in co-cultures of MM PC with osteoclasts (n=5) attenuated the stimulatory effect of osteoclasts on myeloma cell growth and survival as indicated by 38% and 62% reduction in viable cells and BrdU LI, respectively, and by 24% increase in annexin positive cells.

This study demonstrates that impaired bone formation in myeloma is triggered by induction of apoptosis of osteogenic cells. The results also suggest that osteoclasts impede myeloma growth. Therapeutic approaches that increase osteoblast activity and restore bone turnover level in MM will not just improve bone density and skeletal complications but may also have impact on the disease progression.

We have recently identified macrophage inflammatory protein-1 (MIP-1) as a chemokine produced by myeloma cells that induces human osteoclast (OCL) formation independently of Receptor Activator of NF-Kappa B Ligand (RANKL), and enhances the effects of RANKL and interleukin-6 (IL-6) on OCL formation. Blocking MIP-1 expression in an in vivo model of human Multiple Myeloma (MM) profoundly decreases disease progression and bone destruction. Furthermore, patients with elevated levels of MIP-1α have an extremely poor prognosis. Recently, we demonstrated that abnormal transcriptional regulation of MIP-1 gene expression occurs in myeloma due to an imbalance in the expression levels of the Acute Myeloid Leukemia-1A (AML-1A) and AML-1B transcription factors, which results in enhanced MIP-1 expression. It is our hypothesis that the imbalance in the expression levels of AML-1A and AML-1B in myeloma cells results in abnormal expression of hematopoietic and bone specific genes that contributes to the poor prognosis of MM by enhancing adhesion of tumor cells to stromal cells, the growth of myeloma cells, and stimulating osteoclastic bone resorption. To test this hypothesis, we screened myeloma cell lines and purified plasma cells from myeloma patients for genes known to be regulated by AML-1 including GM-CSF, G-CSF receptor, c-fms, IL-3, IL-7, and Bcl-2. We found that myeloma cells that overexpress AML-1A compared to AML-1B, produced increased amounts of interleukin-3, and IL-7, in addition to MIP-1α, at both the mRNA and protein level. We then evaluated the effects of IL-3 on myeloma cell growth and osteoclastic bone resorption. IL-3 stimulated the growth of IL-6 dependent myeloma cell lines such as KAS 6/1 in the absence of IL-6 and addition of IL-6 to cultures of KAS 6/1 cells in the presence of IL-3 did not further increase their growth. The effects of IL-3 were not due to induction of IL-6 because IL-3 did not induce IL-6 expression by myeloma cell lines, and anti-IL-6 did not block the stimulatory effects of IL-3 on the growth of myeloma cells. Importantly, IL-3 induced osteoclast formation in human marrow cultures and in combination with RANK ligand or MIP-1α markedly increased OCL formation. Time course studies demonstrated that IL-3 was acting at the early proliferative stage of these cultures, increasing the osteoclast precursor pool, which was then induced to differentiate by either RANK ligand or MIP-1α. These results show that the abnormal expression pattern of the AML-1 class of transcription factors in myeloma results in upregulation of genes that may significantly impact on the prognosis of these patients by enhancing the growth of myeloma cells (IL-3), increasing expression of adhesion molecules and homing of myeloma cells to marrow (MIP-1α) and inducing osteoclastic bone destruction (IL-3 and MIP-1α). Studies to characterize genes that are abnormally expressed in myeloma cells due to the imbalance in AML-1A and AML-1B expression should provide important insights into the biology of myeloma and identifying potential therapeutic targets for treating this incurable disease.
Multiple myeloma is associated with the development of a bone disease, which is mediated by increased osteoclastic bone resorption. Until recently, the factors responsible for stimulating the increased osteoclast formation have been unknown. The identification of the ligand for receptor activator of NF B (RANKL) and the demonstration that RANKL plays a critical role in normal osteoclast formation has raised the possibility that abnormal expression of this molecule may contribute to osteoclast formation in myeloma. RANKL exists principally as a membrane-bound molecule, although a soluble form (sRANKL) can be produced. The aim of this study was to determine whether sRANKL was associated with increased osteoclast formation and the development of myeloma bone disease in the 5T2MM and 5T33MM syngeneic models of myeloma.

Male C57BL/KaLwRijHsd mice were injected with 5T2MM or 5T33MM cells, or left un-injected. The myeloma disease was allowed to develop, which took 12 weeks in 5T2MM-bearing mice and 4 weeks in 5T33MM-bearing mice. At these points animals in each group, including the respective un-injected control groups (naïve), were sacrificed. sRANKL and the decoy receptor, osteoprotegerin (OPG), were measured by ELISA and the bone disease was assessed by a combination of radiographic, densitometric and histological analyses. Soluble RANKL was detected in the serum of mice bearing 5T2MM cells (379±58.4pg/ml) but not in the serum of naïve mice or mice bearing 5T33MM cells. Serum concentrations of OPG in naïve animals and 5T2MM bearing animals were not significantly different (1.77±0.13ng/ml vs 1.86±0.08ng/ml, respectively). However, OPG was higher in mice bearing 5T33MM cells when compared to naïve animals (14.97±1.09ng/ml vs 1.33±0.13ng/ml, respectively, p=0.001). Radiographic analysis of the long bones of mice bearing 5T2MM cells demonstrated the presence of osteolytic bone lesions in 5T2MM-bearing animals but not naïve animals (15.25±3.03 vs 0, p<0.05). No lesions were seen in mice bearing 5T33MM cells. Cancellous bone area was significantly decreased in 5T2MM bearing animals when compared to naïve animals (0.20±0.19% vs 3.65±1.09%, respectively, p<0.05). Cancellous bone area in mice bearing 5T33MM cells was not significantly different from naïve animals (9.0±1.7% vs 8.5±0.8%, respectively). Total BMD was also lower in 5T2MM bearing mice when compared to naïve animals (44.8±0.69mg/cm2 vs 47.8±1.19mg/cm2, respectively, p=0.01). There was no difference in BMD in naïve mice and mice bearing 5T33MM cells (45.7±0.7mg/cm2 vs 46.8±1.0mg/cm2, respectively). The proportion of cortical-endosteal bone surface covered by osteoclasts was significantly increased in 5T2MM bearing animals when compared to control (6.42±0.83% vs 1.1±0.73%, respectively, p=0.05). No osteoclasts were found lining the cortical-endosteal bone surface of mice injected with 5T33MM cells.

In conclusion, mice bearing 5T2MM tumor cells have increased serum concentrations of sRANKL and unchanged OPG, suggesting an imbalance in favour of bone resorption. In contrast, mice bearing 5T33MM cells do not have increased osteoclast formation and have no osteolytic disease. Mice bearing 5T33MM cells have no detectable sRANKL in their serum but had increased serum OPG. These data provide compelling evidence that the balance between a soluble form of RANKL and OPG may play a critical role in regulating osteoclast formation and bone disease in myeloma.

**152 A Murine RANK-Murine Fc Construct Inhibits Osteoclast Formation and Prevents the Development of Osteolytic Bone Disease in the 5T2MM Murine Model of Multiple Myeloma**

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Multiple myeloma is commonly associated with the development of an osteolytic bone disease, which is characterised by pathological fractures, bone pain and hypercalcemia. The bone disease is mediated by increased osteoclastic activity, however, until recently, the factors responsible for inducing osteoclast formation in myeloma have been unclear. The ligand for receptor activator of NF B (RANKL) has been shown to play a central role in normal osteoclast formation and this molecule appears to be abnormally expressed in myeloma. RANKL induces osteoclast formation by binding its receptor RANK on the surface of osteoclast precursors. Targeting this interaction may therefore prevent the formation of osteoclasts and the development of myeloma bone disease. The aim of the present study therefore was to determine whether a recombinant murine RANK-murine Fc fusion protein (RANK.Fc) could inhibit the development of osteolytic bone disease in the 5T2MM model of multiple myeloma.

5T2MM murine myeloma cells were injected intravenously (iv) into C57BL/KaLwRij mice and the development of the myeloma disease was monitored by measuring the concentration of paraprotein in the serum. Mice were treated with RANK.Fc (500µg/mouse, iv, three times/week) from the time of tumor cell injection. At 8 weeks all animals had a detectable serum paraprotein and by 12 weeks showed signs of morbidity and were sacrificed. The presence of tumor was measured by assessing serum paraprotein concentrations and determining the proportion of idiootype positive cells in the bone marrow by flow cytometry. The bone disease was assessed by a combination of radiographic, densitometric and histological analyses. All animals injected with 5T2MM cells developed a myeloma bone disease characterised by the presence of pathological fractures, bone pain and hypercalcemia. The bone disease was monitored by measuring the concentration of paraprotein in the serum. Mice were treated with RANK.Fc (500µg/mouse, iv, three times/week) from the time of tumor cell injection. At 8 weeks all animals had a detectable serum paraprotein and by 12 weeks showed signs of morbidity and were sacrificed. The presence of tumor was measured by assessing serum paraprotein concentrations and determining the proportion of idiootype positive cells in the bone marrow by flow cytometry. The bone disease was assessed by a combination of radiographic, densitometric and histological analyses.
RANK interaction could be a valuable approach to treating development of osteolytic bone disease in the 5T2MM model of cells exhibiting a more differentiated (STRO-1-/Alkaline cell differentiation, as evidenced by an increase in the number of zoledronic acid. Zoledronic acid was also found to promote bone more susceptible to the cytostatic and apoptotic effects of taken together these data demonstrate that RANK.Fc inhibits the p=0.06). 5T2+RANK.Fc=0±0%, p<0.001). RANK.Fc treatment had no effect on serum paraprotein concentration (5T2=0.55±0.06g/dl vs 5T2+RANK.Fc=0.51±0.05); however, tumour burden was decreased by 20% (5T2=62.6±3.7% vs 5T2+RANK.Fc=49.9±5.2, p=0.06).

Taken together these data demonstrate that RANK.Fc inhibits the development of osteolytic bone disease in the 5T2MM model of myeloma raising the possibility that targeting the RANKL / RANK interaction could be a valuable approach to treating myeloma bone disease.

153 The Nitrogen-Containing Bisphosphonate, Zoledronic Acid Increases Mineralization of Human Bone-Derived Cells in vitro
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Bisphosphonates (BPs) exhibit high affinity for the hydroxyapatite mineral in bone and are used extensively to treat the skeletal manifestations of myeloma including osteolytic bone disease and hypercalseamia of malignancy. Although the mechanisms by which BPs act are not fully understood, most studies attribute the increase in bone mass observed following BP therapy to their effects on bone-resorbing osteoclasts (OC). However, few studies have investigated whether BPs can act directly on bone forming osteoblasts (OB) to increase their anabolic activity. Using an established model of in vitro OB differentiation, we found that the potent nitrogen-containing BP, zoledronic acid, may enhance the bone forming potential of human adult bone-derived cells (OB-like cells) in vitro by inducing their differentiation. The effects of zoledronic acid on these cells was concentration dependent: at concentrations of 5 µM or greater, zoledronic acid induced both cell death in a proportion of the OB-like cells and cytostasis by blocking cell replication in S and G2/M phase. Cells expressing high levels of the osteoprogenitor antigen, STRO-1, exhibited a greater proliferative potential than STRO-1negative/dim cells, and were more susceptible to the cytostatic and apoptotic effects of zoledronic acid. Zoledronic acid was also found to promote bone cell differentiation, as evidenced by an increase in the number of cells exhibiting a more differentiated (STRO-1-/Alkaline Phophatase+ and STRO-1/-/Alkaline Phophatase-) phenotype. Analysis of gene expression, using semi-quantitative RT-PCR, demonstrated that zoledronic acid treatment resulted in a significant upregulation of osteocalcin (OCN) and bone morphogenetic protein-2 (BMP-2) gene expression. Consistent with an anabolic action of zoledronic acid, in vitro mineralisation studies revealed that it enhanced mineralised matrix formation at concentrations between 5 µM and 25 µM. These results show that, in addition to its direct effects on OC, zoledronic acid also directly affects the proliferation and differentiation of human OB-like cells in vitro. This is the first demonstration that zoledronic acid can enhance bone formation by mediating the differentiation of osteoprogenitor cells into mature OB-like cells.

154 RANKL Expression by Human Myeloma Cells Mediates Osteoclast Formation In Vitro and Correlates With Bone Destruction In Vivo
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Multiple myeloma (MM) is an incurable B cell malignancy able to mediate massive destruction of the axial skeleton. The precise mechanisms responsible for the observed bone pathology remain unclear; nevertheless, it is generally accepted that it is due to MM cell-mediated disruption of the normal equilibrium between bone formation by osteoblasts (OB) and bone resorption by the multinucleated osteoclasts (OC). The aim of this study was to examine the involvement of the TNF-ligand family member, RANKL and its naturally occurring antagonist, osteoprotegerin (OPG) in MM biology. While several studies have shown that MM plasma cells (MPC) secrete factors that cause upregulation of RANKL by stromal osteoblasts, the issue of direct expression of RANKL by MPC remains controversial. An isolated report identified RANKL immunohistochemically in MM cells in biopsy material, contradicting the findings of others. In this report, we demonstrate categorically by flow cytometry, RANKL expression in patient-derived CD38+++/CD45+ and CD38+++/CD45- MPC subpopulations, using two independent anti-RANKL antibodies. By reverse transcription-polymerase chain reaction (RT-PCR) analysis of these subpopulations, fluorescence-sorted to purity, we demonstrate that MPC express transcripts for both transmembrane and soluble RANKL isoforms. By RT-PCR and ELISA, we also demonstrate that MPC do not secrete OPG. In addition, we show that RANKL expressed by MPC is functional, as an in vitro co-culture of CD38+++/CD45+ and CD38+++/CD45- MPC subpopulations with adherent human peripheral blood mononuclear cells (PBMC), resulted in the formation of multinucleate, tartrate-resistant acid phosphatase (TRAP)-positive OC-like cells, capable of forming typical resorption pits. Furthermore, high expression of membrane-associated RANKL by CD38+++/ MPC correlated with the presence of multiple radiological bone lesions in individuals with MM (p < 0.025, Mann Whitney U Test). Together, our data strongly suggests that RANKL expression by MPC confers on them the ability to participate directly in the formation of OCs in vivo and extends our knowledge of the involvement of RANKL and OPG in the formation of the focal osteolytic lesions characteristic of this disease.
Evidence for a Role for Bone Marrow Endothelial Cells in the Development of Myeloma Bone Disease

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The growth of myeloma cells in the local bone marrow environment is associated with the development of osteolytic bone disease; however, the mechanisms involved in the pathogenesis of this bone disease are poorly understood. Recently, the ligand for receptor activator of NF-κB (RANKL) osteoprotegerin (OPG) system has been reported to play an important role in the development of normal osteoclasts and this system may be abnormally regulated in patients with myeloma. Different cell types are present in the bone marrow microenvironment and may express components of the RANKL/OPG system. Endothelial cells are one such cell type and these cells can be found in close association with bone, making them ideally placed to influence bone turnover. Furthermore, myeloma cells promote angiogenesis, suggesting that local proliferation of endothelial cells may play an important role in the development of multiple myeloma. Therefore, the aim of the present study was to determine whether murine bone marrow endothelial cells express RANKL and OPG and whether myeloma cells can regulate expression of these molecules.

RT-PCR and flow cytometric analysis demonstrated the presence of OPG mRNA in the murine bone marrow endothelial cells STR10 and STR12. ELISA confirmed that these endothelial cells release OPG. In contrast, OPG could not be detected in the supernatant of cultures of LESVO lung endothelial cells. The 5T33MMvvt cells, an in vitro growing, but clonally identical variant of 5T33MMvivo cells were shown to express OPG mRNA but OPG protein was not detected in the culture supernatant. RANKL was also shown to be expressed by STR10 and STR12 cells.

Media conditioned by STR10 and STR12 cells and containing OPG was able to inhibit the ability of tartrate resistant acid phosphatase positive osteoclasts to resorb a mineralised substrate in vitro. The addition of 5T33MMvvt cells to either STR10 or STR12 cells resulted in a cell number-dependent decrease in OPG production, as determined by ELISA. Quantitative RT-PCR confirmed that expression of the mRNA for OPG was decreased in STR10 and STR12 cells. When myeloma cells and endothelial cells were physically separated a decrease in OPG was still observed. To establish whether a soluble factor was responsible for the decrease in OPG, medium conditioned by myeloma cells was added to the endothelial cells. Surprisingly, no decrease in OPG production was seen, suggesting that close contact but not necessarily physical contact between myeloma cells and endothelial cells is required to down-regulate OPG production. In conclusion, these data demonstrate that bone marrow endothelial cells express RANKL and produce OPG and that production of OPG by these cells may be able to decrease bone resorption in vitro. Myeloma cells decrease OPG production in endothelial cells raising the possibility that this could contribute to the development of myeloma bone disease.

Osteoprotegerin and sRANKL serum levels in multiple myeloma patients

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Receptor activator of NF-κB (RANK) is a TNF receptor superfamily member expressed on the surface of osteoclasts and their precursors that mediates their differentiation, survival, and activation upon interaction with its ligand, RANKL, expressed by osteoblasts, and stromal cells. RANKL is produced as a membrane bound protein and cleaved into a soluble form by a metalloprotease. The primary secreted form is produced by activated T-lymphocytes. Osteoprotegerin (OPG) is a secreted TNFR, it acts as a decoy receptor for RANKL and inhibitor of RANK-RANKL interaction. Recent studies suggest that MM triggers osteoclastogenesis by disrupting the balance between RANKL and its natural inhibitor, OPG. Therefore in this study we analyzed the concentrations of serum OPG and sRANKL in MM patients at diagnosis. Determinations of serum OPG and sRANKL concentrations were performed in 25 healthy subjects and 94 MM patients (15 at stage I, 12-II, 55-IIIA, 12-IIIB acc. to D.S; 67 had lytic lesions at skeletal X-ray survey and 10 had hypercalcemia; monoclonal protein IgG was in 61 patients, IgA-22, IgD-1, Bence Jones-8, non-secretory-2) by means of ELISA method using Osteoprotegerin ELISA and sRANKL ELISA kits (Biomedica GmbH, Wien, Austria). In the whole group of MM patients, OPG concentrations in particular patients ranged from 40 to 371 pg/ml with a mean concentration of 111±62, median 94 pg/ml while in healthy persons OPG levels ranged from 49 to 130 pg/ml, mean 77±22, median 74 pg/ml (p=0,002). In MM patients with renal failure mean level of OPG was 172±87, median 145 pg/ml while in MM patients with normal renal function they were 101±51 and 85 pg/ml respectively (p=0.0002). In MM patients with hypercalcemia mean level of OPG was 169±98, median 143 pg/ml while in MM patients with normal serum calcium level they were 104±54 and 88 pg/ml respectively (p=0,01). The differences in OPG concentrations depending on occurrence of osteolyis were not statistically significant: in patients with osteolysis 116±64 median 99 pg/ml and in those without it 99±57, median 84 pg/ml (p=0,1). In MM patients serum concentrations of OPG increased significantly with age (r=0,42; p=0,00002). There was a positive correlation with OPG and β2M serum concentrations (r=0,32; p=0,002). In MM patients sRANKL concentrations in particular patients ranged from 0 to 63 pg/ml with a mean concentration of 2,77±7,7 pg/ml, while in healthy persons sRANKL levels ranged from 0 to 43 pg/ml, mean 5,0±10,1 pg/ml.

Conclusions: In MM patients at diagnosis serum OPG levels are not reduced; in contrast in 20% of patients are elevated and it may be a compensative reaction in relation to increased bone destruction. Significantly increased OPG concentrations in MM patients with renal failure may be related to its decreased elimination. In the majority of MM patients serum sRANKL is undetectable.
The Nitrogen-Containing Bisphosphonate, Zoledronic Acid Regulates RANKL Expression in Human Osteoblast-Like Cells, by Activating TNF-alpha Converting Enzyme (TACE)

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Bisphosphonates (BPs) exhibit high affinity for hydroxyapatite mineral in bone and are used extensively to treat the osteolytic bone disease and hypercalcaemia seen in most patients with myeloma. The BP-mediated inhibition of bone resorption has been attributed mainly to their effects on the proliferation and apoptosis of bone-resorbing osteoclasts (OCs). In the present study, we examined the effect of the nitrogen containing BP, zoledronic acid (ZOL), on the expression of RANKL and OPG, critical factors in the regulation of OC formation and activation, in primary human osteoblast (OB)-like cells. Our studies show that ZOL, whilst not significantly affecting RANKL or OPG gene expression, markedly increased OPG protein secretion and reduced membrane RANKL protein expression. The reduction in membrane RANKL expression was preceded by a marked increase in the expression of the metalloprotease-disintegrin TNF-alpha converting enzyme (TACE) in OB-like cells. In addition, the decreased membrane expression of RANKL could be partially reversed by a TACE inhibitor, TAPI-2. ZOL not only caused an increase in TACE expression, but also appeared to mediate a redistribution of the TACE protein from a nuclear to perinuclear compartment in OB-like cells, raising the possibility that TACE may function intracellularly to cleave RANKL protein prior to its expression at the cell surface. Therefore, our studies indicate that ZOL, in addition to its direct effects on mature OCs, may inhibit the recruitment of pre-OCs by decreasing the level of membrane-associated RANKL expression in OB-like cells.

Zoledronic Acid inhibits the proliferation and induces apoptosis of bone marrow stromal cells in multiple myeloma
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Bisphosphonates are commonly used to prevent bone resorption in multiple myeloma. In vitro studies have demonstrated their capacity of inhibiting the proliferation of a variety of human tumor cells lines as well as decreasing their viability and inducing apoptosis. On the contrary, few evidences are reported on their effects on stromal cells, in particular in myeloma patients. To explore the anti-tumor activity of bisphosphonates against clonal plasma cells and to test their impact on bone marrow microenvironment, we investigated the cytostatic and apoptotic effects of Zoledronate on the human myeloma cell line RPMI 8226 and bone marrow stromal cells (BMSCs) isolated from seven patients with active multiple myeloma. Bone marrow collected from MM patients was diluted with an equal volume of Dulbecco's Phosphate Buffered Saline (PBS), separated on Ficoll-Hypaque (density = 1.077). Mononuclear cells were then incubated in culture flask 175 in medium "MyelocultTM" with added 100 U/ml Penicillin, 100 µg/ml Streptomycin and Hydrocortison at 37°C in 5% CO2. The cultures were weekly fed by replacing 50% of the supernatant with fresh culture medium, until a confluent adherent cell monolayer was obtained (2-3 weeks). The anti-proliferative effect of Zoledronate was evaluated at escalating concentrations of the drug (10µM-500µM) for 72h, using the MTT assay on BMSCs and on 8226 cell line. To determine whether the reduction in cell proliferation observed with zoledronic acid treatment was sustained by apoptotic death, 8226 cells and BMSCs were analyzed by flow cytometric detection of fluorescein labelled Annexin V.

Our results show that zoledronic acid induces a dose-dependent inhibition of proliferation of BMSCs and of 8226 cells; furthermore, the addition of 5ng/ml rhIL-6 to myeloma cells does not revert the antiproliferative effect of Zoledronate. Apoptosis induced by Zoledronate in human myeloma cell line was daily evaluated at concentration of 10-4 M or 5x10-4 M for five days. The percentage of apoptotic cells was time- and dose-dependent; in particular a stronger effect was observed at the concentration of 500µM. Given the ability of Zoledronate to significantly reduce cell proliferation of BMSCs, we also performed flow cytometric analysis of apoptotic cells. After 3 days of treatment with bisphosphonate, approximately 7% of the adherent cells were found to be positive for Annexin V binding at 10-5 M of Zoledronate, compared to 3-4% in untreated controls; the percentage of apoptotic cells increased significantly at a concentration of 10-4 M.

In conclusion, these results demonstrate an antiproliferative and pro-apoptotic activity in vitro of zoledronic acid on bone marrow stromal cells and on myeloma cell lines, and support a possible anti-tumor effect in vivo of this drug.

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OXP Can Protect Human Myeloma Cells Against TRAIL-Induced Apoptosis; A Role for OPG as a Paracrine Survival Factor in Multiple Myeloma?
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Multiple myeloma is a haematological malignancy in which the tumour cells grow preferentially within the bone marrow microenvironment. Within this local environment myeloma cells can interact with a range of different cell types including osteoblasts, bone marrow stromal cells (BMSCs) and osteoclasts. These interactions are critical both for tumour growth and the development of the associated bone disease. Osteoprotegerin (OPG) is a member of the tumour necrosis factor (TNF) receptor superfamily. OPG binds to the ligand for receptor activator of nuclear factor B (RANKL), and prevents the interaction between RANKL and RANK, thus inhibiting osteoclast formation and bone resorption. The RANKL/OPG system appears to play an important role in the development of myeloma bone disease. However, it is unclear whether OPG may be able to bind to other TNF family members, such as TNF-related apoptosis-inducing ligand (TRAIL), and, by inhibiting their activity, function as a survival factor for myeloma cells. The aim of the present study was to determine whether OPG released from osteoblasts could protect human myeloma cells against TRAIL-induced apoptosis. Apoptotic cells were identified by characteristic changes in nuclear morphology and by a fluorescence in situ nick translation assay. Apoptosis was measured in mononuclear cells isolated from the bone marrow of patients with multiple myeloma or cocultures of myeloma cells and osteoblasts by two colour flow cytometry. Myeloma cells were identified by Ig light chain expression and apoptosis by a fluorescence in situ nick translation assay. Recombinant OPG significantly protected RPMI 8226 and NCI H929 myeloma cells against TRAIL-induced apoptosis in a dose-dependent manner (p<0.001). Recombinant OPG was also shown to inhibit TRAIL-induced apoptosis of primary human myeloma cells isolated from the bone marrow of a patient with multiple myeloma (p<0.05). Recombinant
OGP had no effect on constitutive levels of apoptosis in human myeloma cell lines or primary myeloma cells. Media conditioned by MG63 osteoblast-like cells was also found to significantly protect human myeloma cells from TRAIL-induced apoptosis in a concentration-dependent manner (p<0.05). This effect was inhibited by soluble RANKL or a neutralizing antibody to OPG (p<0.05), confirming that the protective soluble factor released from MG63 cells was OPG. TRAIL-induced apoptosis of OCI H929 myeloma cells was prevented when myeloma cells were cultured in the presence of MG63 osteoblast-like cells (p<0.05). These results demonstrate that OPG can function as a soluble decoy receptor for TRAIL and can inhibit TRAIL-induced apoptosis of myeloma cells in vitro. In addition, cells of the bone marrow microenvironment can provide a source of OPG which can protect myeloma cells against TRAIL-induced apoptosis. These observations raise the possibility that, in addition to regulating bone resorption, osteoblast-derived OPG may function as a paracrine survival factor for myeloma cells.

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Osteoclasts enhance myeloma growth and survival: a vicious cycle between bone destruction and myeloma expansion

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Multiple myeloma (MM) generates devastating bone destruction by osteoclasts (OCs) induced by MM cells in their close vicinity. We and others have identified and reported that CC chemokines macrophage inflammatory protein (MIP)-1 alpha and MIP-1 beta are among major OC activating factors secreted by MM cells (Abe M, et al. Blood 100: 2195-2202, 2002). Since MM cells almost exclusively expand in the bone marrow and preferentially grow in bone destructive lesions, close interactions of MM cells with bone cells may be critical to the tumor expansion as well as the development of the bone disease. Accordingly, we have reported that OCs generated from human peripheral blood mononuclear cells (PBMC) by addition of sRANK ligand and M-CSF enhanced survival of primary MM cells as well as growth of MM cell lines (ASBMR 2000, 2001, 2002, 3rd CBHD 2001). In the present study, we further investigated a role as well as a mechanism of OC-mediated MM cell growth and survival. Interestingly, even though bone marrow stromal cells also supported MM cell expansion, the stimulatory effect of PBMC-derived OCs on MM growth was much stronger than that of stromal cells (4.8 vs. 1.8 folds from the baseline in U266 cells, respectively). Furthermore, the OCs almost completely abrogated apoptosis of RPMI8226 cells induced by serum reduction (1%), and efficiently enhanced growth of the MM cells survived after exposure to doxorubicin, suggesting protective effects of OCs on MM cells. Importantly, such OC effects were mostly abrogated by inhibition of a cellular contact between MM cells and OCs by membrane filters. To further elucidate the mechanism of OC-mediated MM growth enhancement, we first examined a role of IL-6, and found that direct contact with MM cells induced production of IL-6 by OCs. However, the OC-mediated MM growth enhancement was only partially inhibited by an anti-IL-6 neutralizing antibody, suggesting a limited contribution of IL-6. We then screened OC-derived factors and focused on osteopontin (OPN), a major extracellular noncollagenous matrix protein, because OCs produce OPN and MM cells constitutively expressed OPN receptors on their surface including alphabeta3 integrin, VLA-4, and CD44 which have been shown to be involved in growth and survival signals. We found that PBMC-derived OCs produced OPN at a large amount (140-fold higher than stromal cells at a per cell basis) and their production of OPN was further up-regulated by MM cell contact. Consistently, exogenous OPN significantly enhanced MM cell growth in concert with IL-6. However, anti-OPN antibody only partially suppressed the OC effect even in combination with anti-IL-6. Moreover, isolated rabbit OCs and murine osteoclastic cell lines also significantly enhanced growth of human MM cells which do not respond to non-human IL-6, indicating additional involvement of IL-6-independent mechanism. The present study suggests that OCs induced by MM cells in turn contribute to the establishment of a microenvironment suitable for MM cell expansion at least partially through the induction of IL-6 and OPN secretion by OCs, thereby forming a vicious cycle that leads to extensive bone destruction and MM expansion.

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A NEUROTROPHIN AXIS IN MYELOMA BONE PAIN

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Skeletal pain is a frequent source of morbidity for patients with MM, yet the mechanisms responsible remain poorly understood. We find that malignant plasma cells express BDNF (brain-derived neurotrophic factor), a neurotrophin known to cause hyperalgesia by potentiating the activity of nociceptive neurons. We propose that BDNF amplifies MM skeletal pain by sensitizing nociceptive neurons. We also find that BDNF inhibits stromal expression of OPG and propose a role for BDNF in the etiology of MM-associated bone destruction. In addition, we find that malignant plasma cells express the receptor for BDNF, trkB, and present data to suggest that BDNF promotes MM survival, both indirectly through effects on marrow stroma and angiogenesis, and directly through autocrine stimulation of MM itself.

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Killing of osteoblasts by myeloma cells: a TRAIL to bone lesions?

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In multiple myeloma (MM), neoplastic plasma cells accumulate in the bone marrow where their survival, proliferation and apoptosis are controlled at multiple levels by interaction with the bone marrow microenvironment. Myeloma cells actively control these interactions in that they activate stromal and endothelial cells for production of survival factors such as interleukin-6 (IL-6), and suppress other cell types such as erythroblasts, normal B-cell progenitors and T-cells. In the present study, we identified primary osteoblasts as additional potential targets for myeloma cell-mediated suppression which was partly dependent on the death receptor ligand TRAIL and involved a small, heat-stable factor. Beside killing of osteoblasts, supernatants from myeloma cell line cultures sensitized osteoblasts to cell death mediated by recombinant TRAIL, whereas supernatants from primary osteoblast cultures protected myeloma cells from TRAIL-mediated apoptosis. The suppression of bone-forming cells by myeloma cells might contribute to the frequent development of bone lesions in MM patients. In addition, the production of a factor by myeloma cells which sensitizes normal bone cells to TRAIL-induced apoptosis might represent an important drawback for safe application of TRAIL in multiple myeloma.
7. New prognostic criteria for classification and monitoring MM

7.1 Prognostic markers

163 Prevalence and prognostic significance of sMUC-1 levels in plasma cell dyscrasias

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High serum sMUC-1 levels have been detected in adenocarcinoma patients and seem to correlate with tumour burden. It has been shown that patients with multiple myeloma (MM) have high peripheral blood and bone marrow levels, and that the latter directly correlate with tumour mass. To define the prevalence of high sMUC-1 levels in patients with plasma cell dyscrasias, we tested 89 monoclonal gammapathy of undetermined significance (MGUS), 76 MM and six plasma cell leukemia (PCL), admitted consecutively to our Institution during the last ten years. Peripheral blood samples were collected at the time of diagnosis or at any time during follow-up from MM and PCL patients; for MM and PCL patients were analyzed stored serum aliquots, collected at the time of diagnosis. Samples obtained from 65, age and sex matched, healthy subjects, attending our Transfusion Department, were also evaluated. All of the samples were tested using Immunolite B27,29 antibody against MUC-1 protein. The sera from the MM/PCL patients were also tested using Abbot IMX CA15.3, Boehringer Mannheim Enzymmun CA15-3 and Centrocor CA15-3 in accordance with the manufacturers’ protocols. High sMUC-1 levels were found in 11/89 subjects with MGUS (12.4%), 13/76 with MM (17.1%) and 3/6 with PCL, while in the healthy control group only one subject had high levels (1.5%) (p=0.001). The mean sMUC-1 levels were significantly higher in the Monoclonal Component carrying patients than in the healthy subjects (43.2 vs 26 U/ml; p=0.001). The median follow-up of the 82 MM/PCL cases was 30 months (range 6 -114), during which 51 patients died: 39/66 (59%) with normal and 12/16 (75%) with high sMUC-1 levels. The median overall survival (OS) was 44 months. There was a difference in OS between the MM/PCL patients with normal or increased sMUC-1 levels: median OS 49 vs 25 months, 3-year OS 63% and 25% (p=0.036). Furthermore, increased sMUC-1 levels in MM patients correlated with some features associated with high tumour burden, such as anemia and high serum LDH levels. Finally we tested the hypothesis that the various antibodies used in sMUC-1 assays recognise distinct antigen epitopes and their different degrees of reactivity may depend on the extent of the glycosylation of the CA15.3 antigen, as reported by many authors. In our MM patients evaluated using different methods, we found differences in absolute values but multiple comparisons (Bonferroni’s test) showed a high degree of correlation.

In conclusion, this study shows that a subset of MM and MGUS patients, and most PCL cases, had increased sMUC-1 levels. The frequent presence of high MUC-1 levels in PCL patients suggest a possible relationship between them and tumor malignancy (cell proliferation, genetic instability). In patients with MM, high sMUC-1 levels identify a group with a poor prognosis, showing a possible role of MUC-1 in tumor progression. Given the short follow-up of the MGUS patients (median 12 months; range 6 -30) and the absence of any MM transformation during this time, no conclusions can be drawn concerning a possible correlation between high sMUC levels and the relative risk of MM evolution.

164 Serum MUC1 as a Marker of disease status in Multiple Myeloma (MM) Patients Receiving Thalidomide ± Interferon-α-2b

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Objective: MUC-1 is a glycosylated transmembrane protein normally found on the luminal surface of secretory glands. Serum MUC-1 (measured by serum CA15-3 assay) has also been shown to correlate with tumour burden. It has been shown that patients with multiple myeloma (MM) have high peripheral blood and bone marrow levels, and that the latter directly correlate with tumour mass. To define the prevalence of high sMUC-1 levels in patients with plasma cell dyscrasias, we tested 89 monoclonal gammapathy of undetermined significance (MGUS), 76 MM and six plasma cell leukemia (PCL), admitted consecutively to our Institution during the last ten years.

Peripheral blood samples were collected at the time of diagnosis or at any time during follow-up from MMUS patients; for MM and PCL patients were analyzed stored serum aliquots, collected at the time of diagnosis. Samples obtained from 65, age and sex matched, healthy subjects, attending our Transfusion Department, were also evaluated. All of the samples were tested using Immunolite B27,29 antibody against MUC-1 protein. The sera from the MM/PCL patients were also tested using Abbot IMX CA15.3, Boehringer Mannheim Enzymmun CA15-3 and Centrocor CA15-3 in accordance with the manufacturers’ protocols. High sMUC-1 levels were found in 11/89 subjects with MGUS (12.4%), 13/76 with MM (17.1%) and 3/6 with PCL, while in the healthy control group only one subject had high levels (1.5%) (p=0.001). The mean sMUC-1 levels were significantly higher in the Monoclonal Component carrying patients than in the healthy subjects (43.2 vs 26 U/ml; p=0.001). The median follow-up of the 82 MM/PCL cases was 30 months (range 6 -114), during which 51 patients died: 39/66 (59%) with normal and 12/16 (75%) with high sMUC-1 levels. The median overall survival (OS) was 44 months. There was a difference in OS between the MM/PCL patients with normal or increased sMUC-1 levels: median OS 49 vs 25 months, 3-year OS 63% and 25% (p=0.036).

Furthermore, increased sMUC-1 levels in MM patients correlated with some features associated with high tumour burden, such as anemia and high serum LDH levels. Finally we tested the hypothesis that the various antibodies used in sMUC-1 assays recognise distinct antigen epitopes and their different degrees of reactivity may depend on the extent of the glycosylation of the CA15.3 antigen, as reported by many authors. In our MM patients evaluated using different methods, we found differences in absolute values but multiple comparisons (Bonferroni’s test) showed a high degree of correlation.

In conclusion, this study shows that a subset of MM and MGUS patients, and most PCL cases, had increased sMUC-1 levels. The frequent presence of high MUC-1 levels in PCL patients suggest a possible relationship between them and tumor malignancy (cell proliferation, genetic instability). In patients with MM, high sMUC-1 levels identify a group with a poor prognosis, showing a possible role of MUC-1 in tumor progression. Given the short follow-up of the MGUS patients (median 12 months; range 6 -30) and the absence of any MM transformation during this time, no conclusions can be drawn concerning a possible correlation between high sMUC levels and the relative risk of MM evolution.
thalidomide: RR 35% cf. 14% (P =0.084). Among the 20 responders, the median baseline CA 153 was 28 U/ml (range 5-80), and fell to a significantly lower median of 19U/ml (range 6-42) at first response (p=0.03) Among all 29 pts who developed PD on thalidomide, including 9 who initially responded, there was no significant difference between the median baseline level and the median at PD.

Of the 9 pts who developed PD after initial response/SD to thalidomide, there was a significant rise in CA15-3 at PD: median at response = 13(6-34) cf median at PD = 25(8-52); P=0.037. 18/62 pts (29%) had clinically significant changes in CA153, meaning the serum level changed by >25% and fell or rose in conjunction with other recognised markers of disease.

Conclusions: Our results suggest that elevated serum CA 15-3 levels are a useful and novel marker in high-risk MM pts that may be predictive of response to thalidomide and may also be of value in monitoring disease.

165 Serum C-terminal telopeptide of collagen type I (ICTP) and urinary N-terminal telopeptide of collagen type I (Ntx) are useful parameters for monitoring myeloma bone disease

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Background: Within recent years, several serum and urine based biochemical assays for markers of bone resorption and bone formation have been introduced. In particular, assays of bone resorption might be useful in multiple myeloma (MM), but few studies have investigated their clinical value. Earlier, we have shown that serum C-terminal telopeptide of collagen type I (ICTP) and urinary N-terminal telopeptide of collagen type I (Ntx) correlate with the histomorphometrically assessed bone resorption activity in MM and that elevated pre-treatment levels of S-ICTP and U-Ntx are predictive for early progression of the bone disease in myeloma patients.

Aim: This study was performed to evaluate the clinical usefulness of analyses of biochemical markers of bone metabolism in monitoring the bone disease in MM.

Methods: Assays for measuring ICTP in serum and Ntx in urine were employed for assessment of bone resorption, and assays for measuring serum levels of the C-terminal and N-terminal propeptides of procollagen type I (PICP and PINP, respectively), bone-specific alkaline phosphatase (bAP), and osteocalcin were measured as indicators of bone formation. Thirty patients with newly diagnosed MM were included before start of treatment and were followed for a median of 24 months. All patients received standard cyclic melphalan-prednisone treatment. Serum and urine samples were collected each 6 weeks before initiating chemotherapy. X-rays of the skeleton were performed each 6 months and when indicated by symptoms. The X-rays were scored blindly.

Results: Elevated levels of serum ICTP and urinary Ntx over time were highly predictive for progression of the bone disease within the observation period. The markers of bone formation were less informative. In Cox proportional hazards model, serum ICTP showed the highest predictive significance, but could be replaced by Ntx, which should be preferable in patients with impaired renal function. For prediction of bone events, S-ICTP and U-Ntx were superior to the M component measurements, which in fact did not correlate with progression of the bone disease.

Discussion and Conclusion: The introduction of bisphosphonates, osteoprotegerin, and proteasome inhibitors in the treatment of MM bone disease highlights the need of non-invasive methods for monitoring end-organ damage in bone. A clinical valid non-invasive marker of bone resorption would allow individualised treatment. Our study indicates that S-ICTP and U-Ntx are sensitive and predictive markers of the bone disease in MM.

166 Critical role of receptor activator of nuclear factor kB ligand (RANKL)/osteoprotegerin (OPG) pathway on bone disease and survival in patients with multiple myeloma.

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Bone disease is a major cause of morbidity in multiple myeloma (MM). Through the interactions between myeloma and stromal cells, osteoclasts are activated resulting in an increased resorptive activity, which is illustrated by the elevated levels of N-telopeptide of type-I collagen (NTX), and of tartrate-resistant acid phosphatase isofrom-5b (TRACP-5b), an enzyme which is produced only by activated osteoclasts. The receptor activator of nuclear factor-kB ligand (RANKL) and osteoprotegerin (OPG) pathway has been found to be the dominant mediator of osteoclastogenesis and possibly promotes growth of myeloma cells. The aim of this study was to evaluate the role of soluble RANKL (sRANKL), OPG and markers of bone remodelling [NTX, TRACP-5b, bone-alkaline phosphatase (bALP), and osteocalcin (OC)] in bone disease and survival in MM.

A total of 121 newly diagnosed patients (61M/60F; median age: 68 years) with MM were studied. Ten patients had no lytic lesions or osteoporosis only; 28 patients had 1-3 osteolytic lesions, while 83 patients had >3 osteolytic lesions and/or a pathological fracture. The above markers were also measured in 46, age and sex matched, healthy controls. Patients with MM had elevated mean sRANKL, TRACP-5b and NTX values and decreased levels of OPG, OC and bALP compared with controls. The ratio of sRANKL/OPG was also significantly higher in MM patients. There was a strong correlation between the ratio sRANKL/OPG and the extent of bone disease (p<0.001), stage of MM (p<0.001), levels of TRACP-5b (p<0.001), NTX (p<0.001), IL-6 (p<0.001), and beta2-microglobulin (p<0.001). The median overall survival for the 121 patients was 56.9 months. Eighty-two patients had received only conventional chemotherapy, while 39 patients had undergone autologous stem cell transplantation. The multivariate analysis revealed that only the ratio of sRANKL/OPG, and the levels of beta2-microglobulin and CRP were independent prognostic factors for survival. Based on these 3 factors, we created a risk score (Hammersmith Prognostic Index): sRANKL/OPG ratio of <1 and beta2-microglobulin levels of ≤3 mg/l was given a score of 1 point; sRANKL/OPG ratio of between 1-3 and CRP levels of ≤10 mg/l had 2 points; 3 points were given for CRP levels of >10 mg/l and beta2-microglobulin of >3 mg/l, and 4 points for sRANKL/OPG
ratio of $>3$. This system has subdivided our patients into three groups. The low-risk group included 26 patients (score $<6$), the intermediate group 55 patients (score 6-8), and the high-risk group 40 patients (score $>8$). The 5-year probability of survival for each group is illustrated in the Figure. We compared this scoring system with that proposed by Bataille et al using beta2-microglobulin and CRP as prognostic values. Our risk score appears to be more discriminating in identifying a very good risk group, a superior intermediate risk group. Not only do these results confirm for the first time in humans the importance of the RANKL/OPG system in the development of bone disease but they also highlight the role of this pathway in the biology of plasma cell growth as reflected by its influence on survival.

167 Low serum level of soluble tumor necrosis factor receptor p55 predicts for response to thalidomide in advanced myeloma.

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65 patients with primary or secondary resistance to melphalan/ prednisone or other types of chemotherapy were treated with thalidomide at doses escalating from 200 mg to 800 mg (Nordic Myeloma Study Group trial #10). The mean age of the patients was 63.4 +/- 11 years. Serum taken before start of treatment was available from 34 patients and analyzed for soluble TNF receptors (sTNFR) p55 and p75. In addition, serum taken after 3, 12-16, and 20-24 weeks of treatment was available from 16 patients for analysis. Patients were grouped into immunoglobulin heavy chains (Harlan Sera-Lab Ltd, England). Main pts characteristics: median age = 63 y; stage I, II, III, DS, 36, 19 and 45%, respectively; 64, 27 and 6% with IgG, IgA and light chain M-component, respectively; 51% with at least one bone lesion; 31% chromosome 13 deletion (n=70); median level of beta2m and creatinine were 2.8 mg/L and 10 mg/L, respectively. Thirty-seven pts received an intensive treatment (44%). Median +/- se of the follow-up, overall survival and progression-free survival times were 51.7 +/- 3.1, 48.7 +/- 7.3 and 30.0 +/- 4.0 months (mo.), respectively. HLA-Is and HLA-Gs median values (range) were 839ng/ml (206-7913) and 28ng/ml (4.8-124.5), respectively. Both marker levels were not correlated to the creatinine level (P >.400). The factors predictive of a poor survival in Cox model univariate analysis were: high level of beta2m (2.5 and 4.0 mg/L, P=0.001), stage II or III DS (P=0.001); Hb <12g/dL (P=0.002); bone lesions (P=0.005); albumin <= 35 g/L (P=0.007); HLA-Is >= 2100 ng/ml; bone marrow plasmacytosis >13% (P=0.010); chromosome 13 deletion (P=0.069); age > 65y (P=0.131). When coupling HLA-Is and beta2m levels, a very efficient prognostic score was derived (P <0.001). Beta2m score was 0, 1 and 2 for beta2m <2.5 to 4.0 and >= 4.0 mg/L, respectively. HLA-Is score was 0 and 1 for HLA-Is <2100 and >= 2100, respectively. When adding these scores, median +/- se of survival time was (mo.) 14.1 +/- 4.3 with an overall score of 3, 27.8 +/- 4.1 with 2, and 47.8 +/- 2.7 with 1. The median survival time was not reached at 95 mo. with score 0. For pts with a given level of beta2m, relative risk of death was 2.2 (95% CI 1.6-3.0) for pts with HLA-Is < 2100 as compared to those <2100. When taking into account conventional and intensive treatment as a time-dependent covariate in the Cox model, this prognostic score was still very effective.

This study demonstrates that HLA-Is gives additional prognostic information to beta2m alone leading to a powerful prognostic score. The relative efficiency of this score among pts according to conventional or intensive therapy remains to be evaluated in a larger cohort of MM pts.

168 Total soluble HLA class I (HLA-Is): a new prognostic factor in Multiple Myeloma


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Assessment of survival prognostic factors in multiple myeloma (MM) patients (pts) remains an important issue for the selection of the best treatment. Serum beta-2 microglobulin (beta2m) which is the light chain of the HLA class I molecular complex (HLA-I) is still one of the most powerful survival prognostic factor. The aim of this study was to assess the levels and prognostic roles of the total soluble class I (HLA-Is) and soluble HLA-Gs in MM, especially relatively to beta2m. Serum was collected from 101 pts before any treatment. HLA-Is, and HLA-Gs concentrations were measured using a specific sandwich ELISA using MEM-G/9, a monoclonal antibody (mAb) detecting HLA-Gs (Exbio, Czech rep) or W6/32, a mAb recognizing a determinant of beta2m associated HLA class I heavy chains (Harlan Sera-Lab Ltd, England). Main pts characteristics: median age = 63 y; stage I, II, III, DS, 36, 19 and 45%, respectively; 64, 27 and 6% with IgG, IgA and light chain M-component, respectively; 51% with at least one bone lesion; 31% chromosome 13 deletion (n=70); median level of beta2m and creatinine were 2.8 mg/L and 10 mg/L, respectively. Thirty-seven pts received an intensive treatment (44%). Median +/- se of the follow-up, overall survival and progression-free survival times were 51.7 +/- 3.1, 48.7 +/- 7.3 and 30.0 +/- 4.0 months (mo.), respectively. HLA-Is and HLA-Gs median values (range) were 839ng/ml (206-7913) and 28ng/ml (4.8-124.5), respectively. Both marker levels were not correlated to the creatinine level (P >.400). The factors predictive of a poor survival in Cox model univariate analysis were: high level of beta2m (2.5 and 4.0 mg/L, P=0.001), stage II or III DS (P=0.001); Hb <12g/dL (P=0.002); bone lesions (P=0.005); albumin <= 35 g/L (P=0.007); HLA-Is >= 2100 ng/ml; bone marrow plasmacytosis >13% (P=0.010); chromosome 13 deletion (P=0.069); age > 65y (P=0.131). When coupling HLA-Is and beta2m levels, a very efficient prognostic score was derived (P <0.001). Beta2m score was 0, 1 and 2 for beta2m <2.5 to 4.0 and >= 4.0 mg/L, respectively. HLA-Is score was 0 and 1 for HLA-Is <2100 and >= 2100, respectively. When adding these scores, median +/- se of survival time was (mo.) 14.1 +/- 4.3 with an overall score of 3, 27.8 +/- 4.1 with 2, and 47.8 +/- 2.7 with 1. The median survival time was not reached at 95 mo. with score 0. For pts with a given level of beta2m, relative risk of death was 2.2 (95% CI 1.6-3.0) for pts with HLA-Is < 2100 as compared to those <2100. When taking into account conventional and intensive treatment as a time-dependent covariate in the Cox model, this prognostic score was still very effective.
SOLUBLE CD138: A NEW IMPORTANT MARKER IN DIAGNOSIS OF MULTIPLE MYELOMA

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Objectives: Multiple myeloma (MM) is a B-cell malignancy characterised by the progressive accumulation of clonal malignant plasma cells. Previous studies have shown that syndecan-1 (sCD138) is shed from the surface of myeloma cells into serum and that this marker is a new independent prognostic parameter in MM.

Methods: In our study we evaluated value of sCD138 in serum samples drawn at diagnosis from 14 MGUS patients and 37 MM patients, 17 patients were treated by high-dose chemotherapy regimen. For determination of sCD138 level we used a rapid and simple ELISA procedure (Diaclone, France).

Results: Mean serum levels of sCD138 in MGUS patients were 32.4 (median 35.1) ng/ml and 1004.4 (median 202.1) ng/ml in MM patients (p=0.001). Multiple myeloma patients with high level of sCD138 at diagnosis (>500 ng/ml) had worse prognosis (p = 0.049) despite of good response to chemotherapy in some of them.

Conclusions: We present our first experience with the use of the new important prognostic marker in diagnosis of MM. We verified its very strong prognostic significance. In our opinion the determination of sCD138 can also be recommended as helpful marker for differential diagnosis of monoclonal gammapathies.

170 Serum free light chain concentrations and their use for disease monitoring in multiple myeloma patients with intact immunoglobulin monoclonal proteins

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Introduction: Automated serum assays specific for free immunoglobulin light chains (fLC) have shown elevated serum fLC concentrations in all of 224 cases of light chain multiple myeloma and 68% of 28 cases of nonsecretory multiple myeloma. In this retrospective study, we determined the proportion of myeloma patients producing intact immunoglobulin monoclonal proteins who also had elevated levels of serum fLC. In addition, fLC levels in some of these patients, were measured during the course of cytotoxic chemotherapy and their changes compared with those of other disease markers.

Materials & Methods: fLC levels were measured in presentation sera from 492 subjects entered into the UK MRC myeloma trials (314 IgG, 142 IgA & 36 IgD) and compared with levels in 282 normal individuals. Assays were also performed on 37 sera from patients being treated for Waldenström’s macroglobulinemia (WM) and 5 IgE myeloma sera. fLC levels in serial serum samples from 17 myeloma trial patients (12 IgG & 5 IgA) were measured and where possible, compared with measures of total immunoglobulins, monoclonal immunoglobulins (by electrophoresis and densitometry), 2 microglobulin and bone marrow plasma cell counts. The intervals between samples varied from 3 weeks to 1 year. All fLC assays were performed on the Behring NephelometerTMII.

Results: In the presentation sera, elevated fLC levels were observed in 84% (265/314) of the IgG myelomas, 92% (130/142) of the IgA, 94% (34/36) IgD, 100% (5/5) IgE and 89% of the WM sera.

In the serial samples, all patients showed some fall in their monoclonal immunoglobulins, total immunoglobulins and fLC after initial chemotherapy. The range of change in the fLC concentrations was typically several-fold greater than for the intact immunoglobulin. For the majority of IgG myelomas, the timing of the samples revealed that fLC concentrations stabilised within the normal range in advance of the intact immunoglobulin but this was apparent in only 1 of 5 IgA patients. There was an initial fall in 2 microglobulin levels in only 5/17 patients but the speed of normalisation matched that of the fLC. In 5 patients, bone marrow plasma cell counts had been made while the fLC concentration was within the normal range but monoclonal IgG was still present by electrophoresis; in all these cases the plasma cell counts were <5%.

In 4 of 7 patients, concentrations of intact monoclonal immunoglobulin and fLC increased together at relapse while intact IgA increased earlier in 2 cases and fLC increased before monoclonal IgG in one case.

Conclusions: It is clear that nearly all multiple myeloma patients have elevated levels of serum fLC. Monitoring serum fLC levels provides a swifter indication of response to therapy than measuring monoclonal or total intact immunoglobulins. This is a consequence of the shorter half-life and the large clinical range of fLC concentrations. This is more apparent when compared with IgG monoclonal proteins, which have the longest serum half-lives (approximately 20 days). Potentially the most valuable use for fLC measurements could be in rapidly assessing the efficacy of salvage treatment regimens.

171 Frequent measurement of total immunoglobulin and serum free light chains in myeloma patients during peripheral blood stem cell transplantation

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Introduction: Assays specific for free immunoglobulin light chains are useful in monitoring light chain multiple myeloma and nonsecretory multiple myeloma. It has also been postulated that the short serum half-life of free light chains could be more useful than intact immunoglobulin, for measuring early responses to therapy. In this study, daily measurements of total immunoglobulin and free light chain have been compared in four myeloma patients undergoing autologous peripheral blood stem cell transplantation.

Methods and Materials: The four patients all had multiple myeloma with production of intact immunoglobulin monoclonal protein and monoclonal fLC (IgGκ, IgGλ, IgAκ & IgAλ). Daily sampling was initiated at the time of high dose melphalan treatment (200 mg/m2) with stem cell transplantation 24 hours later. Serum samples were collected over 10-24 days. Measurement of both κ and λ serum free light chains was performed on the Behring NephelometerTMII and total immunoglobulin measurement (IgG or IgA) was performed on the Behring Nephelometric Analyser. Half-life curves were plotted for free light chains and intact immunoglobulins.

Results and Discussion: All four patients showed reductions in both free light chains and intact immunoglobulin levels after melphalan treatment. There was considerable variation in the estimated serum half-lives for the monoclonal immunoglobulins and free light chains between the four patients.
established. In two patients, the melphalan treatment produced no finding.

It was notable that the flc half lives in the patients producing IgG plasma cells.

Patient 1 (IgG/κ); IgG – 16.5 days, free κ - 5 days
Patient 2 (IgG/κ); IgG – 10 days, free λ - 5 days
Patient 3 (IgA/κ); IgA - 2.5 days, free κ 5 days
Patient 4 (IgA/λ); IgA – 3.5 days, free λ - 1.5 days
The estimated half-lives for the free light chain were always shorter than for the intact immunoglobulin. This difference was greatest for the IgG monoclonal proteins in accordance with the known serum half lives in normal individuals (IgG1,IgG2 & IgG4, 12 -21 days; IgG3, 7–8 days; IgA, 6 days and free light chain 2-6 hours). The shorter half-life of the free light chains should provide a more accurate estimation of the rate of tumour kill. Half-life estimates were quite different between the 4 patients but the relationship to prognosis has yet to be established. In two patients, the melphalan treatment produced no reduction in the concentration of the alternate (non-tumour) light chain while in a third, the concentrations of both light chains decreased at the same rate. By monitoring the concentrations of both light chain types, it should be possible to gain an estimate of the differential killing rates of the tumour and non-tumour plasma cells.

It was notable that the flc half lives in the patients producing IgG monoclonal proteins were considerably longer than in the patients with IgA monoclonal proteins. Further patients need to be examined to determine whether this is chance or a consistent finding.

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Prognostic significance of immuneparesis in progression of solitary bone plasmacytoma
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Prognostic factors for patients with plasmacytoma are not fully understood. This study aims to further define factors predicting outcome for patients diagnosed with a solitary plasmacytoma. Twenty-nine patients were studied retrospectively, including 23 with solitary bone plasmacytoma (SBP) and 6 with solitary extramedullary plasmacytoma (SEP). Median age was 54 years (range 21 to 81) and 70 years (range 52 to 88) respectively. Thirteen patients with SBP had axial disease (56%). All patients with SBP received radiotherapy (median dose 40 Gy range 20 to 50 Gy), 10 had prior surgery and 4 prior VAD chemotherapy. Five of 6 SEP patients received radiotherapy (median dose 48 Gy; range 27 – 50 Gy) and 1 had oral melphalan chemotherapy.

Results: SBP patients: Serum paraprotein was present in 16 of 22, BJP in 4 of 16 and immuneparesis in 5 of 21 evaluable patients. β2 microglobulin was normal in 14 of 15 patients, and raised in 1. Local relapse occurred in 2 patients at 8 and 31 months. Radiotherapy dose, diagnostic paraprotein level, disappearance of paraprotein at 1 year, and immuneparesis at diagnosis did not predict relapse. Eight patients progressed to MM at a median of 18 months (range 6 to 48 months), including one patient with a distant relapse at 18 months, progressing to MM at 4 years. Median follow up from progression to MM is 20 months.

Immuneparesis at diagnosis predicted progression to MM (p=0.0112). Of 5 patients with immuneparesis 4 progressed to MM the other to amyloidosis. Paraprotein disappeared at one year following treatment in 3 patients, none of whom developed MM (not significant). Axial site of disease, radiotherapy dose, serum paraprotein at diagnosis and β2 microglobulin did not predict progression to MM.

Twenty-one of 23 patients remain alive at a median of 3 years (range 2 months to 11 years). Two patients died. One died of unrelated causes. One progressed to MM (at 6 months, died at 10 months).

SEP patients: M protein was present in 2 patients (1 serum, 1 BJP), 3 were non-secretory, and 1 not assessed. None were immuneparesed. BJP disappeared in 1 patient; follow up paraproteins were not recorded in the other patients.

Three died of unrelated causes. Three patients are alive at a median of 18 years (range 2 to 22 years). Relapse occurred in 2 patients. One had local relapse at 15 years (treated with radiotherapy), and remains alive at 18 years after diagnosis. The other patient relapsed with multifocal plasmacytomas, spanning 2 to 13 years following diagnosis and remains alive at 22 years (treated with radiotherapy and chemotherapy). Neither has evidence of MM, and both are disease free.

Conclusion: Results support immuneparesis at diagnosis as predicting progression to MM. Site of disease (axial skeleton), presence of the t(11;14) or trisomy 11 and elevated cyclin D1 expression, as evaluated by FISH analyses were predictive of progression. In the present study we analyzed the frequency and the clinical and prognostic relevance of cyclin D1 expression, as evaluated by FISH analyses were performed in an attempt to investigate the presence of chromosome 11 and 13 abnormalities. Patients harboring the t(11;14) had significantly higher cyclin D1 mRNA levels than those with trisomy 11 (P<0.0001). Therefore, a close relationship between the presence of the t(11;14) and trisomy 11 and elevated cyclin D1 mRNA levels was found in ≥ 90% of patients, with the exception of a single case showing a very low expression of cyclin D1, but no evidence of 11q abnormalities. In contrast, in the same 46 patients no correlation was found between cyclin D1 mRNA
leukines may be considered as their most useful property as than the value of a single interleukin. Prognostic value of evaluation of all immunological parameters could be more useful in differential diagnosis of borderline cases. However, concomitant with some cellular subsets may be an additional element in the

overexpressed cyclin D1 had a significantly longer duration of remission compared to patients who did not (median, 41 vs. 26 months, respectively) (P = 0.02). As a result, median event-free survival (EFS) was longer in group A than in group B (33 vs. 24 months, respectively); the difference between the two groups was of borderline significance (P = 0.055). It is concluded that cyclin D1 overexpression 1) is a common molecular abnormality in de novo MM; 2) is closely related to 11q abnormalities and 3) does not predict for poor prognosis, as previously emphasized, rather identifying a subset of patients who are more likely to have longer duration of remission and extended EFS following autotransplant(s).

Supported by MIUR, FIRB project RBAN01E9A_001 (M. Cavo), Università di Bologna, Progetti di Ricerca ex-60% (M. Cavo) and Fondazione Carisbo.

174 The network of cytokines in multiple myeloma: diagnostic, prognostic and therapeutic implications

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The study of the network of cytokines seems to be useful in multiple myeloma. IL-6 and IL-2 with their soluble receptors, IL-3, IL-4, IL-10, IL-11 have been examined. Among target cells, growth of normal and myeloma plasmacells is supported by IL-6 together with IL-3, IL-4 and probably IL-8 and IL-10. Differential diagnosis between multiple myeloma and monoclonal gammapathies of undetermined significance is generally based on clinical and laboratory parameters such as the value of monoclonal component, bone marrow plasma cell proportion, the serum level of different classes of immunoglobulins and bone lesions. Nevertheless, the evaluation of the serum level of IL-6, soluble IL-6 receptor, soluble IL-2 receptor together with the activity exerted by IL-3 and IL-4 on some cellular subsets may be an additional element in the differential diagnosis of border-line cases. However, concomitant evaluation of all immunological parameters could be more useful than the value of a single interleukin. Prognostic value of interleukins may be considered as their most useful property as all the interleukins involved in multiple myeloma exhibit this significant value in different manners. Serum levels of IL-6, soluble IL-6 receptor, soluble IL-2 receptor and the expression of membrane-bound IL-2 receptors, both on bone marrow plasmacells and on peripheral blood mononuclear cells, are correlated with the disease activity and the disease stage. In addition, IL-6 and sIL-6R serum level are correlated with the duration of disease-free survival as a high value at the time of diagnosis is connected to a short duration of survival. However, some laboratory parameters may express the same prognostic value as high β2 microglobulin and LDH serum levels together with the high value of plasma cell labelling index are correlated with the disease activity. Furthermore, if the evaluation is performed at the time of diagnosis, high values of these parameters are related to a short duration of disease-free survival. A correlation between laboratory parameters and the serum level of several cytokines was demonstrated. Therefore, the real advantage of prognostic evaluation of cytokines is reserved to patients who do not exhibit uniform results of β2 microglobulin and LDH serum levels or better to border-line cases. Finally, most studies indicate that interferons are mainly used in the immunotherapy of multiple myeloma as the effectiveness of anti-IL-6 antibodies or anti-idiotypic vaccines has not been confirmed.

175 LEVELS OF SERUM PHOSPHOLIPIDS AS PROCESS OF SIGNAL TRANSDUCTION IN PATIENTS WITH MYELOMA

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Myeloma is very interesting field for investigation of disturbances lipids as represent of limfoproliferative malignant disorders. According biology of disease, phospholipids disturbances is very interesting in myeloma, as processes of signal transduction in deficit synthesis of pathological proteins. Correlation between phospholipids an precese of signal transduction is the aim of this investigation.

This study included 57 patients with myeloma and 20 healthy persons as control group. Serum phospholipidslipids was presented by total serum phospholipids, phosphatidylethanolamin/ PE/, sphingophospholipids, lysolecitin, phosphatidichololin in all patients and control group. Biochemical method of level detection was thin layer chromatography/TLC/. Corelation between phospholipid levels in differrent group of patients according of Durie end Salmon classification was the aim of study and statistical analyse presented facts:

- significant difference between group of patients and control group was very strong
- difference between group of patients accord D/S classifications was strong
- levels of lyzolecitin was higher in group of patients in III group ac.D/S classification
- phosphatidylethanolamin is higher in patients with myeloma v.s control group
- sphingophospholipids is higher in terminal phase of disease ac.D/S classification

Synthesis of this results shown that phospholipids in patient with myeloma are veru predictabl and prognostic factor of progresiviv disease. Process of signal transduction is definitly damaged and depend of phase according D/S classification.
We have also found that serum level of HGF was the best marker to examine progression of disease in multiple myeloma patients (p<0.001). Our study showed that examination of plasma levels of pro-inflammatory and angiogenic cytokines in MM might be at clinical value in the near future. Presently the use several anti-angiogenesis agents are resulted in clinical and laboratory remission even in relapsed and/or resistant MM patients.

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Progressive Multiple Myeloma is associated with increased serum VEGF
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Multiple Myeloma (MM) is associated with an increased risk of deep venous thrombosis (DVT) especially during treatment with Doxorubicin or Thalidomide. Recent studies have indicated that in patients with MM, the plasma concentrations of the factor VIII and the von Willebrand factor (vWF) are frequently increased, compared to healthy controls. This may contribute to the pro-thrombotic state in patients with MM. In the current study the clinical role of Vascular Endothelial Growth Factor (VEGF) as a mediator of the pro-thrombotic state was investigated in patients at different stages of MM as compared with healthy individuals. VEGF was determined by an ELISA assay in normal blood donors (n=13), patients with MGUS (n=6), patients with MM at diagnosis (n=8), MM in first remission (n=11), and progressive MM at relapse (n=18). Mean VEGF levels were at progression/relapse 2.65 mg/L, as compared to 1.33 mg/L in controls, 1.22 mg/L in MGUS, 1.57 mg/L at diagnosis of MM and 1.42 mg/L in remission. VEGF levels were significantly correlated with the level of the M-component in serum. Although vWF and FVIIIc is significantly higher in patients with MM compared to healthy controls, there was no correlation of VEGF with FVIIIc or vWFc. Patients with progressive disease who were treated with Thalidomide had higher levels of VEGF (3.76 mg/L) as compared with patients treated with other medication (2.35 mg/L). These data indicate that VEGF serum levels increase with the development of progressive disease, especially after the use of the anti-angiogenic drug Thalidomide. An update of therapeutic monitoring of VEGF during treatment will be provided during the meeting.
evaluated at diagnosis and at various time points during therapy. By study design, all patients received four months of combined thalidomide (100mg/d for two weeks and 200mg/d thereafter) and dexamethasone (40mg/d, on d 1-4, 9-12, 17-20/28 d on odd cycles and on d 1-4/28 d on even cycles) therapy (THAL-DEX) as inducetion of remission before peripheral blood stem cell (PBSC) collection with high-dose cyclophosphamide and subsequent double autotransplants upon treatment with melphalan 200mg/sqm. Zoledronic acid (ZOLE acid) was administered at 4mg/28d for at least 9 months. Data from 21 patients (10M, 11F, median age = 53 years) have been collected so far. At diagnosis, all bone resorption markers were increased in more than half of the patients, while BAP and osteocalcin were decreased in 29% and 18% of the patients, respectively. Both urinary NTX (p=0.039) and serum crosslaps (p=0.000) were positively correlated with the extent of skeletal involvement, graded according to the number and the size of osteolytic bone lesions assessed in whole skeleton X-ray. After 4 months of therapy with THAL-DEX and ZOLE acid a significant decrease in mean urinary NTX (58.6 ±9.5 SE nmol/mmol crea vs 21.2±5.1 SE, p=0.003) and serum crosslaps ( 5992±1213 SE pmol/L vs 2239± 598 SE ) was observed. Other resorption markers were also reduced, though not significantly. In patients who responded favorably to THAL-DEX, reduction in bone resorption markers paralleled the decrease in M protein concentration. A slight decrease in bone formation markers was also detected, possibly as a result of DEX therapy; however, this finding needs to be confirmed at a subsequent analysis performed at the end of the whole treatment program. It is concluded that among all the markers of bone turnover, serum crosslaps and urinary NTX are the ones most strictly related to actual bone resorption and to the extent of bone involvement, as evaluated at X-ray survey. Combined THAL-DEX and ZOLE acid administered as primary therapy for patients with newly diagnosed and symptomatic MM seem to be highly effective in reducing bone resorption, although the relative merits of each of these drugs cannot yet be determined.

Supported in part by MIUR, FIRB project RBAU012E9A_001 (M. Cavo)

179 Prognostic value of circulating levels of angiogenic cytokines fibroblast growth factor (FGF-2), hepatocyte growth factor (HGF) and vascular endothelial growth factor (VEGF) in patients with multiple myeloma

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Introduction: Increased bone marrow angiogenesis has been identified as an important factor for growth and disease progression in multiple myeloma. It was recently shown, that progression from MGUS to advanced stages of multiple myeloma is accompanied by an increase in bone marrow microvessel density (MVD). Furthermore MVD has been shown to be an independent prognostic factor for overall survival, as evaluated at X-ray survey. Combined THAL-DEX and ZOLE acid administered as primary therapy for patients with newly diagnosed and symptomatic MM seem to be highly effective in reducing bone resorption, although the relative merits of each of these drugs cannot yet be determined.

Supported in part by MIUR, FIRB project RBAU012E9A_001 (M. Cavo)

Figure. Prognostic relevance of elevated levels of FGF-2, HGF and VEGF for overall survival in multiple myeloma patients. Conclusions: Our data showed for the first time that FGF-2, HGF and VEGF were found to be the most powerful independent prognostic factors (hazard ratio 2.67 and 3.24, respectively).
Clinical study on the bone lesions for 93 patients with multiple myeloma

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Background. Multiple Myeloma (MM) is an incurable plasmocytic dyscrasia associated with bone lesions and having a major impact on the quality of life of these patients.

Aims. We investigated the incidence of the bone lesions in multiple myeloma (MM), the course of those lesions and we also evaluated the relationships of skeletal symptoms with prognostic factors.

Methods. The subjects were 93 patients, aged 45 years or more (median age 62 years). They consisted of 54 men and 39 women. According to the Durie-Salmon staging 72.05% of patients were in stage III. Regarding the type of plasma cell morphology, the lot was shared as following: 9.09% plasmablastic group, 18.18% immature group, 15.9% intermediate group and 56.81% mature group (Greipp).

The occurrence rate of osteoporosis plus osteolytic lesions was higher in elderly patients over 65 years (69.3%) than that in non-elderly patients (51.3%) (p < 0.045). The bone lesions were most observed in lumbar vertebrae (53.69%), cranial bone (46.7%), thoracic vertebrae (40.4%) and ribs (27.9%). The occurrence rate of bone lesion in lumbar vertebrae was higher in elderly patients (59.7%) than that in non-elderly patients (45.6%) (p < 0.05). There was a significant difference in the rate of plasma cells in the bone marrow between the patients with and without pathologic fracture (p < 0.05). Significant differences of survival times were found between non-elderly MM patients with and without pathological bone fractures (p < 0.05). The rate of the bone lesions and their gravity is higher in the patients with immature and plasmablastic morphology (p < 0.06).

Conclusions. The bone marrow represent an unsolved problem with major impact on the life quality for the patients with MM. Their incidence ans extension seem more important in elderly patients and in those who have aggressive morphology.

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FREQUENCY AND PROGNOSTIC RELEVANCE OF P53 EXPRESSION IN MYELOMA MULTIPLE (MM)

Spain

Objective: The aim of this study was to investigate the expression of protein p53 in MM de novo and analysis the prognostic influence in survival and response to treatment.

Patients and Methods: A total of 73 new patients with MM were included. In aspirate of bone marrow from MM we study the immunochemistry expression of p53 in plasma cells and considered positive when more than 5% of plasma cells were nuclear positive expression. The most important clinical and biological parameters were included. The cumulative survival probability and cumulative response probability into positive cases were calculated by men Kaplan Meier estimator. Univariate and multivariate Cox models were used to identify possible predictor of poor survival and resistance to treatment.

Results: Mean age (SD) 77y. (11). Males 59%. Histological subtypes: Bence-Jones MM (n=13), Non-Secretor (n=2), IgG-κ (n=17), IgG-λ (n=15), IgA-κ (n=13), IgA-λ (n=13). 46% (n=24) were positive for p53 expression. Different schedules of treatment were administered: MFL+PDN (n=39), VCMP/VBAD (n=21), VAD (n=4) and no treated 9 cases. Response was 63% (33% objective, 11% complete and 19% partial). Mean survival 32m (0.5-54) and cumulative survival probability at 3y. was 43% (IC95% 36-51%). Multivariate Cox model LDL> 5 μkat/L, albumin < 30g/L and Durie stage III were independent factors for shortened survival. Multivariate analysis to resistance to treatment albumin< 30 g/L and positive expression of protein p53 were significantly.

Conclusion: These findings suggest that p53 immunostaining in routine bone marrow aspirate may be helpful for detection of MM with potentially resistance to treatment.

Methylation status of FHIT and its clinical impact

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Introduction: Hypermethylation of CpG island is one of the mechanisms for gene inactivation. Aberrant methylation of tumor suppressor genes (TSG) has been reported in multiple myeloma (MM), and has been discussed about its relationship to pathogenesis, disease progression and prognosis. For example, it has been reported that methylation of p16 or DAP kinase gene was related to poor prognosis. We investigated methylation status of FHIT (fragile histidine triad) gene, which was a putative TSG, and evaluated the clinical impact of its methylation in MM. Patients: Forty-eight patients were investigated in this study. Forty of 48 patients were de novo cases, and the other 8 patients were transferred from other hospitals, where they had already received one to three courses of chemotherapy. The characteristics of patients were as follows: gender; male vs. female=26 vs. 22, age; 42 to 82 years (median 63), clinical stage; I=2, II=13, III=23, immunoglobulin class of M-protein; IgG=29, IgA=9, Bence-Jones type=8, IgD=1, non-secretary type=1, types of light chain; κ=34, λ=14. Materials and method: Bone marrow (BM) was obtained before treatment, or at administration to our institution in the patients who had already received chemotherapy. Mononuclear cells in BM were separated by a density-gradient centrifugation, and stored at -150C. Genomic DNA was extracted from the above mononuclear cells, and detected methylation of the FHIT gene by using the methylation-specific PCR. Briefly, DNA was treated with sodium bisulfite, and then, amplified using two sets of primers. One set was for unmethylated FHIT alleles, while another for methylated FHIT alleles. If the FHIT gene in a sample was methylated, the sample was able to be amplified by the primer set for the methylated alleles. The survival time was calculated from the day when BM
was obtained. Results: Methylation of the FHIT gene was observed in 21(44%) of the 48 patients. Statistical correlation between the methylation of the FHIT gene and any clinical variable was not seen. The estimated 50% survival time of FHIT methylation group and unmethylation group were 20.2 months and 30.0 months, respectively (p=0.0042). By using univariate analysis, the following variables had adverse prognostic features: methylation of FHIT gene (p=0.0042), advanced age (61 years old, p=0.0384), bad performance status (ECOG performance status 0 to 2, p=0.0004), advanced stage (β, p=0.0040), low level of hemoglobin (β 8.5 g/dl, p=0.0042), low level of serum albumin (β 3.5 g/dl, p=0.0001), elevated level of serum C-reactive protein (>0.5mg/dl, p=0.0031), elevated level of serum β2-microglobulin (>6.5 mg/l, p<0.0001), and treatment not including high-dose chemotherapy supported by stem cell transplantation (p=0.0404).

By using multivariate analysis, methylation of FHIT gene (p=0.0128), bad performance status (ECOG performance status α to γ, p=0.0299), and elevated level of serum β2-microglobulin (>6.5 mg/l, p=0.0327) had statistical significance. Conclusion: These findings suggest that aberrant methylation of the FHIT gene may be an independent adverse prognostic factor for patients with MM.

183 SOCS-1 gene methylation is frequent but does not appear to have prognostic value in patients with multiple myeloma

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Background. SOCS-1 is a negative regulator of the Jak/STAT signalling pathway. Aberrant methylation of SOCS-1 was initially shown in hepatocellular carcinoma (Yoshikawa, 2001). Recently, Galm and co-workers found SOCS-1 hypermethylation in 23/35 (63.9%) patients (pts) with multiple myeloma (MM) (Galm, 2002). In order to investigate the possible influence of SOCS-1 methylation on the clinical outcome of MM pts, we analyzed SOCS-1 gene methylation using methylation specific PCR (MSP) in a series of MM pts with long term follow-up.

Patients and Methods. Fifty-one previously untreated MM pts were included in the study. Median age was 66 years (range 36-81). There were 33 males and 18 females. Clinical staging was: stage I, 8 (15.7%); II, 12 (23.5%); III, 31 (60.8%). M-component was Ig G in 30 (58.8%), Ig A in 15 (29.4%), Ig G +Ig A in one, stage I, 8 (15.7%); II, 12 (23.5%); III, 31 (60.8%). M-component was Ig G in 30 (58.8%), Ig A in 15 (29.4%), Ig G +Ig A in one, Bence Jones in 3 (5.9%). Two pts (3.9%) had myeloma without M-component. Thirty-one patients (60.8%) were treated with melphalan-prednisone, 15 (29.4%) with intensive protocol, and 5 (9.8%) were not treated. Bone marrow mononuclear cells from MM pts were isolated by Ficoll Hypaque sedimentation and extracted DNA was modified by bisulfite. SOCS-1 gene promoter regions were amplified with DNA methylated and unmethylated specific primers as previously described (Yoshikawa, 2001).

Results. Fifty-one samples of MM bone marrow cells were analyzed by MSP. Median time follow-up was 9 years. Selective methylation of SOCS-1 was found in 38/51 pts (74.5%). No correlation could be made between SOCS-1 methylation and gender, age, isotype, level of M-component, stage of the disease, serum levels of albumin, creatinin, calcium, β2-microglobulin, LDH, C-reactive protein, or response to treatment. Overall survival was not significantly different between pts with methylated or unmethylated SOCS-1 gene (p= 0.58, log-rank test). In contrast pts presenting an elevated β2-microglobulin level had a significantly poorer prognosis (p= 0.033).

Conclusion. Methylation of SOCS-1 is frequent in MM, occurring at frequencies of 75% in our series, and does not appear to have any significant prognostic value.

184 Plasma Cell Proliferation Index As A Clinical Prognosticator For Relapsed Multiple Myeloma

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The plasma cell labeling index (PCLI) is a measure of plasma cell proliferative activity and has been shown to predict poor prognosis in newly diagnosed multiple myeloma (MM) patients. Other reports have demonstrated that PCLI is an independent prognostic indicator for patients with stable-plateau MM. Major drawbacks of this technique are that it is time-consuming, requires a degree of subjectivity in its interpretation, and is not readily available at all institutions. The plasma cell proliferation index (PCPI) is a double staining immunohistochemistry technique that allows identification of proliferating malignant plasma cells in a core biopsy by dual staining Ki-67/CD-138. It is far less time-consuming and can be consistently performed by a trained pathologist. High-dose pulsed dexamethasone (Dex) is one of the preferred treatments for relapsed MM patients and is currently the accepted gold standard against which investigational drugs are being tested. The predictors of outcome for relapsed patients while on Dex treatment have not been well established. We are therefore prospectively evaluating the PCPI in patients with relapsed MM receiving Dex in consecutive, prospective clinical trials. We intend to test its value as a predictor of response and time to progression (PD). Time to progression is defined as date of PD - date of Dex initiation. PCPI and cytogenetics results are being evaluated before Dex initiation in all enrolled patients. Sixteen patients with relapsed MM were evaluated. Eighty-one percent (n=13) and 19% (n=3) had IgG and IgA subtypes, respectively. Of the 16 patients, 56% (n=9) showed progressive disease at a median time of 87 days. The median PCPI for those patients with PD was 2.8, while the PCPI for those that did not show PD was 0.80. Cox-regression analysis revealed a 1.5 times greater likelihood of progression per unit increase in PCPI (p=0.07). In accordance with other studies, an abnormal cytogenetics profile predicted a worse prognosis (p=0.03). Our results, albeit in a small but homogeneous cohort of patients, supports the clinical utility of the PCPI in predicting which patients will progress after high-dose Dex treatment. Accrual continues and further analysis will be presented at the meeting.
7.2 Imaging studies

185 99mTc-MIBI SCINTIGRAPHY PATTERNS IN MULTIPLE MIELOMA (MM) AND MONOCLONAL GAMMOPATHY OF UNKNOWN SIGNIFICANCE (MGUS).

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Aim of this study: This study has been designed in order to find out the usefulness of 99mTc-MIBI in determining the activity and extension of MM and to differentiate MM from MGUS.

Materials and Methods: We have studied a total of 65 patients with the following diagnosis: MM (43), Waldenström’s (6), Macroglobulinemia (1), and MGUS (21). In several patients two or more scintigraphies were performed (2-4) at different times.

The first scan was made before therapy in 58 patients, post chemotherapy in 4 patients and post autologous peripheral stem cell transplantation in 3 patients. Stage disease was recorded according to Durie & Salmon criteria; for this purpose MGUS was classified as "no diagnostic criteria of MM". Bone radiographic lesions were classified as normal (0), osteoporosis (1), more than 4 osteolytic lesions (3) and between 1 and 3 (2). Whole body scans were obtained 10 minutes after injection of 740 MBq of 99mTc-MIBI, using dual head camera. Scans were classified (according L.Pace) into four patterns: Normal (N, only physiological uptake), diffuse (D, presence of bone marrow uptake), and diffuse plus focal (D+F). The diffuse uptake was scored according to extension and intensity. Other biochemical data with prognostic significance have been studied: Monoclonal protein, Beta2microglobulin protein, C, LDH and albumin.

Results: Only 3 of 21 MGUS have been slightly positive (+) for 99mTcMIBI (1 F and 2 D). The difference of MIBI score among the different clinical stages (including MGUS) is significative (p<0.0001, KW test), but the sensitivity for positive diagnosis is only 72.5 %. The WM patient had ++ intensity score . One patient with non-secretory MM had normal intensity and pattern of MIBI uptake in two consecutive scans, even in the presence of active spine and paravertebral lesion, documented with CT and MNR. The highest intensity MIBI score (+++) has been observed only in osteolytic lesions grade two (4 patients) and three (5 patients). The difference of MIBI score among the different degrees of bone lesions is significative (p<0.0001; KW test). The mean of beta 2 microglobulin (=B5g/ml) increases with bone lesions grade two (4 patients) and three (5 patients). The difference of MIBI score among the different degrees of bone lesions is significative (p<0.0001; KW test). The mean of beta 2 microglobulin (=B5g/ml) increases with the MIBI intensity score (1.9 - normal; 3.3 - +; 3.7 - ++ and 9.6- +++)(p =3D 0.001, KW test). The % of plasma cells in bone marrow also increases with the MIBI intensity score (9.6%; 25.2%; 23.2% and 40.1% respectively) (p =3D 0.006; KW test).

Focusing on the follow up of individual patients we have observed that MIBI-1 uptakes correlates with the degree of activity of MM with exceptions (one patient had persistent + activity despite being in CR with negative immunofixation). All the MGUS with normal uptake remain normal in the absence of MM. One patient became surprisingly +++ MIBI score with normal bone marrow three weeks before he suffered a sudden relapse. Several radiographic residual osteolytic lesion became MIBI (-) after chemotherapy.

Conclusions: the 99mTc-MIBI scanning is a useful tool in the evaluation of MM patients. There are discordant facts that need further studies.

186 Imaging Bone Disease in Multiple Myeloma – Comparison of Modalities

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Introduction: Magnetic resonance imaging (MRI) provides assessment of focal myelomatous bone disease as well as additional prognostic information through assessment of abnormal bone marrow patterns. MRI is, however, a costly and limited resource. Alternatively, technetium-99m-sestamibi scintigraphy (MIBI) is widely available, and, although typically used for cardiac imaging, is known to demonstrate uptake in myeloma. Systematic comparison of MIBI to other imaging modalities is lacking. Inter and intra-observer variability for these techniques is unknown.

Objective: To determine the sensitivity of MIBI as compared to MRI for the detection of skeletal disease due to multiple myeloma. To determine observer variability of MIBI and MRI.

Methodology: Between August 2000 and February 2003, 41 patients (25 male, 16 female) with multiple myeloma were enrolled. Median age was 61 years (range 37-83). 34 were newly diagnosed and 7 relapsed requiring alteration in treatment. In addition to clinical, laboratory, and radiographic evaluation, patients underwent MRI and MIBI imaging. MRI evaluation of the entire spine, pelvis, proximal humeri and femora was performed with T2 weighted fat saturated sequences. MIBI imaging included whole body anterior and posterior planar images. Tomographic MIBI evaluation was performed with separate tomograms of the chest, abdomen and pelvis. Image sets for each modality were evaluated blindly by two separate experienced radiologists using predefined criteria. Discrepancies in interpretation of images were resolved at a later session.

Results: Imaging in both modalities was performed on 39 of 41 patients. MIBI patterns included focal in 8 patients, variegated in 3 patients and diffuse in 28; for the purposes of analysis diffuse and variegated were considered together. Potential information obtained from MIBI includes pattern, intensity and extent of involvement. Abnormal MIBI findings were demonstrated in 31 patients with a normal pattern in 8. Correlation coefficient for the modalities was 0.69 with a p value of<.0001.

Despite a relatively brief median follow-up of 12 months, clear trends in survival analysis were seen for those patients with a variegated or diffuse MRI pattern compared to a focal pattern and for those with a normal marrow pattern on MRI compared with abnormal. In addition, extent of disease by MIBI, defined as proximal versus distal, was associated with median survivals not yet reached in the proximal group and 12.6 months in the distal group.

Observer variability was analyzed with the kappa method. Correlation between radiologists was very good with kappa values of 0.86 for MRI and 0.76-0.78 for MIBI. Kappa value for intra-observer variability for MIBI was 0.87-0.92.

Conclusion: MIBI is a sensitive imaging technique for myeloma and provides information on abnormal marrow pattern similar to MRI. We have demonstrated excellent intra and inter-observer variability for these techniques implying the potential for generalizability. With a relatively short median follow-up survival trends are seen and may be borne out.
Both F-18 FDG PET and Tc-99m sestamibi are informative imaging modalities which frequently aid the clinical management of patients with Multiple Myeloma (MM).

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Objective: Multiple myeloma (MM) may be difficult to assess due to: absent/small volume paraprotein; minimal bone marrow infiltration/biopsy sampling variation; persisting pain or abnormalities on plain skeletal surveys (SS) following therapy which may not represent active disease. In previous small studies, both FDG-PET (PET) and Tc-99m sestamibi scans have identified sites of occult bony and soft tissue disease in MM. This analysis aims to compare the results of PET and MIBI in MM.

Methods: Over Jan 1999–Aug 2002, 34 pts had PET, 21 of whom had concurrent MIBI. Medical records and scan results were reviewed to assess: (a) ability of the scans to identify otherwise occult disease; (b) concordance between the scans; and (c) impact on management.

Results: Disease state was: newly diagnosed (8pts), remission (3pts), relapsed/refractory (17pts), MGUS (2pts); isolated plasmacytoma (4pts). 16 had scans at diagnosis or as baseline, 13 for suspected progression (PD), 3 for re-staging, and 2 to investigate persistent fever. 14 (41%) had difficult to assess for suspected progression (PD), 3 for re-staging, and 2 to plasmacytoma (4pts). 16 had scans at diagnosis or as baseline, 13 with myeloma, and confirmed with further imaging or biopsy in 5. 3 cases showed unexpected additional sites in pts thought to have limited/stable disease. Additional imaging or biopsy confirmed all were true positives. In 11/21 cases (52%), additional sites of disease not seen on routine SS. Additional sites were soft tissue in 4 cases, and bony in 10. 11 of these 14 cases were pts with known active disease at other sites. The additional sites were consistent with myeloma, and confirmed with further imaging or biopsy in 5. 3 cases showed unexpected additional sites in pts thought to have limited/stable disease. Additional imaging or biopsy confirmed all were true positives. In 11/21 cases (52%), additional sites of disease not seen on routine SS, were identified on concurrent MIBI. Additional sites were soft tissue in 3, and bony in 10 (both in 2). 7 of 11 cases were pts with known active disease at other sites. The additional sites were consistent with myeloma, and confirmed with further imaging or biopsy in 4. 4 cases showed unexpected additional sites in pts thought to have limited/stable disease. Additional imaging/biopsy confirmed 2 true positives and 2 false positives. MIBI generally detected more disease sites than PET: median # sites/case = 1 (0-4) vs 3 (0-8); P=0.01. PET and MIBI were concordant in 8 cases (6 both negative scans). In 10/21 cases, MIBI detected additional sites to PET (all bony). In 3 cases, PET detected additional sites to MIBI (2 bony, 1 soft tissue). In 15/34 cases (44%), scan results led to a management change. In 8 cases, stable or responsive disease was confirmed and pts continued their treatment plan. In 2 cases, radiotherapy fields were altered to encompass active disease sites. In 2 cases, treatment was changed due to detection of PD. In 3 cases of pts thought to have only isolated plasmacytoma, a diagnosis of MM was made and systemic therapy commenced.

Conclusions: PET and MIBI are useful additional diagnostic tools for detecting otherwise occult MM sites. Their use should be considered in the work-up of pts with presumed solitary plasmacytomas to exclude the presence of MM. MIBI detects more additional disease sites than PET. However, PET scans have detected sites of soft tissue disease not seen with other imaging modalities.

188 Prospective Evaluation of 460 Patients from Total Therapy II – Identification of Characteristics on Baseline MRI Examinations of Prognostic Significance – Importance of Focal Lesions (FL) in Multiple Myeloma (MM)

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MM frequently presents with focal plasmacytoma lesions (FL) superimposed on diffuse infiltration of the marrow which can be recognized on MRI (T1-weighted and STIR images). These MRI-FL have previously been shown to contain malignant plasma cells morphologically, by flow cytometry, cytogenetics and FISH, and by gene expression profiling. These lesions can be associated with or progress to osteolytic lesions (OL) recognized on standard X-rays, and can progress when treatment is ineffective. To evaluate the significance of diffuse and FL patterns on MRI in MM patients enrolled in TT II (intensive remission induction, tandem autotransplants with melphalan 200 mg/m2, consolidation chemotherapy for 1 year and interferon maintenance, up-front randomization to +/- thalidomide), 460 baseline MRI examinations of the axial skeleton were prospectively evaluated in terms of signal characteristics on T1-weighted and short-tau inversion recovery (STIR) sequences for the presence of hyper-, iso- or hypointense background, homogeneous versus heterogeneous overall signal, and number of FL present (FL being a sharply circumscribed region thought to be myeloma measuring ≥5 mm in diameter). The only statistically significant (p<0.01) prognostic characteristic of baseline MRI was the number of FL in the spine and pelvis, revealing significantly superior event-free survival (EFS) and overall survival (OS) with < 5 FL. Four-year estimates of EFS were 68% for < 5 FL, 54% for 5–20 FL, and 37% for FL ≥ 21 (p=0.0002). Similarly, 4 yr OS was 78% with < 5 FL compared to 62% for 5–20 FL and 35% for FL ≥ 21 (p=0.001). Examination with Cox multivariate regression for potential associations with standard prognostic factors (SPF) such as β2M ≥ 4.0, CRP ≥ 4.0, LDH ≥ 190, cytogenetic abnormalities (CA), albumin < 3.5, platelets < 150K, HGB < 10, and creatinine ≥ 2 revealed superior significance of number of FL > 5 in OS (HR 2.9, CI 1.2, 3.1, p=0.008) and EFS (HR 1.9, CI 1.3, 2.8, p=0.002), and of any CA in OS (HR 2.9, CI 1.7, 4.8, p<0.001) and EFS (HR 2.7, CI 1.8, 4.0, p<0.001). Interestingly, while a variety of CA were present (T01p, Del08, Del13, Del17, and Del20), all patients with Del20 had FL ≥ 5, whereas no patients with FL < 5 had Del20 (p=0.001). Follow-up of OS in 296 patients and EFS in 280 at a one-year landmark following enrollment demonstrate this correlation with number of FL to continue (OS p=0.0009, EFS p=0.0001). We conclude that the presence of MRI defined FL ≥ 5, present in 43% at baseline, often without associated OL, identifies a MM entity with distinct biological and clinical characteristics, including inferior survival. These patients are at high-risk, and should be targeted for novel treatments as well as detailed biological investigation.
Factors Predicting Occult Spinal Cord Compression in Multiple Myeloma

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Introduction: Bone involvement in multiple myeloma (MM) can lead to significant morbidity, including potentially irreversible neurologic deficits if spinal cord compression occurs. During a study comparing MRI to TcMIBI scan for bone involvement in MM, we encountered patients with evidence of compression of the spinal cord (SCC) or subarachnoid space (SASC) on MRI, but without neurologic signs or symptoms.

Methods: 41 patients with newly diagnosed MM or relapsed disease requiring treatment were enrolled in a prospective study comparing MRI to TcMIBI for extent of bone involvement, between August 2000 and February 2003. Imaging was performed at enrolment, and repeated at time of stable disease or 100 days post autologous stem cell transplant. MRI scans were reviewed for evidence of MM involvement in the vertebral canal resulting in either bone fragment or tumour contacting the spinal cord (SCC) or impressing on the subarachnoid space (SASC). All charts were then reviewed for clinical manifestations of SCC/SASC. Clinical markers including patient age, hemoglobin, serum calcium, percentage plasma cells in bone marrow, M-protein, extent of disease on skeletal survey, and presence of back pain were investigated for correlation with presence of SCC/SASC.

Results: 38 patients completed MRI at baseline, one of whom had symptomatic SCC. Of 37 patients without neurologic symptoms at presentation, 10 patients (27%) had occult SCC/SASC using MRI. One additional patient asymptomatic and with negative MRI at baseline developed symptomatic SCC with positive MRI at 10 months, making the overall rate of SCC/SASC 32% (12/38). Presence of back pain at baseline (11/12 with positive MRI vs 11/26 with negative MRI, Fisher exact p =0.0042), baseline hemoglobin (120.8 ± 11/12 with positive MRI vs 11/26 with positive MRI, p=0.010), and baseline serum calcium (2.51±0.18 vs 2.37±0.18, p=0.019) were predictive of a positive MRI at baseline or follow-up. Age at presentation, %plasma cells in bone marrow, M-protein, and extent of disease on skeletal survey were not predictive.

Conclusions: In MM patients, we have identified a 27% rate of occult SCC/SASC. In univariate analysis, patients with back pain, elevated serum calcium, and near-normal hemoglobin were more likely to have SCC/SASC on MRI. These predictors may be useful for identifying patients at risk of developing potentially irreversible neurologic complications of MM. The skeletal survey, a standard test for staging multiple myeloma, was not predictive of SCC/SASC. Longer follow-up is required to determine if a positive baseline MRI is predictive of development of symptomatic cord compression.

Utility of the MR. in the evaluation of plasma cell bone marrow infiltration in patients with Multiple Myeloma before double PBSCT

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Background: Multiple Myeloma (MM) is a neoplastic disease characterized by scattered bone marrow plasma cells infiltration. The information about mapping of disease is not complete when making solely aspirated or marrow biopsy. The MRI has demonstrated its utility in the evaluation of the bone marrow infiltration by their capability to distinguish water from fat and this technique permit to obtain a map of changes in the hematopoietic distribution. In MRI three patterns of bone marrow infiltration are described: diffuse, variegated and focal. Diffuse pattern is associated with progressive disease. At diagnosis approximately 20% of patients have not skeletal manifestation, nevertheless MR showed bone marrow infiltration. Besides MRI is useful to evaluate bone marrow response after therapy. The objective of this study is to use the MRI procedure in diagnosis and evaluation of plasma cell bone marrow infiltration after and before PBSC in patients with MM.

Materials and Methods: In twelve consecutive MM patients diagnosed in Haematology Department of Miguel Servet University Hospital between 01/00 and 06/02 a prospective study was carried. All patients received chemotherapy with VBMP/VBAD schedule, subsequently double PBSCST were performed. MRI designed protocol was applied at diagnosis and 4 months after double PBSCST was performed. Type of MRI study: signal intensity sequences in Ta (TR600 ms TE-20) in coronal imaging located in lumbar spine, pelvis and femoral bones. A General Electric System was used (GE MR. max 0.5 Tesla intensity). All the studies were performed and evaluated by the same radiologist. Three patterns of bone marrow infiltration were proposed: 1.- normal, 2.- non-homogeneous, 3.-homogeneous. The non-homogeneous pattern being subclassified in three different subtypes: reticular, mottled and diffuse.

Results: Mean age 56.33 (range 47-63 years), males/females 10/2. Bone marrow biopsy: mean of plasma cells 41.0 (range 0-75%). M-spike component: mean 2.4 (range 0-8.3 g/dL). (Immunohematologic subtype: IgG 6, IgA 3, Bence-Jones 3), 2microglobulin 3.7 (range 1-15.6 mg/dL), haemoglobin 12.3 (range 6.4-15.4 g/dL). The conventional bone X-ray showed generalized osteoporosis in 2 patients, osteolytic lesions 6 patients, vertebral collapse 2 and normal 2 patients. MRI was performed in all patients showing evidence of bone marrow involvement: diffuse 2, reticular 6, mottled 4. After double PBSCST MRI remained being positive in 11 (91.6%): diffuse 2, reticular 4 and mottled 5. Relapse has been observed in 7 patients (58.3%), mean free relapsed survival 19.4 months SD 16.4. Apparently MRI pattern does not seem to be related with free time to relapse.

Conclusion: The MRI is an effective non invasive procedure to evaluate bone marrow replacement and to determine the extent of disease in MM. This procedure will be very useful to evaluate the response to therapy. In our short experience, we have found that MM showed persistence of disease in spite of the negativity of the others evaluated parameters.
Volume localized in vivo proton magnetic resonance spectroscopy (MRS) of the spine in multiple myeloma: variation of fat-water ratio in patients receiving chemotherapy.

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Introduction. Magnetic resonance imaging (MRI) has become the method of choice over other imaging modalities in the noninvasive evaluation of bone marrow disease. By sampling a large volume of bone marrow, it provides information that complements bone marrow aspiration or biopsy in the diagnosis and staging of multiple myeloma (MM). In the post-treatment evaluation of patients with MM, bone marrow MRI may provide important information but assessment of the degree of response is highly subjective. Proton nuclear magnetic resonance spectroscopy (1H MRS) may be able to measure the ratio of water to lipid proton resonance signal intensities and thus reflect the relative percentages of cellular and fatty bone marrow within a defined three-dimensional volume (voxel). These measurements could be used to quantify the degree of cytotractus in MM patients.

Patients and methods. Twelve subjects (six male, median age 76 years, range 57-82) with a newly diagnosed multiple myeloma underwent MRI and 1H MRS of the fifth lumbar vertebral body. Six of them could be re-evaluated after completing six cycles of chemotherapy (2 of them had progressed, 1 presented a partial response, and 3 had achieved a complete response). All measurements were performed with a 1.5-T system (Gyrescan Intera, Philips). Voxel imaging parameters included repetition time of 5000 msec and echo time of 40 msec, voxel size of 2x2x2 cm, 32 measurements were acquired with a spectral bandwidth of 1000 Hz. Spectra at diagnosis and after-treatment of the six patients who completed the study were compared for differences in peak intensities, peak areas and lipid to water ratios (LWR). Age-matched individuals submitted to MRI for reasons not related to bone or bone marrow pathology were used as controls. A McNemar signs test was used to assess statistical significances of the spectral changes.

Results. The initial water peak intensity was initially high in all patients when compared to age-matched reference values and it significantly decreased after treatment (p=0.028). A decrease of the water peak intensity < 50% after treatment was associated to progression (2 cases) or partial response (1 case). Measurements of the water resonance as peak area correlated well with intensity measurements (r=0.93, p=0.001) although variability was higher. The initial lipid peak intensity was decreased in 5/7 cases when compared to age-matched reference values. Increase after treatment was variable (differences not significant with respect to pre-treatment data). Lipid peak area measurements correlated well with lipid peak intensities (r=0.99, p<0.001). Lipid peak intensities or areas <5-fold normal age-matched reference values were associated to disease progression (1 case). LWR of peak intensities and areas correlated well before (r=0.99, p<0.001) and after (r=0.99, p<0.001) treatment. With respect to age-matched reference values, LWR was decreased 2 to 100-fold (mean 30-fold) in MM patients. Failure to increase LWR after treatment was associated to disease progression (1 case).

Conclusions. Proton nuclear magnetic resonance spectroscopy (1H NMR) may be used to assess noninvasively the response to chemotherapy in multiple myeloma patients.

Study supported by grant 01/1108 from Fondo de Investigaciones Sanitarias (FIS).

Ex vivo proton nuclear magnetic resonance spectroscopy (1H NMR) of native myeloma cells. Preliminary assessment of its potential utility to quantify bone marrow infiltration in vivo

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Introduction. Magnetic resonance imaging associated to proton nuclear magnetic resonance spectroscopy (1H NMR) may be able to quantify noninvasively the degree of cytoruction in multiple myeloma (MM) patients. The measurements rely on the ratio of water to lipid proton resonance signal intensities and thus reflect the relative percentages of cellular and fatty bone marrow. However, these measurements are unspecific and may reflect changes in the bone marrow other than MM infiltration. 1H NMR spectroscopy may also give more specific information detecting metabolites related to tumour aggressivity or response to treatment. The 1H NMR spectra of native myeloma cells has not been described yet.

Objective. (1) Determine if high resolution 1H NMR spectra of native myeloma cells can be reproducibly obtained ex vivo and (2) identify marker resonances in the spectra potentially detectable in in vivo studies.

Materials and methods. Mononuclear cells from bone marrow samples were isolated by ficoll-metrizoate gradient techniques, incubated and washed with sterile PBS. A fraction of the sample was used to obtain a global cell count and to assess the proportion of myeloma cells of the overall cellularity (CD19/CD38/CD56 triple labeling). Samples were considered adequate for high resolution 1H NMR analysis if at least 20×106 cells were obtained with at least 50% of them expressing an atypical plasma cell phenotype. Ex vivo 1H NMR spectral analysis. Cells were washed twice with PBS made with D20 (99% isotopic purity), suspended in a final volume of 400 µL, and placed on ice until data acquisition. Samples were analyzed on a 400 MHz high resolution Bruker ARX spectrometer using a WATERGATE sequence and 128 excitations, 16k points and 5 kHz bandwidth. Trimethylsilylproponic acid (TSP), 0.1% solution in D20 was used as reference (0.0 ppm) for each experiment and the approximate relative concentration of metabolites were assessed as a ratio of their highest peak altitude compared to that of TSP. Samples were stored in liquid nitrogen afterwards and perchloric acid (PCA) extracts of frozen samples were used to obtain 1H NMR spectra from the soluble metabolites using a presaturation sequence for residual water.

Results. Six samples of at least 20×106 cells were obtained from untreated patients by bone marrow aspiration (3 cases), biopsy (extramedullary plasmacytoma, 1 case), pericardiocentesis (pericardial effusion of myeloma cells, 1 case) or venopuncture (plasma cell leukemia, 1 case).

Ex-vivo spectra. Intense signals at 3.2 ppm (choline containing compounds) and 3.4 ppm (taurine) were obtained in all samples with a relative intensity to TSP of the peaks ranging from 0.63 to 0.79 for taurine and 1.86 to 2.86 for choline. The assignment of ex
vivo detected resonances was investigated in further detail in the extracts spectra. Conclusions. These preliminary data suggest that several myeloma cell metabolites identified ex vivo in the 1H NMR spectra (i.e. taurine and choline containing compounds), are potentially detectable in vivo and thus could be good candidate markers of MM bone marrow involvement at diagnosis and follow-up. Study supported by grant 01/1108 from Fondo de Investigaciones Sanitarias (FIS).

193 Left Ventricular Diastolic Dysfunction Predicts Myocardial Strain in Cardiac Amyloidosis

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Background: While the degree of left ventricular diastolic dysfunction has been correlated with severity of cardiac amyloidosis, myocardial strain remains relatively unstudied. The purpose of this study was to determine the relationship between left ventricle diastolic dysfunction and myocardial strain in cardiac amyloidosis.

Methods: Eight patients with a diagnosis of cardiac amyloidosis underwent complete echocardiographic study. We used tissue Doppler imaging (TDI) to obtain longitudinal myocardial strain and myocardial strain rate. Patients were classified according to stage of diastolic dysfunction. Baseline clinical characteristics and echocardiographic findings were compared between these two groups.

Results: Three patients were classified as stage I (abnormal relaxation) and five were classified as stage III (restrictive) LV diastolic dysfunction. There were no significant differences in baseline characteristics between these groups. Echocardiographic findings for both groups are demonstrated in Table 1. There was a trend for decreasing myocardial strain as LV diastolic dysfunction worsened. Conclusions: As LV diastolic dysfunction worsens, longitudinal myocardial strain progressively decreases, which may imply decreased long-axis function in amyloidosis. Further larger studies are needed to confirm these findings.

<table>
<thead>
<tr>
<th>Table 1</th>
<th>Stage I LV diastolic dysfunction (n=3)</th>
<th>Stage III LV diastolic dysfunction (n=5)</th>
<th>P value</th>
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</thead>
<tbody>
<tr>
<td>Age (years)</td>
<td>68 ± 5</td>
<td>66 ± 10</td>
<td>0.774</td>
</tr>
<tr>
<td>LVEF (%)</td>
<td>55 ± 0</td>
<td>48 ± 16</td>
<td>0.492</td>
</tr>
<tr>
<td>Mitral Inflow Deceleration time</td>
<td>262 ± 63</td>
<td>146 ± 10</td>
<td>0.005</td>
</tr>
<tr>
<td>Avg Septal Strain (%)</td>
<td>15.4 ± 4.3</td>
<td>7.3 ± 1.6</td>
<td>0.073</td>
</tr>
<tr>
<td>Avg Lateral Strain (%)</td>
<td>9.8 ± 3.0</td>
<td>4.0 ± 2.4</td>
<td>0.053</td>
</tr>
</tbody>
</table>

194 BONE DENSITOMETRY IN PATIENTS WITH MULTIPLE MYELOMA

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Multiple myeloma (MM) is malignant disease characterised by skeletal involvement. Osteolytic lesions and osteoporosis are the major cause of morbidity in MM. Bone densitometry (DEXA) seems to be a useful method for monitoring of metabolic bone disease, predicting fracture risk and for assessment the efficacy of treatment on bone status.

Aims: to assess the bone involvement in patients with newly diagnosed multiple myeloma using DEXA, to assess the impact of degenerative changes and compressive fractures of vertebral bodies in lumbar spine BMD, to compare BMD before and after treatment.

Methods: Analysed group consisted of 106 patients with newly diagnosed MM. The male-to-female ratio was 51:55, the mean age was 59 years in men and 64 years in women. In the clinical stage I by Durie-Salmon were 25 patients, in the stage II were 38 and in III were 43 patients. BMD, T-score and Z-score of lumbar spine and whole body was measured by the LUNAR DPX-L scanner.

Results: Osteoporosis of lumbar spine (T-score below -2.5 SD) was presented in 25% patients, osteopenia in 29% and normal BMD in 46% patients. Bone mineral density expressed as Z-score was very low in lumbar spine, the mean value of Z-score was -1 SD (range -5.2 to +2.6 SD) and also in other trabecular bones (ribs, pelvis and the whole spine), but Z-score of cortical bones (lower and upper extremities) was statistically significantly higher (mean value +0.3 SD). Patients in clinical stage I had better bone mineralization than patients in stage II and III, in lumbar spine and trunk; but, density of arms and legs was significantly higher in clinical stage III. In lumbar spine, BMD was significantly higher in patients with large number of degenerative changes. In contrast, severity of vertebral bodies’ compressive fractures had not impact to measured BMD. In long-term follow-up the bone density of lumbar spine increased in patients treated by bisphosphonates and responding to chemotherapy, did not changed in patients not responding to therapy (or responding, but without bisphosphonates), and decreased in relapsing patients without bisphosphonates.

Summary: DEXA seems to be a sensitive technique for monitoring myeloma bone disease. Lumbar spine is the most often site of bone loss in MM, while long cotrical bones are involved not so often. In patients with clinical stage III is bone mineral density lower in lumbar spine and trunk, but higher in arms and legs. Osteophytes of vertebral bodies has an important impact to BMD, but compressed vertebral bodies did not increased bone density. Bone densitometry is also a useful way how to assess the efficacy of chemotherapeutic and anti-resorptive treatment.

195 Prognostic significance of Technetium –99m-MIBI scintigraphy in multiple myeloma.

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Objectives: The aim of this study was to evaluate the role of 99mTc-MIBI scintigraphy in the detection of myeloma lesions.

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There was done evaluation of intensity of MIBI and known markers of myeloma disease before and after therapy. Methods: 96 consecutive patients with MM and 28 patients with MGUS were evaluated. All patients were examined at the time of diagnosis and 29 patients after chemotherapy. Intensity of MIBI was scored as N-normal, D-diffuse, F-focal. Results: The uptake score of MIBI correlate with clinical status, activity of disease as determined by serum beta-2-microglobulin, monoclonal immunoglobulin level, lyphmidine-kinase, CRP, LDH, telopeptide ICTP, infiltration of bone marrow by plasma cells. (p < 0.05).

Focal type of MIBI scintigraphy indicated worse prognosis and was present in more advanced disease stage II and III according Durie-Salmon classification. In the group of 28 MGUS patients was found diffuse pattern of MIBI only in 2 patients, they are stable during two years observation period. In non secretory myeloma was MIBI reliable method for detection of focal involvement in soft tissues, which was proved by MRI, CT and cytological examination.

Conclusion: The use of 99mTc-MIBI scintigraphy is a reliable tool for the detecting and staging of MM disease, can detect multifocal involvement and is very useful especially in non-secretory and low secretory MM.

Supported by grant IGA Czech Republic, NC 6724-3/2001.

7.3 Prognostic models.

196 Prognostic factors in Multiple Myeloma

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Cancer Research UK Clinical Trials Unit, Department of Immunity and Infection

Patients with myeloma vary greatly in prognosis at presentation and those patients entered into different studies are not necessarily comparable. Lack of comparability particularly applies if studies using intensive chemotherapy are compared with trials using less toxic treatment. It is important to prospectively identify those who could tolerate prolonged chemotherapy and who may benefit from more aggressive regimens. Efforts to improve patient management in this disease have been assisted by identifying a number of prognostic factors, which are now routinely recorded in the UK Medical Research Council (MRC) myelomatisos trials. A number of prognostic indices have been developed in an attempt to divide patients into clearly defined prognostic groups. The Durie-Salmon index is the most widely used system but most MRC patients fall into the poor prognostic group. The Cuzick index and the use of serum 2 microglobulin both provide reliable means for dividing patients into prognostic strata, each containing a useful number of patients. However, there are several other potentially useful prognostic factors that could be incorporated into an improved staging system.

Prognostic factors have been assessed in the 999 patients randomised to receive ABCM (adriamycin, BCNU, cyclophosphamide, and melphalan) combination therapy in the Vth and VIth MRC myeloma trials. The Vth trial compared ABCM (314 patients) versus melphalan (316 patients) (Lancet, 1992 339: 200-205) between October 1982 and May 1986. The VIth trial assessed whether reduction in tumour bulk achieved by corticosteroids was useful given at the start of the ABCM regimen; 343 patients randomised to ABCM, 342 patients randomised to ABMCP between June 1986 and March 1991. Cross-trial survival analysis shows no difference between the ABCM treatments used in the Vth and VIth trials or with the addition of prednisolone. Hence the 999 patients can be pooled for the exploration of prognostic factors.

Log-rank analysis was performed on the twenty-three potential prognostic factors to identify the importance of each factor. Cox regression models were then applied to these factors firstly by grouping them into six main groups: 1) general factors measured by age, sex, performance status and serum albumin; 2) tumour burden or activity measured by bone marrow plasma cells, extramedullary involvement, paraprotein class and serum 2 microglobulin; 3) haemopoietic function measured by anaemia, thrombocytopenia, lymphopenia and neutropenia; 4) skeletal disease measured by bone pain, fractures, hypercalcaemia, serum phosphate, osteolytic lesions and osteoporosis; 5) alkaline phosphatase and other liver enzymes; 6) renal disease measured by serum creatinine and blood urea. This allows the importance of each factor to be considered in a similar group to explore correlations. This reduced the set down to thirteen potential factors which were considered in a final overall model. The final Cox regression model identified serum 2 microglobulin, corrected serum calcium, age, osteolytic lesions, platelets, performance status and bone marrow plasma cells as independent prognostic factors. C-reactive protein was also considered but was not an independent factor. This model is a useful tool to divide the patients into good, intermediate and poor prognostic groups, allowing the prediction of clinically useful prognostic groups.

197 Common Data Elements Development for Myeloma Trials: A Joint Project of the National Cooperative Cancer Clinical Trials Groups and NCI

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1Mayo Clinic, Rochester, MN, USA; 2Blood and Marrow Transplantation, Medical College of Wisconsin, Milwaukee, WI, USA; 3University Health Network, Princess Margaret Hospital, Toronto, ON, Canada; 4 Dana Farber Cancer Institute, Boston, MA, USA; 5EMMES Corporation, Rockville, MD, USA; 6National Cancer Institute, Bethesda, MD, USA.

Successful monitoring, analysis, and presentation of clinical trials are largely dependent on the data collected through the generated study forms set, which identifies the content and timing of protocol-specific data to be collected for each patient. This is challenging for trials in hematologic malignancies that use more than one set of staging and/or response criteria which are ever changing and differ between investigative groups. In an initiative sponsored by the National Cancer Institute (NCI) to standardize and simplify the collection and reporting of data for clinical trials, a Common Data Elements (CDE) committee was organized to generate standardized forms for multiple myeloma, amyloidosis, and Waldenstrom’s macroglobulinemia. This committee included statisticians, physicians, and data managers with representation from the major North American cancer cooperative groups involved in this research (ECOG, NCIC, NCCIT, SWOG, ABMTTR/IBMTR) as well as representatives from NCI and the EMMES Corporation, an NCI contractor. The charge of the CDE project and of our committee was two-fold: to review data collection with the intent of eliminating unnecessary items, and to propose standardized definitions and uniform valid values for the required elements. These case report forms will be used in the phase III trials being implemented through the Cancer Trials
Support Unit (CTSU) with flexibility in adding standardized data elements to address the specific objectives of a study. The CDEs will serve as a foundation for data forms generation in all NCI-sponsored clinical trials across all diseases. A process has also been established to systematically review and revise data collection standards as necessary. The standardized data elements and forms generated for multiple myeloma, amyloidosis, and Waldenström’s macroglobulinemia will be presented, and the CDEs and forms will be made publicly available on the NCI web site:


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A PROSPECTIVE ANALYSIS OF A POPULATION
STUDY 622 UNSELECTED PATIENTS PRESENTING
WITH MULTIPLE MYELOMA IN HAEMATOLOGY UNITS
IN A LARGE UNITED KINGDOM, NATIONAL HEALTH
SERVICE REGION

Phekoo K, Møller H, Richards M, Bevan D, Gillett D, Streetly M, Bell S, Fields P, Schey S on behalf of the South Thames Haematology Specialist Committee

Guys & St Thomas’ NHS Trust, The Thames Cancer Registry, Guy’s & St Thomas’ NHS Trust, St George’s NHS Trust, Kent & Sussex NHS Trust, Guy’s & St Thomas’ NHS Trust, Thames Cancer Registry, Guy’s & St Thomas’ NHS Trust, Guy’s & St Thomas’ NHS Trust

A major limitation of randomised controlled trials is selection bias. We present a population-based analysis of patients diagnosed with Multiple Myeloma (MM).

The setting of the study was the South Thames area (1.5 million inhabitants), managed by 66 haematologists covering 27 Acute NHS Trusts. Haematologists confirmed cases of MM prospectively between 1999-2000, and recorded the paraprotein and the Salmon-Durie stage. Haematology data officers from the Thames Cancer Registry (TCR) collected data on haematological and biochemical laboratory results. The cause and date of death were obtained from the Office of National Statistic by the TCR. Survival analysis was performed using the Wilcoxon test and the Cox Regression model. We present a population-based analysis of patients presenting with MM in this series is 73 years. Most clinical trials have been designed for patients <65 years and treatment strategies should be reviewed in light of this changing epidemiology.

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The Belgian Epidemiological study on Multiple Myeloma

De Plaen, P. (1)Michaux, J.-L. (2)Van Oy en, H. (1)

(1) Scientific Institute of Public Health(2) Université Catholique de Louvain

This study is essentially an evaluation of the incidence rate of the multiple myeloma in three Belgian sub-regions. The inclusion criteria were presence of a monoclonal protein detected at the electrophoresis completed by plasma cells smears and other biological, radiological and pathological types of information. The basis of case inclusion by clinical experts was the clinical staging system proposed by Durie and Salmon.

The study started on the 1 May 1994. All new cases of multiple myeloma were systematically registered in the populations of Hainaut, Namur and Walloon Brabant provinces. Afterwards, the inclusion procedure has been completed by microscopic examination of bone marrow biopsy results. This additional research allowed to include the first non-collected cases by clinical laboratories.

All records were coded. Duplicate recording were recognized when residence (post code), gender, age and monoclonal component were similar.

The study is multicentric. A group of experts uniformised the analytical approach by looking at the different measuring methods in use. The number of multiple myeloma is 495 (474 recent diagnoses and 8 plasmocytoma with evolution to a myeloma during the observation period, from 1 May 1994 until 30 April 1999. The gender ratio is 1.004 (female = 248 and male = 247).


The median age is 69 years, ranging from 35 to 93 years.

We report an incidence of 4.5/100,000 per year. This may reflect improved ascertainment rates in the elderly population by engaging haematologists in the reporting process. Median survival in this series compares favourably (30 vs. 31 months) with a single centre study (Lacy et al. 1999) at the Mayo clinic conducted between 1985-1998. Whilst only 2.2% of patients were entered into clinical trials the overall survival reported in the <65 years cohort is comparable (41.6 vs. 48.5 months) to the MRC Myeloma VII trial. However, the median age of patients presenting with MM in this series is 73 years. Most clinical trials have been designed for patients <65 years and treatment strategies should be reviewed in light of this changing epidemiology.
Presentation and outcome of multiple myeloma in elderly patients: A prospective single center study

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(1) Unité d’Hématologie, Département de Médecine Interne, Centre Hospitalier, Blois, France (2) Centre d’Investigation Clinique, Faculté de Médecine, Tours, France

Few data exist concerning multiple myeloma (MM) in elderly patients (pts). We reported a multicenter retrospective study including pts with MM aged 75 years or more at diagnosis (Rodon et al, Eur J Haematol 2001; 66: 11-17). Median survival was 22 months. Age ≥85 years, Performance Status (PS) ECOG ≥2 and creatinine level ≥120 mmol/l were identified as independent prognostic factors. However, inclusion bias were possible.

In order to confirm these results, we analyzed survival and prognostic factors of all consecutive elderly pts with MM included in a prospective database in a single center from January 1985 to December 2002. One hundred and three pts were included (M: 51, F: 52). Median age at diagnosis was 81 years (range 75-97). Twenty six pts were 85 years or older. According to the Durie and Salmon (DS) staging, 26 pts had stage I, 23 pts stage II and 53 pts stage III MM. Immunochemical subtype was IgG in 58 pts, IgA in 31 pts, light chain in 11 pts, other in 3 pts. Light chain was kappa in 62 pts, lambda in 40 pts, absent in 1 pt. Forty five significant comorbid diseases were recorded (cardiovascular 16, neurological 9, 2nd malignancy 8, other 12) in 41 pts. Infection at diagnosis was found in 23 pts. Twenty seven pts remained untreated [asymptomatic DS stage I: 18 pts, stage II-III: 9 pts (heavy comorbidity: 3, poor condition: 3, other: 3)]. In 76 treated pts, initial treatment was a combination of an alkylating agent and corticosteroids in 69 pts, other in 7 pts. Response was defined as a reduction ≥25% in M spike level. Forty five pts were responders, 10 pts were non responders, early death (ED) (<3 months after diagnosis) occurred in 13 pts and 8 pts were not assessable for response. Median survival of the 103 pts was 14 months [95% confidence interval (95IC) 11.6-27]. Twenty one pts (20.4%) had ED. Survival was 51.1% at 1 year, 27.6% at 3 years. In univariate analysis, age ≥85 years (p<0.001), PS ≥2 (p<0.001), albumin level <30 g/l (p<0.001), infection at diagnosis (p=0.002), high LDH level (p=0.002), beta 2 microglobulin >4 mg/l (p=0.008), creatinine ≥120 mmol/l (p=0.013), platelet count <100x10^9/l (p=0.027) and comorbidity (p=0.042) were unfavourable prognostic factors. In multivariate analysis, PS ≥2 [hazard ratio (HR) 2.84 (95IC 1.65-4.88), p<0.001], creatinine ≥120 mmol/l [HR 2.51 (1.45-4.35), p=0.001], age ≥85 years [HR 2.36 (1.31-4.26), p=0.004] and platelet count <100x10^9/l [HR 2.56 (1.03-6.33), p=0.042] were independent prognostic factors. DS stage II-III was paradoxically favourable compared to stage I [HR 0.51 (0.27-0.97), p=0.039], because of heavy comorbidity in stage I pts with a higher rate of death unrelated to MM (p<0.001).

We conclude that, compared to a previous retrospective multicenter study, the present prospective single center study shows a shorter survival, probably related to the omission of some severely ill pts in the former. We confirm that PS, age and creatinine level are independent prognostic factors. In addition, a low platelet count is also a predictor of short survival.

VALIDATION OF DURIE SALMON STAGE, THE SOUTHWEST ONCOLOGY GROUP (SWOG) AND ECOG STAGING SYSTEM FOR MULTIPLE MYELOMA

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INTRODUCTION. The survival of multiple myeloma (MM) patients varies widely from months to greater than 10 years. The Durie Salmon staging system does not fully explain this survival variation. Other systems have been proposed but not has been widely adopted. A SWOG system employing beta-2 microglobulin (β2M) and albumin (Jacobson et al, ASH 2001) and, recently, an ECOG system employing β2M and platelet count (Greipp et al, ASH 2002) have been employed but their prognostic value in MM has not been well demonstrated.

OBJECTIVE. Validate these new staging systems (SWOG and ECOG).

PATIENTS-METHODS. We have revived data from 63 pretreated MM patients for both serum albumin and β2M and for both platelet count and β2M. Survival was measured from the diagnosis. We have distributed these patients according with Durie Salmon, SWOG and ECOG stage systems and studied their correlation with survival.

RESULTS

<table>
<thead>
<tr>
<th>% of patients</th>
<th>Median survival (months)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Durie-Salmon Stage:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I 10</td>
<td>55.6 ± 20.9</td>
<td>Log-Rank (0,4)</td>
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<tr>
<td>II 27</td>
<td>42.2 ± 7</td>
<td>Breslow (0,7)</td>
</tr>
<tr>
<td>III 63</td>
<td>32 ± 4</td>
<td>Tarone-Ware (0,5)</td>
</tr>
<tr>
<td><strong>SWOG Stage:</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>I 11</td>
<td>52 ± 15</td>
<td>Log-Rank (0,7)</td>
</tr>
<tr>
<td>II 56</td>
<td>38.2 ± 6</td>
<td>Breslow (0,7)</td>
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<tr>
<td>III 29</td>
<td>32.4 ± 6</td>
<td>Tarone-Ware (0,7)</td>
</tr>
<tr>
<td>IV 4</td>
<td>13 ± 5</td>
<td></td>
</tr>
<tr>
<td><strong>ECOG system:</strong></td>
<td>Log-Rank (0,01)</td>
<td></td>
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<tr>
<td>Poor risk*</td>
<td>12</td>
<td>14.1 ± 6.9</td>
</tr>
<tr>
<td>Standard risk</td>
<td>88</td>
<td>38.6 ± 4.6</td>
</tr>
</tbody>
</table>

* Poor risk = platelets < 150,000 and β2M >=4.0.

CONCLUSION. ECOG staging system is a simple, reliable and widely applicable model to identify poor-risk patients.

SURVIVAL ANALYSIS IN ELDERLY PATIENTS WITH MYELOMA. WHAT CAN WE DO TO IMPROVE?

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Background: Multiple myeloma (MM) remains incurable using conventional therapies, raising the need for an alternative approach, especially in the elderly. The increase in the ageing population is going to emphasize the problem. Nowadays, life expectancy in Córdoba, Andalucia (South of Spain) is 75.99 yrs for men and 82.23 for women. Our community hospital attends a rural population of 87,000 inhabitants.

Methods: From 01/93 to 01/03 we have analyzed all patients (pts) diagnosed with MM in our center. We have measured at diagnosis standard prognostic factors like β2 microglobulin...
203 Serum free light chain assay assessment in patients with relapsed refractory myeloma receiving treatment with a Thalidomide analogue (CC-4047).

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Relapsed myeloma remains a very difficult therapeutic challenge. Newer treatments have emerged including immunomodulatory drugs such as the thalidomide analogue CC-4047 (ACTIMID®, Celgene). The ability to monitor disease response and predict which patients may benefit from this therapy is important to planning treatment. A new assay for myeloma assessment is the measurement of serum free light chains (SFL). This has been demonstrated to have greater sensitivity than conventional assays for monitoring disease status and is a much truer reflection of the activity of myeloma cells given the shorter half-life of light chains as compared to whole immunoglobulins.

Method: We undertook a retrospective study of SFL in a cohort of patients undergoing treatment for relapsed myeloma with the immunomodulatory drug CC-4047. 21 patients (age range 47-83 years, median 67 years) were studied, and serum was analysed using a latex enhanced immunoassay (Freelite™). 17/21 patients had IgG kappa and 4/21 IgG lamda paraprotein. The median dose of CC-4047 administered was 2mg. Time points for sampling of serum was pre dose, day 7, day 14 and day 28.

Results: Using conventional assays 12/21 patients had evidence of stable disease (<25% reduction in paraprotein), 6/21 a partial response (25-75% reduction), 2/21 a very good partial response (> 75% reduction) and 1/21 progressive disease. The overall response rate was 38%. 12 patients had SFL analysis pre and at day 7 with 10/12 patients showing a response (<25% reduction). 10 of these 12 patients had day 28 SFL analysis with 3/10 patients continuing to respond, 1 patient showing an increase in SFL and 6 patients showing no further improvement in SFL levels. Paraprotein measurements taken at day 28, showed a decrease in value (>25%) in 8/21 patients (38%). This was compared directly to the free light chain results. Of these 8 patients, 5 had either a day 7 or day 14 sample for sfl analysis, and all 5 patients showed a response in SFL levels by day 14. A further 5 patients showed a delayed response (>28 days) to treatment with a median time to best response of 5 months. In this group 4 patients had day 7 or 14 samples for sfl levels which showed a reduction in 3 patients and no change in 1 patient.

Conclusions: The use of the serum free light chain assay at early time points in the treatment regime may predict for overall response rate. Further studies are being undertaken to validate these findings to see if an early fall in SFL levels can be used to stratify those patients who will respond well to treatment with the IMID CC-4047.

204 PROGNOSIS IN MULTIPLE MYELOMA (MM) UNDER CONVENTIONAL CHEMOTHERAPY. A MULTIVARITE ANALYSIS OF A SERIES INCLUDING 644 PATIENTS.


Background: Many clinical and biological variables are of prognostic value in MM. However, most studies are incomplete and lack on the analysis of several important variables.

Methods: In this study we analyzed the prognostic value of several clinical and biological characteristics at diagnosis and their impact on the overall survival in a series of 644 MM patients treated without high dose therapy.

Results: The univariate analysis of the whole series identified 22 parameters studied at diagnosis that negatively influenced overall survival: age >60 yr, plasma cell (PC) infiltration >10% assessed by flow cytometry (FC) or >20% by morphology, ECOG performance status ≥2, S-phase PC >3%, 2M >6 mg/L, LDH >460 U/L, albumin ≤3.5 g/dL, advanced osteolytic bone lesions, Durie & Salmon stage III, only light chain secretion, light chain subtype, creatinine >2 mg/dL, urea >65 mg/dL, CRP >6 mg/dL, calcium ≥11.5 mg/dL, light chain proteinuria >1.8 g/day, hemoglobin ≤8.5 g/dL, platelets 100-109/L, plasma cell DNA index <1, presence of <13 and absence of >9. After multivariate analysis, only seven factors kept an independent prognostic influence: S-phase PC >3%, BMPC by FC >10%, ECOG 3, age >60 yr, β2M >6 mg/L, LDH >460 U/L and albumin ≤3.5 g/dL. The combination of these seven factors allows making a powerful prognostic system for the whole series discriminating three different prognostic groups.

Conclusions: The present analysis of prognostic factors displayed seven variables with an independent influence on survival in MM reflecting that it remains as a heterogeneous disease. These factors could be categorized into three groups: related to the host age, key biological parameters and stage.
Results: The median level of tumor cells in the BM was 0.04% of a tumor specific target, and quantitation of the tumor load was immunoglobulin heavy chain gene variable sequence was used as unselected, were included in this study. The myeloma followed by autologous PBSCT, either CD34+-selected or

Patients and Method: Sixty-six patients with MM treated by HDT

Background: High dose chemotherapy (HDT) with autologous hematopoietic stem cell transplantation (PBSCST) significantly improves survival in multiple myeloma (MM) patients. In a subgroup of MM patients, early relapses occur after HDT and autologous transplantation. In order to investigate whether the amount of residual tumor cells in the bone marrow (BM) after transplantation can predict the duration of response, a quantitative PCR assay was used to measure tumor load in BM at 3 to 6 months post-transplant.

Patients and Method: Sixty-six patients with MM treated by HDT followed by autologous PBSCST, either CD34+-selected or unselected, were included in this study. The myeloma immunoglobulin heavy chain gene variable sequence was used as a tumor specific target, and quantitation of the tumor load was performed by limiting dilution.

Results: The median level of tumor cells in the BM was 0.04% of the total mononucleated cell fraction at 3 months post-HDT (n=59), and 0.021 at 6 months (n=32). Using maximally selected logrank statistics, a post-HDT BM tumor load cutoff value with respect to progression-free survival (PFS) was identified (p=0.001). The threshold for placing patients into a good or bad prognostic group was found to be 0.015%. Median PFS was 61 vs. 15 months For multivariate analysis, Beta2-microglobulin at diagnosis, number of high-dose therapy cycles (HDT), best response after PBSCT (CR versus PR/MR) and grouping by qPCR result were included in a Cox proportional hazard model as potentially prognostic parameters. Grouping by PCR result was found to be an independent and strong predictor of PFS (hazard ratio, 3.54).

This study demonstrates for the first time a threshold of the post-HDT tumor load with prognostic significance regarding PFS in MM. Quantitative molecular assessment discriminates between low and high risk groups of patients as early as 3 to 6 months after autologous transplantation, and thus helps to identify those patients who are in need of further treatment after autologous transplantation.

205 Quantitation of the post-transplantation tumor load in the bone marrow by PCR with allele-specific oligonucleotide primers is a prognostic parameter in multiple myeloma

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Background: PCR with allele-specific oligonucleotide primers is a prognostic parameter in multiple myeloma. Recently, real-time PCR assays have been developed in order to quantify the number of tumor cells after treatment. However, these strategies are hampered by the presence of somatic hypermutation in VDJH rearrangements from MM patients, which causes mismatches between primers and/or probes and the target, leading to a non accurate quantification of tumor cells. Our group has recently described a 60% incidence of incomplete DJH rearrangements in MM patients, with no or very low rate of somatic hypermutation. In this study, we compare the efficiency of a real-time PCR approach for the analysis of both complete and incomplete IgH rearrangements in eight MM patients using only three JH consensus probes. We were able to design an allele specific oligonucleotide for both the complete and incomplete rearrangement in all patients. DJH rearrangements fulfilled the criteria of effectiveness for real-time PCR in all samples (i.e. no unspecific amplification, detection of less than 10 tumor cells within 105 polyclonal cells and correlation coefficients of standard curves higher than 0.98). By contrast, only three out of eight VDJH rearrangements fulfilled these criteria. Further analyses showed that the remaining five VDHJ rearrangements carried three or more somatic mutations in the probe and primer sites, leading to a dramatic decrease in the melting temperature. These results support the use of incomplete DJHJ rearrangements, instead of complete somatically mutated VDJH rearrangements for investigation of minimal residual disease in multiple myeloma.

206 UNMUTATED INCOMPLETE DJH REARRANGEMENTS AS PREFERENTIAL TARGET FOR REAL-TIME PCR QUANTIFICATION IN MULTIPLE MYELOMA.


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The hypervariable regions of immunoglobulin heavy chain (IgH) rearrangements provide a specific tumor marker in multiple myeloma (MM). Recently, real-time PCR assays have been developed in order to quantite the number of tumor cells after treatment. However, these strategies are hampered by the presence of somatic hypermutation in VDJH rearrangements from MM patients, which causes mismatches between primers and/or probes and the target, leading to a non accurate quantification of tumor cells. Our group has recently described a 60% incidence of incomplete DJH rearrangements in MM patients, with no or very low rate of somatic hypermutation. In this study, we compare the efficiency of a real-time PCR approach for the analysis of both complete and incomplete IgH rearrangements in eight MM patients using only three JH consensus probes. We were able to design an allele specific oligonucleotide for both the complete and incomplete rearrangement in all patients. DJH rearrangements fulfilled the criteria of effectiveness for real-time PCR in all samples (i.e. no unspecific amplification, detection of less than 10 tumor cells within 105 polyclonal cells and correlation coefficients of standard curves higher than 0.98). By contrast, only three out of eight VDJH rearrangements fulfilled these criteria. Further analyses showed that the remaining five VDHJ rearrangements carried three or more somatic mutations in the probe and primer sites, leading to a dramatic decrease in the melting temperature. These results support the use of incomplete DJHJ rearrangements, instead of complete somatically mutated VDJH rearrangements for investigation of minimal residual disease in multiple myeloma.

207 Quantification of minimal residual disease by real-time IgH-PCR using TaqMan chemistry together with LightCycler technology predicts relapse in multiple myeloma


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Background: In multiple myeloma (MM) PCR with allele-specific oligonucleotides (ASO) for immunoglobulin H (IgH) provides a method for detection of minimal residual disease (MRD). Real-time PCR using LightCycler technology offers the opportunity to quantify MRD. In general, two hybridization probes are used for specific detection of the product during real-time PCR. Since the PCR products in IgH-PCR are too small for the opportunity to quantify MRD. In general, two hybridization probes are used for specific detection of the product during real-time PCR. Since the PCR products in IgH-PCR are too small for
plasmids carrying the clonotypic insert were normalized by parallel detection of β-actin copy numbers in the corresponding sample.

Results: Sensitivity was 10E-4 to 10E-5 with linear correlation coefficients of r≥0.98 for all standard curves and r=0.93 and r=0.99 for inter- and intraassay variability. Accuracy was confirmed by spiking experiments with dilutions of U266 myeloma cell line in normal mononuclear cells. For clinical validation we used the method to monitor residual disease in peripheral blood (PB) and bone marrow (BM) of 11 MM patients following autologous and/or allogeneic peripheral blood stem cell transplantation (PBSC T). The intraindividual quantification of myeloma cells in BM and PB samples obtained at the same time showed a median 58-fold higher tumor load in samples from BM in comparison with PB as reflected by an IgH/β-actin ratio of 0.4% and 0.002% (p<0.001), respectively.

Serial analyses of IgH/β-actin showed a significant reduction of clonotypic cells of 2 log comparing pretreatment values and those of best response in BM (median 5% to 0.09%, p=0.003) and PB (median 0.04% to 0%, p=0.03). In 5 patients who were in durable remission stable amounts of clonotypic cells at a low level could be observed in PB. In 6 patients with relapse an increase of IgH/β-actin ratio to 2% in BM and to 0.6% in PB was found at time point of relapse. In 4 of these 6 patients an at least 10-fold increase of the IgH/β-actin ratio in PB was detectable in median 3 months (range: 0.5-6) before relapse.

Conclusion: Quantification of MRD in MM can be performed reliably with real-time IgH-PCR using ASO-TaqMan probes adapted to the LightCycler system. The number of clonotypic cells both in BM and PB correlates with stage of disease and an increase in PB during remission predicts relapse. This method permits the evaluation of the prognostic significance of residual malignant cells after therapy and can be used as a basis for stratification or therapeutic interventions.

208 Real Time Quantitative Polymerase Chain Reaction (RQ-PCR) of DJH / VDJH rearrangements is an useful tool to monitor Minimal Residual Disease (MRD) in Multiple Myeloma (MM) patients undergoing autologous peripheral blood stem cell transplantation (ABPSCT).

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Multiple Myeloma remains an incurable disease although the use of high dose therapy followed by ABPSCT provides a high complete remission rate. However, patients achieving this status can not be cured and many of them will ultimately relapse due to the persistence of MRD. The hypervariable regions of immunoglobulin heavy chain (IgH) rearrangements provide a specific tumor marker in multiple myeloma (MM). Several molecular methods have been used to study MRD in MM, including the use of RT-PCR of such clonospecific rearrangements. However, these strategies have long been hampered by technical pitfalls that have led to a non accurate quantification of tumor cells.

Our group has recently described a new strategy including the use of incomplete DJH rearrangements, which are very frequent in MM patients. Such strategy allows the specific quantification of at least 1 tumor cell within 105 polyclonal cells. In this work we have use this strategy to monitor the presence of monoclonal plasma cells in the bone marrow of 10 patients who achieved complete remission (irrespective to the immunofixation -IFX-result) three months after the use of polychemotherapy and APBSCT (Spanish Protocol GEM-2000). The results correlated with the IFX status, since IFX- patients (n=6) had a mean of 2 clonal cells per 105 normal cells while the patients with positive IFX showed an MRD mean of 12 clonal cells per 105 normal cells (p=0.048). Patients were divided into two groups: MRD+ if they had an MRD was detected higher than 10-4 (n=4), and MRD- if it was lower (n=6). MRD patients were mainly characterized by the presence of less cases of positive IFX (17% vs. 75%) and a better event free survival (32 vs. 11 months, p=0.0046). With a median follow-up of 30 months, no deaths have been observed yet, so no estimations on overall survival can be made.

209 PERIPHERAL BLOOD STEM CELLS CONTAMINATION EVALUATED BY A HIGHLY SENSITIVE MOLECULAR METHOD FAILS TO PREDICT OUTCOME OF AUTOTRANSPLANTED MULTIPLE MYELOMA PATIENTS.

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To evaluate the clinical impact of minimal residual disease in multiple myeloma, apheresis products from 51 autotransplanted patients were tested by fluorescent (GeneScan) PCR. Sixty-nine percent of harvested resulted contaminated when evaluated for IgH rearrangement. Forty-six patients responded to transplant, with 52.9% of CR. The clinical response resulted significantly conditioned by the number of CD34+ reinfused cells. PCR positivity of reinfused harvests was not significantly related to patients’ outcome.

Median OS resulted of 33 months, with a significant advantage for patients transplanted by 12 months from diagnosis. Moreover, OS was longer for patients receiving PCR-negative stem cells, with 72% of patients surviving at 70 months in the group receiving PCR-negative harvests versus 48% in the group transplanted with contaminated precursors (p=n.s.). Ex vivo purging allowed a reduction of contamination up to 3 logs; nevertheless, 80% of purged harvests remained PCR-positive and purging procedure did not condition response or survival rates. Thus, the failure of predictive role of a high sensitive molecular method could be explained by the assumption that in vivo persisting malignant cells are the real source of relapse in MM.
Monitoring of minimal residual disease in the bone marrow of patients with multiple myeloma after allogeneic stem cell transplantation by combined morphological and cytogenetic analysis

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The detection and monitoring of minimal residual disease (MRD) in patients (pts) with multiple myeloma (MM) after allogeneic stem cell transplantation (SCT) is crucial for clinical decision making. The need for DLI or aditional therapy is mainly based on the determination of disease status at that stage. However, the definition of CR and MRD status in MM is often difficult and uncertain. This difficulty also exists when a chromosomal aberration is found in patients bone marrow. FISH is considered the study of choice for these abnormalities, however positive results in the range of up to 10% of bone marrow cells are frequent (background). We recently described the use of the combined morphological and FISH analysis by the DUET™ system (Bioview Ltd, Israel) for more precise determination of the chromosome 13 status in MM pts. By this method we could determine the lineage of the cells carrying chromosome 13q deletion (del13q) and detect the true positive FISH signs from the false positive signs that emerged from non-plasma cells. A minute population of plasma cells with del13q can be identified by this system.

Methods: We monitored MRD by the combined system for del 13q or rearrangement at chromosome 11 and 14 in the BM of 7 MM patients (1 in clinical PR, 6 in clinical CR) after reduced intensity SCT. Complete cytogenetic remission (CCR) was defined by the absence of plasma cells bearing an aberration.

Results: The patient in PR had a positive study for del13q while with 100% donor chimerism defined by microsatellite study. In 4 pts with clinical CR, 2 with 100% chimerism and 2 with mixed chimerism, CCR was defined and maintained for 18, 16, 12 and 7 months. The chimerism status converted to 100% donor in the two patients with mixed chimerism 2 and 5 months later. In one patient whith clinical CR, a CCR was initially defined, but after 6 months the combined study detected 2% of true positie cells. During this period the chimerism status was defined 100% donor. A clinical progression was evident in this patient three months later. One patient in clinical CR had an initial study with 1.5% positive cells while 100% donor chimerism by microsatellite study. Immunofixation test converted positie in this patient 4 months later.

Conclusions: The combined morphology and FISH study can serve as a powerful tool for the precise determination of MRD status in pts with MM after SCT. This method can overcome obstacles in the definition of CR that are specific to this group of patients. Chimerism status may not be an obvious equivalent of the MRD status in patients with MM after SCT with reduced intensity conditioning.
newly diagnosed multiple myeloma as compared to the bone marrow of healthy controls and of patients with MGUS. A few studies, however, have documented bone marrow angiogenesis to be a predictor of survival in patients with newly diagnosed multiple myeloma.

The aims of the study were to:

evaluate three different techniques to estimate bone marrow angiogenesis,
investigate the relation between pro-angiogenic cytokines and bone marrow angiogenesis,
screen relations between bone marrow angiogenesis and known prognostic factors in patients with multiple myeloma, and
look for prognostic impact of estimates of bone marrow angiogenesis in these patients.

Material and Methods: Sixty-seven patients with newly diagnosed multiple myeloma were included in this study. The median follow-up time was 99 months (range 33-156 months).

Paraffin-embedded bone marrow samples, aspirates and/or biopsies, from the time of diagnosis were investigated. Sections of the specimens were stained with hematoxylin and eosin (HE), and immunohistochemically stained for CD34 to identify micro vessels. The marrow’s angiogenic density was determined by three separate methods: 1) vascular grading, 2) micro vessel density (MVD), and 3) Chalkley counting.

The concentrations of interleukin-6 (IL-6), basic fibroblastic growth factor (bFGF), hepatocyte growth factor (HGF) and Syndecan-1 in blood and bone marrow were analysed using commercial kits.

Results: Positive correlations were found between the three different methods: 1) vascular grading, 2) micro vessel density (MVD), and 3) Chalkley counting.

The strategies of interleukin-6 (IL-6), basic fibroblastic growth factor (bFGF), hepatocyte growth factor (HGF) and Syndecan-1 in blood and bone marrow were analysed using commercial kits.

The overall survival was significantly poorer for patients with bone marrows showing a high vascular grade as also demonstrated a higher number of micro vessel profiles per mm2 and higher Chalkley count than patients with bone marrow showing a low or an intermediate vascular grade. No correlations were demonstrated between the estimates of angiogenesis and the concentrations of IL-6, bFGF and HGF in blood and bone marrow. A positive correlation was seen between the estimates of angiogenesis and concentrations of Syndecan-1 in blood. The overall survival was significantly poorer for patients with bone marrows showing a high vascular grade or a high MVD, and furthermore, all three estimates of angiogenesis showed prognostic significance when tested in a multivariate Cox analysis.

Conclusion: Our study documents that the simple technique of vascular grading may represent an efficient alternative to MVD or Chalkley estimation of bone marrow angiogenesis. Moreover, the prognostic evaluation demonstrates that a high vascular grade, a high MVD or a high Chalkley count may represent predictors of poor survival in patients with newly diagnosed multiple myeloma.

213 Incidence, clinical features and risk factors of blood stream infection in multiple myeloma

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Although blood stream infection (BSI) is a major complication of multiple myeloma (MM), investigations assessing its incidence and risk factors are still lacking. We reviewed cases of positive blood cultures in 172 consecutive patients (pts) with MM hospitalized in our institution between January 1987 and January 2003 and included in a prospective database. The incidence of BSI was calculated relative to the number of pt-days of follow-up according to disease phase: asymptomatic phase (asymptomatic and untreated MM), initial symptomatic phase (from diagnosis of symptomatic MM to end of first-line chemotherapy), remission phase (remission after completion of first-line chemotherapy), advanced phase (from 1st relapse to death).

Sixty five episodes of positive blood cultures were identified. Twenty were considered contaminant and excluded. Forty five episodes of BSI occurred in 35 pts (1 episode: 27 pts, 2 episodes: 6 pts, 3 episodes: 2 pts). Twenty three were community-acquired and 22 were nosocomial. Microbiological isolates were gram positive: 21, gram negative: 17, anaerobes: 3, yeast: 1, polymicrobial: 3. Primary source of infection was lung in 20%, urinary tract in 11.1%, other in 17.8%, unknown in 51.1%. Ten episodes (22.2%) occurred during chemotherapy-induced neutropenia (neutrophil count <1000/mm3). The global incidence was 0.43/1000 per pt-day. No significant difference was found according to age at diagnosis (<75 years vs ≥75 years, p=0.058), sex (p=0.48), or immunohematological subtype (IgG vs others, p=0.085). As compared to advanced phase, the incidence of BSI was significantly lower in asymptomatic phase [differential incidence (DI) –0.74/1000 pt-day, 95% confidence interval (95CI) –1.71; –0.33, p=0.004, in initial symptomatic phase [DI –0.46/1000 pt-day (95CI –0.88; –0.06), p=0.032] and in remission phase [DI –0.82/1000 pt-day (95CI –1.19; –0.47), p=0.001]. In treated pts, the incidence of BSI was higher when high-dose dexamethasone was included in the treatment regimen [DI 0.6/1000 pt-day (95CI 0.06; 1.19), p=0.026]. No BSI was recorded during high-dose therapy with autologous stem cell transplantation, performed in 16 pts. Death within 30 days following the diagnosis of BSI occurred in 13 episodes (28.9%). Death rate at the first episode was 14.3% in gram positive BSI, 42.9% in others (p=0.074).

We conclude that BSI mainly occurs in advanced phase of MM. The incidence increases with treatment including high-dose dexamethasone. Mortality is high.
Suppressor of Cytokine Signalling-3 (SOCS3) is a key physiological negative regulator of IL-6

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Because IL-6 is a key modulator of myeloma cell survival and proliferation, identification of intracellular negative regulators of IL-6 signalling is an important step in the process of developing novel therapies. The SOCS family of proteins are attractive candidates as physiological regulators of IL-6 signalling. SOCS1 and SOCS3 are highly homologous and the expression of both is induced by IL-6 in both lymphoid and myeloid cells. In overexpression systems, both can inhibit STAT activation after gp130 ligation by IL-6. However, IL-6 signalling is not perturbed in primary cells from SOCS1 knockout mice. We have therefore concentrated on investigating the role of SOCS3 in IL-6 signalling in primary haemopoietic cells.

A conditional gene targeting strategy was pursued because SOCS3 knockout mice die early in utero of placental failure. We have generated mice in which either haemopoietic or hepatic cells have no functional SOCS3 alleles, by using mice bearing a Lox-P flanked allele of SOCS3 (ki) and a null allele (o), and intercrossing them with mice expressing Cre recombinase selectively in haemopoietic cells (LysMCre or VavCre) or hepatic cells (AlbCre) respectively.

Hepatocytes from AlbCre+SOCS3fl/o do not express SOCS3. Similarly, macrophages from LysMCre+SOCS3fl/o mice and all haemopoietic cells from VavCre+SOCS3fl/o mice are totally deficient in SOCS3. In all SOCS3 deficient cells from these mice, marked abnormalities in IL-6 signalling and cellular responses were observed. Following either in vitro or in vivo stimulation with IL-6, STAT3 phosphorylation was both increased and prolonged. Microarray analysis confirmed this excess signalling resulted in aberrant target gene transcription. Accordingly, cellular responses to IL-6 were amplified in the absence of SOCS3: (i) serum acute phase proteins were increased in AlbCre+SOCS3fl/o mice; (ii) IL-6 inhibition of macrophase proliferation was augmented; and (iii) myeloid progenitor cell proliferation was increased.

These data unequivocally prove that SOCS3 is a key physiological negative regulator of IL-6 signalling. Whether the absence of SOCS3 increases plasmacytoma development or progression in murine models is being addressed.

8. Mouse models for MM

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Multiple myeloma (MM) is characterized by the proliferation of malignant plasma cells (PC’s) in the bone marrow. We have undertaken a collaborative project to target gene deregulation that will contribute to models of plasma cell malignancy in the mouse. Specifically, our project seeks to address three goals: 1) Identify novel transcriptional regulators whose activity is limited to B-lymphoid cells in late developmental stages; 2) Develop new mouse models of B-lymphoid malignancies, including MM, using transcriptionally targeted transgenes; and 3) Develop novel strategies to traffic plasma cytomagenesis in vivo. While no pathognomonic lesion has been identified in MM, upregulation of anti-apoptotic proteins (BCL-XL) and deregulation of growth-promoting oncogenes (MYC and N-RAS) are common in many MM patients. The 3’ kappa immunoglobulin (Ig) light chain enhancer (3’KE) regulates transcription of the kappa Ig gene, and its activity in murine B-cells is restricted to the late stages of B-cell development. The 3’KE was used to create a 3’KE/BCL-XL transgenic mouse. It was expected that this transgene would alter B-cell compartment composition, as the B-cells in late developmental stages would be rendered resistant to apoptosis. Indeed, we found significant increases in these cell populations, and nests of PC’s were found in the bone marrow of 3’KE/BCL-XL mice. Additionally, perivascular foci of PC’s with nuclear atypia occur in multiple soft tissues in aged 3’KE/BCL-XL mice, and other sequelae consistent with excess Ig production are common. To accelerate B-lymphoid malignancies, we crossed the 3’KE/BCL-XL mouse to an E/MYC transgenic mouse (these mice use the IgH E or E enhancers to drive MYC expression). While Eα activity begins early in B-cell development and continues throughout all other developmental stages, Eβ activity is developmentally restricted to late stages of development and is especially influential in governing heavy chain Ig expression in PC’s. Co-expression of BCL-XL and MYC under the context of the 3’KE and the E leads to a highly fatal B-lymphoid malignancy with a median survival of 5.5 weeks. When MYC expression is controlled by the E, however, fatal PC neoplasms in the bone marrow and other lymphoid organs develop with a median survival of 14.5 weeks. To facilitate visualization of normal and malignant PC’s, we are employing the use of the PC-GFP mouse, which uses kappa Ig regulatory elements to drive GFP expression in PC’s. To investigate the role of constitutively activated RAS expression in B-lymphoid malignancy, we have generated a 3’KE/N-RASV12 mouse. To elicit antigen-induced clonal expansion of the genetically altered plasma cells, we are immunizing the genetically engineered mice. In summary, deregulating apoptotic and oncogenic pathways in plasma and other B-cells by using novel transcriptional regulators in genetically engineered mice serves as a good platform to further the understanding of B-lymphoid malignancies, including MM.
IL-6 and Myc promote neoplastic plasma cell development. In developed mice are useful for studying the mechanisms by which expression can be tested.

Conclusions: Our findings demonstrate that IL-6 and Myc are neoplasia by interrupting IL-6 signaling or deregulated Myc addition, they afford a valuable pre-clinical model system in bone marrow involvement was common and often widespread. Approximately 20% of the gene-inserted IgHMycE mouse Ig heavy-chain locus, IgH, just 5' of the intronic enhancer, E3.

Results: All three transgenic mouse strains are characterized by the spontaneous development of plasmacytomas in extramedullary lymphoid tissues with secondary involvement of the bone marrow. Approximately 50% of BALB/c.H2-Ld-IL-6 mice developed IgG plasmacytomas in lymph nodes and spleen by 18 months of age. Virtually all tumors contained Myc-activating chromosomal translocations. Bone marrow infiltration with malignant plasma cells occurred at a late stage of tumor development (plasma cell leukemia). Doubly transgenic λ-MYC/IL-6 mice developed plasmacytomas in the gut-associated lymphoid tissue, often beginning in Peyer’s patches. Bone marrow involvement was variable. The mature plasmacytic phenotype of the λ-MYC mice (without the IL-6 transgene) developed lymphoblastic B-cell lymphoma resembling human Burkitt lymphoma. Approximately 20% of the gene-inserted IgHMycE λ mice developed spontaneous lymph node plasmacytomas by 21 months of age.

Bone marrow involvement was common and often widespread.

Conclusions: Our findings demonstrate that IL-6 and Myc are crucial for plasma cell tumor formation in mice. The newly developed mice are useful for studying the mechanisms by which IL-6 and Myc promote neoplastic plasma cell development. In addition, they afford a valuable pre-clinical model system in which pharmacological approaches to inhibiting plasma cell neoplasia by interrupting IL-6 signaling or deregulated Myc expression can be tested.

Purpose: Accurate mouse models of human plasma cell tumors are needed to study the events that are involved in the initiation and progression of these neoplasms and to test new intervention strategies that might lead to a better outcome. Based on the fact that the cellular oncogene, MYC, and the B-cell growth, differentiation and survival factor, IL-6, are key players in the pathogenesis of human plasma cell tumors including multiple myeloma, we decided to study plasma cell tumor development in mice that express a human IL-6 transgene and/or a human MYC or mouse Myc transgene in B cells.

Mouse models: Three different model systems were utilized to study IL-6 and MYC/Myc driven plasma cell tumor formation in mice. The first model is the BALB/c.H2-Ld-IL-6 congenic strain that harbors a human IL-6 transgene controlled by the widely expressed H2-Ld promoter. The second model is a doubly transgenic mouse that carries in addition to the IL-6 transgene of the first model a human MYC gene driven by the human Igλ enhancer (λ-MYC mice). The third model is a gene-targeted mouse, designated IgH-MycE, which was generated by inserting a histidine-tagged mouse Myc gene, MycHIs, into the mouse Ig heavy-chain locus, IgH, just 5' of the intronic enhancer, E3.

Results: All three transgenic mouse strains are characterized by the spontaneous development of plasmacytomas in extramedullary lymphoid tissues with secondary involvement of the bone marrow. Approximately 50% of BALB/c.H2-Ld-IL-6 mice developed IgG plasmacytomas in lymph nodes and spleen by 18 months of age. Virtually all tumors contained Myc-activating chromosomal translocations. Bone marrow infiltration with malignant plasma cells occurred at a late stage of tumor development (plasma cell leukemia). Doubly transgenic λ-MYC/IL-6 mice developed plasmacytomas in the gut-associated lymphoid tissue, often beginning in Peyer’s patches. Bone marrow involvement was variable. The mature plasmacytic phenotype of the λ-MYC/IL-6 tumors was apparently caused by the constitutive expression of IL-6, as singly transgenic λ-MYC mice (without the IL-6 transgene) developed lymphoblastic B-cell lymphoma resembling human Burkitt lymphoma. Approximately 20% of the gene-inserted IgHMycE λ mice developed spontaneous lymph node plasmacytomas by 21 months of age.

Bone marrow involvement was common and often widespread.

Conclusions: Our findings demonstrate that IL-6 and Myc are crucial for plasma cell tumor formation in mice. The newly developed mice are useful for studying the mechanisms by which IL-6 and Myc promote neoplastic plasma cell development. In addition, they afford a valuable pre-clinical model system in which pharmacological approaches to inhibiting plasma cell neoplasia by interrupting IL-6 signaling or deregulated Myc expression can be tested.
To confirm a direct relation between vaults and MDR and to investigate possible other functions of vaults, we have generated a major vault protein knockout mouse model. The MVP-/- mice are viable, healthy and do not show abnormalities. We investigated the drug sensitivity of MVP-/- embryonic stem (ES) cells and bone marrow derived from the MVP deficient mice to melphalan, doxorubicin, mitoxantrone, etoposide, vincristine, dexamethasone, cisplatin and ara-C. In neither cell type an increased sensitivity was observed as compared to wild-type cells. The activities of MDR efflux proteins P-glycoprotein, MRPI and BCRP were unaffected by MVP disruption. In vivo treatment of MVP wildtype and deficient mice with doxorubicin resulted in similar responses and toxicity.

This study is one of the first to specifically investigate a gene that showed a high level of expression in the myeloma gene array. No specific role of MVP for therapy resistance could be demonstrated.

Supported by a grant from the Multiple Myeloma Research Foundation

219 Whole Body Green Fluorescent Protein (GFP)-Imaging of Myeloma Tumors in Skeleton of Mice In Vivo.

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The prognosis of multiple myeloma (MM) patients post-diagnosis has not improved in the last three decades and there is a continuing and compelling need for development of novel anti-myeloma agents that significantly impact tumor burden. This has hitherto been hindered, in part, by the lack of appropriate preclinical models that faithfully replicate the human disease. Unlike cells in solid tumors, myeloma cells are often spread diffusely throughout the bone marrow cavity and in anti-tumor efficacy studies involving currently available models of disseminated MM, determination of overall myeloma burden using serum titers of the monoclonal paraprotein titers is often equivocal because of the relatively long half-life of immunoglobulins. In an attempt to overcome this, we genetically engineered the murine myeloma 5TGM1 cell line that we originated to stably express enhanced green fluorescent protein (eGFP). 5TGM1 cells, originally subcloned as a stroma-independent variant from the Radl ST33 myeloma, were retrovirally transduced with the LZRS-pBMMNZ vector encoding eGFP under the control of the M-MuLV promoter. Following single cell cloning by fluorescence activated cell sorting (FACS), several stable subclones were isolated and one clone (H1.1+) expressing eGFP at a very high level was further characterized. There was no difference either in the growth rates or monoclonal paraprotein (IgG2b) production between the eGFP-expressing clone and parental 5TGM1 cells. eGFP expression in cultured H1.1+ cells was analyzed by FACS repeatedly and found to be stable in the 4-month period prior to inoculation into mice. H1.1+ cells were inoculated intravenously into 6-9 weeks old syngeneic C57BL/KaLwRij mice through tail veins and whole body optical images of the live mice were obtained using an fluorescence illuminator and a thermoelectrically-cooled color CCD camera weekly thereafter up until sacrifice. Genetically fluorescent 5TGM1 tumors growing in situ in spine, skull and long bones were visualized on high-resolution images. Fluorescent tumor foci were first evident two weeks after inoculation of myeloma cells, and always in calvariae or scapulae. Imaging of freshly isolated whole skeleton and visceral organs post-sacrifice revealed that the myeloma cells homed preferentially to the skeleton in all mice with multifocal fluorescent lesions particularly pronounced in the axial skeleton (skull, iliac crests, scapula, lumbar and thoracic vertebrae, ribs, sternum) but also evident in metaphyseal regions of long bones, consistent with typical tumor distribution in MM patients. There were also smaller fluorescent extra-medullary tumor foci detectable infrequently in spleens, kidneys, and ovaries but not livers of tumor-bearing mice. eGFP-positive STGM1 cells, sorted by FACS from splenic cell harvests, retained the ability to home to bone marrow when re-injected into naïve mice. Fluorescent tumor foci were consistently associated with increased resorptive activity assessed by staining for TRAP activity, a recognized marker of osteoclasts. In conclusion, whole body GFP-imaging facilitates real-time, continuous visual monitoring of myeloma growth and spread within tumor-bearing animals. This ability to externally and non-invasively follow myeloma progression, combined with quantitative histomorphometry, increases the utility of the 5TGM1 model that has already proven to be predictive of efficacy in preclinical studies. This should accelerate evaluation and development of novel anti-myeloma therapies.

220 LAGδ, a SCID-hu Xenograft Model of Multiple Myeloma

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Most SCID murine myeloma models were developed from human multiple myeloma (MM) cell lines. Success has been limited when primary MM cells were used. We have recently developed a new SCID-hu murine model of MM, LAGδ, from the serial passage of an intramuscularly (IM) implanted myeloma patient’s bone marrow (BM) sample. So far, LAGδ has been growing continuously for 8 passages. In this subline, we have not only achieved nearly a 100% passing rate, we also are able to consistently grow up visible intramuscular tumor within 3-4 weeks with a relatively similar growth rate. The mean human IgG (hlG) elevation and tumor growth are approximately 230 mg/dl and 0.38 cm3 per week, respectively. A similar growth pattern was noted when the LAGδ cells were implanted subcutaneously with or without the use of Matrigel. LAGδ cells have also been tested in the intravenous (IV) model. Approximately 70-80% of the implanted mice showed elevated secretion of hlG around 10 weeks after tumor cell inoculation. MM cell infiltration can be detected in mouse BM and other organs as early as 5 weeks after tumor cell implantation. The clonality of the LAGδ myeloma cells was verified by both PCR and protein electrophoresis. The level of calcium was noted to be unchanged in the IM model, but progressively increased in the IV model. There is also a steady increase in osteolytic lesion noted in the IV model that correlates with the increased number of osteoclastic cells present in mouse BM. The clinical relevance of LAGδ SCID-hu xenograft model was tested by injection of bortezomib, a proteasome inhibitor, intravenously into the tail vein of IM model. The mice that received 0.5 mg/kg bortezomib showed significant inhibition of LAGδ MM cell growth, whereas the mice that received 0.05 mg/kg bortezomib showed no inhibition, as measured by hlG elevation and increasing tumor size.
221 Prolonged survival in the 5T2MM murine myeloma model is associated with high proportions of CD45+ myeloma cells

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Although both CD45+ and CD45- multiple myeloma (MM) cells are observed in the bone marrow (BM) of MM patients, their roles in myeloma biology are not completely understood. In our previous works (Asosingh et al, Exp Hematol. 2000; Asosingh et al, Clin Exp Metastasis, 2002) in the 5TMM murine model, we were able to demonstrate that CD45+ 5T2MM cells are the predominant in vivo BM homing population. This was attributable to their high migration and invasion capacities. In contrast to the CD45+ 5T2MM cells, the CD45- 5T2MM cells have a high in vivo proliferative capacity. In a more recent report (Asosingh et al, Blood, 2003) we demonstrated that 5T2MM disease progression is an ongoing differentiation process of CD45+ MM cells into CD45- MM cells. In the current work we provide in vivo data indicating that CD45 subsets in the 5T2MM model have an impact on the outcome of the disease. 92% of the naïve mice injected with 5T2MM cells develop myeloma in 10-12 weeks (terminally diseased) and hind leg paralysis. In these animals the end stage 5T2MM cells are predominant CD45- (23.1 + 11.1% CD45+ MM cells, mean + SD values) in analogy to the common human situation. The remaining 8% of the animals have delayed tumor progression (terminally diseased after 14-24 weeks, p< 0.002) with a complete different feature in the end stage of the disease. In addition to the prolonged survival, these animals had typically a bowed back and never got paralyzed. The MM cells in the BM of these mice were predominant CD45+ (86.1 + 5.8%, mean + SD values, p<0.0001). The underlying mechanisms of these findings are currently unclear. However, the data suggest that CD45 subsets are associated with the final outcome of myeloma disease, suggesting that they have different and crucial roles in myeloma biology.

222 The effect of the broad-spectrum matrix metalloproteinase inhibitor SC-964 on tumor growth, development of osteolytic lesions and angiogenesis in multiple myeloma: A study in the 5T2MM model.

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Matrix metalloproteinases (MMPs) are known to play a role in processes like cancer cell growth, invasion, angiogenesis, metastasis and bone degradation, all of which are important in the pathogenesis of different types of cancer. Therefore, a major interest has developed in the use of MMP inhibitors as anticancer agents. SC-77964 is a potent inhibitor of several MMPs (MMP-2, -3, -8, -9 and –13), but was designed to be highly sparing of MMP-1 in order to avoid inducing the musculoskeletal syndrome. Recent results indicated that SC-77964 delays tumor growth in a variety of models of human cancer (prostate, lung and breast). It has been shown that multiple myeloma cells and bone marrow stromal cells express MMPs. Recently, we demonstrated the secretion of MMP-9 by the 5TMM murine myeloma cells. In the present study, we investigated the effect of SC-77964 in the 5T2MM murine model of myeloma, on tumor development, development of osteolytic lesions and angiogenesis.

Mice were injected with 5T2MM cells and given access to food containing SC-77964 at different concentrations: 25, 200 and 1600 ppm. After 11 days of treatment, SC-77964 was detected in the plasma at a mean concentration (n=8) of 134±37, 921±319 and 3200±774 ng/ml respectively. At each of these concentrations, we could observe an inhibition of MMP-9 secreted by 5T2MM cells when examined by gelatin zymography. Upon development of the first signs of disease (11 weeks after injection of tumor cells), all mice were sacrificed. Aspects of MM disease including tumor burden in the BM, osteolytic lesions and angiogenesis, were assessed to evaluate response.

Tumor load was measured by staining bone marrow with flow cytometry. Treatment of mice injected with 5T2MM with SC-77964 resulted in a 29% (25 ppm), 24% (200 ppm) and 36% (1600 ppm) reduction of tumor burden in the bone marrow. One hind leg was removed from the sacrificed mice and x-rayed to assess the number of osteolytic lesions. We observed a significant reduction in osteolytic lesions of 54%, 47% and 65% in mice treated with respectively 25, 200 and 1600 ppm SC-77964. We have also previously, demonstrated that angiogenesis is induced in the bone marrow of terminally diseased 5T2MM mice by determining microvessel density. Therefore, in the present study fore limbs of the sacrificed mice were used to assess microvessel density. SC-77964 treatment resulted in a decrease of microvessel density of 35% (25 ppm), 25% (200 ppm) and 29% (1600 ppm).

Taken together, these data suggest that inhibition of MMPs has an effect on MM disease. MMP blockage not only resulted in reduction of tumor growth, but also had a significant effect on the development of osteolytic lesions and neovascularization. These results support the potential use of SC-77964 as an anticancer agent in myeloma. Further in vitro experiments will be performed to elucidate the specific role of MMP inhibition in the different processes in MM.

223 Zoledronic Acid inhibits myeloma growth in vitro and in vivo

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Clinical studies with nitrogen-containing bisphosphonates in multiple myeloma (MM) showed not only a reduction of bone involvement but also a survival benefit. This suggests that bisphosphonates could have a direct antiproliferative effect on malignant plasma cells. In vitro experiments with the plasma cell lines INA-6 and JK-6L, established in our laboratory, demonstrated a dose dependent growth inhibition upon treatment with zoledronic acid (ZOL). Zoledronic acid strongly induced apoptosis in both cell lines upon 48 h treatment. However, little is known about the efficacy of bisphosphonates on malignant plasma cells in vivo. Therefore, we tested ZOL in our SCID mouse xenograft model with the strictly IL-6 dependent human plasma cell line INA-6 (The Hematology Journal 2: 42, 2001). The advantage of this model is that the mice harbour extramedullary tumors but do not show significant osteolytic
lesions. Initially, high doses of ZOL (15 µg single dose; 3 times weekly) were tested given by the intravenous (i.v.) or subcutaneous (s.c.) route. Six out of 12 treated animals (4/6 i.v., 2/6 s.c.) did not show any signs of tumor formation at the end of the 84-day observation period. Subsequently, single doses of 2 or 8 µg ZOL, more comparable to the human dose range, were chosen. The treatment schedule of 3 i.v. injections/week was maintained to achieve constant bisphosphonate availability. The 14 day treatment was tolerated without toxicity and the median survival was significantly prolonged in the groups treated (2 µg group: 84 days; 8 µg: 86 days, controls: 47 days). As a marker for tumor growth, serum levels of soluble human IL-6 receptor were measured at day 21 and found to be significantly higher in untreated than in ZOL treated animals. Similar results were obtained when treatment was started at a later time point, starting at day 14 upon tumor cell inoculation. In summary, ZOL possesses significant anti-tumor activity as demonstrated in this study using a unique xenograft model for human plasmacytoma with more than 50 SCID mice involved. Thus, nitrogen-containing bisphosphonates given at frequent intervals may have a therapeutical potential in multiple myeloma beyond the treatment of osteolytic lesions.

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**HSP90 INHIBITORS PROLONG SURVIVAL IN A SCID/NOD MICE MODEL OF DIFFUSE MULTIPLE MYELOMA: THERAPEUTIC IMPLICATIONS**

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The molecular chaperone hsp90 (heat shock protein-90) is critical for the proper 3-dimensional structure and function of kinases and receptors (e.g. HER2/neu, androgen or estrogen receptors) with major role in proliferation/survival of solid tumor cells. While these aforementioned prominent hsp90 targets are not deemed major contributors to multiple myeloma (MM) pathophysiology, we hypothesized that other hsp90 client proteins could be major determinants of MM cell survival against pro-apoptotic stimuli (e.g. anti-cancer drugs). We first studied the in vitro anti-MM effect of a panel of small molecule inhibitors (including geldanamycin and its analogs, e.g. 17-AAG) of the ATP-binding pocket of hsp90. We found that hsp90 inhibitors potently induce apoptosis of both drug-sensitive and -resistant MM cell lines, and tumor cells from patients with relapsed refractory MM (even PS-341- or IMiD-resistant); sensitize tumor cells to other pro-apoptotic agents (e.g. PS-341, Apo2L/TRAIL, HDAC inhibition); suppress cell surface expression and downstream signaling (via PI-3K/Akt and Ras/Raf/MAPK) of both IGF-1R and IL-6R; decrease intracellular levels of key kinases, including Akt, Raf, IKK-α; increase activity of pro-apoptotic Forkhead transcription factors; decrease constitutive and cytokine-induced activity of NF-xB and telomerase; and suppress expression of intracellular anti-apoptotic proteins (e.g. FLIP, XIAP, cIAP2). We then evaluated the in vivo anti-MM activity of 17-AAG in a recently developed model of diffuse GFP+ MM lesions in SCID/NOD mice. RPMI-8226/S MM cells stably transfected with Green Fluorescent Protein (GFP) construct were injected (5x10⁶ cells, i.v. tail injection) in 40 SCID/NOD mice, randomly assigned to 2 groups (of 20 mice each): a) mice receiving 50 mg/kg i.p. of 17-AAG (daily i.p. injections for 5 consecutive days followed by 2 days off therapy, in each cycle) for a total of 4 cycles, or b) control group. Mice in both cohorts were followed up serially by whole body real-time fluorescence imaging, which allows external visualization of GFP+ tumors in internal organs and was previously validated in a separate study. The primary endpoint of this study was overall survival (time between injection of tumor cells and sacrifice for paralysis, moribund state, or death, whichever occurred first). Kaplan-Meier survival analysis showed that 17-AAG treatment significantly prolonged median overall survival of mice (>250 days, with 14/20 of 17-AAG-treated mice surviving at the last interim analysis, vs. 29 days median overall survival for control mice, with 0/20 mice surviving) (p<0.0001, log-rank test). Control mice had diffuse GFP+ lesions (detected by fluorescent imaging and confirmed by histopathology), primarily involving the axial skeleton (thoracic/lumbar spine, skull, pelvis, thoracic cage) and extremities. Soft-tissue plasmacytomas (e.g. subcutaneously) were also observed, but there was no frequent development of visceral metastases in liver, lung or spleen or kidneys. The primary cause of morbidity in control mice was paralysis associated with development of bone vertebral lesions. In contrast, 17-AAG was well tolerated, without vital organ tissue damage in histopathologic analyses. This significant in vivo anti-MM activity of hsp90 inhibitors, coupled with our mechanistic and molecular profiling studies, provide a framework for the upcoming clinical trials of this novel class of agents in MM.
9. Chemotherapy, maintenance, treatment and supportive care

9.1 Chemotherapy

The value of anthracycline sparing by the use of weekly cyclophosphamide after induction with the ABCM regimen: the results of the VIIIth MRC Myeloma trial

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This trial compared the efficacy of two cytotoxic chemotherapy regimens in patients presenting with multiple myeloma, at the age of 65yrs and over, or patients presenting under the age of 65yrs in whom intensive chemotherapy was contraindicated. The treatment options were ABCM to plateau vs 3 courses ABCM then C-weekly to plateau.

ABCM cycle-time 6weeks – adriamycin 30mg/m2 plus BiCNU 30mg/m2 iv on day one; cyclophosphamide 100mg/m2 plus melphalan 6mg/m2 orally days 22, 23, 24 & 25.

C-weekly – cyclophosphamide 400mg/m2 orally on first day of each week; prednisolone 40mg/m2 orally on alternate days for first 6 weeks.

Observations in the VIIIth MRC myelomatosis trial and subsequent pilot studies indicate that changing to weekly cyclophosphamide after starting treatment with ABCM may be as effective as continuing to give ABCM until plateau is reached (Lancet, 1992 339: 200-205).

The modified treatment: 1) Causes less marrow toxicity than either conventional melphalan regimens or ABCM. 2) Uses less adriamycin than continued ABCM and so is less cardiotoxic. 3) Improves treatment options after relapse from plateau. 4) The management of treatment is easier and less expensive than when ABCM is used until plateau. Given these advantages a result showing either equal or improved efficacy of ABCM followed by C-weekly would be taken as an indication for using this modified regimen.

The trial opened for entry on 1 November 1993 and 600 patients were registered into the study, the last in July 2002. Patients were randomised after successfully completing three courses of ABCM at a time that is between 18 and 36 weeks post diagnosis. Of the 600 patients remaining alive at the time of analysis.

Median follow-up for all alive patients is 3.7 years with 94(29%) of 328 patients remaining alive at the time of analysis.

VAD (vincristine, adriamycin, dexamethasone) is an effective and widely used treatment for patients with multiple myeloma (MM). However, its administration via a central venous line causes considerable inconvenience and complications. We previously designed an oral induction regime, Z-Dex (Idarubicin 40mg/m2 & Dexamethasone 160 mg over 4 days) that demonstrated disease efficacies in a phase I/II study. We therefore conducted a randomised study to compare Z-Dex with VAD as induction therapy in newly diagnosed patients with myeloma. 106 patients were randomised (Z-Dex n=52, VAD n=54) to receive 4-6 cycles of Z-Dex (33% 4 cycles, 48% 6 cycles) or VAD (44% 4 cycles, 35% 6 cycles). The median age was 56 yrs (range 37-73) with a male preponderance (F:M 1:1.4) and Durie-Salmon stage II (n=46) and III (n=60) disease. The EBMT definitions of response were used. At this time point, data of 99 patients are evaluable for toxicity (Z-Dex n=48, VAD n=51) and of 91 patients for efficacy (Z-Dex n=41, VAD n=50). Thirty-four patients have died from disease progression (Z-Dex n=9, VAD n=8), regimen-related toxicity (Z-Dex n=1, VAD n=0), infection (Z-Dex n=0, VAD n=3) and other causes (Z-Dex n=8, VAD n=3). Grade 4 neutropenia occurred in 33% of Z-Dex recipients compared with 18% of VAD recipients. 70 of 218 cycles of Z-Dex were delayed: secondary to neutropenia in 23 and not treatment-related in 25 cycles. In contrast, 57 of 231 cycles of VAD were delayed: secondary to neutropenia in 5 and not-treatment related in 26 cycles. Central line problems were reported in 37 patients on 56 cycles, primarily due to infection. 32 patients treated with VAD were hospitalized on 63 occasions for a median of 6 days (range 1-96) compared with 26 patients treated with Z-Dex who were hospitalized on 37 occasions for a median of 9 days (range 2-80). The response to therapy in evaluable patients was: CR VAD 17% cf Z-Dex 9%; PR VAD 56% cf Z-Dex 53%; SD VAD 13% cf Z-Dex 13%. The estimated difference in response (CR/PR: VAD vs Z-Dex) is 11% (95%CI 7-30%, P=0.23). 51% of Z-Dex recipients and 62% of VAD recipients proceed onto high-dose therapy protocols. The data from our preliminary analysis demonstrates that Z-Dex is efficacious in myeloma, similar to VAD, though more CRs were reported with VAD. Z-Dex may be a suitable alternative, oral-based induction regimen for newly diagnosed patients with myeloma, although definitive evidence for equivalence is not provided from the study.
MELPHALAN/PREDNISONE VS. MELPHALAN/DEXAMETASONE AS FIRST LINE TREATMENT IN ELDERLY MULTIPLE MYELOMA PATIENTS


PETHEMA Group

Melphalan/Prednisone (MP) remains as the standard therapy for elderly Multiple Myeloma (MM) patients. High doses Dexamethasone is one of the most active agents in MM. However, there aren't controlled studies to explore the efficacy and safety of the combination of Dexamethasone with Melphalan. For this reason, the PETHEMA group (Spanish Program for the Therapeutic of Malign Hemopathies) designed a multicentric randomized study in order to compare if Melphalan/Dexamethasone (MD) is superior to conventional MP. Response rates, event-free survival (EFS), survival and toxicity have been analyzed.

MATERIALS AND METHODS: Patients: 201 MM patients (proceeding of 27 Spanish Hospitals) > 65 year old or not candidates for autologous transplantation were included in the study. Patients were centrally randomized at diagnosis to receive 6 courses of MP or MD (this with double pulse of Dexamethasone). Patients who achieved any grade of response (Complete Response (CR), Partial Response (PR) or Minimal Response (MR)) received six additional courses of the same schema; then, those achieving CR or PR went into maintenance therapy with Interferon alfa-2b plus Dexamethasone. Both arms were well balanced according to the most relevant clinical-biologic disease characteristics. Median of follow-up was 46.8 months. The study was approved for the local ethical committees.

RESULTS: In 27 cases (10 MP and 17 MD) there were lost of follow-up or major protocol violation. Although the overall response rate was similar in both arms after six courses (MP: 65.4% vs. MD: 65%) (p=0.85) and after twelve courses (MP: 46.1% vs. MD: 46.7%) (p=0.94), the proportion of CR was significantly higher in patients receiving MD than in those treated with MP, and this occurred both after six cycles (MP: 2.6% vs. MD: 9.3%) (p<0.01) and after twelve cycles (MP: 7.7% vs. MD: 22.7%) (p<0.001). This translate in 9 months EFS advantage for MD patients (35.4 m. vs. 26.5 m. for MD and MP, respectively) (p=0.07). Although overall survival (OS) in responding patients was slightly better for MD than MP arm (56 m. vs. 33.8 m.) (p=0.14), when we considered the whole series of patients (both responding and not responding) the median survival was almost the same (p=0.89). Hematological toxicity wasn't different between both arms. Extrahematological toxicity was mainly due to infectious complications or no-controlled hyperglycemias, and it was significantly higher in the MD arm (MP: 9.4%; MD: 28.8% (p<0.001).

CONCLUSIONS: The preliminary results of this randomized study show that MD is superior to MP in terms of CR rates and EFS. However, this not translates into a superior OS and was associated with higher toxicity.

Randomized Comparison Between VMCP/IFN With Continuous Prednisolone and Conventional VMCP/IFN as First-Line Chemotherapy in Multiple Myeloma

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Corticosteroids activate proapototic pathways and exert direct anti-tumor effects in multiple myeloma. Standard chemotherapy is still the treatment of choice in patients unfit to undergo high-dose chemotherapy. In addition, some patients still opt for less aggressive frontline treatment and consider high-dose therapy an option for rescue therapy. In the present trial, 292 patients with multiple myeloma (median age: 67) were randomized to continuous prednisolone-VMCP/IFN (prednisolone PRED 40mg, orally d1-7 and 25mg TIW d8-28; vincristine 2mg, melphalan 15mg/m², cyclophosphamide 450mg/m² i.v. d1; interferon α-2b 3 Mega U TIW s.c.) or conventional VMCP/IFN with Pred 40 mg, d1-7, and 25mg TIW, d8-14). Patients who responded or remained stable after induction therapy were subsequently randomized to IFN maintenance (3 Mega U, TIW) or IFN maintenance with PRED 25mg TIW. Response was evaluated according to the SWOG criteria.

Median follow-up time at evaluation of this study was 25 months. Data were analyzed according to intent to treatment. The overall response rate was 72% in patients on continuous PRED and 68% in patients on the standard VMCP/IFN regimen with a paraprotein reduction of ≥ 75% in 30% and 27% of the patients, respectively. Progression-free survival was 23 months in the continuous and 22 months in the standard arm; overall survival was 37 months in the continuous PRED and 41 months in the control arm. Results of maintenance treatment will be reported after longer follow up. There was a tendency for increased episodes of grade-IV leucopenia, hypertension, and hyperglycemia and a tendency for fewer episodes of nausea, vomiting and psycho-neurological adverse effects in the patients with continuous PRED.

In conclusion, continuous PRED treatment during VMCP/IFN induction chemotherapy did not yield significant advantages in terms of response rate, progression-free survival or overall survival. Overall survival of the entire group of this mainly elderly patient population was 39 months and compares favorably with the outcome obtained in older studies with conventional chemotherapy.
Introduction: There is a known large number of standard chemotherapy protocols in the treatment of multiple myeloma (MM). In this study we evaluated the effectiveness of first line chemotherapy in the treatment of MM and its influence on overall survival.

Methods: One hundred and twenty multiple myeloma patients (61 female and 59 male) treated in Department of Hematooncology in Lublin (Poland) between 1992 and 2001 were enrolled in this study. Patients age ranged from 38 to 79 years (mean value 60.6). In our study group the predominant type of disease was classic multiple myeloma with IgG monoclonal proteins (82.2%).

Results: The best response was observed after VAD protocol - 60% of patients acquired complete remission (CR) or partial remission (PR). Good response (CR+PR) was obtained in 46% of patients after VMBCP protocol, but only in 10.7% of patients after MP protocol. In 16.7% of patients partial remission was observed after single intravenous infusion of cyclophosphamide and melphalan as transplantation protocol according to Palumbo et al. After chemotherapy with Idarubicin we noticed only stabilization of disease parameters. In more than 50% of patients we studied also the expression of adhesion molecules on multiple myeloma cells using flow cytometry technique. The expression of fibronectin receptor CD49e (VLA-5) and laminin receptor CD49f (VLA-6) significantly correlated with effects of chemotherapy. In patients with negative expression of CD49e, the percentage of CR+PR was 47.2, while in group with positive CD49e expression, the percentage of CR+PR was 27.6. Considering the positive and negative expression of CD49f, the percentage of good response was adequately 47.4 vs 25.9. No significant differences in follow-up time between particular treatment protocols were found. In group with VAD, VMBCP and MP protocol follow-up time was adequately 23.6 and 35 months. Statistically significant shorter follow-up time (23.6 months) was observed in patients with positive expression of CD49d (VLA-4) vs 30.8 months in patients with negative CD49d expression.

Conclusion: On the basis of our study we confirmed that none of evaluated first line chemotherapy protocols significantly prolonged overall survival. We found that the high expression of CD49d on the surface of myeloma cells may be considered as poor prognostic factor.
Currently accepted initial chemotherapy management at 6 months when compared with baseline QOL. QOL was measured in consecutive newly diagnosed MM patients using the EORTC QLQ-C30 questionnaire. Patients completed questionnaires at diagnosis (baseline) 3 & 6 months during treatment. Results from 52 evaluable patients with baseline and 6-month QOL scores are reported. Analysis was based on two groups; (i) VAD/C-CAMP regimens directed towards stem cell transplantation, 20 patients and (ii) standard chemotherapy, 32 patients; (ABCM or alkylating agents directed towards “plateau”.

QOL scores for Physical, Emotional and Social Functioning improved in each patient group; the degree of improvement was greater for those patients ingroup (i). Cognitive functioning improved for group (i) but showed a decline for patients in group (ii). Pain and fatigue scores also showed improvements in each group at 6 months.

Global health scores improved by a mean of 15 points for group (i) and by 0.8 points for group (ii) patients. In analysing QOL data using EORTC QLQ C30 hanges of >10 points in scores are considered clinically relevant. In this study it was not possible to show statistical significance for this observation because of the relatively small numbers of patients in each group.

Management of MM with chemotherapy improves QOL. The degree of improvement appears more marked at 6 months using VAD/C-CAMP based chemotherapy approaches when compared with standard chemotherapy. Although the patient groups are not directly comparable in this prospective, non-randomised study, the findings make a case for VAD/C-CAMP based regimes as initial treatment for MM in terms of more rapid symptomatic benefit and consequent QOL gain. Further studies are needed to explore treatment effects over longer time scales and larger numbers of patients are needed.

232 Vinorelbine Plus Dexamethasone For Relapsed Or Refractory Multiple Myeloma

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Multiple myeloma is a malignant hematological neoplasm that remains incurable despite the use of modern treatment options. Even after high-dose chemotherapy and autologous bone marrow transplantation, a high percentage of patients still relapse and eventually die of progressive disease; therefore, new treatment options for relapsed or refractory patients need to be investigated.

Vinorelbine has been evaluated in vitro in myeloma cell lines, displaying proapoptotic and antiproliferative effects. A prospective clinical trial in relapsed multiple myeloma patients was conducted in order to evaluate the efficacy of vinorelbine 25 mg/m² iv, given on days 1 and 4 of each cycle, together with dexamethasone 40 mg iv on days 1-4, and 9-12; treatment cycles were administered every 21 days.

Seventeen patients were included (7 male, 10 female) from March 1998 to January 2003, 13 of which were evaluable. Median age at diagnosis was 52 years (range: 39-69). Median number of previous treatments was 3 (range: 2-4), 9 patients had relapsed following autologous PBSC transplantation, and 4 patients had progressed after thalidomide. Median time from diagnosis was 38 months (range: 16-288). Patients received a median of 9 treatment cycles (range: 2-18). Two patients died of progressive disease after receiving two cycles of treatment.

Results: Overall response rate was 77% (10/13), CR 1/13 (7.6%), PR 7/13 (54%) and minor response (“M” protein reduction greater than 25% but less than 50%, 2/13 (15.4%). One patient had stable disease. Median time to best response was 6 months, and median duration of remission was 7.5 months. Only seven patients were alive at the time of evaluation, with an median overall survival of 18 months.

Toxicity was mainly haematological, with 57% of patients developing grade III/IV neutropenia, and 28% of patients requiring transfusions.

Conclusion: the combination of vinorelbine plus dexamethasone is active in relapsed multiple myeloma patients, with an overall response rate of 77%, and an acceptable toxicity profile.
Multiple myeloma (MM) is conventionally treated with combination chemotherapy using oral melphalan, prednisolone (MP) or infusional vincristine, Adriamycin, and dexamethasone (VAD) followed by autologous bone marrow/stem cell transplant (ABMT). Even though VAD doesn’t offer any survival advantage it is preferred because of less stem cell toxicity and faster responses. The faster response with VAD regimen is because of dexamethasone. For patients who are not candidates for autologous transplant combination of melphalan and dexamethasone (MD) may be a good oral alternative to MP or VAD if it results in comparable responses and time to responses. It is all the more important for country like us where many patients are unable to bear cost of VAD. From November 2000 to September 2002, 22 newly diagnosed patients who were not willing to undergo ABMT, were enrolled onto this study. Patients characteristics: Median age- 55 years (27-75 years), Sex- males 12, Stage distribution- IIIA-16, and IIIB-6 patients. Sixteen patients had bone marrow plasma cells > 25%. Monoclonal band- IgG-13, IgA-2, pure light chain myeloma-7 patients. Beta 2 microglobulin was done in 13 patients and it was high in 9. Treatment- melphalan 6 mg/m2 PO day 1-4, dexamethasone 40 mg PO day 1-4 and 9-12 every 4 weeks with PCP prophylaxis using Septran. Therapy was discontinued if; patient became intolerant, disease progressed, or 12 cycles were completed in responding patients. Results- results are shown in table 1. Two patients achieved CR after 2 cycles.

<table>
<thead>
<tr>
<th>Response</th>
<th>CR</th>
<th>PR</th>
<th>SD</th>
<th>PD</th>
<th>Non evaluable (LFU before assessment)</th>
</tr>
</thead>
<tbody>
<tr>
<td>After 4 cycles</td>
<td>5</td>
<td>9</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
<tr>
<td>Best response</td>
<td>6</td>
<td>8</td>
<td>4</td>
<td>0</td>
<td>4</td>
</tr>
</tbody>
</table>

Toxicity- Treatment was generally well tolerated, still, dexamethasone was stopped in 3 patients because of proximal myopathy. Survival- Currently 5 patients are alive without evidence of disease, 10 are surviving with disease, 6 have died of disease progression, and in 1 patient follow up information is not available. Median survival has not reached. Two years OS and PFS is 63% and 70% respectively. Conclusion- Combination of melphalan and dexamethasone is safe, may be effective oral alternative to infusional VAD in patients not willing to undergo ABMT, and probably superior to MP in time to responses. We, plan to start a randomized trial between MP and MD in patients not willing for ABMT.

A 15 years experience in the treatment of multiple myeloma patients with conventional chemotherapy – the end of one era?

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Despite all the advances in systemic and supportive therapies, multiple myeloma (MM) is still currently an incurable plasma cell malignancy. The aim of study was to present results of a long-term follow up (15yrs) of MM patients treated with conventional chemotherapy followed with interferon-alpha (IFN-a) maintenance therapy.

Patients and methods: The study included 120pts with de novo diagnosed MM between 1988 and 2003. The group consisted of 63 male and 57 female pts, mean age 65yrs (range 27-79). According to the clinical stage (CS) of disease, distribution of MM pts was as follows: I 8pts (6,7%), II 44pts (36,7%), III 68pts (56,7%). Renal impairment existed in 29pts (24,2%). There were 48pts (40%) with IgG kappa monoclonal (M) protein, 36pts (30%) with IgG lambda, 22pts (18,3%) with IgA, 7pts (1,7%) with IgD, and 12pts (16%) with secretion of kappa/lambda light chains. None secretary MM was diagnosed in 4pts (3,3%). Elevated Beta2-microglobulin was registered in 45/60 analyzed pts (75%). Imunohistochemical staining of bone marrow revealed high expression of VEGF, BFGF and Ki-67 in 20/45 analyzed pts (44,4%).

Results: Chemotherapy according to the MP/VMCP regimen was applied through 2-15 cycles (Me 9) at 100pts (83,3%) as the initial MM treatment. Plateau phase was achieved in 55pts (55%) with duration 1-90m (Me 21). During plateau phase, 65/100pts (65%) relapsed and therefore were treated with second line regimen (VAD,M2,Z-Dex). The overall survival for this group of MM pts was 1-142m (Me 42) with five-year survival obtained in 20% of MM pts.Two pts (2%) died due to secondary malignancy. Twenty MM pts (16,7%) in the II and IIICS with renal impairment were treated with 2-9 cycles (Me 6) of VAD protocol. Five pts (25%) died during the treatment. Responses according to the EBMTR/IBMTR criteria were as follows: 3/15 complete responses (20%), 9/15 partial responses (65%), 2/15 minimal responses (13,3%) and 1/15 stable disease (6,7%). Average duration of remission was 18m (7-29). Relapses were observed in 10/15pts (66,7%). Median overall survival (24m) was similar to the survival of MM pts treated with MP/VMP protocol. During plateau phase 29pts (24,2%) received IFN-a maintenance therapy which resulted with evidently longer duration of plateau phase compared to MM pts without IFN-a therapy (Me 21m vs. Me15m).

Conclusion: Treatment results of different chemotherapy regimens for MM, followed by IFN-a maintenance therapy, are very similar and not satisfactory.

Is it the end of one era? A great improvement is achieved applying high-dose therapy with stem-cell support, but without definitive curable effect. New treatment options which target is the MM cell, the MM cell-host interaction offers a great promise to improve patient outcome in MM.

A NEW PROTOCOL OF MULTIPLE MYELOMA: RESULTS OF A PRELIMINARY EVALUATION.

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INTRODUCTION: Multiple myeloma (MM) is a clonal plasma cell proliferative disorder that accounts for 10% of malignant hematologic neoplasms. Combinations of different chemotherapy regimens have not improved survival significantly in relation with the combination of melphalan-prednisone. Others alternative treatments as autologous stem cell transplantation, thalidomide and immunotherapy have been proposed. We followed up a group of 35 patients with active MM treated with a novel total therapy protocol during a two years period.
PURPOSE: To evaluate the outcome of 35 consecutive patients with a novel total myeloma multiple protocol and its relation with classic prognosis features at diagnosis.

PATIENTS AND METHODS: Between January 2000 and December 2002 we studied 35 consecutive patients with multiple myeloma. Patients younger 60 years old were treated with i.v. chemotherapy (regimens containing cis-platin, etoposide, anthracyclines, vincristine and corticosteroids). Chemotherapy responders and refractory patients with response to oral thalidomide plus dexamethasone and/or rituximab were underwent to autologous transplantation followed of oral thalidomide. Patients with resistance were treated with a dose-scaling schedule of thalidomide. Patients between 60 and 65 years old were aleatorized into i.v. chemotherapy followed of thalidomide or melphalan-prednison; patients older 65 years old were given the Alexanian protocol. Response was assessed by monoclonal protein quantitation, bone marrow plasma cells percentage and lytic lesions evolution.

RESULTS: Response and survival: Five patients(18,5%) had obtained complete response (CR); 15 (55,5%) patients had partial response; 7 patients (25,9%) were non responders. Only 4 patients underwent autologous transplantation and 12 patients were given thalidomide (3 of 5 responders). 8 patients died (22,8%); patients between 55-60 years aged had higher mortality, probably because the chemotherapy related toxicity (median age (22,8%); patients between 55-60 years aged had higher mortality, probably because the chemotherapy related toxicity (median age alive vs died was 62 vs 58); neutropenia infections were the cause of superior mortality in this group. No death was registered in patients with melphalan/prednison, though higher chemoresistence was observed (40% vs 22%).

Prognosis factors at diagnosis: No significative difference was observed between outcome and initial evaluated features (hemoglobin level, platelets cipher, beta-2 microglobulin, renal failure, state at diagnosis, lytic lesions, tumoral mass, bone marrow plasma cell percentage, previous treatment and age). However,poorer responding patients had lower hemoglobin levels (median 12,5 g/dl in CR vs 9,6 g/dl in NR ) and higher beta-2 microglobulin levels (median 3,5mg/dl in CR vs 8,8 mg/dl in NR).Supervivence was influence by clinic stage at diagnosis (11% mortality in IIA stadied patients vs 22% in III-A stage).

CONCLUSIONS: Despite of short following up, high reponse rate is obtained being thalidomide an effective drug in maintenance of remission. Patients 55-60 years aged are more vulnerable to infections in postchemotherapy neutropenia, a cause of higher mortality; a new i.v. chemotherapy strategy should be planned for them. No classic features as renal failure are clearly correlate with patients outcome. Previously treated patients have the same probability of response that newly diagnosed patients.

237 The diversity of relapse and refractory disease in multiple myeloma indicates an urgent need for larger randomized studies of more homogenous populations.

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In spite of the improved survival and increased knowledge of the biology of multiple myeloma all patients will eventually relapse and later meet the problem of refractory disease to any type of treatment. The physician of such patients has to meet the challenge of having to choose between several regimens of treatment from combination chemotherapy (CCT), a second or third course of high-dose chemotherapy with stem cell support, high-dose or continuous steroids, thalidomide and soon newer treatment principles as IMIDs and proteasome inhibitors. From reviewing the literature only few randomized studies have been found and a ‘gold standard’ is missing. Most of the phase II studies are relatively small and include a heterogeneous population of relapse, refractory disease, and even some after multiple treatment regimens including both autologous and allogeneic transplantsations. The major reason for the lack of help from evidence based studies is this heterogeneity of the patients. The Mayo clinic demonstrated the importance of the number of prior treatment regimens at the recent ASH meeting1, while the Spanish registry underlines the diversity of relapse after high-dose therapy with stem cell support 2.

We have looked at the significance of the time for relapse after stem cell transplantation among our 159 patients who were treated according to standard condition of the Nordic Myeloma Study Group3. We found a significant shorter survival after relapse among patients progressing within the first year of transplant (median OS 7,5 months, N=21) compared to patients progressing later (median OS 30,2 months, N=31) (p=0.03). We therefore request that future studies on the treatment of relapsing and refractory disease are designed to either unveil the heterogeneity by narrow the inclusion criteria e.g. by including a larger number of centers or by ensuring a stratified inclusion in randomized studies with enough patients to draw conclusions for subgroups too.

We recommend that the included patients are as homogenous as possible with respect to:
Relapse vs. refractory disease
Number of prior treatment regimens
Time to progression after stem cell transplantation

Pattern of relapse (insidious form, classical form, plasmaactoma form and the leukaemia form)

This is an important precondition for informing the patients in a proper way instead of giving a professional judgment.

Reference List


238 VECD for refractory and resistant multiple myeloma

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Background. The VAD regimen (infusional vincristine, doxorubicin and intermittent high-dose dexamethasone) is widely considered the standard salvage chemotherapy for multiple myeloma resistant to alkylating agents and is increasingly used for induction in previously untreated patients prior to high-dose chemotherapy. VAD chemotherapy needs 4 - days continuous intravenous (CIV) infusion of vincristine and doxorubicin.
Methods. We investigated the VECD protocol, a VAD-based regimen using bolus injections of vincristine 1.5 mg day 1 and epirubicin 20 mg/m2 days 2 and 3 with 1 h infusions of cyclophosphamide 200 mg/m2 days 1-3 and oral dexamethasone 20 mg/m2 days 1-5 as induction and salvage treatment in multiple myeloma. The cycles were repeated every 3 weeks.

Results. 22 patients were treated on study and received median 3 cycles (range 2-8). No patient achieved a CR. The overall rate of PR was 15/36 (42%). Patients achieved maximal response after a median of 4 (range 3-6) courses. PR rates were 40% (3/8) in patients with primary refractory disease, 50% (7/14) in patients with secondary refractory disease, and 50% (7/14) in patients receiving 2nd or subsequent relapse therapy. Two patients died during their initial cycle of therapy from rapidly progressive disease and sepsis. The overall median survival was 24 weeks with a 1-year survival of 33.3% (95% confidence interval of 20-46%). Myelosuppression was the most frequent adverse event with NCI grade 2 neutropenia and/or thrombocytopenia in 15% of first cycles, grade 3 in 20%, and grade 4 in 65%. Two-thirds of patients had at least one episode of grade 3 or 4 sepsis. In 15% of septic episodes positive blood cultures were obtained. Overt cardiotoxicity was seen in two patients.

Conclusion. VECD appears to be an effective regimen for salvage therapy in multiple myeloma. Based on the limited number of patients treated the results are comparable to those reported for VAD, with the advantage that the infusional application of vincristine and the anthracycline is omitted. E-mail: mbadea@crainova.pcnnet.ro

239 Chemotherapeutical regimen Ifosfamide + Epirubicine (IE) – an effective salvage and mobilisation regimen for multiple myeloma patients, who insufficiently responded to induction therapy VAD or VID. One centre experience

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Background: Chemotherapeutical regimen IE has been used as an induction and mobilisation therapy for newly diagnosed multiple myeloma patients. Sufficient antmyeloma effect and mobilisation property of this regimen had been already proved. We decided to verify its effectiveness in multiple myeloma patients, who insufficiently responded to induction therapy VAD or VID.

Patients and methods: From 1/01 to 1/03 10 patients with multiple myeloma received IE therapy – their median age was 55 years (range 50-65 years); clinical stage I – 1x, II – 7x, III – 2x; type of paraprotein IgA – 2x, IgG 7x, light chains – 1x.

type of paraprotein IgA – 2x, IgG 7x, light chains – 1x.

Results: 9 patients underwent 3 courses and 1 patient 4 courses of IE therapy. Therapeutical response was noticed in 8 cases (CR 1x, PR 7x) and none response in 2 cases. Haematological toxicity grade III or IV (according to WHO classification) appeared in 4 cases. Non-haematological toxicity of this grade did not appear.

Regimen IE + G-CSF (10 ug/kg/day) was used for mobilisation of peripheral blood stem cells during 2nd course of therapy in 3 patients, during 3rd course of therapy in 6 patients and during 4th course of therapy in 1 patient. Yield was sufficient in all cases except one.

Medial follow up was 17 months (10-24 months). All patients received further therapy (2xHD Mel +HSCT 7x, 1xHD Mel + HSCT 2x, thalidomide + dexamethasone 1x). Recently 8 patients are in remission (2xCR, 6xPR), and 1 patient has stable disease. One patient died early after transplantation.

Conclusion: Chemotherapeutical regimen IE has satisfactory antmyeloma effect and good mobilisation property for multiple myeloma patients, who insufficiently responded to induction therapy VAD or VID.

240 Retrospective analysis of response to vincristine-doxorubicin-dexamethasone (VAD) chemotherapy before autologous transplantation in multiple myeloma

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Background: The combination VAD (vincristine, doxorubicin and either mepiprednisolone or dexamethasone in equivalent doses) is used in our centre as the initial chemotherapy in myeloma patients planned to proceed to high dose therapy and autologous haematopoetic stem cell transplantation. We retrospectively analysed the monoclonal protein responses and survival data in 62 patients treated in this way. The aim was to assess the relative response to components of the pre-transplant chemotherapy and its relation to the overall disease response.

Methods: Clinical and laboratory data were reviewed for the 62 patients. Skeletal imaging results were not available and so response rates are referred to as unconfirmed. Defined events for survival analysis were rising paraprotein, commencement of salvage regimen or death. The median age of patients was 55 years. Only two patients had prior chemotherapy, while 29 had prior or concomitant local radiotherapy.

Results: The average reduction in paraprotein after the first cycle of VAD was 37% in serum and 69% in urine. After the third cycle, the reduction was 59% and 89% respectively. The unconfirmed complete remission rate (uCR) was 6% and for partial remission (uPR) 60%. Following cyclophosphamide mobilisation the uCR rate rose to 11%, while the uPR rate was unchanged.

Greater than 50% reduction in monoclonal protein after the first cycle of VAD was observed in 39.6% of patients. This group had a significantly better event-free survival (EFS at 3 years 53.6% vs. 12.0%; p=0.05) than those with <50% reduction. The same association was no longer significant when comparing response groups after the third cycle of VAD. The OS curves for these two groups did not differ at 3 years. Similarly, there was no significant correlation between uCR, uPR or non-responders with regard to event-free or overall survival.

Discussion: These results indicate that the greatest measurable response in paraprotein is observed after the first cycle of chemotherapy. This initial response appears to be predictive of EFS and may be used as a marker for risk stratification. As most of these patients were treated de novo it is likely that this response reflects the underlying chemosensitivity of the disease.

The absence of correlation between early paraprotein response to treatment and overall survival is likely to reflect the small number of patients. Efficacy of salvage regimens means that longer follow-up is required to establish effect on overall survival.

We conclude that monoclonal protein response is a potential prognostic marker which is readily available and which may be used in conjunction with other accepted markers such as...
cytogenetics and β2 microglobulin to guide alternative chemotherapy regimens in patients identified as high risk. Future strategies might include alternative preparatory regimens or post-transplant therapies, such as consolidation with non-myeloablative allogeneic transplantation or interferon maintenance.

241 Single Agent Dexamethasone for Induction in Patients with Multiple Myeloma Undergoing Autologous Stem Cell Transplants

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Background: Randomized clinical trials have demonstrated that autologous stem cell transplantation (SCT) offers a survival advantage for patients with multiple myeloma (MM). Outside of SCT, none of the combination chemotherapy regimens have shown any survival advantage compared to standard melphalan/prednisone combination. The combination of vincristine, doxorubicin, and dexamethasone (VAD) is the most commonly utilized induction therapy for myeloma prior to stem cell collection and SCT. Within this combination, dexamethasone (Dex) is probably the most active agent. It is not clear, if the addition of vincristine and Adriamycin to Dex offers benefit to patients proceeding to PBSC after induction therapy. We retrospectively evaluated our experience using Dex as a single agent for pre-SCT induction therapy, to address this question.

Patients and Methods: 108 patients with MM, who underwent SCT between April 1995 and April 2002, with at least 3 months post-transplant follow up, form the subjects for this study. 36 patients who received dexamethasone (Dex) as a single agent for induction therapy are compared to a historical group of 72 patients who received initial therapy with VAD. Patient data was obtained from a prospectively maintained database and from patient clinical records. Single agent dexamethasone was given at 40 mg/day on days 1-4, 9-12, and 17-20 for four cycles. Results were defined using standard criteria.

Responses were defined using standard criteria.

Results: The study cohort of 108 patients had a median age of 59 years (range 29-72), 58% were males. Baseline demographic and clinical characteristics were similar in the two groups except for higher Hb in the VAD group. 74% of patients in the VAD group had a PR to the induction therapy compared to 63% in the Dex group (P=0.25). Overall response rates including minimal responses (25-49% reduction in M-protein) were 86% for the VAD group and 74% for the Dex group (P=0.13). Patients in the Dex group collected more CD34 cells (median 10.8 X 106/Kg vs 8.5 X 106/Kg, P=0.004). All patients in the Dex group received melphalan only conditioning compared to 57% in the VAD group, the rest receiving Melphalan/TBI. Time to engraftment for neutrophil (>500) and platelets (>50K) was shorter for the VAD group, P < 0.01. All patients in the VAD group and 33 patients (94%) in the Dex group achieved an objective response at the completion of the transplant. Conclusion: The response to induction therapy with dexamethasone alone results in similar response rates to those seen with VAD. When combined with the fact that response to transplant is no different between these groups, it makes a case for using Dexamethasone alone as initial therapy for patients planning to proceed to transplant. This regimen obviates the need for catheter placement for chemotherapy as well as decreases the risk of neutropenic infections and thrombotic events seen with cytotoxic chemotherapy as well as avoids the inconvenience of continuous infusion chemotherapy which often needs inpatient care.

242 An Open, Randomized, Controlled, Phase II, single centre, two-period cross over study to compare the quality of life (QoL) and toxicity experienced on PEG Interferon (P-IFN) with interferon-alpha2b (IFN) in patients with multiple myeloma (MM) maintained on a steady dose of IFN

B Sirohi, R Powles, D Lawrence, H Hollis, E Salmon, D Heming, G Patel, M Das, S Kulkarni Royal Marsden NHS Trust

Subject compliance with IFN dosing during the prolonged treatment courses of maintenance therapy for patients with MM is an important factor for achieving the intended clinical benefit. The significantly longer half-life of P-IFN (Schering-Plough) allows once-weekly dosing rather than thrice weekly Consenting, eligible MM patients who had been receiving IFN maintenance therapy for at least six weeks were randomly (1:1) allocated to receive P-IFN for three months followed by IFN for 3 months, or to continue with IFN for 3 months followed by P-IFN for 3 months (crossover design). Patients were assessed for toxicity using the NCI-Common Toxicity Criteria throughout the study and were asked to complete an EORTC QLQ-C30 and EORTC-QLQ MY24 (Stead et al; Br J Haematol, 1999;104:605-611) questionnaire to assess their QoL before receiving the first randomised treatment and at 3 and 6 months. The dose of P-IFN was equivalent to IFN. The main aim of the study was to compare the Global QoL score from QLQ-C30 on P-IFN and IFN. The study enrolled 60 patients (Median age, 51 years (31-70)17F, 43M, 80% stage III). At the time of enrolment, 55% patients were in complete remission and 23% in partial remission and 22% were minimal responders to previous therapy . 54 (90%) patients completed all the three Qol questionnaires (baseline, 3 and 6 month) and all patients completed at least 2 questionnaires. The data from the EORTC-QLQ-C30 has been analysed Scores for each scale in the EORTC-QLQ-C30 questionnaire were calculated as suggested in the scoring manual. A higher score on functional scores i.e positive difference indicates P-IFN is better and a lower score on symptom scales i.e negative difference indicates P-IFN is better. P-IFN was associated with a statistically significantly better global QoL score (mean difference 8.33; 95%CI 4.2-14.98; P=0.001). Also there was a statistically significant improvement in the functional scales-physical (mean diff±5.52,P=0.03), emotional (mean diff±5.5,P=0.04), social (mean diff±8.49,P=0.001) with P-IFN. The fatigue (Mean diff ª10.19;P=0.001), pain (mean diff ª6.79; P=0.02) and appetite (mean diff ª9.26; P=0.004) symptom scales were less in patients while they were on P-IFN. Though EORTC rates 10 or more as clinically important, our figures of below 10 may reflect the relative low level of symptoms relating to non-pregnated IFN. P-IFN did not have an impact on cognitive function, or the nausea, insomnia, constipation, diarrhea and financial worries symptom scales. There was no evidence of any period or crossover effects. These data suggest that patients on P-IFN have a statistically significantly better quality of life and should become standard of care for maintenance therapy in patients with myeloma and dose escalation studies should be done to see an even better impact on survival. We await the results of the QLQ-MY24.
9.2 Renal complications.

243 MERIT – A new RCT of adjunctive plasma exchange in patients with newly diagnosed multiple myeloma and acute renal failure (ISRCTN37161699)

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Introduction: MERIT (Myeloma Renal Impairment Trial) is a new multicentre randomised controlled phase III trial for patients with newly diagnosed multiple myeloma and acute renal failure, developed on behalf of the UK Myeloma Forum and the UK Renal Association. The trial is funded jointly by the Leukaemia Research Foundation and Cancer Research UK and aims to recruit a total of 286 patients in five years.

Aims: The primary aim of the trial is to determine whether the addition of plasma exchange to chemotherapy increases the likelihood of renal recovery in patients presenting with acute renal failure and newly diagnosed multiple myeloma. It is designed to detect an absolute improvement in recovery of renal function of 20% (from 20% to 40%) at the 5% (2 sided) level of significance. The primary endpoint will be the proportion of patients alive and dialysis-independent at 100 days.

Secondary aims are to assess the value of plasma exchange on overall survival and quality of life, the value of renal histology in predicting recovery of renal function and the value of serum free light chain assay in determining response of the myeloma and recovery of renal function.

Why is the trial necessary?: Severe renal impairment is a serious complication of multiple myeloma, contributing greatly to the morbidity of the disease, and occurs in up to 20% of patients at presentation. 25-50% of these patients will not respond to standard measures, such as rehydration and correction of hypercalcemia. Overall, approximately 5% of all myeloma patients will require long-term dialysis. Light chains produced by the myeloma cells are thought to be responsible for much of the renal damage, on the basis of a number of experimental models. Plasma exchange removes free light chain and there are several anecdotal reports of improvement in renal function in patients following treatment. However, only two small randomised controlled trials have been published and they reached opposing conclusions regarding the value of plasma exchange.

Main eligibility criteria

- Newly diagnosed myeloma
- Acute renal failure (creatinine >500µmol/l, urine output <400ml/day or requiring dialysis)
- Aged 18 years or over
- Written informed consent
- No previous chemotherapy for myeloma
- No significant intrinsic renal disease unrelated to myeloma

Intervention: All patients will receive two 4-day courses of dexamethasone (d1-12), followed by four cycles of vincristine, Adriamycin and dexamethasone (VAD) (d17-83), with appropriate supportive therapy. Patients will be randomised to undergo 7 plasma exchanges within the first two weeks of entry (at least 4 within the first week), or to be treated with drugs alone.

Method of plasma exchange will be by either cytocentrifugation or plasma filtration, according to local practice). Treatment after 100 days will be according to local preference.

Current situation: The trial is currently in set-up, with a view to starting recruitment in June 2003. We welcome participation from all interested centres. Further details about the trial and how to participate will be provided in the poster.

244 A retrospective analysis of the renal failure in multiple myeloma

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Background. Multiple myeloma (MM) is characterized by plasma cell infiltration in the bone marrow, suppression of normal haematopoiesis, destruction of bone and renal failure.

Methods. The study included 93 patients (55 men and 29 women) with multiple myeloma followed up between October 1983 and June 2002. The age range was 45 to 81 years (mean age 63.4).

55.95% of patients had a monoclonal IgG component, 25% had IgA, 16.6% had only light chains in urine, 1.19% had IgD and 1.19% was nonsecretory myeloma. 65.47% of patients were in stage III, 27.38% in stage II and 7.14 in stage I.

Results. 28.75% of patients presented RF in the onset of the disease. 75% of patients with RF are in stage III, while only 61.66% of the patients without RF are in stage III. 40% of patients with RF have IgA or light chains MM, while only 21.27% of patients with IgG presented RF (Mantel-Haenszel: Chi=3.36 and p=0.000). In 37.5% of patients with MM and RF was identified a precipitation factor of RF.

25% of patients with RF died within the first 2 months from the diagnosis. The 1 year survival of the patients with MM and persistent RF was 16.66%, compared to 66.66% for the patients whose urea and creatinine levels recovered, Fisher exact p=0.03. However, when patients dying within the first 2 months of treatment were excluded, no significant differences in the response or survival rates were found between patients with RF and those with normal renal function. The same is also true for MM patients with persistent RF and patients with recovered RF.

The urea and creatinine levels recovered in another 25%. The renal function recovery was positively influenced by the creatinine level of less than 3.5 mg% at presentation (66.66% in patients with reversible RF and only 16.66% in patients with persistent RF - Fisher exact p=0.04 - by the calcium level of less than 11 mg% (61.11% of patients with reversible RF had a calcium level of less than 11mg%, versus 16.66% of patients with persistent RF (Fisher exact p=0.07). In 66.66% of MM patients with recovered renal function was identified a RF releasing factor, while this factor was present in only 27.77% of patients with persistent RF. Six patients with RF were dialysed; in two of them the renal function recovered, 3 remained in chronic dialysis program and one has died.

Conclusions. Renal failure was present in over one fourth of patients with MM. Renal failure is more frequent in patients with light chains and IgA MM than in IgG MM. The factors associated with renal function recovery were the degree of RF and presence of hypercalcemia.
9.3 Treatment of bone disease.

245 THERAPY WITH BISPHOSPHONATES ENHANCES OVERALL SURVIVAL IN PATIENTS WITH MULTIPLE MYELOMA

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INTRODUCTION. Bisphosphonates (BPs), which are routinely used in multiple myeloma (MM) patients, provide significant protection against skeletal complications and hypercalcemia. BPs have been shown to cause cell cycle arrest and apoptosis in MM cells. However, most in vivo studies fail to reproduce the anti-tumor effect of BPs and little is known about the efficacy of BPs on malignant plasma cells in vivo.

OBJECTIVE. The main goal of this study was to investigate the effect of the BPs in response to chemotherapy and survival in patients with MM.

PATIENTS-METHODS. We have reviewed 72 patients (median age 69 y; 35 F, 37 M; 31 Ig G, 29 Ig A, 11 Bence-Jones and 1 non-secretor; 7 stage I, 15 stage II and 50 stage III disease) with newly-diagnosed myeloma of whom 65 received chemotherapy (melphalan and prednisone in 44, polychemotherapy in 16 and autotransplantation in 5 patients) and 7 MM I stage were not treated with the actual protocol. Thirty of these patients were regularly treated with BPs (pamidronate 90 mg/monthly). We compared two groups of patients: treated with BPs (30) and the historical patients not treated (42) that showed significant differences in the following parameters: age, sex, stage, bone lytic lesions, M-band Ig class, percentage of peripheral blood plasma cells, hemoglobin, percentage of bone marrow plasma cells, creatinine, calcium, LDH, beta-2-microglobulin, RCP and proteinuria.

RESULTS. The results of chemotherapy was higher in MM patients with BPs (65.4%) than in the other patients (44.7%), but this difference was not significant. The overall survival was significantly better in patients with BPs treatment (49 ± 9.9 months vs 26 ± 3.7 months, p = 0.03).

CONCLUSION. The association of BPs to the treatment displays an important benefit in the overall survival of MM patients. This effect could be attributed to this potential anti-tumor activity in vivo.

246 Bisphosphonates in Multiple Myeloma: A single institution experience of 30 patients with Advanced Disease

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A Leukemia Lymphoma & Myeloma Clinic was established at the SMS Medical College Hospital, Jaipur, India in 1992. The present work reports the use of bisphosphonates in 30 newly diagnosed patients of advanced Multiple Myeloma (Salmon Durie stage III) registered at this clinic. There were 26 patients with stage IIIA and 4 patients with stage IIIB myeloma. There were 18 males and 12 females with the mean age of 54 years (range 38 to 72 years). Twenty two patients were given 6 cycles of standard infusional VAD (Vincristin, Adriamycin & Dexamethasone) Chemotherapy while 8 patients received 12 cycles of intermittent oral M+P (Melphalan + Prednisolone). Twenty four patients were given 90 mg of Pamidronate every 4 weekly as a 3 hour intravenous infusion while 6 patients received 4 mg of Zoledronic acid as a half hour infusion. Bisphosphonates were continued for 12 months and then stopped. Patients were evaluated monthly. Effectively of the bisphosphonates was assessed by tabulation of all skeletal events, incidence of pathological fractures, incidence of radiotherapy to painful bony disease and evaluation of analgesic use for bone pains and its severity as assessed by a detailed questionnaire.

Overall response rate (RR) was 73% (22 out of 30) with a RR of 86% (19 out of 22) in the VAD group and 37% (3 out of 8) in the M+P group. Four (18%) patients in the VAD group should a complete response. All patients have been followed-up for a median period of 17 months (range 11 to 42 months).

There were 5 (16%) skeletal events, 3 (10%) pathological fractures and 4 (13%) patients required radiotherapy to bony lesions. Bony pain and analgesic use was significantly reduced within the first two months of treatment in 14 (47%) patients and was significantly reduced in 22 patients (73%) after six months of treatment. Only one patient had hypercalcemia at presentation which resolved after one cycle of chemotherapy and Zoledronic acid. Except for allergic rash in one patient after Pamidronate, there were no adverse events reported after bisphosphonate treatment.

Even though this is a small study with relatively short follow-up, we conclude that bisphosphonate treatment is well tolerated and effective in reducing bone pains and skeletal morbidity in patients of advanced multiple myeloma.

247 Cost of skeletal complications in patients with multiple myeloma

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Background: Multiple myeloma patients often experience skeletal-related complications including pathological fracture, hypercalcemia, pain requiring surgery, radiotherapy, or opioid analgesics, or spinal cord compression (collectively, skeletal-related events [SREs]). SREs may result in increased morbidity and medical care costs. In the Netherlands, the estimated average cost attributable to SREs in prostate cancer patients in 1998 was 6,973 Euros (7,300 $US 2002).1 The cost of SREs in multiple myeloma patients is unknown. Methods: The objective of this study was to estimate the costs of SREs in US multiple myeloma patients. We used data from large health-insurance claims database spanning 7/94-6/02 and linked mortality data from the US Social Security Administration. Study subjects included all persons with (1) >2 encounters with a diagnosis of multiple myeloma; and (2) presence of >1 SRE. SREs were identified based on the occurrence, on or after the date of first diagnosis of multiple myeloma; and (2) presence of >1 SRE. SREs were identified based on the occurrence, on or after the date of first diagnosis of multiple myeloma, of (1) >1 encounter with a diagnosis of pathological fracture, spinal cord compression, or hypercalcemia, or (2) >1 bone surgery or radiotherapy procedure, or (3) initiation of opioid analgesic therapy. The primary measure of interest was the expected lifetime cost of SRE-related care, which was estimated using Kaplan-Meier methods. Results: We identified 835 multiple myeloma patients, of whom 352 (42%) experienced >1 SRE. Mean age of patients with SREs was 66 years. Median survival from date of first SRE was 28 months. Expected lifetime...
cost of SRE-related care was $10,247 per patient (95% CI $7,921-$12,573); 74% of these costs were incurred within 6 months of the first SRE-related claim. Conclusions: The economic burden of SREs in multiple myeloma patients is substantial. Coupled with evidence from randomized trials demonstrating the benefits of potent intravenous bisphosphonates in reducing the incidence of SREs, our findings suggest that early intervention with these agents may play an important role in improving outcomes in multiple myeloma patients.


248 Safety and efficacy of pamidronate and zoledronic acid in multiple myeloma patients are comparable
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Randomized, double-blind study was conducted to compare the efficacy and safety of zoledronic acid and pamidronate for treating myeloma bone disease. Since March 1999 the efficacy and safety of pamidronate and zoledronic acid is evaluated in MM patients all receiving anti-myeloma chemotherapy acc. to VMCP/VBAP regimen. Nine patients with stage III myeloma and osteolytic lesions (3 female, 6 male, median age 57 years, range 52-67), were randomly assigned (1:1:1 ratio) to treatment with either 4 or 8 mg of zoledronic acid via 15-minute intravenous infusion or 90mg of pamidronate via 2-hour intravenous infusion every 3 to 4 weeks for 12 months. In extension phase of the study (June 2000 – February 2003) patients did not received bisphosphonates. Results. In 7 patients 18 cycles of assessed treatment was administered to each of them and one patient received 16 cycles. One patient died after receiving 12 pamidronate therapy cycles at 11 month of the trial duration. The patient’s death occurred during the progression of plasma cell proliferation due to acute left ventricle cardiac failure. During the 12-month-period of bisphosphonate treatment and in extension phase skeletal related events (SRE) and progression of osteolysis occurred with the same frequency in 3 treatment groups. One patient experienced spinal cord compression and received radiation to bone and 2 patients experienced vertebral fracture. Time from study entry to the first SRE was 304 days in pamidronate and 366 and 392 days in 4 and 8 mg zoledronic acid group, respectively. The skeletal morbidity rate was identical in all treatment groups. Adverse events: single hypocalcemic events occurred in 2 patients, mild hypertransaminasemia was observed all treatment groups. We recorded, for the study group and the control group, respectively, a CR incidence of 80% (4/5 patients) vs 57% (8/14) (p=0.3) at 12 months after ASCT; 80% (4/5 patients) vs 21.5% (3/14 patients) (p=0.04) at 24 months after ASCT and 40% (2/5 patients ) vs 21% (3/14 patients) (p=0.11) at 36 months from transplant. At a median follow-up of 24 months (range 12-63) from the 2nd ASCT the study group and the control group showed an OS of 100% vs 79% (p=NS) and a median CR duration of 52 months (range 12-60) vs 15 (range 2-30 ) (p= NS) respectively. In conclusion this preliminary observation on a substantial benefit of Pamidronate after 2 years post ASCT needs to be confirmed on a large number of patients.

250 Kyphoplasty enhances function and structural alignment in multiple myeloma
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Multiple myeloma is associated with vertebral compression fractures and secondary kyphosis. These pathological fractures result in pain and functional disability. Kyphoplasty that utilizes minimal invasive balloon tamps to reduce osteoporotic vertebral
fractures has been applied to multiple myeloma. This investigation tests the hypothesis that kyphoplasty can significantly restore vertebral height and spinal function. Eighteen consecutive patients with vertebral fractured secondary to multiple myeloma underwent kyphoplasty balloon reduction and stabilization with PMMA. Patients were evaluated prior to treatment and post-operatively with the validated general Oswestry back questionnaire. 7 patients with 20 fractures were measured for height restoration on long kyphosis (36 inch) standing films. Over 90% of the fractures were older than 3 months by history and MRI. By Oswestry analysis the average pre-op score was 48.94 and the average post-op score 32.70 with and average improvement of 16.23 (p = .002). The enhanced function was greatest for patients with the poorest pre-op Oswestry scores and of little improvement for patients with initial scores better than 28. An average height restoration of 25.3% (p < .001) and 34.9% (p < .001) was achieved in the anterior and mediolateral vertebral body respectively. No patients underwent a major complication. These results will be correlated with stage of disease, cytogenetics, B2M, LDH, proliferation index, treatment with bisphosphonates and response to systemic therapy. Kyphoplasty has proven safe and efficacious in treating both acute and chronic vertebral compression fractures secondary to myeloma. This balloon tamp methodology results in vertebral height restoration and enhanced function with no major complications. This prospective cohort study established the functional and structural benefits of kyphoplasty for patients with pathological vertebral fractures related to multiple myeloma.

9.4 Treatment of anemia

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Once weekly epoetin beta (NeoRecormon®) is as effective as three times weekly administration in anaemic patients with multiple myeloma

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Background: Once weekly administration of subcutaneous (SC) epoetin beta (NeoRecormon®) has been shown to be as effective as three times weekly (tiw) administration in increasing haemoglobin (Hb) levels and reducing transfusion requirements in studies of patients with mixed haematological malignancies. However, the effects of epoetin beta once weekly have not been reported specifically for patients with multiple myeloma.

Aim: Data from the NOW (NeoRecormon® Once Weekly) study were analysed to compare the efficacy and tolerability of SC epoetin beta 30 000 IU once weekly compared with 10 000 IU tiw in patients with multiple myeloma.

Methods: Patients with anaemia (Hb 9–11 g/dl) associated with multiple myeloma and serum erythropoietin ≤100 mU/mL (indicating defective endogenous erythropoietin production) were randomised to receive open-label treatment with SC epoetin beta 30 000 IU once weekly or 10 000 IU tiw for 16 weeks. Primary endpoint was the difference in the time-adjusted area under the Hb concentration-time curve (Hb-AUC) from weeks 5–16 (protocol population). Secondary endpoints (intent-to-treat population) included: the percentage of patients with an Hb response (Hb increase ≥2 g/dl from baseline without transfusion in the previous 6 weeks), and percentage of patients with corrected anaemia (Hb nadir ≥11 or ≥12 g/dl at 4 week intervals).

Results: A total of 161 patients with multiple myeloma were enrolled in the study (once weekly, n = 78; tiw, n = 80), over half of whom had advanced stage disease (Durie-Salmon stage IIIA/B = 52%). The least squares mean difference in Hb-AUC between the once weekly and tiw treatment groups was -0.14 (95% CI: -0.56, 0.28). Similar proportions of patients responded to therapy with once weekly and tiw treatment (69% vs 78%). Median time to response was 71 and 37 days, respectively, with the differences due to the 2-weekly assessment period. The percentages of patients with corrected anaemia were also similar in both once weekly and tiw treatment groups (Hb nadir ≥11 g/dl, 76% vs 82% or Hb nadir ≥12 g/dl, 59% vs 63%). Epoetin beta was well tolerated in both groups, and no antibodies to epoetin beta were detected in any of the patients.

Conclusion: Epoetin beta administered once weekly appears to be as effective and well tolerated as tiw in anaemic patients with multiple myeloma and defective endogenous erythropoietin production.

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FATIGUE AND HEMOGLOBIN LEVEL IN MULTIPLE MYELOMA PATIENTS: RESULTS OF A CROSS-SECTIONAL STUDY


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Fatigue is common in myeloma patients. We conducted a cross-sectional study to examine fatigue in multiple myeloma (MM) patients. The impact of this symptom on patients’ quality of life (QOL) was evaluated using a specific fatigue questionnaire. We examined the relationships between hemoglobin (Hb) level, QOL, and other patient characteristics in this population (eg, sex, age, disease duration, response phase, Durie and Salmon stage, previous/concomitant cancer therapy, concurrent disease, transfusion dependence, recombinant human erythropoietin use). We used the Functional Assessment of Cancer Therapy-Anemia (FACT-An) scale, which includes 13 items comprising the FACT-An Fatigue subscale and 7 comprising non-fatigue items, to evaluate QOL. The study was conducted in 24 Italian centers; between November 2001 and March 2002, 1071 patients were included (51% male, 49% female) aged 31–94 yrs (mean, 65 yrs). MM was newly diagnosed in 22% of the patients; response phase in the others was: remission 57%, relapse 15%, and no response 6%. Durie and Salmon staging was: I 15%, II 27%, III 58%. In all, 78% of the patients had received one or more treatments for MM, and 76% were currently being treated. Mean Hb was 11.9±1.9 g/dL. Significant differences in Hb level were related to sex (women 11.6, men 12.2 g/dL), age (<50 yrs 12.4, 51-60 yrs 12.2, 61-70 yrs 11.8, >70 yrs 11.6 g/dL), MM treatment response (no response or relapse 11.0, remission 12.4 g/dL), staging (I 12.5, II 11.9, III 11.7 g/dL), and concurrent chemotherapy (yes 11.3, no 12.3 g/dL). Mean FACT-An scores were 56.4±13.2 overall, 36.4±10.1 for the FACT-An Fatigue subscale, and 20.0±3.9 for the non-fatigue items. FACT-An scores were directly related to Hb level (Spearman r = 0.279, P = 0.0001), increasing from 48.4 for patients with Hb ≤10 g/dL to 59.6 for...
those with Hb >13 g/dL. Analysis of covariance showed that this relationship was not explained by the effect of covariates. A higher correlation with Hb was observed for the FACT-An Fatigue subscale than for the non-fatigue items (Spearman r=0.277, P=0.001 vs 0.214, P=0.0001, respectively). FACT-An scores were significantly lower in older patients, particularly older women (from 62.0 if ≤50 yrs to 51.2 if >70 yrs for women vs 60.5 to 56.3 for men, respectively); in patients with no response or in relapse (50.1 vs remission 58.3); and in patients with concurrent disease (52.7 vs 58.7 without concurrent disease). The differences between the subgroups in Hb level and other covariates partially explained these effects. After adjusting for Hb and other covariates (yes 56.8, no 55.9) the effect of concomitant chemotherapy (yes 58.3, no 53.5) was no longer significant. In this large study population we demonstrate a significant correlation between fatigue and Hb level in MM patients. Hemoglobin level appears to play an important role in determining some aspects of QoL. The treatment of anemia should be a consideration in the global management of patients with MM.

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In multiple myeloma, the extent of skeletal disease and response to therapy are more important predictors for quality of life than hemoglobin levels

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Prospective trials have demonstrated a statistically significant effect of erythropoietin treatment on Hb levels and quality-of-life (QoL) in the anaemia of malignant disease. However, the effect on QoL scores seems to be modest. In order to explore the relationship between Hb and QoL, we pooled data from 745 patients taking part in two recent NMSG trials (4/90 and 5/94) who had completed the QoL questionnaire EORTC QLQ-C30 prior to treatment and at regular intervals during follow-up. By means of this questionnaire, several QoL domains, e.g. physical functioning, global quality of life, fatigue, pain and dyspnea, were assessed by a 0-100 scale where differences of 5-10 are considered small, 11-20 moderate and above 20 large. QoL scores at diagnosis were outcome variables in linear regression analysis, with Hb, age, gender, Durie & Salmon stage, extent of skeletal disease, beta2-microglobulin, creatinine, calcium and albumin as predictor variables. At 12 months of follow-up, the effect of Hb on QoL scores was adjusted for age, gender and the degree of response to therapy (complete, partial or minimal response, no response or progressive/relapsed disease). P-values < 0.01 were required for statistical significance.

In univariate analysis of data obtained at baseline, Hb was significantly related only to fatigue (p<0.001) and dyspnea (p<0.001). In multiple regression, Hb remained an independent predictor for fatigue (p=0.006) and dyspnea (p=0.007). The beta coefficient (+1.89) indicates that a rise of Hb from 8 to 13 g/100ml on the average would lower the fatigue score by 9 on the 0-100 scale (mean score: 50). The most important independent predictors for physical functioning as well as fatigue at diagnosis were extent of skeletal disease (p<0.001), s-calcium (p=0.001) and gender (p=0.001). Compared to patients with normal skeletal x-rays, the mean predicted score of patients with extensive skeletal disease was 26 points lower for physical functioning (mean score: 50) and 13 points higher for fatigue.

At 12 months, univariate analysis showed that Hb was related to the scores for physical functioning (p<0.001), global quality of life (p<0.01), fatigue (p<0.001) and dyspnea (p=0.006). When adjusted for age and gender, the effect of Hb was significant for physical functioning (p=0.004) and borderline significant (p=0.01) for fatigue. When adjusted for response to therapy in addition to age and gender, the effect of Hb on these scores was no longer significant. Response to therapy emerged as the strongest independent predictor for QoL scores at 12 months. Patients who had obtained a complete or partial response had a predicted score for physical functioning that was 17 points higher on the 0-100 scale than patients without objective response, and 24 points higher than patients in relapse or progressive disease.

Conclusion: This study confirms that Hb is an independent predictor for some QoL variables, most notably fatigue, dyspnea and physical functioning. However, the impact of anaemia on QoL is weak compared to other factors. In newly diagnosed multiple myeloma, the extent of skeletal disease was a stronger predictor for QoL scores. During treatment, response to therapy was the most important explanatory variable.
were not changes in the rate of TNF, VEGF, beta-2-MG, immunoglobulins, platelets neither other parameters. Conclusion: The treatment with high dose of rHuEpo, corrects the anemia in the patients with myeloma and it also interferes with the biology from this illness when diminishing, for unknown mechanisms, the rate of IL-6 and of PCR. The TNF-alpha and VEGF don't modify after the treatment with rHuEpo.

Project 2PR02A006 financed by the Direzione General de Enseñanzas Universitarias e Investigacion de la Consejeria de Educacion, Ciencia y Tecnologia de la Junta de Extremadura.

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High dose Erythropoietin in Elderly Multiple Myeloma(MM) patients with anemia.


Anemia occurs in almost all patients (pts) during the course of MM and inadequate levels of erythropoietin can be detected in at least 50% of them. Recombinant Epo (rh-Epo) therapy became an useful component in the treatment strategy of MM giving a response in 50-60% of patients when administered at conventional dosage of 10,000 IU s.c. 3 times/weeks. The aim of the present study was to investigate: 1) the efficacy in improving Hb level and quality of life and 2) feasibility of early high dose rh-Epo (40,000 IU, twice a week) during induction treatment of elderly (<60 yrs) MM patients with Hb level <10g/dl. As induction chemotherapy, all patients received melphalan and prednisone; high-doses rh-Epo - were administered for 3 weeks, concomitant iron (300 mg iv, day 0) and folic acid therapy was given. Responders patients continued rh-Epo, according to conventional schedule , as maintenance treatment until disease progression.

Hb, transferrin saturation index and reticulocyte count have been evaluated weekly, during the first three weeks, whereas serum Epo level assay has been carried out at start of chemotherapy, then after 3, 15 and 15 weeks. Quality of life by fact-an test is done at starting therapy, after 3 weeks and, successively, monthly. Response to rh-Epo treatment has been considered complete (CR) if Hb level increases to > 12g/dl, major (MR) - stable increase of 2 gr/dl , and minor (mR) - Hb increase >1 < 2 gr/dl . Patients, who did not achieve any improvement in Hb and/or maintained the same transfusion requirement were considered non responders.

From December 2001 to August 2002, 9 patients (7 females and 2 males, median age 68 yrs, range 60-73 yrs) entered in this study: 4 patients were at diagnosis and 5 in first progression stage, 6 had disease stage IIA, 2 IA and 1 II B at diagnosis. Before rh-Epo therapy median Hb level was 8 gr/dl (range 6,9-9,5), median reticulocyte count was 0,8% (range 03-2), median transferrin saturation index was 43% (range 13-65). Three patients required supportive therapy: 2,2 and 3 RBC unit/month, respectively. The median fact-an evaluation was 42,7.

After three weeks of treatment ( 6 high doses rh-Epo), 8/9 patients (88%) were responders (4 MR and 4 aMR), the median Hb level was 10,1 gr/dl (range 8,4-11,3) and median increase Hb level was 1,5 gr/dl (range 0,7-3,5). Only 1 pt resulted refractory, even if in this case supportive therapy requirement reduced from 3 to 1 RBC unit/month. No difference was observed between patients treated at diagnosis or in progression phase. Only one patient (at diagnosis) increased significantly Fact-an evaluation (>30% );total median point 43,5.

After the first month of maintenance with conventional doses, all responder pts maintained the Hb improvement (median Hb level 10,5 gr/dl, range 9,5-12 g/dl) achieved during induction. As to February 2003, after a median follow-up of 11,8 months (range 6-13 months), 6 pts continued to be responders, (3 MR and 3 mR) and 2 relapsed after 4 and 6 months respectively. The only refractory patient died, because of disease progression, after 7 months of follow-up.

These results show that, in the treatment of elderly MM patients, early HD rh-Epo seems to be an effective and safe approach in inducing a rapid and stable increase of Hb level, which can be successively maintained with conventional rh-Epo doses. These data, even if promising, need to be confirmed in a larger cohort of patients, with longer follow-up, to better evaluate the real impact of this rh-Epo schedule both on disease outcome and quality of life.

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Rapid and sustained response of disease-related anemia in patients with multiple myeloma by high doses of epoetin alpha: the updated results of an open, non-comparative, single centre pilot study.

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The results of a pilot experience on the management of the multiple myeloma (MM)-related anemia, evaluating the efficacy and tolerability of a new regimen of high doses (HD) of erythropoietin-alpha (epoetin alpha, 40,000 IU), are reported. Ten patients with a median age of 72 (45-87) years entered the study. Nine patients were in IIIA and one in IIA disease stage. Median time from diagnosis was 38 months (range: 1-74 months). Five patients presented very advanced MM, three a stable response and two were at the onset of the disease. The median previous lines of anti-myeloma treatments were 2 (range: 0-5). All patients received anti-myeloma treatments according to the phase of disease.

The mean hemoglobin (Hb) and serum erythropoietin levels were 8.5 ± 0.7 g/dl and 43±17 mU/ml respectively. Epoetin alpha 40,000 IU, was given twice weekly (TW), SC, for 4 weeks (8 weeks in non- responders) or shorter period if patients achieved an Hb level >12 g/dl. After the induction phase, the dosing interval was progressively longed. The responders received epoetin alpha 40,000 U once weekly (OW) for two weeks and then the same dose was given every two weeks. An I.V. iron supplementation was given on regular basis (125 mg weekly) during the induction phase. The responders received the same dose of I.V. iron if presenting hypochromic erythrocytes >10% of the total or transferrin saturation <20%.

Major and minor responses were defined as no need for transfusions and an increase of the Hb level by >2 gr/dl or by >1 gr/dl respectively. Overall, 9 (90%) of 10 patients responded to the treatment: 2 achieved a minor response and 7 a major response. Out of these, 2, 3, 2 and 1 achieved the response within the first, the 2nd, the 3rd and the 4th week respectively, reaching a mean (±SD) Hb level of 12.6±1.5 gr/dl; one patient showed a late response, becoming transfusion independent after 7 weeks. Out of the 9 responders, 8 stopped the treatment: 6, presenting a stably maintained erythroid response, died after 18, 24, 26,42, 46 and 58 weeks respectively; the seventh stopped epoetin alpha after 10 weeks and was undergone to an allogeneic peripheral blood stem cell transplantation; the remaining patient lacked the response to epoetin alpha after 52 weeks. To date, two patients have a
Early treatment of anaemia in multiple myeloma with rh-EPO

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Anaemia is frequently observed in patients affected by multiple myeloma both at diagnosis and during therapy. In the treatment of this disease the action of rh-EPO is well known: EPO is able in fact to increase the erythropoietic progenitor cells survival bearing proper EPO receptors (CFU-E and proerythroblasts). The “conventional dose” is based on the administration of rh-EPO (10 000 UI x 3/weekly) (OR 50-60%). The better result (OR 80-90%) was obtained using high rh-EPO doses (40 000 UI x 2/weekly) is probably due to a higher recruitment followed by proliferation and maturation of BFU-E. We tested the efficacy of a weekly treatment with rh-EPO in order to take advantage of the EPO “peak” dose on the progenitor cells maintaining a monthly conventional treatment (16 0000 UI/monthly vs 32 0000 UI/monthly). Moreover, we started treatment during the early phase of disease (if Hb<12g/dl) in order to use the residual bone marrow erythroid supplies. 14 patients with MM have been treated. The median of the age was 59 years (range 54-73); 4 patients were in IA stage of the disease, 2 in IIA, 3 in IIB, 2 in IIIB. 4 patients were naive, 6 were pretreated (1 refractory and 5 relapsed, 2 of these relapsed after ABMT). 4 of the patients were submitted at last to 2 cycles therapy. The patients were treated with Thalido (4), Thalido+Dex (5), Mp (3), Dex+Edx (2). Rh-EPO treatment was carried on if Hb<12g/dl or in case of an Hb decrease of at least 2g/dl during therapy. Therapy was then continued till an increase of Hb up to 2g/dl: in this stage gap of rh-EPO administration was increased to reach the minimum permanent maintenance dose when Hb>12g/dl. The treatment was then suspended when the Hb levels were >13 g/dl. The response was considered major (MR) for Hb>12g/dl or for a steadily increase >2 g/dl, minor (mR) for Hb increase >1<2 g/dl. After 2 months treatment 11 out of 14 patients (78%) obtained a MR and 3/14 (22%) an mR. The median Hb increased from 9,7 (9,5-11,7) to 12,5 (11,4-13,5). The median increase was 2,1g/dl. The major increase has been observed in patients treated with Thal-Dex (this was probably due to the anti TNF effect and to the absence of bone marrow toxicity). Patients (follow-up 6 months) maintain a steady response with 40000 UI / 2-4 monthly.

These data (OR 100%) are in favour of our early treatment of anaemia (both in terms of rate of response and in terms of total rh-EPO UI) and seems to strengthen the hypothesis that a high concentration of rh-EPO to a plasma levels might recruit a higher number of erythroid progenitor cells (BFU-E) which carry , to a minor extent, proper EPO receptors.

Anaemia associated to Multiple Myeloma: Response to Epoetin alpha.

Background: Anaemia is a common finding in Multiple Myeloma (MM) patients, being observed in about 60%. Several mechanisms as bone marrow infiltration, low levels of endogenous erythropoietin due to renal damage, IL-6 increase and chemotherapy toxicity are involved in its origin. Epoetin alpha (Epo-a) therapy has been reported useful to improve both haemoglobin levels and quality of life (QOL) in these patients.

Design of study: a randomised, prospective, observational study was performed in a cohort of MM patients. The main objective of study was to evaluate the response of the anaemic syndrome to Epo-a and subsequently the response to chemotherapy.

Patients: 91 MM diagnosed in the Haematology Department of Miguel Servet University Hospital and followed up along 2002 were included in the study by intention to treat.

Methods: Inclusion criteria in study: haemoglobin levels <12 g/dl in males and <11 g/dl in females. Normal serum concentration of iron, folates and cyanocobalamine were required. Patients were randomised to receive A) Epo-a 10.000 U sc three times per week (No 46) and B) red cells package transfusion when haemoglobin concentration was lower than 9.0 g/dl (No 45). Response criteria to Epo-a: Increase of haemoglobin levels at least 1 g/dl after 8 weeks under therapy. Clinical response criteria: CR: disappearance of M-spike by electrophoresis. PR: Decrease at least >50% of M-spike. Minimal response: Decrease of 25-50% of M-spike. F: No response. Statistical analysis: descriptive and frequency distribution analysis were performed. Comparison between groups was done by y2 test.

Results: A total of 64 patients could be evaluated: arm A (No 35) arm B (No 34), mean age: (68,3 SD 10,5 years) gender: 38 females and 31 males and subtype: IgG 31 (IA-7, IIA-13, IIB-1, IIA-10); IgA 17 (IA-4, IIA-6, IIB-6, IIB-1), Bence Jones 17 (IA-2, IIA-4, IIB-3, IIA-8), IgG+BJ IIA-1, IgA+BJ IIB-1, Non secretory IIA-2. Chemotherapy: VB CMP/VBAD (26), Melphalan/Prednisone (9). In A group, objective response to Epo-a was achieved in 27 patients (77%); two patients showed haemoglobin increase (<1 g/dl), 4 failed to therapy (11%) and the remaining 2 were non valuable. In B group 13 patients (38%) showed a dependence on red-cells package transfusion, (mean units required: 1.5 SD 2.6), 5 patients received only one transfusion. Clinical response: A group: Objective response 29 (82.8%) F: 4 (11.4%), NV: 2 (5.7%). B group: Objective response 28 (82.3%); F: 3 (8.8%) NV: 3 (8.8%). Better QOL indicators have been observed in A group.

Conclusions: In our experience Epo-a has been effective therapy to improve both haemoglobin levels and QOL. No differences in response to chemotherapy have been found in both groups.
10. Stem and cell transplantation

10.1 Prognostic factors and results with single ASCT.

259 HIGH-DOSE THERAPY AUTOTRANSPLANTATION/INTENSIFICATION IN MULTIPLE MYELOMA (MM): PRETRANSPLANT PREDICTORS OF COMPLETE REMISSION (CR)


Institute of Hemato-Oncology, Hematology Department, BMT Section, Blood Bank. Hospital Clinic IDIBAPS, Barcelona.

Background: High-dose therapy (HDT) followed by stem cell support is widely used as intensification treatment in patients with MM responsive to the initial chemotherapy. However, there is growing evidence that only patients achieving CR benefit from this approach.

Aim: To identify pretransplant predictors of CR in responding myeloma patients intensified with HDT.

Patients and Methods: From June 1992 to August 2001, 59 patients (37 M, 22 F; median age 54 years, range 38-69) with chemotherapy disease received myeloablative therapy. The M-protein type was IgG in 32, IgA in 13, light-chain in 12 and IgD and non-secretor one case each. The initial chemotherapy consisted of MP (6 cases), alternating combination chemotherapy VCM/PV/BAP or BVMCP/VBAD (44) and VAD or VBAD (11).

The intensification regimen consisted of: Mel-140/TBI 12 Gys (21), MEL-200 (23) or busulphan-based regimens (15).

Results: The response rate to the initial chemotherapy was: CR 8%, PR 70% and MR 22%. Complete remission after HDT was achieved in 22 of the 59 patients (37%). No patient died from transplant-related toxicity or while in response. The median EFS and OS from the initiation of therapy were 41 and 68 months, respectively. Patients who achieved CR had an EFS (median, 47 months; p= 0.006) significantly longer than those attaining a CR. The pretreatment features significantly associated to CR were a low M-protein size (serum ≤10 g/L and urine < 0.5 g/24 h) (p< 0.0001) and a percentage of bone marrow plasma cells ≤ 5 % (p= 0.03). There was also a trend towards a lower CR rate in patients with a Hb level < 10 g/dL (p= 0.055).

At the logistic regression analysis only the M-protein size retained its statistical significance (p= 0.008).

Conclusion: These results confirm the achievement of CR after HDT as the crucial step for long-lasting disease control and prolonged survival in MM. The critical factor predicting CR is the tumour burden at the time of transplant measured by the M-protein size.

260 Population–based study of the ‘young’ myeloma population: only a minority of potentially eligible patients with myeloma receive stem cell transplantation; a study of the contributory factors.

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*Belfast City Hospital for Northern Ireland Haematology Group, Royal Victoria Infirmary, Newcastle-Upon-Tyne for the Northern Regional Haematology Group.

Autologous transplantation is recognised as the standard of care for younger patients with myeloma. Clinical trials do not represent fully the spectrum of patients who present with myeloma in this age group. The aim of this survey was to identify the number of patients, eligible by age, who had failed to receive a transplant, the reasons for this, what proportion of these patients might in different circumstances have been transplanted and to identify if there was unmet need for transplantation. A preliminary six month survey was undertaken by identifying all eligible (by age) transplant recipients in two regions (a) in Northern Ireland and (b) Northern Region (England) using the well established haematological malignancy data registries in these regions. A one page questionnaire was completed for each of the patients who had not received peripheral blood stem cell transplantation. We found that only 50% of patients aged 60 or under had received a transplant. Early death, co-morbidity, poor response to therapy and failed peripheral blood stem cell harvests were the main reasons for non-transplantation. Less than 20% of patients between 60 and 70 were transplanted with co-morbidity and patient choice being the main reasons for non-transplantation. We have now extended this survey to take in all patients aged under 65 years diagnosed with myeloma between January 2000 and December 2001 in these two regions. 48 and 134 patients have been transplanted prior to March 2003. The wide range of reasons for non-transplantation in the remaining 86 cases will be presented in detail.

261 Autologous Transplantation in Rare Myelomas; an EBMT study of patient characteristics, transplant rated factors and outcome

TCM Morris*, M. Drake*, T. Löppönen**, B. Bjorkstrand**, G. Gahtron**, J. Apperley+*Huddinge University Hospital, Stockholm, Sweden; +Royal Post Graduate Medical School, Hammersmich Hospital, London

Most myelomas are IgG, IgA or Bence-Jones Protein only idiotypes. In contrast, IgD and non-secretory (NS) myelomas are uncommon while IgM and IgE myelomas are very rare. While survival in IgD myeloma is generally accepted to be poor than for common myelomas, the situation for NS, IgM and IgE myeloma is less clear; little is known about the impact of conventional transplant strategies on the outcome of any of these myelomas. Most myelomas are IgG, IgA or Bence-Jones Protein only idiotypes. In contrast, IgD and non-secretory (NS) myelomas are uncommon while IgM and IgE myelomas are very rare. While survival in IgD myeloma is generally accepted to be poor than for common myelomas, the situation for NS, IgM and IgE myeloma is less clear; little is known about the impact of conventional transplant strategies on the outcome of any of these myelomas. We have therefore used the European Blood & Marrow Transplant Group (EBMT) myeloma database to study these patients and their outcome. The analysis is restricted to patients in whom Med B forms have been submitted; these contain considerably more information than the Med A (registration) forms but not all fields are completed by all investigators. A total of 10467 Med B records were studied from which 580 patients with rare myelomas were identified; common myelomas 9887, IgD 135; IgE 5, IgM 10; NS 415. IgD and NS myeloma were both at a more advanced stage at diagnosis with a greater
proportion of bone abnormality. IgD myelomas also had higher β2 microglobulin levels than the common myelomas. IgD myelomas presented with a significant reduction in haemoglobin whereas NS had significantly raised haemoglobins in comparison to the common myelomas. There was no difference in the median time from diagnosis to transplantation for IgD, NS and common myelomas. Both IgD and NS myeloma had a higher incidence of complete remission at conditioning than the common myelomas. The median survival for patients with IgD myeloma was 42.5 months (p = 0.003 log rank test) while that for NS was 50.0 months (p = 0.80). 11 patients with IgM myeloma were identified, (9 male 2 female) 8 (of 9) had stage 111 disease, all but one had PBSC transplant with none receiving TBI. Median time from diagnosis to transplant was 8 months, survival ranged from 0.4 to 30.2 months. There were 5 patients with IgE myeloma, (3 male, 2 female), 2 patients had bone marrow transplants and 3 peripheral blood stem cells, only 1 received TBI, the median time to transplantation was 7 months and survival ranged from 0.3 to 33.9 months.

These data sets confirm that patients with IgD myeloma have a poor prognosis even if treated with transplantation and both IgE and IgM would appear to be similar; in contrast NS myeloma appears to have a similar outcome to the common myelomas.

### 262 PROGNOSTIC FACTORS AT THE TIME OF SINGLE AUTOTRANSPLANTATION WITH 200 MG/M2 MELPHALAN: A SINGLE-CENTER STUDY OF 451 MYELOMA (MM) PATIENTS

**Bhawna Sirohi, Ray Powles, Jayesh Mehta, Samar Kulkarni, Clive Horton, Radovan Saso, Seema Singhal Royal Marsden NHS Trust**

McElwain & Powles (Lancet, 1983;2:822-4) pioneered the use of high-dose melphalan to the common myelomas. High-dose melphalan 200mg/m2 (HDM200) has been documented complete remission for the first time in these patients. McElwain & Powles (Lancet, 1983;2:822-4) pioneered the use of high-dose melphalan to the common myelomas. High-dose melphalan 200mg/m2 (HDM200) has been documented complete remission for the first time in these patients. High-dose melphalan 200mg/m2 (HDM200) has been documented complete remission for the first time in these patients. High-dose melphalan 200mg/m2 (HDM200) has been documented complete remission for the first time in these patients. High-dose melphalan 200mg/m2 (HDM200) has been documented complete remission for the first time in these patients. High-dose melphalan 200mg/m2 (HDM200) has been documented complete remission for the first time in these patients. High-dose melphalan 200mg/m2 (HDM200) has been documented complete remission for the first time in these patients.

**Table:**

<table>
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<tr>
<th>Event</th>
<th>Adverse Variables at HDM200</th>
<th>RR</th>
<th>95% CI</th>
<th>P value</th>
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<tr>
<td>OS</td>
<td>Age ≥ 53</td>
<td>1.63</td>
<td>1.22-2.18</td>
<td>&lt;0.0001</td>
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<td></td>
<td>Alb (µM) ≥ 39</td>
<td>0.63</td>
<td>0.47-0.84</td>
<td>&lt;0.0001</td>
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<tr>
<td></td>
<td>β2M &gt; 2.3</td>
<td>1.47</td>
<td>1.1-1.96</td>
<td>0.006</td>
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<tr>
<td>EFS</td>
<td>Not in CR at HDM200</td>
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<td>0.48-0.94</td>
<td>0.004</td>
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<tr>
<td></td>
<td>Age ≥ 53</td>
<td>1.36</td>
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<td>Relapse</td>
<td>Not in CR at HDM200</td>
<td>0.69</td>
<td>0.49-0.99</td>
<td>0.03</td>
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<tr>
<td>NRM</td>
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<td>0.24</td>
<td>0.08-0.71</td>
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<td></td>
<td>β2M &gt; 2.3</td>
<td>3.49</td>
<td>1.37-8.86</td>
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</table>

Given the importance of achieving CR at the time of high-dose therapy, intensification of induction treatment may be of benefit and makes the case for administering induction therapy to maximum response. No other factor influenced relapse. We conclude that HDM200 results in excellent outcome in myeloma and should be considered as the standard of care and this also raises questions about the use of tandem-autotransplantation as routine therapy.

### 263 IMPACT OF AGE ON SURVIVAL AFTER INTENSIVE THERAPY IN NEWLY DIAGNOSED MYELOMA.


Intensive therapy, including autologous stem cell transplantation (ASCT), is superior to conventional therapy in newly diagnosed myeloma patients below 60 years. Above that age the value is less clear.

In 1998 the NMSG initiated a population based, prospective trial (#7/98) aiming to study the effect of age on event-free survival and survival, and to compare survival to a conventionally treated historic control group. Newly diagnosed patients up to the age of 65 years were included in an intensive therapy protocol with four phases; I) VAD x 3-4; II) Cyclophosphamide 2g/sqm, G-CSF (filgrastim) and stem cell harvest; III) Melphalan 200 mg/sqm, stem cell infusion and G-CSF; IV) Interferon maintenance. Double ASCT was optional.

From Jan 1998 to June 2000, 452 patients were registered. 414 (92%) were included in the intensive therapy protocol (=Intensive Therapy Group (ITG)). 294 were below 60 years (=ITG-60) and 120 were 60-64 years (=ITG-65).

The historic control group was derived from a previous population based NMSG trial (#4/90) which included patients from Jan 1998 to June 2000. 452 patients were registered. 414 (92%) were included in the intensive therapy protocol (=Intensive Therapy Group (ITG)). 294 were below 60 years (=ITG-60) and 120 were 60-64 years (=ITG-65).

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Current 25 pts are alive after a median of 40 ms (9-62) and 18 complications (21%) were observed.

In patients 60-64 years survival was prolonged with a risk ratio of 0.58 (95%CI 0.38-0.87; p=0.01). Survival at 3 years was 65% (54-75%) in the ITG-65 and 47% (38-57%) in the CG-65. However, after correction for differences in prognostic factors between the groups the survival advantage only reached borderline significance (risk ratio 0.65, 95%CI 0.31-1.03; p=0.06). To conclude, in this population-based study patient age was found to influence outcome after intensive therapy. Intensive therapy prolongs survival also at ages between 60 and 65, but with less superiority than in younger patients.

**265**

**Autologous Peripheral Blood Stem Cell Transplantation For Primary Refractory Multiple Myeloma**

Shaji Kumar, Martha Q Lacy, Angela Dispensieri, S. Vincent Rajkumar, Rafael Fonseca, Susan Geyer, Nancy Iturria, Cristine Allmer, Thomas E Witzig, John A Lust, Philip R Greipp, Robert A Kyle, Mark R Litzow, and Morie A Gertz.

**Objectives:** We have analysed newly diagnosed MM patients (pts) (A group) receiving DAV 1st line therapy and stem cell autograft (ASCT) after a intensified conditioning regimen with idarubicin, busulphan and melphalan (IDA-BU-MEL); we have evaluated the toxicity and the impact on overall survival (OS) and progression-free survival (PFS), comparing the results with historical controls receiving BU-MEL regimen (B group).

**Methods:** The pts A were 32 (M=22), median age 53 ys (30-60). At diagnosis 28 were stage>II. Paraproteins were detected in all patients. At the time of ASCT 7 pts were already in complete remission (CR) and 25 in partial remission (PR).

Pts received a median of 5,4x106 CD34+ cells/Kg (2,2-23,1) collected after Cy (1,2 g/m2, d 1,3) and Dex, followed by daily subcutaneous rhG-CSF 5 microg/Kg.

The conditioning regimen consisted of IDA 21 mg/m2 by continuous infusion over 24 hours on d-12 to–11, BU 4 mg/kg/d on d–8 to d–5 and MEL 60 mg/m2 i.v. on d–4; ASCT was performed on d 0.

On d+1 from ASCT rhG-CSF was started at dose of 5 mcg/Kg/day and continued until granulocyte (PMN) count exceeded 1x10^9/L.

Maintenance treatment with recombinant alpha2-interferon was started after the complete recovery and continued at dose of 3 MU 3 times a week until relapse.

**Results:** In pts A the recovery was reached at a median of 10 d (8-15) for PMN>0,5x10^9/L and at 16 d (9-74) for platelets (PLTs) >50x10^9/L. The median duration of profound neutropenia was 12 d (8-14); 31/32 pts had fever: 25 agents were isolated in 18 pts. 30/32 oral mucositis grade >III WHO and 7 extrahematological complications (21%) were observed.

Currently 25 pts are alive after a median of 40 ms (9-62) and 18 are progression-free after a median of 32 ms (9-60); 13 pts relapsed after a median of 22 ms (5-47).

5 ys OS is 73% and PFS is 37%.

Data were compared with the results obtained in 38 pts receiving ASCT after BU-MEL regimen. Patients’ details were comparable in both series.

The comparison with the historical control showed an increased toxicity of the intensified regimen, in particular concerning duration of neutropenia (p<0,001), mucositis, infections (p<0,01), PLTs requirement (p<0,001) and duration of hospitalisation (p<0,05), while 5-ys OS and PFS were comparable in the 2 groups (73% vs 78% and 37% vs 48%).

Conclusions: Although we can confirm the feasibility of IDA-BU-MEL, there isn’t a real benefit in OS and PFS in comparison with BU-MEL which appears better tolerated.
disease. The lack of response to initial induction therapy does not appear to prevent these patients from obtaining a meaningful response to the SCT. Longer follow-up will be needed to ascertain if the responses in the refractory patients are durable. Meanwhile, patients refractory to initial therapy should be offered early SCT.

266 Autologous stem cell transplantation for multiple myeloma – a 12 year single centre experience

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Dept of Haematology, Southampton University Hospitals, Southampton, UK

We present the results of a single centre experience with autologous stem cell transplantation (ASCT) for multiple myeloma.

Methods and Results: EBMT registry data was reviewed for patients with multiple myeloma who underwent autologous stem cell transplantation at Southampton bone marrow transplant unit, UK, from 1990 to 2001, receiving melphalan 200mg/m2 (day –1) and intravenous methylprednisone 1.5g (day +1 to +5) as the conditioning regime. A total of 75 patients were identified. The patient characteristics were: males - 43(57%), females - 32(43%), IgG - 36(48%), IgA - 20(26.6%), Light chains -18(24%) and Non-secretory – 1(1.3%). The median age at transplantation was 57.9 years (29.5 –63.5 yrs). 74(98%) patients received peripheral blood stem cells and one patient (2%) received bone marrow. All patients received a single autograft. Median time to neutrophil engraftment was 15 days (8-40days). The transplant related mortality was 2.6% (2 patients) due to infection(s) in the immediate post transplant period. The response to ASCT was: complete remission (CR)-19(25 %), partial remission (PR)-53(71%) and unknown in 3(4%) patients.

The median follow up was 5.07yrs. The median overall survival (OS) of patients in PR was 6.7yrs(0.5 - 12.4 yrs) while it has not yet been reached for the whole series and for patients in CR. The mean OS was: all patients - 8.8yrs(0.5-12.4 yrs) and patients in CR - 8.7yrs(0.6-9.4yrs). There was no statistical difference between the OS of patients in CR vs. PS (log rank p = 0.08). 48(64%) patients are still in remission, 25(33%) have relapsed and the status of 2(3%) remains unknown. There was no significant difference in OS in patients with: IgG vs. IgA; kappa vs. lambda; or presence or absence of light chain disease.

Conclusion: The present findings appear to be in keeping with the published literature though the OS appears to be superior compared to studies using single agent high dose melphalan as conditioning. The use of methylprednisone in the conditioning regime does not appear cause any additional toxicity. There appears to be a trend for superior OS in patients achieving a CR though this was not statistically significant. There also appears to be a small cohort of long term survivors, irrespective of the remission status (CR or PR), supporting a concept of “functional cure”. These findings may suggest a role for addition of methylprednisone to the standard melphalan conditioning. Further studies are needed to clarify the disease biology of the patients with a “functional cure”.

267 A Multicentre Randomised Study of High Dose or Intermediate Dose Melphalan Consolidation of Initial VAD / VAMP Therapy of Myeloma.

for the Southern England Collaborative Trials Group. *Royal United Hospital, Bath, BA1 3NG, UK. Tel (44) 1225-824488, Fax (44) 1225-461044, email charles.singer@ruh-bath.swest.nhs.uk

Introduction: VAD plus high dose melphalan has become the standard treatment for younger patients with myeloma but is not curative and has significant toxicity. More therapy is always required subsequently and few patients are able to tolerate several intensive regimens. This study compares high dose melphalan (HDM) and intermediate dose melphalan (IDM) as consolidation after an initial response to VAMP/VAD.

Methods: 59 newly diagnosed patients with myeloma, aged < 65 years treated with 4-6 cycles of VAMP or VAD were randomised to HDM or IDM after cyclophosphamide (CTX; 1.5 - 4g/m2) and peripheral blood stem cell (PBSC) harvest. Patients then received HDM (200mg/m2) plus PBSC re-infusion or IDM (80 mg/m2) plus G-CSF (Lenograstim, Chugai). Maintenance interferon-alpha (Intron-A, 3MU/m three times weekly, Schering-Plough) was given to both arms.

Results: 39 patients were male; median age 55 (39-64); 37 had IgG, 12 IgA, 1 IgD serum paraproteins; 5 LC & 4 NS. 11 received VAD rather than VAMP. 10 received < 4g/m2 CTX. 29 was given to both arms.

Alpha interferon (Intron-A, 3MU/m three times weekly, Schering-Plough) was given to both arms.

Results: 39 patients were male; median age 55 (39-64); 37 had IgG, 12 IgA, 1 IgD serum paraproteins; 5 LC & 4 NS. 11 received VAD rather than VAMP. 10 received < 4g/m2 CTX. 29 was given to both arms.

Follow-up: HDM: progression in 13/30 (43%) at a median 22 (2-43) mo from HDM; 21/30 (70%) are alive; median overall survival 30 (2-87) mo from HDM; 6/9 deaths were due to myeloma.

IDM: progression in 20/29 (69%) at a median 17 (4-84) mo from IDM; 18/29 (62%) are alive; median overall survival 40 (5-98) mo from IDM; all 11 deaths were due to myeloma.

Conclusions: HDM offers a higher chance of achieving CR and a more prolonged response but there is no clear evidence of superior survival to IDM (80 mg/m2). IDM is a reasonable alternative for those patients in whom PBSC harvest is unsuccessful.
268
High Dose Therapy (HDT) supported with Autologous Blood Stem Cell (ABSC) transplantation: long term follow-up of 3 prospective studies including 455 patients with Multiple Myeloma (MM)

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Between 1986 and 1995, we initiated 3 prospective studies designed to assess the interest of HDT and ABSC transplantation in the treatment of MM.

The first one was a phase II study in which 63 young patients (median age 44 yrs) with stage III or stage II MM were included (Blood, 1993, 82, 2005-9).

Thereafter, patients up to 56 years of age with successful ABSC collection (185 out of 202 initially enrolled patients) were randomly assigned to receive HDT and ABSC transplantation or conventional chemotherapy (CCT). In the later group, HDT and ABSC transplantation was intended to be systematically performed in case of primary resistance to CCT or at relapse in responders (Blood, 1998; 92, 3131-36).

The third study randomly compared HDT and ABSC transplantation with CCT in 190 patients aged between 55 and 66 years (Blood, 1999; 94, 396a, abst. 1754). In this study, HDT protocol consisted of melphalan (MLP) 200 mg/m2 (or MLP140 + busulfan) whereas HDT included a 12 Gy total body irradiation (TBI) in the 2 other studies.

At the reference date of 01/01/2003, median follow-up of all 455 patients enrolled in the 3 studies reached 13.8, 10.3 and 8.2 years, respectively. Considering the rate of long-term survival as crucial in evaluating the treatment, we up-dated all data aiming to precise the rate and characteristics of long-term survivors. Results of the analysis will be presented at the meeting.

269
High-Dose Melphalan (MEL) 280mg/m2 plus Amifostine Cytoprotection and ASCT as Part of Initial Therapy in Patients with Multiple Myeloma

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Recurrent multiple myeloma (MM) after ASCT remains a significant problem in the treatment of this malignancy. One approach has been to administer a cytotoxic regimen more intensive than the usual melphalan (MEL) 200mg/m2 given before a single ASCT. The use of tandem transplant represents one effort in this regard that has produced encouraging results. We have evaluated a different strategy in which we increased the dose of MEL to 280mg/m2 before a single ASCT; this dose has previously been shown to be well tolerated when given with amifostine (AF) to reduce non-hematopoietic toxicity (Proc ASCO, 2001:24).

The current study was performed to evaluate this regimen in MM patients (pts) undergoing ASCT as part of initial therapy. Between 05/99 and 12/02, 43 pts received AF 740mg/m2 IV over 5-15 min 24 hr and 15min before MEL 280mg/m2 (given IV over 15 min); stem cells were reinfused 24 hrs after MEL. Median age was 58 yrs (32-65 yrs); 28 were male. Ig subtypes were as follows: IgG (24 pts), IgA (13 pts), light chain (4 pts) and non-secretory (2 pts). Median pre-ASCT beta 2-microglobulin level was 1.64 mg/L. Thirty-nine were in CR/PR. Priming therapy for blood stem cell collection included G-CSF alone in 6, cyclophosphamide (CY)+G-CSF +/-GM-CSF in 12 and CY+etoposide +G-CSF in 25. Median CD34+ cell dose was 6.97x106/kg. No significant hypotension, but occasional hypocalcemia and nausea/vomiting, were noted with AF. The median day to recovery of an ANC of 0.5 x 10^9/L was 12 (range 6-28) and day of the last platelet transfusion was 16 (range 4-34) post-ASCT; 2 pts had delayed engraftment. Median hospitalization was 19 days (range 8-34 days). Using the Seattle criteria for regimen-related toxicity (RRT), grade (gr) I mucosal toxicity was seen in 14 and gr II (requirement for continuous IV narcotics) in 12 pts evaluable to date. In addition, reversible gr II cardiac (2 pts), bladder (2 pts), CNS (1 pt) and renal (1 pt) RRT were seen, but no gr III or fatal toxicity was observed. One pt with bacteremia needed temporary hemodialysis. Best post-ASCT response in evaluable pts includes CR in 25/40 (63%), PR with bacteremia needed temporary hemodialysis. 2 pts had delayed engraftment. Median hospitalization was 19 days (range 8-34 days).

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AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION FOR MULTIPLE MYELOMA: CLINICAL RESULTS FROM A SINGLE CENTER.

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Background: Autologous peripheral blood stem cell transplantation (APBSCT) has become a routine treatment for symptomatic patients with multiple myeloma (MM). We have retrospectively analyzed our center experience.

Patients and methods: Between June 1995 and May 2002, 30 patients with symptomatic MM (22 males/8 females), median age was 63,8 years (46-72), were treated with high-dose therapy rescued with APBSCT. The median time from diagnosis to transplant was 19,3 months (5-72). Fourteen (46,2%) patients had IgG, 9 (29,7%) IgA, 6 (19.8%) Bence-Jones and 1 (3,3%) IgM monoclonal component. Stage according Durie-Salmon was: I in 2 patients (6,6%), II in 11patients (36,3%)and III in 17 patients (56,1%). Prior to APBSCT 21 (69,3%) cases had received only one line of chemotherapy and 9 (29,7%) two o more lines. At the time of transplant 3 patients (10%) patients were in complete remission (CR), 23(75,9%) in partial remission (PR), 3(10%) in minor response and 1 (3,3%) was non responding. Mobilization of stem cells was performed with chemotherapy + G-CSF 5 µg/Kg/day in 18 procedures, G-CSF 10 µg/Kg/day in 11 procedures and G-CSF 10 g/Kg twice a day in 11 procedures,
yielding a median of 3x10^6/kg CD34 cells and 35.3x10^4/Kg CFU-GM. The conditioning regimen was melphalan 200 in 26 cases (86.8%) and busulfan plus melphalan in 4 (13.2%).

Results: The median days to recovery of neutrophils (> 0.5x10^9/L) was 11.2 days (10-13), to recovery of platelets (>1x10^9/L) was 9.4 days (9.0-27), and to recovery of platelets (>20x10^9/L) was 13.1 days (10-36) and to recovery of platelets (>50x10^9/L) was 15.2 days (12-50). Transplant related mortality occurred in 1 patient (3.3%). Following the autograft 12 patients (41.2%) were in CR, 14 (48.1%) in PR and 3 (10.3%) in progression status. Three months after APBSCT 18 (62%) patients received recombinant alpha interferon and steroid as maintenance therapy. With a median follow-up of 69.6 months (96-13),16 patients (53.3%) remain alive and 8 (26.6%) are free of progression. The overall survival (OS) of all patients was 41.9 months (SE ,CI 95% 29-54) and the median duration of progression free survival (PFS) was 24.8 months (SE 3.9 CI 95% 17-32).

Comments: We conclude that APBSCT is a safe and effective therapy in MM patients. Our results are similar to obtained by other groups in terms of mortality, overall survival and event-free survival.

271 Long term survival after high dose chemotherapy and stem cell transplantation for multiple myeloma - two case reports

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Multiple myeloma is not considered curable with standard chemotherapy. High dose chemotherapy (HD-CT) followed by stem cell transplantation (SCT) improves survival in selected patients (pts.). After SCT 50-60% of myeloma pts. achieve remission, minimal residual disease causes relapse in most pts, predictors for long term survivors are not yet clear.

Among 18 pts (12 male, 6 female, median age 56 years, range 35-62) transplanted for myeloma in our institution 2 pts with long term remission can be identified: we report two female patients, age at diagnosis 35 and 57 years, both presenting with stage IIIB disease and elevated serum creatinine (3.5 mg/dl and 5.6 mg/dl); multiple bone lesions were apparent in one case at time of diagnosis, in the other at time of progression. Both patients had Bence-Jones proteinuria, paraprotein was IgG lambda in one case, light chain kappa in the other, in whom disease was complicated by amyloidosis of the tongue apparent at time of case, light chain kappa in the other, in whom disease was Bence-Jones proteinuria, paraprotein was IgG lambda in one case.

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272 A Multi-Center Randomized Phase Two Trial of Thalidomide and Prednisone as Maintenance Therapy For Multiple Myeloma Following Autologous Stem Cell Transplant.

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Purpose: Multiple myeloma (MM) patients benefit from high dose chemotherapy however relapse is inevitable and novel means of maintaining remission are required. A multi-center, randomized phase II trial was therefore conducted to assess the tolerability of combined thalidomide and prednisone maintenance in MM.

Patients and Methods: Eligibility required transplant within one year of treatment onset and maintenance commenced 60-100 days post stem cell infusion. All patients received prednisone 50mg p.o. on alternate days and thalidomide at a starting dose of either 200 mg or 400mg p.o. daily. The primary endpoint was the incidence of dropout or dose reduction due to treatment toxicity within 6 months.

Results: 67 patients were enrolled. Median follow-up is 9.6 months. The primary endpoint was reached by 14 of 45 patients on the thalidomide 200mg arm (31%; CI 20.7 - 47.4%) and 14 of 22 patients on the thalidomide 400mg arm (63%; CI 45.4 - 83%). Allowing for dose reduction, 76% (34/45) of patients assigned to thalidomide 200mg and 41% (9/22) of patients assigned to thalidomide 400mg remained on maintenance therapy 17.8 months after registration. Common toxicities included neuropathy 54%, constipation 42%, fatigue 37%, dizziness 34%, infection 30%, sedation 22%, mouth dryness 22%, skin rash 20% and edema 19%. Symptomatic DVT was observed in 7.5% of patients.
Conclusion: Only the thalidomide 200mg arm of this trial met our definition of a tolerable maintenance therapy i.e. no dose reductions or discontinuation in at least 65% of patients for a minimum of 6 months. This regimen has therefore been selected for further study in a Phase III randomized trial.

10.2 Conditioning regimens, stem cell collection, toxicity and immune reconstitution

273 TWO DOSE-INTENSIVE MELPHALAN REGIMENS (100 mg/m² versus 200 mg/m²) IN MULTIPLE MYELOMA PATIENTS.

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In multiple myeloma the superiority of high-dose melphalan (usually 200 mg/m², MEL200) followed by stem cell support versus standard therapy has been showed in several trials. It increases complete remission (CR) rate, extends event-free survival (EFS), and overall survival (OS) from 3 to 5 years. The median age for transplanted patients ranged from 49 to 52 years in the major clinical trials. Recently the clinical impact of intermediate dose-intensive melphalan has been evaluated. Melphalan 60 mg/m² was superior to melphalan 30 mg/m² in refractory patients. Melphalan 100 mg/m² (MEL100) was superior to standard oral melphalan and prednisone in newly diagnosed patients. In both studies, the median age was 63-64 years, health-care support was similar to that required for intravenous conventional chemotherapy.

Both MEL100 and MEL200 are clearly superior to standard-dose melphalan, however their comparative toxicities and outcomes are unclear. In this study we treated patients with similar disease characteristics with MEL100 or MEL200: their toxicities and outcomes were compared.

Ninety patients at diagnosis were treated with two MEL100 courses between 1994 and 2001. The clinical outcome was compared with a control group of 90 pair mates matched for serum microglobulin levels and Durie and Salmon clinical stage. These patients were treated at diagnosis with MEL200 courses.

Clinical characteristics were similar in both groups except for age that was significantly different (p < 0.001). Transplant-related mortality was 4% for MEL100 and 5% for MEL200 (p = NS). Complete remission (CR) was 35% after MEL100, 48% after MEL200 (p = 0.08). Median event-free survival (EFS) was 32 months in the MEL100 group, 42 months in the MEL200 group (p = 0.005), but overall survival (OS) was 67 months for MEL100 and 75 months for MEL200 (p = NS). After MEL100, the duration of hospitalisation (p = 0.05), of severe neutropenia (p = 0.0001) and of thrombocytopenia (p = 0.0001), transfusion requirements (p = 0.002), incidence of mucositis (p = 0.016) and fever of unknown origin (p = 0.008) were all significantly reduced. Despite a significant age difference, MEL100 was less toxic than MEL200, MEL100 was inferior to MEL200 in terms of EFS but not in terms of OS. This difference might be related to the deliver of more effective salvage regimens in patients relapsing after MEL100.

274 A NEW CONDITIONING REGIMEN INVOLVING TOTAL MARROW IRRADIATION, BUSULFAN AND CYCLOPHOSPHAMIDE FOLLOWED BY AUTO-SCT EVALUATED IN A PHASE III AND IN COMPARISON TO TANDEM MELPHALAN IN A PHASE III STUDY


The overall survival of patients with advanced multiple myeloma (MM) undergoing high-dose chemotherapy and autologous stem cell transplantation (SCT) depends mainly on the quality of response. Thus, to improve the response rate, a new intensified highdose chemoradiotherapy was evaluated in a phase I/II study. After induction chemotherapy 89 patients (median age 51, range 32 - 60 years) with MM stage II/III received a conditioning regimen with total marrow irradiation (9 Gy), busulfan (12 mg/kg) and cyclophosphamide (120 mg/kg) followed by SCT.

Regimen-related toxicity according to WHO criteria and response rates defined by EBMT/IBMTR were analysed. The main toxicity was mucositis grade III/IV in 76%, and fever grade > I in 75% of patients. Three patients developed reversible venoocclusive disease. Transplant-related mortality was 2%. Among patients with de novo and pretreated MM a CR rate of 48% and 41%, respectively, was documented. With a median follow-up of 45 months, the actuarial median duration of EFS and OS after transplant were 29 and 61 months for the whole group, 36 and 85 months for patients with de novo MM, respectively. Administration of this intensified conditioning regimen was associated with a tolerable toxicity, a high response rate and long EFS and OS. Thus, conditioning therapy with TMI/Bu/Cy was compared to tandem melphalan followed by autologous SCT in a European Multicenter Phase III Study involving 49 centers. 294 patients were recruited. Patients received 4 cycles of induction therapy (ID) and stem cell mobilization (IEV and G-CSF 5 mg/kg). 215 patients who completed the induction therapy, achieved at least SD and mobilized > 4 x 10⁶ CD34+ cells /kg underwent randomization to either receive TMI/Bu/Cy or tandem high dose melphalan therapy. Preliminary data from this study will be presented at the meeting.

275 Increased, but manageable, Cardiovascular Toxicity during High-Dose Chemotherapy and Autologous Peripheral Blood Stem Cell Transplantation for patients aged ≥60 years.

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Introduction: High-dose therapy (HDT) for lymphoma (NHL) and multiple myeloma (MM) is considered a feasible option for
Atrial fibrillation was the most frequent cardiovascular toxicity - pulmonary 20% 13% 0.36 - cardiac 50% 10% <0.0001 - renal 10% 3% 0.17

Methods: Patients aged ≥60 yrs at HDT were identified from the transplant database (Jan 1997-Aug 2002). Medical records were retrospectively analysed to assess efficacy and safety. Event-free and overall survival rates were compared with a control group, matched by disease type, chemotherapy sensitivity and conditioning regimen. NHL pts were also matched by international Prognostic Index (IPI) score.

Results: 40 patients aged ≥60 yrs were identified. Median age was 65(range:60-76) with 22 MM and 18 NHL. Median number prior chemotherapy regimens was 2(1-9) and performance status ECOG 1(0-2). Median IPI for NHL was 2(1-4), 17/18(94%) having aggressive histology. 50%had ≥1 comorbidities. 35% had cardiovascular comorbidity vs 18% of controls (P=0.075). Indications for transplant in MM pts were relapsed/refractory disease in 7(32%) and high-risk disease (CR1/PR1) in 15(68%).

Conditioning regimen for MM pts was high-dose melphalan(140-200mg/m) ± amifostine. 13 pts with NHL received BEAM, 4 received Busulphan/Melphalan and 1 received CBV (cyclo/BCNU/VP-16). CD34+ mobilisation was achieved in NHL with cyclophosphamide 4g/m2 and G-CSF (10µg/kg/day). Median CD34+ cells collected was 6.68 x106/kg (range 0.66-23.78) and similar in controls (P=0.44).

Response rates(RR) following HDT for MM were: 418% complete response (CR) and 1882% partial response (PR), giving an overall response rate (ORR) of 100%, vs 77% for controls; (P=0.02). RR for NHL were: 8 CR(44%), 4 PR(22%), 2 progressive disease(11%), 1 stable disease(6%) and 3 not evaluable(17%), giving ORR of 67%, vs 83% for controls; (P=0.3). Transplant-related mortality (TRM) was 8% compared to 5% in controls (P=0.6). Median time to neutrophils >0.5 x109/L = 11d vs 10d (P=0.32), >1.0 x109/L = 12d vs 11d (P=0.07), platelets >20 x109/L = 14d vs 15d (P=0.24), >50 x109/L = 19d vs 22d (P=0.26). Median hospital stay 17 days (range 13-73) was similar in controls (P=0.11).

Important toxicities >60 yrs <60 yrs P value

Median days IV antibiotics 12(2-61) 11(3-73) 0.54

Pts given IV amphotericin 15% 23% 0.39

Pts given supplemental feeding 70% 63% 0.37

Median days supplemental feeding 5.0(5-7) 4.0(5-1) 0.67

ICI grade 3/4:

-mucositis 83% 83% 1.0

-renal 10% 3% 0.17

-cardiac 50% 10% <0.0001

-pulmonary 20% 13% 0.36

Atrial fibrillation was the most frequent cardiovascular toxicity (9pts). 5 had pre-existing cardiovascular morbidity, 4 episodes occurred during electrolyte disturbance±anaemia, and 5 during sepsis. All pts were controlled with digoxin ± amiodarone. 7 pts fully recovered, 2 subsequently found to be hyperthyroid. 2 pts died from septic shock. At median follow-up 15mo, there is no difference in median EFS(25mo vs 25mo:p=0.49) or OS(39mo vs 50mo:p=0.46).

Conclusions: HDT is feasible and effective in selected patients ≥60 yrs with MM and NHL. Pts ≥60 yrs are more susceptible to cardiovascular toxicities, particularly atrial fibrillation, but have similar or better response rates following HDT and similar long-term outcomes to younger pts.

276 Oral mucositis (OM) and analgesic treatment in patients with myeloma undergoing peripheral blood stem cell transplantation. A single centre experience.

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Aim: To analyze the episodes of OM and analgesic treatment and nutritional support in patients with MM undergoing peripheral blood stem cell transplantation (PB SCT) in our center between 1995 and 2002. Evaluation of physician objectivity using WHO classification and its correspondence with analgesic requirements. Patients and methods: Retrospective review of 56 clinical files (M:37:F:19), median age at the moment of PBSCT 56 yrs (range: 34-68). Status at PBSCT was objective response (OR) 45 patients (80,3%), partial response (PR) 9 (16%) and complete response (CR) 2 (3,7%). WHO classification of mucositis was applied. About analgesic treatment those were the options: dipyrone ev, tramadol ev, morphine ev, dipyrone and tramadol ev or dipyrone and morphine ev.

Results: 38 patients (70,4%) presented OM (M:26:F:12), the distribution was: grade 0 (16), grade 1 (5), grade 2 (9), grade 3 (5) and grade 4 (19). 25 patients needed nutritional support, two of them peripheral nutrition (1 grade 3, 1 grade 4) and the others total parenteral nutrition: 18 grade 4, 3 grade 3 and 1 grade 2. Analgesic treatment and OM grade are described in the table below:

<table>
<thead>
<tr>
<th>ANALGESIC TREATMENT</th>
<th>G 0</th>
<th>G 1</th>
<th>G 2</th>
<th>G 3</th>
<th>G 4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dipyrone ev</td>
<td>11</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Tramadol ev</td>
<td>11</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Morphine ev</td>
<td>11</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Dipyrone+Morphine ev</td>
<td>11</td>
<td>5</td>
<td>2</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

Conclusion: 70,4% of patients in our serie presented OM, similar to previously described in literature. We have observed a statistical correlation between analgesic treatment required and grade of OM assigned by a physician.

277 The immune reconstitution after Autologous Peripheral Blood Stem Cell Transplantation (aPBSCT) for Hodgkin Disease, Multiple Myeloma and non Hodgkin Lymphoma: a comparison.

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Persistent total T cell reduction, CD4/CD8 imbalance, defective T cell proliferation and defects in B cell function, have been reported after aPBSCT. We have studied the lymphocyte recovery in 149 patients (75 NHL, 45 MM, 29 HD) undergoing selected or unselected aPBSCT over 7 years after stem cell infusion. Peripheral blood sample were obtained before conditioning regimen, after transplantation on day 15, 30, 60, 90, 120 and yearly for 7 consecutive determinations. We studied eight subclasses of lymphocytes (total lymphocytes, CD3, CD4, CD8, CD4/CD8 ratio, CD19, CD3HLA-DR, CD16/56) for a total
of 4590 single subset determinations in 601 controls. We did no
found statistically significant differences in the number of
reinfused CD34+cells/kg between patients receiving selected or
unselected CD34+ PBSC. All patients achieved lymphocyte
genfraftment; median time to achieve a count of 500x10^6/L was 14
days for HD, 22 for MM and 20 for NHL. Total lymphocytes
count was significantly higher in HD group (730x10^6/L) than in
MM (270x10^6/L) and NHL (220x10^6/L) on day 15; then, from day
60, it reached stable values beyond 1000x10^6/L in all groups. CD3
cells recovery was similar in the 3 groups without reaching
normal values but with a trend toward normalization until the 3rd
year; thereafter the values returned into the normal range. CD4+
counts remained below the normal value throughout the
observation period with a trend toward normalization in the 3
groups. Normal values were reaching for HD and NHL on the 6th
year and on the 7th year for the MM. Supressor (CD8) T-cells,
decreased after transplant, as from day 60 returned to normal
values or beyond the normal range without statistical differences
among the groups. The persistent reduction in CD4 cells and the
recovery of the CD8 cells subset determined a persistent reduction
of the CD4/CD8 ratio. CD19 B cells recovered from day 30,
achieving normal range on day 120 in the 3 groups; after 1 year
all values settled beyond the normal range; only at baseline time
the MM-group showed significant increase of CD19 lymphocytes
in comparison with the others two groups. Effector cells,
including CD3/HLA-DR and CD16/56, showed a similar trend
during the study period. CD16/56 remained below the normal
range until day 15 in the 3 groups and increased markedly
reaching values beyond the normal range from day 30 with the
maximum value on the 5th year in MM and on the 6th year in HD
and NHL groups. The immunological recovery after PBSCT was
similar in the 3 groups of lymphoproliferative disorders. These
data suggest that CD4 and CD4/CD8 could reach stable normal
value resulting in a complete lymphocyte recovery in a longer
follow-up.

278
Peripheral Blood Stem Cell (PBSC) Mobilization for
Autologous Transplantation in Multiple Myeloma (MM):
Comparison of four different schemes in 115 patients
from a single center.

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Cannata, E. Martí, S. Nistal, S. Osorio, A. Figuera, M.J.
Alegre, A. Granda, B. Aguado, C. Aláez, C. Blázquez, J.
Cannata, E. Martí, S. Nistal, S. Osorio, A. Figuera, M.J.
Fernández-Villalta and J.M. Fernández-Raïlada.
Hematology Department. Hospital Universitario de la Princesa.
Madrid, Spain.

Introduction and Objectives: Autologous PBSC transplantation is
the treatment of choice for symptomatic MM patients under 70
years of age as intensification after induction treatment. However,
the best method to mobilizing and collecting progenitor cells
is not well defined the collectees results could be influenced by
several variables related with disease status and with the
technical procedures used.

Patients and Methods: We present our single Centre experience
comparing four methods used for PBSC mobilization and collection
cell for autologous PBSCT in MM. From 1989 we
have included 115 patients with MM (58M/57F), median of age
was 50 years (28-70); 6 stage I, 24 stage II and 85 stage III.
Median interval between diagnosis and mobilization was 9.5
months (2-142). Mobilization schemes were as follows: Group 1:
Cyclophosphamide 4 g/m2 iv + GM-CSF 8 mcgr/kg/d sc
(Molgramostim, Leucomax®- Schering Plough); Group 2:
Cyclophosphamide 2-4 g/m2 iv + G-CSF(filgrastim,
Neupogen®, Amgen) sc; Group 3: G-CSF alone 10 mcgr/Kg/d sc
x 5 (Group 3A) y 20 mcgr/Kg/d sc x 5 (Group 3b). Group 4:
Cyclophosphamide 2 g/m2 iv + G-CSF 10 mcgr/Kg/d sc + SCF
20 mcgr/Kg/d sc.

Lymphopheritopheresis were started on day 5 in the cytokine alone
schemes and after leucocyte recovery > 4x10^9/L in the
chemotherapy+cytokine schemes.

Results: Different results are showe in the table. ( Patients
characteristics included in each group did not show significant

<table>
<thead>
<tr>
<th>Group</th>
<th>Pheresis number</th>
<th>CD34 x10^6/kg</th>
<th>MNC x10^6/kg</th>
<th>CFU x10^6/kg</th>
<th>Graft &gt; 0.5 neut/ x 10^9/L</th>
<th>Graft &gt;20 neut/ x 10^9/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>Group 1</td>
<td>1</td>
<td>21 (1-13)</td>
<td>3.1 (1-16.4)</td>
<td>7.7 (2-15)</td>
<td>4.8 (0.5-69.5)</td>
<td>12 (7-25)</td>
</tr>
<tr>
<td>Group 2</td>
<td>2</td>
<td>20 (1-2)</td>
<td>3.9 (0.5-14.7)</td>
<td>6.6 (2-14.8)</td>
<td>13.8 (0.5-37)</td>
<td>11 (7-14)</td>
</tr>
<tr>
<td>Group 3</td>
<td>3</td>
<td>67 (2-14)</td>
<td>6.2 (0.0-21.4)</td>
<td>18.3 (2-25)</td>
<td>12 (7-25)</td>
<td>12 (7-1)</td>
</tr>
<tr>
<td>Group 4</td>
<td>4</td>
<td>56 (2-14)</td>
<td>1.2 (0.0-1.2)</td>
<td>6.2 (2-15.2)</td>
<td>12 (7-25)</td>
<td>12 (7-1)</td>
</tr>
</tbody>
</table>

*p<0.005

The main variables that influence the results, apart from
mobilization scheme, were previous treatment with melphalan
and the number of lines of previous chemotherapy. These two
variables showed the major negative impact.

Comments and Conclusions: The methods with best results
regarding rates of CD34+/kg harvesting include chemotherapy
followed by the cytokine G-CSF. However, the use of G-CSF
alone is enough to reach the minimal CD34/kg targeted rate of
2x10^6/kg. This method simplifies the collections, reducing the
morbidity and the cost of the global procedure. Patients with
previous treatment including alkilants as melphalan may have
compromised the hemopoietic reserve for PBSCT mobilization.

The increase of G-CSF dosage, until 20 g/kg, did not showed in
our experience a significant increase in the CD34+ cells
collected, although new studies with much cases are needed to
define this point. SCF after chemotherapy and G-CSF was a
scheme that showed optimal results although more experience
and much cases, including testing ambulatory administration, are
also needed, considering its secondary effects and the scarce
experience with this drug.

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Intermediate-Dose Cyclophosphamide and Granulocyte
Colony-Stimulating Factor (G-CSF) is a Valid
Alternative to High Dose Cyclophosphamide for
Mobilizing Peripheral Blood CD34+ Cells in Patients
with Multiple Myeloma.

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High doses (7g/m2) cyclophosphamide (CTX) followed by
recombinant human granulocyte colony-stimulating factor (G-
CSF) is an effective mobilizing regimen for collecting peripheral
blood stem cells (PBSC). However, this regimen can cause severe
toxicity requiring hospitalization and increasing costs. In this
study, we retrospectively evaluated the efficacy and safety of a
PBSC mobilizing regimen utilizing CTX at 1.2 gr/m2 on days 1 and 3 and associated to dexametasone 40 mg daily (from day 1 to day 4) in 38 newly diagnosed MM patients (group A). Results were compared with those obtained in a previous cohort of 25 newly diagnosed MM patients (group B) in whom PBSC were mobilized using 7 gr/m2 of CTX. Subcutaneous administration of G-CSF (5 µg/kg/day) was started in all patients 24 hours after the last dose of dexametasone (group A) or CTX (group B) administration. Both consecutive cohorts of patients were comparable for age, immunoglobulins subtype, stage, disease status, time from diagnosis, previous chemo-radiotherapy regimens.

The efficacy of the two different mobilizing regimens as for the number of CD34+ collected cells was evaluated only after the first leukapheresis considering that, differently from patients of group B, a target of ≥ 2 x 106 CD34+ cells/Kg for patients of group A was required. This target was reached after a median of 1 (range 1-3) and 2 (range 1-5) leukapheresis in patients of group A and B, respectively. Failure of mobilization was observed in 2 patients of the group A and 1 of the B. The median number of CD34+ cells collected at the time of the first leukapheresis was not significant different between the two groups. A statistically significant reduction in G-CSF utilization was observed in group A as compared to Group B. No deaths in both groups were observed during mobilization and all patients of group B were hospitalized. The median duration of neutropenia (< 0.5 x 10^9/L) and thrombocytopenia (< 20 x 10^9/L) was 5 (range 3-8) vs 9 days (range 5-14) and 6 (range 5-10) vs 13 days (range 9-18) for patients of group A and B, respectively. Severe hepatotoxicity after 5 days from CD34+ cells reinfusion was the cause of death in one patient of group B. As for fever, 15/38 (39.5%) patients in group A and 20/25 (80%) in group B had a cause of death in one patient of group B. As for fever, 15/38 (39.5%) patients in group A and 20/25 (80%) in group B had a body temperature > 38°C (2; 10:03; 2P < 0.02). The duration of neutropenia and thrombocytopenia following PBSC infusion as well as the hospitalization time and the response rate were the same in both groups. Noteworthy, for the relevant economic implications, all patients in group A required no hospitalization and all but two collected a satisfactory number of PBSC. However, a longer follow-up is required for clarifying whether or not survival duration will be different between the two groups. This will be relevant for planning future mobilization strategies.

280 DCEP IS MORE EFFECTIVE THAN hd-CTX AS MOBILIZING REGIMEN in multiple myeloma WITH GOOD antitumor activity

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Introduction. We previously reported the higher efficacy in mobilizing PBSC and the lower toxicity of DCEP (dexamethasone 40 mg x 4 d and 4-day continuous infusion of daily doses of cyclophosphamide 400 mg/m2, etoposide 40 mg/m2, and cisplatin 10 mg/m2), with respect to HD-CTX. Few information, however, are available about its anti-tumor activity.

Aims of the study. 1) to confirm on a large population the efficacy of DCEP as mobilizing regimen; 2) to compare its anti-tumor activity with that of HD-CTX in previously untreated MM patients.

Patients and methods. From 1996 to 2002, 203 MM patients were consecutively enrolled in high-dose programs including single or double autologous transplantation. Two different mobilizing regimens were used: from 1996 to 1999 patients (n=61) were mobilized with HD-CTX (group 1); from January 2000 we adopted the DCEP protocol as mobilizing regimen in all MM patients for whom high-dose therapy was indicated (n=142; group II).

We evaluated the mobilizing capacity of DCEP on 142 MM pts (M 74, F 68, median age 55 yrs), and the toxicity related to this regimen. We compared the response to the induction/mobilizing phase on a total of 142 evaluable pts: 40 pts of group 1 treated with VAD chemotherapy (3 cycles) followed by a single cycle of HD-CTX (4 g/mq) and 102 pts of group 2 treated with VAD chemotherapy (2 cycles) followed by DCEP (2 cycles). Results. At the start of mobilization with DCEP, 101 pts (71%) were responsive to VAD; 11 pts (7%) were previously treated with alkylating agents; the median interval from first treatment was 3 mos (range1-54). First leukapheresis was performed after a median of 13 days (8-18) from chemotherapy. The patients collecting ≥4x10^6/kg were 92 (64.8%), while poor mobilizers (≤2x10^6/kg) were 5 (3.5%). The analysis of toxicity showed thrombocytopenia (PLT<100x10^3/ l) in 11 pts (7.7%), neutropenia (N<500/ l) in 17 pts (12%); no patient had fever, nor transfusional need. Responses of untreated pts were as follows: CR+VGPR: 36% for group 1 vs 25% for group 2; PR 32% vs 27%; NR 32% vs 48%. The differences between the two groups were not statistically significant (p=0.2).

Discussion. In this study we confirm in a large cohort of pts that DCEP is effective as mobilizing regimen. A high proportion of pts collected an adequate number of progenitor CD34+ cells with only minimal haematological toxicity never requiring hospitalization. The analysis of the clinical efficacy of the block VAD/DCEP compared with VAD/HD-CTX shows an improvement of the pre-transplant response in the VAD/DCEP group, although the difference does not reach statistical significance. In conclusion, these results indicate that DCEP chemotherapy is very effective in mobilizing stem cells with good anti-tumor activity.

281 COMPARISON OF THREE G-CSF SCHEDULES FOR PERIPHERAL BLOOD PROGENITOR CELL MOBILIZATION IN MULTIPLE MYELOMA PATIENTS

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Background: Autologous stem cell transplantation (ASCT) has an established role in the treatment of multiple myeloma patients (MM). Mobilization of peripheral blood stem cells is an integral component of current treatment protocols for MM. However the optimal mobilization schedule is not universally agreed. Aim: It is not yet clear whether a combination of chemotherapy and growth factors (G-CSF) is superior to only G-CSF. So far we have studied three variants of peripheral blood stem cell mobilization in MM patients, to compare the efficacy and toxicity.

Patients and Methods: We have retrospectively analyzed 31 patients (50 procedures) with MM who were transplanted between 1995 and 2002 in a single center. Group A (n=18): cyclophosphamide 3g/m2 and G-CSF 5 g/kg/daily, Group B (n=11): G-CSF 10 g/kg/daily and Group C (n=11): G-CSF 10 g/kg twice a day. The groups were comparable for age, sex,
stage of myeloma, response to initial chemotherapy and number of courses of prior chemotherapy. The parameters observed were:

number of mononuclear cells (MNC) and CD34+ cells, number of CFU-GM, number of leukaphereses and side effects.

Results: The median number of MNC x108/Kg were 5.6 (range 1.5-11.6) for the A group, 8.6 (range 1.8-13.4) for the B group and 13.9 (range 10.2-22) for the C group. The median number of CD34+ cells x106/Kg collected were 2 (range 0.1-5.3) for the A group, 2.1 (range 0.01-7) for the B group and 4.9 (range 2.6-7.6) for the C group. The median number of CFU-GM x104/Kg were 23.1 (range 1.3-56) for the A group, 17.5 (range 6.4-28) for the B group and 65.5 (range 31-132) for the C group. There were no statistically significant differences in number of leukaphereses between all the groups. No major side effects were observed in the A and B groups. In C group pain bone were observed in 4 cases. Significantly more CD34+ cells and CFU-GM cells were harvested in group C than A and B. There was no significant difference in the engraftment parameters.

Conclusion: We conclude that mobilization of peripheral blood stem cell with G-CSF at dose of 10 - g/Kg twice a day results in significantly more CD34+ and CFU-GM than the other groups; the three regimens are similar in terms of hematoletic recovery after infusion.

282 Individual quality assessment of autografting by probability evaluation: Models estimated from analysis of graft related end points in 522 patients with newly diagnosed Multiple Myeloma.

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Pretransplant quality assessment of autografts is an important step in prediction of posttransplant support, complications and safety. Previously, disease and therapy related variables and the impact of graft related factors have been described by weighty end points as delayed engraftment, graft failure, regimen related death etc. However, in actual clinical practice these observations are rare. Today, haematological toxicity is considered one of the more secondary end points important for graft evaluation – which in todays practice focuses primary on health economic end points. Such end points graded binary as acceptable or unacceptable has allowed us to estimate clinically relevant prognostic models for quality assessment of autografting as a whole.

In the Nordic area 522 newly diagnosed, symptomatic MM patients were enrolled into a prospective, population based registration study of high dose therapy and autologous transplantation. Data from this cohore of patients were analysed to illustrate the benefit of combining demographic, disease and graft related variables in predictive models for individual quality assessment of autografting by help of clinical end point describing short-term efficacy, toxicity and safety.

The model which estimated efficacy of autografting by graded posttransplant primary end points (time on antibiotics and use of transfusions) contained six independent variables including age, sex, WHO performance status, length of priming, days of harvest and graft CD34+ cell number. The model which estimated toxicity by graded secondary end points (time to blood cell recovery) included six independent variables at diagnosis (marrow plasma cell percentage, serum creatinine, M component isotype and WHO performance status), following therapy (response to induction therapy) and graft CD34+ cell number. The model evaluating safety by graded tertiary end points (early disease recurrence or death) included known prognostic variables at diagnosis (haemoglobin, serum beta-2 microglobulin, Durie-Samon staging) and length of priming but not CD34+ cell number.

In conclusion binary graded clinical end points of supportive care, complications and safety related to autografting depends upon pretransplant variables of which only the number of CD34+ cells may be improved by increasing the numbers of apheresis. However, this needs to be confirmed in future prospective multicenter studies, including strict clinical guidelines, before recommendations for intervention can be established.

Acknowledgement: This study is supported by research grants from the Nordic Cancer Union (grant no 56-9350/56-9351 and 56 100 02-9101/9102 95) and Danish Cancer Society (grant no 945 100-15).

Clinical data were based on the NMSG database (study NMSG #5/94 and #7978) from Oncologi Centrum in Lund, Sweden.

283 PRELIMINARY RESULTS FROM A PHASE III STUDY OF HIGH DOSE 153-SAMARIUM EDTMP (153-SMEDMTP) WITH FIXED DOSE MELPHALAN PERIPHERAL STEM CELL TRANSPLANTATION (PBSCT) FOR MULTIPLE MYELOMA (MM)

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Abstract: Background: High dose chemoradiotherapy improves the quality of life, prolongs the time to progression, and prolongs survival of patients with myeloma (MM). 153-Sm EDTMP is a therapeutic radioisotope linked to a diphosphonate compound that binds tightly to bone. It has a short range beta emission, a 1.9 day half-life and very rapid clearance from non-osseous tissues. Methods: MM patients who were candidates for autologous PBSCT were eligible. For the phase I portion of the trial, patients were treated with a fixed dose of melphalan (200 mg/m2) and an escalating dose of the 153-Sm EDTMP. The original four dose levels were 6, 12, 19.8, and 30 mCi/kg. Because of variability of bone uptake of 153-Sm EDTMP noted after the first 12 patients, the protocol was modified to include a trace dose of 153-Sm EDTMP with a 24-hour whole body uptake measurement. For an additional 6 Phase I patients and all Phase II patients, the 153-Sm EDTMP administered activity was calculated to deliver 40 Gy to the marrow. The 53-Sm EDTMP was given on day -1 and Melphalan was given day -1. The goal of the Phase II study was to achieve a combined CR and VGPR rate of at least 50%. Results: Fifty-five patients have been treated. Median age at PBSCT was 55, range 38-74. Forty-one (75%) were treated with upfront transplant and 25% at relapse. Response categories pretransplant were: primary responsive (33; 60%); primary refractory (8; 15%); resistant relapse (5; 9%); and sensitive or untested relapse (9; 16%). Thirteen and twenty-four patients, respectively, had a PCLI  1 and a B2M  2.7 prior to PBSCT mobilization/transplant. At the Phase II dose level, median days to ANC > 0.5 platelet > 20 and platelet > 50 x 10(9)/L were 13 (95% CI 12-14), 13 (95% CI 11-18) and 25 (95% CI 16-53) days, respectively. There was one death due to peri-engraftment syndrome; the patient refused to be intubated and expired despite treatment with high dose corticosteroids. All patients engrafted
their leukocytes and platelets. No dose limiting toxicity or cases of thrombotic thrombocytopenic purpura, radiation nephritis or bladder toxicity have been observed during the 10.1 months median follow-up, range 0.25-32 months. Forty-nine patients are evaluable for response and five are too early to be evaluable for response. For the 18 patients in the phase I cohort, continued or new CR, VGPR, and PR rates were as follows: 28% (5/18), 39% (7/18), and 28% (5/18), respectively. There was one minimal response. Conclusions: Addition of high dose 153-Sm EDTMP to the Melphalan conditioning regimen appears to be safe and well tolerated. The additional therapeutic benefit is not known but the preliminary very good and complete response rates are promising.

284 A Phase II/II Clinical Trial of High-dose Melphalan, Topotecan and VP-16 Phosphate (MTV) Followed by Autologous Stem Cell Rescue in Patients with Multiple Myeloma.


Pre-clinical data suggest that myeloma cell kill may be optimal when cells are exposed to drugs in the sequence, alkylator → topoisomerase (topo) I inhibitor → topo II inhibitor. To explore this paradigm, a 30 min infusion of melphalan (50 mg/m2 x d 3) was immediately followed by the same day by a 30 min infusion of dose-escalated topotecan (TPT) each d x 3d (days -7,-6,-5), followed by a 4h infusion of VP-16P days -4 and -3 (1200 mg/m2/d total etoposide equivalents x 2d). CD34+ cells (≥ 2x106/kg) collected after cyclophosphamide priming (50 mg/kg/d x 2d) were given on day 0. Eighty-two patients (ages 27-69; 48% female) were treated, and the maximum tolerated dose (MTD) of TPT was found to be 20 mg/m2 total dose (dose level 4 (DL)). The dose limiting toxicity was grade 4 mucositis and enteritis. The median time to an ANC >500/l was day 10 (range, 8-15), and day 18 (range, 12-20+) for a platelet count >50,000/l. 100-day non-relapse mortality was 4.1%. The MTD was expanded to 30 patients, and a modified DL 4 (4M) that did not include VP-16P enrolled 22 patients. Grade 4 mucositis decreased from 80% (DL 4) to 13% at 4M. The overall response rate in 81 evaluable patients was 53.1% (33.3% CR and 19.8% PR), with 34.6% stable disease. The overall and event free survival were significantly increased in those that received TPT (DLs 2-4), compared to the 10 patients in DL 1 that did not receive TPT. Analyses of pre-treatment and d-4 plasma cells obtained from bone marrow aspirates by confocal microscopy showed: (1) 5- to 7-fold higher levels of topo I (compared to topo II α and β) in pre-treatment and d-4 plasma cells, (2) significantly increased whole cell topo I, Iα and Iβ protein in plasma cells at d-4, (3) significantly more cytoplasmic Iα and Iβ at d-4, and (4) increased breast cancer resistance protein (BCRP) expression in d-4 plasma cells compared to pre-treatment cells. Drug levels of TPT, VP-16P and melphalan were obtained in all patients and correlated with response and topo levels/location. In summary, DNA topoisomerase I appears to be a relevant target in the treatment of myeloma. Drug resistance may be related to cytoplasmic trafficking of topoisomerases and increased expression of BCRP. A follow-up study of high-dose melphalan + dose-escalated TPT, stratified for age (≤ or > 60 yrs), has enrolled 20 patients with minimal mucositis at a total TPT dose thus far of 54 mg/m2 (younger group) [TPT and VP-16P were provided by GlaxoSmithKline and Bristol-Myers Squibb, respectively. Supported in part by NIH grants CA82050 and CA82533].

285 Melphalan vs Busulphan plus Melphalan in the conditioning for ABMT in the treatment of patients with multiple myeloma

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30 patients out of 101 with stage III multiple myeloma that have undergone transplantation at our Institution over the past 12 years were included in this study. The selection criteria included: age less than 70 years, creatinine less than 200 mg/ml, cardiac ejection fraction >50%, DLCO >50%, no active infection disease or other co-morbidity conditions. 16 patients were treated with Melphalan 180 mg/m2 (arm A) and 14 with Busulphan 16 mg/Kg + Melphalan 100 mg/m2 (arm B) for ABMT conditioning. The median age at transplantation was 52.2 years (range 34-64) for arm A and 49.5 years (range 34-60) for arm B. In the arm A were included 9 male and 7 female and in the arm B 9 male and 5 female. All patients received 3 cycles of VAD and were mobilized with Cyclophosphamide 7 gr/m2 and rHuG-CSF (10 µg/Kg b.w./die). The dose of CD34+ cells infused range from 1.5 to 15.6 x106/Kg (median 9.2 x106/Kg) for the arm A, and from 1.5 to 15.8 x106/Kg (median 8.9 x106/Kg) for the arm B. Days to achieve engraftment (neutrophils > 500/µl) were similar in the two arms (arm A: median 11, range 9-20; arm B: median 11, range 9-21), all the patients received rHuG-CSF 10 µg/Kg b.w./die after reinfusion of PBSC. No difference in the incidence of transplant-related infective and non-infective complication were evidenced in the two arms and no transplant-related deaths were observed. The best percentage of response (complete or partial response) were observed in the arm B (85% vs 75%), 2 progression after ABMT were observed in the arm A.

All patients received maintaining therapy with Interferon 3 MU x 3/week after ABMT. Overall survival at 5 years were 45% in the arm A (median 3.6 years) and 64% in the arm B (median 4.7 years) (p <0.05). Overall survival at 5 years were 49% in the arm A (median 3.4 years) and 56% in the arm B (median 4.1 years) (p = 0.7). In conclusion, the conditioning regimen with Busulphan (16 mg/Kg) and Melphalan (100 mg/m2) are well tolerated as well as the conditioning with Melphalan alone and gave best results in terms of response to treatment and disease free survival but have no effect in the overall survival that was not significatively increased in the arm B.
CD34+ selection of PBSC has been used in MM as a mean to reduce relapse linked with tumor cells contamination and thus improving outcome. However, a clinical benefit has not been demonstrated at the onset of this study. From May 1995 to November 1999, 127 pts from 17 EBMT centers with newly diagnosed advanced MM were included into this phase III trial and 112 pts are analysed. Responders to 3 cycles of VAD were randomized to receive a CD34+ selected graft (arm A, n=55) or an unselected graft (arm B, n=57). PBPC were harvested following mobilization with cyclophosphamide 4g/m² and G-CSF (Filgrastim). Conditioning regimen in both arms was TBI and following mobilization with cyclophosphamide 4g/m² and G-CSF an unselected graft (arm B, n=57). PBPC were harvested randomized to receive a CD34+ selected graft (arm A, n=55) or

An EBMT phase III randomized study evaluating CD34 + selection of autologous transplants in patients with newly diagnosed myeloma.


The median number of CD34+ cells reinfused was 7.2x10⁶ CD34 cells/kg (1.4 – 50.4) in arm A and 14.4x10⁶ CD34 cells/kg (1.8-99.2) in arm B. The median time to neutrophil engraftment (ANC>O.5x10⁹/l) was 10 days in arm A (8-14) and 10 days in arm B (8-21). The median time to platelets engraftment (ptls>20x10⁹/l) was 11 days in both arms but one patient in arm A never reached 20x10⁹/l platelets without supportive transfusions. 13 episodes of serious infections between the time of neutrophil engraftment and day 100 were reported in arm A compared to only 1 in arm B. All the infections were viral except 1 bacterial and 1 protozoal. For 3 patients in arm A, these infections were fatal (parainfluenza, CMV and myocarditis of infective etiology). The overall transplant mortality was 2.7% (3 patients in arm A). There was no significant difference in CR rate at 1 year as defined by EBMT/IBMTR/ABMTR criteria (16% in arm A and 15% in arm B). There is so far no significant difference in EFS and OS. Median follow-up is 47 months in both arms. Probability of OS at 3 years is 71% in arm A and 81.5% in arm B. The 3 year relapse risk is 54.05% in arm A vs. 30.5% in arm B. In summary, CD34+ selection resulted in a 1.9 tumor cell depletion without delay in hematological recovery. However, long term follow up analysis of these patients shows no significant clinical benefit for progression free survival. Moreover, the increased incidence of bacterial and viral life threatening infections in the CD34+ selected arm, similar to those for allogeneic BMT recipients, legitimate systematic prophylaxis regimens and raise clinical concerns. Altogether, these results suggest new approaches in CD34+ selection to circumvent immune recovery delay.

An EBMT phase III randomized study evaluating CD34 + selection of autologous transplants in patients with newly diagnosed myeloma.


There were no grade 3-4 toxicities within the first 100 days. However, 27 patients experienced Grade 2-3 hemorrhagic cystitis, with all but 1 case occurring among patients who had not received continuous bladder irrigation at the time of drug administration. Seven patients experienced severe thrombotic microangiopathy manifest as TTP/HUS. All 7 patients had received doses of at least 35 Gy to marrow. Thirty percent of patients experienced Grade 2 to 4 renal toxicity, usually at doses targeting over 40 Gy to the marrow. Survival of patients receiving <30 Gy to marrow was higher than patients who received >30 Gy due to fewer late toxicities.

In order to obtain further information regarding the dosimetry and pharmacokinetics of 166Ho-DOTMP, a trial was conducted among 12 patients with multiple myeloma. Eight patients with responsive disease and 4 patients with relapsed, stable or refractory disease were treated. Patients received 2 consecutive tracer doses of 166Ho-DOTMP to evaluate intra-patient variability of skeletal uptake. Data from the first tracer dose was used to determine the dosage required to deliver a target dose of
25 Gy to marrow. The tracer dose was followed by a therapeutic dose of 166Ho-DOTMP. Patients received a median dose of 674 mCi/m2 (range 551-860), followed, 6 days later (range 4-8) by melphalan 200 mg/m2. Autologous stem cells were reinfused two days later. Engraftment was prompt with recovery of neutrophils at a median of 12 days (range 9-15) and platelets to 20,000/ul at 10 days (range 6-15). No grade 4 adverse events were noted. Two episodes of grade 3 mucositis and 1 episode of grade 3 neutropenic fever were seen. Six other serious adverse events occurred, none related to holmium.

Gamma camera imaging confirmed skeletal localization of 166Ho-DOTMP, ranging from 19% to 39% of the injected dose. The highest calculated doses to bone surfaces, red marrow and bladder wall were 4608 cGy, 2522 cGy and 1498 cGy respectively. PK and dosimetry were reproducible within 15% between the 2 tracer doses. Skeletal uptake did not appear to be affected by disease status. Kidney dosimetry was difficult to measure reproducibly because of low activity in kidneys; however, the median dose to kidneys was estimated to be about 0.9 cGy/mCi.

Assessment of response after transplant is ongoing at this time. 166Ho-DOTMP is a promising therapy for patients with multiple myeloma and merits further evaluation. A prospective randomized trial comparing high dose melphalan alone with the combination of 166Ho-DOTMP + melphalan is planned.

10.3 Double ASCT

LONG-TERM FOLLOW-UP OF A SINGLE CENTRE POPULATION OF AUTOTRANPLANTED MULTIPLE MYELOMA PATIENTS: LOW INCIDENCE OF TOXICITY AND NO ADVANTAGE FOR DOUBLE TRANSPLANTATION OR PURGED GRAFT.

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Between January ’96 and December ’02 84 patients with multiple myeloma (MM) received one or two courses of high-dose therapy in our Centre. Median age was 55 years (range 31-65), 42 were female and 42 male, 14 patients (17%) were in stage IA, 7 (8%) in stage II A, 54 (64%) in stage III A and 9 cases (11%) in stage IIIB. M-Component (MC) was IgG in forty-seven patients (56%), IgA in 15 patients (18%), IgM in 2 (2%), Bence Jones in 16 cases (19%) whereas 4 patients (5%) were non-secretory. Nine patients (11%) were pretreated with at least two lines of conventional therapy whereas the other 75 cases proceeded directly to high-dose therapy after 4 courses of VAD induction therapy. PBSC was collected after Cyclophosphamide + G-CSF at the dose of 7g/m2 (65 patients) or 4 g/m2 (19 patients); in 49 patients (58%) the leukapheresis products were processed to positively select CD34+ cells using an avidin-biotin immunoaffinity device (CEPRATE, CellPro). The first course of melphalan was administered in 24 patients with melphalan 200 mg/m2. Melphalan, the second course was administered in 24 patients with melphalan 200 mg/m2. Median time between diagnosis and the autotransplantation was 10 months (6-68) for the first course and 16 months for the second course, median number of CD 34 positive cells reinfused was respectively 2.7 x10^6/Kg and 3.2 x 10^6/Kg. Median time to 1 x 10^6 neutrophils was 13 days (10-31) and to 50 x 10^6 platelets was 26 days (12-210). Forty-eight patients (57%) had a WHO grade I-II mucositis, 22 patients (26%) had a WHO grade III-IV mucositis, 44 (52%) a febrile episode with evidence of bacteremia in 9 cases. Two patients (2%) were dead 32 days and 95 days after transplantation for graft failure and mycotic infection respectively. Long-term complications included 1 deep venous thrombosis, 5 herpes zoster, 3 bacterial pneumonias, 1 pseudomembranous colitis and 3 myocardial infarctions, all but the heart complications occurring within 10 months after ASCT. Haematologic engraftment and extramedullary toxicity did not differ significantly after the first procedure in comparison with the second one and after reinfusion of positively selected CD 34+ cells in comparison with unselected PBSC. Forty-six objective responses (55%), all partial, were achieved after the induction therapy, 53 partial responses (64%) and 15 complete responses (18%) were obtained after the first ASCT, 14 partial responses (58%) and 6 (25%) complete responses were achieved among the 24 patients undergoing the second procedure. After a median follow-up of 37 months (86-102) after ASCT, PFS and OS for the whole population were respectively 56% (95%CI: 22% - 55%) and 40% (95%: 10% - 72%), without any significant difference between one or two courses of myeloablative treatment and between selected and unselected PBSC reinfused.

We conclude that ASCT was a safe and effective procedure after a long-term follow-up of a single centre population mainly constituted by newly diagnosed MM and no clear advantage emerged for a double ASCT or reinfusion of positively selected PBSC.

289 Statistical Analysis of EBMT Registry Data of Single Vs Double Peripheral Stem Cell Transplantation (pbsc) in Myeloma and its timing; clinical findings and methodological limitations

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EBMT registry data show that double pbsc transplantation is common therapeutic strategy in myeloma with over 40% of 7452 transplant patients having received or planned to receive a second transplant between January 1993 and March 2002. Of these patients 2665 were described as “planned” (to receive a second transplant) and this group was compared with the remaining patients on an intention to treat basis. This approach was chosen because a direct comparison of patients actually receiving one transplant against those receiving two transplants is not methodologically (statistically) valid: in order to receive a double transplant patients must first of all survive sufficiently long. Even though the transplant rate in the planned group failed to reach 60% (55% of planned had the 2nd SCT before their relapse, while 58% of the planned group had a 2nd SCT in any case) the median survival from transplantation was 60 months for the planned compared to 51 months for the remainder group. However while the Hazard Ratio (HR) of the planned group is 0.89 (CI 0.79-1.00 p=0.05) before 70 months the position is reversed after 70 months with the HR being 3.01 (1.07-8.46 p=0.04). Using a separate approach we ignored the information on “planning” and instead described the data in terms of hazard ratios for the main outcomes of interest (Relapsed, Treatment Related Mortality and Overall Survival) with respect to the
occurrence of second transplantation and the timing of this intervention. A time-dependent multivariate Cox analysis shows that for patients having a second transplant in first remission there is a marked increase in the HR for TRM if the transplant is performed after 12 months while the HR for relapse incidence is not as beneficial as when the transplant is performed earlier. As regards survival after relapse, performing a second transplant after this event does not appear to provide an advantage, although if the second transplantation was given before relapse there is an increased HR. Further prospective studies of single and double transplant strategies are required to identify the best therapeutic stratagems for patients with myeloma.

290 Long-Term Event Free Survival (≥ 5 years) in Patients with Myeloma Planned to Receive a Single Autotransplant: Better or Comparable results Compared to Tandem Autotransplants

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Between February 1985 and October 2001, 451 consecutive myeloma patients received a single autotransplant conditioning with high-dose melphalan 200mg/m2(Sirohi et al, Proc Am Soc Clin Oncol 2002; 21:269a). For this study, only those patients transplanted ≥ 5 years prior to the date of analysis (26 April 2002) were included (n=266). The median age of patients was 50 years (31-69); 92F; 174M; 93% IgG; 68% ≥ 2 bone lesions; 76% stage III disease; median creatinine at autograft 83 µmol/L (46-352); median B2M 2.3mg/L (0.1-16.4); 169 (60%) remained in or attained complete remission after autograft; 75 partial remission; 13 non-responders and 9 (4%) died. The median OS and EFS of these patients was 5.8 years and 2.3 years respectively. The EFS curve shows a stable plateau after 7-8 years comprising 26 (10%) such patients who remain event-free. This data is comparable to the 515 patients presented by Tricot et al for tandem transplantation as shown in the Table.

<table>
<thead>
<tr>
<th>Variable</th>
<th>RMH (n=266)</th>
<th>Tricot et al (n=515)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Transplants</td>
<td>Single</td>
<td>All received single , 77% received a tandem autograft</td>
</tr>
<tr>
<td>Age ≥ 50 years</td>
<td>53%</td>
<td>58%</td>
</tr>
<tr>
<td>Stage III disease</td>
<td>76%</td>
<td>48%</td>
</tr>
<tr>
<td>Median OS</td>
<td>5.6 years (10days-16years)</td>
<td>3.5 years</td>
</tr>
<tr>
<td>Median EFS</td>
<td>2.3 years (10 days-16years)</td>
<td>1.4 years</td>
</tr>
<tr>
<td>7-year OS/EFS</td>
<td>42%/22%</td>
<td>31%/18%</td>
</tr>
<tr>
<td>Patients event-free at ≥5years</td>
<td>28%</td>
<td>24%</td>
</tr>
</tbody>
</table>

Based on previously described prognostic variables chosen by recursive partitioning, there was a group of 85/272 (31%; B2M missing in 4 ) patients with age <58 years, albumin ≥ 40mmol/L, B2M ≤ 4mg/L who had an amazingly long median OS/EFS of 8.6/3.7 years compared to 5/2 years in those without these factors (P=0.00003; P=0.004) respectively. These data suggest that long-term results of two single-centre studies are comparable and in patients with myeloma though it is possible that with tandem autotransplants, the depth of remission may be better, with a larger number of patients remaining event-free at 10 years.

291 The Upgrade in Response Rate from PR to CR is a Determinant Factor for the Favourable Outcome Effect of a Second Transplant in Multiple Myeloma. Update of a Tandem Transplants Phase II GELTAMO/GEM Study

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Between 1994 and 1999, 88 multiple myeloma (MM) patients in response after MEL200-ASCT1 were included in a phase II study to evaluate a tandem autologous stem cell transplantation (ASCT) conditioned, the first with melphalan 200 mg/m2 (MEL200-ASCT1) and the second with cyclophosphamide, etoposide and BCNU (CBV-ASCT2). A control group of MM patients with stable response to a single MEL200-ASCT was selected from the Spanish Registry for MM transplantation to compare outcomes. After MEL200-ASCT1 27 patients (31%) achieved CR (negative immunofixation, EBMT Criteria). Of the remaining patients, 16 (33%) achieved CR with CBV-ASCT2 with a final CR in evaluable patients of 53% (48% CR in the global series). The results of a previous analysis updated as of July 2001 suggested that patients in PR after the first transplant who entered CR after CBV-ASCT2 are significantly benefited as compared to patients who remained in PR. This study updates the information with a longer follow-up.

At the moment of update the median follow-up since MEL200-ASCT1 for living patients is 58 months. The median OS and EFS for the entire group was respectively 70 months and 41 months (previous study OS and EFS, 68 and 39 months, respectively) the OS rate at 5 years was 61% ([95% CI, 51% to 71%]), previous 5y OS 55% and the EFS rate was 29% ([95% CI, 15% to 39%], previous 5y EFS 28%). The status of CR after CBV-ASCT2 was the most important prognostic factor for OS and EFS. Comparing OS and EFS between CR and PR after the second procedure, the outcome was significantly better in the CR group, but no significant differences in either EFS or OS were detected when the patients in CR after MEL200-ASCT1 were compared with the patients in PR after ASCT1-MEL200 who went on to obtain CR after the second procedure. Adjusted multivariate analyses showed improved OS [OR 1.6, (95% CI, 1.2 to 2.1)] and EFS [OR 1.7, (95% CI, 1.4 to 2.2)] for the tandem series as compared to the control series treated with a single MEL200-ASCT. However, a comparison of the 42 tandem patients who attained final CR with the patients in the control group who attained CR with a single ASCT showed no significant differences in either EFS or OS. For tandem patients in final PR, the differences were again not significant. In multivariate analysis, final CR (or PR) in the tandem group vs. CR (or PR) in the reference group were not significant for either OS or EFS. In summary, this update confirms the findings of our original study in that the benefit of a second high-dose therapy course depends on its capacity to obtain CR on patients in
non-CR after the first transplant and highlights the importance of obtaining CR as strictly defined by immunofixation.

292 Prospects of the current protocols of the Spanish Myeloma Group (GEM-Grupo Español Mieloma)

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For the GEM Group Grupo Español de Mieloma

The GEM group is the result of integration of the PETHEMA and the Stem Cell transplant Myeloma / GEL-TAMO study groups. The current national protocol was activated in January 2000 and the design was based on the previous experience of both supporting groups. For this reason we used VBMCP/VBAD (x 6 cycles) as induction therapy, because in previous PETHEMA studies this resulted in an optional debulking chemotherapy (1st aim: to determine the response rate to this induction regimen).

Stem cells have been collected after 4th cycle (2nd aim: to see if the use of short courses of alkylating agents hampers stem cell collection). After this, all patients received high dose chemotherapy followed by PB stem cell support. Conditioning consisted of Busulphan-Melphalan (BUMEL), because it was superior to Melphalan 200 in our previous retrospective study (Br. J. H. 2002) (3rd aim: to confirm the efficacy and toxicity of BUMEL). Patients that achieved CR went into maintenance therapy, reserving the second potential transplant for relapse. Among CR patients we will compare the outcome of those with both EF- and immunofixation- (CR1) versus CR2 patients (EF-but IF-±) (4th aim). Patients in PR or MR receive a second transplant, either autologous (conditioned with CBV) or mini-allogeneic (conditioned with Fludar-Melphalan), based on the availability of an HLA-identical donor (5th aim: to compare the efficacy and toxicity of these two procedures). In all patients, cytogenetic/FISH studies have been performed at diagnosis. Moreover in patients in CR after TRx, MRD investigation is being carried out.

So far, 732 patients have entered into the study. The overall response rate to induction chemotherapy is 93% (14% CR1, 18% CR2, 61% PR, 7% stable or progressive disease). Following the first transplant, 57% of patients have achieved CR (39% CR1 and 18% CR2) while 34% were in PR. After the initial 200 patients were transplanted an alarm emerged about a possible increase in VOD toxicity associated with BUMEL (5 out of 12 deaths), and accordingly, the conditioning regimen was switched to Melphalan 200. Data about this adverse event as well as stem cell collection efficacy (93% success with a median of 3.6 x 106 CD34 cells/ kg and 5.3 x 106 CD34 cells/kg for percentil 75) after chemotherapy, including low doses of melphalan, will be presented.

293 T2 model - a pilot study for the evaluation of the experimental therapy of multiple myeloma relapsing after the first autologous stem cell transplantation. Report of the prospective non-randomized pilot study of the Czech Myeloma Group

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Background: High doses of chemotherapy followed by autologous transplantation became widespreadly indicated as upfront therapy for patients with multiple myeloma (MM) in the last decade, with very good tolerance and low mortality (2-3%). Therapy of relapsing MM is still considered to be experimental.

Design of study: The principle of T2 model is repeated transplantation therapy with testing different experimental approaches in patients with MM relapsing/progressing after the 1st autologous transplantation (AT). The patients (pts) are treated with the same + something more - same or very similar induction and reinduction treatment, same myeloablative regimen in the 1st and the 2nd AT and a different maintenance, experimental therapy after the 2nd transplantation. The evaluation of the results of each therapeutic approach uses intra-individual analysis - the comparison of event free survival I (EFS I) (after the 1st AT) and EFS II (after the 2nd AT) in one patient, therefore the inter-individual differences are excluded.

Subjects: 32 pts with relapsing/progressing MM after the 1st AT were included in the pilot study between January 1997 and January 2003, median follow-up was 75,2 months.

Methods and results: Pts were matched to the following groups of experimental therapy: autologous transplantation with IL-2 activated PBSC - 10 pts (31,2 %), pamidronate -4 pts (12,5 %), bortezomib -15 pts (46,9 %), consolidation chemotherapy CED - 3 pt (9,4 %). A sensitivity to reinduction chemotherapy C-VAD (4 cycles) was more than 90 %, the response to the 2nd transplantation according to the 1st one was in 23 % better (7 pts), in 50 % same (16 pts) and in 27 % worse (9 pts). The toxicity of the 1st and second transplantation was similar and usually did not exceed grade II (SWOG criteria), there were no significant differences instead of clinically irrelevant hematological toxicity. Transplant-related mortality was 3 % (1/32). EFS II is known in 22 pts. Total of 8 pts have achieved prolongation of EFS II versus EFS I, 2 in IL-2 activated graft group, 1 in pamidronate group, 1 in consolidation chemotherapy CED group and 4 in thalidomide group. In the whole group median of EFS I was 15,7 months, median of EFS II was 12,9 months, median of overall survival (OS) was 79,1 months, 63 % pts (20/32) were alive (till 31st January 2003).

Conclusions: Repeated transplantation is one of the most powerful approaches in treatment of relapsing MM; toxicity is acceptable, also engraftment is similar to the 1st AT. Testing of new perspective approaches by T2 model may bring a fundamental benefit.
SECON TD TRANSPLANT AS TREATMENT OF RELAPSE AFTER A FIRST AUTOLOGOUS TRANSPLANT IN MULTIPLE MYELOMA. RESULTS OF THE SPANISH REGISTRY


REGISTRY OF HSCT IN MULTIPLE MYELOMA.

Objective To evaluate the results of hemopoietic stem cells transplantation (HSCT) as treatment of relapse after a first autologous transplantation in patients included in the Spanish Registry of HSCT in multiple myeloma.

Patients The registry included 1287 patients. 80 have received some modality of HSCT as treatment of relapse after a first autologous transplant. Median age at second transplant was 52 years (25-70). Interval between 1º y 2º HSCT: 28,5 months (3-103). Clinical status at 2º HSCT was CR (13%), PR (36%), Minimal response (7%), stable disease (3%) and progression (41%).

Autologous HSCT: Stem cell source was peripheral blood in 57 patients, bone marrow in 5 and both in 1. Peripheral blood stem cells were mobilized with G-CSF (47%), Cyclophosphamide+G-CSF (45%) and Cyclophosphamide+GM-CSF (8%). Median number of apheresis was 3 (1–8), CD34 harvested 2.87 x 10^6/kg (0.56–16.7). Conditioning treatment was Melphalan 200 (27%), Busulfan/Melphanal (23%), CBV (20%), BUCY (10%), Melphalan 140/TBI (7%), Cyclophosphamide/TBI (5%), and others (8%).

Allogeneic HSCT: Stem cell source was marrow in 9 patients, and peripheral blood in 8. 14 were conventional allogeneic transplantations and 3 received a nonmyeloablative conditioning. Median number of CD34 cells infused was 3.59 x 10^6/kg (0.33–12.5). Conditioning treatment was Cyclophosphamide/TBI (27%), Fludarabine/Melphanal (20%), Melphanal 200 (13%), Melphalan 140/TBI (13%), BUCY (13%), CBV (7%), others (7%).

Results Autologous HSCT: 85% of patients recovered neutrophils > 500/mm3 in 11 days (7-40) and platelets > 20,000/mm3 in 14 days (8-214). Response to HSCT was CR (33%), PR (28%), Minimal response (9%), stable disease (10%), progression (12%) and early death (8%). Median survival from the second transplant is 23 months (CI 95% 14,8-31,2) with 30% of patients alive at 3 years.

Consecutive HSCT: 71 % of patients recovered neutrophils > 500/mm3 in 16 days (7-24) and platelets > 20,000/mm3 in 14 days (7-32). Response to HSCT was CR (31%), PR (31%), Minimal response (12%), stable disease (6%) and early death (38%). Median survival from the second transplant is 5 months (CI 95% 0,4-9,6) with 17% of patients alive at 3 years.

Conclusions Autologous HSCT as treatment of relapse after a first transplantation is feasible and has an acceptable toxicity. A significative fraction of patients (30%) are alive at 3 years. Allogeneic transplantation, most of them with conventional conditioning treatment, has shown a high toxic mortality and short survival.

10.4 Transplantation in other plasma cell disorders

Reducing the dose of melphalan used for stem cell transplantation in amyloidosis is associated with a lower response rate

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High dose chemotheraphy with stem cell transplantation is increasingly being used for eligible patients with immunoglobulin light chain amyloidosis (AL). Morbidity and mortality in this patient group with multiorgan dysfunction is high and mortality hovers at 15%. In an attempt to render more patients eligible for stem cell transplantation, a risk adapted strategy has been developed to categorize patients into those eligible for full dose chemotherapy and to treat those patients who would be at inordinate risk for complications with lower doses. An important issue revolves around whether de-escalation of the conditioning dose for patients with poor performance status, multiorgan AL involvement, mild renal insufficiency and cardiomyopathy results in a reduction in overall survival. A total of 125 patients received high dose therapy with stem cell reconstitution for AL. Patients who received melphalan 140/m2 plus total body radiation 1,200 cGy and those receiving melphalan 200 mg/m2 were considered a higher dose group. Those receiving melphalan 140 mg/m2 or melphalan 100 mg/m2 were considered a lower dose group. Patients who died as a direct result of complications of transplant were considered non-responders. Responses were categorized as hematologic responses with criteria identical to those for patients with multiple myeloma who undergo stem cell transplantation and organ which requires direct evidence of improved organ function. The table gives the patients categorized based on their conditioning pre-transplant and whether they achieved a hematologic response and organ-based response both or no response.

<table>
<thead>
<tr>
<th>Conditioning</th>
<th>Death Before D100</th>
<th>Hematologic Response</th>
<th>Organ Response</th>
<th>Both</th>
<th>No Response</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mel-TBI</td>
<td>17</td>
<td>3</td>
<td>4</td>
<td>7</td>
<td>3</td>
</tr>
<tr>
<td>Mel-200</td>
<td>66</td>
<td>7</td>
<td>15</td>
<td>7</td>
<td>24</td>
</tr>
<tr>
<td>Mel-140</td>
<td>31</td>
<td>5</td>
<td>7</td>
<td>1</td>
<td>6</td>
</tr>
<tr>
<td>Mel-100</td>
<td>11</td>
<td>3</td>
<td>2</td>
<td>10</td>
<td>5</td>
</tr>
</tbody>
</table>

Statistical analysis showed that there were significant differences between the four groups with the Mel-TBI, Mel-200 and Mel-140, Mel-100 showing response rates of 72% and 48% respectively. Treatment related mortality was not different among the four groups. When the groups were categorized as high dose therapy (N=83) and lower dose therapy (N=42) the response rate was significantly higher in the higher dose group (P=01). We conclude that reducing the dose of Melphalan renders more patients with amyloidosis eligible for stem cell transplant, but their response rate is significantly lower. Efforts to reduce treatment-related toxicity to permit a greater proportion of patients to receive a full dose of conditioning chemotherapy are warranted.
Background: The POEMS syndrome is characterized by peripheral neuropathy (PN), clonal plasma cell disorder (PCD), organomegaly, endocrinopathy, skin changes, edema, sclerotic bone lesions, and thrombocytosis. Patients have a low bone marrow (BM) plasma cell (PC) burden and a survival superior to patients with multiple myeloma (MM). Based on the improved response rates observed with PBSCT in patients with other PCD, autologous PBSCT may be an attractive treatment option for patients with POEMS syndrome.

Methods: Between March 1999 and February 2003, 11 patients with POEMS syndrome have undergone PBSCT at Mayo Clinic Rochester and Jacksonville. Seven received melphalan 200 mg/m², three melphalan 140 mg/m², and one BEAM as conditioning regimens. All received a minimum of 4.4 x 10⁶ CD34 autologous PBSC/kg. Standard supportive care with prophylactic antibiotics and growth factor support was provided. Results: All but one had a severe pulmonary compromise, requiring biphasic positive airway pressure throughout his transplant course. Of the 8 evaluable patients, all had a hematologic response and 7 have had neurologic improvement at a median follow-up of 6 months.

Primary amyloidosis is a poor-prognosis disease with limited response to conventional chemotherapy. High-dose melphalan followed by autologous hematopoietic stem-cell rescue results in a significant response rate, although it is hampered by a significant toxicity.

Aim: To analyze the long-term results of a series of 17 consecutive patients with AL undergoing peripheral blood ASCT at our institution.

Patients and methods: 17 patients (53% female; median age, 53, range: 33 – 66) diagnosed with systemic AL who underwent ASCT during a 5-year period (11/97 – 11/02) were included in the analysis. Median interval from diagnosis to ASCT was 9 months (2 – 63) and seven patients had received previous chemotherapy (melphalan/prednisone, 4; VBMCP + melphalan/prednisone, 1; VAD, 1; fludarabine, 1). The light chain was of lambda type in 82% of cases. One patient presented with a concomitant Waldenström’s macroglobulinemia and a non-clonally related follicular lymphoma. The median number of involved organs was 2, including renal (14 patients), heart (13), liver (7), and peripheral nervous system (5). All patients were mobilized with G-CSF (dose ranging from 10 to 24 µg/kg) and conditioned with high-dose melphalan (200 mg/m² in 13 pts, 140 mg/m² in 4).

Results: Six patients (35%) died in the peritransplant period due to multiorgan failure (n=2), hemorrhage (n=1), arrhythmia (n=2), and/or erythrocytosis, and/or thrombocytosis, and/or 8 had sclerotic bone lesions (diffuse in 5, solitary lesion in 3). Using the Bardwick Criteria, the median number of POEMS features was 5 (range 2-5). Using the Mayo Criteria which includes the five criteria of the Bardwick acronym, but also sclerotic bone lesions, Castleman Disease, extravascular volume overload, and papilledema, the median number of features was 6 (range 3-8). The median number of therapeutic regimens prior to PBSCT was 3 (range 0-6); two of the three patients with a solitary bone lesion were progressing despite external beam radiation. From first symptoms and from diagnosis of POEMS the median times to transplant were 27 and 10 months (ranges 4-180 and 2-180), respectively. Two patients had not had prior cerebrovascular accidents and one a history of transient ischemic attacks. All but one patient had significantly abnormal pre-transplant pulmonary function tests. There was one transplant-related death in a patient who did not engraft after an episode of a culture negative systemic inflammatory response syndrome on day +8; he died 115 days after his transplant. This same patient and another had temporary renal failure, requiring dialysis. Four of the 11 patients spent time in the intensive care unit, and 3 required intubation for respiratory compromise. One patient required biphasic positive airway pressure throughout his transplant course. Of the 8 evaluable patients, all have had a complete hematologic response and 7 have had neurologic improvement at a median follow-up of 6 months. Other symptoms including fatigue, organomegaly, pulmonary compromise, hyperpigmentation and extravascular volume overload have improved substantially in affected patients. Conclusions: PBSCT for POEMS syndrome may result in a high hematologic response rate and improvement in peripheral neuropathy and systemic symptoms, but may also be associated with significant morbidity.
Primary plasma cell leukaemia (PCL) is a rare disorder, characterised by plasma cells circulating in the peripheral blood at absolute numbers > 2.0 \times 10^9/l or greater than 20% of peripheral leucocytes. The disorder is frequently advanced at presentation and is often rapidly progressive with a median survival of 8-12 months with conventional chemotherapy, significantly shorter than for multiple myeloma. High dose therapy and autologous haemopoietic transplantation (ASCT) has been reported to produce prolonged survival but as with conventional therapy, may produce inferior outcome relative to that seen in multiple myeloma, as reported in our previous study of 56 patients with PCL. Here, we report an EBMT registry study of an extended series of 135 patients with PCL and 9887 patients with multiple myeloma undergoing first ASCT. Data was obtained from Med-A (limited data set) and Med-B (extended data set) forms. Patient groups were comparable with regard to gender and performance status pre-transplantation. PCL patients were significantly younger at diagnosis (51.9 v 53.6 years, p = 0.022) and transplantation (52.7 v 55.1 years, p = 0.002). There were significant differences in presenting serum creatinine (114 v 91 micro mol/l, p = 0.021) and haemoglobin (8.75 v 10.9 g/dl, p = 0.000), while beta 2 microglobulin was raised at diagnosis in 71.8 v 30.8% of PCL patients (p = 0.000) respectively. PCL patients were more likely than myeloma patients to have normal bone structure (54.1% v 32.1%, p = 0.02). No difference was seen in levels of M protein, calcium or albumen at diagnosis. There was a non-significant trend to higher stage at diagnosis in the PCL patients with 64.5% of common-type myeloma patients and 78.7% of PCL patients in Stage III (p = 0.077). There was a tendency for more favourable disease status pre-transplantation in the PCL patients with 32% and 28.8% of PCL patients and 16.1% and 15% of myeloma patients in complete remission at mobilisation (p = 0.074) and transplant conditioning (p = 0.000) respectively. PCL patients were transplanted a median of 6.57 months from diagnosis compared to 8.25 months for the myeloma group (p = 0.000). Use of TBI in the conditioning regime and graft-type (marrow, peripheral blood or both) were similar for the groups. Survival of PCL patients post-ASCT was markedly inferior to that of myeloma patients with a median survival of 20.9 months (95% confidence intervals 5.71 - 36.09 months) versus 54.93 months (52.05 - 57.82, p = 0.000). These findings confirm that ASCT may produce prolonged survival in PCL. However, as for conventional therapy, outcomes are significantly inferior to those in multiple myeloma despite the fact that any group of PCL patients achieving autograft are biologically pre-selected through early morbidity and mortality during initial chemotherapy. Further study of novel treatment strategies is required to produce progress in the treatment of this aggressive disease.

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**Autologous transplantation in primary plasma cell leukaemia: an EBMT study comparing presenting features and outcome with common-type myeloma**


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Primary plasma cell leukaemia (PCL) is a rare disorder, characterised by plasma cells circulating in the peripheral blood at absolute numbers > 2.0 x 10^9/l or greater than 20% of peripheral leucocytes. The disorder is frequently advanced at presentation and is often rapidly progressive with a median survival of 8-12 months with conventional chemotherapy, significantly shorter than for multiple myeloma. High dose therapy and autologous haemopoietic transplantation (ASCT) has been reported to produce prolonged survival but as with conventional therapy, may produce inferior outcome relative to that seen in multiple myeloma, as reported in our previous study of 56 patients with PCL. Here, we report an EBMT registry study of an extended series of 135 patients with PCL and 9887 patients with multiple myeloma undergoing first ASCT. Data was obtained from Med-A (limited data set) and Med-B (extended data set) forms. Patient groups were comparable with regard to gender and performance status pre-transplantation. PCL patients were significantly younger at diagnosis (51.9 v 53.6 years, p = 0.022) and transplantation (52.7 v 55.1 years, p = 0.002). There were significant differences in presenting serum creatinine (114 v 91 micro mol/l, p = 0.021) and haemoglobin (8.75 v 10.9 g/dl, p = 0.000), while beta 2 microglobulin was raised at diagnosis in 71.8 v 30.8% of PCL patients (p = 0.000) respectively. PCL patients were more likely than myeloma patients to have normal bone structure (54.1% v 32.1%, p = 0.02). No difference was seen in levels of M protein, calcium or albumen at diagnosis. There was a non-significant trend to higher stage at diagnosis in the PCL patients with 64.5% of common-type myeloma patients and 78.7% of PCL patients in Stage III (p = 0.077). There was a tendency for more favourable disease status pre-transplantation in the PCL patients with 32% and 28.8% of PCL patients and 16.1% and 15% of myeloma patients in complete remission at mobilisation (p = 0.074) and transplant conditioning (p = 0.000) respectively. PCL patients were transplanted a median of 6.57 months from diagnosis compared to 8.25 months for the myeloma group (p = 0.000). Use of TBI in the conditioning regime and graft-type (marrow, peripheral blood or both) were similar for the groups. Survival of PCL patients post-ASCT was markedly inferior to that of myeloma patients with a median survival of 20.9 months (95% confidence intervals 5.71 - 36.09 months) versus 54.93 months (52.05 - 57.82, p = 0.000). These findings confirm that ASCT may produce prolonged survival in PCL. However, as for conventional therapy, outcomes are significantly inferior to those in multiple myeloma despite the fact that any group of PCL patients achieving autograft are biologically pre-selected through early morbidity and mortality during initial chemotherapy. Further study of novel treatment strategies is required to produce progress in the treatment of this aggressive disease.

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**Efficacy of thalidomide treatment after up-front high dose chemotherapy followed by autologous peripheral stem cell transplantation in AL amyloidosis**

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Introduction: Primary amyloidosis (P.A) is a rare plasma cell dyscrasia in which insoluble immunoglobulin light chain fragments are produced and polymerize into fibrils that deposit extracellularly, causing visceral organ dysfunction. The most important prognostic factor is the presence of cardiac involvement.

Case: A 51-year-old woman was admitted because of systemic oedema and proteinuria. Her blood pressure was 120/90 mmHg, weight 63.7 Kg. Urinalysis demonstrated severe proteinuria at 3.58g/day. Blood urea and creatinine were 30 and 0.8 mg/dl, respectively. Total serum protein/albumin was 4.5/2 g/dl. Blood and urine Immunoelectrophoresis demonstrated two light chains. The level of total and LDL cholesterol was 432 and 328 mg/dl. The blood test normal for WBC, platelet and RBC. Cardiac echography as normal. Kidney biopsy showed amyloid deposition (congo red histology and immunofluorescence study) leading to a diagnosis of AL amyloidosis.

Myelogram and Bone Rx examination were negative. The proteinurie rapidly increased to 6.315 mg/dl. High dose Melphalan (200 mg/m2) followed by autologous stem cell Tx was performed in augustus 2001, 8 month after diagnosis. The havest was performed under GCSF alone. After the Tx, proteinuria initially regressed to 4.8 g/24h. Because this patient suffered from HTA, Lisinopril was given at dose of 20 mg/day.

Quickly, proteinuria raised to 6.2 g/24H 5 month after the PBSC; aspirin at 100 mg/ day and sartan at 50 mg were added. Thalidomide 400 mg/day was startet 6 month after Tx, and slowly the doses of Thal was reduced to 100 mg/day. Thalidomide 400 mg/day was startet 6 month after Tx, and slowly the doses of Thal was reduced to 100 mg/day. After the Tx, proteinuria initially regressed to 4.8 g/24h. Because this patient suffered from HTA, Lisinopril was given at dose of 20 mg/day.

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Remarkable Neurological Improvement of POEMS Syndrome Associated Severe Paraproteinemic Polyneuropathy Following High-Dose Melphalan Therapy With Autologous Stem Cell Transplantation: Report of Two Cases

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High-dose melphalan therapy with autologous peripheral blood stem cell transplantation (PBSCT) has recently been reported to improve neurological disability in patients with POEMS syndrome. Two additional patients with rapidly progressive paraproteinemic polyneuropathy are documented:

**Patient 1**
- A 38-year-old male with a 5-year history of progressive proximal and distal weakness, distal sensory loss, and areflexia, making him wheelchair-bound within two years.
- The patient exhibited an IgG lambda monoclonal gammapathy of undetermined significance. Concomitant hepatosplenomegaly and endocrinopathy (hypothyroidism and hypogonadism) were suggestive of POEMS syndrome. High-dose glucocorticoids, plasmapheresis and immunoglobulins were ineffective but six cycles of pulsed cyclophosphamide caused transient slowing of progression, maintained with cyclosporin for 18 months but followed again by rapid progression.
- Patient 2 was a 41-year-old male with an 8-month history of a rapidly deteriorating sensorimotor length-dependent polyneuropathy progressing to proximal muscle groups with loss of ambulation within few months. This patient had a small monoclonal IgA-lambda gammapathy (8 g/L), 20% bone marrow infiltration with monotypic plasma cells, hepatosplenomegaly and endocrinopathy, as well. In addition, deep vein thrombosis (DVT) of the vena cava inferior led to edema of the lower extremities requiring continuous anticoagulation. Subsequent to initial plasmapheresis, this patient received standard myeloma induction regimen of four cycles anthracycline/pulsed dexamethasone with a paraprotein response but with little effect on neurological disability.
- In both patients, neurophysiology revealed a severe demyelinating and axonal sensorimotor polyradiculoneuropathy. Amyloidosis was excluded by appropriate staining of bone marrow and rectum biopsies.
- Both patients were treated with high-dose melphalan (200 mg/m²) and PBSCT subsequent to an alkylating agent-based stem cell mobilization with granulocyte colony-stimulating factor (G-CSF, 10 µg/kg/d). The procedure was well tolerated. Hematologic recovery was timely and sustained. Applying the EBMT/IBMTR/ABMTR criteria, response was partial in patient 1 and complete in patient 2. Clinically, both patients rapidly improved in neurological function, regaining normal strength in proximal muscle groups as well as remarkable improvement in the hands. However, nerve conduction studies after one year showed little change.
- After more than one year of follow-up, patient 1 still requires a wheelchair for longer distances due to persisting sensory ataxia and paresis of distal leg muscles while his residual paraprotein remained stable. Patient 2 is ambulatory with only residual paresis of peroneal muscles despite reappearance of a serum paraprotein on immuno fixation (no monoclonal paraprotein on routine serum electrophoresis).
- The rapid clinical improvement in our two patients further supports a potential role for high-dose melphalan chemotherapy and autologous stem cell transplantation as rescue treatment for patients with rapidly deteriorating severe paraproteinemic neuropathy and possibly other immune-mediated polyneuropathies. Procedure-related risks seem not to be increased in comparison with other myeloma patients.

10.5 Allogeneic transplants

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Myeloablative versus reduced-intensity conditioning followed by allogeneic stem cell transplantation in multiple myeloma patients

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Multiple myeloma (MM) is still an incurable disease and allogeneic stem cell transplantation (allo-SCT) is a potentially curative strategy. Nevertheless, despite the high rate of clinical and molecular remissions, allo-SCT is still characterised by a relevant transplant-related mortality (TRM), which significantly
affects the clinical outcome. Recent developments on transplant strategies have shown that a reduction in the intensity of the conditioning can decrease the TRM while retaining an antitumor effect. However, it is still unknown if reduced-intensity regimens can offer the same opportunities of cure as myeloablative ones. We have compared the results of two clinical trials: in the first trial (myeloablative conditioning protocol, MCP) 30 patients underwent allo-SCT mainly at diagnosis, conditioning regimen consisted of busulfan 12-16 mg/kg and melphalan 140 mg/m2, all patients received peripheral blood stem cells from HLA identical siblings and immune suppression consisted of cyclosporine and short-course methotrexate; in the second trial (reduced intensity conditioning, RIC) 14 patients were treated with allo-SCT mainly with a disease relapsed or resistant to autologous stem cell transplantation. In this trial conditioning regimen consisted of thiota 5mg/kg, cyclophosphamide 60mg/kg and fludarabine 60mg/kg, and antithymocyte globulin 7.5mg/kg or alemtuzumab 30mg and GVHD prophylaxis consisted of cyclosporine and methotrexate. MCP patients had a median age of 48 years (range 31-55) and 15 (50%) had a chemosensitive disease at the time of transplant. RIC patients had a median age of 56 years (range 48-69) and 6 (43%) were transplanted with a chemosensitive disease. All patients of both protocols engrafted promptly. Acute GVHD grade I°-IV° occurred in 26 (87%) of 30 MCP patients and in 4 (28%) of 14 RIC patients (P=0.008), while grade III°-IV° aGVHD occurred in 5 (17%) and 1 (7%) patients respectively (P=0.65). Chronic GVHD developed in 17/24 (71%) evaluable MCP patients and in 4/12 (33%) RIC patients (P=0.02). TRM at 100 days was 16% in MCP and 0% in RIC (P=0.10), while overall TRM was 30% in MCP and 7% in RIC (P=0.09). Median follow-up for MCP patients is 35 months (range 1-75), and 24 of them (80%) attained a complete remission (CR). Median follow-up for RIC patients is 12 months (range 3-27), and 5 patients (36%) attained CR: 3 of them after cyclosporine withdrawal and/or DLI. MCP seem superior to RIC in terms of attainment of CR (80% vs. 36%, P=0.006). Overall survival at 2 years is 75% for MCP and 65% for RIC. Our data on a limited series of patients suggest the following conclusions: i) there is a trend for a lower TRM in RIC transplants, especially considering that patients were older and heavily pretreated; ii) RIC with partial in vivo T-cell depletion can decrease GVHD incidence; iii) patients receiving MCP at diagnosis were more likely to attain a CR. Thus, RIC regimens appear promising and further investigation is warranted.

302 Adoptive Immunotherapy with Donor Lymphocyte Infusions (DLI) and IL-2 after High-Dose Therapy and Autologous Stem Cell Transplantation for High-Risk Multiple Myeloma.

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The outcome of high-dose therapy (HDT) with autologous stem cell transplantation (AutoSCT) in patients (pts) with relapsed/refractory myeloma is disappointing (median overall survival 19 months, SWOG S8993). DLI can result in a powerful graft-vs-myeloma (GvM) effect in pts who have relapsed after allogeneic transplantation. Taking advantage of the immunosuppression associated with the immediate post-transplant period, we explored the use of DLI as the means to induce a GvM effect after HDT and AutoSCT rescue. Seven pts (median age 52, range 31 to 62) with primary refractory (<75% response after VAD x 4, n=3) or relapsed (n=4, sensitive 2, refractory 1, untested 1) multiple myeloma (MM) were accrued to the study between 8/1999 and 7/2000. Pre-transplant, all patients had a demonstrable monoclonal protein in serum and/or urine. Six of the seven pts had a -2 microglobulin >3.5 mcg/dL. HDT consisted of melphalan (200 mg/m2), idarubicin (45 mg/m2) and etoposide (1200 mg/m2)(MIDET) on d-9 to d-6, with AutoSCT rescue on d0. DLI (total dose of CD3+ cells 0.5 to 1 x 10^7/kg) was given fractionated on d+1 (20%), d+5 (30%) and d+10 (50%), with IL-2 as a CIVI at 1 x 106IU/d from d+1 to d+12. Donors were selected among relatives on the basis of best available HLA match: 3/6 (n=4), 4/6 (n=1), 5/6 (n=1) and 6/6 (n=1). A syndrome of hyper-acute graft-versus-host disease (GVHD) developed in 6 of the 7 pts, including high fevers, skin rash (Grade II skin biopsies in 5/6) and elevated serum bilirubin. No specific therapy was given for GVHD, which resolved within days. Engraftment kinetics were compared to those of 6 MM pts in 1st remission who received MIDET/AutoSCT but without DLI/IL-2 (controls). While both groups received a similar number of CD34+ cells (median 2.96 x 3.3 x 106/kg, pNS) and recovered neutrophils in a similar pattern (median d+13.5 vs d+12, pNS), recovery of platelets was delayed in patients receiving DLI/IL-2: d+23.5 vs d+14 in controls (P=.04). One patient failed to engraft by d+18 and received, per protocol, a backup AutoSC infusion. VNTR (sensitivity 5%) evaluated from d+7 to d+201, failed to detect donor cells in peripheral blood. One patient died from complications of a pulmonary hemorrhage on d+55, by d+46 her serum monoclonal IgG was not longer detectable. Five patients achieved a complete remission. Remarkably, with a median follow up of 36 months, all 6 remaining pts are alive and in continuous remission from 31 to 42 months post transplant. DLI/IL-2 in the setting of HDT/ASC is biologically active, resulting in self-limiting GHVD, and rarely in autologous graft rejection. An allogeneic GvM effect is suggested by the high response rate in this cohort of refractory and relapsed pts. The use of haploidentical donors could make this approach available to nearly all pts undergoing HDT/ASC for MM.

303 Long-Term Follow-Up of Previously Untreated Symptomatic Myeloma Patients Treated with Myeloablative Therapy and Sibling-Matched Allogeneic Transplantation of the SWOG Study 9321.

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A long-term follow-up is provided on the myeloablative sibling-matched allotransplant arm of a multi-institutional randomized study which enrolled 38 newly diagnosed patients with symptomatic myeloma between 1994 and 1997. Patients were treated initially with 2-4 cycles of VAD followed by high-dose cyclophosphamide (4.5 g/m2) and proceeded, in the absence of progressive disease, to receive melphalan 140 mg/m2 + fractionated TBI of 1200 cGy given over 4 days, followed by infusion of marrow cells from HLA A, B, & DR matched siblings. Prevention of graft-versus-host disease (GVHD) was performed.
with cyclosporine A and methotrexate. Of the 38 patients enrolled, 37 proceeded to allotransplant within 6 weeks following cyclophosphamide. The median age was 47 years (range: 31-55), 68% were male. The median survival from study enrollment is 31 months with 22 deaths, but 6-year survival is 42%. Of the 16 patients evaluable for response, 11 achieved complete remission (normal marrow; no paraprotein; negative immunofixation) or very good partial response (normal marrow; >90% decrease in serum paraprotein; negative BJ proteinuria) and response (>50% decrease in marrow plasmacytosis; >75% decrease in serum paraproteins; >90% decrease in BJ proteinuria). Seven patients relapsed during the first 3 years, with a 2-year estimate of relapse of 20%. Ten patients are event-free now for more than 5 years, with a 6-year projection of 90% decrease in BJ proteinuria). Seven patients relapsed during the first 3 years, with a 2-year estimate of relapse of 20%. Ten patients are event-free now for more than 5 years, with a 6-year projection of 26%. The major cause of failure, however, was transplant-related mortality (TRM), with 13 deaths within 6 months (1-year estimate of 36%), resulting in closure of the enrollment to this arm in 1997. Of the transplant-related deaths, infection (n = 8) was the most common cause and was associated with acute GVHD. No further events after 3 years post-transplant were observed suggesting the possibility of long-term disease control, but at the expense of high initial TRM.

304 Chronic but not acute Graft versus host disease improves outcome in multiple myeloma patients after nonmyeloablative allogeneic transplantation

Spanish Collaborative Group for Non-myeloablative Transplantation.

A graft versus myeloma effect (GVM) has been observed in MM patients after allogeneic transplantation. This effect has been used to design less toxic regimens such as non-myeloablative transplants (NMT) maintaining antitumor efficacy through the GVM effect. In the present study we have evaluated the outcome of 29 MM patients receiving fludarabine and melphalan-based NMT. Event free survival (EFS) at 24 months was 33% being significantly higher for patients who developed aGVHD as compared to those who did not (51% vs 0% respectively; p=0.02; Hazard Rate (HR) = 3.16 (95% CI=1.09-9.15, p=0.03)) as well as for patients transplanted in response (CR/PR) or stable disease (SD) as compared to those with refractory/progressive disease at transplant (43% vs 0% respectively, p=0.02). Overall survival (OS) at 24 months was 60% ranging from 72% vs 42% for patients who did and did not develop cGVHD, respectively (p=0.1) and 63% vs 41% for patients in CR/PR or SD vs refractory/progressive disease at transplant, respectively (p=0.013). After a median follow up of 366 days 13 patients are in complete or partial remission (45% overall response rate). Nine patients have died, 3 of them due to disease progression and 6 (21%) due to transplant related mortality (TRM). Actuarial incidence of TRM was 37% for patients who developed aGVHD vs 13% for those who did not (log rank p=0.04). Among patients evaluable for eGVHD, these figures were 13% and 33% for patients with and without cGVHD, respectively (log rank p=0.22). The present study suggests that graft versus myeloma (GVM) effect is the main weapon for disease control after NMT in MM patients and the efficacy of this immune effect depends on tumor burden before transplant.

(Supported by Grant from Spanish FIS G03/136)

305 Results of Reduced Intensity Conditioning Allogeneic Transplantation in Myeloma – A report from the EBMT

For the Chronic Leukaemia Working Party of the EBMT.

The more widespread use of conventional allogeneic transplant for myeloma has been limited by the substantial procedure related mortality. Reduced intensity conditioning has been widely adopted with the demonstration of its feasibility and apparent lower mortality. Evidence for efficacy is limited, as the majority of series are small and frequently diagnostically heterogeneous. Data were collected for myeloma patients from 38 EBMT centres on a total of 256 transplants performed between 11/97 and 07/00. The median age was 52 years (32-66), 61% were male of whom 43% received stem cells from a female donor. 70% had stage III disease at diagnosis. 194 were matched siblings, 40 were unrelated and 9 were from mismatched or other related donors. 73% were CMV IgG positive. At the time of transplant 7.5% were in CR, 54% PR and 29% had progressive disease. The median time to transplant was 20 months (2 months – 11 years). Conditioning regimens varied but included fludarabine in 95%. The most frequent regimens were Fludarabine + TBI, Fludarabine, Busulphan + ATG and Fludarabine, Melphalan ± Campath. The cell source was peripheral blood stem cells (PBSC) in 205 and bone marrow (BM) in 51 patients. The time to neutrophil and platelet engraftment was faster for PBSC recipients (14 and 13 days vs 17 and 21 days respectively). Acute GVHD (grade II-IV) occurred in 31% and cGVHD in 49% of 195 assessable patients (extensive in 25%). The 1 and 2 year the TRM was 23.5% and 26%. Factors associated with an increased TRM were more than one prior transplant (RR 1.9), >1 year from diagnosis (RR2.7) and male patients with a female donor (RR 2.3). At 2 years the overall and progression free survival was 50% and 39%. Patients in remission prior to transplant had a better 2 year overall survival (1st CR/PR 74%, >1st CR/PR 49%, no remission 38% p=0.006). 2 year survival was worse in male patients receiving cells from a female donor (36% vs 54%) and in patients who had had more than one prior transplant (28% vs 54%). Similar factors were predictive for progression free survival at 2 years namely > 1 prior transplant (20% vs 45% p< 0.001) and remission status at conditioning (1st remission 62% vs no remission 35% p=0.003)

Disease progression and treatment related mortality remain challenges in allogeneic transplants for myeloma even with reduced intensity conditioning. It is, however, possible to identify patients where the risk may be acceptable and who may derive real benefit.

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Outcomes in patients with refractory relapse multiple myeloma after reduced intensity conditioning regimen allograft or myeloablative autologous stem cell transplantation.

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Introduction: Stem cell transplantation (SCT) has been shown to improve survival in patients with multiple myeloma (MM). Patients with refractory relapse (RR) MM have a poor prognosis with standard conditioning allografts due to high transplant related mortality. Myeloablative autologous SCT (ASCT) and reduced intensity conditioning allografts (RIC) have been explored in this heavily treated group to improve survival. To determine the feasibility and efficacy of ASCT and RIC we retrospectively evaluated our experience in 51 RR MM patients.

Patients and Methods: From 12/89 to 8/02, twenty-nine patients received ASCT and twenty-two received RIC at the University of Texas MD Anderson Cancer Center. Among ASCT and RIC, median age was 53 years (range 31-70) and 51 years (range 46-65); median time to transplant since diagnostic was 26 months (range 5-104) and 33 months (range 12-135); median number of previous treatments was 4 (range 2-9) and 5 (2-9) respectively. In the RIC group 17 patients (77%) had failed prior autograft. Among ASCT and RIC, median beta 2 microglobulin prior to SCT was 3.4 mg/dl (range 1.3-20.3) and 5.0 mg/dl (2.3-5.8) respectively. Cytogenetics studies prior to SCT were performed in 73% (37/51) of the patients: 46% (17/37) were diploid and 54% (20/37) showed random and complex karyotypes, mostly involving chromosomes 11 and 13. For ASCT, 52% received Thiotepa/Busulfan/ Cyclophosphamide (Cy) as conditioning; 38% Melphalan (Mel) 200 mg/m2 and 10% Cy/Topotecan/Mel. For RIC, 81% received Fludarabine (F) 30 mg/m2 for 4 days with Mel 140 mg/m2 as conditioning; 9% F/Mel 180 mg/m2 and 10% F/Mel 100 mg/m2 or Cy/F.

Results: Forty-eight patients achieved absolute neutrophils counts (ANC) ≥0.5*10^9/l, one RIC and 2 ASCT failed to recover. Median time for ANC recovery was 12 days (range 8-24) and 10 days (range 8-24) for RIC and ASCT respectively. Early death (<60 days post SCT without evaluation) was 14% in each group. Acute graft-vs-host disease (GVHD) grade I-IV was presented in 73% (37/51) of the patients: 46% (17/37) were diploid and 54% (20/37) showed random and complex karyotypes, mostly involving chromosomes 11 and 13. For ASCT, 52% received Thiotepa/Busulfan/ Cyclophosphamide (Cy) as conditioning; 38% Melphalan (Mel) 200 mg/m2 and 10% Cy/Topotecan/Mel. For RIC, 81% received Fludarabine (F) 30 mg/m2 for 4 days with Mel 140 mg/m2 as conditioning; 9% F/Mel 180 mg/m2 and 10% F/Mel 100 mg/m2 or Cy/F.

Acute GVHD (grade II-IV) was presented in 57% (12/21) patients, 43% (9/21) grade ≥ II. Chronic GVHD was presented in 53% (9/17). Among ASCT and RIC; overall response was 62% (18/29) and 59% (13/22); complete response was 17% (5/29) and 27% (6/22); median follow up was 8.9 months (range 0.4-60) and 8.2 months (0.2-43); overall survival (OS) at 12 months was 53±10% and 47±11% (p=0.53), respectively. When initial cytogenetics was available, OS at 2 years was 55±12% and 12±10% (p=0.03) for diploid cytogenetics and random/complex ones respectively. Among responders, event free survival at 12 months was 47±12% and 23±12% (p=0.31) for ASCT and RIC respectively. Non relapse mortality (NRM) at 100 days was 11% and 14%, NRM at 6 months was 16% and 30% (p=0.44), for ASCT and RIC respectively.

Conclusions: ASCT and RIC are feasible strategies for patients with RR-MM, however relapse or unresponsive disease remains the most important cause of treatment failure. Patients with diploid cytogenetic may represent a better responsive group. Novel therapeutic approaches need to be explored in this patient population including combination of novel agents as preparative regimen or maintenance therapy.

Follow-up of patients with progressive MM undergoing allografts after reduced intensity conditioning


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Following allogeneic stem cell transplantation (SCT) 30 – 50% of patients with advanced multiple myeloma remain disease-free at 3–6 years with cases of sustained molecular remissions. Unfortunately transplant–related mortality in the first 100 days was 30–50%, ranging up to 70% in patients with an relapse after an autograft. In an effort to reduce TRM, low intensity conditioning regimens have been developed to limit systemic toxicity.

In this study allogeneic SCT after reduced-intensity conditioning was evaluated in 22 patients (median age 53, range 36-66 years) with heavily pretreated multiple myeloma relapsing after autografting. At the time of allografting, 9 (41%) patients had chemorefractory disease, 5 (23%) patients were in untreated progression after autografting and eight (36%) were chemosensitive (MR or PR) after salvage chemotherapy. FISH analysis showed deletion 13q14 in 6 of 10 patients studied. The reduced-intensity conditioning regimen consisted of fludarabine (150 mg/m²), cyclophosphamide (40 mg/kg) and 2 Gy TBI. Seven patients received a transplant from an HLA-identical sibling and 15 patients from an unrelated donor including 3/22 (14%) from an HLA-mismatched unrelated donor (2 HLA-DRB1 subclass mismatch, 1 HLA-A mismatch). GVHD prophylaxis consisted of serotherapy with ATG (3 x 10 mg/kg) and ciclosporin CSA (n=12) or CSA plus mycophenolate mofetil (n=10).

All 22 patients engrafted and 8 of 21 (38%) evaluable patients developed aGVHD grade II-IV and 1/21 (5%) grade III-IV. Seven patients developed chronic GVHD. Inspite of heavy pretreatment and advanced age non-relapse mortality was only 5/22 (23%) (intracerebral hemorrhage n=1, aGVHD n=1, disseminated, toxoplasmosis n=2, EBV-LPD n=1). In 15 of 20 evaluable patients at least a minimal response was observed with 13 (65%) achieving a PR/CR. Six of these 13 (46%) patients remain alive and progression-free a median of 24 (range 8-36) months post-allografting. Two patients with deletion 13q14 have a progression-free survival > 1 year. Long-term disease control was only achieved in patients with chemosensitive disease before allografting. Estimated 2 years overall and event-free survival was respectively 25.5% and 22.0% for the whole patient group, and 62.5% and 57.1% for patients with chemosensitive disease. Chemorrefractory disease prior to allogeneic stem cell transplantation (p=0.0182) and absence of cGvHD (p=0.069) were associated with shorter event-free survival. Thus long-term disease control can be achieved, but is restricted to patients responding to prior salvage chemotherapy.
11. Role of novel therapies targeting the myeloma cells and its marrow microenvironment

11.1 Thalidomide in refractory MM

Thalidomide (THAL) shows antitumour efficacy in refractory and relapsed patients with multiple myeloma (MM) and has proved to be one of the most potent agent for treatment of MM in the last 30 years. We present here the results of THAL therapy in 191 refractory and relapsed MM patients (median age 59.7 years; range, 32 – 79 years) enrolled to the study from March 1999 to October 2002 in 7 Polish haematological centers. They received a median of 3 prior regimens (range, 2 – 6 regimens) including autologous stem cell transplantation in 29 cases. THAL was administered at a dose of 200 mg increased by 100 mg every week up to well tolerated dose (maximum 400 mg). 175 patients received THAL as monotherapy and 16 patients received THAL with dexamethasone. Major response was defined as ≥75% monoclonal protein (M-protein) reduction in serum and/or in urine. Partial and minor responses were defined as ≥50% and ≥25% M-protein reduction respectively. The stable disease was considered when M-protein reduction was less than 25% without evidence of disease progression. Clinical response was observed in 107 patients (56%) including major response in 27 patients (14.1%). In 18 patients (9.4%) we confirmed nearly a complete remission with M-protein reduction more than 90%. Then 11 patients from this group (6%) were treated with autologous peripheral blood stem cell transplantation (autoPBSC). 84 patients (44%) did not respond to the therapy. Overall survival (OS) time in the responders was 48.8 months (median 42.4).

Statistical analysis showed that mean survival time from the beginning of THAL therapy was 14 months (median 10.3) and the event-free survival (EFS) was on average 10.3 months. Responses occurred within a median time of 6 weeks. Cumulative dose of THAL in the whole group of patients was on average 28.7 g after 3 months of treatment. There was no correlation between the clinical response and the cumulative dose of THAL, what is suggested by some authors. No treatment-related mortality was observed. After median follow-up of 8.6 months (range 0.3 to 39 months) 124/191 patients are alive, whereas 67/191 patients died. Somnolence, constipation and fatigues were the most common side effects – in over 65% of patients in the early stage of THAL treatment. These symptoms tend to resolve after 2-3 weeks of therapy. After 8-10 months of treatment the most frequent problem was peripheral sensory polyneuropathy observed in 59% of patients. THAL treatment was stopped at the first sign of neuropathy. The sensory neuropathy improved in the majority of pts after cessation of treatment but it persisted several weeks in 8 pts and in 3 pts it was irreversible.

The results of our study indicate that THAL might be able to improve the outcome in refractory and relapsed patients with multiple myeloma and should be the treatment of choice in these cases even though its unclear ways of action are still not fully understood.

309 Treatment of advanced multiple myeloma (MM) with thalidomide (THAL). Long term follow-up in a prospective study of 121 patients.


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The aim of this prospective study was to describe the efficacy, tolerance and prognostic factors of therapy with THAL in patients with advanced MM.

Patients and methods: We studied prospectively 121 patients (62 male, 59 female, median age 65.4 years) who received THAL for advanced MM (40 refractory, 81 in relapse). The median time from MM diagnosis to initiation of THAL therapy was 4.5 years. Thirty nine patients (32 %) had previously been treated with at least one autologous bone marrow transplantation and 81 patients (67%) with conventional therapy. After informed consent THAL was started at a dose of 200 mg/d and increased to 400 mg/d after 2 weeks. Response was evaluated after 2 months of treatment on the level of the monoclonal component. In 39 patients dosage of seric THAL by HPLC was performed after 2 months of treatment. Overall survival and event-free survival were evaluated in all patients 24 months after inclusion.

Results: After 2 months of treatment, thirty eight patients (31.4 %) had at least a minor response (decrease of monoclonal component ≥ 25%), 39 (32.2%) were non responders and 7 (5%) non-evaluable. Main toxicities were somnolence (77.1 %) and constipation (61.2%). Neuropathological examination was abnormal in 30.8 % of patients, after 2 months of THAL versus 18.6 % before treatment. Median seric THAL level was 0.82 ± 0.79 (0.1 – 3.03) in non responding patients and 0.66 ± 0.49 (0.19) in responding patients. At 1 year and 2 years overall survival rates were 47.5 ± 8.9 % and 30.0 ± 8.2 % respectively and event-free survival rates 33.3 ± 8.5 % and 15.0 ± 6.4 %. In multivariate Cox regression analysis the adverse prognostic factors were refractory status at inclusion, Beta 2 microglobulin > 3 mg/l and platelets < 100 000/mm3.

Conclusion: In a prospective study of advanced MM treated with THAL we observed a response rate of 32 %. We did not find any relationship between response and levels of seric THAL. Finally the best prognostic factors were status at inclusion (refractory or relapse), Beta 2 microglobulin level and platelet count.
MM is an incurable disease showing a poor prognosis due to the limited therapeutic options. Among the new therapeutic approaches tested, Thal with its antiangiogenic properties, has re-emerged as a promising anti-cancer agent in many refractory malignancies. Between March 2000 and July 2002, 80 refractory or relapsed MM patients were treated with Thal. Median age was 63.5 (range 33-84) years; 49 were males and 31 females; 1 patient had serum creatinine > 2 mg/dl and 49 patients were IgG, 21 IgA, and 10 light chains MM; 5 patients had PCL. As for disease status: 36 were refractory (defined as progression while on therapy) to at least two lines of therapies and 44 were ≥ 2 relapses. Median follow-up before inclusion in this study was 36 months (range 5-104). Thal, kindly provided by Grunenthal Stolberg Germany, was administered as a single agent, through a compassionate-use protocol, starting at 100 mg daily subsequently increasing by 100 mg every other week, to a maximum of 800 mg/day or according to the maximum tolerated dose. The median daily dose of Thal administered to all patients was 500 mg (range 100-600 mg).

None of the five PCL responded, even though two of them had reduction in circulating PC. Considering that 4 patients died during the first 10 days of the treatment and 4 stopped the therapy during the first month, because of side effects, 72 (treated for at least one month) were considered valuable for response defined as decline in the monoclonal protein level > 25%. Among the evaluable 72 MM patients 46 (63.5%) were responding to treatment (14 achieved a decline in the MC > 25%, 17 > 50% and 15 > 75%). The median interval between the start of treatment and the response was 1 month (range 1-9) and the duration of response was 13 months. A total of 22 patients relapsed and 17 died for progression disease, 21 patients did not respond to Thal and 15 of them died. Median survival for the responding patients was not achieved yet and was 12.5 and 5 months for the overall population and for no responding patients, respectively. The most frequently side effects, registered in our patients, during the first weeks, were lethargy (12.5%), weakness or fatigue (87.5%), constipation (77.5%), skin rash (7.5%), mood change or depression (27.5%) These side effects were easily manageable and reversible by reducing or discontinuing the therapy. More serious side effects observed in those patients treated for long time were peripheral neuropathy (13.75%), bradycardia (3.75%) such us hypothyroidism and deep vein thrombosis (1.25%). The response obtained in our patients adds further evidence concerning the efficacy of this drug in resistant and refractory MM patients but PCL were not affected by Thal. The different effectiveness of Thal may be can depend of aggressively of tumor. In conclusion Thal is a true anti-myeoma drug even thought its toxicity must be taken into account in designing new clinical trials.

## RESPONSE TO THALIDOMIDE IN MULTIPLE MYELOMA: IMPACT OF ANGIOGENIC FACTORS AND EXTRAMEDULLARY INVOLVEMENT


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Background: Thalidomide produces a response rate ranging from 32 to 64% in patients with refractory / relapsed MM. However, its efficacy in patients with extramedullary plasmacytomas (EMP) remains controversial. It is postulated that thalidomide has antiangiogenic and immunomodulatory effects, mediated by several cytokines such as VEGF, bFGF, HGF, IL-6 and TNFalpha.

Aims: 1) to ascertain whether serum levels of different angiogenic cytokines can predict response to thalidomide 2) to assess the response of patients with EMP.

Patients and methods: From November 1999 to December 2002, 38 patients with refractory / relapsed MM were treated with thalidomide. Eleven patients had EMP when therapy was initiated. Serum specimens were obtained in all patients before treatment was started and at the time of maximum response in responding patients or at thalidomide discontinuation in those patients showing no response. Serum levels of VEGF, HGF and bFGF were determined in 18 patients whereas IL-6 and TNFalpha where measured in 19 patients.

Results: Seventeen of the 38 patients (45%) responded to thalidomide. Eight (21%) achieved a partial response while 9 (24%) had minimal response. The response rate was significantly higher in patients without EMP (63% vs 9%, p= 0.0029). Three of the 11 patients with EMP achieved a minimal response according to the serum M-protein decrease but had an increase in their soft-tissue masses. In addition, two patients without EMP when therapy was started developed soft-tissue plasmacytomas while on thalidomide therapy. VEGF serum levels were significantly higher in patients who achieved a response. In contrast, baseline serum levels of HGF were significantly lower in responding patients. Neither, VEGF nor HGF serum levels showed correlation with the presence or absence of EMP. Baseline TNFalpha serum levels were significantly lower in responding patients and in those without EMP. The serum levels of bFGF and IL-6 did not correlate with response to treatment or presence of EMP.

Conclusions: Baseline high levels of VEGF and low levels of HGF and TNFalpha predict response to thalidomide whereas only TNFalpha correlates with the presence of EMP. Patients with EMP have a poor response to thalidomide. The lack of efficacy on soft-tissue plasmacytomas supports a bone marrow microenvironment-mediated mechanism of thalidomide antimyeloma effect.
Definitively, this drug should be considered between the therapeutic options for rescue of MM relapsing after transplant.
Its optimal effects are observed when associated to dexamethasone. The stable and long duration response, observed in some patients at low dose for long term treatment, suggests a possible role of this drug as maintenance treatment that need to be explored. In our series, patients with del cr13 showed poor response but some cases with multiple plasmocytomas responded.
The toxicity presented with this drug make necessary the introduction of new thalidomide analogs with similar effects but without that toxicity (Immunomodulatory drugs: IMID as Revimid®, CC5013). The exact dose, timing and treatment duration of these agents and its more efficacious combinations need to be explored in randomized controlled trials. Also is necessary to evaluate its potential role in smoldering MM to prevent or retard evolution to open MM; in symptomatic de novo patients instead of standard induction regimen and also as maintenance treatment modulating minimal residual disease.

### 313 Thalidomide in relapsed and refractory multiple myeloma.: a retrospective analysis of efficacy and toxicity

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Thalidomide and its analogues have emerged as a novel class of therapeutics in multiple myeloma. The aim of this retrospective analysis was to evaluate the activity and toxicity of thalidomide in relapsed and refractory myeloma patients at the University hospital of Ghent. From April 2000 to November 2002, 40 patients (16 males/24 females) with relapsed/refractory myeloma have been treated with thalidomide. Before starting thalidomide different schemes of chemotherapy - like VAD, VCMP, platinum - have been treated with thalidomide. Before starting thalidomide different schemes of chemotherapy - like VAD, VCMP, platinum - were used associated to polichemotherapy following the DECP 24 h i.v. infusion scheme (dexamethasone, etoposide, cyclophosphamide, and platinum). Patients with bone lesions also received bisphosphonates iv monthly.

Results: The mean age was 62 years (range 39 to 83 y). The myeloma subtypes were distributed as follows: IgG (n=25), IgA (n=8), light chain (n=6), non secreting (n=1).

On an intent-to-treat basis, the 40 patients were evaluable for response after 6 weeks of therapy. Overall response was 37.5% (n=15/40). Four patients showed ≥50% reduction in serum or urinary M-protein concentration, eleven patients showed >25% tumor reduction. Nine patients had stable disease.

On an intent-to-treat basis, overall response after 3 months of therapy was also 37.5% (n=15/40). Seven patients had ≥50% reduction in serum or urinary M-protein concentration, eight patients reached ≥25% tumor reduction, four patients could be considered having stable disease.

Thalidomide has been considered a new class of active agents in multiple myeloma with a response rate ranging from 30% to 40% in refractory patients and from 70% to 90% when associated to dexamethasone in symptomatic de novo patients. We present the experience of a Spanish multicentre registry using this drug in MM patients as rescue for relapse after hematopoietic transplantation.

Results: 61 patients were evaluable for response*: 29 patients achieved favourable response (47%) See Table. Median treatment duration was 5 months (0.5-36). Only 1 patient, of 7 known cases with del cr13, showed favourable response. However, 7 out of 15 patients with multiple plasmocytomas showed objective response.

Main toxicity > grade 1 according WHO, was: constipation (50%), somnolence (33%) and peripheral neuropathy (12%). 2 cases presented venous thromboembolism; one with APCR coagulative test positive; and 4 patient showed cardiotoxicity. One case of TRM probably for cardiac failure was observed. Treatment was interrupted due to toxicity in 6 cases (10%). After a median follow up of 14 months (2-30) the OS and EFS estimated at 2 years was 50% and 20% respectively.

#### Table of Results

<table>
<thead>
<tr>
<th>Response (Reduction of M Component)</th>
<th>N Patients (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Complete Response</td>
<td>2(2%)</td>
</tr>
<tr>
<td>Objective response (&gt;50%)</td>
<td>12 (20%)</td>
</tr>
<tr>
<td>Minimal Response (25%-50%)</td>
<td>15 (25%)</td>
</tr>
<tr>
<td>Total favourable response</td>
<td>29 (47%)</td>
</tr>
<tr>
<td>Non response-Stable disease</td>
<td>9 (15%)</td>
</tr>
<tr>
<td>Progression</td>
<td>23 (38%)</td>
</tr>
<tr>
<td>Median duration of Response (months)</td>
<td>7 (2-26)</td>
</tr>
</tbody>
</table>

*Patients evaluable (at least 6 weeks on treatment)

Comments and Conclusions. Oral thalidomide alone or combined with steroids or chemotherapy induces a considerable response rate in this population of MM with poor prognostic.
37.5%. Patients who do not achieve a 25% reduction in monoclonal protein at 6 weeks are unlikely to respond later on. 32.5% of patients needed to interrupt therapy with thalidomide early or late because of severe adverse events.

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**Long Term Treatment with low dose of Thalidomide in Refractory Multiple Myeloma: Preliminary Results**

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Introduction. Thalidomide has been shown to be active in relapsed and refractory patients with multiple myeloma and its current role as a first line agent in the induction treatment is currently being investigated. The role and clinical results of thalidomide as maintenance treatment at low dose for prolonging response is not known. The potential toxic effects of this drug has limited its use as long term, however there is a rational for its use in this setting: As the number of treatment lines increases in MM patients, including intensification schemes, the response phase becomes progressively shorter suggesting development of multidrugs resistance (MDR). Thalidomide could maintain the response or plateau phase acting at different pathogenic levels. We present the preliminary experience of a preliminary group of patients that received thalidomide in a long term period.

Patients and treatment: Eighth patients with MM that had received oral Thalidomide as rescue for relapse after autologous hematopoietic transplantation and that showed favourable response were intended to keep on treatment with thalidomide at low dose to prolong response. The initial treatment included oral Thalimodide® 100 mg (Grunnenthal, Germany): 200 mg/d, escalating doses every 14 d, according tolerance until a maximum daily dose of 800 mg. Median dose received was 400 mg/d. In 4 patients thalidomide were used alone. Rest of the patients received this drug associated to Dexamethasone ( 20 mg x 4 every 21-28 d). 2-3 weeks after observing the maximum response thalidomide was reduced and maintained for long term at low dose 50-100 mg/d continuously or on alternate weeks, according tolerance, until relapse or progression. Neurological examinations and study of thyroid hormones levels were periodically performed.

Results. Three patients progressed after 6-9 months on treatment and five patients were evaluable for “long term” follow up wit at least 10 months of treatment. One patient maintain CR, two cases objective response and two patients presents stable disease. (Anecdotically one patient (3) on dialysis recovered from renal failure after 24 months on treatment). Median duration of treatment was 12 months (12-30). Somnolence, cutaneous rash and peripheral mild neuropathy that improved alternating the low dose were the main secondary effects.

Comments and Conclusions. Long term treatment with low dose of thalidomide presents an acceptable tolerance. The stable and long duration of responses, observed in this group of patients, suggest a possible role of this drug as maintenance treatment. This role of thalidomide at low dose in the long term, prolonging the response phase of multiple myeloma, needs to be studied in randomized trials.

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**EFFICACY OF THALIDOMIDE ALONE IN 25 RELAPSED OR REFRACTORY MULTIPLE MYELOMA PATIENTS.**

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Thalidomide is an active agent in the treatment of relapsed or refractory myeloma. In this retrospective study, responses and time of responses were observed with thalidomide alone. Dexamethasone was added in a second time if necessary. Our study population comprised 25 patients (median age 60 years) who received directly thalidomide alone for relapsed or refractory myeloma between January 2000 and January 2002. Responses were defined according to M-component reduction in serum or in urines at 21st and 90th median days. Median time from myeloma diagnosis to onset of thalidomide therapy was 26 months (range 9-76 months). Patients had either prior conventional therapy alone (n=8) or intensive treatment with a single or a tandem transplantation (n=17). All the patients had received 2 or more previous treatments before thalidomide had been instituted. Thalidomide usually began in oral dose of 100 to 200 mg every evening, increased at weakly intervals when tolerable. At the reference date, the median follow up from the start of thalidomide treatment was 17.5 months. All responses occured with dose ranging from 100 to 400 mg. Complete responses were observed. At the 21st median day, 9 patients (36%) achieved Partial Response (PR) : 2 PR>50% and 7 PR>25%. At the 90th median day, a dexamethasone addition was necessary for 6 of them. PR were recorded in 14 patients (56%) : 6 PR>50% and 8 PR>25%. Major adverse effects included somnolence and sedation (43%), peripheral neuropathy (38%), dizziness (31%) and constipation (24%). We observed a good response after only 21 days of treatment, which is increased after 3 months. However efficacy of thalidomide is short (median time 7 months) and an association is frequently required to maintain or increase response.

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**THALIDOMIDE ALONE OR WITH DEXAMETHASONE IN THE MANAGEMENT OF MULTIPLE MYELOMA: OUR EXPERIENCE.**


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INTRODUCTION: Multiple myeloma (MM) remains an incurable malignance, as a results of innate drug resistance present at diagnosis. Thalidomide(THAL) is a novel antimonyeloma agent because of its multiple, including antiangiogenic, antitumor mechanisms. This drug, alone or with dexamethasone, was given in several trials in patients with refractory or relapsed MM after stem cell transplantation or conventional chemotheraphy, as well as a response maintenance therapy.

PURPOSE: To evaluate the activity of thalidomide as a drug included in a new protocol for patients diagnosed of MM.

PATIENTS AND METHODS: The study included a group of 13 consecutive patients diagnosed of multiple myeloma and treated with THAL alone or in combination with dexamethasone in a dose-scaling schedule as a part of a novel total MM therapeutic protocol, between January 2000 and December 2002. The median age was 56 years (range, 31-71 years); of them 8 were males and 5 females. M-component isotype was IgG in 9 patients, IgA in 2
patients, light chain in 1 patients and 1 patient was diagnosed of non-secretor myeloma. At the onset of THAL, 2 (15%) patients were in complete remission (CR), 3 patients (23%) in partial response (PR) and 8 patients (62%) were refractory to treatment. Four patients had mass tumoral previously. Response was assessed by monoclonal protein quantitation in serum or urine, bone marrow plasma cells percentage and mass tumoral disappearance. Parameters as hemoglobin level, neutrophils and platelets ciphers before and after THAL, side effect were evaluated.

RESULTS: CR was seen in 4 patients(37%), PR was achieved in 5 patients(45%) and 2 patients (18%) were refractory to treatment. Comparing to the state of disease at the onset of THAL, 44% of obtained response should be attributable to this drug. Two deaths were registered before evaluation (in one patient infection was the cause and in the other was progression of MM). Two responding patients (18%) had relapsed after evaluation in a extramedullar mode and died due to myeloma progression; both of them had tumoral mass previously to THAL treatment. Median of follow up was 11 months (range 4-22).

Although side effects with THAL were frequent (69%), they were nearly always mild and reversible beeing of grade 1 or 2 degrees (fatigue, sedation, numbness, unsteadiness). Only one patient required cessation of THAL due to intolerance. Concerning blood count, lower neutrophils ciphers had been observed after THAL treatment although no significative difference was observed(median of group before THAL was 2.6 x 10^9/L vs 2.2 x10^9/L after THAL).

CONCLUSIONS: In our experience THAL alone or with dexamethasone is a good alternative as salvage therapy in patients with refractory myeloma without severe side effects. In patients with high tumoral burden this treatment should be completed with others therapy strategies.

317 Combination therapy with thalidomide plus oral melphalan compared with Thalidomide alone for advanced multiple myeloma

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To date, few therapeutic options are available for patients with relapsed MM, especially when the relapse occurs after stem cell transplantation. In this context, several trials demonstrated that thalidomide alone or in combination with dexamethasone produce response rates of about 30% and 50%, respectively. We report our experience using a combination of thalidomide and oral melphalan compared with thalidomide alone in a case-control study including patients with advanced MM.

From May 2000 to July 2002, 27 patients were treated with thalidomide plus oral melphalan (TM group) and 23 patients with thalidomide alone (T group). Patients were not excluded because of either poor performance status or cardiopulmonary, renal and liver disfunctions. The initial dose of thalidomide was planned to be 100 mg p.o. daily at bedtime, escalated weekly by 100 mg increments until a maximum dose of 600 mg daily continuously until side effects or disease progression were documented. Melphalan was administered intermittently at a dose of 0.20 mg/kg/d orally for four days every 28 days for almost one course after greatest response or until severe toxicity. No patients received antithrombotic prophylaxis.

Prognostic features such as 2-microglobulin, hemoglobin level, prior regimens, prior high-dose therapy and disease history did not significantly differ between the two groups of patients while age was significantly lower in the TM group (69 vs 74 years; p = 0.042) and median follow-up was significantly longer in the TM group (13 vs 10 months; p = 0.022). Rate of paraprotein decrease ≥ 50% was significantly higher in the TM group compared with T group (63% vs 26% p = 0.009). Remarkably, ≥ 75% paraprotein decrease was obtained in 4 patients (15%; ¾ true CR) of TM patients compared with only 1 (4%; no true CR) of T group. The median time to remission was significantly shorter in the TM group (4 weeks vs 7 weeks; p = 0.0312). Multivariate analysis selected only TM therapy (p = 0.008) as factor associated with better response. After a median follow-up of 13 months (range 6-32), 18 patients (36%) had disease progression and 11 (22%) died. Eight patients died of disease progression, and 1 patient died for pulmonary embolism, infection and heath failure, respectively. PFS was significantly longer in TM group compared with T group (median NR vs 13 months; 61% vs 45% at 2 years; p= 0.0356) whereas OS did not most likely because of a median follow-up significantly shorter in the T group. In the multivariate analysis only response ≥ 50% was associated with higher PFS at two years (66% vs 42%; p= 0.0464) and only Hb ≥ 10.5 mg/dl significantly affected longer OS (76% vs 49% at 2 years; p = 0.0449).

Fourteen patients (28%) stopped and 24 (48%) reduced thalidomide because of side effects. In the TM group, 4 patients (15%) delayed the administration of melphalan because of hematologic toxicity. The main side effects attributable to thalidomide were constipation (72%), somnolence (38%), asthenia (24%) and sensory peripheral neuropathy (44%). This latter adverse event was cause of thalidomide withdrawal in 7 patients (14%). Central nervous system adverse effects (dizziness, numbness, headache) were found in 8 patients (16%) although severe toxicity was rare. No differences were found between the two groups with respect to the above side effects while rate of deep venous thrombosis (11% vs 4%; p = 0.614) and grade 3 (WHO) leukopenia (30% vs 13%; p= 0.073) were higher in the TM group.

This study suggests that oral melphalan added to thalidomide improves response rate and PFS in advanced poor prognosis multiple myeloma without increasing severe toxicity. Consequently, combination of thalidomide plus oral melphalan should be further investigated in the context of controlled studies.

318 Thalidomide in Relapsed/Refractory Multiple Myeloma: Experience of a Single Center

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Background: Thalidomide (THAL) has significant activity both as a single agent and in combination with other therapies in patients with de novo and advanced multiple myeloma. We presented our results treating advanced disease with THAL alone or in combination.

Methods: We treated patients with relapsed/refractory myeloma using dose-escalated THAL up to maximum dose of 800 mg/day. THAL was given orally for 2 weeks at 200 mg/d, increasing of 200 mg/day each 2 weeks if tolerated. In those patients with no
response, dose was reduced to 400 mg/day and combined with dexamethasone (DEX) and/or chemotherapy (CH). All patients, who discontinued THAL for good response and those who reduced dose because unacceptable toxicity, relapsed. Adjuvant bisphosphonate therapy was done in all and haematopoietic growth factors when necessary. We evaluated response using the following criteria: Complete Response (CR: ≥ 90% paraprotein reduction, no progression of bone lesions and no anaemia), Major Response (MjR: ≥ 75% and < 90% paraprotein reduction), Partial Response (PR: ≥ 50% and < 75% paraprotein reduction), Minor Response (MnR: ≥ 25% and < 50% paraprotein reduction) and Stable Disease (SD:<25% paraprotein reduction). We consider No Responders those patients with Stable and Progressive Disease (PD).

Results: From July 2000 to January 2003, thirty-seven patients were enrolled. Twenty-nine (15 men, 14 women) were valuable with median age 57 years (range:42-85); the median number of prior therapies was 4 (range:1-5); the median interval from diagnosis to THAL therapy was 38 months (range:10-136). Eleven patients received THAL as a single agent, thirteen received a combination THAL+DEX and five THAL+CH. The median time of treatment was 3 months (range:2-24) and the median daily dose of THAL was 400 mg. We obtained CR in 3 patients (11%), MjR in 5 (17%), PR in 5 (17%), MnR in 5 (17%) and SD in 4 (14%). Seven patients (24%) had PD. The overall response rate (CR+MjR+PR+MnR) is 62%. The relation between dose of THAL and response seems not linear: a)dose escalation from 200 to 800 mg/d was achieved in 55% of the patients but not all have respond; b) some have respond with lower dose. Seven patients had died: four with PD and three with infection. The most common observed toxicities attributable to THAL included constipation (66%), fatigue (38%), sedation (64%), peripheral neuropathy (48%) and venous thromboembolism (17%). In our series the estimated overall survival to THAL is at this time 64.8% (SD±11.2%, median not reached yet).

Conclusion: THAL, in monotherapy or associated with DEX and/or CH, is active in relapsed/refractory multiple myeloma. Optimal THAL dosing appears to be variable and side effects can be dose limiting suggesting that therapy should be individualized for each patient. The duration of treatment, the criteria for adding DEX and/or CH and the potential synergism of bisphosphonates and erythropoietin remain hot points not yet answered successfully.

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High Grade B-Cell Neoplasms Arising After Discontinuation of Thalidomide Therapy
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Plasma cell dyscrasias (PCD) have been reported to rarely undergo transformation into high-grade B-cell neoplasms (Richter syndrome). The relationship between PCD and the subsequent development of high-grade B-cell malignancies remains unclear and controversial. Some data suggest a clonal evolution in which the same neoplastic clone undergoes de-differentiation and loses the ability to secrete clonotypic immunoproprotein while transforming to a more aggressive neoplasm. Other data suggest that they are genetically unrelated and represent independent transformation events. We report 5 cases of patients that have developed high-grade B-cell neoplasms (large cell lymphoma and anaplastic myeloma) after discontinuation of thalidomide-based treatment due to toxicity. In one instance (patient # 5) the patient was treated with thalidomide for IgGk myeloma, displaying a partial response. Due to untoward neuropathy and somnolence treatment was withheld. Four months later he developed abdominal pain and cervical adenopathy which upon biopsy showed diffuse large B-cell lymphoma (B-cell DLCL). LDH was within normal limits and paraprotein had risen to baseline. CT and PET scan imaging showed extensive cervical, mediastinal and retroperitoneal adenopathy. Bone marrow revealed involvement by myeloma but not by lymphoma. Lymph node immuno-histochemistry revealed an IgAk neoplasm. Based on sequencing of the clones of PCR products, the myeloma and lymphoma belonged to two different clones. Other cases of confirmed clonal evolution have been summarized in the Table I. We have therefore identified 2 classes of transformation after thalidomide withdrawal. In one, the B-cell DLCL transformation appears to be an independent clonal process in which thalidomide’s direct role, albeit conjectural, remains intriguing. The second type (patients #1-4) clearly indicates clonal evolution. Although thalidomide’s efficacy in PCD has been widely accepted we have recognized a short progression free interval and a rapid rebound phenomenon upon withdrawal of therapy. The cases presented here suggest that thalidomide withdrawal may be directly linked to clonal transformation, perhaps by a surge in cytokine-mediated processes.

Table I: Transformation of PCD to aggressive B-cell neoplasms after discontinuation of thalidomide

<table>
<thead>
<tr>
<th>Pt. #</th>
<th>Diagnosis</th>
<th>Stage</th>
<th>Treatment</th>
<th>Response</th>
<th>Months to Transformation</th>
<th>Transformation</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>IgG MM</td>
<td>IIIA</td>
<td>BLT-D</td>
<td>NCR</td>
<td>12</td>
<td>Anaplastic MM k</td>
</tr>
<tr>
<td>2</td>
<td>IgG L MM</td>
<td>IIIIB</td>
<td>BLT-D</td>
<td>SD&lt;1</td>
<td>15</td>
<td>Anaplastic MM l</td>
</tr>
<tr>
<td>3</td>
<td>IgG L MM</td>
<td>IIIA</td>
<td>BLT-D</td>
<td>CR</td>
<td>15</td>
<td>Anaplastic MM l</td>
</tr>
<tr>
<td>4</td>
<td>WM</td>
<td>N/A</td>
<td>BLT-D</td>
<td>MR</td>
<td>4</td>
<td>B-cell DLCL k</td>
</tr>
<tr>
<td>5</td>
<td>IgG MM</td>
<td>IIIA</td>
<td>T</td>
<td>SD&lt;1</td>
<td>4</td>
<td>B-cell DLCL</td>
</tr>
</tbody>
</table>

NCR=near complete response, SD=stable disease, CR=complete response, MR=major response

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The addition of clarithromycin (BIAXINTM) significantly improves the response to dexamethasone in chemotherapy-naive multiple myeloma patients: results of a prospective, sequential, randomized trial.

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We have previously reported the clinical activity of the combination of clarithromycin (BiaxinTM)(B), low-dose thalidomide (LT), and dexamethasone (D) (BLTD) in patients with multiple myeloma (MM). The importance of B in this regimen has not been clear, however, a surprising finding that we and others have observed is that patients whose disease is refractory to T or D alone may respond to the BLTD combination, suggesting that a synergistic effect might be involved. To better define the role of each component of the BLTD program we initiated a prospective, randomized trial which compares the safety and efficacy of standard pulsed D to LTD as induction therapy. Patients who achieve less than 50% drop in paraprotein after 8 weeks of treatment would then go on to receive B in an attempt to further reduce tumor mass. Accrual is ongoing but to date 14 patients are evaluable for response and toxicity. Patients have been randomized to either D or LTD: age of 60 years (50-76), hemoglobin of 11.0 (8.4-15.7),
platelets of 207 (76-402), B2M of 3.2 (1.5-5.1), CRP of 1.9 (<3-5.9), creatinine of 1.3 (0.7-2.3), albumin of 3.4 (2.7-4.4), and calcium of 9.3 (7.9-10.8). Sixty six percent of the patients are stage III, 17% are stage II, and 17% are stage IIB. Response rates are summarized in the table below. Patients randomized to D displayed a steeper fall in the paraprotein at 8 weeks of treatment than the LTD patients who appear to have a more gradual response (A=5.53 vs Δ-2.27). 36% of patients (D=2, LTD=4) have had a limited response (<50% drop in paraprotein) to induction therapy after 8 weeks and therefore B was added to their regimen. B has produced further tumor mass reduction at 16 (median 45.75% SD 25) and 20 weeks (median 54.5% SD 21.4). Furthermore, the drop in the paraprotein, as attested by Δpre B vs Δ post B, was steeper after the addition of B in both the D (-3.89 vs -6.16) and LTD (-2.7 vs -3.34) arms. Steroid related morbidities have been more apparent after B was initiated. Preliminary evidence suggests that B contributes to tumor mass reduction by potentiating dexamethasone’s effect. Pharmacokinetic studies are underway to further clarify this issue. Contrary to expectations, thromboembolic events have been similar in both groups (D=2 LTD=1). This represents the first clear evidence that B can augment the response to dexamethasone when given alone or in combination with Thalidomide to patients with MM.

321 Thalidomide allowed an adequate collection of peripheral blood stem cells and a safe autologous stem cell transplantation in VAD-refractory multiple myeloma patients.

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Multiple myeloma (MM) patients with resistant or progressive disease after induction therapy are poor candidates for adequate collection of peripheral blood stem cell (PBSC) progenitors and for autologous stem cell transplantation (ASCT). Since thalidomide emerged as an active drug in previously pretreated relapsing MM patients, we evaluated if thalidomide treatment could obtain clinical and histological responses in patients with refractory disease after induction therapy and allowed them to proceed to collection of PBSC and ASCT. Ten newly diagnosed MM patients with a median age of 60 years, who had a resistant or progressive disease after a median of 4 monthly courses of VAD-based chemotherapy, were treated with a maximum daily dose of 400 mg thalidomide and followed monthly, until they achieved the greatest reduction of M-protein and marrow plasma cell infiltration. The patients who achieved at least a partial response, proceeded to PBSC collection after cyclophosphamide plus G-CSF and to myeloablative treatment with busulfan and melphalan. Seven patients were responsive: after a median of 16 weeks of thalidomide therapy, they presented the maximum cytoriduction, with a median decrease of 60% of M-component level and of 84% of bone marrow plasma cell infiltration in comparison with the values prior to thalidomide treatment. Median daily dose was 400 mg, as planned, in 8 out 10 patients; in 2 cases peripheral neuropathy and mialgias lead to dose reduction to 300 mg and 200 mg respectively. Collection of PBSC was successful and adequate in all responsive patients, who underwent ASCT with a rapid and eventless engraftment. At a median follow-up of 5 months, one patient achieved a complete remission, the other 6 had 5% or less marrow plasma cell infiltration with the persistence of a positive serum immunofixation.

We conclude that thalidomide can be a suitable preparative regimen to PBSC collection and ASCT for patients who demonstrated an initial resistance or progression after conventional chemotherapy.

322 Low dose thalidomide is an effective therapy for relaps after stem cell transplantation in multiple myeloma

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Rationale: Thalidomide is an active and safe salvage therapy for advanced stages of multiple myeloma. Optimal dose and schedule of administration, however, remain to be determined. Recent studies have not been able to demonstrate a significant gain of therapeutic effects at the expense of increasing adverse events as thalidomide dosage was escalated. We therefore analysed the outcome of low dose thalidomide therapy in our cohort of multiple myeloma patients who had relapsed after high dose chemotherapy and stem cell transplantation. Patients and methods: Eleven patients (6 male and 5 female, median age 58, range 46-62) who had relapsed after autologous stem cell transplantation (n=10) or were resistant to allogeneic stem cell transplantation (n=1) were included in the study. Bone marrow specimens available from the time of diagnosis were studied by interphase fluorescence in situ hybridisation (FISH) to determine the status of chromosome 13 in multiple myeloma cells. The median thalidomide dose administered was 100mg/d (range 100-400mg). Five patients received thalidomide as monotherapy, four patients received a combination of thalidomide and dexamethasone (approximately every 4 weeks 40mg per day for 4 days). Dexamethasone was initiated when we did not observe objective response to thalidomide alone after at least 6 months. Two patients who were resistant to VAD received VAD combined with thalidomide. Response was defined as a decrease of paraprotein or Bence-Jones protein level by more than 25% (>50% and >75% respectively) in two following observations at least four weeks apart. Results: We could observe an overall response (paraprotein reduction of >50%) in 8 of 11 (73%) patients who had relapsed after stem cell transplantation. Three of 5 (60%) patients in the thalidomide monotherapy group, three of 4 (75%) patients in the thalidomide plus dexamethasone group and both patients in the thalidomide plus VAD group responded. In one patient thalidomide had to be withdrawn after only 13 days because of neuropathy. All 6 patients with a normal chromosome 13 status responded with a paraprotein reduction of >50%.

Conclusion: Thalidomide treatment appears to be very effective particularly in patients who have been pretreated with high dose chemotherapy and stem cell transplantation when the tumor burden is still low. Our results provide some evidence that the response to thalidomide might be also dependant on prognostic parameters such as deletion of the 13q chromosome although we could not observe statistical significance which might be due to the small patient numbers. Larger prospective studies are necessary to fully answer these questions.
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Thalidomide treatment of patients with advanced multiple myeloma
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Background: Thalidomide activity in the treatment of advanced myeloma is confirmed by number of studies. It is administered as single agent or in combinations with other therapies. The aim of study was to present results of Thalidomide treatment used in patients with advanced myeloma. Patients and methods: During period November 2001 – January 2003, 11pts (8M/3F, mean age 57yrs, range 48 – 69yrs) were treated with Thalidomide. According to the clinical stage of disease, distribution was as follows: IIA 2pts (18,2%), IIIA 5pts (45,4%), and IIIB 4pts (36,4%). There were 5pts (45,4%) with IgG kappa monoclonal protein; 4pts with IgG lambda (36,4%); 1pts with IgA lambda (9,1%); and 1pts with secretion of lambda light chain (9,1%). Highly elevated Beta2 microglobulin (>3mg/l) was registered in 8pts (72,7%). Immunohistochemical stainings of bone marrow biopsy revealed high expression of VEGF and Ki-67 in 5/6 analyzed pts. The group consisted of 2pts (18,2%) with refractory disease and 9pts (81,8%) with relapsing myeloma. All the patients were heavily pretreated with at least three types of conventional chemotherapy (MP/VMCP, VAD, M2, Z-Dex). Thalidomide as monotherapy was administered in 7pts (63,6%) at median dose of 400mg/day (range 200-600mg/day). At the same dosage, in 4pts (36,4%) Thalidomide was used in combination with high doses of Dexamethasone (HD-Dexa 40mg/d, I-IVd, XV-XVIIId for 6 cycles).

Results: Median follow-up was 12m for both pts groups (range 3-15m). The commonly observed side effects were: neuropathy in 6pts (54,6%), sleepiness in 4pts (36,4%), constipation in 1pts (9,1%) and skin dryness in 2pts (18,2%). No case of deep vein thrombosis was observed. Responses according to the EBMT/IBMTR guidelines in 7pts treated with Thalidomide as monotherapy were: 2 partial responses (28,6%), 3 minimal responses (42,8%), and 1 stable disease (14,3%). One patient had a progressive disease (14,3%). In the group of 4pts treated with Combination Thalidomide+HD-Dexa, distribution of responses was as follows: 1 partial response (25%), 2 minimal responses (50%) and 1 stable disease (25%). Median survival was 6,5months (range 3-15m) and one year survival was registered in 30% pts.

Conclusion: The activity of Thalidomide, used as a single agent or in combination with Dexamethasone, is significant in the treatment of patients with advanced myeloma. The optimal dose is still uncertain and although it has a significant side-effect profile, Thalidomide is a good prototype of a whole new class of anti-myeloma agents.

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LOW-DOSE THALIDOMIDE AND DEXAMETHASONE IMPROVES SURVIVAL IN MULTIPLE MYELOMA PATIENTS.
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Oral melphalan and prednisone has been the standard treatment of multiple myeloma for more than 30 years. High-dose chemotherapy and autologous stem cell transplantation improves clinical outcome. Relapses, however, constantly occur and resistance to chemotherapy is the major cause of death. The search for new/old drug has led to the selection of thalidomide. We evaluated the efficacy of low dose thalidomide (THAL) plus dexamethasone (DEX) in patients with relapsed or refractory multiple myeloma.

One hundred and twenty patients (median age 63), that had relapsed or were refractory to chemotherapy, started treatment with THAL 100 mg/day (continuous) and DEX 40 mg (days 1-4 of each month) between July 1999 and October 2001. Their clinical outcome was compared to a control group of 120 patients (median age 62) selected from relapsed or refractory patients treated with conventional chemotherapy (CC). Clinical characteristics were quite homogeneous in the two groups. Results were showed separately on patients receiving THAL-DEX or CC after one line of chemotherapy only (early stages of disease), and those treated after two or more lines of chemotherapy (late stages of disease). THAL-DEX regimen significantly improved outcome in patients treated after one line of chemotherapy only. Myeloma protein reduction 50%-100% was observed in 56% of the THAL-DEX group and in 46% of the CC group. The probability of progression-free survival (PFS) for 3 years was 38% in the THAL-DEX group and 6% in the CC group (p=0.0024). The estimated survival for 3 years was 60% in THAL-DEX group and 26% in CC group (p=0.0016).

Clinical outcome was similar in patients receiving THAL-DEX or CC after two or more line of chemotherapy. Myeloma protein reduction 50%-100% was observed in 46% of the THAL-DEX group and in 42% of the CC group. The probability of PFS for 3 years was 11% in the THAL-DEX group and 3% in the CC group (p=0.23). The estimated survival for 3 years was 22% in THAL-DEX group and 12% in CC group (p=0.45).

Most adverse effects were recorded as WHO grade I. 12% of patients displayed a grade II toxicity and 4% grade III. Constipation was relatively frequent (17% of patients). Sedation was recorded in 13% of patients, and 7% showed confusion. Tingling and numbness were observed in 11% of patients as grade I, in 8% as grade II. Tremors and incoordinations were present in 6% of patients and were generally mild. Discontinuation was required in 18% of patients, mainly due to neurologic toxicity (11%). In the earlier phases of disease, THAL-DEX was superior to CC. In the more advanced stages of disease THAL-DEX was equivalent to CC. This regimen is not myelotoxic, postpones the delivery of chemotherapy, and therefore the development of resistant disease, that is the major cause of death in the more advanced stages of myeloma.
Low Dose Thalidomide plus Dexamethasone Schedules Produce Equivalent Results with Less Toxicity than Higher Doses in Both Frontline and Relapse Myeloma

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Starting in October 1998, low dose thalidomide (50-100mg q.d. H.S.) was used for myeloma treatment with evaluation of response, time to progression, tolerance and overall survival. 83 patients are available for analysis. 36 relapse patients initially received low dose thalidomide alone, then had dexamethasone added; 47 patients received low dose thalidomide/dexamethasone: 21 as frontline and 26 at relapse. In the first 36 patients starting with thalidomide alone, there was dose escalation of thalidomide (50, 100, 150, 200). In the subsequent patients and in the dexamethasone combinations, the dosage was 50 or 100mg. Eleven of 36 patients started on thalidomide monotherapy had dexamethasone added. 6/11 (55%) responded (> 50% regression) with added dexamethasone. 13/21 frontline patients (62%) responded: 2 patients had < 50% regression; 3 had to stop therapy; 2 are too early to evaluate, and 1 developed progressive amyloidosis. No patients had significant coagulation/thrombotic problems. Of the 26 relapsed/refractory patients, 14 had > 50% regression (54%). A total of 13 patients had daily Biaxin (Clarithromycin) added either to improve partial or incomplete response and/or avert relapse. 9/13 (69%) had added benefit with induction of no detectable residual disease in 3 patients. However, because of enhanced steroid toxicity, the dexamethasone was switched to a 1 day per week schedule, also used as maintenance for other patients. The limiting toxicity for all patients was progressive peripheral neuropathy requiring thalidomide dose reduction or discontinuation of drug, despite Vitamin B6, alpha lipoic acid and other measures. One patient developed severe erythromelalgia after 2 years of thalidomide maintenance (50mg q.o.d.).

Currently, 22% of the original low dose thalidomide monotherapy responding patients are alive at > 4 years from start of therapy. It is too early to assess median time to first progression with low dose thalidomide/dexamethasone, but it will exceed 1 year.

Low dose thalidomide combination therapy is very effective and well tolerated. Durable remissions and long term survival are achievable. Further comparisons with higher dose schedules, with regard to response, toxicity and survival, are required.

ThaCyDex in relapsed/refractory multiple myeloma.

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Introduction: The association of Thalidomide, Cyclophosphamide and Dexamethasone (ThaCyDex) has been shown to be effective in a short series of relapsed/refractory multiple myeloma (MM). However, its real efficacy in large series of patients, has not been analysed more in some of these schemes cyclophosphamide was used i.v. and toxicity was rather high. In the present work we have evaluated the efficacy and the tolerability of ThaCyDex in oral formulations in a series of 59 patients.

Material and methods: The protocol included the administration of thalidomide at escalating doses (200 to 800 mg/day) according to its tolerability, daily oral cyclophosphamide (50 mg/day) and pulsed dexamethasone (40 mg/day, four days every three weeks).

Results: With a median follow-up of 2 years, fifty-four patients were valuable for response, 5 patients were not valid to evaluate the response due to early rejection of therapy in two cases, two cases of thalidomide toxicity and one main protocol violation. At three months of therapy 47 patients (87%) responded to the therapy, including 29 cases (57%) with a >50% M-component reduction (two of them with a complete remission). Only 15 patients have progressed so far, giving a projected progression fee survival of 65% at 3 years, resulting in a projected overall survival of 55% at 3 years. Causes of dead were disease progression (n=10), infection (n=3), sudden death (n=2) and unknown (n=1). Event free survival was 61%. Adverse effects were moderate and generally well tolerated. Infection were recorded in six patients, five patients requiring hospitalisation for intravenous antibiotic therapy. No cases of thrombocytopenia grade >2 were noted. Other effects included grades ≤2 constipation (25%), somnolence (33%), dizziness (12%) or paresthesias (13%). In addition two cases of deep venous thrombosis and one embolism pulmonary were noted.

Conclusion: This study shows that ThaCyDex is a feasible and effective therapeutic approach for patients with relapsed/refractory MM.

Thalidomide Treatment for Relapsed Multiple Myeloma May Promote Progression of Secondary 5q- Myelodysplasia / Acute Myeloid Leukemia

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Introduction: Patients treated for symptomatic multiple myeloma (MM) carry a significant risk to subsequent myelodysplasia (MDS) or secondary acute myeloid leukemia (sAML) development. Prolonged preceding standard therapy with alkylating agents is the likely cause of MDS after autotransplantation (Govindarajan et al, 1996) with del(5q) being the characteristic cytogenetic abnormality (Amiel et al, 1999). It has been hypothesized that thalidomide may paradoxical effect concomitant MM and MDS in heavily pretreated patients (Badros et al, 2002).

Material and Methods: We treated 60 patients with primary refractory or relapsed MM with two to six monthly courses combining hyperfractionated cyclophosphamide (300 mg/m² IV over 3 h q 12 h x 6, days 1 – 5, total dose 1800 mg/m²) with pulsed dexamethasone (20 mg/m²/d PO, days 1 – 4, 9 – 12, 17 – 20) and once daily thalidomide at individually escalating doses (100 to 400 mg/d) depending on tolerability (HyperCDT). Responding patients were maintained on daily thalidomide and monthly dexamethasone pulses. All patients were recommended pretherapeutic bone marrow examinations for cytology and cytogenetics (conventional karyotyping and interphase FISH studies).

Results: 4/44 patients (9%) with MM in relapse and prior myeloma therapy > 50 months revealed cytologic evidence of MDS/sAML two to four months after study entry on bone marrow specimens obtained for prolonged refractory cytopenia after chemotherapy. Three of four patients had been pretreated with HyperCDT. 8/13 patients (62%) with sAML did not respond to HyperCDT and all relapsed over 2 months after the last cycle of HyperCDT. Of these, 3/8 patients received conventional chemotherapy and 5 patients were referred to autologous transplantation.
with oral melphalan/prednisone during the early 90s. In retrospect, these three patients had a del(5q) chromosome abnormality besides a complex aberrant karyotype already at study entry. AML was the cause of death in these patients 3, 4, and 9 months after AML diagnosis. In the fourth patient, the only pretreatment consisted of a VAD-induction followed by two high-dose therapies with melphalan and autologous transplants. This patient had a normal karyotype, when refractory cytopenia with multilineage dysplasia was diagnosed. Twenty four months later still on thalidomide maintenance treatment he had progressed to refractory anemia with excess of blasts with his myeloma remaining in remission. In all four patients with MDS/AML, neither myelodysplasia, nor an excess of myeloblasts were detectable on cytologic evaluation of the bone marrow specimens obtained at study entry when plasma cell infiltration of the bone marrow was predominant. None of the remaining 40 patients with relapsed MM and informative pretreatment karyotype had evidence of cytogenetic abnormalities specific for MDS/AML.

Conclusion: Despite published data indicating efficacy of thalidomide in subsets of patients with MDS or AML (Zorat et al, 2001; Steins et al, 2002; Strupp et al, 2002), our observations suggest that thalidomide, at least in combination with dexamethasone and cyclophosphamide, does not control or even adversely affects concurrent MDS/AAML with 5q- abnormality. Cytogenetic screening by conventional karyotyping or FISH with special regard for this aberration is warranted prior to start of therapy. Use of thalidomide should be considered with caution in patients with suspicious cytogenetic abnormalities.

328 Oral hyperfractionated cyclophosphamide and intermittent thalidomide-dexamethasone (pulsed CTD) for previously treated patients with multiple myeloma.

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Thalidomide is an oral agent with significant activity in one-third of patients with refractory myeloma. However, long term continuous administration of thalidomide can be associated with significant side effects such as deep vein thrombosis and peripheral neuropathy. Furthermore, it is not clear whether continuous administration of thalidomide is necessary for its antimaloma effect. We performed a phase II study with a combination which was based on the intermittent administration of thalidomide. Materials and Methods: Fifty-three patients, median age 64 years (25%-70 years) with previously treated, primary refractory (27%) or relapsed (73%) myeloma, (32% had previously failed high dose chemotherapy), received oral cyclophosphamide 150 mg/m2 every 12 hours before meals on day 1 – 5, thalidomide 400 mg p.o. in the evening on days 1 to 5 and 14 to 18 and dexamethasone 20 mg/m2 in the morning after breakfast on days 1 to 5 and 14 to 18 (CTD). The CTD combination was repeated every 28 days for three courses. Subsequently responding patients were scheduled to receive maintenance treatment with monthly courses of CTD administered only for the first five days of each month. Results: On an intention to treat basis 32 patients (60%) achieved a partial response with a median time to response of 1.5 month. Among the 43 thalidomide-naïve patients, 67% responded. Three of the 10 patients who were previously treated with thalidomide and dexamethasone responded to CTD. Three of the six patients with extramedullary disease responded to the regimen. Toxicities were mild or moderate and the cumulative incidence of deep vein thrombosis and peripheral neuropathy was 4% and 2% respectively. The median time to progression for responding patients was 12 months and the median overall survival for all patients was 17.5 months. Conclusion: The oral, outpatient, pulsed CTD regimen is associated with significant activity in patients with previously treated multiple myeloma. The incidence of deep vein thrombosis and peripheral neuropathy appears to be lower than expected when thalidomide is being administered on a continuous basis. Low serum albumin and high levels of serum LDH were associated with shorter time to progression. A Cox regression analysis indicated that high serum LDH and impaired performance status were associated with poorer survival.

329 Doxil, Vincristine, Decadron and Thalidomide (DVd-T) for Relapsed/Refractory Multiple Myeloma (RMM)

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Dv is an effective and well tolerated regimen in newly diagnosed MM pts. However, in RMM pts only 22% and 5% of the pts achieved ≥50% and ≥90% reduction in the M-Protein respectively. The pts whom achieved the >90% decrease in the M-Protein on DVd had a durable response. Thal/Dex in a similar group of pts results in 60% overall response with rare cases achieving ≥90% reduction in the M-protein. Biologically Thalidomide has a direct anti myeloma effect in addition to its ability to modulate integrins. This interrupts the interaction between the myeloma cell and the bone marrow stroma resulting in a significant decrease in the supportive cytokine environment rendering the myeloma cell vulnerable and sensitized to different chemotherapeutic agents. Study objectives are to evaluate the role of Thalidomide in increasing the rate as well as the quality of the response to DVd in addition to assessing the tolerability of the combination in RMM. 45 RMM pts are currently enrolled. Median age is 63.5 years; PS is ≤3. Mean β2M, and albumin are 6.6, & 3.2 mg/dl respectively. On day 1 of each cycle Doxil was given at 40 mg/ m2 IVPB; Vincristine at 2 mg IVP & Decadron at 40 mg PO daily X 4 days. Thalidomide was started at 50 mg a day, to be increased by 50 mg a day every week to the maximum tolerated dose & not to exceed 400 mg a day. DVd was repeated every 4 weeks, for a minimum of 6 cycles & 2 cycles after best response. Thereafter pts were maintained on prednisone 50 mg every other day and the maximum tolerated dose of Thalidomide until disease progression. Response was assessed according to SWOG criteria. However, for complete remission (CR) we required in addition to the standard SWOG criteria, the bone marrow to show polyclonal plasma cells by immune staining. Following an increased incidence of neutropenia, infections, oral herpes simplex activation, and Deep venous Thrombosis (DVT’S) in the first 20 patients; the protocol was amended to initiate all pts on prophylactic amoxicillin 250mg BID, acyclovir 400 mg BID until completion of chemotherapy, GM-CSF or G-CSF if the total WBC was less than 5000/ L on day 1 of therapy, and Aspirin 81mg daily. Overall response (>50% reduction in the monoclonal protein occurred in 34 pts (76%). Complete remission (Disappearance of the M-protein by immune fixation, and the presence of polyclonal plasma cells in the bone marrow by immune staining) is achieved.
in 5 pts (11%), M protein decreased by ≥90% - < CR, ≥75% - < 90%, ≥50% - <75% in 15 (33%), 4 (9%) & 10 (22%) pts, respectively. 4 pts had stable disease (≥25% - <50%) and 7 pts progressed on therapy. Median time to initial response (i.e. at least a ≥50% reduction in M- and/or urine protein levels) was 1.8 months (range 0.9-5.6 months). Median time to best response was 4.0 months (range 0.9-6.3 months). Toxicity prior to amending the protocol included 4 cases of pneumonia, 1 septic arthritis, 2 GI bleeds, 18 of these first 20 pts had Grade 3/4 neutropenia, and 10 pts with thrombocytopenia. Following the above mentioned precautions these cytopenia related complications has been reduced to only 1/15 grade 3 neutropenia and fevers. DVT's occurred in 5 of the first 20 pts, and after the implementation of low dose aspirin 2/25 pts developed DVT, one of which was below knee, requiring no specific therapy or treatment course modifications. Grade 3 paraesthesia was noted in 20% of the pts necessitating Vincristine dose reduction or eliminating Vincristine from the regimen. DVT-d in RMM following supportive care modifications is well tolerated. Compared to historical data in a similar pt population receiving Dvd or Thal/Dex, the response rate (76% >50% reduction in M-protein) and the quality of response (45% near CR and CR) is significantly enhanced by combining both regimens, and reducing steroids.

330 Hyperfractionated Cyclophosphamide in Combination With Pulsed Dexamethasone and Thalidomide (HyperCDT) in Primary Refractory or Relapsed Multiple Myeloma

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Introduction: We report the final results of a phase II trial evaluating the role of thalidomide in combination with puls dexamethasone and hyperfractionated cyclophosphamide for remission induction and maintenance therapy in patients with advanced multiple myeloma (MM).

Material and Methods: 60 patients with primary refractory or relapsed MM were treated with 2 to 6 monthly courses of hyperfractionated cyclophosphamide (300 mg/m² IV over 3 h q 12 h x 6 doses, days 1 – 3, total dose 1800 mg/m²) combined with pulsed dexamethasone (20 mg/m²/d PO, days 1 – 4, 9 – 12, 17 – 20) and once daily thalidomide at individually escalating doses ranging from 100 to 400 mg/d depending on tolerability. Responding patients were maintained on daily thalidomide and monthly dexamethasone pulses. 16/60 patients in this study had primary refractory disease; 44/60 patients had relapsed after high-dose melphalan with autotransplant (29/60 untested relapse, 15/60 resistant relapse). Patient characteristics included median age 58 years; age > 60, 43 %; B2M > 3.0 mg/L, 56 %; CRP > 3.0 mg/L, 14 %; and prior standard therapy > 12 mo, 72 %.

Results: We observed 4 % CR, 68 % PR, and 12 % of patients with a minor response; total response rate 84 % (EBMT/IBMTR/ABMTR criteria). Based on an intention-to-treat analysis event-free survival was 11 months with an overall survival of 19 months. No single disease characteristic at baseline was predictive for response or survival at 1 years, however cytogenetics and plasma cell labeling index were not available for all patients. Following chemotherapy, 67 % of patients experienced grade 4 neutropenia during at least one treatment cycle; 9 % grade 4 thrombocytopenia. There were 26 % grade 3/4 infections; two early deaths due to neutropenic infection after the first treatment cycle. Presumably thalidomide related side effects included neuropathy (40 % grade 2; 16 % grade 3), 17 % grade 2 constipation, 5 % edema, 5 % bradycardia, 3 % skin reaction, and 5 patients (8 %) with deep vein thrombosis (DVT). DVTs were not related to known thrombophilic risk factors.

Conclusion: Although highly effective in relapsed or refractory myeloma, efﬁcacy and tolerability of the HyperCDT regimen will have to be compared to thalidomide/dexamethasone combined with oral cyclophosphamide at an either continuous low-dose (García-Sanz et al, 2002) or hyperfractionated intermediate-dose schedule (Dimopoulos et al, 2001).

331 Discordant response or progression in patients with refractory myeloma treated with thalidomide based regimens.

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Response to treatment in secretory myeloma is most commonly assessed with the reduction of the paraprotein levels. This usually correlates with reciprocal reduction of the bone marrow plasma cells and the size of extramedullary sites. Thalidomide based regimens (TBR) are now widely used for the treatment of refractory multiple myeloma and have shown significant activity in newly diagnosed patients. In some patients, we observed discrepancies between the reduction of the paraprotein levels and the plasma cell infiltrate in the bone marrow and/or extramedullary sites after treatment with TBR. The purpose of this study was the assessment of the incidence and analysis of this phenomenon in all myeloma patients treated with TBR in our Institution.

Patients and methods: We studied responses and pattern of progression of all patients who received TBRs and had a follow up time of at least 6 months. Partial response (PR) was defined as at least >50% reduction of serum myeloma protein production and/or >90% reduction of Bence Jones protein excretion and minor response a >25% reduction of the serum myeloma protein. Relapse was defined as the earliest of >25% increase of myeloma protein from lowest level, new lytic bone lesions, marrow plasmacytosis to >10% or hypercalcemia.

Results: Between 7/99 and 7/02 we treated 93 patients with refractory or relapsed myeloma with TBR. Fifty-six patients (60%) achieved either partial or minor response. Three of them (3%) had at least 25% reduction of the paraprotein levels (1 had >50% reduction) but at the time of best response the bone marrow was still infiltrated with plasma cells (PC) [from 25% (prior to treatment PC) → to 90% (post treatment PC), 85%→80% and 40%→50% in each of the 3 cases respectively]. Four additional patients (4%) after achieving a PR (3 of them with >75% reduction of serum paraprotein) which lasted between 5 and 7 months, relapsed with bone marrow plasmacytosis (all...
cases), soft tissue plasmacytoma (2 cases) and mediastinal lymphadenopathy (1 case), without increase of serum and/or urine monoclonal protein.

Conclusion: Our data indicate that after treatment with TBR in some patients with reduction of monoclonal protein the bone marrow plasmacytosis may persist. Furthermore some patients with both monoclonal protein and bone marrow response may progress in the bone marrow with or without extramedullary involvement but without a concomitant increase of paraprotein. If our data are confirmed, they may have practical implications for assessment of response and follow up of patients treated with TBR.

332 Preliminary Efficacy of a Phase III trial of Oblimersen Sodium (G3139, bcl-2 antisense oligonucleotide) combined with Dexamethasone and Thalidomide in Patients with Relapsed Multiple Myeloma

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Bcl-2 plays a major role in drug resistance in MM. In an attempt to overcome drug resistance and increase remission rate in relapsed/refractory MM patients, we administered G3139 to patients with Relapsed Multiple Myeloma

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Thalidomide (Thal) is a promising new drug for relapsed/refractory multiple myeloma (MM). Literature data show prolonged survival in patients (pts) treated with Thal, but the optimal schedule, dosage and association with other drugs is under investigation. The association of Thal with dexamethasone (Dex) seems to be highly effective in advanced MM, including pts previously resistant to Dex and chemotherapy. Between January 2001 and January 2003 thirty pts (8 females and 22 males) with relapsed/refractory MM were enrolled in an open label trial of oral dose Thal (100-200 mg/day) plus Dex (40 mg, days 1-4, every month). The main pre-Thal treatment characteristic were the following: median age 64 years (range 54-80); median B2M 3.3 mg/L (range 1.06-14.2); median bone marrow plasma cell infiltration 30% (range 4-90). Eight pts were previously treated with autologous stem cell transplantation, while 22 pts had received more than two chemotherapy regimens.

Median time from MM diagnosis to Thal treatment was 30 months (range 5-124). A total of 28 pts were evaluable with a median follow up of 6 months (range 1-20), while 2 pts were not evaluable because of early withdrawal (<15 days) due to progression and intolerance, respectively. Twenty-two pts (78.6%) responded to the therapy, including 16 cases (57.2%) with a >50% M-component reduction, while 6 pts had disease progression. Median time to response was 2 months (1-3). Projected EFS and OS at eleven months were 28% and 63%, respectively. As to side effects, treatment was discontinued in 6 pts due to neurotoxicity (4 cases) and deep venous thrombosis (2 cases). Otherwise, low dose Thal plus Dex was well tolerated and only minor toxicities were recorded in six pts (mild somnolence and constipation). Data analysis did not show any correlation between pre-treatment characteristics and treatment response.

In conclusion, our results confirm that low dose Thal plus Dex is highly effective and feasible in pts with relapsed/refractory MM. Further studies and a longer follow-up are warranted to evaluate the duration of the favorable responses, the effect on survival and possible long-term side effects.

333 Low dose thalidomide plus dexamethasone for relapsed/refractory multiple myeloma: a single center experience.

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Thalidomide (Thal) is a promising new drug for relapsed/refractory multiple myeloma (MM). Literature data show prolonged survival in patients (pts) treated with Thal, but the optimal schedule, dosage and association with other drugs is under investigation. The association of Thal with dexamethasone (Dex) seems to be highly effective in advanced MM, including pts previously resistant to Dex and chemotherapy. Between January 2001 and January 2003 thirty pts (8 females and 22 males) with relapsed/refractory MM were enrolled in an open label trial of oral dose Thal (100-200 mg/day) plus Dex (40 mg, days 1-4, every month). The main pre-Thal treatment characteristic were the following: median age 64 years (range 54-80); median B2M 3.3 mg/L (range 1.06-14.2); median bone marrow plasma cell infiltration 30% (range 4-90). Eight pts were previously treated with autologous stem cell transplantation, while 22 pts had received more than two chemotherapy regimens.

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334 Salvage therapy with Cyclophosphamide, Dexamethasone and Thalidomide (CDT) is a well-tolerated and effective regimen in advanced relapsed/refractory myeloma

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The combination of Thalidomide and Dexamethasone produces a response rate of 30% to 40% in refractory myeloma and 80% to 90% in de novo disease. We have carried out a pilot study to assess the efficacy and tolerability of the combination of cyclophosphamide, dexamethasone and thalidomide (CDT) in myeloma patients who have previously failed multiple treatment lines.

Patients and Methods: Twenty-three patients (16 Males, 8 Females, mean age 55 years, range 37 to 70 years) were treated with CDT between March 2002 and February 2003. The average
time from diagnosis was 3.7 years and the mean number of previous treatment lines was four. Seven patients had previously undergone low intensity allogeneic stem cell transplantation followed by donor lymphocyte infusions, 13 had received autologous stem cell transplantation and 5 patients had only had chemotherapy. Thalidomide was started at a daily dose of 50 mg and escalated up to the mean dose of 200 mg/day. Dexamethasone was administered at 40 mg p.o daily for four days monthly, and Cyclophosphamide was given at the target dose of 400mg/m2 p.o weekly. Thirteen patients with neutropenia at start of treatment were commenced on G-CSF support. Eight patients who were transfusion dependent were commenced on Erythropoietin.

Results: Nine patients developed infections (8 developed chest infection and one patient developed an abscess, all successfully treated with iv antibiotics). All patients with no contraindications for anticoagulation were on prophylaxis with warfarin 1mg daily. No patients developed thrombocytopenia or thromboembolic episodes. Thyroid function was monitored and remained normal for euthyroid patients. Two patients who were on thyroxine had to have the thyroxine dose increased during CDT treatment. Fourteen patients experienced thalidomide related toxicity (grade I neuropathy –sensory, grade II depressed level of consciousness and grade II constipation). CDT was administered for up to 6 courses in responding. Fourteen patients (61%) had very good and rapid response to treatment with >90% reduction of paraprotein at a mean of 2.5 months. These patients completed six courses and remained on thalidomide maintenance 100mg with sustained response at median time of 5 months follow-up (range 3 to12 months). Three patients (13%) had minimal response with at least 25% reduction of paraprotein, 5 patients (22%) showed no change with stable disease and one patient died from disease progression. All patients with severe anaemia became transfusion independent following response. Six patients with severe renal impairment received treatment with no increased toxicity and 3 showed marked improvement in renal function. Two of the good responders have successfully undergone a second stem cell autograft.

Conclusion: CDT is a well-tolerated oral regimen, which has high activity in relapsed/refractory myeloma. These preliminary results are encouraging considering the prognosis and therapeutic options available for this group of patients. Treatment related toxicity was low and a significant proportion can be salvaged. Longer follow-up and more patients are required to determine the true value of this approach.

335 Thalidomide in combination with Dexametasone and Cyclophosphamide for relapsed/refractory multiple myeloma
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INTRODUCTION: The antimumoral effect of thalidomide (Thal) alone has been demonstrated in several clinical trials. Recent data indicate that Thal can increase the therapeutic effect of chemotherapy and might be able to overcome drug resistance. Response rates of 25% with Thal used as a single agent, and up to 75%, when used in combination with other agents, have been observed. The optimal schedule, dosage and association with other drugs is still not established. MATERIAL AND METHODS: Between October 2001 and December 2002 twelve patients (pts) (10 M, 2 F) with relapsed/refractory MM were enrolled in an open-label trial of oral low dose Thal (100-200 mg/day) plus Dex (40 mg, day 1-4, every month) and cyclophosphamide (500 mg iv/week). Main pre-treatment characteristics were the following: median age 66 years (range 54-74); median B2M 3.3 mg/L (range 1.2-14.2); median bone marrow plasma cell infiltration 32.5% (range 4-80). Median time from MM diagnosis to treatment was 60 months (range 6-132). All pts were heavily pre-treated. In particular, 9 pts received a median of 3 pre-treatment chemotherapy regimens (range 1-5) and 3 underwent autologous stem cell transplantation. In addition 11 pts showed disease progression during previous treatment with Thal alone or combined with Dex. The EBMT/IBMR/ABMT criteria were used for definition of response. RESULTS: Adverse effects were moderate (grade <or=2). No cases of thrombocytopenia grade >or=2 were observed, while 2 pts experienced neutropenia requiring supportive treatment with G-CSF. Other side effects included grade <or=2 constipation (30%), somnolence (35%) or dizziness (9%). No cases of deep venous thrombosis were observed. With a median follow-up of 3 months (2-10), 12 patients were evaluable for response: 10 (75%) responded to this association therapy, including 5 (42%) with a >50% M-component reduction; while 3 (25%) showed disease progression. At present 8 pts are alive and maintaining the response for 1-8 months; while 4 pts died due to disease progression, including one progressed after a 5 months response. CONCLUSION: These results show that the Thal plus Dex and cyclophosphamide combination is active and feasible in heavily pre-treated multiple myeloma pts, including those relapsing after Thal+Dex therapy. Further studies and a longer follow-up are warranted to evaluate the duration of favorable responses, the effect on survival and possible long-term side effects.

336 Salvage treatment with thalidomide, dexametason and zoledronate in advanced multiple myeloma: the pattern and the outcome of relapsed/refractory disease.
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Thalidomide (thal) plus dexamethasone (dex) is an effective-proven salvage treatment in MM, allowing to obtain a response in an about one third of pre-treated patients. Zoledronate is an active agent in bone disease and it’s maybe provided of direct anti-myeloma effects. This combined therapy represent the most innovative current approach in the management of advanced MM.

However, the illness relapse is therefore unavoidable and the patients, having few therapeutic options, can only benefit of supportive measures. The pattern and the outcome of relapsed/refractory disease in 21 consecutive patients (8 M / 13 F, median age 74 yrs) with pre-treated advanced MM receiving a salvage treatment with thal, dex and zoledronate, are reported. Thal was given at a median dose 150 (100-400) mg/day, dex and zoledronate were respectively given at the dose of 40 mg/day LV or P.O. for 4 days and of 4 mg as single LV. infusion both every 4 weeks. Eight out of 21 (38%) showed a stable disease,5 (24%) progressed and 8 (38%) responded. Out of the 8 responders, one deceased without relapse (congestive heart failure) and 7 relapsed. Median duration of
response was 8 (2 – 12) months. To date, 4 patients are alive: 3 with stable MM and 1 with progressive disease. The median overall survival was 8 months (1 – 16).

We have analysed the clinical pattern of relapsed/progressed disease in our series. Out of the 7 relapses, 3 cases presented untreated and very extended pulmonary (2) and frontal bone (1) plasmocytes.

The remaining 4 patients presented progressive increase of paraprotein levels associated with plasmacytic BM infiltration and new osteolytic lesions. The extramedullary manifestation were treated with radiotherapy and in all relapsers a weekly dose (500 mg) of cyclofosfamide was added to the current therapy. All relapers died after 2 (1 – 3) months.

Out of the 5 patient with disease progression, 4 presented the typical haematological features and 1 a large plasmacytoma, extended from the 5th lumbar spine to the iliac region. This patient, alive from 15 months, received radiotherapy, weekly cyclofosphamide and regular courses of dex, as above reported, achieving a partial control of disease, lasting from 5 months. Median survival after progression was 2 (1 – 15) months. Although the small number of patients, the occurrence of extramedullary disease, found in 4/12 (33%) patients, suggests a role of a resistant and more aggressive clone, who has lacked the regulatory adhesion molecule-mediated mechanisms, causing metastasis to distant sites, as the lung, maybe favoured by its fine microcirculation.

Our experience, outlining the poor prognosis of the relapsed/progressed MM patients receiving the reported salvage therapy, suggests the need of further studies on the efficacy of new drugs in modifying the clinical history of the advanced MM.

338 Clarithromycin, Dexamethasone and low dose Thalidomide (CDT) is effective therapy in relapsed/refractory myeloma; a Phase II study and quality of life survey.

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We have previously shown that Clarithromycin has a modest anti myeloma effect1 and more recently a combination of Clarithromycin, Dexamethasone and Thalidomide has been shown to have significant activity in patients with myeloma2. We report a very high response rate in a phase II trial of this combination in which the object was to minimise the doses of both Thalidomide and Dexamethasone. Sixteen patients (10 M, 6 F) with relapsed (12) or refractory (4) myeloma, age range 52 – 83, from whom informed consent was obtained, were given Clarithromycin 250mg bd continuously, Dexamethasone 10 mg daily for 4 days, once every 4 weeks and Thalidomide 50 mg nocte. An additional 4 days of 10 mg of Dexamethasone was given on days 15–18 only. Dose escalation of Thalidomide to 200mg by 50mg increments was permitted but only 3 patients reached 150mg and 3 patients 100mg. All patients received IV bisphosphonates once every four weeks. Results: Three patients had complete remission, 2 very good partial response, 8 partial response and 2 minimal response and one patient progressive disease. Toxicities TID therapy was stopped because of severe somnolence. Of seven patients protein after 1 cycle of TID therapy but in two patients it was to 75 years) and five were male. In myeloma subtypes, five were IgG, two were IgA and two were Bence Johns protein type. All of patients were relapsing despite combination chemotherapy contained high dose dexamethasone and two or more treatment regimens were administered before therapy. One patient was highly aggressive relapsed after tandem autologous peripheral blood stem cell transplantation.

Results: All of nine patients achieved over 25% reduction of M-protein after 1 cycle of TID therapy but in two patients it was stopped because of severe somnolence. Of seven patients evaluable for response after 3 cycles of therapy, five patients achieved 50% reduction, one patient 25% reduction of M-protein and one patient progressive disease. Toxicities TID therapy was equally effective in patients with or without prior-resistance to dexamethasone-based regimes. These data suggest that TID is a feasible and promising therapeutic approach for advanced multiple myeloma patients.

337 The combination therapy of thalidomide, incadronate and dexamethasone (TID) for relapsed or refractory multiple myeloma

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Introduction: Thalidomide has proven to be a useful drug for treatment of refractory and relapsed MM patients and the efficacy has been reported up to 35% in several clinical trials. Thalidomide has been reported to restore the sensitivity of myeloma cells to other drugs and to enhance the anti-myeloma activity of dexamethasone, and in clinical study the combination of thalidomide with dexamethasone have been more effective than thalidomide alone. It is reported that nitrogen-containing bisphosphonates, such as incadronate, have direct anti-myeloma effect, and inhibit the mevalonate pathway and prevent post-translational prenylation of GTP-binding proteins for example Ras. Based on these observations, we investigated the combination effect of thalidomide, incadronate and dexamethasone (TID therapy) as clinical phase 2 trial for relapsed or refractory MM patients, in order to assess its efficacy and toxicity.

Patients and methods: Nine patients with relapsed or refractory MM were treated after informed consent was obtained from patients. The protocol scheduled the administration of thalidomide at doses from 100mg to 300mg/day, incadronate at 10mg/day iv weekly and dexamethasone at 12mg/day four days every three weeks. Patients’ median age was 69 years (range: 52 to 75 years) and five were male. In myeloma subtypes, five were IgG, two were IgA and two were Bence Johns protein type. All of patients were relapsing despite combination chemotherapy contained high dose dexamethasone and two or more treatment regimens were administered before therapy. One patient was highly aggressive relapsed after tandem autologous peripheral blood stem cell transplantation.

Results: All of nine patients achieved over 25% reduction of M-protein after 1 cycle of TID therapy but in two patients it was stopped because of severe somnolence. Of seven patients evaluable for response after 3 cycles of therapy, five patients achieved 50% reduction, one patient 25% reduction of M-protein and one patient progressive disease. Toxicities TID therapy was equally effective in patients with or without prior-resistance to dexamethasone-based regimes. These data suggest that TID is a feasible and promising therapeutic approach for advanced multiple myeloma patients.

References:
1 Morris et al, J Medical Oncology, 2001;18: 79-84
2 Coleman et al, Leukaemia & Lymphoma, 2002; 43: 1777-1782
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Thalidomide, Clarithromycin and Bisphosphonates in a Unique Maintenance Chemotherapy in Patients with Multiple Myeloma
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Introduction: The majority of multiple myeloma patients relapse within 3 years. By using less toxic agents as maintenance we hope to improve the survival rate.

Methodology: All patients with multiple myeloma from 1999 until present, once induced in remission, were maintained on Thalidomide 200mg/day p.o. Bisphosphonates IV. every fourth week. Clarithromycin 500mg/day p.o. Both bisphosphonates were used in standard doses. The addition of Clarithromycin to Thalidomide has helped to change the pharmacodynamics of Thalidomide. Basically it will interfere with its excretion.

Results: 22 patients were eligible. There were 12 males and 10 females. There was an average age of 62 years for the males, and 60 years for the females. Eight patients have been taking this combination for greater than 3 years without any adverse effects. The longest follow-up has been 4 years. Three patients were dropped, 2 due to neuropathy and 1 due to syncrype. 3 patients have died, 2 with unrelated causes, and 1 with myeloma. Of 19 patients, including the 3 who died, only one patient has active disease. All others are in remission with a median of 18 months.

Conclusion: The Thalidomide, Clarithromycin, and Bisphosphonates are an effective combination in patients with multiple myeloma in regard to keeping these patients in remission.

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Thalidomide and Celecoxib for patients with multiple myeloma (MM) – a promising combination
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Introduction: Thalidomide (Thal) has proven efficacy in the management of MM. However response rate (RR) when used as a single agent is low. Thal has been shown to downregulate COX-2 mRNA, and COX-2 inhibitors have anti-angiogenic effects. Commencing August 2001, we performed a prospective Phase-II open-label, multi-centre(n=7) study combining Thal and Celecoxib (Thal/Cel) in patients(pts) with relapsed/refractory MM. The primary objective was to determine the RR and compare this with our previous trial using single agent Thal +/- interferon (Mileshkin et al, Blood in press). Further objectives were to determine event-free (EFS) and overall survival (OS), as well as the toxicity profile.

Methods: Eligible pts had relapsed or refractory MM, platelets ≥50 x 109/L and serum creatinine ≤ 1.5 x upper limit of normal. Pts commenced Cel 400mg bid plus Thal 200 mg/d. After 14 days, Thal was escalated by 200 mg/d every 14d to a maximum of 800 mg/d, or an individually maximum tolerated dose (iMTD) ≤800mg. The combination was to continue while tolerated or until progressive disease(PD). Cel was dose reduced for oedema or elevations in creatinine. Pts continue on Thal alone, if Cel is ceased due to toxicity. The trial is ongoing.

Results: An interim analysis was performed in February 2003. 39 pts (17 females, 22 males) with a median age 68 (range:43-84) had completed a median 20 weeks treatment (range 1-67). Median follow-up was 8 months (2-17). Median WHO performance status was 1 (0-2) with median prior chemotherapy regimens of 2 (range:1-8). At study entry, 15 pts (38%) had an elevated B2M, elevated CRP (n=20:51%), elevated LDH (n=5:13%), ANC <1.5 x 109/L (n=7:18%), Hb <100 g/L (n=30:77%). Median iMTD of Thal was 400mg/d and mean daily dose of Cel was 446mg. Responses for all pts were: PR 36%, stable disease 56%, progressive disease 3%, not evaluable 5%. Estimated predicted RR at 6 months was 26% (SE 7%). Median EFS and OS have not been reached, with 25 pts (64%) alive without progression at the time of analysis. Estimated 12 month EFS was 57% (95%CI:42-78) and 12 month OS was 63% (95%CI:47-86) for all pts. By comparison, our previous trial of Thal ± interferon reported an ORR of 28%, with estimated 12 month EFS of 23% (95%CI:14-34) and 12 month OS of 56% (95%CI:44-67). At the time of analysis, 22 pts had ceased Thal due to PD in 45% and toxicity in 23%. 22 pts had ceased Cel, due to toxicity in 50%. Important grade 3/4 toxicities seen above expected of single-agent Thal were elevated creatinine (10%) and oedema (5%). There were no treatment-related deaths. Less than expected constipation was seen.

Conclusions: These preliminary results suggest Thal/Celeb is tolerable in the majority, but elevations in creatinine and oedema are important additional toxicities over Thal alone. Although this was not a direct comparison with the Thal +/- interferon combination in our previous trial, given the promising RR (36% vs 28%) and predicted 1 year EFS (57 vs 23%), the trial will continue to accrue to 65 pts.

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Treatment of Relapsed or Refractory Multiple Myeloma with Thalidomide, Dexamethasone and Clarithromycin.
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Background: thalidomide is an effective treatment for relapse multiple myeloma leading to 30% of responses. This rate of responses may be improved by the combination with dexamethasone (DXM). Clarithromycin also reduces serum level of IL6, which promote the proliferation of plasma cells. In order to confirm the benefit of the combination of these three agents we prospectively treated relapsed or refractory multiple myeloma pts, with thalidomide combined to DXM and clarithromycin.

Materials and methods: Between June 2001 and January 2003, 12 pts were enrolled in this study. The median age was 65 years (range 49-88). There were 3 primary refractory diseases, 3 refractory relapses and 6 relapses off treatment. Four pts were previously treated and progressed on thalidomide. The median number of line treatments was 3.3 (2-7). Thalidomide was started at 100mg/day and progressively increased every two weeks according to the tolerance of pts. DXM was administrated at the dose of 20mg/m2 at the day 1-4, 9-12, 17-20 during the first month and then four days/month. Clarithromycin was give at 500 mg twice daily continuously and dropped to 250mg in case of toxicity. The trial is ongoing.

Results: all patient (100%) had a at least a reduction of 25% of the MP. Complete remission, defined as a disappearance of MP and a normalisation of bone marrow, was seen in 5 pts (42%). Major response (75-99% reduction of the MP) was observed in 1
342 DT-PACE is highly effective in plasma cell leukemia

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Plasma cell leukemia (PCL) is a rare form of plasma cell neoplasm with poor prognosis. PCL evolving from long standing multiple myeloma (MM) is considered a terminal event for refractory/refractory MM and is characterized by a fulminant course and is frequently not responsive to any treatment modality. The optimal regimens for the treatment of PCL have not been firmly established. Recently, combination therapy DT-PACE has been shown to be highly effective in patients with relapsed/refractory multiple myeloma. We report here two cases of PCL treated successfully with DT-PACE. In both cases, PCL developed during the course of treatment of advanced MM. #1 with 4 cycles of VAD (first line therapy) and #2 with 5 cycles of thalidomide and Decadron (third line therapy), to which both patients had initial at least partial response. Prior to the treatment with DT-PACE, patients showed progressive disease with circulating plasma cells meeting criteria for PCL, chromosome 13 abnormalities, and elevated beta2-microglobulin. In both cases, patients cleared circulating plasma cells within 2 weeks of the first cycle of DT-PACE. Both patients achieved near CR after 2 cycles of DT-PACE. Overall, the treatment was well tolerated. Patient #1, who presented with high LDH, pneumonia, large bilateral plural effusions, and profound cytopenias developed early tumor lysis syndrome during the first cycle of therapy. Subsequent therapies consisted of allogeneic stem cell transplant (case #1) and autologous stem cell transplant. Both patients remain in remission. These results suggest that TD-PACE, which has established high efficacy in aggressive/refractory multiple myeloma, appears very effective in PCL. Moreover, the results support further previous promising outcomes of treatment of PCL with intensive chemotherapy.

343 Combination of Thalidomide, Cyclophosphamide, and Dexamethasone in refractory-relapsed multiple myeloma

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Cyclophosphamide (CTX) is a drug with activity against myeloma and, when used at low doses, it has anti-angiogenic activity (Browder T, Cancer Res 2000; 60: 1878). For these reasons we elected this drug for a combination with Thalidomide (THAL) and Dexamethasone (DEX) in refractory multiple myeloma. Forty consecutive patients were included in this study. Seven were relapsing and 33 were resistant or progressing after previous treatment. Time elapsed between diagnosis and entering this study was 23 months (range 2-70). At time of starting this protocol, five patients were at stage I, 3 at stage II and 32 at stage III. Median percentage of bone marrow plasma cells infiltration was 50% (range 10-100), median Hb value was 10.2 g/dl (range 6.6-13.7), median WBC count 2.9 x10^9/l (range 6.6-7.2), median PLT count 159 x10^9/l (range 48-347). DEX was given at the fixed dose of 40 mg/die for 4 days every month, while THAL at 200 mg every evening and CTX 100 mg every morning continuously. Eight patients did not receive DEXA because of diabetes and in 6 additional patients it was discontinued because of side effects. Nine patients discontinued treatment with THAL (five of them definitely) because of skin rash, peripheral neuropathy (4 patients), poor compliance in a patient affected by Alzheimer’s disease, pneumonia, FUO, dizziness. CTX was temporarily discontinued in 23 patients for leukopenia (20 patients), nausea, hematuria, Alzheimer’s disease. Other side effects of the combination included constipation, nausea, somnolence, asthenia, fever diarhoea. Response to treatment was evaluated by the percentage of reduction of the monoclonal component (MC). Two patients were not evaluable: one died for progression of disease a few days after starting treatment, and the other refused treatment because developed a skin reaction to THAL two days after starting treatment. Among the remaining 38 patients, 3 (8%) were considered as non responsive. Eight patients (21%) had a reduction of MC < 50 %, 8 patients (21 %) < 75 %, and 19 patients (50 %) > 75%. Of the latter, 8 (21 %) achieved a complete response (100 % reduction of MC) and in these patients bone marrow evaluation showed the disappearance of plasma cells infiltration. Median time between start of treatment and evaluation of response was 7 months (range 2-26). No differences of response were observed among different Ig isotypes. After a median follow up time of 13 months, 12 of the responding patients (32 %) have experienced a relapse after a median time of 5 months (range 2-19) from evaluation of response. In conclusion, the combination of THAL-CTX-DEX seems to be a very active and well tolerated scheme against refractory myeloma. The high rate of discontinuation of CTX should be evaluated in the light of the poor bone marrow reserve of patients included in this study (median WBC 2.9). The duration of treatment seems to be a crucial point, since some patients have relapsed soon after stopping treatment. Future studies are also necessary to evaluate the weight of CTX in this combination and to explore the possibility of using this treatment as first line therapy.
COMBINATION OF THALIDOMIDE, DEXAMETHASONE AND ZOLEDRONATE FOR THE TREATMENT OF MYELOMA PATIENTS RELAPSED AFTER FRONTLINE AUTOLOGOUS STEM CELL TRANSPLANTATION


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Autologous peripheral blood stem cell transplantation (PBSCT) is currently considered the first line therapy of choice for a large number of patients affected by multiple myeloma (MM). However, PBSCT does not cure MM and relapse is the rule. Thus, strategies for treating MM patients relapsed after PBSCT are strongly awaited. Thalidomide induces significant responses in about one third of patients with pre-treated MM. Even higher rates of response have been reported with the association of thalidomide and dexamethasone, another agent with well known efficacy in MM, due to the possible synergistic activity of these drugs. Zoledronate is a new generation bisphosphonate, which has been demonstrated to be active on bone disease in MM, but which also seems to exert a direct anti-myeloma effect. Based on these data, we performed a pilot study by administering thalidomide, in combination with dexamethasone and zoledronate, to 20 patients with MM (12 males and 8 females, mean age 59 years, range 36-68), who had relapsed after PBSCT performed as frontline treatment. Thalidomide was given at the dose of 200 mg/d per os, at bedtime, after a week in which the patients received only 100 mg/d, to test tolerance. Dexamethasone was given at the dose of 40 mg i.v. or p.o. for 4 days every 4 weeks. Zoledronate was administered at the dose of 4 mg every 4 weeks, as 15' i.v. infusion. The treatment was performed exclusively on an outpatient basis. Nineteen patients received at least 12 weeks of therapy. Somnolence, sedation, oedema, constipation and skin rash, alone or in combination, were the most relevant side effects observed, occurring in 9 patients. The reduction of thalidomide dose to 50-100 mg/d resolved adverse events in these cases. Two additional patients showed hypertension and hyperglycaemia, which disappeared after reduction of the steroid dose to 20 mg. Asymptomatic hypocalcemia was also observed in 6 patients and was corrected by substitutive therapy. No patient developed thrombotic complications, but one patient interrupted early the trial, due to severe pancytopenia. Among the 19 evaluable patients, four did not show any modification of hematological parameters after 12 weeks and stopped the treatment. Four additional patients showed progressive disease (in two cases after an initial moderate reduction of M-component) and were also considered unresponsive. The remaining 11 patients (57.8%) evidenced a significant response, according to conventional criteria. In particular, a reduction of M-component > 25%, > 50% and >75% was observed in 2, 6 and 2 out of patients, respectively, while one subject achieved complete remission, with complete disappearance of the paraprotein at immunofixation. Among remitters, two heavily anemic patients also interrupted their transfusional support. Accordingly to hematological response, a clear ameloration of bone pain and decrease of marrow plasma cell infiltration occurred. Median progression-free and overall survival are still not reached after 19 months from start of salvage therapy. Interestingly, in 3 patients progression-free survival was longer than that observed after PBSCT. Our data suggest that this possibly synergistic association may have significant efficacy and manageable side effects as salvage therapy in MM patients relapsed after PBSCT.

Cuadruple Maintenance Treatment (Bisfosfonates + Interferon + Dexamethasone + Thalidomide) after Autologous Peripheral Blood Stem Cell Transplantion (APBSCT) in Múltiple Myeloma (MM): Preliminary Experience

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Introduction: The favourable results of APBSCT in MM, (high CR rate, low toxicity and longer event free survival and overall survival), are counteracted by the fact that relapses and progresses are inevitables even with consolidation regimens. Due to this circumstance, different strategies must be designed aiming to extend the anti-myeloma effect achieved with APBSCT to prevent relapses including different maintenance treatment (1). We present our preliminary experience with a cuadruple maintenance therapy scheme including Bisfosfonates + INF 2b + Dexamethasone + Thalidomide. We decide to include thalidomide to previous triple maintenance because this drug has been recently proven to have an impact on myeloma, even at low dose, through multiple mechanisms. Its role as maintenance treatment posttransplant is still unknown.

Objectives: 1) Evaluate the toxicity and applicability of a prolonged cuadruple maintenance therapy post-APBSCT in MM including low dose of oral thalidomide 2) Analyze its effects on the clinical evolution posttransplantation (EFS, OS and rate of relapses.

Patient and Methods: Criteria of inclusion: 1) Diagnosis of symptomatic MM grade II-III according Durie Salmon criteria. 2) Previous autologous PBSCT transplantation with stable hematopoietic engraftment: >75 x 10e9/L platelets, > 1.5 x 10e9/L neutrophils, 4) ECOG < 2; 3) Complete remission or partial response posttransplantation or 5) Patients rescued with standard dose of thalidomide for relapsed with previous triple maintenance treatment.

Patients included: Six patients with symptomatic MM (1 stage II and 5 stage III), median of age 58 years. 4 M, 2 F, have been included in the study. 4 patients received intensification with BUMEL (Busulphan 12 mg/Kg orally and Melphalan 140 mg/m² i.v.) and two with Melphalan 200 mg/m² iv). Three patients following triple maintenance treatment were rescued with thalidomide for signs of progression before introducing the cuadruple maintenance therapy.

Cuadruple Maintenance Treatment: Bisfosfonates: Pamidronate 90 mgs iv or Zolendronate (Zometa®, Novartis) 4 mg iv/month, minimum x 12 months + INF 2b (Intron® Schering-Plough) 3 MU s.c.3 x w until relapse or progression + Dexamethasone 20 mg orally x 4 days every 6 weeks, minimum x 12 months + Thalidomide (Thalidomide® Grunnenthal-Germany) 50-100 mg/d according tolerance, until relapse or progression.

Results: Maintenance treatment including low dose oral thalidomide was initiated at a median of 46 days post-transplant (range 23-138 days) in the three patients without previous treatment after transplant. The median leukocyte and platelet counts at the moment of thalidomide initiation were 4800/mL (range 2800-5900/mL) and 125,000/mL (range 75,000-240,000/mL), respectively. Thalidomide was started at 50 mg daily increasing the dose to 100 mg/day according tolerance. No patient failed to tolerate this dose of thalidomide. The most common adverse effects were constipation, dry skin and somnolence No grade 3-4 adverse effects were documented.
Neutropenia, previously reported as a rare adverse effect in this setting, was not seen to date in our cohort. We have not observed progression of disease, although the median follow up is still short (10 months; range 5-30 months).

Conclusions and Comments: Cuadruple maintenance treatment with bisfosfonates + INF + Dexamethasone + Thalidomide presents an acceptable tolerance. The different effects of each drug could induce a theoretical multiple control over MRD by acting at different levels on the pathogenesis of MM. Thalidomide seems to be a safe drug in the post-transplant setting, perhaps adding effect to the response achieved post-transplant without major toxicity. Longer follow up and future randomized trials will be needed to validate the role of thalidomide and its long-term effect when used as maintenance therapy in the post-transplant setting and in prolonging the plateau phase of MM.

References:
A Alegre et al. Triple Maintenance Therapy (Pamidronate + Interferon + Dexamethasone) Post- Autologous Peripheral Blood Stem Cell Transplant ion (APBSCT) in Multiple Myeloma (MM) VII International Myeloma Workshop, Banff, Canada, 2001,145, PS7

346 Maintenance or Salvage Therapy with Thalidomide Enhances Overall Survival following Autologous Hematopoietic Progenitor Cell Transplantation for Multiple Myeloma.

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Autologous hematopoietic progenitor cell transplant (HPCT) has been proven to prolong survival for patients with multiple myeloma, and is a standard part of therapy for most newly diagnosed patients. While this therapy is well tolerated, ultimately it is palliative, as nearly all patients will eventually relapse. We have retrospectively analyzed the impact of maintenance therapy (planned) or salvage therapy with thalidomide (Thal) and its impact on overall survival following HPCT. Methods: 54 patients received busulfan, cyclophosphamide, and etoposide (Bu/Cy/VP-16) and 58 received melphalan 200mg/m2 (MEL 200). All of the patients who received MEL 200 also received PBSC grafts, while 25 of the 54 recipients of Bu/Cy/VP-16 received BM grafts. Patients were evaluated for disease status, overall survival, and relapse free survival based upon the last known documented follow up or restaging (for disease status). 12 patients received the combination of Thal-INF, 22 patients received Thal alone, 27 patients received interferon alone, and 45 patients chose to receive no maintenance therapy at all. Thal doses ranged between 100 and 400mg/day (median dose of 200mg/d) for a median duration of 8.5 months. Interferon doses were 3 million units SQ TIW for between 6 and 12 months post transplant as tolerated. Results: Statistical comparisons of both overall survival and progression free survival demonstrated no difference between patients who received either conditioning regimen. Median follow-up for the group as a whole was 18.5 months, 13.1 months for the group receiving MEL200, and 35 months for the group receiving Bu/Cy/VP-16. Median survival for the group as a whole was 51 months, with a predicted 5 year OS of 49%. We then evaluated the impact of post transplant therapy on overall survival. Median survival for the group receiving Thal or Thal/INF was 113 months, INF alone was 51 months, and the group that received neither Thal nor INF was 29 months (P=.0003 log rank test). In a multivariate analysis, factors which were significant for overall survival included response to transplant (p=.001), age <55 (p=.038), creatinine <1.5 (p=.001), the use of thalidomide at any point after transplant (p=.005), and the combined use of interferon and thalidomide after transplant (p=.035). Conclusion: The use of post transplant maintenance therapy can impact OS following autologous HPCT. Our analysis is the first to demonstrate a survival advantage for patients receiving post transplant thalidomide or the combination of Thal/INF. Further prospective studies evaluating the impact of post transplant Thal are warranted to validate our results. A pilot study evaluating the combination of Thal/INF is planned. Survival analysis will be further updated an additional 6 months prior to presentation.

11.3 Thalidomide in untreated MM and mechanism of action

347 A PHASE II STUDY ON EFFICACY AND TOLERABILITY OF COMBINATION CHEMOTHERAPY WITH VAD REGIMEN CONTAINING LIPOSOMAL DOXORUBICIN AND THALIDOMIDE IN NEWLY DIAGNOSED MULTIPLE MYELOMA (MM) PATIENTS

For the Greek Myeloma Study Group.

Background. In MM patients the VAD regime is considered the standard initial treatment especially if high dose chemotherapy with autologous stem cell rescue is planned. Increased cardiac toxicity due to doxorubicin is a limiting factor. The new liposomal formulation of doxorubicin, administered as a single dose, is safer, and has an extending circulation time, which probably results in more antimyeloma effect. Recently Thalidomide has shown a significant antimyeloma activity. The purpose of this multicenter phase II study was the assessment of efficacy and toxicity of the combination of VAD-liposomal doxorubicin and thalidomide in newly diagnosed MM patients. Patients. Since March 2001, thirty-nine (20 males and 19 females) newly diagnosed patients, with median age 68 years (range 43-78) were entered into the study. Treatment. The treatment consisted of liposomal doxorubicin (40mg/m2) iv day 1, vincristine (2.0mg) iv day 1 and dexamethasone orally (40 mg) days 1-4. Thalidomide was given daily at a dose of 200mg continuously. The regimen was administered every 4 weeks. Methods. Response to treatment according to the usual criteria and toxicity according to NCI criteria were evaluated before every cycle. In patients with progressive disease and unacceptable toxicity the treatment was discontinued. Reevaluation was performed after four cycles of treatment and responders could continue with high dose chemotherapy or with the initial regimen until the maximum response. Results. Overall response rate was 74%. Four patients (10%) achieved complete and 25 (64%) objective response. Three patients (8%) showed minor response and seven patients (18%) progressed during treatment. Median follow-up was 10 months (range 2-21). Event-free survival and overall survival at 22 months were 55% (CI 13.17-18.05) and 74% (CI 15.33-19.72) respectively. During follow-up 2 responders progressed, 15 and 19 months from diagnosis. Eight patients died. Six of them
experienced early death during the first three months of treatment due to disease progression or neutropenic infection. Two responders died one after disease progression and one during ABMT. Treatment was generally well tolerated. Most of the patients experienced somnolence, constipation and tremor, which were mild. None of the patients discontinued thalidomide treatment. In 75% of the patients less than grade 2 (one grade 3) neurotoxicity was observed. Palmar-plantar erythrodysesthesia, grade 2, observed in 4 patients (10%). Uncomplicated deep venous thrombosis occurred in 4 patients (10%). Severe, grade 3, hematologic toxicity observed in 6 patients (15%).

Conclusions. Combination of VAD- liposomal doxorubicin containing regimen with thalidomide is an effective and well tolerated treatment for newly diagnosed patients with MM. A phase III clinical trial comparing this combination with the standard VAD regimen is warranted.

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Treatement of Multiple Myeloma with Thalidomide, Dexamethasone, and Zoledronate in an Inner-City Setting: An Interim Analysis

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The role of angiogenesis at molecular and cellular levels in the pathogenesis and treatment of myeloma remains uncertain. The antiangiogenic agent thalidomide in combination with dexamethasone constitutes an effective therapy for newly-diagnosed myeloma as a pretransplant regimen, and provides rescue for patients in relapse. This Phase II clinical trial assessed the effectiveness of thalidomide and dexamethasone as first-line therapy for myeloma in combination with the bisphosphonate zoledronate, which mitigates bone resorption and possibly tumor growth and angiogenesis. Our intention is also to assess the effectiveness of long-term treatment with this drug combination, since access to stem cell transplantation is limited for some patients.

Trial: Patients with newly diagnosed symptomatic myeloma were enrolled. The TDZ treatment regimen consisted of thalidomide, 100 mg/day, p.o.; dexamethasone, 10-40 mg/day, p.o., as tolerated, on Days 1-4, 9-12, and 17-20 monthly for six months, then on Days 1-4 monthly; and zoledronate, 4 mg monthly, i.v. Response was defined as a decrease in serum or urine monoclonal (M) protein by 50% or greater.

Patients: The current cohort was drawn from the first 20 enrollees, 15 (13F/2M) of whom have been compliant and therefore could be evaluated; the remaining 20% failed to collect the PBSC.

Results: Of the 15 patients, 10 (67%) had a criterional decrease in serum or urine M protein (> 75% in 7 patients and 50-75% in 3 patients). In addition, 4 patients had reductions of 25-50%. One patient with multiple plasmacytomas deteriorated. In addition to decreases in serum M protein and urine M protein, treatment with TDZ decreased serum β2 microglobulin (P < .001 for all). Treatment effects retained significance after adjustment for multiple comparisons. Neither renal function (serum BUN, creatinine) nor calcium levels changed during this study. Importantly, response to treatment was unaffected by HIV status in these patients. Side effects of treatment included peripheral edema (four patients) and elevation of blood sugar (two patients) that necessitated dose reduction in dexamethasone. Thromboembolic episodes that resolved on anticoagulant treatment occurred in two patients; subsequently daily treatment with aspirin was initiated for all subjects. In addition, Grade I neuropathy occurred in one patient and rash in another.

Conclusions: TDZ had significant activity against newly diagnosed myeloma in 67% of patients, as determined by a decrease in M protein of at least 50%. These effects occurred using lower doses of thalidomide than previously reported, and are possibly attributable to the addition of zoledronate to the regimen. HIV status did not affect response. These findings underscore the effectiveness of this combination of anti-myeloma agents, at least in the short term. Long-term outcome is currently being studied.

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Combined thalidomide-dexamethasone as first-line therapy for newly diagnosed multiple myeloma

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Thalidomide has emerged as an active agent in the treatment of advanced and refractory multiple myeloma (MM) and is currently under investigation also as primary therapy for patients with newly diagnosed disease. In January 2002, we started a phase II multicenter study with combined thalidomide and dexamethasone (THAL-DEX) as first-line induction of remission (x 4 months) before collection of autologous peripheral blood stem cells (PBSC) with high-dose cyclophosphamide (HD-CTX) and two subsequent autotransplants (Tx-1 and Tx-2) to support two sequential courses of l.v. melphalan at 200 mg/m2 (ME1-1 and ME1-2). By study design, treatment with THAL-DEX was interrupted the day before HD-CTX and was resumed upon collection of PBSC; similarly, THAL-DEX was discontinued the day before ME1-1 and was re instituted following postTx-1 hematological recovery. The starting dose of thalidomide was 100 mg/d, with a subsequent increase to 200 mg/d after 14 days; the monthly dose of dexamethasone was 40 mg/d on days 1 to 4, with courses repeated on days 9 to 12 and 17 to 20 on odd cycles. Primary objective of the study was to investigate the toxicity profile, antimyeloma activity and effect on PBSC collection of combined THAL-DEX as primary therapy for symptomatic and/or progressive MM. As of January 2003, 75 patients below the age of 65 were enrolled in the study; at the time the present analysis was performed, 43 patients could be evaluated for toxicity and antimyeloma activity of THAL-DEX. More than 70% of these 43 patients were in advanced clinical stage and approximately 50% had high-risk MM (β2-M ≥ 2.5 mg/L and/or chromosomal 13 abnormalities). Toxicities most frequently seen with THAL-DEX included constipation (91%), sedation (66%), tremors (66%), neuropathy (47%), fatigue (56%) and DVT (16%). On intent-to-treat basis, response to combined THAL-DEX was observed in 34 patients (79%) (complete, 5%; partial, 23%; minimal, 7%), including 23% who attained ≥ 90% reduction in tumor cell mass. Nine patients (21%), including 2 who died too early, were classified as treatment failures. Collection of PBSC was adequate (median, 8x106 CD34+ cells/kg) and prompt (median, 2 days) in 80% of patients who could be evaluated; the remaining 20% failed to collect the
minimum target cell dose (≥ 4x10^6 CD34+ cells/kg). It is concluded that orally administered THAL-DEX as primary therapy for MM has definite antitumor activity and doesn’t seem to adversely affect subsequent collection of PBSC. Based on these preliminary observations, THAL-DEX deserves further investigation as an alternative to combined chemotherapy (i.e., VAD) administered in an attempt to reduce myeloma cell mass, particularly in patients who are candidates to autotransplantation.

Supported in part by MIUR, progetto FIRB RBAU012E9A_001 (M. Cavo), Università di Bologna, Progetti di Ricerca ex-60% (M. Cavo) and Fondazione Carisbo.


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Background: Thalidomide has proven efficacy in multiple myeloma. However, its mode of action is unclear, and it may be anti-angiogenic or may promote apoptosis. We have investigated the changes to the expression of genes involved with these cellular processes following culture with thalidomide in the multiple myeloma U266 MM cell line. Methods: Cells were cultured with s-thalidomide (0 - 1000 µM; Cellgene Corp., USA), and cell parameters, including apoptosis, were assessed on day 3. RNA was also extracted from cells cultured for 24 hr with IC50 of s-thalidomide, and their gene expression profiles established by microarray methodologies. Results: Reductions in cell viability was observed in U266 cells cultured with s-thalidomide (IC50: 357 µM), which were mirrored by significant increases in apoptosis (day 3: 9.3 ± 0.6% vs. 3.3 ± 0.9% on day 0; p<0.001). The table below shows the changes in expression of the key genes involved with apoptosis or with angiogenesis.

<table>
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<th>Gene</th>
<th>VEGF</th>
<th>FLT-1</th>
<th>β-FGFR</th>
<th>FGF-2</th>
<th>MMP</th>
<th>IL-6</th>
<th>myc</th>
</tr>
</thead>
<tbody>
<tr>
<td>ΔFold</td>
<td>0</td>
<td>0.6</td>
<td>3</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>apoptosis</td>
<td>p53</td>
<td>ras</td>
<td>bcl-2</td>
<td>bcl-xl</td>
<td>TNF-α</td>
<td>lκB</td>
<td>NF-κB</td>
</tr>
<tr>
<td>ΔFold</td>
<td>2.1</td>
<td>10.1</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>3.5</td>
</tr>
</tbody>
</table>

Immunoblotting analyses of treated cells revealed that changes in protein levels did not always correlate with gene expression. As an example, s-thalidomide resulted in a reduction in IL-6 protein level but no change to the gene expression. Conclusion: Our data suggests that both angiogenic and apoptotic genes and proteins are affected by s-thalidomide. There is evidence that microarray analyses should not be used alone, as epigenetic factors, not considered by the technique, may eventually alter protein level and functionality.

351 THALIDOMIDE AND ZOLEDRONIC ACID IN MULTIPLE MYELOMA (MM)

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Objective: Bisphosphonates are used in MM to prevent skeletal complications. There is evidence from in vitro studies that they may also induce tumor cell apoptosis and that they interact synergistically with other antineoplastic agents. We studied the effect of zoledronic acid (ZA) on growth of myeloma cell lines, alone or in the presence of thalidomide in order to detect putative synergistic effects. Materials-Methods: The myeloma cell lines used were RPMI8226, U266B1, HS-Sultan and IM9. The monocytic cell lines THP1 and U937, the T-leukemia Jurkat and Peripheral Blood Mononuclear Cells (PBMCs) from healthy volunteers were used as controls. To study the effect of the drugs on cellular growth, all cell-types were cultured for 6 days (PBMCs for 3 d) in the presence or absence of ZA ([range]=10-1000 µM) and/or thalidomide ([range]=0.1-1000 µM), and the cells/well were counted and viability was estimated by trypan blue exclusion. Alternatively, the cells were cultured as above for 24h, RNA was extracted, and expression of the anti-apoptotic genes bcl2 and bclxl, and pro-apoptotic genes bak, fas and fasL was studied by RT-PCR.

Results: Thalidomide inhibited the cell growth of all MM cell lines by 86-97% at the concentration of 500 µM, and of U266B1 (alone) by 63% at 100 µM; thalidomide also inhibited the growth of the control cell lines by 91-97% at 500 µM, and THP1 (alone) by 21% at 100 µM. ZA inhibited the cell growth of the MM cell lines by 27-80% at 50-100 µM, and by 85-97% at 500 µM. ZA also inhibited the growth of the control cell lines by 15-65% at 50-100 µM, and 82-93% at 500 µM. The lowest concentrations of the drugs that in combination resulted in the maximum inhibition of cellular division (about 80-90%) in all cell lines tested was [10µM thalidomide+100µM ZA]=smallest effective combination dose]. The drugs alone or together did not significantly affect the viability of PBMCs.

The results of the drug effect at the smallest effective combination dose on the expression of the apoptotic genes studied are shown below:

<table>
<thead>
<tr>
<th>Cell type</th>
<th>Drug effect on relative intensity of bak over: bcl-2 + bclxl</th>
<th>Drug effect on relative intensity of the fas/fasL system</th>
</tr>
</thead>
<tbody>
<tr>
<td>RPMI8226</td>
<td>+137</td>
<td>+313</td>
</tr>
<tr>
<td>U266B1</td>
<td>+430</td>
<td>-35</td>
</tr>
<tr>
<td>IM-9</td>
<td>+350</td>
<td>+343</td>
</tr>
<tr>
<td>HS-Sultan</td>
<td>-375</td>
<td>-124</td>
</tr>
<tr>
<td>THP1</td>
<td>+193</td>
<td>+166</td>
</tr>
<tr>
<td>U937</td>
<td>+539</td>
<td>+443</td>
</tr>
<tr>
<td>Jurkat</td>
<td>+777</td>
<td>-125</td>
</tr>
<tr>
<td>PBMCs</td>
<td>-75</td>
<td>+153</td>
</tr>
</tbody>
</table>

Intensity of gene expression was measured in pixels.

Discussion and Conclusions: Thalidomide enhances significantly the inhibitory effect of zoledronic acid on cellular growth of the MM and tumor cell lines studied. Both drugs are no toxic to normal PBMCs. The smallest effective combination dose of the drugs was found to be [10µM thalidomide+100µM zoledronic acid]. From the observed effects of the drugs on the expression of the apoptotic genes studied, we hypothesize that they enter the cytoplasm and alter the conformational binding of Bak to Bclxl or Bcl-2 proteins, leading thus the MM cells to apoptosis and/or induce the fas-fasL apoptotic pathway. Apoptosis of the cell line HS-Sultan involves another-yet unknown-apoptotic pathway.

352 Thalidomide-dexamethasone as primary therapy for multiple myeloma of high tumor mass


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The combination of thalidomide with intermittent, high-dose dexamethasone (TD) has induced remission in approximately 65-75% of newly diagnosed patients with multiple myeloma of low or intermediate tumor mass. Oral administration has been acceptably tolerated and convenient, with the 18% rate of thromboembolic complications virtually prevented by therapeutic anticoagulation. We assessed this program in 17 consecutive,
untreated patients with high tumor mass (Hgb <8.5 gm/dl or calcium >11.5 mg/dl), a disease stage for which similar patients had previously received a VAD-based regimen. Treatment consisted of thalidomide 150-200 mg q hs and dexamethasone 20 mg/m2 on days 1-4, 9-12, 17-20. Anticoagulation was provided by warfarin (INR 2.0-3.0) or, preferably, low molecular weight heparin. Results were compared with those observed in 50 matched patients with high tumor mass who received high-dose fractionated cyclophosphamide (2.4 gms/m2) with VAD (HCVAD) by continuous infusion through a central venous catheter (1996-2001); this program included prophylactic prophylactic neutopenic and oral antibiotics. For both TD and HCVAD, clinical features were similar (Hgb <8.5 gms/dl in 71 vs 80%, corrected serum calcium >11.5 mg/dl in 41 vs 62%, median β2M 7.1 vs 8.9 mg/L). With either program, early deaths occurred in 6%, but HCVAD induced grade 4 neutropenia in all patients of whom 20% were hospitalized for serious infection; grades 3 or 4 thrombocytopenia also occurred in 70% of patients. None treated with TD developed similar reduction of blood counts, but nonneutropenic infections required hospitalization in 3 patients (17%); whether prophylactic antibiotics may reduce the frequency of such infection is not clear. Other serious complications included one patient with DVT and PE and one patient with dehydration. Response rates were similar with either program (71% with TD vs 67% with HCVAD), the median time to remission was similarly rapid (0.6 month), and CR occurred in 2 patients in each group (p=0.18). One-half of patients received intensive therapy with autologous blood stem cell support after TD or HCVAD, exclusions based on medical and/or socioeconomic factors. The median survival was 26 months with HCVAD and is projected at a similar duration with TD. While similarly effective against myeloma, TD was superior to HCVAD for patients with advanced disease because a central venous catheter was not required and severe neutropenia and thrombocytopenia were avoided.

353 EFFECT OF THALIDOMIDE TREATMENT ON VEGF, bFGF AND HGF SECRETION IN MULTIPLE MYELOMA PATIENTS

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Introduction: Increased angiogenesis has recently been recognized in active multiple myeloma (MM). Vascular endothelial growth factor (VEGF), basic fibroblast growth factor (bFGF) and hepatocyte growth factor (HGF) are key mediators of angiogenesis. Thalidomide (Thal) is a drug with well established antitumor activity in relapsed and refractory to chemotherapy or relapsed patients. BM MVD has been the most studied in this respect. Recently it has been reported, that bone marrow angiogenesis is correlated with clinical stage of disease and the plasma cell labeling index and often predicts the response to treatment. Other angiogenic cytokines, such as angiopoietin and vascular endothelial growth factor (VEGF) or platelet-derived growth factor (PDGF), may be involved in the angiogenic process. The purpose of the current study was to investigate microvessel density (MVD) in bone marrow in MM patients treated with thalidomide.

Materials and methods: We evaluated MVD from bone marrow (BM) samples obtained before and after 6 months of thalidomide treatment in patients with refractory to chemotherapy or relapsed patients. BM MVD was examined using immunohistochemical staining for vonWillebrand factor (vWF) and anti-CD34 monoclonal antibodies. MVD was estimated by determining the average number of vessels in three hot spots examined at x 400 magnification.

Results: In immunohistochemical vWF stained paraffin embedded bone marrow biopsies of 20 patients with multiple myeloma median MVD was 34.7 vessels/mm2 with a range of 3-85 vessels/mm2 before thalidomide treatment and after thal therapy median bone marrow MVD was 19.4 vessels/mm2 with a range of 5-48 vessels/mm2. Median bone marrow MVD measured in immunohistochemical CD34 stained bone marrow biopsies was 35.5 vessels/mm2 (range 2-90) before thalidomide treatment and after therapy median bone marrow MVD was 21.2 vessels/mm2 (range 5-45).

Before Thal treatment, in responding patients group median MVD measured in immunohistochemical vWF stained bone marrow biopsies was 31.1 vessels/mm2 (range 3-59 vessels/mm2) and in non-responding group 38.8 (12-85 vessels/mm2). After Thal treatment in responding group median MVD was 19.3 (range 5-42), and in non-responding group was 19.4 vessels/mm2 (range 5-48).
Before Thal treatment in responding patients group median MVD measured in immunohistochemical CD34 stained bone marrow biopsies was 32.1 vessels/mm² (range 2 - 63) vessels/mm², and in non-responding patients group was 39.3 vessels/mm² (range 17 - 90 vessels/mm²). After Thal treatment in responding patients group median MVD was 20.1 vessels/mm² (range 5 - 40 vessels/mm²), and in non-responding patients 22.6 vessels/mm² (range 7-45 vessels/mm²).

Conclusions: We found no statistical significant differences in bone marrow MVD after thalidomide treatment in relapsed or refractory to chemotherapy MM patients. Microvessels in the bone marrow appear to persist even after therapy with thalidomide an agent with postulated antiangiogenic properties, however the lack of resolution of microvessels may not be an accurate way to measure the effect of antiangiogenic therapy.

11.4 Thalidomide: side effects

355 Venous thrombo-embolism in patients with MM treated with high dose chemotherapy and thalidomide.

Preliminary results of a prospective HOVON/GMMG phase III trial.
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For the Dutch-Belgium Hemato-Oncology Cooperative Group (HOVON)

A high incidence of venous thrombo-embolism (VTE) up to 30% is reported when thalidomide is combined with chemotherapy. In an ongoing Dutch/Belgium/German multicenter study (HOVON50 MM/GMMG HD-3), patients with MM are randomized to receive induction therapy consisting of 3 cycles of vincristine, doxorubicin and dexamethasone (arm A) or 3 cycles of vincristine, doxorubicin and thalidomide (arm B). Thalidomide, starting dose of 200 mg/day and may be escalated to maximum 400 mg, is administered from day 1 throughout the 3 cycles and is combined with VTE prophylaxis (Nadoparine 2850 IE/day). Following induction PBSC are collected after mobilization with cyclophosphamide, doxorubicin, dexamethason and G-CSF (CAD). Patients then may proceed to High Dose Melphalan (HDM 200 mg/m²). As maintenance therapy patients continue with α-interferon 3 × 10⁶ IU/day s.c. 3 times a week (arm A) or thalidomide 50 mg/day (arm B) until relapse or progression. Patients with an HLA-identical may proceed to nonmyeloablative Allo-SCT after HDM. From November 2001 till February 2003, 150 patients have been included in the HOVON-50 trial, of whom 75 have been assigned to arm B. Sixty patients completed the induction therapy with VAD or TAD.

Ten cases of VTE have been recorded, 5 in arm A and 5 in arm B (incidence 8.3%) which all occurred in the induction phase. All 5 patients in arm A had DVT of the leg, one patient also presented with signs of a pulmonary embolism. Two patients had additional risk factors (long travel and an ankle fracture), no additional risk factors were found in the other 3 patients.

In arm B, three patients presented with DVT of the leg, one with pulmonary embolism and one patient with thrombosis of the arm. All patients used nadroparine prophylaxis, except one patient with atrial fibrillation who used acenocumarol. He developed a DVT of the leg after the oral anticoagulation was stopped 5 days earlier to perform a bone marrow biopsy. In the other 4 patients no additional risk factors could be identified.

In conclusion, 8.3% of the patients in treatment arm A developed VTE during induction therapy, which is comparable with the incidence reported before in patients with intensive chemotherapy (about 10%). However, the patients receiving both thalidomide and doxorubicin seem to have a lower incidence of VTE when compared with the incidence reported in previous publications (16 to 30%). We therefore conclude that the use of Nadoparin prophylaxis seems effective in reducing the occurrence of VTE in patients with MM during intensive chemotherapy and use of thalidomide.

356 Preliminary analysis of a double blind randomised study comparing Thalidomide (Thal) or placebo in combination with a (V)AD-like regimen in relapsing patients with Multiple Myeloma (MM)

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For the group "Myélome-Autogreffe", Caen, Créteil, Limoges, Paris, France.

In 2002, we initiated a prospective multicenter randomised double blind trial in which myeloma patients in first or second relapse after high dose (HDT) or conventional chemotherapy (CCT) were randomly assigned to receive a (V)AD-like regimen (without vincristine) combined with thalidomide (Thal arm) or placebo. In both arms, patients received monthly courses of doxorubicin (9 mg/m²/day by continuous infusion, Day 1 to 4) and dexamethasone (40 mg/day Day 1 to 4 and day 14 to 17 during the first 2 cycles) for a maximum duration of one year. Thalidomide (or placebo) was administrated at a daily dose of 200 mg during 15 days with serial increments at 15 days interval according to tolerance to a maximum dose of 400 mg po qhs. Screening for deep venous thrombosis (DVT) was performed monthly by Doppler ultrasound during the first 3 months of treatment and later when appropriate. There was no systematic thrombosis prevention.

At randomisation, characteristics of the first forty five enrolled patients were the following: median age 63 years; IgG, IgA and BJ alone MM, 58, 22 and 10%, respectively; in first relapse after CCT 33%, after HDT 51%; median beta 2 microglobulin level 3.3 mg/L. Five patients had a past-story of DVT or pulmonary embolism (PE). None of these presented a thrombotic event whereas DVT was diagnosed in 8 patients, 7 in the Thal arm (28%), one in the placebo arm (5%). DVT were usually observed during the first two months on therapy, were bilateral in 3 cases and were complicated by PE in 3 cases. Patients were treated by heparin derivative followed by full-dose oral anti-vitamin K. Thalidomide or placebo was stopped during one month. After that, patients were either maintained on or withdrawn from the study according to outcome and results of control venous Doppler.

During follow-up, 3 patients died (MM progression n=2, septic shock n=1), none because of DVT or PE. Four patients were withdrawn from the study because of a thrombotic event, three in the thal arm and one in the control group. Otherwise, treatment complications that caused patient’s withdrawal were vertigo and dyspepsia (n=1, control arm), tremor and severe neutropenia (one each, thal arm).

Preliminary results of this study confirm the high venous toxicity of the combination of doxorubicin, dexamethasone and thalidomide. Accordingly, the study design was modified by excluding doxorubicin and the trial is ongoing comparing dexamethasone plus thalidomide with dexamethasone plus placebo.
Thalidomide has anti-angiogenesis activity and is effective in many patients with multiple myeloma. It is however associated with an increased of thrombosis, especially in patients who have received prior anthracycline chemotherapy. Anti-angiogenesis and thrombosis are both endothelial-related activities, and we therefore speculated that the interaction of both agents on endothelium could promote thrombogenic activity. We therefore evaluated in vitro effects of thalidomide on intact cultured endothelium and cultured endothelial cells. Thalidomide could promote thrombogenic activity. We therefore speculated that the interaction of both agents on endothelium could promote thrombogenic activity. We therefore evaluated in vitro effects of thalidomide on intact cultured endothelium and cultured endothelial cells injured by pre-incubation with doxorubicin. Endothelial cells (human coronary artery endothelial cells) were purchased from Clonetics Corporation and grown in endothelial basal medium with 5% heat-inactivated fetal bovine serum. Cells were treated with varying doses of doxorubicin (1-2µL) for 1 to 48 hours and cells were then harvested by scraping and centrifugation. Cell lysates, supernatant, and extract were prepared. Cell viability was measured by MTT assay. Caspase-3 activity was done by measurement of fluorescent leaving group after cleavage at the Asp residue using fluorescent spectrofluorometry. PAR-1 receptor assay was done using PAR-1 antibody in an enhanced chemiluminescence assay system. Immunostaining and fluorescent microscopy were done on paraformaldehyde-fixed cells on coverslips incubated with FITC-conjugated phalloidin followed by nuclear staining with propidium iodide. Cells were imaged using fluorescent microscopy at 400x magnification. Results showed caspase-3 activity was increased within one hour after doxorubicin treatment, continued to increase for up to 8 hours, and then stabilized. Doxorubicin caused a significant dose-dependent increase in caspase-3 activity when used in concentrations from 0 to 6µmol/L. Thalidomide alone did not induce caspase-3 activity. Cell pretreated with doxorubicin (6µmol/L) for 6 hours, followed by thalidomide incubation, resulted in decreased caspase-3 activity. Doxorubicin caused a progressive time-dependent decrease in cell viability from 1 to 48 hours (77% to 25%); whereas thalidomide treatment alone did not alter cell viability. When endothelial cells were co-incubated with doxorubicin and thalidomide, there was less decrease in cell viability than when endothelial cells were treated with doxorubicin alone (76 to 51% vs 77 to 29% cell viability). Doxorubicin caused endothelial cell apoptosis within 24 hours, and this was prevented by treatment with thalidomide (40 g/ml). When thalidomide was incubated with cells exposed to doxorubicin, there was immediate and marked formation of neotubules and pro-angiogenesis. Neotubule and pro-angiogenesis effects were not seen when thalidomide was incubated with untreated endothelial cells. The neotubule formation was related to thrombin receptor activation since thrombin receptor activation occurred in doxorubicin-treated, but not in untreated, endothelial cells, as measured by immunoblotting using PAR-1 antibody. These findings suggest that thalidomide may be procoagulant and proangiogenic through stimulation of thrombin receptors of doxorubicin-injured endothelium and may explain the increased hypercoagulability in patients treated with anthracycline chemotherapy followed by thalidomide.
We had 3 cases of severe thrombocytopenia not related to the multiple myeloma and cleared up after discontinuation of therapy.

Case 1: A 51-year-old female presented with stage I A IgG multiple myeloma in 1991; she achieved a complete remission after a double autologous stem cells transplantation in 1998. She relapsed in 1999. She was begun on thalidomide (100 mg/die) in January 2001. Her paraprotein decrease from 1500 mg/dl to 900 mg/dl, the bone marrow plasmocytosis decreased to 15%. The therapy was well tolerated, she had moderate constipation as a secondary effect. In August 2002 she developed thrombocytopenia (24,000/cc). The bone marrow aspiration showed few marrow cells and 5% plasmocytosis. Her platelet count reached a normal value after discontinuation of thalidomide.

Case 2: A 66-year-old male presented with stage III A IgGk multiple myeloma in 1999, refractory to different lines of chemotherapy; he was given Thalidomide (100 mg/die) and prednisone in May 2002. His paraprotein decreased from 7700 mg/dl to 1290 mg/dl and he achieved a better quality of life. He developed thrombocytopenia in February 2003; plt= 2000/cc. Case 3: A 75-year-old male presented with stage III A IgGk multiple myeloma in 1999 refractory to standard chemotherapy; he was begun on thalidomide (100 mg/die) in November 2001. His paraprotein decreased from 8530 mg/dl to 3700 mg/dl. Because of lethargy and neutropenia, as an adverse side effect the thalidomide was reduced to 50 mg/die. In February 2003 he developed thrombocytopenia (plt 5000/cc). The platelets count increased (75,000/cc) after thalidomide discontinuation and commencement of low doses of prednisone therapy.

In all patients the bone marrow aspiration showed few marrow cells especially megacaryocytes and 10-20% plasmocytosis. Her platelet count reached a normal value after discontinuation of thalidomide.

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In all patients the bone marrow aspiration showed few marrow cells especially megacaryocytes and 10-20% plasmocytosis.
**EOSINOPHILIA IS A VERY COMMON FINDING IN MYELOMA PATIENTS TREATED WITH THALIDOMIDE AND CYCLOPHOSPHAMIDE**

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Although thalidomide represents one of the major improvements in the treatment of multiple myeloma its mechanism of action remains to be fully elucidated. Moreover, thalidomide side effects are quite variable among different series and their incidence and severity appears to be different if this drug is used alone or in combination. Among modifications of hematological parameters, increase in eosinophil count has been reported in conjunction to other side effects induced by thalidomide. However, by treating patient with thalidomide, we observed a high frequency of eosinophilia. On these basis we retrospectively examined medical records of 39 patients affected by resistant or refractory multiple myeloma treated with a combination of thalidomide 200 mg, cyclophosphamide 100 mg, both daily, and dexamethasone 40 mg for 4 days every month. Before starting therapy, median eosinophilic count of the whole group was 0.52 x10e9/l and it increased to 0.85 x10e9/l after the first month (p 0.08), 1.30 x10e9/l after the second month (p 0.01), and 1.12 x10e9/l after the third month (p 0.001). Mean determination of eosinophil count recorded during entire duration of treatment was higher than pretreatment count in 31 out of 39 patients. Three patients only did not respond to this combination therapy and their median eosinophil count during treatment was 0.26 x10e9/l vs 1.12 x10e9/l of responding patients. However, no difference in the eosinophil count or in the magnitude of increase was observed among the groups of patients with different degree of response (minimal or complete response). In addition, no correlation was found between percentage of plasma cell bone marrow infiltration and the eosinophil count or the entity of its increase. None of the common thalidomide side effects were correlated to the eosinophilia.

In conclusion, we found that in myeloma patients treated with a combination of thalidomide, cyclophosphamid and desamethasone, increase of eosinophil count was a very common event. This finding cannot be ascribed to desamethasone because actually it may induce eosinopenia. On the contrary, the combination of thalidomide and cyclophosphamid may be responsible for eosinophilia and the increase of eosinophil count could be related to thalidomide mechanism of action. In fact, in non responding patients eosinophil count was lower than in responsive ones but the number of the former is too small to be significant.

**EVALUATION OF THROMBOPHYLIC STATES IN MYELOMA PATIENTS RECEIVING THALIDOMIDE**

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Fundación Jiménez Díaz. Hematology Department. UAM *Grant from Fundación Conchita Rábago

Introduction: An increased incidence of deep venous thrombosis (DVT) has been described in multiple myeloma (MM). Its pathogenesis is multifactorial but the presence of other states of inherited or acquired thrombophilia can increase this hypercoagulability. Thalidomide has been used in refractory MM. An increased risk of thrombosis has been reported in combination with other chemotherapeutic agents. We present a patient with MM, homozygous carrier of the C677T mutation of the MTHFR gene who developed DVT and pulmonary embolism (PE) during thalidomide treatment.

Case Report: A 60-year-old male with stage IIIA Bence Jones (BJ) MM was treated at diagnosis with conventional chemotherapy and he obtained a complete response. During the treatment, the patient suffered DVT in the right subclavain vein with a central venous catheter. After relapse, thalidomide was initiated with a 200mg/day dose with 15-day cycles of dexamethasone. The thalidomide was increased 200mg/day every two weeks as tolerated. With the 400mg/day dose, he presented constipation and drowsiness. Upon reaching 800mg/day, he had a slight polyneuropathy which ceded with 600mg/day. In the 7th month of treatment, the patient presented progressive dyspnea without other symptoms. Patient was obese, eunoeic at rest and had normal cardiopulmonary examination. He presented slight malleolar oedema without signs of DVT. Laboratory studies: haemoglobin 10.7g/dl, normal leukocyte, platelet count, PT, TTPA and biochemical; fibrinogen 480mg/dl; proteinuria 700mg/24 hours without evidence of BJ. D-Dimer (ELISA): 2428µg/l (68-494µg/l). Chest X-ray normal. Pulmonary perfusion scintigraphy and helical CT were suggestive of PE. An echodoppler of the lower limbs detected thrombosis of the right popliteal vein. With the diagnosis of PE and DVT, anticoagulant treatment was initiated. Thrombophilia study detected he was homozygous for the C677T mutation of the MTHFR. High serum levels of homocysteine, 26 IU/ml (normal <15 IU/ml), were also observed. Factor V Leiden and prothrombin G20201A were negatives and functional antithrombin III and plasminogen were in normal range. Lupus anticoagulant and anticoatheripline antibodies were negative. The patient had no family history of thrombosis.

Discussion: The percentage of DVT associated with thalidomide and chemotherapy treatments including dexamethasone varies (4-28%) and a lower percentage of PE cases has been reported. This rate is greater than in MM patients not treated with thalidomide. A strong association exists between DVT and exposure to doxorubicin-thalidomide combination, but thrombotic mechanism seems to be multifactorial. No increase in the risk of thrombosis has been observed in MM treated with thalidomide as the only agent (2%). In our patient, the potential risk factors were: MM, older age, obesity, immobility and genetic factors (MTHFR C677T homozygous with high levels of homocysteine), in addition with the treatment with thalidomide and dexamethasone. All cases of thrombosis described during the treatment with thalidomide have evolved favourably with anticoagulant treatment, as in our case. For this reason, the development of thrombosis is not an absolute contraindication for continuing the treatment. Patients with personal and family history of thrombosis must be investigated for genetic and acquired prothrombotic factors before they begin thalidomide treatment. Therefore, prophylactic anticoagulation should be considered in selected cases.
In patients with advanced and refractory multiple myeloma (MM) treated with thalidomide ± glucocorticoids, the risk of deep vein thrombosis (DVT) didn’t exceed 5%. Recently, an increase in the frequency of DVT, up to the range of 21-28%, was reported with the use of thalidomide and doxorubicin-containing regimens as primary therapy for newly diagnosed disease. In 2002, we started a phase II study with thalidomide and dexamethasone as first-line induction of remission for patients with de novo MM (Cavo et al, IX International Workshop on MM). The starting dose of thalidomide was 100 mg/d, with a subsequent increase to 200 mg/d after 14 days; the monthly dose of dexamethasone was 40 mg/d on days 1 to 4, with courses repeated on days 9 to 12 and 17 to 20 on odd cycles. Among the first 19 patients who entered the study (group A) and received a median of 4 months of thalidomide-dexamethasone, 5 (26%) had symptomatic DVT, of whom 1 with associated non-fatal pulmonary embolism. DVT was documented by doppler ultrasonography and developed in the lower extremities at the first month of therapy in 2 patients, at the second month in 1 patient, at the third month in 1 patient and at the end of the fourth month of therapy in the last patient. Based on this unexpectedly high frequency of DVT, treatment protocol was amended and the use of fixed low-dose warfarin (1.25 mg/day) as prophylaxis against DVT was instituted. Forty-three consecutive patients entered the amended study and received a median of 4 months of thalidomide-dexamethasone (combination therapy). Comparison between patients in groups A and B revealed that they were well balanced with respect to risk factors for both thrombosis and MM, including abnormalities of chromosome 11 and 13. Among the 43 patients included in group B, DVT in the lower extremities was documented by doppler ultrasonography in 4 patients (9%). It is worthy of note that in 2 of these 4 patients DVT occurred 10 and 12 months after starting treatment and was not linked to an elevated FVIII:c.

Results of the analysis excluded primary hypercoagulable states in all the 5 patients who had DVT in group A, whereas 1 patient out of the 4 who had DVT in group B was found to be heterozygous for Factor V Leiden. It is concluded that in patients with de novo MM receiving first-line combined thalidomide-dexamethasone 1) therapy carries an increased risk of DVT; 2) the hypercoagulable state is generally not related to identifiable prothrombotic abnormalities; 3) fixed low-dose warfarin may provide an effective and well manageable prophylactic measure to reduce the risk of thrombosis.

Supported in part by MIUR, progetto FIRB RBAU012E9A_001 (M. Cavo), Università di Bologna, Progetti di Ricerca ex-60% (M. Cavo) and Fondazione Carisbo.

Thalidomide exhibits potent anti-tumour activity in myeloma, but in combination with chemotherapy leads to venous or arterial thromboembolism in up to 20% of patients. Other anti-angiogenic therapies under investigation have been similarly prothrombotic, through unknown mechanisms. Elevations of FVIII coagulant activity (FVIII:c), von Willebrand factor antigen (vWF:Ag), or acquired resistance to activated protein C (aPC-R) have been implicated in this process. We performed a pilot study to investigate these parameters in patients on thalidomide plus chemotherapy during the first 4 months when thromboembolic events are known to occur with the highest frequency. Eleven patients with mesothelioma, currently enrolled in a parallel non-randomised phase-II study were studied. Six received thalidomide alone, and five received thalidomide, cisplatin and gemcitabine (combination therapy). Four patients on combination therapy developed thromboembolic events in the first 3 weeks (2 with pulmonary emboli, one DVT/ pulmonary embolus, and one upper limb DVT), with no thrombotic events in the thalidomide arm. All patients in this study showed increased levels of vWF:Ag, with marked elevations in the 4 patients with thromboembolic events (193-205%, normal range 50-150%). Pretreatment vWF:Ag levels were high in three patients (195–196%), and baseline FVIII:c was markedly elevated in two patients (2.10–2.96 IU/mL; RR 0.50–1.50 IU/mL), suggesting a pre-existing prothrombotic state. Only one patient with thrombosis had normal pre-treatment vWF:Ag (143%), and levels reached 194% during treatment, coinciding with the thrombotic event. To determine the effect of thalidomide therapy on procoagulant parameters, patients with high baseline vWF:Ag and FVIII:c (5 and 2 cases respectively) were omitted from the following analysis.

Patients with normal pre-treatment procoagulant levels demonstrated significantly increased vWF:Ag levels after starting thalidomide (mean vWF:Ag, 113% pre-treatment; 155% at one month, p < 0.02; 170% at two months, p < 0.001) with high levels still apparent at 4 months (mean 144%). Thalidomide also increased FVIII:c (mean pre-treatment FVIII:c, 0.92 IU/mL, 1.16, 1.24, 1.46 and 1.43 IU/mL at 1, 2, 4 and 8 months respectively), which was statistically significant at 4 months (p < 0.05). Elevated FVIII:c is a known cause of acquired activated protein C resistance (aPC-R), so aPC-R was assessed by two methods (APTT and DRVVT-based). One patient with thrombosis had a clearly abnormal aPC-R associated with heterozygosity for Factor V Leiden. Two additional patients (one with thrombosis) showed aPC-R not associated with Factor V Leiden; however, this preceded treatment and was not linked to an elevated FVIII:c. These preliminary data suggest that many patients who develop thrombosis on thalidomide/combination therapy have pre-existing prothrombotic risks, including elevated coagulation factors and acquired aPC-R. In addition, patients with normal coagulation parameters appear to develop high FVIII:c and vWF on thalidomide, typically within 4 weeks of therapy. Further studies are urgently needed to define the mechanisms of thalidomide-induced thrombosis, both to identify those patients at high risk, and to develop safer therapeutic approaches.
11.5 CC 4047, PS 341 and arsenic trioxide

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CC4047 (Actimid), a new immunomodulatory agent is well tolerated and has anti-myeloma activity in patients with relapsed / refractory multiple myeloma

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The anti-myeloma activity of thalidomide is well established. However side effects such as somnolence, constipation and neuropathy are common thereby limiting its usefulness. CC4047 (Actimid) is a synthetic analogue of thalidomide with immunomodulatory activity. It has been observed to have a 5000 fold greater inhibition of Tumour Necrosis Factor Alpha activity than thalidomide and an excellent toxicity and safety profile in human volunteer studies. We present the results of a Phase I dose escalation study of this agent (1mg, 2mg, 5mg, 10mg) in patients with relapsed/refractory myeloma to identify the maximum tolerated dose of CC4047 when given orally for 4 weeks. The secondary endpoint was disease response.

24 patients with a median age 66 years (range 49 – 82) and a median of 3 (range 1 – 6) previous treatment regimens including previous high dose therapy (5 patients) and/or thalidomide (7 patients) entered the study. During the study period the maximum tolerated dose was established at 2mg/day. Grade IV neutropaenia developed in 2/3 patients on 10mg/day, 1/6 patients on 5mg/day and 2/9 patients on 2mg/day. Neutropaenia resolved in all patients within 4 weeks of discontinuation of treatment. Furthermore 1 patient developed a lower limb deep venous thrombosis (DVT) at 3 weeks and was withdrawn from the study (the patient was subsequently shown to have malignant melanoma related lymphadenopathy proximal to the site of thrombosis). Following the study period 20/24 patients continued treatment on a compassionate use basis (patients on 10mg during trial reduced dose to 5mg). Treatment was withdrawn in 7 of these patients (4 due to neutropaenia, 2 due to DVT, 1 due to renal failure) and 1 patient withdrew from the study. The median duration of treatment was 25 weeks (3-96 weeks).

All 24 patients are eligible for assessment of response on an intention to treat basis. 13/24 patients (54%) achieved >50% reduction in paraprotein, of these 4 patients (16%) had complete disappearance of paraprotein and 2 patients (8%) had a 75-99% reduction in paraprotein. A further 3/24 patients (24%) achieved a 25-50% reduction and 8/24 patients (33%) had stable disease for at least 4 weeks. The median time to achieve maximum response (>25% reduction) was 23 weeks (4 – 51) and the median duration of response was 11 weeks (0 – 53). Although quality of life was not formally assessed most patients reported a subjective improvement in wellbeing irrespective of response. 6/24 (25%) patients developed progressive disease whilst on treatment (1 previous CR, 1 previous VGPR, 2 previous PR and 2 stable disease).

Conclusion: CC4047 is well tolerated with acceptable side effect profile. There is good evidence for anti-myeloma activity. Studies are ongoing to further define the optimum dosing schedule and further studies are justified to fully assess the efficacy of this drug both as a single agent or in combination with other treatments.
The clinical development of the proteasome inhibitor, bortezomib (VELCADETM) for the treatment of late stage multiple myeloma has included a transcriptional profiling-based pharmacogenomics strategy to identify genomic predictors of response and further elucidate the mechanism of action of the drug. Through a collaborative effort with the Phase II SUMMIT clinical trial sites, patient bone marrow aspirates were subjected to a rapid negative selection enrichment protocol to purify myeloma cells before being frozen and shipped for centralized pharmacogenomics assessment. Sixty-two percent of 202 patients agreed to the optional expression profiling analysis. The intrinsic gene expression patterns of these samples were determined on Affymetrix U-133 oligonucleotide microarrays prior to treatment with bortezomib. Multiple bioinformatic marker selection algorithms with supervised learning were used to identify genes expression patterns that characterized the differences between responders and non-responders to bortezomib using the Blade response criteria. Predictive accuracy as determined by 5 fold cross validation was significant (71-78%, range) in multiple models. These pharmacogenomic studies of late stage multiple myeloma patients is continuing with the evaluation of new samples obtained from patients in the Phase III clinical trial designed to further discover new markers and validate the current predictive gene sets.

**Effects of PS-341 and PS-1145 on cytokine-mediated proliferation and anti-apoptosis in myeloma cells.**

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Several cytokines have been implicated in the pathogenesis of multiple myeloma (MM). Interleukin (IL)-6 is an important growth and anti-apoptotic factor for MM cells. However, other cytokines may substitute for IL-6, for example tumor necrosis factor (TNF), IL-10, IL-15, insulin-like growth factor (IGF)-1 or IL-21. For instance, in the human myeloma cell line OH-2, combinations of TNF with IL-10, IL-15 or IL-21 give synergistic effects on proliferation and a stronger response than IL-6 alone.

We examined in vitro effects of the proteasome inhibitor PS-341 and the IκB kinase inhibitor PS-1145 on the cytokine-dependent myeloma cell lines OH-2 and IH-1 as well as on the cytokine-independent cell line RPMI 8226. We wanted to see if the two substances discriminated between MM cells stimulated to grow by different cytokines and combinations of different cytokines. We examined proliferation by 3H-thymidine incorporation and induction of apoptosis by Annexin V-FITC/propidium iodide flow cytometry.

PS-341 inhibited proliferation and induced apoptosis in all cell lines, irrespective of which cytokine stimulated the growth. In the proliferation assay ED50 was 1.3 nM. PS-1145 inhibited TNF-induced proliferation of OH-2 cells by more than 50% in a dose-dependent manner, whereas growth stimulated by other cytokines was inhibited less than 25%. In the apoptosis assay, PS-1145 totally inhibited the anti-apoptotic effect of TNF in OH-2 cells, whereas little or no inhibition was seen against anti-apoptosis mediated by IL-6 or IL-21. When growth of OH-2 cells was supported by a combination of TNF and other cytokines, proliferation or anti-apoptosis was inhibited to the level seen in controls without TNF. Because of the striking synergy between TNF and other cytokines in OH-2 cells, the effect of PS-1145 was most pronounced under these conditions, both in absolute and in relative sense (e.g. more than 60% reduction of proliferation stimulated by TNF and IL-6). Conversely, in the IH-1 cell line, which is not growth-stimulated by TNF, PS-1145 gave a less than 10% reduction in proliferation of cells stimulated with a combination of TNF and IL-6.

In the cytokine-independent cell line RPMI-8226, PS-1145 was pro-apoptotic only in the presence of TNF, showing that TNF became cytotoxic when added together with PS-1145. In conclusion, PS-341 induces apoptosis irrespective of which cytokine is promoting growth of the cells, suggesting that its pro-apoptotic effect is not linked to any specific signaling pathway. In contrast, PS-1145 seems to be specific for TNF-mediated growth, and in fact, TNF itself becomes cytotoxic in the presence of PS-1145. The anti-proliferative effect of PS-1145 is most conspicuous when TNF is combined with other growth-promoting cytokines. We have earlier suggested that TNF might be more important to myeloma growth as a synergistic stimulant than its effect as a single agent suggests. Inhibition of TNF-induced and NF-κB-mediated proliferation and anti-apoptosis is promising, suggesting a place for PS-1145 or other agents targeting NF-κB in treatment of MM.

**Proteasome inhibitors induce growth inhibition and apoptosis in human myeloma cells irrespective of chromosome 13 deletion**

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The proteasome is a ATP ubiquitin-dependent protease which plays a crucial role in degrading cytosolic and nuclear proteins, which are strongly involved in basic cellular functions, like the cell cycle regulation, transcription of growth factors and apoptosis. The inhibition of the ubiquitin-proteasome pathway leads to an uncoordinated expression and degradation of several short living proteins required for appropriate cell cycle progression and survival in malignant cells and activates apoptosis. In this study, we investigated the effects of cell-permeable proteasome inhibitors MG-132, MG-262, PSI and lactacystin on multiple myeloma cell lines OPM-2, U266, RPMI8226 and freshly isolated plasma cells with or without deletion of chromosome 13 from patients with multiple myeloma or plasma cell leukemia, and CD34+ human hematopoietic stem cells. The effects of proteasome inhibitors on cell cycle progression, cell growth and apoptosis were determined.

Using the MTT-assay, we found PSI with the half maximal cytotoxicity (IC50) of 5.7 nM to be the most potent proteasome inhibitor among those tested, followed by MG-262 (31 nM), MG-132 (177 nM) and lactacystin (>1 µM). The growth inhibition occurred despite of deletion of chromosome 13, there was no significant difference for IC50 between myeloma cells with and
without chromosome 13. Mean IC50 for PSI was 5.4 and 6.1 for myeloma cells with and without chromosome 13 deletion, respectively. Mean IC50 for MG-262 was 24 and 38 for myeloma cells with and without chromosome 13 deletion, respectively. Cell cycle arrest occurred either in G0, G1 or in G2-M in a dose and time dependent manner, as shown by 7-AAD, Ki-67 staining and flow cytometry. Sub-apoptotic dosages led to a partial loss of Ki-67 antigen and arrested cells in the G0, whereas higher dosages led to a reduction of Ki-67 levels, growth inhibition and induction of apoptosis. As shown by Annexin-V staining, apoptosis was partially dependent on activation of caspase-3, since Ac-DEVD-cho, a caspase-3 inhibitor, could reduce the apoptosis significantly. The cytotoxicity of the four proteasome inhibitors tested was significantly lower on human progenitor stem cells than in myeloma cells. In conclusion, our results show that proteasome inhibitors induce cell cycle alterations, growth inhibition and apoptosis in human myeloma cells and importantly, chromosome 13 status did not affect the response to proteasome inhibitor treatment.

371 Phase I study of the proteasome inhibitor bortezomib and pegylated liposomal doxorubicin in patients with advanced hematologic malignancies


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Introduction: The proteasome is involved in intracellular ubiquitin-dependent and –independent protein degradation, and is a novel target for cancer therapy. Bortezomib (VELCADE™, formerly PS-341), a novel specific and selective inhibitor of the proteasome, has shown significant single-agent activity against multiple myeloma in both preclinical and clinical studies. Proteasome inhibitors also block several survival pathways activated by anthracyclines, including nuclear factor- B and p44/42 mitogen activated protein kinase, that may limit their own effectiveness, suggesting such combinations might have enhanced anti-tumor efficacy. Study Aims: We therefore sought to evaluate the maximum tolerated dose (MTD), dose limiting toxicity (DLT), pharmacokinetics, and pharmacodynamics of bortezomib and pegylated, liposomal doxorubicin (Doxil®) in patients with hematologic malignancies. Methods: Bortezomib was given as an intravenous bolus at doses that were to range from 0.90 to 1.50 mg/m2 on days 1, 4, 8, and 11 of a 3 week cycle. Doxil® was given as an intravenous infusion following bortezomib on day 4 at 30 mg/m2. The MTD was defined based on toxicities occurring during cycle 1, while responses were evaluated every 2 cycles. Patients: 25 patients have been enrolled and treated to date, with 18 of these having had advanced multiple myeloma. Results: A mean of 3.9 cycles (range 1-10) has been administered, with 22 patients evaluable for toxicity. At the 0.90 mg/m2 dose level a patient with Crohn’s disease had grade 3 diarrhea, hypotension, confusion and syncope, but no other DLTs were seen at this level, nor at 1.05, 1.20, and 1.30 mg/m2. At the 1.40 mg/m2 level one patient with multiple myeloma and plasma cell leukemia had prolonged grade 4 neutropenia, but additional patients in this cohort did not experience a DLT. All other non-hematologic drug-related toxicities during cycle 1 have been grade 1 or 2 in intensity. Grade 3 or 4 toxicities in later cycles included thrombocytopenia, neutropenia, febrile neutropenia, fatigue, neuropathy, and palmar plantar erythrodysesthesia. Of 13 evaluable multiple myeloma patients complete responses (CR) have been observed in 4, near-CR with a residual paraprotein seen only by immunofixation in 1, partial responses in 3, a minor response has been seen in 1 patient, and 3 patients have had stable disease, while one patient with non-secretory myeloma progressed by marrow criteria. Six of these patients, including two of the CRs, had disease that previously progressed or didn’t respond to anthracycline-based therapy. One patient with multiple cytogenetic abnormalities, including a deletion of chromosome 13, achieved a CR after four cycles with clearance of cytogenetic abnormalities after two cycles. Five of these patients are continuing treatment, the MTD has yet to be defined, and accrual is continuing at the 1.50 mg/m2 dose level. Conclusions: Early results from this study suggest that bortezomib/Doxil® may be well tolerated and active in patients with advanced multiple myeloma, and phase II testing of this regimen may be warranted.

372 Identification of Molecular Markers that Determine Sensitivity (or Resistance) to PS-341 / Velcade in Multiple Myeloma

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Phase I study of the proteasome inhibitor bortezomib (VelcadeTM/Bortezomib) is a highly potent therapeutic agent for multiple myeloma. Although some of its downstream molecular targets have been defined, the mechanisms of sensitivity and resistance to PS-341 are not known. We compared sensitivity of cell lines derived from various hematologic malignancies to PS-341. Our preliminary experiments showed that two B-cell lines, both derived from patients with diffuse histiocytic lymphoma (DHL), had remarkably different responses to PS-341 treatment. While the SUDHL-6 cell line was very sensitive (IC50 = 2.5 nM), the SUDHL-4 cell line had a 20-fold higher IC50 (>50nM). Interestingly, PS-341-resistant SUDHL-4 cells survived (for at least 48 hours) in the presence of 20nM PS-341, despite the fact that greater than 80% of the proteasome chymotrypsin-like activity was inhibited by 6 hours. Proteasome inhibition in resistant SUDHL-4 cells was also confirmed by intracellular accumulation of p27Kip1, a proteasome substrate, within 6 hours after treatment with 20nM PS-341. Since the two cell lines are phenotypically quite similar (CD20-positive, CD138-negative) and derived from a similar developmental stage, we are using these cell lines as models to determine markers of PS-341 sensitivity and resistance. We have profiled these two cell lines as well as the myeloma cell line MM.1S, to define changes in gene expression before and after PS-341 treatment. We are currently analyzing these data to identify genes, which are uniquely altered in SUDHL-4 or SUDHL-6 cells upon PS-341 treatment, to define molecular mechanisms conferring the “resistant” versus “sensitive” phenotype.
A Phase II Trial Using Bortezomib (VELCADETM Formerly PS-341) and Melphalan Combination Therapy (Vc+M) to Treat Patients with Relapsed or Refractory Multiple Myeloma (MM)

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Background: The proteasome inhibitor, Bortezomib (VELCADETM formerly PS-341), is being evaluated in clinical studies for the treatment of MM. A recent phase II trial reported efficacy of bortezomib in treating MM patients who either relapsed from or were refractory to previous treatments. By combining a non-cytotoxic bortezomib dose with the chemotherapeutic agents melphalan, doxorubicin, or mitoxantrone, we demonstrated in vitro that the highly chemoresistant MM cell-lines can be sensitized to chemotherapeutic agents at markedly lower concentrations than were necessary to kill these cells without bortezomib. This result indicates that the anti-myeloma effects can be achieved when lower doses of bortezomib and chemotherapeutic agents are used together, thereby providing a new therapeutic approach to minimize the toxicity and overcome the chemoresistance. Methods: The primary objective is to determine the response rate and safety and tolerability of Vc+M therapy in patients with MM. The secondary objectives are to assess the time to response, progression-free survival, and overall survival. Patients who relapsed from or were refractory to their previous anti-myeloma treatments are eligible for the study. The patients who failed prior melphalan or bortezomib therapy are also eligible for the study. Patients received a fixed dose of bortezomib intravenously at 0.7 mg/m2/dose on days 1, 4, 8 and 11 of 28-day cycle for up to 8 cycles. At the same time, melphalan was given orally in 3-patient cohorts with escalating doses, starting at 0.025 mg/kg to maximal 0.25 mg/kg, four times a week every 4 weeks. The MTD of melphalan will be determined when used together with bortezomib. Results: To date 15 patients have been enrolled. Median age of patients was 55 years (range from 33 to 70). All patients have received multiple different treatments prior to this study, ranging from 3 to 7. Of the 3 patients in the first dose level who have received 8 cycles of treatment, 2 had major responses and 1 had a minor response based on reduction of serum and urine para-protein. Of the 3 patients in the second dose level who have received 5 cycles of treatment, 1 had a partial response and 2 had stable disease. Three patients in the third dose level have received 4 cycles of treatment. Among them, one had partial response and 2 had stable disease. Three patients in the forth dose level and 3 patients in the fifth dose level have just received their second and third cycles of treatment and are too early to be evaluated. Up to now, the mediate follow-up period is approximately 3 months. Among all the patients who responded to the treatment, 3 patients (1 with major response, partial response and minor response, respectively) have relapsed. The treatment has been well tolerated with minimal neurotoxicity. No grade 4 toxicity was observed and one patient developed a grade 3 transient ischemic attack. The remaining toxicities were either grade 1 or 2 in 8 patients: Conclusion: These preliminary results suggest that combination of low doses of bortezomib and melphalan are effective in treating refractory and relapsed MM patients and may be a promising new treatment for relapsing myeloma.

Preliminary Findings in a Phase 1/2 Study of Trisenox(R) (arsenic trioxide) Dosed Twice Weekly in Patients with Advanced Multiple Myeloma

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Background: Trisenox(R) (arsenic trioxide) injection is highly effective for the treatment of relapsed or refractory acute promyelocytic leukemia (APL). Trisenox(R) has unique, multifaceted mechanisms of action offering a scientific rationale for investigation in diseases other than APL. At clinically relevant concentrations, it causes apoptosis in various tumor cell lines and has anti-angiogenic effects in vitro and in vivo. Human-myeloma-derived cell lines and freshly isolated myeloma cells are particularly sensitive to Trisenox(R), and there is no apparent cross-resistance in myeloma cell lines that are resistant to other agents. A number of clinical trials are investigating ATO in MM. Methods: This ongoing, single-center, phase 1/2 trial is being done to evaluate the safety and efficacy of ATO given twice weekly as a single agent to patients who have relapsed from conventional therapies for multiple myeloma. The study also will describe safety and efficacy of ATO given with high-dose corticosteroids to patients whose disease progresses after ATO. Results: To date 15 patients have been enrolled. One patient did not meet entry criteria for this study. Trisenox(R) was well tolerated with only one drug-related SAE of epistaxis reported. Preliminary Assessment: In patients with advanced MM, Trisenox(R) shows signs of activity, both as a single agent and in combination with steroids, and is tolerated at the 0.25mg/kg dose. The dose was increased recently to 0.35 mg/kg using the same schedule; to date 3 patients have received the higher dose with one patient showing an objective response. In addition to this study, further clinical trials are planned using Trisenox(R) in combination with low dose oral melphalan. These additional trials are based on the tolerability of Trisenox(R) and studies that show the chemosensitization effects of Trisenox(R) on myeloma cell lines.

Mechanisms of action and determinants of sensitivity for arsenic trioxide in multiple myeloma

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Arsenic trioxide (ATO) is emerging as a standard therapy for refractory acute promyelocytic leukemia. We have reported that the combination of ATO and ascorbic acid is an effective strategy in chemoresistant myeloma cell lines and in plasma cells from
Melphalan, Arsenic Trioxide (ATO) and Ascorbic Acid Combination Therapy (MAC) in Refractory and Relapsing Multiple Myeloma (MM)

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Pre-clinical studies in our laboratory have shown that the addition of arsenic trioxide to chemotherapy can greatly reduce the concentration of chemotherapy required to kill myeloma tumor cells compared to chemotherapy alone. As a result on this, we evaluated a combination of oral melphalan (0.05-0.1 mg/kg qd for four days every six weeks), ATO (0.25 mg/kg IV qd for the first four days followed by twice weekly infusions) and ascorbic acid (1 g IV qd for the first four days followed by twice weekly infusions) to treat eight patients with refractory MM (ages 47-63; two males and six females; IgG subtype (n=3), IgA subtype (n=3) and light chain disease (n=2) associated renal failure (n=5; serum creatinine 2.1, 3.1, 5.1, 5.2, and 6.1 mg/dL)) for a duration ranging from 14-37 weeks. The patients had failed multiple prior therapeutic regimens including high dose glucocorticoids (n=6), VAD (n=6), melphalan (n=3), autologous peripheral stem cell transplantation (n=3), thalidomide (n=6) and PS-341 (n=3) prior to MAC administration. At the time of initiation of MAC therapy, all the patients were on oral glucocorticoids (oral methylprednisolone, prednisone or dexamethasone) which was maintained during MAC therapy. Seven of the eight patients responded with a reduction of serum paraprotein from 25-45% and 26-58% reduction in the urine M-protein. Importantly, in four of the five patients with renal failure, renal functions improved with a reduction of serum creatinine from 39-56% reduction in the serum creatinine (from 2.3 to 1.4 mg/dL, 5.1 to 2.4 mg/dL, 5.1 mg/dL to 2.9 mg/dL, and 6.2 to 2.7 mg/dL). One patient with profound hypercalcemia despite treatment with zolendronic acid normalized her serum calcium after one cycle of MAC therapy. Two of the eight patients progressed after responding for 21 and 26 weeks. MAC was generally well tolerated. One patient with light chain disease with associated renal failure failed to respond to MAC treatment. The most common adverse events noted with this combination were marrow suppression (anemia n=8, leukopenia n=6 and thrombocytopenia n=4), prolongation of the QT interval (n=3), gastrointestinal symptomatology (n=3), reactivation of zoster (n=2), headache (n=2), pulmonary edema (n=1), hyperpigmentation (n=1) and neuropathy (n=1). The combination of arsenic trioxide, ascorbic acid and low-dose oral melphalan appears to be effective and well tolerated treatment for patients with refractory and relapsing myeloma, including patients with renal failure. Because of these encouraging results, a large Phase II trial is beginning.

377 A Peptide Trivalent Arsenical Induces Apoptosis in Myeloma Cell Lines

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High-dose chemotherapy and autologous transplantation are unable to prevent disease progression in the great majority of patients with multiple myeloma. Novel therapies which target the interactions between malignant plasma cells and the bone marrow microenvironment, such as thalidomide, proteasome inhibitors and arsenic trioxide, have the potential to achieve durable remissions, even in patients whose disease has become refractory to standard chemotherapy. In previous reports, arsenic trioxide (As2O3) has been shown to induce apoptosis in a range of myeloma cell lines via caspase-9 activation. This effect can be augmented in vitro by the concurrent addition of ascorbic acid, which depletes intracellular glutathione. Clinical trials of As2O3 are underway in myeloma, but as with thalidomide, drug toxicity may limit its utility in older patients. “Second-generation” agents with reduced toxicity are urgently needed to improve patient outcomes.
As reported for MM cells, PS341 caused activation of endogenous apoptotic routes in PCL cells. These results open a new avenue for the treatment of patients with primary PCL who are usually refractory to conventional therapies.

379 Arsenic trioxide (Trisenox®), Ascorbic acid (AA), and Dexamethasone (Dex) pulses-TAD for relapsed refractory progressive multiple myeloma patients

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Trisenox® (arsenic trioxide; ATO) is a novel anticancer agent whose unique, multifaceted mechanisms of action offer a scientific rationale for investigation in many hematologic malignancies. At clinically relevant concentrations it causes apoptosis in human myeloma-derived cell lines. Freshly isolated myeloma cells are particularly sensitive to Trisenox®, and no apparent cross-resistance was seen in myeloma cell lines that are resistant to other agents. Two phase II studies of Trisenox® in advanced, heavily treated myeloma reported ≥25% decreases (40-50% of treated individuals) in serum M-protein concentrations. Available data from both studies suggest that long term therapy might result in enhanced quality of responses. More recently it has been shown that ATO sensitizes myeloma cells to Dex in vitro. In addition, the group at the University of Miami has shown that ascorbic acid (AA) potentiates the effect of ATO on different plasma cell lines as well as human cell lines. We therefore initiated a phase II trial combining ATO with Dex and AA. Multiple myeloma patients with active, progressive disease who have failed no more than 2 different regimens were eligible for enrollment. The treatment regimen is as follows. Cycle 1: Week 1, loading with ATO at 0.25mg/kg IV d1-5, AA 1000mg IV within 30 minutes after each ATO infusion and Dex 40 mg orally d 1-4. Weeks 2 through 12; ATO at 0.25mg/kg IV twice weekly, AA 1000mg IV within 30 minutes after each ATO infusion, and Dex 40 mg orally days 11-14, 29-32, 39-42, 57-60, & 67-70. Weeks 13 through 15, is a rest period. Cycles 2 and 3 are the same as cycle 1 except the Dex frequency is reduced to once a month as follows; Dex 40 mg orally days 1-4, 29-32, 57-60, & 67-70. 15 patients are currently enrolled, and 14 are evaluable for response and toxicity. Median age is 64, β2 microglobulin 3.3mg/dl, and median serum albumin is 3.7. 14 patients completed cycle 1 (3 months), and 4 completed cycle 2 (6 months). The regimen was well tolerated with one patient experiencing grade 4 bony pain, and one patient each experiencing grade 3 hyperglycemia, headaches, gram- positive bacteremia, and fatigue. 6 patients (42%) achieved >50% reduction in M-protein following cycle 1 of therapy with one patient achieving near complete remission, Seven patients had stabilization of their disease process. 1 of the stable disease patients progressed in the midst of the second cycle. Preliminary results suggest the combination is active in this group of relapsed myeloma patients. This initial response could be partly explained by the use of the Dex. Historically, single agent Dex responses are not durable; longer follow up will elucidate the effect of Trisenox®’s role in sensitizing myeloma cells, to Dex as reflected in response and durability.

We attached the trivalent arsenical, phenylarsenonic acid, to the free thiol of glutathione, a GlyCysGlu tripeptide, to produce 4-(N-(S-glutathionylacetyl)amino) phenylarsenoxide (GSAO). The water-solubility of GSAO restricts its entry into cells, which we thought might improve the efficacy/toxicity ratio. The corresponding pentavalent arsenical, 4-(N-(S-glutathionylacetyl)amino)phenylarsonic acid (GSAA), was used as the control for GSAO. The effects of As2O3, GSAO and GSA on the viability of three cultured human myeloma cell lines (ARH77, RPMI 8226 and U266) was measured. Apoptosis of myeloma cells was assessed by flow cytometry, using binding of Annexin V-FITC and 7-aminactinomycin D (7AAD). Annexin V binds to anionic phospholipids exposed early in apoptosis, while 7AAD staining of nuclear DNA indicates a subsequent loss of membrane integrity. Consistent with previous reports, 0.01mM As2O3 induced apoptosis of all three cell lines, which was augmented in the presence of 0.1mM ascorbic acid. Incubation with GSAO for 24h also induced greater than 90% apoptosis at 0.5mM in the ARH77 and U266 cell lines, and at a concentration of 0.1mM for RPMI-8226 cells. In both ARH77 and U266 cells, the addition of 0.1mM ascorbic acid did not increase the effect of GSAO. The control compound, GSAA, did not induce apoptosis in the cell lines. These data indicate that GSAO is toxic for myeloma cells, although it is not as potent as As2O3. The lack of augmentation by ascorbic acid suggests that GSAO’s mechanism of action is different to that of As2O3. There were no signs or symptoms of toxicity from thrice weekly S.C. administration of 10 mg/kg GSAO to mice for 22 weeks. We now plan to compare the efficacy versus toxicity of GSAO and As2O3 in a mouse model of myeloma.

378 Proliferation Studies and Biochemical Analysis of PS341 in Cells from Primary Plasma Cell Leukemia Patients.

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Primary plasma cell leukemia(PCL) is a rare disorder characterized by increased numbers of circulating plasma cells, common organ and tissue infiltration and very poor prognosis. Novel treatment strategies are required in order to improve the outcome of these patients. We report here the in vitro effect of the proteasome inhibitor PS341 on fresh PC obtained from patients with primary PCL. The effect of PS341 was compared with that of four other drugs: dexamethasone, the Erk pathway inhibitor PD98059, the Akt pathway inhibitor LY294002, and the farnesyltransferase inhibitor FPTIII. The PC were characterized by immunophenotyping and molecular cytogenetics showing complex structural and numerical chromosomal changes. In both patients PS341 caused a potent inhibition of PC growth as measured by an MTT assay: 10nM PS341 induced a reduction of cell viability to 20% (control untreated cells being considered as 100%) with an IC50 around 2 nM. This inhibition was dose dependent. In patient 1, but not in patient 2, the FPTIII caused a marked effect on cell viability (<25%). Erk and Akt pathway inhibitors as well as dexamethasone showed a marginal effect on PC growth (approx. 70 % cell viability). Biochemical analysis of the action of PS341 indicated that the drug caused caspase 3 and PARP cleavage in peripheral PCL cells from two patients. In addition, PS341 did not substantially affect the levels of classical cell cycle inhibitors such as p21 or p27, or the Akt protein. Thus, as reported for MM cells, PS341 caused activation of endogenous apoptotic routes in PCL cells. These results open a new avenue for the treatment of patients with primary PCL who are usually refractory to conventional therapies.
380 EFFECT OF STI571 ON MYELOMA CELL PROLIFERATION
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Multiple myeloma (MM) is a plasma cell disease characterized by the accumulation of monoclonal plasma cells in the bone marrow. In spite of important recent advances in the understanding of MM pathophysiology, the outcome of MM patients is still poor. As occurs with most other tumors, several avenues of research are being explored in order to improve treatment. We have investigated the effect of STI571 on MM cells. This drug inhibits the kinase activity of Abl, the platelet-derived growth factor receptor (PDGFR) and c-Kit. STI571 was able to block proliferation of MM cell lines and myeloma cells from patients. In MM cells STI571 inhibited cell cycle progression. Microarray studies of cell cycle proteins as well as Western blot analyses showed that STI571 increased the levels of p21 and p16, but decreased p27. STI571 inhibited the proliferation of MM cells resistant to dexamethasone or melphalan, and had an additive effect when combined with dexamethasone on MM cells partially sensitive to the steroid drug. This opens the interesting possibility that association of STI571 to conventional treatments used in myeloma treatment may be of benefit in the therapy of this disease.

(Supported by Grant from Spanish FIS G03/136).

381 PHASE I/II STUDY WITH BENDAMUSTINE HYDROCHLORIDE IN PATIENTS WITH PROGRESSING MM AFTER 1-2 CYCLES OF HIGH DOSE THERAPY AND AUTOLOGOUS STEM CELL TRANSPLANTATION
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High-dose therapy followed by autologous bone marrow transplantation improved response rates, disease-free survival, and overall survival in comparative studies with standard chemotherapy in patients with advanced multiple myeloma. However, the chance of definitive cure with this treatment modality is low, especially in patients with bad risk parameters. Thus, most of the patients will relapse following high dose chemotherapy and there is an increased need for treatment strategies in patients with multiple myeloma progressing after high dose chemotherapy and autologous stem cell transplantation.

Bendamustine hydrochloride is an alkylating agent chemically related to chlorambucil. It has been used as monotherapy or in combination with other agents in the treatment of a range of solid tumours and malignant lymphomas. Clinical studies evaluating bendamustine hydrochloride in patients with multiple myeloma have been performed since 1975 and the drug has been shown to be effective as monotherapy as well as in combination with prednisolone in patients with de novo and pretreated multiple myeloma. Here we present a dose-finding phase I/II study of bendamustine hydrochloride in patients progressing after 1 or 2 cycles of high dose chemo(radio)therapy and autologous stem cell transplantation. Patients up to the age of 70 years with a WBC > 3000/µl and a platelet count of > 100 000/µl were eligible if the creatinine level was below 2x and the serum bilirubine and transaminase levels < 3x the normal values. The drug was administered intravenously on 2 consecutive days over 30 min. and repeated in the same dosage very 4 weeks for a minimum of 2 and a maximum of 6 cycles. 4 patients received 2x 60 mg/m2 (2 – 6 cycles), 8 patients 2x 70 mg/m2 (1-5 cycles) and 6 patients 2x 80 mg/m2 (2 – 4 cycles) and 4 patients were started on 2x 90 mg/m2. None of the 20 evaluable patients developed dose-limiting hematotoxicity as defined by an ANC < 0.5 x 109/l for > 7 days or an ANC < 0.1 x 109/l for > 3 days or a platelet count < 25 000/µl. One patient who did not take Pcp-prophylaxis developed a PCP, no other serious infectious complications were observed. 8 (40%) patients responded (MR or PR), whereas 6 patients each showed stable or progressive disease, respectively. Thus, bendamustine hydrochloride in dosages up to 2x90 mg/m2 every 4 weeks seems to be very well tolerated even in patients who had undergone one or two cycles of high dose chemo(radio)therapy and autologous SCT. Thus we will continue the dose escalation and will evaluate an improvement of the efficacy of bendamustine (RR 40%) if applied in higher dosages in patients with progressive disease after autologous SCT.

382 Treatment with farnesyl transferase inhibitor FPT III inhibits BMSC-mediated Ras/MAPK activation and induces apoptosis in multiple myeloma cells
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Introduction: It has been shown that Ras signaling plays a crucial role in the pathogenesis of some hematological malignancies. Activation of the Ras/MAPK pathway can be achieved through various stimuli, e.g. cytokines (IL-6), and certain mutations in the Ras genes. It is thought that at early stages the growth of multiple myeloma (MM) cells is dependent on support from the bone marrow (BM) microenvironment, which at later stages, through the accumulation of mutations, e.g. in the Ras genes, is no longer required. Therefore, pharmacological interference with Ras signaling could be of considerable therapeutic interest. Farnesyl transferase inhibitors (FTI) are a novel class of drugs that have been developed to inhibit the growth of Ras-dependent tumors. They can block the farnesylation of Ras, which is an essential posttranslational modification for its activation, and consequently inhibit Ras-triggered pathways (such as the MAPK/ERK1,2 pathway). Here we asked, whether activation of the Ras/MAPK pathway through constitutively active Ras or through the presence of BM stromal cells (BMSCs) contributes to the survival of MM cells.

Experimental model: Two human MM cell lines that either harbour an activating Ras mutation (INA-6) or contain wild-type Ras (U266) were treated with the FTI FPT III to determine the role of their Ras status for their survival. Additionally, the IL-6-dependent cell line INA-6 or primary MM cells were cultured either in the presence or absence of BMSCs, and FTI-mediated effects were analyzed.

Results: Constitutive activation of the Ras/MAPK pathway was found in the INA-6 as well as in the U266 cell line. Co-culturing INA-6 cells with BMSCs resulted in enhanced constitutive phosphorylation of ERK1 and ERK2. The FTI FPT III effectively inhibited BMSC-mediated Ras/MAPK activation and induces apoptosis in multiple myeloma cells.
blocked the translocation of Ras to the plasma membrane, Ras activation and ERK1,2 phosphorylation in both cell lines and also in the INA-6/BMSC co-culture model. Treatment with FTI induced apoptosis of MM cells (cell lines and primary cells) and was equally effective in the presence or absence of BMSCs. Conversely, the human Burkitt lymphoma cell line Namalwa, which lacks constitutive ERK1,2 activity, was resistant against FTI-induced apoptosis.

Conclusions: Treatment of MM cells with FPT III blocks the Ras/MAPK pathway and induces apoptosis in the absence and in the presence of BMSCs. Our experiments support the hypothesis that the sensitivity of MM cells to FTIs depends on the activation status of the Ras/MAPK pathway rather than on the mutation status of Ras. These findings underscore the potential of FTIs for the development of novel therapeutic strategies for the treatment of MM.

383 Effect of farnesyl transferase inhibitor R115777 alone or in combination with incadronate on the growth of fresh and cloned myeloma cells in vitro
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Multiple myeloma (MM) is a malignancy of B cells characterized by the monoclonal proliferation of malignant plasma cells. Several chemotherapeutic regimens and high-dose chemotherapy supported by autologous stem cell transplantation (auto-SCT) have been employed to improve the survival of patients with MM but many patients relapsed for a long time follow up period. Therefore there is an obvious need for new therapeutic strategies. Ras gene mutations occur in 30% to 40% of multiple myeloma (MM), and farnesylation is the first step in the posttranslational modification of Ras proteins. Zarnestra TM (R115777) is one of the potent farnesyl transferase inhibitors (FTIs), but is less effective to K-Ras mutation, which is one of the most common mutations in MM, than N- and H-Ras mutations. It has recently been reported that bisphosphonates have direct antitumor effect in vitro and in vivo, and nitrogen-containing bisphosphonates, such as incadronate, inhibit the mevalonate pathway and prevent post-translational prenylation of GTP-binding proteins. Mevalonate pathway is essential for the biosynthesis of sterols and long chain isoprenoid lipids, including farnesylpyrophosphate (FPP) and geranylgeranylpyrophosphate (GGPP) which are substrates for the FTase and geranylgeranyl transferase (GGTase) 30. Based on these observations, we examined the effect of R115777 alone or in combination with incadronate on the growth of fresh and cloned myeloma cells in vitro. R115777 inhibited the growth of fresh and cloned myeloma cells dose-dependently. Flow cytometric analysis using annexin V and 7AAD showed that R115777 induced apoptosis of two of three myeloma cell lines at the concentration of 1.0×10-8 mol/L. The inhibition of cell growth was intensified when R115777 was combined with incadronate at 1.0×10-6 mol/L and 1.0×10-5 mol/L. R115777 appears to be a potent inducer of apoptosis in fresh and cloned myeloma cells, and the effect was intensified in combination with incadronate. R115777 alone or in combination with incadronate might have some benefit in the treatment of myeloma patients.

384 MOLECULAR CHARACTERISTICS OF APOPTOSIS INDUCED BY THE FARNESYLTRANSFERASE INHIBITOR (FTI) BMS-214662 IN HUMAN MYELOMA CELLS
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We have studied the molecular mechanism of apoptosis induced in vitro, by the FTI BMS-214662, in several human multiple myeloma (MM) cell lines (RPMI-8226, NCI-H929, U266 and IM-9). In MM cells treated with BMS-214662 farnesylation of cytosolic chaperone HDJ-2 and of lamin A was clearly observed. BMS-214662 treatment induced the typical morphology of apoptosis, including condensation and fragmentation of chromatin, in all MM cell lines at low doses (0.3-1 µM) after 24 h incubation. Cell death was not prevented by co-treatment with the protein synthesis inhibitor cyclohexamide. Apoptosis was accompanied by cell shrinking, phosphatidylserine exposure in plasma membrane, loss of mitochondrial membrane potential (Δψm), activation of caspases 9 and 3 and traslocation of Apoptosis-Inducing Factor (AIF) from mitochondria to nucleus. Cotreatment of cells with the pan-caspase inhibitor Z-VAD-fmk prevented development of apoptotic morphology and death in RPMI8226 and U266 cells. Z-VAD-fmk attenuated some morphological and biochemical characteristics of apoptosis in NCI-H929, but did not prevent cell death. Selective inhibitors of caspase-3 or caspase-9 did not block cell death in any cell line tested. Levels of antiapoptotic proteins Bcl-2 and Mcl-1 decreased in IM-9, RPMI-8226 and U266 cells upon treatment with BMS-214662. This FTI caused a concomitant increase in levels of pro-apoptotic BH3-only proteins Bik and Bim during treatment. Multidomain, proapoptotic Bax protein levels also increased in U266 treated with BMS-214662. These results support the potential usefulness of FTIs as new therapeutic agents for the therapy of MM.

385 Rituximab-mediated signaling and sensitization of 8226/S and 8226/Dox40 MM cell lines to paclitaxel-mediated apoptosis.
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Rituximab is a chimeric mouse anti-human CD20 monoclonal Antibody that has been approved by the FDA for the treatment of patients with low grade and follicular NHL. The response rate is approximately 50%. Following initial treatment, patients with MM develop chemo-resistance. Thus, there is a need for alternative therapeutic approaches. Previous findings from our laboratory have demonstrated that Rituximab sensitizes NHL cell lines to various drug-mediated apoptosis (Clinical Cancer Research, 7: 709, 2001, Cancer Research, 61: 5137, 2001). The objective of this study was to investigate whether Rituximab has an effect on MM. We have used the drug sensitive 8226/S parental and the doxorubicin-resistant variant 8226/Dox40 cell lines as a model system. A fraction of the cells (~20%) expressed CD20 as determined by flow cytometry. Treatment of the 8226/S and 8226/Dox40 cells with Rituximab (20 ng/ml-24h) resulted in
significant inhibition of cell proliferation. Further, pretreatment of the 8226/S and 8226/Dox40 cells with Rituximab followed by treatment with paclitaxel (10nM) for an additional 24h resulted in significant potentiation of cytotoxicity and synergy was achieved. The synergy in cytotoxicity was determined to be due to the induction of apoptosis. The mechanism by which Rituximab sensitized the 8226/Dox40 cells to paclitaxel-mediated apoptosis was investigated. Using western blot analysis it was found that treatment with Rituximab (20 µg/ml-24h) resulted in selective down-regulation of anti-apoptotic Bcl-xL expression with little effect on other gene products involved in apoptosis (c-IAP1, c-IAP2, XIAP, survivin, Mcl-1, Bcl-2). In addition, treatment with paclitaxel (10nM) arrested the cells at the G2/M phase of the cell cycle and resulted in the down-regulation of Mcl-1, Bcl-2, Bcl-xL, c-IAP1 and survivin. These various gene modifications by Rituximab and paclitaxel resulted in functional complementation and the induction of the apoptotic signaling pathway. These findings suggest that signal I mediated by Rituximab and resulting in down regulation of anti-apoptotic Bcl-xL was important to circumvent the resistance of 8226/Dox40 cells to paclitaxel (signal II)-mediated apoptosis. Further, even though the expression of CD20 was low, nevertheless, the combination treatment resulted in significant percentage of cells undergoing apoptosis. The present findings also demonstrate that Rituximab-mediated sensitization of the 8226/Dox40 cells to paclitaxel mediated apoptosis is independent of the MDR phenotype of the cells, as the functionality of the MDR pump was unaffected by single or combination treatments. These findings are of potential clinical significance. (Supported in part by the Jonsson Comprehensive Cancer Center At UCLA (A. J.).

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“An Antibody-avidin Fusion Protein Targeting the Human Transferrin Receptor Inhibits the Growth and Induces Apoptosis in Eight Human Malignant Plasma Cell Lines”

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We have previously reported that an anti-rat transferrin receptor (TfR) IgG3-avidin fusion protein exhibits anti-proliferative/pro-apoptotic activity against the rat myeloma cell line Y3-Agl2.3 and the rat T-cell lymphoma cell line C58(NT)D.1, GOVAR.1. This activity was not observed in two rat cell lines of nonhematopoietic lineage (bladder carcinoma BC47 and gliosarcoma 9L) suggesting the potential use of anti-TfR IgG3-avidin for the treatment of hematopoietic malignancies. In addition, an anti-human TfR-IgG3-avidin fusion protein (anti-hTfR-Av) was shown to inhibit proliferation and induce apoptosis in human erythroleukemia cell line K562 (Ng et al., PNAS, 2002, 99:10706). Recent studies demonstrated that anti-hTfR-Av also inhibits the growth and induces apoptosis in the human malignant plasma cell lines 8226/S, 8226/Dox40 [doxorubicin-resistant and also multidrug resistant], U266, MM.1S, OCI-MY5, S6B45, ARH-77, and IM-9, although different levels of sensitivity were observed. Interesting, such anti-proliferative/pro-apoptotic activity seems not to be correlated with the level of TfR expressed on the surface of the cells. We are currently investigating the mechanism by which anti-hTfR-Av mediates its anti-proliferative/pro-apoptotic activity. We will also determine if it can be used in combination with various agents currently used in the treatment of hematopoietic malignancies. Importantly, anti-hTfR-Av can be further loaded with biotinylated anti-cancer drugs, which may increase its intrinsic anti-cancer activity. These approaches may lead to more effective therapeutic strategies for both eradication of plasma cell malignancies such as multiple myeloma and ex vivo purging of plasma cancer cells in autologous transplantation.

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A chimeric humanized anti-CD40 antibody renders human multiple myeloma (MM) cells refractory to the mitogenic and protective effects of IL-6

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CD40 is expressed on B-cell malignancies including human multiple myeloma (MM) and a variety of carcinomas, and there is considerable interest in using CD40 activating agents as novel cancer therapies. Recently, a humanized anti-CD40 Ab (SGN-14) demonstrates a significant anti-tumor effect in SCID mice xenografted with human MM (Cancer Res. 60, 3225-3231, 2000). In this study, we examined the therapeutic impact of SGN-14 in human MM using MM.1S cells (CD138+++/CD40+) and plasma cell leukemia patient cells. SGN-14 (0.01-100 µg/ml) did not significantly stimulate proliferation of CD40-expressing MM.1S and MM.1R or two patient plasma cell leukemia cells (p > 0.1 for all samples tested). SGN-14 (5 µg/ml) neither significantly induce AKT nor NF-κB activation in MM.1S cells, in contrast to 5 µg/ml sCD40L-treated counterparts. ERK activation was induced by SGN-14, although to a lesser extent than sCD40L. SGN-14 did not block nor enhance AKT/NF-κB activation induced by sCD40L at the same concentration (5 µg/ml). Notably, 24-hr pretreatment of cells with SGN-14 blocked sCD40L-mediated PI3K/AKT and ERK activation. In contrast, cells treated with sCD40L for 24 h still retained the ability to further activate downstream signaling pathways in response to sCD40L. Importantly, pretreatment of MM.1S cells with SGN-14 rendered them refractory to further proliferation induced by IL-6 (p < 0.05): the two-fold increase of DNA synthesis triggered by Dex and IMiDs (0.01-10 µM) in cells pretreated with SGN-14 for 24-48 hrs. Using oligonucleotide microarray analysis, we first found differential gene expression profile between SGN-14-treated vs sCD40L-treated MM.1S cells and identified an approximately three-fold reduction of interleukin 6 receptor (IL6R) expression in SGN-14-treated MM.1S cells over 6-24 hr. Currently, the mechanism by which SGN-14 down regulates IL6R and possibly CD40 receptor is under further investigation. In addition, VEGF secretion was inhibited in the presence of SGN-14 (0.01-10 µg/ml) in MM.1S and MM.1R cells, as well as two plasma cell leukemia patient cells, correlating with reduced baseline migration of plasma leukemia cells in the presence of SGN-14. SGN-14 also induces antibody-dependent cell-mediated cytotoxicity and enhanced tumor cell lysis by effectors treated
with IMiD (1 μM). Finally, in the presence of cycloheximide (chs) to inhibit de novo protein synthesis, Dex-sensitive MM.1S and Dex-resistant MM.1R cells responded to a similar killing (50-60%), suggesting that proapoptotic mechanisms induced by SGN-14 depend on endogenous production of cytotoxic cytokines for induction of cell death. From oligomicroarray analysis, we further identified a 3-5 fold inhibition in anti-apoptotic genes (Mcl-1, Bcl-XL, FLIP) and an increase of apoptosis proteins (Apo2L/TRAIL, p53-regulated apoptosis-inducing protein 1, apoptosis protease activating factor). Taken together, these results further support targeting CD40 in human MM with SGN-14.

388 Therapeutic Potential of Amyloid-Reactive Monoclonal Antibodies in Primary (AL) Amyloidosis

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We have previously reported that certain of our murine (m) anti-human light chain monoclonal antibodies (mAbs) recognized an epitope common to AL and other types of amyloid fibrils (Hrncic R, et al, 2000, Am J Path 157:1239-1246). Based on this evidence, one such antibody, 11-1F4, was administered to mice bearing AL amyloidomas, induced by the subcutaneous injection of human AL extracts. The mAb bound to the amyloid and initiated an Fc-mediated cellular inflammatory response that led to the rapid elimination of the induced tumors. To develop this reagent for clinical use, the 11-1F4 mAb was chimerized and its activity compared to that of the unmodified antibody. The chimeric (c) 11-1F4 mAb was produced in CHOdhfr-stable mammalian cell lines that had been transfected with a supervector S261 the conjugate by the cells. Antibody-DM1 conjugates that target antibody (mAb). The antibody-DM1 conjugate acts as a pro-drug potent and selective when covalently linked to a monoclonal drugs. A novel maytansinoid, DM1, was developed, that is very selectively improve the therapeutic anti-cancer efficacy of these Immunoconjugates of cytotoxic drugs have the potential to further support targeting CD40 in human MM with SGN-14.

390 Inhibitors of the mevalonate pathway as potential therapeutic agents in multiple myeloma.

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Clinical studies have suggested that in addition to preventing osteoclast-mediated osteolytic bone disease, bisphosphonates (BPs) may induce a reduction of the tumour burden and prolong the survival of MM patients. Evidence from in vitro studies by a number of groups indicates that nitrogen-containing BPs such as Zometa interfere with osteoclast recruitment, differentiation and action, and induce apoptotic cell death of these cells by disrupting the mevalonate pathway. A large body of evidence indicates that the mevalonate pathway plays an important role in cell growth and survival. Mevalonate is synthesized intracellularly from 3’hydroxy-3-methylglutaryl coenzyme A (HMGCoA) in a process catalysed by HMGCoA reductase, the rate-limiting enzyme in this pathway. Mevalonate metabolism yields a series of isoprenoid compounds that are incorporated into cholesterol, isopentenyl adenine, prenylated proteins and other end products essential for cell growth. Data generated in our laboratory has demonstrated that inhibitors of the mevalonate pathway vary in their ability to inhibit cellular proliferation and/or induce cell death in myeloma cell lines. We found that the HMGCoA reductase inhibitor fluvastatin inhibits proliferation of a number of myeloma cell lines of different clinical origin more effectively than the farnesyl transferase inhibitor SCH66336 or Zometa, an antigen present on multiple myeloma (MM) cells could therefore be tested as a novel strategy for anti-myeloma treatment. CD56 (N-CAM) is expressed on malignant plasma cells in a subpopulation of MM patients. Hu-N901 is a humanized mAb that binds to CD56 with high affinity. We therefore evaluated the cytotoxicity and specificity of a conjugate of hu-N901 with DM1, huN901-DM1, on a panel of cell lines, that included three CD56-expressing MM cell lines, OPM1, OPM2 and U266, two CD56-negative MM cell lines, LP-1, MM-AS, and a CD56-negative Waldenstrom’s macroglobulinemia (WS) cell line, WSU-WM. The cytotoxicity of the conjugate was evaluated by the MTT assay. HuN901-DM1 treatment decreased survival of CD56+ cell lines (IC50: 50-200 nM after a 48 h exposure), but did not affect CD56- MM or WS cell lines. In contrast, the cytotoxicity of non-conjugated DM1 was equally detected (following a 24 h exposure) in both CD56+ and CD56- cells (5-200 nM). The exposure of cells to the non-conjugated mAb huN901 (6-60 nM, up to 96 h exposure) did not produce any cytotoxic effects. We also examined the cytotoxicity of non-conjugated mAb, huN901-DM1 and DM1 for CD56+ and CD56- cell lines in the presence of bone marrow stromal cells (BMSC). Non-conjugated mAb did not induce any detectable cytotoxicity while huN901-DM1 showed specific activity against CD56+ cells, but not against BMSC. DM1 induced toxicity in all cells including BMSC. To further evaluate the specific activity of huN901-DM1 in a co-culture setting, we evaluated the activity of the immunocomjugate against CD56+ and CD56- cells. By flow cytometry, we detected that huN901-DM1 selectively depleted CD56+ cells. Moreover, induction of apoptosis was detected by Annexin-V in CD56+ cells exposed to huN901-DM1 after 24 h. In vivo efficacy of huN901-DM1 is presently under investigation in hu-SCID MM mice. In conclusion, our data suggest that huN901-DM1 may have therapeutic potential in the treatment of MM.
which acts to prevent the synthesis of geranyl diphasphate, a precursor of FPP and GPP, from mevalonate. After 3 days culture, fluvastatin concentrations as low as 2.5µM significantly inhibited proliferation of all cell lines except RPMI-8226 (p<0.05 by paired student’s t-test). Concentrations of 25µM and 50µM significantly inhibited proliferation in all cell lines (p<0.05 by paired student’s t-test), with inhibition at 50µM ranging from 45% for U266 to >90% for OPM-2. SCH66336 significantly inhibited proliferation of LP-1, RPMI-8226 and NCI-H929 cell lines (p<0.05 by paired student’s t-test) but not U266 and NCI-H929 at a concentration of 5µM; with inhibition ranging from less than 1% for OPM-2 to 33% for NCI-H929. Zometa only significantly inhibited the proliferation of RPMI-8226 and OPM-2 myeloma cell lines (p<0.05 by paired student’s t-test) at a concentration of 100µM. The reduction of cellular proliferation at this concentration ranged from 9% for NCI-H929 to 86% for RPMI-8226. Immunoblot analysis of whole cell lysates from MM cells following 72hr treatment with inhibitors of the mevalonate pathway indicated changes in prenylation status of treated cells compared to untreated cells. Antibodies to Rap 1, Rab 5 and pre-lamin A were used, as Rap 1 is modified by type I geranylgeranylation and Rab 5 is modified by type II geranylgeranylation, while a change in the size of lamin A is indicative of a change in farnesylation status. Isobologram analysis to investigate possible synergistic interactions between inhibitors of the mevalonate pathway produced interesting results. Combining SCH66336 with either fluvastatin or zoledronic acid produced results varying from antagonistic, to additive, to synergistic depending on the MM cell line studied. In contrast, fluvastatin and zoledronic acid were found to be highly synergistic in inducing MM cell death. Combinations of fluvastatin or zoledronic acid with the common therapeutic dexamethasone also proved to be highly synergistic in inducing MM cell death.

In summary our data indicates that inhibitors of the mevalonate pathway vary in their ability to inhibit cellular proliferation and/or induce cell death in myeloma cell lines. The HMGCoA reductase inhibitor fluvastatin was more effective than Zometa or SCH66336 in inhibiting proliferation of a number of myeloma cell lines of different clinical origin, suggesting that fluvastatin is a potential therapeutic agent for multiple myeloma that warrants further investigation. Interestingly, combining Zometa and fluvastatin worked synergistically to inhibit proliferation of all myeloma cell lines studied while SCH66336 potentially antagonized the activity of fluvastatin or zoledronic acid in some cell lines. The reason for this varying interaction need to be further investigated.

391 THIAZOLIDINEDIONES/PPAR-å AGONISTS: A NOVEL CLASS OF AGENTS WITH ANTI-MYELOMA ACTIVITY

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Thiazolidinediones (TZDs) constitute a class of pharmacological agents which are ligands to the nuclear receptor PPAR-å (peroxisome proliferator-activated receptor-å). TZDs induce adipocyte differentiation and increase insulin sensitivity, while recent studies have shown their activity against PPAR-å-expressing solid tumors (e.g. prostate cancer, liposarcoma etc.). The effect of TZDs against human multiple myeloma (MM) cells was studied with MTT survival assays, PI cell cycle analyses, annexin V/PI staining and Apo2.7-PE staining. We found that ciglitazone and rosiglitazone, at concentrations relevant to those achieved in diabetic patients treated with standard doses of TZDs, induced growth arrest and apoptosis in a panel of 25 MM cell lines (including both cells sensitive and resistant to dexamethasone (Dex), anthracyclines, alkylating agents, Apo2L/TRAIL, thalidomide and its immunomodulatory derivatives, IMiDs), as well as tumor cells derived from MM patients, including patients resistant to IMiDs or the proteasome inhibitor PS-341. The expression of PPAR-å in our panel of cell lines (as assessed by immunoblotting analyses) was variable, but did not correlate with the sensitivity of MM cells to TZDs, or to other agents, either conventional or novel. Furthermore, in our panel of cell lines, the patterns of sensitivity of MM cells to TZDs did not correlate with sensitivity or resistance to Dex, Dtx, thalidomide or its derivatives, PS-341, or Apo2L/TRAIL, suggesting that TZDs exert their anti-MM effects through molecular sequelae highly distinct from other anti-MM agents, conventional or novel. Indeed, the gene expression profile of ciglitazone-treated MM cells was notable for the much less pronounced degree of transcriptional changes in comparison to those triggered by other anti-MM agents (such as the proteasome inhibitor PS-341 or steroids), suggesting that TZD-induced effects on MM cells are mediated, at least in part, by post-transcriptional/ post-translational events. Based on high-throughput proteomic analyses of the signaling state of TZD-treated MM cells and subsequent confirmatory immunoblotting studies, we found that TZDs induced phosphorylation of Thr446 and Thr451 at the activation loop of interferon-inducible serine/threonine protein kinase R (PKR, or double stranded RNA (dsRNA)- dependent kinase). Phosphorylated (activated) PKR phosphorylates and inactivates the å subunit of eukaryotic initiation factor 2 (eIF2-å). This TZD-induced phosphorylation of PKR and eIF2å leads to inhibition of translation initiation, and may account, at least in part, for TZD-induced growth arrest/apoptosis. Furthermore, ciglitazone downregulated the expression of intracellular inhibitors of apoptosis, including FLIP and cIAP2, and sensitized MM cells to pro-apoptotic stimuli, including Apo2L/TRAIL. Importantly, TZDs suppressed the
expression of RANKL in MM cells and suppressed cytokine-induced production of VEGF by viable MM cells, indicating that TZDs may not only directly target MM cells, but can also abrogate their interactions with the local BM microenvironment, by inhibiting the stimulation of bone resorption and MM-associated neo-vascularization. The comprehensive direct anti-tumor sequelae and indirect microenvironmental effects of TZDs and, most importantly, their highly favorable profile of side effects, even in the setting of chronic administration of these agents, provide a strong pre-clinical and clinical rationale for trials of TZDs to improve the outcome of patients with MM.

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ANTI-MYELOMA ACTIVITY OF HMG-CoA INHIBITORS (STATINS): FRAMEWORK FOR CLINICAL APPLICATIONS.

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3-Hydroxy-3-methylglurul-CoA (HMG-CoA) reductase inhibitors, including lovastatin, simvastatin, and atorvastatin, have been extensively used for treatment of hypercholesterolemia, and recent studies have also suggested that this class of agents may have anti-tumor activity. The median age of patients affected with MM and WM is >60 years, an age-group where statins are extensively prescribed for hypercholesterolemia, and are well-tolerated. In this context, we studied if HMG-CoA inhibitors have direct anti-tumor activity against MM or WM cells. We found that lovastatin, at low µM and sub-µM concentrations, induces growth arrest and apoptosis in tumor cells (10/10 samples) freshly isolated from relapsed refractory MM patients, including patients resistant to novel immunomodulatory thalidomide derivatives (IMiDs) or the proteasome inhibitor PS-341; in a wide panel of 25 MM cell lines, including those resistant to dexamethasone (Dex), antracyclines, thalidomide/IMiDs, or Apo2L/TRAIL; as well as WM patient tumor cells and the WM-WSU cell line model. Importantly, lovastatin overcomes the anti-apoptotic effects conferred upon MM-1S cells by forced overexpression of bcl-2 or constitutively active Akt. Transcriptomic profiling and proteomic analysis of the signaling state of lovastatin-treated MM cells and subsequent mechanistic studies revealed that lovastatin suppresses constitutive and cytokine (IGF-1, or TNF-α)-stimulated activation of NF-κB; downregulates the levels of several intracellular inhibitors of apoptosis (IAPs); and abrogates constitutive and cytokine (IGF-1, IL-6)-induced expression of proteasome subunits and proteasome activity. Consequently, lovastatin sensitizes MM cells to other pro-apoptotic stimuli, including Dex, Doxo, Apo2L/TRAIL, and PS-341. These pre-studies in support of the anti-MM activity of statins, as well as the significant clinical experience acquired through safe long-term administration of statins for hypercholesterolemia, provide the framework for the ongoing pilot clinical trials of lovastatin therapy for MM patients, with the ultimate goal to provide the contextual framework for more extended studies on the role of this class of agents in the therapeutic management of plasma cell dyscrasias.

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Bendamustin - new therapeutic option for relapsed and refractory multiple myeloma

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Multiple myeloma (MM) is a malignancy of terminally differentiated B-lymphocytes. It is characterized by the clonal proliferation of plasma cells that are innately resistant to standard doses of chemotherapy. Despite modern treatment modalities, including high-dose chemotherapy with stem cell support, multiple myeloma remains incurable in most cases. New treatment modalities are being evaluated to improve response rates and to achieve a cure in MM. Bendamustin is a bifunctional alkylating agent and has been evaluated mainly in the treatment of NHL, multiple myeloma, CLL and breast cancer first in Germany. We used Bendamustin/Prednisone regimen in 16 patients with relapsed and refractory disease for a 2 year period (Bendamustin 100mg/m2 day 1 and 2 plus Prednisone 60mg/m2 days 1 to 4). This regimen results in a clinical response in approximately 50% of patients and a median survival of approximately 20 months. The toxicities profile differs somewhat from that of other alkylating agents. In summary, bendamustin is an effective and well-tolerated drug in the palliative treatment of NHL, including multiple myeloma.
of B and its metabolites, however, was low. Only 20 and 5% of the administered B dose were recovered in urine in patients of Group 1 and 2. B and its metabolites are dialysed in the same quantities as renally eliminated in non-dialysed patients with end-stage renal disease. By means of anova, no differences were found between the two patient-groups in plasma kinetics of B and its metabolites. The toxicities that occurred were salivary gland dysfunction causing dry mouth and taste disturbances, nausea and vomiting, moderate myelotoxicity of CTC grade 2/3 leuko-and thrombocytopenia and worsening of pre-existing lymphocytopenia. These toxicities are well known and manageable. With the exception of a higher frequency of moderate leuko- and thrombocytopenia in patients with renal insufficiency, no difference in toxicity was observed between the two patient groups. Toxic effects of B on liver and kidney function were not observed.

No dose reductions will be necessary in patients with normal liver function and end-stage renal disease, including dialysis-dependent patients, thus B can be applied at 120 mg/m² iv over 30 minutes on days 1 and 2 at 4-week intervals. This may be an advantage over melphalan.

### Table 1. Plasma chitotriosidase after frequent plasmapheresis with HES*

<table>
<thead>
<tr>
<th>Patient</th>
<th>Age (y)</th>
<th>Total HES (l)</th>
<th>HES (l/month)</th>
<th>Creatinine clearance (mL/min)</th>
<th>Plasma chitotriosidase (nmol/h/mL)</th>
<th>Bone marrow biopsy</th>
</tr>
</thead>
<tbody>
<tr>
<td>1 (f)</td>
<td>43</td>
<td>130</td>
<td>6.5</td>
<td>61</td>
<td>6130</td>
<td>Foam cells</td>
</tr>
<tr>
<td>2 (m)</td>
<td>54</td>
<td>18</td>
<td>3.6</td>
<td>88</td>
<td>422</td>
<td>Foam cells</td>
</tr>
<tr>
<td>3 (f)</td>
<td>75</td>
<td>16</td>
<td>2.2</td>
<td>73</td>
<td>307</td>
<td>Foam cells</td>
</tr>
<tr>
<td>4 (f)</td>
<td>54</td>
<td>14</td>
<td>4.7</td>
<td>84</td>
<td>242</td>
<td>Foam cells</td>
</tr>
<tr>
<td>5 (m)</td>
<td>23</td>
<td>425</td>
<td>2.2</td>
<td>120</td>
<td>206</td>
<td>Foam cells</td>
</tr>
<tr>
<td>6 (m)</td>
<td>51</td>
<td>33.1</td>
<td>4.1</td>
<td>112</td>
<td>200</td>
<td>Foam cells</td>
</tr>
<tr>
<td>7 (f)</td>
<td>54</td>
<td>31.5</td>
<td>1.4</td>
<td>78</td>
<td>165</td>
<td>Foam cells</td>
</tr>
<tr>
<td>8 (f)</td>
<td>75</td>
<td>10</td>
<td>10</td>
<td>119</td>
<td>93</td>
<td>Foam cells</td>
</tr>
<tr>
<td>9 (f)</td>
<td>34</td>
<td>67.5</td>
<td>1.6</td>
<td>187</td>
<td>78</td>
<td>Foam cells</td>
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<tr>
<td>10 (m)</td>
<td>66</td>
<td>25.5</td>
<td>1.1</td>
<td>85</td>
<td>60</td>
<td>Foam cells</td>
</tr>
<tr>
<td>11 (m)</td>
<td>34</td>
<td>31.5</td>
<td>4.5</td>
<td>116</td>
<td>59</td>
<td>Foam cells</td>
</tr>
</tbody>
</table>

Moderate leuko- and thrombocytopenia in patients with renal insufficiency, no difference in toxicity was observed between the two patient groups. Toxic effects of B on liver and kidney function were not observed.

No dose reductions will be necessary in patients with normal liver function and end-stage renal disease, including dialysis-dependent patients, thus B can be applied at 120 mg/m² iv over 30 minutes on days 1 and 2 at 4-week intervals. This may be an advantage over melphalan.

### 395 FREQUENT PLASMAPHERESIS WITH HYDROXYETHYL-STARCH (HES) IN MONOCLONAL GAMMOPATHY: RESULTS IN TISSUE INFILTRATION WITH ACTIVATED FOAMY MACROPHAGES

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Hydroxy-ethyl starch (HES, Fresenius) is a chemically modified cornstarch. Because of the reported advantages of synthetic plasma expanders, it has been suggested that HES-products might emerge as a standard replacement fluid in plasmapheresis. The kinetics of HES elimination depend on enzymatic degradation (α-glucosidases/α-amylases) in blood, tissues, and the reticuloendothelial system, followed by urinary excretion. However, tissue and lysosomal storage in macrophages can occur. In Gaucher disease, similar foamy macrophages excrute chitotriosidase (CT) and the level of plasma CT reflects the extent of tissue infiltration. We have investigated the clinical applicability of plasma CT concentration as a parameter of tissue accumulation of HES in patients undergoing chronic plasmapheresis for monoclonal gammopathy or other reasons. Eleven patients receiving frequent plasmapheresis with HES were identified. The cumulative HES dose and renal function (creatinine clearance) were calculated and plasma CT was determined (table 1). When available, bone marrow aspirates were reviewed.

All patients with impaired renal function, who were exposed to a high dose (liters/month) exhibited an increase in plasma CT (normal range 4-195 nmol/h/mL). Foamy macrophages were observed in all available bone marrow biopsies regardless of the plasma CT. One patient (no-1) even developed a severe acquired lysosomal storage disease causing malnutrition and weight loss, myelofibrosis, polynuropathy, organomegaly and ascites due to massive tissue infiltration with foamy macrophages. In this patient, plasma CT increased after a cumulative dose of 20 liters and reached the range of Gaucher disease at a cumulative dose of 85 litres. Conventional plasma expanders which had been used prior to HES exposition had not altered the normal plasma CT concentration in this patient. After cessation of HES, plasmapheresis with conventional expanders did not result in a decrease in plasma CT. This was confirmed by bone marrow biopsies performed a year later, which still revealed massive foamy macrophage infiltration. Based on these results we conclude that in patients with impaired renal function, frequent plasmapheresis with HES results in tissue infiltration with activated, CT secreting foamy macrophages. We believe that the level of plasma CT can be used to monitor tissue storage of HES. Furthermore, excessive administration of HES will result in a severe acquired lysosomal storage disease.

### 396 APOMINE™, A NOVEL INHIBITOR OF THE MEVALONATE/isoprenoid Pathway, PROMOTES APOPTOSIS OF MYELOMA CELLS IN VITRO AND IS ASSOCIATED WITH A MODULATION OF MYELOMA DISEASE IN VIVO

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The process of isoprenylation plays an important role in regulating the function of proteins that are critical in the growth and survival of myeloma cells. Inhibiting the pathways responsible for this process therefore represents a novel approach to controlling the growth of myeloma cells. APOMINE, a novel 1,1 bisphosphonate ester, increases the rate of degradation of HMGCoA reductase, thereby inhibiting the mevalonate pathway and preventing protein prenylation. The aim of the present study was to determine whether APOMINE could induce apoptosis in myeloma cells, influence osteoclast formation and bone resorption in vitro and modulate the myeloma disease in vivo. Treatment of the human myeloma cell lines NCI H929, RPMI 8226 and JJN-3 with 2-20µM APOMINE induced a dose-dependent increase in apoptosis, as identified by characteristic changes in nuclear morphology and by an in situ nick translation assay (p<0.001). APOMINE had no effect on the accumulation of cells in the S phase of the cell cycle; which we have previously seen with bisphosphonic acids. To investigate the effect of APOMINE in vivo, 5T2MM murine myeloma cells were injected intravenously into C57BL/KaLwRij mice. After 8 weeks all animals injected with tumour cells had detectable serum paraprotein and were treated with APOMINE in the diet (200mg/kg) (n=10), or vehicle (n=10) for a further 4 weeks when all animals were sacrificed. All animals injected with tumour...
cells and treated with vehicle had radiographically detectable osteolytic bone lesions (p<0.001), decreased cancellous bone volume (p<0.05), decreased bone mineral density and an increase in osteoclast number (p<0.01). APOMINE treatment caused a 12% decrease in serum paraprotein and an 18% decrease in tumour burden. APOMINE prevented the development of osteolytic lesions by 59% (p<0.05) and was associated with a partial protective effect on cancellous bone volume. APOMINE had no significant effect on osteoclast number, which was seen previously with bisphosphonics in this model. To determine whether APOMINE could affect osteoclast formation and bone resorption directly, peripheral blood mononuclear cells were treated with APOMINE (10 M) or a bisphosphonic acid (10 M) in the presence of RANKL, M-CSF, dexamethasone and 1,25-dihydroxy vitamin D3. While a bisphosphonic acid completely prevented the formation of TRAP-positive multinucleated osteoclasts and bone resorption, APOMINE, at the concentrations used, had no significant effect on osteoclast formation or bone resorption. These data demonstrate that APOMINE is able to promote myeloma cell apoptosis in vitro and inhibit the development of lytic bone lesions in vivo. Interestingly, in this model APOMINE had no effect on osteoclasts in vitro or in vivo at the concentrations studied, suggesting that the effects of APOMINE on development of myeloma may be a direct effect on myeloma cells or via cells, other than osteoclasts, in the bone marrow microenvironment. Inhibiting protein prenylation with APOMINE may represent a novel therapeutic approach in the treatment of multiple myeloma.

397 Melphalan, Prednisone and Liposomal Doxorubicin (MPDL) is a feasible and promising regimen in elderly Multiple Myeloma patients.

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In Multiple Myeloma patients not eligible for high dose therapy (HDT), Melphalan & Prednisone (MP) has remained as the standard therapy for over 30 years. Accordingly, new treatment strategies are needed in order to improve the outcome of these patients, that represent ~50% of Myeloma population. Adramycin is an active drug in Myeloma that has been used in several polychemotherapy combinations (VAD, VBAD, etc.) but it is associated with cardiac toxicity which hampers it use on old MM patients: The new Liposomal Adriamycin (Caelyx®) is associated with cardiac toxicity which hampers its use in old MM patients. Inhibiting myeloma cell apoptosis in vitro and inhibit the development of lytic bone lesions in vivo. Interestingly, in this model APOMINE had no effect on osteoclasts in vitro or in vivo at the concentrations studied, suggesting that the effects of APOMINE on development of myeloma may be a direct effect on myeloma cells or via cells, other than osteoclasts, in the bone marrow microenvironment. Inhibiting protein prenylation with APOMINE may represent a novel therapeutic approach in the treatment of multiple myeloma.

398 A Phase 2 Study of Bcl-2 Antisense (Oblimersen Sodium, G3139) Combined with Vincristine, Adriamycin and Dexamethasone (VAD) in Patients with Refractory Multiple Myeloma

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Aims and Methods: Bcl-2 expression in myeloma cells prevents apoptosis induced by dexamethasone and doxorubicin (Adriamycin). Oblimersen sodium (G3139, GenasenseTM) is a phosphorothioate antisense oligodeoxynucleotide (ODN) complementary to the first 6 codons of the Bcl-2 mRNA. We have previously shown that time- and dose-dependent uptake of G3139 is associated with sequence-specific reduction (>75%) of Bcl-2 mRNA levels after 2 days in ex vivo purified myeloma cells. Ex vivo treatment with G3139 led to a sequence-specific reduction of Bcl-2 protein levels within 4 days of exposure in 10 out of 11 clinical samples from patients with chemosensitive and multidrug-resistant disease and sensitized the cells to both dexamethasone and doxorubicin-induced apoptosis (Leukemia 2003; 17(1): 211-219). Based on these findings, a phase 2 study was started to examine the clinical benefit and safety of oblimersen sodium administration in patients with VAD-refractory myeloma. In a 28-day cycle, G3139 (7 mg/kg/day) was given as a continuous intravenous infusion (day 1-7) in combination with VAD (day 4-7).

Results: To date, 8 patients with documented Bcl-2 protein expression in myeloma cells have been treated with 2-4 cycles. Median age was 54 years (range 40-64 years). All patients were heavily pretreated, including 6 with VAD-refractory disease after autologous stem cell transplantation. One patient was refractory to VAD and allogeneic stem cell transplantation, and one patient had VAD-refractory disease after VAD/IDM (intermediate-dose melphalan). In addition, 6 patients were resistant to thalidomide and dexamethasone. Four patients (50 %) achieved a partial remission after 3 cycles of therapy, which persists in 2 patients 30 months.
and 10 months after start of therapy, whereas 2 patients relapsed after 4 and 6 months. One patient had stable disease, 2 progressed during treatment, and 1 patient is too early to evaluate. The most common side effect reported was grade 2 fatigue. One patient had WHO grade 4 thrombocytopenia/neutropenia and grade 3 infection. However, this individual had a 99% bone marrow infiltration with plasmablastic myeloma cells. Treatment resulted in a reduction of Bcl-2 protein expression levels in peripheral blood lymphocytes (mean reduction on days 4 and 7 was 23% and 18% for T cells and 43% and 30% for B cells, respectively) and circulating myeloma cells (mean reduction on days 4 and 7 was 18% and 17%, respectively) as determined by flow cytometry.

Conclusion: These data indicate that G3139 can successfully downregulate Bcl-2 in vivo in myeloma cells and may be of value for the treatment of refractory multiple myeloma. This trial continues to enroll patients.

399 Metabolism of a cholesterol-rich microemulsion (LDE) in patients with multiple myeloma and a preliminary clinical study of LDE as drug vehicle for the treatment of the disease.

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Purpose: Previously, we showed that cholesterol-rich microemulsions that binds to LDL receptors have the ability to concentrate in acute myeloid leukemia cells and in ovarian and breast carcinomas. Thus, LDE may be used as vehicle of drugs directed against neoplastic cells. Indeed, we subsequently showed that when Carmustine was associated with LDE the toxicity of the drug was pronouncedly reduced in patients with advanced cancers. The present study aimed to verify whether LDE may be taken-up by multiple myeloma cells and whether multiple myeloma patients may be responsive to treatment with LDE associated with carmustine. Methods: 131 consecutive recently diagnosed volunteer multiple myeloma patients classified as clinical stage IIIA had their plasma lipid profile determined. LDE plasma kinetics were performed in 14 of them. The uptake of LDE by a multiple myeloma cell lineage was evaluated. Finally, an exploratory clinical study of LDE-carmustine (carmustine dose: 180 mg/m2 body surface every 4 weeks) was performed in 7 untreated multiple myeloma patients. Results: LDL cholesterol was smaller in the 131 multiple myeloma patients than in the healthy controls and the fractional clearance rate (FCR, in min-1) was twice greater in the 14 multiple myeloma patients than in 14 paired control healthy subjects. Moreover, entry of LDE was shown to be mediated by LDL receptors in the multiple myeloma cells. Taken together, those data indicate that LDE may target multiple myeloma. The exploratory clinical study showed that gammaglobulin decreased 10-70% (36%) after 3 cycles and 25-75% (44%) after 6 cycles. Furthermore, there was amelioration of the symptoms in all patients. Cholesterol concentration increased post-treatment, suggesting that the treatment resulted in at least partial destruction of neoplastic cells with receptor upregulation. Side effects of the treatment were negligible. Conclusion: Because it targets multiple myeloma and when associated with an antineoplastic agent it produces therapeutic responses in patients with lesser side-effects, LDE has potential for use as drug vehicle in the treatment of the disease.

400 Gliotoxin (GLT) and dehydroxymethlepoxyquinomicin (DHMEQ) induce apoptosis in multiple myeloma (MM) cells by causing endoplasmic reticulum (ER) stress.

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Introduction: Proteasome inhibitors (PI) have been reported to be useful in the treatment of MM. One of the mechanisms of PI action is thought to increase the amount of IκB, thereby decreasing NF-κB migration of NF-κB to nucleus, indicating that PI serves as NF-κB inhibitors. Considering that NF-κB represents a key molecule in B cell lineage, we asked whether PI and NF-κB inhibitors induced apoptosis in MM cells.

Materials and Methods: A proteasome inhibitor, GLT, and two NF-κB inhibitors, BAY11-7082 (an IκB phosphorylation inhibitor) and DHMEQ (an inhibitor of NF-κB nuclear translocation; a gift from K. Umezawa) were employed. Apoptosis occurring in an MM cell line, U266, was examined using May-Giemsa staining and Annexin V/PI staining. Profiles of IκB, phosphorylated-IκB, XBP-1, BiP/GRP78 and caspase 12 were also examined using western blot.

Results: All three agents used induced apoptosis in U266 cells, among which GLT was the most potent apoptosis-inducer and IκB phosphorylation-potentiator, followed by BAY11-7082 and DHMEQ. When U266 cells were treated with GLT or DHMEQ, the cleavage of Caspase-12 was readily seen, a salient feature of endoplasmic reticulum (ER) stress, whereas other indicators employed showed no changes as determined using western blot.

Conclusions: The present data suggest that proteasome inhibitors and NF-κB inhibitors could induce apoptosis in MM cells through causing ER stress. The data might explain how such agents exert their clinically favorable effects in patients with MM. (This work was supported in part by International Myeloma Foundation)

401 Inhibition of Multiple Myeloma Cell Growth and Bone Resorption By Silence TRAF6 mRNA

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Although recently advances in the management of Multiple myeloma (MM) include the use of high-dose chemotherapy followed by autologous or allogeneic transplantation of hematopoietic stem cells, the patients can be prolonged survival to 4-5 years. Myeloma remains incurable. However, RNA interference (RNAi) is emerging as one of the most promising RNA-based treatments. RNAi is the process of sequence specific, post-transcriptional gene silencing, initiated by complementary double strand RNA (dsRNA). RNAi can be used to interfere with the expression and action of targeted genes involved in tumor genesis. RNAi can also used to silence the genes that are involved in the promotion of MM cell growth and proliferation, and the biological function of osteoclast cells. Our previous studies showed that increased DNA-binding activity of the transcription factor nuclear factor kappa B (NF-κB) is associated with enhanced tumor cell survival in MM. Tumor necrosis factor receptor-associated factor6 (TRAF6) is essential for the activation of NF-κB signaling in plasma cells and the Jun NH2-
terminal kinase (JNK) pathway which controls osteoclast proliferation. The important function of TRAF6 in signal transduction provides us an ideal target for enhancing the cell killing of MM cells and inhibiting bone turnover initiated by osteoclast activity. We have generated two human TRAF6 dsRNA by construction in vitro for Target 1 TRAF6 mRNA sequence 5'-AAACTGTGAAAA ACAGCTGTGG-3' and Target 2 TRAF6 mRNA Sequence 5'-AGTATGAATGCCCCATCTGC - 3'. The apoptosis and cell proliferation assays showed that Target-1 dsRNA inhibited MM cell proliferation in a dose-dependent manner. The Target-2 dsRNA did not produce detectable inhibition of MM cell proliferation. Only the Target-1 dsRNA induced MM cell apoptosis. In contrast, the Target-2 dsRNA and the transfection reagent control vector did not induce cell apoptosis. We tested that whether silence Target-1 TRAF6 mRNA could inhibit NF-kB gene expression induced by IL-1. The 8226 MM cells were transduced of either 100nM TRAF6 or 100nM GAPDH dsRNA for 72 hours and then the cells were incubated with IL-1 for 0, 15, 30, and 60min. The cells were lysates and the total RNA or protein was detected by RT-PCR or western blot. The result showed that amount of NF-kB mRNA or activated NF-kB was significantly increased after 30 minute IL-1 stimulation while NF-kB dropped to normal level after 60 minutes. In contrast, in cells that received TRAF6 dsRNA, the amount of phosphor-NF-kB is no changed. It has been clear that NF-kB specific response to TRAF6 knocked down by silence TRAF6 mRNA. We sought further to determine whether TRAF6 RNAi could inhibit NF-kB trigger function on luciferase activity. Using luciferase vector to monitor the activation of NF-kB signal transduction pathway. PNF-kB-Luc is designed to measure the binding of NF-kB to k enhancer, providing a direct measurement of activation this pathway. PTAL-Luc is ideal for use as a negative control vector. In view of the results showed that IL-1 stimulation of the double transfected cells at 30 minute result in a significant induction of luciferase by NF-kB releasing. Furthermore, this induction was abolished when TRAF6 mRNA was silenced by dsRNA. Multiple nuclear cells (pre-osteoclast) were decreased after silence TRAF6 when monocytes stimulated with RANKL and m-SCF. For future studies, we would like to use SCID-hu murine models of human myeloma to further develop this therapeutic strategy in relevant pre-clinical in vivo models. The in vitro studies outlined in this proposal will certainly significantly advance the understanding of this adapter protein in the pathogenesis and give us more impetus to target this protein for future therapeutic manipulation for patients with multiple myeloma.

12. **Vaccination strategies in multiple myeloma**

402

**NY-ESO-1 AND MAGE-A3 ARE HIGHLY EXPRESSED IN MYELOMA PATIENTS WITH ABNORMAL CYTOGENETICS AND/OR RELAPSE**

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Despite recent successes with chemotherapy and tandem autologous PBSCT, myeloma remains largely incurable. This highlights the need for novel, synergistic therapies, such as immunotherapy, which may reduce myeloma relapse. Using micro-array and immunohistochemical (IHC) staining, we have examined the expression of two potential targets for immunotherapy, NY-ESO-1 and MAGE-3. Purified myeloma cells were studied by micro-array (n=335), micro-array and IHC on corresponding biopsy (n=20), or IHC alone (n=19, 35 biopsies). Micro-array analysis of 335 patients revealed that expression of NY-ESO-1 and MAGE-A3 is related to the stage of myeloma. The frequency of expression of NY-ESO-1 and MAGE-A3 in MGUS, smoldering myeloma and newly diagnosed myeloma patients eligible for Total Therapy II (TT II) was 4.5%, 5.9% and 20.5% (NY-ESO-1) and 9.1%, 11.8% and 26.2% respectively (MAGE-A3). The presence of abnormal cytogenetics influenced the expression of both antigens. Newly diagnosed TT II trial patients with abnormal cytogenetics were more likely to express NY-ESO-1 and MAGE-A3 cRNA (NY-ESO-1 60% vs. 30.9%, p=0.004; MAGE-A3 65.8% vs. 27.5%, p=1.16x10-6). The percentage NY-ESO-1/MAGE-3 positive plasma cells at the protein level (IHC) was similarly correlated with abnormal cytogenetics. Both antigens (micro-array) were expressed in >50% of relapsed patients (NY-ESO-1 50%, p=8.8x10-9 and MAGE-A3 58.3%, p= 4.5x10-9). Strikingly, 100% patients with abnormal cytogenetics at relapse expressed NY-ESO-1 and MAGE-A3 (p=6.09x10-6 & 3.25x10-6). All these biopsies also stained positive by IHC for NY-ESO-1 and MAGE-A3. 13 patients were studied at diagnosis and at relapse. All biopsies positive at diagnosis by IHC for NY-ESO-1 or MAGE-A3 were positive for both antigens at relapse. The percentage NY-ESO-1 and MAGE-A3 positive myeloma cells in the biopsies was high (>50-90% positive plasma cells; 89% biopsies for NY-ESO-1 and 87% MAGE-A3). Double staining experiments are in progress to test whether the same myeloma cells are positive for both NY-ESO-1 and MAGE-A3. Expression of NY-ESO-1 and MAGE-A3 could be upregulated in myeloma cell-lines by incubating with the hypomethylating agent, decitabine. There was a significant increase in expression of NY-ESO-1 and MAGE-A3 after incubation with decitabine of the NY-ESO-1 and MAGE-A3 negative myeloma cell line U-937 by real-time PCR. In conclusion, NY-ESO-1 and MAGE-A3 are highly expressed in myeloma with abnormal cytogenetics and relapsed myeloma. These antigens can therefore be used as immunotherapy for myeloma with abnormal cytogenetics. Alternatively, NY-ESO-
terminal kinase (JNK) pathway which controls osteoclast proliferation. The important function of TRAF6 in signal transduction provides us an ideal target for enhancing the cell killing of MM cells and inhibiting bone turnover initiated by osteoclast activity. We have generated two human TRAF6 dsRNA by construction in vitro for Target 1 TRAF6 mRNA sequence 5'-AAACTGTGAAA ACAGCTGTTG-3' and Target 2 TRAF6 mRNA Sequence 5'- AGTATGAATGCCCCATCTG - 3'. The apoptosis and cell proliferation assays showed that Target-1 dsRNA inhibited MM cell proliferation in a dose-dependent manner. The Target-2 dsRNA did not produce detectable inhibition of MM cell proliferation. Only the Target-1 dsRNA induced MM cell apoptosis. In contrast, the Target-2 dsRNA and the transfection reagent control vector did not induce cell apoptosis. We tested whether silence Target-1 TRAF6 mRNA could inhibit NF-kB gene expression induced by IL-1. The 8226 MM cells were transduced of either 100nM TRAF6 or 100nM GAPDH dsRNA for 72 hours and then the cells were incubated with IL-1 for 0, 15, 30, and 60min. The cells were lysates and the total RNA or protein was detected by RT-PCR or western blot. The result showed that amount of NF-kB mRNA or activated NF-kB was significantly increased after 30 minute IL-1 stimulation while NF-kB dropped to normal level after 60 minutes. In contrast, in cells that received TRAF6 dsRNA, the amount of phosphor-NF-kB is no changed. It has been clear that NF-kB specific response to TRAF6 knocked down by silence TRAF6 mRNA. We sought further to determine whether TRAF6 RNAi could inhibit NF-kB trigger function on luciferase activity. Using luciferase vector to monitor the activation of NF-kB signal transduction pathway. PNF-kB-Luc is designed to measure the binding of NF-kB to k enhancer, providing a direct measurement of activation this pathway. PTAL-Luc is ideal for use as a negative control vector. In view of the results showed that IL-1 stimulation of the double transfected cells at 30 minute result in a significant induction of luciferase by NF-kB releasing. Furthermore, this induction was abolished when TRAF6 mRNA was silenced by dsRNA. Multiple nuclear cells (pre-osteoclast) were decreased after silence TRAF6 when monocytes stimulated with RANKL and m-SCF. For future studies, we would like to use SCID-hu murine models of human myeloma to further develop this therapeutic strategy in relevant pre-clinical in vivo models. The in vitro studies outlined in this proposal will certainly significantly advance the understanding of this adapter protein in the pathogenesis and give us more impetus to target this protein for future therapeutic manipulation for patients with multiple myeloma.

12. Vaccination strategies in multiple myeloma

402 NY-ESO-1 AND MAGE-A3 ARE HIGHLY EXPRESSED IN MYELOMA PATIENTS WITH ABNORMAL CYTOGENETICS AND/OR RELAPSE

Sushil K. Gupta, Fenguang Zhan, Pei Lin, Jan V. Droogenbroeck*, Ramesh B. Batchu, Friedel Nollet*, Susann M Szmania, Amberly Moreno, Nancy Rosen, Guilio Spagnoli†, Bart Barlogie, Guido Tricot, John Shaughnessy and Frits van Rhee

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Despite recent successes with chemotherapy and tandem autologous PBSC, myeloma remains largely incurable. This highlights the need for novel, synergistic therapies, such as immunotherapy, which may reduce myeloma relapse. Using micro-array and immunohistochemical (IHC) staining, we have examined the expression of two potential targets for immunotherapy, NY-ESO-1 and MAGE-3. Purified myeloma cells were studied by micro-array (n=335), micro-array and IHC on corresponding biopsy (n=20), or IHC alone (n=19, 35 biopsies). Micro-array analysis of 335 patients revealed that expression of NY-ESO-1 and MAGE-A3 is related to the stage of myeloma. The frequency of expression of NY-ESO-1 and MAGE-A3 in MGUS, smoldering myeloma and newly diagnosed myeloma patients eligible for Total Therapy II (TT II) was 4.5%, 5.9% and 20.5% (NY-ESO-1 and 9.1%, 11.8% and 26.2% respectively (MAGE-A3). The presence of abnormal cytogenetics influenced the expression of both antigens. Newly diagnosed TT II trial patients with abnormal cytogenetics were more likely to express NY-ESO-1 and MAGE-A3 cRNA (NY-ESO-1 60% vs. 30.9%, p=0.004; MAGE-A3 65.8% vs. 27.5%, p=1.16x10-6). The percentage NY-ESO-1/MAGE-3 positive plasma cells at the protein level (IHC) was similarly correlated with abnormal cytogenetics. Both antigens (micro-array) were expressed in >50% of relapsed patients (NY-ESO-1 50%, p=8.8x10-9 and MAGE-A3 58.3%, p= 4.5x10-9). Strikingly, 100% patients with abnormal cytogenetics at relapse expressed NY-ESO-1 and MAGE-A3 (p=6.09x10-6 & 3.25x10-6). All these biopsies also stained positive by IHC for NY-ESO-1 and MAGE-A3. 13 patients were studied at diagnosis and at relapse. All biopsies positive at diagnosis by IHC for NY-ESO-1 or MAGE-A3 were positive for both antigens at relapse. The percentage NY-ESO-1 and MAGE-A3 positive myeloma cells in the biopsies was high (>50-90% positive plasma cells; 89% biopsies for NY-ESO-1 and 87% MAGE-A3). Double staining experiments are in progress to test whether the same myeloma cells are positive for both NY-ESO-1 and MAGE-A3. Expression of NY-ESO-1 and MAGE-A3 could be upregulated in myeloma cell-lines by incubating with the hypomethylating agent, decitabine. There was a significant increase in expression of NY-ESO-1 and MAGE-A3 after incubation with decitabine of the NY-ESO-1 and MAGE-A3 negative myeloma cell line U-937 by real-time PCR. In conclusion, NY-ESO-1 and MAGE-A3 are highly expressed in myeloma with abnormal cytogenetics and relapsed myeloma. These antigens can therefore be used as immunotherapy for myeloma with abnormal cytogenetics. Alternatively, NY-ESO-
1/MAGE-A3 immunotherapy can be used as secondary prevention to prevent relapse/disease progression in NY-ESO-1/MAGE-A3 negative patients receiving chemotherapy or in smoldering/indolent myeloma.

403 Dendritic cell numbers and their subsets during treatment of multiple myeloma

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Background and objective: In this study, the proportion of dendritic cell (DC) subsets (DC1, DC2) in peripheral blood of patients with multiple myeloma (MM) was evaluated before and during treatment.

Design and Methods: Flow cytometric determination of DC subsets in peripheral blood was based on positive expression of the surface antigen CD83 in combination with HLA-DR and either CD11c or CD123.

Results: No significant differences were found in initial values between the group of healthy volunteers (n = 15; mean count of CD83+ cells was 0.62±0.06%; ratio DC1/DC2 = 2.5) and the group of patients before treatment (n = 15; 0.59±0.13% CD83+; DC1/DC2 = 2.17). In a group of patients (n = 10) treated with VAD regimen (vincristine, Adriamycin, dexamethasone), the mean percentage of DC after induction treatment (0.84±0.4% CD83+ cells; DC1/DC2 = 1.59) was higher than initial values. Administration of G-CSF reduced the total DC numbers (0.66±0.6%; DC1/DC2 = 3.71). The lowest total DC counts were in the apheresis products (0.36±0.2%; DC1/DC2 = 4.75). Administration of GM-CSF increased DC numbers (0.56±0.3%; DC1/DC2 = 1.59). Pretreatment DC values were achieved within six months after transplantation (0.83±0.6%; DC1/DC2 = 3.92).

Interpretation and Conclusions: The highest number of total DC was found after induction treatment and within six months of transplantation. The ratio DC1/DC2 showed a the highest value in the apheresis products and in peripheral blood within the first six months after transplantation.

404 IL-12 corrects the dendritic cell defect of patients with myeloma

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The poor response to immunotherapy in patients with multiple myeloma indicates that a better understanding of the immune defects in this disease is required before more effective therapeutic strategies can be developed. Recently we reported that high potency (CMRF44+) dendritic cells (DC) in the peripheral blood of patients with multiple myeloma failed to upregulate the expression of the B7 costimulatory molecules, CD80 and CD86, in response to an appropriate signal from huCD40LT. During antigen presentation to T cells the level of expression of the B7 molecules provides an important “second signal” that determines the fate of each cell – apoptosis, anergy or productive immunity. We have demonstrated that this defect is caused by TGF-1 and IL-10 produced by malignant plasma cells and that it is possible to neutralise the defect in vitro with anti-TGF-1. If this defect has an impact on immunotherapy strategies, it would be important to identify a more suitable agent than anti-TGF-1 for in vivo use. The number of high potency DC (CMRF44+, CD14−, CD19−, PI−) in the blood of patients with myeloma (0.03-0.8% of mononuclear cells; n= 26) was not significantly different from normal controls (0.05-0.8% of MNCs; n=13). The expression of the costimulatory molecules CD80 and CD86 on blood DC of these patients (2917% and 8510% of MNCs respectively) was also normal (2917% and 8616% of MNCs). Incubation with huCD40LT stimulated upregulation of CD80 expression on the DC and B cells of normal controls but there was either reduced or no upregulation of CD80 on the DC of the patients with myeloma. Less than 10% of malignant plasma cells expressed CD80 and huCD40LT failed to significantly upregulate CD80 expression on mature plasma cells (n=6). Upregulation of CD80 on DC of normal controls was inhibited by rTGF-1 in a dose dependent manner. CD86 expression on DC was high both before (86%) and after (80%) stimulation. IL-12 but not interferon-γ could replace anti-TGF-1 as an agent capable of neutralising the failure to stimulate DC80 upregulation by huCD40LT. DC subsets stimulated by IL-12 were predominantly myeloid (CD11c+ and CD123−), suggesting that they could initiate a Th1 type response. Thus patients with myeloma have a normal number of DC but may fail to upregulate CD80 expression in the presence of huCD40LT due to tumor-derived TGF-1 and/or IL-10. This defect can be corrected with IL-12.

405 IN VITRO STIMULATION OF LYMPHOCYTES FROM MULTIPLE MYELOMA PATIENTS WITH AUTOLOGOUS DENDRITIC CELLS PULSED WITH PLASMOCYTE LYSATES.

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Tumor vaccination using dendritic cells (DCs) pulsed with tumor antigens has proven useful mainly in melanomas, prostate cancers, and lymphomas. Experimental data in mouse models or in vitro human models have established that multiple myeloma (MM) is immunogenic and can induce an immune T response when the tumor antigen is adequately presented to T lymphocytes (MM) is immunogenic and can induce an immune response. In a pre-clinical study, we are evaluating the possibility to generate in MM cytotoxic T lymphocytes directed against the autologous patient’s tumor cells, i.e. the plasmocytes in a protocol that fits GMP procedures. Until now, 8 patients (pts) have been collected. Plasmocytes were isolated from bone marrow at diagnosis (except for 2 pts, for whom the monoclonal immunoglobulin was used as tumor antigen), selected with anti-CD138 magnetic beads and cryopreserved. Monocytes and lymphocytes were purified by elutriation from apheresis products of pts at remission. Monocytes were differentiated into DCs by culture during 5 days in teflon bags in the presence of GM-CSF+IL-13+fetal calf serum. DCs were pulsed with autologous plasmocyte lysates or monoclonal Ig, matured using poly-IC+anti-CD40, and then cocultured with autologous T lymphocytes. The cytotoxicity of T lymphocytes against autologous plasmocytes (or Ig-pulsed DCs) was tested using a 51chromium-release assay. The production of interferon-γ by stimulated...
lymphocytes was tested by Ellispot. The entire experiment could be achieved for only 5 pts. Immature DCs had the same phenotype than DCs obtained from healthy donors, i.e. CD14-, CD1a+, HLA-DR++, CD86++, CD40++, CD83-. Until now, only one patient’s lymphocytes showed a specific antitumor activity. In conclusion, although feasible, our procedure is not easy to achieve for all patients. However, we now have experience, and think it would help us to get relevant data to confirm the encouraging results recently reported by Dhodapkar et al (PNAS, 2002) and Wen et al (Blood, 2002)

406 INDUCTION OF MYELOMA SPECIFIC CYTOTOXIC T CELLS USING DENDRITIC CELLS TRANSFECTED WITH TUMOR-DERIVED RNA

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We investigated myeloma RNA transfection of dendritic cells (DC) to induce myeloma specific cytotoxic T cell (CTL) responses in vitro. By this methodology we hope to bypass the need for the identification of shared MM associated antigens or specific antigens such as idiotype (Id).

Monocyte derived DC from buffy coats, which were matched in the HLA class I haplotype to the myeloma cell lines LP-1(HLA-A3/A24) and U266 (HLA-A2/A3), were used for RNA transfection. DC were electroporated with total myeloma cell line RNA and were used as antigen presenting cells for the induction of myeloma specific CTL. After a single restimulation with RNA transfected DC, cytotoxic activity of induced T cells was analyzed in 51-Cr release assays.

We found that myeloma RNA transfected DC induce CTL that lyse the LP-1 myeloma cells. Cell line specificity was demonstrated by cold target inhibition assay and MHC class I restriction was revealed by antibody blocking studies. Total LP-1 RNA transfected DC also served as suitable targets in a 51-Cr release assay, being equivalent to the respective original tumor cell line.

To analyze whether Id specific cytotoxicity contributed to the robust lysis observed, we purified Id protein from LP-1 supernatants and used autologous Id pulsed DC as targets in 51-Cr release assays. Interestingly, LP-1 specific CTL showed no specificity for the idiotype.

U266 specific CTL were also successfully induced by the same methodology. We furthermore analyzed whether MUC1 specificity added to the lytic activity of U266 (MUC1+) specific CTL. As corresponding epitopes we tested the recently described HLA-A2 restricted peptides M1.1 and M1.2 and found a striking fine specificity for M1.2, assuming a possible immunodominance of this peptide.

We report here on the successful induction of myeloma specific CTL by RNA transfection of DC, which could serve as a potential vaccine.

407 IL-15 generated Dendritic Cell Vaccines to Elicit Specific Immunity to Multiple Myeloma

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We have evaluated the anti-tumor efficacy of IL-15-induced Dendritic Cell (DC) vaccines against multiple myeloma (MM) in preclinical studies. MM is a malignancy involving the uncontrolled expansion of clonal B plasma cells secreting a monoclonal idiotypic immunoglobulin (Idlg). This unique antigen contains epitopes that can be specifically recognized by MHC-restricted T cells. When fed to Dendritic Cells (DC), peptides derived from Ig protein can be presented in MHC complexes on the cell surface of the antigen-presenting cell, thus serving as a highly-specific immunogen. We have recently shown that DC can be generated from CD34+ stem cell precursors in cultures containing IL-15. IL-15-induced DCs appear to represent most powerful stimulators of T cell proliferation in mixed lymphocyte reactions (MLR) than DC generated in the presence of GM-CSF and TNF (S. Bykovskaya e.a., 1999). In current work we evaluated the effects of rIL-15 to enhance the ability of DCs to stimulate MM-specific T cells in vitro as a prelude to subsequent clinical development.

CD14+ monocytes were isolated from both MM patient’s and normal donor PBMCs using antibody-coated magnetic beads (MiltenyBiotec) and cultured with the two sets of cytokines: rhGM-CSF + rhIL4 or rhGM-CSF + rhIL-15. Increased frequencies of DCs bearing the CD1a and CD83 maturation markers were noted for cultures containing IL-15. MM patient’s DCs generated in the presence of IL-15 induce enhanced primary responses in CD8+ T cells. MM patient’s T cells were isolated by specific antibody coated-MACS, co-cultured with autologous DCs generated with both IL-4 and IL-15-supplemented medium, and pulsed with the patient’s Id Ig purified by Protein A affinity chromatography from the patient’s serum. Our data suggest specific proliferation of CD8+ T cells in both stimulation groups, however, IL-15-generated DC induced higher rates of proliferation vs. DC cultured in presence of IL-4. We next evaluated specific T cells for secretion of interferon gamma (IFNγ) in response to autologous DCs pulsed with the autologous Id Ig protein. CD14+-derived DCs generated in medium supplemented with both sets of cytokines were pulsed by autologous Id Ig and cultured with the patient’s own T cells. T cells were then analyzed for IFN secretion in ELISPOT assays. IL-15-generated DCs were superior in stimulating the development of specific Th1-type responses, particularly among CD8+ T cell responders.

The results of this study should provide the basis for a perspective clinical vaccine formulation designed to treat MM.

408 DNA fusion vaccine against a cancer testis antigen provides protection in a murine tumor model: relevance for human multiple myeloma

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Modern chemotherapeutic regimens and transplantation strategies are able to bring multiple myeloma (MM) into remission, and open the possibility of active vaccination strategies to raise immunity against tumor cells. In this strategy, vaccination with naked DNA provides an attractive option, able to activate both innate and specific immune responses against encoded tumor associated antigen (TAA). For myeloma, the tumor specific idiotype is one such target. We have shown previously that fusing V gene sequences in a single chain variable fragment (scFv) format to a pathogen-derived sequence from tetanus toxin (fragment C, FrC) promotes idiotype specific CD4+ T cell responses, which provide protection from tumor challenge in the
To test vaccine design, we used the murine P815 mastocytoma CD8+ T cells when delivered as DNA fusion vaccines in MM. The potential CTA-derived CD8+ T cell epitopes, recognized by S270, were enriched dendritic precursor cells, incubated with unmodified product shipped to Dendreon Corporation where it was processed after stem cell transplantation. Median M protein was 3.0 g/dl.

Regimens was three, and 36% of patients had progressive disease that elicit antigen-specific immune responses in vitro and in vivo. This vaccine design facilitates the induction of immune responses to intracellular antigens, which are likely to be presented by the class I pathway. For myeloma, the cancer testis antigen (CTAs) have emerged as potential intracellular targets in early or late stage disease. As CTA expression is restricted in normal cells to the tests and placenta, which lack class I molecules, they provide an additional advantage of being tumor specific. Several potential CTA-derived CD8+ T cell epitopes, recognized by various HLA haplotypes have been described.

We have explored the potential of CTAs as targets for cytotoxic CD8+ T cells when delivered as DNA fusion vaccines in MM. To test vaccine design, we used the murine P815 mastocytoma tumor model, which expresses the P1A gene. This gene is silent in normal murine tissues excepting the tests and placenta, and therefore mirrors human CTA expression. P1A encodes a well-defined MHC class I H2-L(d) CTL motif. A pDOM.epitope vaccine incorporating this motif was constructed. A single vaccination led to detection of epitope specific, IFN- positive CTLs ex vivo, which could be expanded on re-stimulation in vitro. These CTLs were able to kill P815 tumor cells in an epitope specific manner. Importantly, in protection experiments approximately 50% of vaccinated mice were protected from tumor challenge using this vaccine. Our data therefore suggest that effective immunotherapeutic intervention in myeloma may be possible using DNA fusion vaccines encoding CTA epitopes.

**5T33 murine myeloma model. The validation of this DNA fusion vaccine design led to a phase I/II clinical trial which is in progress, and underscores the value of pre-clinical models.** As MM cells are MHC class I+ve, activation of cytotoxic CD8+ T cells may also be important. For this, a new DNA vaccine design has been engineered, incorporating the first domain of FrC (pDOM) fused to a defined CTL epitope. Here, potentially competitive MHC class I-binding epitopes from FrC have been removed, improving presentation of the TAA-derived epitope. This vaccine design facilitates the induction of immune responses to intracellular antigens, which are likely to be presented by the class I pathway. For myeloma, the cancer testis antigen (CTAs) have emerged as potential intracellular targets in early or late stage disease. As CTA expression is restricted in normal cells to the tests and placenta, which lack class I molecules, they provide an additional advantage of being tumor specific. Several potential CTA-derived CD8+ T cell epitopes, recognized by various HLA haplotypes have been described.

We have explored the potential of CTAs as targets for cytotoxic CD8+ T cells when delivered as DNA fusion vaccines in MM. To test vaccine design, we used the murine P815 mastocytoma tumor model, which expresses the P1A gene. This gene is silent in normal murine tissues excepting the tests and placenta, and therefore mirrors human CTA expression. P1A encodes a well-defined MHC class I H2-L(d) CTL motif. A pDOM.epitope vaccine incorporating this motif was constructed. A single vaccination led to detection of epitope specific, IFN- positive CTLs ex vivo, which could be expanded on re-stimulation in vitro. These CTLs were able to kill P815 tumor cells in an epitope specific manner. Importantly, in protection experiments approximately 50% of vaccinated mice were protected from tumor challenge using this vaccine. Our data therefore suggest that effective immunotherapeutic intervention in myeloma may be possible using DNA fusion vaccines encoding CTA epitopes.

**409 Vaccine Therapy of Advanced Refractory Multiple Myeloma with Idiotype-Pulsed Dendritic Cells (Mylovenge™) Final Report.**

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Introduction. Dendritic cells are potent antigen presenting cells that elicit antigen-specific immune responses in vitro and in vivo. This report presents a phase I/II trial of idiotype-pulsed autologous dendritic cells (Mylovenge) for treatment of multiple myeloma. Forty-two patients with advanced refractory myeloma were treated. The median number of prior chemotherapy regimens was three, and 36% of patients had progressive disease after stem cell transplantation. Median M protein was 3.0 g/dl.

Treatment Regimen: Patients underwent a leukapheresis and the product shipped to Dendreon Corporation where it was processed to enrich dendritic precursor cells, incubated with unmodified autologous serum containing idiotype for 40 hours. The cells harvested were returned to the clinical site. Mylovenge was infused in either weeks 0, 4, 8, and 24 or weeks 0, 2, 4, and 24. The mean dose was 350 ± 328 x 106 dendritic cells per infusion.

Results: Treatment related adverse events occurred in 10 of 134 infusions (7.5%)and two (1%), episodes of dyspnea were grade 3-4 in severity. Mylovenge treatment induced idiotype-specific T cell immune responses in 43.3% of evaluable patients without pre-existing immunity. Development of immunity correlated with improved time to disease progression. No complete or partial remissions were observed. However nine of these heavily pre-treated patients had disease stabilization or minor responses for more than 36 weeks. The overall median time to progression was 32 weeks.

Conclusion: The data indicate that Mylovenge induces idiotype specific immunity and disease stabilization in patients with refractory myeloma. Further testing in patients with lower tumor burden is warranted.

**410 DENDRITIC CELLS VACCINATION POST-PBSCT IN MULTIPLE MYELOMA: PRELIMINARY CLINICAL EXPERIENCE.**

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INTRODUCTION: The curability of multiple myeloma is only possible by means of allogeneic transplant, which is a procedure available just for a few patients and criticized because of its high mortality. After our group preliminary experience in follicular lymphoma (Haematologica 2002; 87:400-407), we have started a program of idiotype vaccination after autologous peripheral blood stem cell transplantation (PBSCT) with dendritic cells pulsed with the purified paraprotein from patients with myeloma. Here we communicate our initial experience in the selection and vaccination of patients.

PATIENTS AND METHODS: We choose patients who were diagnosed of myeloma and who were suitable for PBSCT. We isolate the paraprotein from a sample at diagnosis and after treatment with VAD courses, we perform the mobilization and collection of PBSCT, obtaining a purified fraction of CD34+ in order to generate dendritic cells. After reevaluation at +3 months post-PBSCT, the patients in minimal residual disease state began the vaccination program: 3 subcutaneous doses of dendritic cells pulsed with the purified paraprotein every two weeks, 5 sc doses of paraprotein + KLH + GM-CSF monthly, 1 boost dose of pulsed dendritic cells six months after the beginning of vaccination and a final boost x 2 doses of paraprotein + KLH + GM-CSF.

RESULTS: We have evaluated 15 patients suitable for the vaccination program. PBSCT could not be performed in three cases due to previous complications, and three are still receiving chemotherapy treatment. PBSCT was performed in 9 patients: 1 died of refractory disease, two are waiting for reevaluation, 2 have not reached enough response and 4 cases have begun the vaccination program (3 in CR and 1 in a very good PR). So far, we have generated enough dendritic cells in all of them; 1 has finished the vaccination and 3 are still receiving it without local or systemic adverse effects.

CONCLUSION: The vaccination treatment seems to be feasible and it does not seems to cause toxicity. One of the problems for the development of the project is the loss of patients before vaccination. (Supported by grant FIS 01/0913).
Dendritic Cell-Based Idiotype Vaccination for Primary Systemic Amyloidosis

Mayo Clinic

Introduction: Immunotherapy is most likely to work in a setting of low tumor burden, making vaccine strategies attractive as therapy for primary systemic amyloidosis (AL). A clinical trial of dendritic cell-based idiotype vaccination as therapy for AL was undertaken.

Methods: Between September 1998 and December 2001 a novel immunotherapeutic, APC8020 (Mylovenge®), was studied as therapy after for AL. Mylovenge™ is prepared from autologous antigen presenting cells (APC), including dendritic cells, partially purified from an unmobilized leukapheresis product by gradient density isolation and then incubated for two days with autologous serum containing M protein. Treatment was given intravenously in weeks 0, 2, 4, and 16. Eligibility criteria included: Histologic diagnosis of amyloidosis, quantifiable M-protein in the serum, age >18 years, ECOG Performance Status (PS) 0-2, leukocytes >1,500/µL, platelets >50,000/µL, bilirubin >5 x the upper limits of normal, creatinine >5.0 mg/dL, adequate venous access for apheresis. Responses were evaluated according to our previously published criteria.

Results: Fifteen patients were enrolled. Median age was 61 years (range 42-76 years). Thirteen had prior therapy (range 1-5 prior regimens). Biopsy positive sites included: liver (1), kidney (7), gastrointestinal tract (3), bone marrow (7), fat (10), lung (1). Associated monoclonal proteins included G (3), G (7), M (2), M (2) and A (1). Previous therapies included: stem cell transplant in 4 and chemotherapy in 11. Organ involvement included: cardiac (4), pulmonary (1), renal (8), peripheral nerve (4), autonomic nerve (1). Four patients had concurrent malignancies, two with multiple myeloma and two with Waldenstrom’s macroglobulinemia. Fifty-five infusions were done in the 15 patients. Toxicity was modest. There were ten adverse events, of which four were attributed to the underlying disease. Best responses consisted of: major response (1), minor response (1), stable disease (11) and progression (2). The major response consisted of >50% drop in 24 hour urine albumin. The minor response consisted of a dramatic improvement of painful peripheral neuropathy resulting in an improvement of performance status (ECOG PS 2 to PS0). Eight patients have progressed and seven have died. With a median follow-up of 23 months in surviving patients, the median time to progression is 9.4 months (95% CI: 7.1 - not yet reached). Five patients have remained progression free for > 24 months (42+, 25+, 43+, 25+, 24+ months).

Conclusions: DC vaccination with idiotype is clinically feasible and safe. Early clinical results appear promising.

VACCINATION IN MULTIPLE MYELOMA PATIENTS: LACK OF RESPONSE IN ADVANCED AND REFRACTORY PATIENTS.

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Background: Patients (pts) with multiple myeloma are at high risk of infectious complications and vaccination against Streptococcus pneumoniae is usually recommended. Response to vaccine is known to be low in this population. However some studies using idiotype vaccination after high dose chemotherapy reported immune responses. We thus evaluated prospectively the immune status against Clostridium tetani and Streptococcus pneumoniae in pts with MM. We also evaluated the antibody responses after vaccination against S.Pneumoniae in pts who have no protective level antibody.

Material and methods: 27 pts were included. Serum was analysed for antibody levels against S. pneumoniae and C. tetani in all pts. For pts with no or weak level of antibody, vaccination was performed and responses to vaccination was evaluated by the increase of antibody level after 4-6 weeks. We stratified this population into 2 groups: pts who had ≤ 2 different line (n=14) of therapy and a group with more than two previous treatments (n=13).

Results: there were 18 men and 9 women. The median age was 64 years (49-88). All pts had a protective titre against C.tetani and vaccination was not required. Four pts (%) had a weak but protective level of antibodies against S. pneumoniae before vaccination. 24 pts were evaluable for their responses to vaccination.

Results: in the group with ≤2 lines of treatment: 11 (92%) pts increase their level of antibody to a protective level of antibody and one pt (8%) failed to respond to vaccination. In the heavily treated group 8 pts (73%) have no or insufficient response to vaccine and 3pts (27%) responded to vaccination. The rate of efficiency responses to vaccine was thus significantly lower in the heavy treated group compared to the other group (p=0.0028, RR=0.141). No refractory pts response to vaccination. Four pts lose their protective level against S.Pneumoniae less than 2 years after the vaccination.

Conclusion: although our series is too small to draw conclusion, it suggest that pts with multiple myeloma have a weak protection against S.Pneumoniae. The covering for Tetanos in this population is still sufficient without a new vaccination. Response to vaccination is strongly influenced by the number of administrated treatment. Some patients lose prematurely their protection and need of earlier vaccination than every 5 years should be envisaged. Response to immunotherapeutique strategies is likely to be better in patient in the early phases of their disease and is probably ineffective.

Immune responses to tetanus vaccination in myeloma patients post autologous transplantation provide information on timing of vaccine-based immunotherapy

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Autologous stem cell transplantation (ASCT) with high-dose melphalan conditioning prolongs survival in myeloma patients. However, most patients eventually relapse and die from residual
tumor detectable following transplantation. Vaccine based strategies aimed at inducing a tumor-specific immune response at the time of minimal residual disease are attractive in this setting but rely on adequate immune reconstitution. When this is achieved depends on the underlying disease, conditioning, stem cell source (marrow or PBSC) and whether any manipulation of graft has been used (purging or CD 34+ selection).

The aim of this study was to assess the ability of patients with plasma cell disorders to generate antibody and cellular responses to tetanus toxoid (TT) vaccination (0.5ml). Immune responses were measured pre and 1 month post-vaccination. Our cohort consisted of 15 myeloma patients at least 15 months post-ASCT, 1 patient who was 3 months post ASCT, 3 patients in plateau phase after conventional chemotherapy, 2 patients with smouldering myeloma and 8 patients with MGUS. The responses of these patients were compared with those of 15 healthy volunteers. Anti-TT antibodies were measured by ELISA and cellular responses by a lymphoproliferation assay and interferon γ elispot.

Each autograft patient had received melphalan, 200mg/m2 as conditioning, followed by an unmanipulated peripheral blood stem cell transplant. The median time since transplantation was 31 months (range 15-81). Only one patient was vaccinated with TT in the post-transplant period prior to this study.

RESULTS: Similar to the fifteen healthy volunteers, transplant recipients who were at least 15 months post-ASCT, boosted their TT-antibodies post-vaccination. This included three who initially had non-protective levels (<0.01 IU/ml). The patient who was three months post transplant, was the only subject who failed to have a humoral response to vaccination. The myeloma patients in plateau phase and with smouldering disease also boosted their antibody levels, as did those with MGUS. The lymphoproliferative assay was positive (stimulation index, SI>3) pre-vaccination in 5 /15 myeloma patients who were at least 15 months post-transplant. Each of these 5 raised their SI post-vaccination. Overall, in this transplant group the assay was positive in 12/15 post-vaccination. Each of these 5 raised their SI post-vaccination. The lymphoproliferative assay was negative both pre and post-transplantation. In the plateau group, the lymphoproliferation assay was positive in 1/3 patients pre-vaccination and positive in 3/3 post-vaccination. Elispot results are available for one of this group and were positive both pre and post-vaccination. Five of the 8 MGUS patients had a positive lymphoproliferation assay pre-vaccination and all were positive post-vaccination. These results present new information on functional immune reconstitution post-ASCT in myeloma and help to determine the timing for vaccine-based immunotherapy in this setting.