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MGUS, WALDENSTRÖM’S MACROGLOBULINEMIA AND MULTIPLE MYELOMA: DIAGNOSTIC AND THERAPEUTIC UPDATE
Milan, November 12, 2004
Guest Editor: Enrica Morra
information for authors, readers and subscribers

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The origin and power of a name

Ancient Greek

αἷμα [aima] = blood;
αἷματος [aimatos] = of blood,
λόγος [logos] = reasoning

Scientific Latin

haematologicus (adjective) = related to blood

Scientific Latin

haematologica (adjective, plural and neuter, used as a noun) = hematological subjects

Modern English

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Monoclonal gammopathies: natural history and risk of transformation

Natural history: prognostic value of clinico-pathological definitions

The term asymptomatic monoclonal gammopathy (MG) means the presence of a monoclonal component (MC) without evidence of multiple myeloma (MM), Waldenström’s macroglobulinemia (WM), or other malignant lymphoproliferative disease (MLD). Although the majority of cases will remain asymptomatic during long-term follow-up, subsets of asymptomatic MG have been found to evolve to overt MLD, with a variable frequency. 

The separation of asymptomatic MG by means of MC and bone marrow (BM) plasma cell (PC) infiltration cut-offs, as suggested by Durie & Salmon, unequivocally manages to define smoldering MM as prognostically different from monoclonal gammopathy of undetermined significance (MGUS). Indeed, by analyzing 1,104 cases of MGUS and 127 smoldering MM diagnosed in our Center from July 1975 to March 1998 (Table 1), we detected a higher risk of evolution in patients with smoldering MM than in those with MGUS (p < 0.0001), with prevalences of transformation of 19.7% and 5.8%, respectively.

A variety of diagnostic criteria were previously used to separate IgM-MGUS from smouldering WM in the setting of the heterogeneous group of IgM-asymptomatic MG, and a reliable distinction of subpopulations of IgM-asymptomatic MG with different probabilities of evolution into active disease was lacking for a long time. According to the Consensus Panel Recommendations from the 2nd International Workshop on WM held in Athens in September 2002, in the absence of symptoms and irrespective of the serum IgM MC level, BM findings would be considered the only reliable parameter for distinguishing smouldering WM from IgM-MGUS. In order to determine whether this arbitrary definition allowed the identification of two different prognostic subgroups, we compared 34 cases of smouldering WM, characterized by unequivocal histopathological evidence of lymphoplasmacytic (LP) non Hodgkin’s lymphoma (NHL) with an intertrabecular (nodular, interstitial or diffuse) BM infiltration pattern, with 138 cases of IgM-MGUS, characterized by the absence of morphological evidence of BM infiltrates, or equivocal evidence of BM infiltrates without confirmatory phenotypic studies. Although overall survival curves did not differ significantly (p=0.76) between the two subgroups, event-free survival (EFS) at 5 and 10 years was 95% (95% CI, 87-98%) and 83% (95% CI, 71-90%), respectively, in IgM-MGUS, and 77% (95% CI, 56-89%) and 42% (95% CI, 19-64%), respectively, in smouldering WM (p=0.0001) (Figure 1). The prevalences of transformation were 10.1% in IgM-MGUS and 38.2% in smouldering WM. Moreover, IgM-MGUS and smouldering WM differed significantly for IgM size, hemoglobin (Hb) level, erythrocyte sedimentation rate (ESR) level, degree of BM LP-NHL infiltration, proportion of patients with polyclonal serum Ig reduction and proportion with lymphocytosis (Table 2). These data suggested that BM histopathological evidence of lymphoma identifies a subgroup of asymptomatic patients with a high probability of evolution to overt MLD. Therefore, patients with an IgM gammopathy and BM infiltration by LP-NHL confirmed by immunophenotypic studies can no longer be considered to have a benign gammopathy.

Risk factors for transformation into MLD

Smouldering multiple myeloma

Only a few studies have evaluated risk factors for disease evolution in patients with smouldering MM. Weber et al., in 1997 demonstrated that IgA isotype, serum MC greater than 3 g/dL and Bence Jones (BJ) protein excretion greater than 50 mg/24 h independently predicted early progression of disease in patients with smouldering MM. Similarly, variables associated with an increased probability of evolution in our 127 patients with smouldering MM were the...
MGUS, Waldenström’s Macroglobulinemia and Multiple Myeloma

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>IgG (n=811)</th>
<th>IgA (n=114)</th>
<th>IgM (n=130)</th>
<th>IgD (n=1)</th>
<th>MC Pair (n=48)</th>
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<th>IgA (n=31)</th>
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<td>-</td>
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<td>% of Pts with Two Serum Polyclonal Ig Reduction</td>
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<td>% of BM PC (BM LP)</td>
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<td>8-128</td>
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<td>Median</td>
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<td>15</td>
<td>59</td>
<td>72</td>
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IgA isotype, detectable BJ proteinuria and > 10% BMPC levels. Recently, Rosinol et al., observed that a progressive increase of serum MC and the IgA isotype characterized a subgroup of patients with smoldering MM in whom the probability of disease evolution was high. Smoldering Waldenström's macroglobulinemia

By analyzing 27 patients diagnosed as having smoldering WM on the basis of IgM MC size greater than 3 g/dL, and/or a BM LP infiltration of 30% or greater, and/or a diffuse infiltration pattern on BM biopsy, we found high MC size and low Hb level to independent-
ly predict the risk of transformation (20). Similarly, Alexanian et al. found Hb levels < 11.5 g/dL, IgM MC greater than 3 g/dL and high β2-microglobulin (β2M) levels to correlate with the risk of evolution.21 The finding that high MC levels constituted a prognostic factor in smoldering WM contrasted with previous data,10,11 probably because of different criteria for selecting patients (in the majority of studies paraprotein levels greater than 5 g/dL had been chosen for the diagnosis of smoldering WM).

In an attempt to define prognostic subgroups of asymptomatic IgM-MG, we previously collected data from 452 patients with any serum IgM MC size and any degree or pattern of BM LP infiltration, but without evidence of overt WM or other MLD, both at diagnosis and for at least 12 months thereafter.22 At multivariate analysis, three risk groups were defined on the basis of IgM MC size, Hb level, and absolute lymphocyte count (ALC) > 4×10^9/L. After the 2nd International Workshop on WM, we separated, from among the previously analyzed cases, 384 patients with asymptomatic IgM-MG defined according to the new criteria (i.e., patients with any size of serum IgM MC, any degree of BM LP infiltration, any LP infiltration pattern except for a paratrabecular pattern on BM biopsy, no symptoms attributable to either IgM MC or tumor infiltration, and no evolution to overt WM or other MLD for at least 12 months from diagnosis) from 74 patients with IgM-related disorder (i.e., those patients with any size of serum IgM MC, symptoms attributable to the IgM MC such as cryoglobulinemia, and/or peripheral neuropathy, and/or cold agglutinin hemolytic anemia, and/or idiopathic thrombocytopenic purpura, no overt evidence of lymphoma, and no evolution to overt WM or other MLD for at least 12 months from diagnosis), the latter now being recognized as a distinct clinico-pathological entity.16 The differential clinical characteristics of the two groups are reported in Table 3. In asymptomatic IgM-MG, at univariate analysis MC level (p=0.0001), Hb level (p=0.0002), ALC > 4×10^9/L (p=0.0015), ESR level ≥ 40 mm/h (p=0.0035) and degree of BM LP-NHL infiltration (p<0.0001) were significantly associated with the probability of disease evolution, while BJ proteinuria (p=0.067) and a diffuse BM infiltration pattern (p=0.081) were associated with a trend for an increased risk of transformation.17 Absolute neutrophil counts < 1.8×10^6/L, serum β2-M

Table 2. Differential clinical characteristics of 138 patients with IgM-MGUS and 34 with smoldering WM at diagnosis.

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>IgM-MGUS</th>
<th>Smoldering WM</th>
<th>p value</th>
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<td>Serum MC, g/dL</td>
<td>Median</td>
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<td>One/Two Normal Ig Reduction, % of Pts</td>
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<td>29.4/8.8</td>
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<td>Hb, g/dL</td>
<td>Median</td>
<td>14.2</td>
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<td>Range</td>
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<tr>
<td>ALC &gt; 4×10^9/L</td>
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<td>12.9</td>
<td>0.09</td>
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<td>ESR, mm/h</td>
<td>Median</td>
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<td>70</td>
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<td>Range</td>
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<td>Serum ≤2-M, µg/mL</td>
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<td>Range</td>
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<tr>
<td>BM LP Infiltration, %</td>
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<tr>
<td>Range</td>
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<td>20-90</td>
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levels and reduced normal Ig levels were not associated with the probability of disease. At multivariate analysis, IgM size (p = 0.005) and lymphocytosis (p = 0.0001) independently predicted malignant evolution, while Hb level was associated with a trend for a higher risk of progression (p = 0.076) (Table 2). Assuming a label (x) for each variable [x1 = MC in mg/dL (log transformed), x2 = Hb in g/dL, x3 = 1 if lymphocytes >4×10^9/L, x3 = 0 if lymphocytes ≤4×10^9/L, x4 = 1 if detectable BJ proteinuria, x4 = 0 if undetectable BJ proteinuria, and x5 = ESR in mm/h], we calculated a prognostic index (PI = 1.2636x1 - 0.2684x2 + 2.4165x3 - 0.1190x4 + 0.4071x5) for each patient and identified 3 risk groups on the basis of PI distribution tertiles. The low-risk subgroup (1st tertile, PI ≥ 8.97) had EFS rates at 5 and 10 years of 100% and 89% (95% CI, 60%-97%), respectively; the intermediate-risk subgroup (2nd tertile, 8.97 < PI ≤ 10.06) had EFS rates at 5 and 10 years of 95% (95% CI, 85-98%) and 83% (95% CI, 64-93%), respectively; the high-risk group (3rd tertile, PI > 10.06) had EFS rates at 5 and 10 years of 85% (95% CI, 72-92%) and 44% (95% CI, 24-63%), respectively. The EFS of patients with a PI in the 3rd tertile significantly differed (p < 0.0001) from that of patients whose PI was in the first two tertiles pooled together in the upper curve.
we found that variables for one polyclonal serum Ig reduction, x5 By evaluating IgM-MGUS apart, pre-
+ 1.05 
+ 0.94 
3,4,22 
40 mm/h, 
for BMPC% 
for BMPC% e 10, 
+ 1.90 
Gertz MA, Fonseca R, Rajkumar SV. Wal-
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In contrast, 
show 
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eris I, Weber D, Delasalle K, Gika D, et al. Waldenström’s macroglobulinemia: clin-
ical features, complications, and manage-
16. Owen RG, Treon SP, Al-Katib A, Fonseca

Monoclonal gammopathies of undetermined significance

In our 1,014 MGUS patients, we found that variables associated with an increased probability of disease evolu-
tion were: the Ig A and IgM isotypes, serum MC lev-
els > 1.92 g/dL, detectable BJ proteinuria, the reduction of one or two serum polyclonal Ig, the ESR and BM PC levels. At multivariate analysis, BM PC infiltration, the presence of BJ proteinuria, polyclonal serum Ig reduction and ESR were independently associated with MGUS malignant transformation.

Assuming a label (x) for each variable (x: for BMPC% from 6 to 9, x: for BMPC% ≤ 10, x: for detectable BJ proteinuria, x: for one polyclonal serum Ig reduction, x: for two polyclonal serum Ig reductions, x: for ESR > 29 ≤ 40 mm/h, x: for ESR > 40 mm/h), we were able to calculate a PI ( = 0.37x 1 + 1.90x 2 + 1.05x 3 + 0.67x 4 + 3.01x 5 − 0.29x 6 + 0.94x 7) for each patient. Four different risk groups were identified, based on quartiles of PI distribution. This model allowed identification of patients in whom the risk of evolution is higher (4th quartile, prognostic index > 2.10) (Figure 3). The prog-
nostic value of detectable BJ proteinuria, reduction of normal Ig and BM PC levels were shown by others, as was no prognostic significance for age, sex, serum β2-M levels or serum albumin levels. In contrast, other authors found serum paraprotein levels to be a factor influencing the probability of malignant conversion in MGUS. By evaluating IgM-MGUS apart, preliminary unpublished data from our series, re-defined retrospectively according to the new criteria, show that prognostic factors for evolution largely overlap not only those found in IgG-IgA-MGUS, but also those found in asymptomatic IgM-MG as a whole, suggesting that IgM-MGUS could be best considered as the first step of a continuous spectrum of an indolent lymphoproliferative disease.


Waldenström's macroglobulinemia: clínico-pathological definition, prognostic markers and treatment recommendations

Clinico-pathological definition

Waldenström’s macroglobulinemia (WM) is a lymphoproliferative disorder characterized by bone marrow infiltration and by production of monoclonal immunoglobulin M (IgM). During the Second International Workshop on WM (September 2002, Athens, Greece) there was a consensus that Waldenström’s macroglobulinemia should be regarded as a distinct clínico-pathological entity and its diagnosis should be confined to those patients with bone marrow infiltration by small lymphocytes showing evidence of plasmacytoid/plasma cell differentiation who also have serum IgM monoclonal protein. Monoclonal IgM can be detected in the serum of patients with a variety of B-cell malignancies including chronic lymphocytic leukemia, small cell lymphocytic lymphoma as well as in monoclonal gammopathy of undetermined significance (MGUS). In the former disorders, the serum levels of monoclonal IgM are usually lower than those seen in WM. However, the concentration of IgM varies widely in WM and it is not possible to define a concentration that reliably distinguishes WM from other lymphoproliferative disorders. Thus the diagnosis of WM can be made irrespective of serum IgM concentration provided there is demonstration of bone marrow infiltration by lymphoplasmacytic lymphoma. A bone marrow biopsy is important for the diagnosis of WM. The pattern of infiltration is usually intertrabecular, most often diffuse, interstitial or mixed. A paratrabecular pattern of infiltration is unusual. The diagnosis of WM is supported by immunophenotypic study with flow cytometry and/or immunochemistry. Over 90% of patients have the following immunophenotypic profile: surface IgM+, CD19+, CD22+, CD25+. Expression of CD5, CD10 and CD23 may be seen in 10% to 20% of patients and should not preclude the diagnosis of WM. However in such patients the possibility of chronic lymphocytic leukemia or mantle cell lymphoma should be taken into account. Most patients with WM present clinical manifestations which are related to direct tumor infiltration of various organs and tissues and to the amount and specific properties of monoclonal IgM. Such patients should be classified as having symptomatic WM and usually need prompt initiation of treatment (Table 1). However, some patients who fulfill the diagnostic criteria of WM are being diagnosed by chance and do not have symptoms or signs attributable to the disease. These patients should be classified as having asymptomatic WM and should not be treated at diagnosis because they may remain stable for several months or years (Table 1). Recent studies have indicated that prognostic factors for early progression are the presence of even mild anemia and of serum monoclonal protein levels >30 g/L. It should be noted that initiation of therapy should not be based on serum monoclonal protein levels per se, since these may not correlate with clinical manifestations of WM. However, a serum monoclonal protein level >50 g/L is associated with a significant risk of hyperviscosity. Initiation of therapy is appropriate for patients who present or develop any of the complications shown in Table 2. Some patients may have symptoms due to the biological effects of the monoclonal IgM but no clear evidence of lymphomatous infiltration of the bone marrow or elsewhere. Such patients are now given the diagnosis of IgM-related disorders and may suffer from peripheral neuropathy, cryoglobulinemia, cold-agglutinin anemia, or amyloidosis. These patients usually have low levels of monoclonal IgM produced by a small clone of lymphocytes which is usually undetected by light microscopy and usually needs prompt treatment. Furthermore, several individuals are discovered by chance with asymptomatic serum monoclonal IgM <30 g/L, hemoglobin >120 g/L, no morphological evidence of lymphomatous marrow infiltration and absence of any
MGUS, Waldenström’s Macroglobulinemia and Multiple Myeloma

Table 1. Classification of WM and related disorders.

<table>
<thead>
<tr>
<th></th>
<th>Symptomatic WM</th>
<th>Asymptomatic WM</th>
<th>IgM-Related Disorders</th>
<th>IgM MGUS</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum IgM</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Bone marrow infiltration by light microscopy</td>
<td>+</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Symptoms due to tumor infiltration</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Symptoms due to IgM</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
</tbody>
</table>

Table 2. Clinical and laboratory indicators of the need to initiate therapy in WM.

- Hemoglobin <10 g/dL
- Platelet count <100x10^9/L
- Bulky adenopathy
- Symptomatic splenomegaly and/or hepatomegaly
- Infiltration of organs and/or tissues
- Fever, night sweats, weight loss
- Symptoms and signs of hyperviscosity
- Symptomatic neuropathy
- Amyloidosis
- Symptomatic cryoglobulinemia
- Cold-agglutinin anemia
- Evidence of disease transformation

Symptoms and signs attributable to monoclonal IgM. Such individuals are classified with a diagnosis of MGUS of IgM type. This condition is the most common diagnosis among individuals with a monoclonal IgM (Table 1). In some studies impaired performance status, presence of B-symptoms and hypoalbuminemia are significant adverse prognostic factors. Elevated serum β2-microglobulin is emerging as an important prognostic factor. This variable reflects tumor burden and also correlates with age. An International Working Group has already collected baseline data on more than 600 patients with WM who required treatment and will develop a prognostic index score for this disease.

It should be noted that 20-30% of patients with WM die of unrelated causes. Future studies of prognostic factors should take this parameter into consideration and should focus on cause-specific survival rather than overall survival. Most patients with WM die of progressive disease that has become refractory to treatment. Occasional patients may die of complications of myelodysplastic syndrome or acute myelogenous leukemia associated with prolonged use of alkylating agents. In approximately 5% of patients the disease may transform into a diffuse large cell lymphoma (Richter’s syndrome) which is usually fatal.

Treatment recommendations

The three main choices for front-line treatment of symptomatic patients with WM are alkylating agents, purine nucleoside analogs and the anti-CD20 monoclonal antibody rituximab. There are no data from prospective randomized studies to recommend the use of one first-line agent over another. Furthermore, it is difficult to compare the results of phase II studies for various reasons. Being a rare disease, most treatment studies of WM have included a small number of patients. In several studies the indication for treatment is not clearly stated and thus it is likely that some asymptomatic patients may have been included. Lastly, interpretation of treatment results has been hampered by the fact that different response criteria have been used.
**Alkylating agents**

Over the last 40 years, the standard primary therapy for WM has been the administration of oral alkylating agents, usually chlorambucil. Administration of chlorambucil either intermittently at high doses or continuously at lower or higher doses has induced partial responses (defined as at least 50% reduction of serum monoclonal protein concentration) in approximately 50% of patients. The addition of corticosteroids does not appear to improve the response rate or survival. These drugs should be avoided unless the patient presents or develops autoimmune anemia or thrombocytopenia. Response to chlorambucil is slow and several months are required to determine the chemosensitivity of the disease. Thus, chlorambucil is usually administered for long periods of time (up to 2 years in some studies). Combinations of alkylating agents with or without a vinca alkaloid, a nitrosourea or an anthracycline have also been used for the primary treatment of WM.\(^7\)\(^,\)\(^8\) Despite the lack of prospective randomized trials there is no evidence of a survival advantage after the administration of these combinations.

**Nucleoside analogs**

Over the last 10 years several phase II studies have evaluated the activity of the purine nucleoside analogs, fludarabine and cladribine, in previously untreated patients with WM.\(^4\)\(^,\)\(^18\)\(^,\)\(^23\) Objective response rates have ranged from 40% to 90%. Treatment with these agents may be associated with a faster response rate than that seen after treatment with alkylating agents; the median time to response has ranged between 1.2 months and 5.8 months.\(^19\)\(^,\)\(^23\) The addition of cyclophosphamide to cladribine has resulted in an objective response rate of 84% and in a median duration of response of 36 months.\(^24\)

Both fludarabine and cladribine have been administered to patients with primary or secondary resistance to alkylating agents. Approximately one-third of patients respond to either nucleoside analogue. These agents are more effective when administered to patients with primary refractoriness to alkylating agents or to patients relapsing off treatment. Patients treated during refractory relapse not only have a lower likelihood of response but are also at higher risk of myelotoxicity and immunosuppression.\(^25\)\(^,\)\(^30\) There is preliminary evidence that the combination of fludarabine and cyclophosphamide may be associated with improved response rates in patients with resistance macroglobulinemia.

**Rituximab**

Rituximab, an anti-CD20 monoclonal antibody, has been administered to both previously treated and untreated patients with WM. When rituximab is given at a standard dose of 375 mg/m\(^2\)/week IV for 4 weeks, 30-40% of patients achieve at least a partial response. The median time to response after rituximab is 3 to 4 months.\(^26\)\(^-\)\(^37\) This agent appears to be equally effective in previously untreated and pretreated patients. In some studies, patients with high levels of serum monoclonal protein (serum monoclonal protein >40 g/L or serum IgM >6000 mg/L) had a lower probability of response to rituximab.

Treatment with rituximab is well tolerated: mild infusion-related symptoms such as fever, chills and headache are noted in one-quarter of patients and myelosuppression is negligible. Thus rituximab may represent the treatment of choice for patients who present with or develop heavily infiltrated bone marrow with cytopenias. Furthermore, this agent is a suitable treatment for patients who are candidates for high dose therapy with autologous blood stem cell transplantation. Clinicians should be aware that in 30-40% of patients a transient increase of serum IgM may be noted 1 to 3 weeks after initiation of rituximab. This *IgM flare* may continue for several weeks and may place the patients at risk of symptomatic hyperviscosity. However the development of an *IgM flare* is not associated with a poorer response since serum IgM will return to its baseline value in most patients.\(^24\)\(^,\)\(^38\)

In view of their single-agent activity and non-overlapping toxicities, the combination of nucleoside analogs and rituximab has a sound rationale. Phase II studies indicate that such combinations, with either cladribine or fludarabine, are associated with at least partial responses in more than 80% of patients.\(^24\)\(^,\)\(^39\)

**High dose therapy**

High-dose therapy supported by autologous stem cell transplantation (ASCT) has been administered to a small number of patients with WM. Various preparative regimens have been used, including high dose chemotherapy with or without total body irradiation. High dose therapy was well tolerated with a treatment-related mortality of <5%. Most patients are treated during a late phase of their disease after refractoriness to conventional chemotherapy has developed. Despite this, objective responses have been documented in more than 80% of patients, including complete responses in up to 30% of patients.\(^40\)\(^,\)\(^44\)

In view of the small number of patients included in each series, the generally short follow-up and the heterogeneous population of patients, it is difficult to assess the duration of response after ASCT. Outside the context of a clinical trial, this treatment modality can be offered to patients under 70 years of age with disease resistant to conventional chemotherapy and rituximab, provided that adequate numbers of stem cell donors are available.
cells can be collected. In such patients high-dose melphalan may be an effective. The role of high dose therapy with ASCT as part of front-line treatment in WM needs to be evaluated.

Allogeneic transplantation has been used for a small number of young patients with WM. Despite its significant activity and the complete responses attained, the recommendation of this modality is restricted by its high treatment-related mortality (≤40%). Non-myeloablative allogeneic transplantation needs evaluation in this disease.

Biological agents
There are limited data suggesting that interferon α may be associated with hematologic improvement and with reduction of monoclonal protein in some patients. Thalidomide with or without dexamethasone and clarithromycin may be active in one-quarter of patients with WM. Bortezomib, a reversible proteasome inhibitor is under evaluation in WM.

Plasmapheresis
Plasmapheresis should be used in patients presenting with or developing symptoms and signs of hyperviscosity syndrome. IgM is a large pentameric molecule; 70% remains within the vasculature and 50% of circulating IgM can be cleared with one exchange. Plasmapheresis may also be of value in patients presenting with or developing severe cryoglobulinemia and rapidly progressing peripheral neuropathy. Because plasmapheresis does not affect tumor growth and IgM production, concurrent administration of systemic therapy is required for long-term control of these complications.

Splenectomy
Anecdotal reports have indicated that some patients with resistant macroglobulinemia and significant splenomegaly benefit from splenectomy. This benefit consists not only of improvement of cytopenia but also in a reduction of monoclonal protein. With currently available data and without prospective studies it is difficult to recommend this procedure as part of the treatment strategy for WM.

Conclusions
The choice of primary treatment for patients with symptomatic WM depends on several variables. These include the patient’s age, whether the patient is a candidate for autologous stem cell transplantation, the presence of cytopenias (thrombocytopenia in particular), the need for rapid disease control and the presence of co-morbid conditions. Ongoing and future trials will assess the impact of combinations that include all three type of active agents i.e. nucleoside analogs, alkylating agents, and rituximab. Large studies focusing on prognostic factors are needed in order to identify, at diagnosis, patients with a poor prognosis who could be candidates for high dose therapy with autologous stem cell transplantation as part of their first-line therapy.

References
15. Merlini G, Baldini L, Broglio C, Correlli M,


38. Treon SP, Wasi P, Emmanouilides C. Combination therapy with rituximab and fludarabine is highly active in Waldenström's macroglobulinemia. Blood 2002; 100:211[abstract].


48. Ghobrial IM, Fonseca R, Greipp PR, The
New therapeutic options in the treatment of Waldenström’s macroglobulinemia

In Waldenström’s macroglobulinemia (WM) therapy is currently reserved for those patients who are symptomatic. Conventional treatment consists of alkylating agents, with or without corticosteroids. Both chlorambucil and cyclophosphamide, generally given in small daily doses, are effective in producing regression of lymphadenopathy, splenomegaly and in reducing IgM levels. In most series the reported response rate to alkylating agents is approximately 60% with a median survival time of about 60 months.\(^1,2\) This therapeutic approach should be considered palliative rather than curative as complete remissions are rarely obtained and most of the patients will relapse and develop resistance to treatment with alkylating agents. Several studies have attempted to improve the outcome of patients by administering alkylating agents in combination with a vinca alkaloid and anthracyclines even if no obvious benefit has been found.\(^3\)

The treatment of WM has changed greatly since the introduction of purine analogs. Fludarabine (FAMP) and 2-chlorodeoxyadenosine (2-CdA) were shown to be effective in patients pre-treated with alkylating agents and even in those with refractory disease.\(^4-9\) Response rates to purine analogs as first-line chemotherapy range from 38% to 85%.\(^7,10,11\) Discrepancies in response rates between different studies could be due to the small number of patients enrolled in the trials and in differences of response criteria. In order to improve the results obtained with purine analogs, associations with other cytotoxic drugs have been studied. Based on in vitro evidence of synergistic effects and on the promising results obtained in other lymphoproliferative disorders combinations of FAMP or 2-CdA with cyclophosphamide are also being utilized in WM.\(^12-15\) Preliminary results show that even if combination chemotherapy can produce high response rates and long sustained responses, complete remission are not achieved. Significant advances in the development of monoclonal antibodies have improved targeting of leukemic cells with acceptable toxicity. Rituximab, a chimeric human-mouse antibody that recognizes and binds to CD20, was demonstrated to be effective in WM when administered as a single-agent and to have a good tolerability.\(^16-18\) Another humanized monoclonal antibody with specificity toward the CD22 surface antigen, epratuzumab, is now under investigation in WM patients. Therapeutic strategies including the administration of monoclonal antibodies in association with chemotherapy or after chemotherapy may improve the quality of response.\(^19,20\) The increase in the incidence of complete remission rates is the first step to obtain long-term control of the disease.

Combination programs of immunotherapy and chemotherapy eradicated minimal residual disease, leading to prolonged, disease-free survival, in other lymphoproliferative disorders.\(^21\) Furthermore, the possibility of performing in vivo purging of the disease after chemo-immunotherapy could improve the results of autologous stem cell procedures.\(^22\)

**Purine analogs**

**Fludarabine phosphate**

There is increasing evidence that the purine analogs which are active in low grade lymphomas and chronic lymphocytic leukemia (CLL) are also active in WM patients. Purine analogs have been used not only in the setting of patients who are resistant to alkylating agents but also as first line chemotherapy, yielding higher response rates. Several studies have addressed the efficacy of fludarabine monophosphate (FAMP) in the treatment of previously treated WM patients. Most of these trials (Table 1) are small phase II studies and their inclusion and response criteria differ. The response rate observed in previously treated patients ranges from 30 to 50%, being highest in patients who are still sensitive to their primary therapy. Dimopoulos et al. observed a higher response rate in patients with primary refractory disease (43%) than in...
patients with refractory relapse (17%). The French Macroglobulinemia Cooperative Group reported data on 71 previously treated patients; 21 patients (30%) showed a response after FAMP treatment with a time to treatment failure of 32 months.

In the literature there is only one randomized trial comparing the efficacy of FAMP treatment alone versus the combination of cyclophosphamide, doxorubicin and prednisolone (CAP) in patients in first relapse or with primary refractory disease following treatment with alkylating agents. Nineteen one patients were enrolled in this trial; the response rate in the patients treated in the FAMP arm was higher (30% versus 11%) and the responses were also more durable (19 months versus 3 months).

Higher response rates have been observed after FAMP treatment in the setting of previously untreated patients. A remission rate of 74% was obtained after 6 cycles of FAMP given as first-line chemotherapy in the 19 patients enrolled in a phase II multicenter European trial. Similar results were obtained by Thalhammer-Scherer et al. who used FAMP treatment in a small study of 6 previously untreated patients; in 5 cases there was a marked decrease (>75%) of the IgM levels with responses lasting from 20 to 55 months. The largest phase II study of FAMP in both untreated and previously treated patients was published by the South West Oncology Group (SWOG) in 2001 and the results were recently updated. The overall response rate among the 118 untreated patients after 4 cycles of FAMP (30 mg/m² iv daily for 5 consecutive days) was of 38%, with 3% of patients obtaining a CR. The 5-year OS and PFS rates were 62% and 49%, respectively. Only a slightly higher response rate was observed in this trial when comparing the overall responses obtained in the untreated group of patients and those who had been previously treated (37% versus 30%, p = 0.33). The difference in the response rate between the SWOG trial and the other cited studies could be related not only to the small size of patients considered in the latter but also to differences in patients’ characteristics and response criteria.

In the SWOG study those patients who had responded after four courses of FAMP received a further four cycles of treatment. In most cases responses occurred within 3 to 6 months of treatment initiation but responses were also observed after 6 months and 1 year. Other authors have recently focused their interest on the timing of response after FAMP treatment. The response rate one month after completing therapy in the 16 patients treated at the Royal Marsden Hospital was 56% with 25% CR and 31% PR. During the subsequent follow-up, without further therapy, 7 delayed responses were observed (44%), one patient achieving a maximum and stable 84% reduction of paraprotein 25 months after FAMP plus cyclophosphamide treatment. These data suggest that at least 6–12 months after the beginning of therapy are required to determine chemosensitivity and to assess the maximum response of WM patients treated with FAMP or FAMP in combination with cyclophosphamide.

Preclinical studies suggested that FAMP and cyclophosphamide had added or synergistic activity based on the inhibition of DNA repair enzymes by the purine analogs. The combination of FAMP and cyclophosphamide has been extensively used in the treatment of low grade non-Hodgkin’s lymphomas and CLL and has been shown to be effective both in untreated and pre-treated patients. The association of FAMP and cyclophosphamide has also been shown to be active in WM patients. Dimopoulos et al. reported partial responses in 55% of 11 patients, most of whom had been resistant to previous treatment, after the administration of four courses of FAMP 25 mg/m² and cyclophosphamide 250 mg/m² for three consecutive days. Only 2 of the 6 responding patients showed disease progression after a median follow-up of 28 months. Similarly, a partial response rate of 71% was obtained in 21 patients, 8 previously untreated, after 4 courses of the same regimen with a higher dosage of cyclophosphamide (300 mg/m²). In none of these series was a CR recorded.

2-chlorodeoxyadenosine

The nucleoside analog 2-chlorodeoxyadenosine (2-CdA) has been effective in several lymphoid malignan-

Table 1. Response rates to fludarabine monophosphate treatment (selected references).

<table>
<thead>
<tr>
<th>Study</th>
<th>Prior treatment</th>
<th>N. pts</th>
<th>Overall response (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimopoulos, 1993</td>
<td>1st refractory</td>
<td>14</td>
<td>43</td>
</tr>
<tr>
<td>Re: refractory relapse</td>
<td></td>
<td>12</td>
<td>17</td>
</tr>
<tr>
<td>Leblond, 1998</td>
<td>1st refractory</td>
<td>8</td>
<td>30</td>
</tr>
<tr>
<td>Refractory relapse</td>
<td></td>
<td>63</td>
<td></td>
</tr>
<tr>
<td>Leblond, 2001</td>
<td>1st refractory or</td>
<td>92</td>
<td>FAMP</td>
</tr>
<tr>
<td>2001</td>
<td>first relapse</td>
<td></td>
<td>treatment : 30</td>
</tr>
<tr>
<td>(FAMP versus CAP)</td>
<td>treatment: 11</td>
<td></td>
<td>CAP treatment: 11</td>
</tr>
<tr>
<td>Dhodapkar, 2001</td>
<td>None</td>
<td>118</td>
<td>38 (CR3%)</td>
</tr>
<tr>
<td>Prior treatment</td>
<td>64</td>
<td>33</td>
<td></td>
</tr>
<tr>
<td>Thalhammer, 2000</td>
<td>None</td>
<td>6</td>
<td>71</td>
</tr>
<tr>
<td>Prior treatment</td>
<td>1</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Foran, 1999</td>
<td>None</td>
<td>19</td>
<td>74</td>
</tr>
</tbody>
</table>

a. The nucleoside analog 2-chlorodeoxyadenosine (2-CdA) has been effective in several lymphoid malignan-

haematologica 2004; 89(supplement 11):November 2004
cies with the most striking results occurring in patients with hairy cell leukemia, in whom prolonged remissions occur after just one single course of therapy.28,29 Dimopoulos et al. first described the activity of 2-CdA in patients with WM, most of whom were resistant to alkylating agent-based combinations (results updated in 19958); an overall response rate of 43% was achieved after the administration of 2 courses of 2-CdA at the dosage of 0.1 mg/kg/day given as continuous infusion over 24 hours for 7 days (Table 2). Based on this encouraging experience, in a subsequent study the same authors administered 2-CdA at the same dosage to 26 previously untreated patients. A higher frequency of response was obtained among this group of patients, as 22 patients (85%) showed a response including three patients who achieved a CR.30 Fridrik et al. also reported a very high number of responses in a small series of 10 not previously treated patients (CR+PR 80%).31 The difference in terms of response rate among previously treated patients compared to untreated patients was not further confirmed by Dellanoy et al.32 or Lewandowsky et al.33 The discrepancy between the results obtained in these studies may be related to the different number of courses administered in the setting of pretreated patients.

Differences in 2-CdA administration mode seem not to have any influence on the treatment outcome; bolus administration is also effective producing complete responses in 5% and partial responses in 50% with a median duration of 28 months.34 In a population of 25 heavily pretreated patients 2-CdA given as subcutaneous bolus injection, for a total dose of 0.5 mg/kg per cycle over 5 days for a maximum of six cycles, was shown to be well tolerated and active, leading to partial remissions a 40% of the patients.35 Recently, Weber et al. reported their experience evaluating the administration of 2-CdA in combination treatment for symptomatic patients with previously untreated WM.36 Combination therapy of 2-CdA with cyclophosphamide, and more recently rituximab, led to response rates of 84% and 94%, respectively. Although combination therapy did not improve the response rate over that achieved by 2-CdA alone, a possible improvement of median remission duration was detected, although a longer follow-up is required to assess this issue. The reason for the inferior response obtained after administering 2-CdA in combination with prednisone remains unclear (Table 3).

### Table 2. Response rates to 2-chlorodeoxyadenosine (2-CdA) as single agent treatment.

<table>
<thead>
<tr>
<th>Study</th>
<th>Number of patients treated</th>
<th>No. courses</th>
<th>Treatment modality</th>
<th>Response CR+PR %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimopoulos, 1995</td>
<td>–</td>
<td>46</td>
<td>2</td>
<td>43</td>
</tr>
<tr>
<td>Dimopoulos, 1994</td>
<td>26</td>
<td>–</td>
<td>2</td>
<td>85</td>
</tr>
<tr>
<td>Dellanoy, 1999</td>
<td>5</td>
<td>13</td>
<td>2 (1-6)</td>
<td>39</td>
</tr>
<tr>
<td>Fridrik, 1997</td>
<td>10</td>
<td>–</td>
<td>4</td>
<td>80</td>
</tr>
<tr>
<td>Lewandowsky, 2000</td>
<td>11</td>
<td>14</td>
<td>3 (1-5)</td>
<td>64</td>
</tr>
<tr>
<td>Betticher, 1997</td>
<td>1</td>
<td>24</td>
<td>3 (1-6)</td>
<td>40</td>
</tr>
</tbody>
</table>

### Table 3. Responses to 2-CdA in combination treatment according to regimen (Weber et al.).36

<table>
<thead>
<tr>
<th>Regimen</th>
<th>2-CdA n=16</th>
<th>2-CdA/pred n=20</th>
<th>2-CdA/Cy n=37</th>
<th>2CdA/Cy rituximab n=17</th>
</tr>
</thead>
<tbody>
<tr>
<td>CR no (%)</td>
<td>3 (19)</td>
<td>1 (5)</td>
<td>2 (5)</td>
<td>3 (18)</td>
</tr>
<tr>
<td>PR no (%)</td>
<td>12 (75)</td>
<td>11 (55)</td>
<td>29 (79)</td>
<td>13 (76)</td>
</tr>
<tr>
<td>Median Remission Duration (mos (range))</td>
<td>23 (2-86)</td>
<td>9 (4-74)</td>
<td>36 (5-64)</td>
<td>NR (2-33+)</td>
</tr>
</tbody>
</table>

CR: complete remission; PR: partial remission; pred: prednisone; Cy: cyclophosphamide; NR: not reached.
Table 3. Responses to 2-CdA in combination treatment according to regimen (Weber et al.).

<table>
<thead>
<tr>
<th></th>
<th>2-CdA</th>
<th>2-CdA/pred</th>
<th>2-CdA/Cy</th>
<th>2-CdA/Cy/rituximab</th>
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<tbody>
<tr>
<td></td>
<td>n=16</td>
<td>n=20</td>
<td>n=37</td>
<td>n=17</td>
</tr>
<tr>
<td>CR n. (%)</td>
<td>3 (19)</td>
<td>1 (5)</td>
<td>2 (5)</td>
<td>3 (18)</td>
</tr>
<tr>
<td>PR n. (%)</td>
<td>12 (75)</td>
<td>11 (55)</td>
<td>29 (79)</td>
<td>13 (76)</td>
</tr>
<tr>
<td>Median remission duration months (range)</td>
<td>23 (2-86)</td>
<td>9 (4-74)</td>
<td>36 (5-64)</td>
<td>NR (2-33+)</td>
</tr>
</tbody>
</table>

CR: complete remission; PR: partial remission; pred: prednisone; Cy: cyclophosphamide; NR: not reached.

Monoclonal antibodies

Rituximab

CD20 is a B-cell-specific antigen that is present from the pre-B stage to the mature B-cell stage of B-cell differentiation. The expression of CD20 continues through B-cell maturation until the plasmocytoid immunoblast phase; it is only weakly expressed on plasma cells. The CD20 antigen is a non-glycosylated protein deeply anchored in the B-cell membrane with amino and carboxyl termini extending into the cytoplasm. Less than 10% of the protein is expressed on the cell surface. CD20 has four cell membrane-spanning domains and probably functions as a calcium channel, although its function is still largely unknown. It is thought to be involved in B-cell activation and regulation of B-cell growth; binding of CD20 by anti-CD20 antibody can affect cell cycle progression. The amino sequence of CD20 is similar to that of the α-subunit of the high affinity IgE receptor; these proteins are members of a family of proteins that play a role in signal transduction. CD20 appears to participate in signaling through cross-linking, and CD40 and/or the MHC class II molecules may play a role in this cross-linking process. In some cell lines anti CD20 antibodies can induce apoptosis. CD20 is expressed on most malignant B cells, with nearly 90% of B-cell lymphomas expressing this antigen. Flow cytometric studies have shown that CD20 is present on malignant plasma cells in 20% of patients with multiple myeloma, up to 50% of patients with plasma cell leukemia and 75 to 100% of WM patients. Therefore CD20 is an appropriate target for the treatment of WM because this antigen is present on circulating clonotypic B cells as well as on most bone marrow lymphoplasmocytic cells.

Rituximab is a monoclonal antibody with a mouse/Fab human/Fc chimeric construction directed against CD20. Rituximab binds to human CD20 with high affinity and is able to bind to C1q human complement through its Fc receptor and mediate both complement-directed and antibody-directed cell-mediated cytoxicity. Its clinical efficacy is also related to its capability of inducing apoptosis and direct inhibition of proliferation. Binding of CD20 by antibody does not appear to induce antigen modulation or internalization, thus making the CD20 antigen an excellent therapeutic agent.

Rituximab has been successfully used in the treatment of non-Hodgkin’s lymphoma alone or in combination chemotherapy. Byrd et al. first reported a 57% response rate in 7 heavily pretreated patients with WM after the administration of rituximab as a single agent. The median progression-free survival for those patients was 6.6 months (range 2.2-29+ months). Other small series appeared in literature providing evidence of the activity of rituximab in WM.

In 2001 Treon et al. published the results of a large retrospective series of 30 WM patients treated with rituximab as a single agent. The monoclonal antibody was administered at the dosage of 375 mg/m² either weekly or three times a week; the median number of Rituximab infusions received by all patients was 4 (range 1-11). In this series of patients rituximab was demonstrated to be active since 27% and 33% of patients achieved a partial and a minor response, respectively, with a median time of treatment failure of 8 months (range 3-20+). An important finding in this study was the beneficial changes in hematologic parameters observed after rituximab treatment; increases in mean hematocrit and mean platelet counts were noted for 63.3% and 50% of all patients.

Based on these encouraging results Dimopoulos et al. conducted a phase II study to evaluate the efficacy of rituximab in previously treated and untreated WM patients. Rituximab in this study was administered at a dosage of 375 mg/m² weekly for 4 consecutive weeks. Patients without evidence of progressive disease received, three months later, four further infusions of the drug. Using strict criteria to define response, which required more than 50% reduction of serum monoclonal protein and tumor infiltration at all involved sites, 44% of the 21 treated patients showed a response. The response rate was similar when comparing the group of untreated patients (50%) with those who had been previously treated (40%). The median time to progression observed in this series of patients was 16 months longer than that observed by Treon et al (7 months). It is unclear whether the difference in the time to progression in the two studies was due to different patients’ characteristics or to the effect of a higher number of rituximab.
infusions administered in the trial by Dimopoulos et al. Similar response rates (52.2%) were reported in the preliminary results of an ECOG phase II study presented at the American Society of Hematology (ASH) meeting in 2003. Both previously untreated and treated patients were enrolled in this trial and treatment consisted of rituximab 375 mg/m² weekly for 4 consecutive weeks. Objective responses were observed more frequently in the previously untreated group (35.3%) than in the previously treated group (20%).

Paradoxical spikes in serum IgM levels may occur in WM patients following IgM treatment. Treon et al. observed this phenomenon, with an increase > 25% in 8 of the 11 patients for whom serial serum IgM samples were available within 3 months following therapy. The mechanism of this effect is still unknown and under investigation; it is suggested that it may be related to CD20 signals triggered by rituximab. In any case, during rituximab treatment all patients, particularly those with a high serum viscosity, should undergo careful clinical and laboratory monitoring. Moreover, patients should not be taken off therapy within the first weeks of treatment solely on the basis of an increased serum IgM level. In all studies, as in several case reports, there has been a remarkable improvement of peripheral neuropathy or a stabilization of this condition after rituximab treatment.

**Epratuzumab**

CD22 is a B cell-specific transmembrane protein of the Ig super-family with seven Ig-like domains. CD22 seems to have two distinct functions. First, it is associated with the B-cell receptor and inhibits the BCR signal, as has been demonstrated by characterization of CD22-deficient mice. B cells of these mice show increased Ca²⁺ responses when stimulated by anti-IgM. The inhibitory effect of CD22 is due to phosphorylation of three Ig-like tyrosine-based inhibitory motifs on its intracytoplasmatic region. These motifs are phosphorylated upon B-lymphocyte antigen receptor cross-linking and can bind the tyrosine phosphatase SHP-1 as a negative regulator of signaling from BCR. Furthermore, CD22 belongs to a family of adhesion molecules, the Siglec family (sialic acid binding Ig-like lectins) and it is also referred to as Siglec-2. CD22 controls the homing of recirculating B cells back to the bone marrow by binding to ligands which are expressed on sinusoidal endothelium. It is not so clear how these two apparently distinct functions of CD22 are linked. Binding to ligands on the B-cell surface may affect the subcellular localization and accessibility of the intracellular domain of CD22 and thereby control its inhibitory function. The B cells of CD22-deficient mice have a shorter life span and enhanced apoptosis, thus indicating a key role of this antigen in B-cell development and survival.

CD22 can internalize rapidly from the cell surface to the cytoplasm after it has bound with its natural ligands or antibodies. Recent studies have shown that CD22 may also internalize constitutively on unstimulated B-cell lines. CD22 is detected in the cytoplasm early in B-cell development (in the late pro-B-cell stage) and appears on the cell surface simultaneously with IgD and is found in most mature B cells. The expression, which is strongly correlated with IgD, is lost with the terminal differentiation of B cells and is absent on plasma cells. Kern et al. showed by standardized fluorescence quantification that peripheral blood B cells from healthy donors expressed on average 20,047±1,082 CD22 molecules, that is almost the 70% of the CD20 molecules expressed by the same cells. As regards the presence of CD22 in the lymphoid tissues, this antigen is weakly expressed in the B cells of the germinal center while it is more strongly expressed in follicular, mantle and marginal zone B cells.

The vast majority of B-cell malignancies express CD22. Clonal lymphocytes from WM patients are characterized by the constant expression of CD22 as well as of CD19 and CD20. Epratuzumab (mLL2, formerly EPB-2) is a mouse monoclonal antibody, developed by Immunomedics, that binds specifically to CD22. Immunohistological evaluation revealed that this antibody recognized B cells within the spleen and lymph nodes but did not react with antigen unrelated to B cells in normal tissue specimens; flow cytometry showed no reactivity with platelets, red blood cells, monocytes or granulocytes in normal peripheral blood. In vitro monoclonal antibodies (mAb) specifically blocking the interaction of CD22 with its ligand binding site have been shown to induce cell death through apoptosis in several Burkitt’s lymphoma cell lines; furthermore the same antibodies have shown an antitumor effect in vivo in animal models. A humanized anti CD22 mAb (hLL2, epratuzumab) has been developed on the basis of mLL2. This is more suitable for clinical use. The humanized antibody contains the original murine sequence only at the antigen binding site as the complementary-determining regions (CDR) of mLL2 were subsequently grafted into a human IgG1 genetic backbone. Approximately 95% of the molecule is of human origin, greatly reducing the potential for immunogenicity: Human IgG1, the isotype of hLL2, has the ability to mediate ADCC and to fix complement and it is also possible that hLL2 could inactivate the physiological function of CD22.

The antitumor activity of epratuzumab has been demonstrated in a recent phase I/II clinical trial in which patients with recurrent indolent or aggressive non-Hodgkin’s lymphoma received escalating doses of the
Six cohorts of patients were treated with 4 consecutive weekly epratuzumab infusions at doses of 120, 240, 360, 480, 600 or 1000 mg/m²/week. Evaluation of treatment response was confounded by the wide range of histological subtypes and dose levels studied; however, all clinical responses were observed at the 240 to 600 mg/m²/week levels and in the follicular and diffuse large B-cell histologies only.

Epratuzumab had a tolerable safety profile in this study and no dose-limiting toxicity was observed. Transient mild or moderate infusion-related reactions occurred primarily during the first infusion. No consistent changes were seen in red blood cell, platelet or absolute neutrophil counts, or in immunoglobulin or T-cell levels. In patients without leukemic involvement peripheral B-cell levels decreased following epratuzumab therapy and remained decreased for at least 3-6 months. Pharmacokinetic data revealed that the blood levels of epratuzumab increased in a dose-dependent manner after the fourth week infusion and remained in circulation with a half life of 23 days.

The tolerability of this compound and the efficacy demonstrated in preliminary studies in the indolent lymphoproliferative disorders, prompted performance of a phase II study of epratuzumab in patients with WM, in which we are currently involved.

Treatment consists of epratuzumab infusions over approximately 30-60 minutes administered at a dose of 360 mg/m² once weekly for 4 consecutive weeks.

Since anti-CD20 mAb have mechanisms of action distinct from those of other cytotoxic drugs or targeted therapies, they are good candidates for combination treatment.

Preliminary data from a small single center study showed that the combination of rituximab and epratuzumab, at doses of 375 and 360 mg/m², respectively, once weekly for 4 consecutive weeks, is well tolerated and efficacious in patients with non-Hodgkin's lymphomas. Ten of the 16 patients treated with the combination of the two antibodies reached an objective response (9 CR and 1 PR). The CR rate observed in this series was higher than that in a historical series of similar patients treated with rituximab alone.

The ability of CD22 to internalize rapidly also makes it an ideal candidate for intracellular delivery of cytotoxic agents or radioimmunoconjugates. Preclinical studies and early clinical evaluation suggested that anti-CD22 antibodies conjugated to ricin A toxin and pseudomonas exotoxin had strong antilymphoma effects in heavily pretreated patients with B-cell malignancies and hairy cell leukemia.

Preliminary clinical evaluation of epratuzumab and its parental murine version, LL2, as radioimmunoconjugates have also demonstrated antilymphoma activity.

Radioimmunoconjugates

In an effort to increase the efficacy by targeting radiation in the tumor site, monoclonal antibodies have been conjugated to radioisotopes. Radioimmunotherapy is a novel treatment modality that combines the benefits of radiotherapy and immunotherapy, enabling multiple sites of disseminated disease to be treated simultaneously and effectively, while minimizing toxicity to normal tissues.

Two of these antibodies target CD20: yttrium-90 (Y-90)-labeled ibritumomab tiuxetan (zevalin) and tositumomab/iodine-131 (I-131)-labeled tositumomab (be/xar). Other agents target either CD22 (Y-90 epratuzumab) or human leukocyte antigen (HLA)-DR (I-131 Lym-1).

The first radioimmunoconjugate to be approved by the US Food and Drug Administration (FDA) for the treatment of cancer was zevalin; this consists of the anti-CD20 monoclonal antibody ibritumomab covalently linked to tiuxetan for chelation of 90Y. The high energy of the β particles emitted by 90Y (2.3 MeV) achieves a wide-ranging cross-fire effect. Approximately 90% of the energy is deposited within 5 mm of the radiation source, which kills not only antibody-bound cells but also neighboring malignant cells within a diameter of up to 12 mm. The half-life of 90Y matches the in vivo biological half-life of the monoclonal antibody (64 h), with negligible excretion of 90Y in urine.

Phase I and II studies demonstrated that zevalin is effective in the treatment of patients with lymphomas with low-grade histology.

A major phase III randomized trial directly compared 90Y-ibritumomab tiuxetan with the unlabeled anti-CD20 mAb rituximab. Results demonstrated that OR and CR rates were significantly higher with zevalin compared with rituximab (OR rate 80% vs. 56%, p = 0.002; CR rate 30% vs. 16%, p = 0.004). Time to progression (TTP) was 11.2 vs. 10.1 months (p = 0.173). The primary toxicity associated with zevalin treatment is reversible myelosuppression, which correlates with the degree of bone marrow involvement at baseline. An integrated analysis of safety data from 5 zevalin studies showed that only 7% of patients were hospitalized due to infection during the treatment period.

In addition, treatment with zevalin did not lead to an increase in the risk of developing secondary malignancies (myelodysplastic syndrome/acute myeloid leukemia), or preclude subsequent therapy upon relapse. Up to now only two patients with WM have been treated with zevalin; they had been enrolled in the non-Hodgkin lymphoma’s study and had to have adenopathy and a limited (<25%) bone marrow involvement. Both patients responded to treatment, one achieving CR lasting more than 20 months, and the other a PR.
A phase I dose escalation trial has been designed to evaluate the use of zevalin in patients with WM and bone marrow involvement of 20% to 50%.

This study will provide valuable information regarding the efficacy of the drug in this disease in the setting of significant bone marrow involvement. Furthermore, this study will also focus on the tolerability of retreatment as patients may receive the drug every 12 weeks up to a maximum cumulative dose of 0.4 mCi/kg.

**Thalidomide/immunomodulatory agents**

Angiogenesis is important for the progression of several hematologic malignancies; studies show that angiogenesis is increased in the late phase of multiple myeloma compared to earlier stages. This study demonstrated that only 30% of WM patients have increased angiogenesis (intermediate or high-grade) and a weak correlation was found between mean microvessel density and extent of bone marrow infiltration. Even if according to these observations angiogenesis does not appear to be a major factor in WM, there is a rationale for studying anti-angiogenic agents in this disease, since the activity of these agents is limited only to the inhibition of angiogenesis but includes other possible mechanisms of action such as:

1. **Modulation of expression of adhesion molecules** (ICAM-1 and LFA-1).
2. **Modulation of several cytokines and inflammatory mediators** (TNFα, IL-1β, IFNγ, IL-6, IL-10, IL-12, COX).
3. **Immunomodulation via induction of secretion of interferon-γ and IL-2**.
4. **Natural killer cell and CD8 lymphocyte activation**.
5. **Down-regulation of vascular endothelial growth factor or basic-fibroblast growth factor**.

Furthermore, in vitro studies in myeloma cells demonstrated that thalidomide and its analogs may be pro-apoptotic both through direct effect and indirectly via down-regulation of pro-apoptotic cytokines in the bone marrow microenvironment.

Recently thalidomide has shown to be effective in approximately 30%-60% of patients with refractory myeloma when administered alone or in combination with dexamethasone or chemotherapy.

The activity of thalidomide as a single agent in the treatment of WM patients was assessed in a phase II study. Twenty patients, 10 previously untreated, were enrolled in the trial and received thalidomide initially at the dosage of 200 mg with dose escalation in 200 mg increments every 14 days to a maximum of 600 mg. Five of the 20 patients achieved a partial response (25%) while 5 patients showed stable disease. Responses occurred in two of the 10 pretreated patients and in three of the 10 previously untreated ones; none of the patients treated during resistant relapse had a response. Time to response was very short, being between 0.8 to 2.8 months, suggesting that thalidomide may have a direct cytotoxic effect on tumor cells. Only in five patients was it possible to escalate the dosage to 600 mg; among the five responding patients the maximum dose of thalidomide administered was 400 mg in 4 cases and 200 mg in one case.

Based on the efficacy of thalidomide demonstrated in this study and considering that this treatment is not generally associated with myelosuppression, further studies were performed combining thalidomide with low dose chemotherapy.

In vitro studies demonstrated that macrolides, including clarithromycin, can suppress the synthesis of several cytokines, such as tumor necrosis factor α and granulocyte colony-stimulating factor, and can also inhibit tumor-induced angiogenesis.

Coleman et al. first reported that the combination of clarithromycin, dexamethasone and thalidomide was active in 6 of 7 pretreated patients with WM. Dimopoulos et al. recently administered clarithromycin at a dose of 500 mg orally twice daily continuously, thalidomide 200 mg daily and dexamethasone 40 mg once a week in pretreated patients. The preliminary results of this trial were presented at the Second International Workshop on WM in 2003; the study is still ongoing.

Three of the 12 patients enrolled so far have had a partial response and interestingly two of them were previously resistant to thalidomide as a single agent. This observation indicates that clarithromycin may have an antitumor effect and its activity may be synergistic with that of thalidomide. The research on immunomodulatory drugs, sharing the properties of thalidomide described above, led to the development of second generation drugs: revimid and actimid. These drugs are two to three orders of magnitude more potent than thalidomide and have a better safety profile.

Currently phase II and phase III trials are ongoing in order to evaluate the efficacy of revimid in relapsed and refractory multiple myeloma. As the preliminary results of this trial are promising this drug should be tested in WM in the future.

**High dose chemotherapy followed by autologous or allogeneic stem cell transplantation**

Although nucleoside analogs have been shown to produce high response rates in patients with WM and results with monoclonal antibodies seem to be promising, the impact on survival of such treatments is still uncertain and most of the patients will relapse and die of disease progression. High dose chemotherapy with
autologous or allogeneic stem cell transplantation can be very effective in similar disorders such as multiple myeloma and indolent lymphomas but has rarely been used in WM.

Limited experiences have been reported in literature regarding autologous stem cell transplantation and most of the series included patients with refractory disease or in relapse regardless of the response to salvage treatment.

At the University of Arkansas, six patients with WM received high dose melphalan with unpurged peripheral blood stem cells (PBSC) most of them achieved sustained PR, only one patient achieved a CR. Four of these patients were transplanted while in untreated relapse or in progressive disease.

Dreger et al. administered a treatment consisting of two or three cycles of Dexa-BEAM followed by myeloablative therapy with fractionated TBI 2 Gy and cyclophosphamide 60 mg/kg/d on two consecutive days to 7 MW patients. All patients received PBSC, in five cases ex vivo B-cell depletion of the leukapheresis product was performed. Although in this study the treatment could be regarded as more intensive when compared to that in the study by Desikan et al., it did not appear to be substantially more effective as only two patients showed a CR 6 months after transplant.

Both studies demonstrated that high dose chemotherapy with autologous stem cell support is feasible with a low incidence of life-threatening complications. The authors outlined that even if cure cannot be achieved, significant disease responses, providing long-term symptom-free intervals, have been observed even in patients with advanced refractory disease treated many months or years after diagnosis.

Based on successful studies of tandem transplants in MM the South West Oncology Group (SWOG) conducted a small pilot study in WM patients. Six patients were initially treated with pulsed dexamethasone followed by PBSC mobilization with cyclophosphamide and etoposide. The conditioning regimen for the tandem transplants was melphalan 200 mg/m² and patients were to receive interferon for maintenance therapy. With a median follow-up of 29 months four patients received the second transplant; no treatment-related deaths had occurred. Five of the six patients, evaluable at the time of presenting data, achieved a PR; interestingly no additional CR or PR were observed following the second autologous transplant. At the second International Workshop on WM in 2003 the French Group presented results achieved in nineteen patients who underwent high dose chemotherapy followed by unmanipulated PBSC. The procedure was found be feasible with a low toxicity even in patients up to 60 years old. The overall response rate was high, reaching 95%, with 10 patients (53%) achieving a major response (very good PR or CR).

The experience of these groups supports the feasibility and the efficacy of high dose chemotherapy followed by autologous transplantation for patients with WM even in those of advanced age. Although all

### Table 4. Results of allogeneic stem cell transplantation.

<table>
<thead>
<tr>
<th>Study</th>
<th>N. pts.</th>
<th>Age yr</th>
<th>Disease Status</th>
<th>Regimen</th>
<th>Response</th>
<th>OS</th>
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<tr>
<td>Anagnostopoulos, 2001</td>
<td>3</td>
<td>51</td>
<td>Refractory Relapse</td>
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<td>NE</td>
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<tr>
<td></td>
<td>30</td>
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<td>60</td>
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<td>Reduced Intensity</td>
<td>NR</td>
<td>36</td>
</tr>
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<td>Martino, 1999</td>
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<td>Sensitive Relapse</td>
<td>Myeloablative</td>
<td>PR</td>
<td>112+</td>
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<tr>
<td></td>
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<td>Refractory Relapse</td>
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<td>CR</td>
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<td>Myeloablative</td>
<td>CR</td>
<td>74+</td>
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<td>Myeloablative</td>
<td>CR</td>
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<td>50+</td>
</tr>
<tr>
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<td>23+</td>
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CR: complete remission; PR: partial remission; OS: overall survival; VGPR: very good partial remission; NE: not evaluable.
authors reported high remission rates, many issues remain to be clarified and studies on larger number of patients are needed to evaluate the real impact of this approach on survival.

Autologous stem cell transplantation in these series of patients did not lead to complete eradication of the disease; additional interventions are needed to ameliorate the role of high dose chemotherapy. The future direction includes evaluation of in vivo purging of the disease with purine analog-containing regimens and the peritransplant use of the monoclonal antibodies.

Allogeneic transplantation could provide the benefits of a progenitor cell source free of tumor cell contamination, furthermore, as observed in other lymphoproliferative disorders, a graft-versus-malignancy effect may be exploited.

A limited number of patients who underwent an allogeneic SCT have been reported in literature; their data are summarized in Table 4.

All the 15 patients were heavily pretreated; the preparative regimen was fully myeloablative in 12 cases and reduced intensity or non-myeloablative in 3 cases. At the time of presenting data 9 patients were alive, 3 in PR and 6 in CR (median follow-up 50 months, range 3–112).23,25 The transplant related mortality rate reported among the 10 patients of the French study is 40%.20 With these data it is not possible to define a role for allogeneic transplant in the treatment of WM patients, so for now this procedure should be considered for patients with severe prognostic factors failing to respond or relapsing after purine analogs in combination with chemotherapy and immunotherapy. Novel treatment strategies using reduced intensity preparative regimens are reasonable investigational treatment options.

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Neuropathy and monoclonal gammopathy

Neuropathy has been known to be associated with monoclonal gammopathy for several years, but it was not until the early 80's that the clinical and pathogenetic relevance of this association started to be thoroughly investigated. In particular in 1981, Kelly et al. drew attention to the frequent association of neuropathy with monoclonal gammopathy found in 10% of patients with neuropathy of unknown etiology, while in 1980 Latov et al. demonstrated that a patient with neuropathy and IgM monoclonal gammopathy of undetermined significance (MGUS) had reactivity of the M-protein with a nerve antigen later characterized as myelin-associated glycoprotein (MAG).3

The prevalence of symptomatic neuropathy in patients with monoclonal gammopathy has been reported to be up to 35% in some series.4 This figure varies however according to the hematologic diseases and, for the same disease, from series to series, depending on the criteria used to define the presence of neuropathy. In two large series of patients with MGUS for instance, the prevalence of a symptomatic neuropathy ranged from 8%5 to 36%.4 In both series the prevalence of neuropathy was significantly higher in patients with IgM than with IgG or IgA MGUS, reinforcing the hypothesis, at least for IgM monoclonal gammopathy, of a possible pathogenetic role of IgM M-proteins in the neuropathy.

Neuropathy and IgM monoclonal gammopathy

IgM monoclonal gammopathies represent approximately 10 to 15% of all monoclonal gammopathies. Since monoclonal gammopathies occur in 1% of the population above 50 years old and in 3% of those above 70 years old, the prevalence of IgM monoclonal gammopathy in these cohorts may be of 100 up to 450 per 100,000. A symptomatic neuropathy has been reported in up to 50% of patients with IgM monoclonal gammopathy, even if in our series of 60 consecutive patients it was found in 28% (unpublished results), being more frequent in Waldenström's macroglobulinemia (WM) (32%) than in IgM MGUS (15%).3 Combining the above mentioned figures, the prevalence of a symptomatic neuropathy associated with IgM monoclonal gammopathy in the population above 50 years may be at least 20 cases per 100,000 population.

Different forms of neuropathy have been associated with IgM monoclonal gammopathy, possibly reflecting different pathogenetic mechanisms.7 Cranial nerve palsies, mononeuropathies or mononeuritis multiplex have been reported in WM and lymphoma and were related to lymphoplasmacytic infiltration of nerves, amyloid deposition, cryoglobulinemic vasculitis or microangiopathy of endoneurial vessels. The vast majority of these patients, as well as of those with IgM MGUS do, however, have a chronic progressive, symmetric and predominantly distal neuropathy which is occasionally related to endoneurial accumulation of the M-protein, or diffuse microangiopathy but most frequently to reactivity of the M-protein with a number of neural antigens including MAG, cytoskeletal proteins, chondroitin sulfate C (Ch5-C), sulfatide and several gangliosides. Overall these reactivities are found in at least two thirds of patients with neuropathy and IgM monoclonal gammopathy being more frequent in MGUS (84%) than in WM (38%).8 Some of these IgM reactivities have been associated with homogeneous neuropathic features which will be briefly reviewed here.

Neuropathy associated with anti-MAG IgM

In almost 50% of patients with neuropathy associated with IgM monoclonal gammopathy the M-protein reacts with MAG and other nerve glycoconjugates (P0, PMP22, SGPG and SGLPG) that, like MAG,
have the HNK-1 carbohydrate epitope. Thus, the prevalence of this neuropathy in the population above 50 years old is at least 10 per 100,000. Almost 80% of patients with anti-MAG IgM have IgM MGUS while most remaining patients have an otherwise asymptomatic WM so that the finding of anti-MAG IgM in a patient with monoclonal gammopathy is prognostically indicative of the presence and development of malignancy.

The neuropathy in patients with high levels of anti-MAG IgM antibodies is quite homogeneous. The majority of affected patients are men who present their first neuropathy symptoms in their sixth or seventh decade. The neuropathy is characterized by a distal and symmetric, predominantly deep sensory involvement, with gait ataxia and postural tremor in the upper limbs. Motor impairment is usually less prominent and often appears later. The term distal acquired demyelinating symmetric (DADS) neuropathy has been recently proposed to distinguish this clinical presentation from that of chronic inflammatory demyelinating polyneuropathy (CIDP) in which a less definite distal to proximal gradient and a more pronounced motor impairment are often encountered. The neuropathy usually runs a slowly progressive course with most of the patients having a long-term favorable functional prognosis with only a minority of them becoming disabled after several years of disease (25% at 10 years, 50% after 15 years).

Results of electrophysiological studies are consistent with a demyelinating neuropathy showing markedly reduced motor and sensory conduction velocities often in the range of 15 to 25 m/sec, with an even more pronounced delay of distal motor latencies. Morphological studies on sural nerve biopsies confirm the demyelinating nature of the neuropathy with typical widely spaced myelin lamellae in over 90% of patients examined by ultrastructural studies, and deposits of IgM and complement around the myelin sheaths by immunohistochemistry.

Several data support the pathogenetic role of anti-MAG IgM in the neuropathy: (i) high titers of anti-MAG IgM antibodies (> 1/10,000 in most laboratories) are not only invariably associated with an homogeneous clinical pattern but significantly predict the development of clinically symptomatic neuropathy in asymptomatic patients with IgM monoclonal gammopathy; (ii) as already mentioned pathological studies on nerve biopsies show segmental demyelination with deposits of IgM M-protein and complement on nerve myelin, i.e. the target of the anti-neural reactivity; (iii) therapeutic reduction of anti-MAG IgM, though difficult to achieve, is often correlated with clinical improvement; even if the reverse does not always occur; (iv) complement-mediated demyelination of nerve has been experimentally induced in animals by intraneural or systemic injection of anti-MAG IgM M-proteins.

Several therapies directed at reducing the presumably pathogenic IgM paraprotein or B-cell clone have been used in these patients, including steroids, plasma exchange, cytotoxic agents such as cyclophosphamide, chlorambucil, fludarabine and, more recently, cladribine, high-dose intravenous immunoglobulin (IVIg) and interferon-α. Although almost 50% of patients have been reported to improve, at least temporarily, after one of these therapies, their effect on the long-term prognosis of the neuropathy remains unclear as the follow-up exceeded two years in only few studies. These data would be particularly important in consideration of the usually slow progression and relatively favourable prognosis of the neuropathy associated with anti-MAG IgM, and the frequent adverse effects of most of these therapies. In addition very few randomized controlled trials have been performed in these patients with only one showing at the most a modest and short-term efficacy of IVIg.

This issue has been specifically addressed by a Cochrane review that concluded that available trials do not provide enough evidence to recommend any particular immunotherapy in this neuropathy.

Recently a number of open pilot trials have addressed the effect of a humanized monoclonal antibody (rituximab) directed against the CD20 antigen, which is variably expressed on the surface of normal and monoclonal B lymphocytes. Renaud et al. treated 21 patients with neuropathy associated with IgM antibodies to neural antigens including 7 with anti-MAG IgM. More than 80% of the patients were reported to have a consistent improvement in muscle strength both at one and two years of treatment as compared to no improvement in a parallel control group of 13 untreated patients. Improvement correlated with a dramatic and persistent decrease of IgM levels, antibody titers and circulating B cells. It is not clear however from the study whether this therapy also improved the sensory ataxic impairment which is the predominant and most disabling feature in most patients with anti-MAG associated neuropathy.

Similarly promising results were obtained by et al. who reported on the effect of rituximab in an open phase II study on 9 patients with chronic polyneuropathy associated with IgM monoclonal gammopathy with anti-MAG reactivity. According to the authors, 6 patients had a meaningful clinical improvement even though probably only two of them had a consistent improvement while the other four had a marginal improvement. Also in this
study there was a pronounced and persistent decrease of IgM levels, antibody titers and circulating B cells whereas motor conduction velocities improved in 5 patients. However, none of these parameters correlated with the degree of clinical improvement. Although the results of all these studies are probably less promising than one would expect from the mechanism of action of rituximab, they seem to provide enough evidence that this therapy may be effective in some patients with this neuropathy.

**Neuropathy with anti-glycosaminoglycan IgM**

After the initial report from Sherman et al. of two patients with a slowly progressive sensorimotor neuropathy, epidermolysis and IgM M-protein reacting with chondroitin sulfate C (ChS-C), a few other patients with this reactivity and IgM MGUS have been reported, most often associated with a chronic sensory or sensorimotor neuropathy. Morphological studies of nerve biopsies in these patients showed axonal degeneration with deposits of IgM in the endoneurium and, in one patient, at Schmidt–Lanterman incisures. One patient improved with therapeutic reduction of IgM levels suggesting a possible pathogenetic role for this reactivity in the neuropathy. The frequency of these antibodies is, however, too low (<2%) in our series of almost 150 patients with neuropathy and IgM M-protein to justify widespread searches for them, also considering the scarce sensitivity and reliability of tests currently used for their detection.

More recently Pestronk et al. reported on five patients with an often painful, predominantly sensory (pan modal), distal neuropathy mostly affecting unmyelinated axons, associated with IgM monoclonal gammopathy. These patients had high titers of IgM antibodies to trisulfated heparin disaccharide (TS-HDS), the most abundant disaccharide component of heparin oligosaccharides, which is also present in heparan-sulfate glycosaminoglycan. In the two examined patients, immunohistochemistry of sural nerves showed the presence of IgM deposits around perineurial (and perimysial) veins. Although this reactivity occurred, according to the authors, in 8% of their 220 patients with serum IgM M-protein, its possible pathogenetic or clinical relevance remains to be established.

**Neuropathy with anti-sulfatide IgM**

Since the original report from Pestronk et al. anti-sulfatide antibodies, mostly IgM, have been reported in several patients with neuropathy, half of whom had IgM monoclonal gammopathy. This is probably the second most frequent anti-neural reactivity detected in patients with neuropathy and IgM monoclonal gammopathy (6%). While most initially reported patients had a chronic progressive, predominantly sensory axonal neuropathy or selective small fiber neuropathy with normal electrophysiological or nerve biopsy studies often presenting with painful paresthesias, in subsequent reports this reactivity has been associated with sensorimotor demyelinating neuropathy. Morphological studies on sural nerve biopsies showed that some patients had abnormally spaced myelin lamellae with myelin deposits of the M-protein and complement. Myelin deposits of IgM were not found in other patients, in whom IgM bound to dorsal root ganglia, possibly revealing a different site of attack for the antibodies. Few data are available on the clinical response to treatment in these patients. Although clinical improvement in our positive patients did not correlate with antibody reduction, the possible diagnostic if not pathogenetic relevance of these antibodies was supported by their strict association with the immune-mediated neuropathy.

**Neuropathy with IgM reactivity with cytoskeletal proteins**

IgM reactivities with vimentin, β-tubulin and the 200kDa NF have been occasionally reported in patients with neuropathy and IgM monoclonal gammopathy. Both patients with anti-vimentin IgM had a sensorimotor demyelinating neuropathy, while the patient with IgM reacting with β-tubulin had a relapsing–remitting chronic inflammatory demyelinating neuropathy (CIDP). This reactivity was also frequently observed by ELISA in CIDP patients without IgM monoclonal gammopathy, but this finding was not later confirmed by immunoblot. Patients with anti-NF IgM had a clinically and electrophysiologically heterogeneous neuropathy frequently associated with other concomitant causes for the neuropathy casting some doubts on the pathogenetic relevance of this reactivity.

**Neuropathy with anti-ganglioside IgM**

Several patients with IgM monoclonal gammopathy reacting with GM1 have been reported, including patients with multifocal motor neuropathy (MMN), other predominantly motor neuropathies and occasionally motor neuron disease or sensorimotor neuropathy. In the vast majority of patients however, anti-GM1 IgM antibodies are not associated with IgM monoclonal gammopathy but are considered a marker for MMN since they are found in 30–50% of patients with MMN and IgM M-protein is found only in 10%. Conversely, no more than 2% of our patients with neuropathy and IgM gammopathy have...
anti-GM1 IgM antibodies, so that even if all positive patients have a motor neuropathy, the relation between these antibodies and the neuropathy associated with IgM monoclonal gammopathy is quite loose.

A number of studies have been published on a peculiar form of neuropathy associated with an IgM monoclonal gammopathy reacting with gangliosides containing disialosyl groups including GQ1b, GD1b, GT1b, GD3 and GD2. This subject has been reviewed by Willison et al., who collected a series of 18 patients with this reactivity observed in approximately 2% of the patients with neuropathy and IgM monoclonal gammopathy. Most of these patients had a homogeneous clinical pattern differing from that associated with other reactivities including signs of a large-fiber chronic sensory neuropathy with prominent ataxia, usually mild or no weakness, recurrent ophthalmoplegia and cold agglutinin activity of the M-protein which often binds to the Pr2 antigen on red cell membranes. Willison proposed the acronym CANOMAD (Chronic Ataxic Neuropathy with Ophthalmoplegia, M-protein, cold Agglutinins and anti-Disialosyl antibodies) for this syndrome. In most of these patients electrophysiological and morphological studies were consistent with a demyelinating process. In none of them were myelin deposits of the M-protein found in sural nerve, but in one case inflammatory cells infiltrates were found. This finding, together with the relapsing course of the neuropathy in most patients, and the frequent occurrence of ophthalmoplegia distinguish this neuropathy from other neuropathies associated with IgM monoclonal gammopathy and is reminiscent of CIDP. This may explain the not so infrequent consistent improvement observed in some of these patients after IVIg therapy, an infrequent occurrence in patients with neuropathy and other anti-neural IgM monoclonal reactivities.

Monoclonal IgM reactivities with other gangliosides have been occasionally reported in these patients including reactivity to GD1a in predominantly motor neuropathy (2% in our series), GM2 and GD1b. The possible pathogenetic and clinical relevance of these and other even less frequent IgM reactivities remains unclear as in none of these patients were IgM deposits found in sural nerves and little is known about their response to immune therapies. At the same time the very small number or reported observations does not allow the clinical phenotype of these reactivities to be clarified so that the search for these reactivities in patients with IgM monoclonal gammopathy may be not clinically justified.

**Neuropathy with IgM not reacting with neural antigens**

In approximately one third of patients with neuropathy associated with IgM monoclonal gammopathy no reactivity of the M-protein with nerve antigens can be detected. Over two thirds of these patients have Waldenström's macroglobulinemia or lymphoma. Several non-immune mechanisms have been implicated in the pathogenesis of the neuropathy in this group of patients including cryoglobulinemia, direct lymphoplasmacytic infiltration of nerves, microangiopathy of vasa nervorum, endoneurial accumulation of the M-protein and amyloidosis. For instance, in a recent study Eurelings et al. found an increased density of T-cells in the sural nerves of one third of patients with an axonal neuropathy associated with monoclonal gammopathy including 4/12 with IgM paraproteinemia. These patients had progressive neuropathy and frequently responded to prednisone suggesting that vasculitis played an important role in their neuropathy. This study highlights the potential clinical usefulness of performing sural nerve biopsy in patients with IgM paraproteinemic neuropathy in whom no other possible mechanism for the neuropathy has emerged from antibody studies. In addition it also explains why steroids, which are often ineffective in patients with anti-MAG or other anti-neural IgM reactivity, may be effective in some patients with IgM paraproteinemic neuropathy. A possible role for microvasculitis in the pathogenesis of this neuropathy is also supported by a study by Turner et al. on a patient with mononeuritis multiplex associated with an IgM-λ secreting B-cell lymphoma. Whatever the mechanism for the neuropathy in this heterogeneous group, treatment directed at reducing IgM M-protein levels was associated in some patients with clinical improvement. Occasionally, other causes for the neuropathy may also be present, suggesting that, at least in some instances, the association of neuropathy with IgM monoclonal gammopathy may be coincidental, a consideration that should be taken in account before using potentially toxic therapies in these patients.

**Neuropathy and IgG monoclonal gammopathy**

While neuropathy associated with IgM monoclonal gammopathy is well characterized, the relationship between neuropathy and IgG M-protein is less clear. Some of the patients with neuropathy and IgG monoclonal gammopathy have or develop typical multiple myeloma and in some of them the neuropathy is the presenting symptom of the hematologic disease. Even more frequently, however neuropathy occurs in patients with an established hematologic diagnosis,
the reported prevalence being 3% to 13%. The neuropathy associated with multiple myeloma is clinically heterogeneous, possibly reflecting the presence of different pathogenetic mechanisms. In approximately half of the patients the neuropathy is caused by light chain amyloidosis with a predominantly sensory distal impairment, postural hypotension and other signs of autonomic impairment, and is often associated with signs of systemic amyloidosis including malabsorption, cardiac and renal dysfunction. Direct nerve or nerve root infiltration by myeloma or compression by bone lesions causes an asymmetric mono or polyneuropathy or radiculopathy often characterized by excruciating pain. Other usually mild forms of neuropathy have been reported in these patients and different mechanisms have been postulated including paraneoplastic or autoimmune effects even if no convincing anti-neural reactivity of IgG M-protein has been demonstrated in these patients. Neuropathy also occurs as a complication of drugs used in myeloma, such as vincristine and thalidomide and may lead to therapy suspension or dose reduction even if this is not always associated with improvement of the neuropathy, particularly in the case of thalidomide.

The features of the neuropathy associated with osteosclerotic myeloma are more typical. Neuropathy is found in up to 50% of the patients and it is often the presenting symptom of the disease. These patients often have a severely disabling predominantly motor demyelinating neuropathy frequently starting with sensory symptoms. This neuropathy is sometime associated with other non-neurological manifestations including organomegaly, endocrinopathy, lymphoadenopathy, ascites, peripheral edema and a very typical reddish brown tanned color of the skin. This constellation of symptoms has been collected under the eponym of POEMS (Polyneuropathy, Organomegaly, Endocrinopathy, M-protein and Skin changes) or Crow-Fukase-syndrome and has been occasionally reported also in patients with non-malignant gammopathies. The possible cause of the frequent association of neuropathy with osteosclerotic myeloma is unclear even if a possible role for cytokines or vascular endothelial growth factor (VEGF), which is frequently increased in these patients, has recently been suggested. The clinical and pathogenetic relevance of this association is supported by the improvement of the neuropathy observed in more than half of the patients who respond to the treatment of the osteosclerotic lesion(s): treatment includes local radiotherapy or resection of the tumor and a variable combination of steroids and melphalan. The majority of patients with neuropathy and IgG M-protein have an IgG MGUS which is usually found during the work-up or even the follow-up of the neuropathy. The prevalence of a symptomatic neuropathy is lower in IgG (3%) than IgM MGUS (15%), possibly explaining the lower representation of IgG in large series of patients with neuropathy and MGUS. Several forms of neuropathy were originally associated with IgG MGUS including chronic relapsing and chronic progressive, demyelinating and axonal polyneuropathy. In three large series of patients with neuropathy and IgG MGUS, including a total of 47 patients, almost half of the patients had a chronic demyelinating neuropathy clinically indistinguishable from CIDP while the remaining had a predominantly sensory axonal or mixed neuropathy indicating that the neuropathy is probably less heterogeneous than previously reported. The possible pathogenetic role of IgG M-proteins in the neuropathy does, however, remain unclear as reactivity of IgG M-proteins with neural antigens or endoneurial deposits of IgG have been reported in very few patients, while the M-protein become manifest several months to years after onset of the neuropathy in over 50% of the patients. Still the improvement observed with immunotherapy in over 60% of reported patients with neuropathy and IgG MGUS, and in a controlled trial of plasma exchange, seems to indicate that even if the IgG MGUS might not be primarily pathogenetic, it may represent a marker of a possibly dysimmune origin of the neuropathy. This is particularly true for those patients with a CIDP-like presentation considering the much less frequent response to immune therapy of those with axonal neuropathy.

**Neuropathy and IgA monoclonal gammopathy**

Only a few patients with neuropathy and IgA monoclonal gammopathy have been reported representing a very small proportion of the patients in large series with neuropathy and monoclonal gammopathy (4% in our series of over 100 patients). Some of these patients have myeloma or a POEMS syndrome (see above) while a few of them have IgA MGUS. The rarity of the latter form of neuropathy is confirmed by the fact that fewer than 30 patients with neuropathy and IgA MGUS have been reported. In one study the prevalence of symptomatic neuropathy in patients with IgA MGUS was 7%. The clinical and electrophysiological features of the neuropathy in these patients are quite heterogeneous making it impossible to identify a prevailing type of presentation except that the neuropathy was clinically progressive in all but one patient in whom the onset had been acute. As in the case of neuropathy associated with IgG MGUS there is little evidence that IgA M-proteins have a primary pathogenic role
in the neuropathy since anti-neural reactivity\(^2\) and endoneurial deposits of IgA M-proteins have only occasionally been reported.\(^3\) Recently however Vallat et al.\(^4\) reported the presence of the typical myelin widening associated with anti-MAG antibodies (see above) in the sural nerve of a patient with chronic demyelinating neuropathy associated with IgA MGUS. In this patient deposits of the IgA M-protein were found in correspondence with the myelin widening. Although the putative antigen bound by the IgA M-protein in nerve was not identified, this study provided the first convincing demonstration of a possible pathogenetic role of the IgA M-protein in the neuropathy.

A few patients have been reported to improve with immune therapies including steroids, plasma exchange,\(^5\) or immunosuppressants\(^6\) but their limited number and the consequent elevated risk of a publication bias are not sufficient to justify the assumption that the identification of an IgA M-protein reveals a dysimmune origin of the neuropathy which might benefit from immune system based therapies.

References


48. Willison HJ, O'Leary CP, Veitch J. Cloning of human anti-GM1 monoclonal IgM antibody activity against intermediate fila


AL amyloidosis

The amyloidoses are disorders of protein conformation and metabolism, in which different unrelated autologous proteins misfold and aggregate into fibrils that deposit in tissues and cause organ dysfunction. At present, at least 21 types of amyloidosis are classified according to the different types of protein that form amyloid fibrils in vivo (Table 1). In AL amyloidosis, the fibrils are formed by the N-terminal fragment of a monoclonal immunoglobulin light chain. Thus, in AL, an underlying plasma cell clone is responsible for the production of the amyloidogenic protein. Light chain amyloidosis is the most common form of systemic amyloidosis in Western countries, with an estimated incidence of 0.8 per 100,000 person years.

The amyloid clone and amyloidogenic light chains

The amyloid clone comprises not only mature bone marrow plasma cells, but also lymphoplasmacytoid cells in the bone marrow and circulating resting B cells. The latter can be induced to differentiate in vitro into mature plasma cells, indicating that circulating clonal B cells may feed the bone marrow population. The bone marrow clone is usually of modest size (median bone marrow plasma cell percentage 7%) and often requires anti-light chain immunostain to be identified. Only a minor cell subset is in the S-phase of the cell cycle. Only 5% of AL patients have associated multiple myeloma and AL also shares certain clinical features, such as the low clonal plasma cell number in the bone marrow, with monoclonal gammopathy of undetermined significance. Using microarray hybridization analysis, the Mayo Clinic Group has recently identified a set of 12 genes that are individually expressed in AL and multiple myeloma and can distinguish the two groups of patients with a 92% accuracy. This set comprises several genes that are known to be downregulated in multiple myeloma plasma cells compared to in normal plasma cells. The comparison of AL plasma cells with normal plasma cells shows that AL has an intermediate gene expression profile between myeloma patients and normal subjects.

Only a small proportion of light chains form amyloid fibrils: in a study of 1384 patients with monoclonal gammapathy followed at the Mayo Clinic for a median time of 15.4 years, amyloidosis occurred in 10 cases. Thus, the ability to form amyloid is probably related to peculiar structural characteristics of the light chain. The \( \lambda \) isotype accounts for the majority of the amyloidogenic light chains and two \( V_\lambda \) genes, 6a and 3r, equally contribute to encoding 42% of amyloidogenic \( \lambda \) light chains. Light chains of the \( \lambda VI \) family are almost invariably associated with amyloidosis. Less is known about amyloid \( \lambda \) light chains. However, the gene families \( V_\lambda I \) and \( V_\lambda IV \) are frequently found rearranged in ALk.

Amyloidogenic light chains are somatically mutated, mutations are uniform within each single clone and most amyloidogenic light chains retain evidence of antigen-driven selection of immunoglobulins, a situation they share with other plasma cell dyscrasias, such as multiple myeloma or Waldenström's macroglobulinemia.

Peculiar characteristics of the amyloidogenic light chains are, at least in part, responsible for the organ tropism of amyloid deposits in AL. A specific antibody activity of certain amyloidogenic light chains against antigens in target tissues was demonstrated only in few cases. Comenzo and co-workers found that 6a light-chains are associated with kidney involvement. This finding was later confirmed by other groups. However, attempts to identify other germline genes with such a strong association with a specific organ involvement have failed.

Diagnosis

Amyloidosis should be considered in the differential diagnosis of non-diabetic
nephrotic syndrome, left ventricular hypertrophy at echocardiography, hepatomegaly with no scan defects and polyneuropathy. The diagnosis of amyloidosis requires the histological demonstration of the amyloid deposits. The highly ordered structure of the amyloid fibrils confers to the deposits stained with Congo red a typical green birefringence under polarized light. Biopsies of the kidney and liver carry a significant risk of bleeding. Fine-needle aspiration of abdominal fat can substitute the biopsy of the organs involved in the great majority of patients with systemic AL. The sensitivity of abdominal fat aspiration in 645 patients referred to our center was 87%. An alternative to organ biopsy can also be minor salivary gland biopsy. The characterization of a biopsy-proven amyloidosis as AL type requires demonstration of a plasma cell clone. Screening electrophoresis is inadequate, since in 56% of patients the serum monoclonal component is not detectable by this technique. All patients should have immunofixation electrophoresis of serum and urine. Six hundred twenty-seven (97%) of 645 patients seen at the Pavia Amyloid Center had a detectable monoclonal component at high-resolution immunofixation.

The measurement of circulating free light chains is a valuable complement to immunofixation and is useful in the follow-up of AL patients after chemotherapy. A monoclonal plasma cell population can be detected in 84% of AL patients on a bone marrow aspirate by immunofluorescence with anti-κ and anti-λ antisera.

Finding amyloid deposits in an organ on biopsy in the presence of monoclonal immunoglobulins is strong but not conclusive evidence of AL. The chance association of a monoclonal gammopathy in an older patient with

<table>
<thead>
<tr>
<th>Amyloid protein</th>
<th>Precursor</th>
<th>Systemic (S) or Localized (L)</th>
<th>Syndrome or Involved tissue</th>
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<tr>
<td>AL</td>
<td>Immunoglobulin light chain</td>
<td>S, L</td>
<td>Primary, Myeloma-associated</td>
</tr>
<tr>
<td>AH</td>
<td>Immunoglobulin heavy chain</td>
<td>S, L</td>
<td>Primary, Myeloma-associated, Familial</td>
</tr>
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<td>Transthyretin</td>
<td>S</td>
<td>Senile systemic</td>
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<td>Aβ2M</td>
<td>β2-microglobulin</td>
<td>S</td>
<td>Hemodialysis</td>
</tr>
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<td>(Apo)serum AA</td>
<td>S</td>
<td>Secondary, reactive</td>
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<td>Apolipoprotein A-I</td>
<td>S</td>
<td>Familial</td>
</tr>
<tr>
<td>AApoA-II</td>
<td>Apolipoprotein A-II</td>
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<td>Familial</td>
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<td>Gelsolin</td>
<td>S</td>
<td>Familial</td>
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<td>Lysozyme</td>
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</tr>
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<td>AFib</td>
<td>Fibrinogen α-chain</td>
<td>S</td>
<td>Familial</td>
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<td>Cystatin C</td>
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<td>AbriPP</td>
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<td>Familial dementia</td>
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<td>Islet amyloid polypeptide</td>
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<td>Islets of Langerhans, Insulinomas</td>
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<td>Lactadherin</td>
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<td>Kerato-epithelin</td>
<td>L</td>
<td>Cornea; familial</td>
</tr>
<tr>
<td>A(tbn)</td>
<td>to be named</td>
<td>L</td>
<td>Pindborg tumors</td>
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<tr>
<td>Alac</td>
<td>Lactoferrin</td>
<td>L</td>
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systemic amyloidosis must be kept in mind, so that additional specialized studies are pursued to exclude the possibility of a monoclonal gammopathy incidentally associated with non-AL (familial, reactive, or senile) amyloidosis. Unequivocal identification of the deposited amyloidogenic protein is essential in order to avoid misdiagnosis and inappropriate treatment. The diagnosis of AL amyloidosis must not be presumed and no patient should receive chemotherapy for a clonal disorder if the diagnosis has not been verified. The diagnostic approach to systemic amyloidoses needs a careful clinical evaluation and refined immunohistochemical and genetic testing. Immunoelectron microscopy unambiguously characterizes the amyloid deposits by co-localizing the specific proteins with the fibrils and can be performed on abdominal fat samples.

**Clinical aspects and prognosis**

In 1986, a national project, the Italian Amyloidosis Study Group, was started to establish a network of clinical centers interested in systemic amyloidoses. The aims of this national effort were to increase the awareness and alertness of medical professionals about these rare conditions, to achieve early diagnoses and to provide the best specific and supportive therapy locally, with the final goal of possibly improving overall survival. A secondary goal was to make the care of AL patients, in particular the critically important supportive care, available locally, increasing its effectiveness through prompt intervention and with obvious logistic advantages for the patients.

So far, 645 patients with AL amyloidosis have been referred to the Pavia Amyloid Center. Their main clinical characteristics are reported in Table 2. Two hundred and seventy-two patients have died; the overall median survival was 46 months (Figure 1) and the cumulative proportion surviving at 10 years was 22%. The cause of death was known in 213 patients, 159 of whom (75%) died of cardiac death (in 46 of them death was sudden). The median survival of patients with heart involvement was significantly shorter than that of patients without cardiac amyloidosis (20 vs 75 months, \(p<0.001\), Figure 2).

In AL amyloidosis the clinical outcome is largely dependent on the presence and extent of heart involvement at presentation. Moreover, cardiac amyloidosis significantly increases the treatment-related morbidity and mortality associated with aggressive therapeutic regimens, such as autologous stem cell transplantation (ASCT). Elevated serum cardiac troponins are related to poor prognosis in AL patients, including those who receive conventional treatment, as well as those eligible for ASCT. Our group reported that the serum N-terminal portion of natriuretic peptide type B (NT-proBNP) is a sensitive marker of myocardial dysfunction in AL and a powerful prognostic determinant. Recently, the Mayo Clinic group proposed a staging system for AL amyloidosis based on serum cardiac troponins and NT-proBNP.

Despite the great prognostic impact of cardiac amyloidosis, hematologic response to treatment confers a survival benefit also in patients who present with heart involvement. Serum NT-proBNP concentration parallels that of the amyloidogenic precursor and is strongly related to clinical symptoms of heart failure in AL. The reduction of the circulating light chain concentration induced by chemotherapy, immediately translates into a reduction of serum NT-proBNP level and improvement of heart failure, before any reduction in amyloid load can be demonstrated at echocardiography. These observations indicate that serum NT-proBNP can be used as a marker of cardiac response to therapy and is a useful tool in the follow-up of amyloid patients. In addition, these findings provide clues to the pathogene-

**Table 2. Clinical characteristics of 645 patients with AL amyloidosis.**

<table>
<thead>
<tr>
<th></th>
<th>N (%)</th>
<th>median</th>
<th>range</th>
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<tbody>
<tr>
<td>Age (years)</td>
<td>62</td>
<td>29-91</td>
<td></td>
</tr>
<tr>
<td>Gender (male)</td>
<td>366 (57)</td>
<td></td>
<td></td>
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<tr>
<td>Organ involvement</td>
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<td></td>
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<tr>
<td>kidney</td>
<td>480 (74)</td>
<td></td>
<td></td>
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<tr>
<td>heart</td>
<td>347 (58)</td>
<td></td>
<td></td>
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<tr>
<td>liver</td>
<td>181 (28)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>PNS*</td>
<td>143 (22)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ANS°</td>
<td>119 (18)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>GI tract</td>
<td>51 (8)</td>
<td></td>
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<tr>
<td>Number of organs involved &gt;1</td>
<td>444 (69)</td>
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</tr>
<tr>
<td>Urine protein loss (g/24h)</td>
<td>3.7</td>
<td>0-56</td>
<td></td>
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<tr>
<td>Serum creatinine (mg/dL)</td>
<td>345 (53)</td>
<td>1.1</td>
<td>0.3-10.3</td>
</tr>
<tr>
<td>Serum creatinine ≥2 mg/dL</td>
<td>115 (18)</td>
<td>13</td>
<td>7-30</td>
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<td>Ejection fraction (%)</td>
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<td>(pmol/L)</td>
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<td>serum NT-proBNP &gt;152 pmol/L</td>
<td>169 (60)</td>
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*Peripheral nervous system, °autonomic nervous system, †101 patients, ‡280 patients. Upper reference limits for NT-proBNP in men and women are, respectively, 10.4 pmol/L and 18 pmol/L in subjects <50 years old; and 26.4 pmol/L and 39.8 pmol/L in individuals >50 years old.
sis of the disease: the NT-proBNP decrease induced by chemotherapy may actually reflect the cardiotoxicity of the amyloidogenic light chains. This is concordant with the observation that patients with amyloidosis caused by transthyretin (who do not have pathogenic circulating light chains) may have severe myocardial infiltration but minimal heart failure, and with the observation that the infusion of light chains from patients with cardiac amyloidosis rapidly caused diastolic dysfunction in isolated mouse hearts. Thus, heart dysfunction in amyloidosis might not only depend on the extent of amyloid deposition, but could be partly related to a toxic effect of amyloidogenic light chains exerted on myocardial cells. This underlines the importance of achieving a rapid reduction in the concentration of the monoclonal component via therapies directed against the amyloidogenic plasma cell clone.

**Therapy**

The current therapeutic approach to systemic amyloidoses is based on the observation that amyloid deposits can be reabsorbed and organ function can be restored if the synthesis of the amyloidogenic precursor is shut down. The aim of therapy in AL amyloidosis is to rapidly reduce the supply of the amyloidogenic monoclonal light chain by suppressing the underlying plasma cell dyscrasia, while using supportive measures to sustain organ functions.

High-dose melphalan followed by ASCT is considered the most effective treatment for AL. However, ASCT is associated with a relatively high treatment-related mortality (current figures 13-14%). The presence of heart failure and multi-organ involvement and conditioning with higher doses of melphalan are associated with higher peri-transplant mortality. Treatment-related mortality was lower in trials performed in single amyloid referral centers, which can accumulate the critical level of experience needed to treat amyloid patients, than in multicenter trials. Indeed, the peri-transplant-related mortality at the Mayo Clinic in 2003 was as low as 6%. Transplant-related morbidity is also very high. In particular, acute renal failure is a frequent (up to 21%) complication. Factors predicting acute renal failure associated with ASCT are pre-transplant creatinine clearance, proteinuria, cardiac amyloidosis, melphalan dose and sepsis. Contrary to the common experience gathered in multiple myeloma, stem cell mobilization and harvest carries a significant risk of death in patients with amyloidosis. In AL, the rate of major complications during blood stem cell mobilization and collection is 15%.

The high toxicity related to stem cell harvest and transplantation in AL compels careful selection of patients. The response rate to ASCT is positively associated with the dose of melphalan infused. The hematologic response varies between 50% and 60% in dif-
ferent trials, with complete remissions in about one third of patients. At our center 22 patients received ASCT. Peri--transplant mortality was 14% and we observed a 59% clonal response rate with 36% complete remissions. The favorable outcome observed with ASCT might be, at least in part, due to patient selection. However, a case-matched control study, comparing patients who underwent ASCT to historical controls who were treated with standard chemotherapy, showed a survival advantage for transplanted patients.25

The association of oral melphalan plus prednisone (MP) was standard therapy for AL for a long time and is now offered to poor risk patients. Two large studies established the efficacy of this approach and demonstrated that colchicine has no role in the treatment of AL, either alone or in association with melphalan.36,37 In the study from the Mayo Clinic group, the response rate to MP was 28% and time to response was longer than 1 year in 30% of cases.27 At our center we used MP to treat 207 consecutive patients with advanced AL who were unable to bear more toxic regimens. A response was observed in 40% of patients after a median time of 7 months and translated into a significant survival advantage (72 vs 18 months, p<0.001). Although MP is the best tolerated regimen, the long time to response may be unaffordable for patients with rapidly progressive disease. The patients who are unable to tolerate prednisone due to severe heart failure may benefit from continuous oral melphalan.38 However, melphalan--based therapy is associated to a 21% actuarial risk of developing a secondary myelodysplastic syndrome.27

A rapid response to therapy is essential in AL amyloidosis. In multiple myeloma, VAD may induce a quick clonal response. However, this regimen presents serious potential concerns in AL patients: vincristine can severely exacerbate autonomic or peripheral neuropathy, doxorubicin cannot be used in patients with heart failure due to its cardiotoxicity and the intensive high-dose dexamethasone regimen can cause severe fluid retention or trigger fatal ventricular arrhythmias. Results obtained in multiple myeloma indicate that dexamethasone accounts for 85% of the plasma cell reduction achieved with VAD.27 A recent multicenter trial showed a 53% hematologic response rate, with 24% complete remissions, with dexamethasone alone (pulsed as in VAD and followed by maintenance with dexamethasone and α interferon).40 The toxicity of this schedule is substantial: treatment--related mortality is 8%. A modified, milder schedule of dexamethasone (40 mg on days 1-4 every 21 days) induced a response in 35% of patients, in a median time of 4 months, without significant toxicity.41

The association of dexamethasone (40 mg on days 1-4 every 28 days) and oral melphalan (MDex) in 46 AL patients, who were ineligible for ASCT due to advanced disease, induced a clonal response in 67% of patients, with 33% complete remissions and improvement of organ function in 48% of cases.42 Despite advanced organ dysfunction, the hematologic response translated into a significant survival benefit. Most importantly, heart failure resolved in 6 of 32 cases. Five patients had severe toxicity but none died. The mortality rate in the first 100 days after treatment was low (4%). The response rate observed in this poor risk series compares favorably with that achieved in unselected patients with MP and pulsed high-dose dexamethasone and also with the results observed in patients selected for the absence of severe heart failure and autonomic neuropathy treated with VAD.

Thalidomide is poorly tolerated in AL amyloidosis, causing fatigue, edema, cognitive difficulties, constipation, neuropathy, syncope due to bradycardia, thromboembolic events and worsening of renal function. The Boston group43 observed severe toxicity in 50% of 16 AL patients treated with thalidomide and the Mayo Clinic group44 reported adverse reactions in 75% of 12 AL patients who received thalidomide, 6 of whom went off study due to side effects. Our group used a combination of thalidomide (100 mg daily, with 100 mg increments up to 400 mg) and dexamethasone (20 mg on days 1-4, every 21 days) in 31 AL patients who did not respond to or relapsed after first line therapy. Only 11 patients (35%) tolerated the target thalidomide dose (400 mg/day). Response to therapy was correlated with the dose of thalidomide. A clonal response was observed in 48% of patients with 19% complete remissions. Median time to response was 3.6 months. Thalidomide--related severe adverse events were frequent (64%), but there was no treatment--related mortality within the first three months. Symptomatic bradycardia emerged as a common (26%) reaction to thalidomide in AL and monthly Holter electrocardiogram monitoring proved useful in detecting bradycardia promptly.

The thalidomide analog, revimid, and the proteasome inhibitor, velcade, which are active in multiple myeloma, represent attractive alternatives in the treatment of AL. The iodinated anthracycline 4'-ido-4'deoxydoxorubicin, used at a low, non--myelosuppressive dose, has produced a response in 15% of 40 AL patients treated in a multicenter trial.45 In an effort to promote amyloid resorption, a compound able to cross--link and clear from the circulation serum SAP, one of the common constituents of amyloid deposits, is under evaluation at the United Kingdom National Amyloidosis Centre. Etanercept was used in 16 AL patients with advanced disease; there were objective improvements in half of them, especially those with
We explored passive immunotherapy with anti-pan B antibodies in chemoresistant AL patients. One patient received a mouse bispecific anti-CD22/anti-saporin antibody and obtained a decrease of bone marrow plasma cell infiltration (8% to 2%) and serum monoclonal component (~45%). Six patients received rituximab and 3 of them achieved a partial hematologic response, which was, however, short-lived. Dendritic cell–based idiotype vaccination was investigated by the Mayo Clinic group, but has shown limited clinical efficacy. Amyloid load can be reduced in mice by passive anti–light chain immunization and a humanized antibody specific for an amyloid-related epitope is being produced for a phase I/II trial in humans.47

Defining the optimal therapeutic strategy

The availability of several effective therapeutic regimens makes it possible to design the best treatment strategy for each patient, aiming at the most rapid and effective suppression of the synthesis of the amyloidogenic light chain at the minimum toxicity cost. Although complete hematologic remission is desirable, reducing the amyloidogenic free light chain by 50–75% is often sufficient to lead to stabilization or regression of amyloid–related organ dysfunction and to improve survival.44 In order to minimize the toxicity and gain precious time for possible alternative treatments, an aggressive follow–up with serial measurements of the monoclonal protein is recommended.

Despite there being no data yet from randomized clinical trials to support the use of one agent over another, some suggestions can be made. Patients <65 years old with normal NT-proBNP and troponin serum concentrations are candidates for ASCT, which should be performed at institutions with expertise in AL amyloidosis. Patients who attain a partial hematologic response, without organ function improvement can be considered for a second ASCT. Patients who are fit enough to bear dexamethasone–based therapy, but who are not eligible for ASCT, can be treated with MDex. However, exposure to melphalan can jeopardize stem cell mobilization and patients who present with a potentially reversible contraindication for ASCT should have their stem cell harvested before MDex, or as an alternative, be treated with the modified mild dexamethasone schedule, carefully weighing the lower response rate versus the preserved possibility of harvesting stem cells.

The toxicity of VAD and VAD–like pulsed dexamethasone is not negligible and must be kept in mind. Patients with heart or peripheral/autonomic nervous system involvement should not receive VAD. Poor risk patients can be treated with MP or included in investigational trials. At our center these patients are treated with a combination of melphalan, intermediate-dose dexamethasone and thalidomide.

Conclusions

In the past few years, advances in molecular biology and immunology have deepened our understanding of AL amyloidosis. New powerful biomarkers (tropinons and NT-proBNP) are now available to assess cardiac dysfunction and prognosis, which will allow better tailoring of therapeutic strategies to individual patients. The availability of serial free light chain quantification will make it easier to determine hematologic response to therapy. These achievements have rendered AL amyloidosis a manageable condition, but an increased awareness of the clinical features of the disease on the part of the physician is still needed to achieve an early and correct diagnosis.

References

New trends in the treatment of multiple myeloma

The results of treatment of multiple myeloma (MM) are disappointing. Melphalan and prednisone (MP) have formed the standard therapy for MM during the last 40 years, giving an overall response rate not exceeding 50% and a median survival of less than 3 years. Numerous attempts have been made to improve these results by using combination chemotherapy and regimens containing high-dose dexamethasone. Although the degree of response significantly increases with increasing doses, it generally does not result in a significant prolongation of survival. Thus, in our Spanish PETHEMA trials we found no significant differences in survival among patients given MP, VCMP/VBAP at standard doses and VCMP/VBAP using higher doses of cyclophosphamide and Adriamycin. In two recently published trials, the combination of thalidomide and dexamethasone has produced partial response rates of 64% and 72% (including 16% complete responses), respectively. However, the long-term results of this combination will only be known after a longer follow-up. In addition to the just mentioned results, the Nordic Myeloma Study Group reported no survival improvement during two decades in conventionally treated younger patients with MM. In fact, the Nordic authors wrote that The history of therapy for myeloma since the introduction of MP is a frustrating story of unconfirmed successes. Unfortunately, they were right. It is the myeloma community's hope that with the incorporation of novel agents with new mechanisms of action along with more innovative treatment strategies the above statement will no longer hold true.

High-dose therapy/stem cell support

The main goals in cancer treatment are to achieve long-term disease control and, eventually, to cure the patient, the achievement of a complete response (CR) being the sine qua non for cure. In this regard, the limited efficacy of conventional treatment prompted the introduction of high-dose therapy followed by stem cell support (HDT/SCT) in an attempt to achieve a greater tumor reduction with a higher CR rate. In MM, CR has been defined as the disappearance of the M-protein in serum and urine by immunofixation in the absence of abnormal bone marrow plasma cells.

Allogeneic transplantation

The first reports on HDT/SCT concerned syngeneic and allogeneic transplant. The results obtained in 25 patients who received a syngeneic graft showed a low transplant-related mortality (TRM) with high CR and a median event-free survival (EFS) and overall survival (OS) of 6 years. Thus, in the rare event that a patient has an identical twin the procedure of choice would be a syngeneic transplant. Although the allogeneic transplant results in a high CR rate with about 10% of patients cured, TRM of about 50%, mainly due to graft-versus-host disease and infectious complications, precludes the procedure. In addition, in patients surviving the procedure the relapse rate is high. In an attempt to decrease TRM and optimize the graft versus myeloma effect, allogeneic transplantation with reduced-intensity conditioning regimens is currently being performed in many institutions. In fact, the conventional conditioned allogeneic transplant (maxi) has been almost universally replaced by the so-called dose-reduced intensity allogeneic transplant (mini). The efficacy of an early non-myeloablative allograft after autologous transplant is an attractive approach which is being investigated in several prospective trials. Nevertheless, all variants of allogeneic transplantation in MM should be considered experimental and performed in the context of clinical trials.

Autologous transplantation

The first studies on HDT/autologous SCT (ASCT) in MM were performed in patients with advanced refractory disease. Although the response rate was encouraging, the median EFS and OS were extreme-
ly short. Thus, patients with relapsed refractory disease generally do not benefit from HDT/ASCT. Considering that the duration of second responses to conventional chemotherapy in patients relapsing off therapy are usually short, there is a general agreement that HDT/ASCT should be considered whenever possible in patients with sensitive relapse. In fact, in a randomized trial designed to assess the optimal timing of HDT/ASCT, patients who underwent a rescue transplant had a survival identical to those in whom the transplant was performed as part of up-front therapy. However, no controlled trials comparing HDT versus continuing conventional therapy have been performed in patients with sensitive relapse.

Perhaps the most crucial issue in autologous transplantation in MM is the establishment of its role as part of up-front therapy. In this regard, there are two randomized trials reporting significantly higher CR rate, EFS and OS with HDT when compared with conventional chemotherapy. However, in two prospective randomized trials HDT was not found to be better than conventional treatment. In addition, the US Intergroup has reported a better EFS (median, 21 vs 25 months, p=0.05) but comparable OS (median 58 vs 53 months) in a large trial comparing HDT versus conventional chemotherapy. The Spanish PETHEMA group found a significantly higher CR in patients with chemosensitive myeloma who had HDT intensification when compared with those who were continued on standard-dose therapy (30% vs. 11%) but the EFS (median, 42 vs 34 months) and OS (median, 65 vs. 67 months) were not significantly different in the two arms. Although the results of HDT/autologous SCT are better than those achieved with standard dose therapy whether HDT is of benefit for the majority of patients or whether the benefit is confined to certain subsets of patients remains to be determined. It seems that the achievement of CR is the crucial step for a long-lasting response and prolonged survival in patients with MM. Two single institution studies performed at the MD Anderson in the US and at the Hospital Clinic in Barcelona showed that patients whose disease responded to the initial chemotherapy and who achieved CR after transplant had significantly longer EFS and OS than those who remained in partial response. Furthermore, patients who did not achieve CR with HDT had similar EFS and OS to comparable patients who met the eligibility criteria for HDT but who did not receive such treatment and were continued on standard therapy. In consequence, identifying factors that can predict the achievement of CR is important in order to predict the patients in whom the chance of benefiting from HDT/ASCT is higher. In the experience of the above mentioned institutions, sensitivity to initial therapy, measured by the M-protein size at the time of transplant, seems to be the most important predictor of CR after autologous transplant.

It has been claimed that patients with primary resistant disease are the most likely to gain benefit from early myeloablative therapy. Thus, the median EFS and OS of 27 patients with primary resistant disease who received a transplant during the first year of initiation of chemotherapy were 3.5 and 6 years, respectively. In 72 patients with primary unresponsive disease, the median EFS and OS were 21 and 47 months, respectively. Similar results were reported in two more recent studies. However, for a meaningful interpretation of the data the two categories of patients generally considered as having primary refractory disease (i.e., primary non-responsive with progressive disease versus minimal response or no change without clinical progression) should have been analyzed separately. In fact, myeloma with minimal response to the initial chemotherapy or non-responding, non-progressive disease, which is usually considered as primary refractory, has a relatively good prognosis when managed with conventional chemotherapy. In this regard, it is of note that in the above mentioned studies the patients who were considered as having primary refractory disease were those failing to achieve a 75% reduction in the M-protein synthetic rate or those who did not reach a 50% reduction in their M-protein size. The Spanish PETHEMA group is currently investigating the efficacy of HDT in non-responders to the initial chemotherapy with stable or progressive disease.

The tandem transplant approach was pioneered by Barlogie et al who showed, in their Total Therapy program, an increasing CR rate with continuing treatment intensification. This observation prompted the design of prospective trials comparing the efficacy of single versus double transplants. The French Intergroup found, in a prospective randomized trial, that tandem autotransplantation improved EFS and OS. Surprisingly, no increase in the CR rate was achieved with the second high-dose procedure. The most interesting finding of this trial was that patients failing to achieve a CR or very good partial response within 3 months after the first transplant had a 7-year survival probability of 11% with a single transplant versus 43% with tandem transplant. From the results of this trial the authors concluded that double transplant should be recommended for patients failing to achieve at least a very good partial response after one transplant. The analysis of the first patients included in the Bologna 96 trial also showed that the response rate (CR plus near-CR) between single and tandem transplants was not significantly different (31% vs. 41%). However, the tandem transplants were associated with a sig-
nificantly longer EFS (21 vs 31 months) and time to progression (23 vs 39 months). As in the IFM trial double autologous transplant was of clinical benefit in patients who failed to respond to the first line conventional chemotherapy and/or in those who did not achieve CR or near CR after the first transplant. In this study, no significant difference in OS was found between single and tandem transplants, but the median follow-up of this series is less than 4 years. The French Myeloma-Autogreffe group also reported the results of single versus tandem transplants. The response rate (CR plus near-CR) was similar in the two arms (37% vs.39%). After a median follow-up of 53 months, no significant differences in EFS and OS were found between the two arms. Finally, in a preliminary report the German group (GMMG-HD2 trial) showed that patients undergoing tandem transplant had a significantly longer EFS than those who received a single transplant (median 23 months vs. not reached). The Spanish PETHHEMA group is also investigating the role of tandem transplants in patients not achieving CR with a single procedure. The definitive role of tandem transplants must await the final results of the trials currently in progress and at the present time this procedure remains investigational. Elderly patients undergoing HDT/ASCT have a higher transplant-related mortality, lower CR rate and also shorter EFS and OS than the younger population. For this reason, HDT/ASCT should be individually considered in elderly myeloma patients. In patients with renal failure at the time of transplant, the TRM and the non-hematologic toxicity, particularly in dialysis-dependent patients receiving MEL-200, is high. We believe that in patients with persistent renal failure, HDT/ASCT should be only performed in younger patients (<50 years) with chemosensitive disease and a good general condition.

One important aspect when considering the results of HDT is whether or not a significant proportion of patients achieve a long EFS with a hope for cure. The long-term results of the Arkansas series and the French experience indicate that the cure of patients with MM remains a difficult challenge, since disease-free survival does not exceed 5% at 10 years even after HDT. In patients in whom the benefit is questionable, the following disadvantages of the high-dose therapy procedure should be considered: 1) immediate toxicity, 2) need for hospitalization, 3) unfounded expectations and 4) economic cost. Thus, alternative approaches should be sought for patients who are unlikely to benefit from HDT. In this regard there are new effective available agents whose mechanisms of action are different from those of the traditional conventional and high-dose therapy (i.e., thalidomide, IMiDs, bortezomib, and others) which are actively being investigated in prospective trials.

**Development of novel therapies**

Angiogenesis is increased in patients with MM and high angiogenic activity is associated with faster disease progression. Thus, it seems that angiogenesis plays a role in the pathogenesis of MM and for this reason antiangiogenic agents, particularly thalidomide, have been introduced into the treatment of this disease.

**Thalidomide**

The response rate to single agent thalidomide in relapsed/refractory patients is about 40%. When combined with dexamethasone, with or without cytotoxic agents, the response rate is between 50 and 60%. We reported that soft-tissue plasmacytomas do not respond to thalidomide and other groups have reported extramedullary progression in patients with serological response. In our updated series, none of the 11 patients with extramedullary plasmacytomas responded to thalidomide while the response rate among 27 patients with no extramedullary disease was 60%. This suggests that the action of thalidomide mainly depends on the site where the tumor cells are growing (bone marrow versus other sites). In fact, although the rationale for the use of thalidomide was its antiangiogenic effect, this drug may also act through many other mechanisms. In the author’s experience, the patients more likely to respond to thalidomide are those who have a more accumulative than proliferative disease (i.e., extensive bone marrow involvement with high-MM protein size and with no large lytic bone lesions and with no soft-tissue masses). In two phase II studies, the administration of thalidomide as a single agent in patients with smoldering multiple myeloma resulted in partial responses in 34 and 36% of them. The results reported with the use of thalidomide in combination with dexamethasone in previously untreated patients are encouraging. Thus, the Mayo Clinic group reported a response rate of 64% and in the MD Anderson experience the response rate was 72%. Interestingly, the last study showed a 16% CR rate and that the response to thalidomide was extremely quick (median less than one month). The Italian group reported a 90% response rate to the combination of melphalan/prednisone and thalidomide with 22% of the responses being complete. Prospective trials comparing thalidomide/dexamethasone versus dexamethasone alone are currently in progress. The role of thalidomide as maintenance therapy is an important issue, which is being investigated in prospective trials. The toxicity of thalidomide can be summarized as follows: 1) sedation, fatigue, constipation, tremor, dizziness, rash, edema and cardiac arrhythmias (bradycardia) that are responsible for early dose adjustments, 2) peripheral neuropathy, limiting the long-term use and 3) deep-vein thrombosis (DVT). The
last complication is usually observed when thalidomide is administered to previously untreated patients in combination with dexamethasone, doxorubicin or melphalan. Anticoagulation with warfarin or low-molecular-weight heparin can decrease the incidence of thalidomide-associated DVT when given in combination with dexamethasone or cytotoxic therapy.

**Immunomodulatory drugs (IMiD)**

Two thalidomide analogs (CC-5013 — revlimid and CC-4047 — actimid), termed immunomodulatory drugs (IMiD), have been shown to have antitymoma activity.48 Thus, in a phase II trial, the use of revlimid resulted in a 38% response rate, including 6% CR, in 83 patients with refractory/relapsed MM. The main toxic effects of revlimide are fatigue, muscle weakness and myelosuppression, the maximal tolerated dose being 25 mg/day. Several phase II trials have been completed and a large phase III randomized trial of CC-5013 plus dexamethasone versus dexamethasone alone in refractory and relapsed patients has recently completed the accrual of patients.49 On the other hand, CC-4047 — actimid — the second IMiD introduced in clinical trials has been investigated in a phase I/II trial in 24 patients with MM refractory to at least one line of treatment.50 Fifty-eight percent of the patients achieved at least an M-protein reduction exceeding 25% with 16% of the patients entering CR. Although the main toxicity was neutropenia, 4 (16%) cases of DVT were observed. The maximal tolerated dose is 2 mg/day.

**Bortezomib**

Bortezomib (Velcade) — dipeptidyl boronic acid, is a selective proteasome inhibitor, formerly known as PS-341, that has recently been approved by the FDA and the EMEA for its use in refractory multiple myeloma. Bortezomib produces proteasome inhibition which recovers in 72 hours. It has an extensive tissue distribution and the average proteasome inhibition is 60%. Greater than 80% proteasome inhibition resulted in unacceptable toxicity in animal models. Bortezomib is administered in a push (3-5 seconds), generally with no premedication. There is no need for a central line since bortezomib is not a vesicant. Due to the duration of proteasome inhibition the interval between doses must be at least 72 hours.

Bortezomib has been used in a large open-label phase II trial (SUMMIT study) including 202 patients with refractory multiple myeloma.51 In this population the median lines of prior therapy was 6 and 91% of the patients were refractory to their last therapy at the time of study entry. The overall response rate was 35% including 4% CR (negative immunofixation) and 6% near CR. The median survival in the last update was 18 months and the median duration of response 13 months. The efficacy of bortezomib versus high-dose dexamethasone in patients with relapsed or refractory MM who had received less than four lines of therapy has been investigated in a large phase III trial (APEX study). This study included 669 patients and the preliminary results have shown a significantly longer time to progression and overall survival as well as a trend toward a lower incidence of severe infections in favor of bortezomib. A number of trials aimed at investigating the possible role of bortezomib, as a single agent and in particular in combination, in earlier phases of the disease are currently in progress. The current/future directions in the investigation of thalidomide, IMiD and bortezomib are summarized in Table 1.

**Arsenic trioxide**

It has been shown that arsenic trioxide (ATO) induces apoptosis in drug-resistant multiple myeloma cell lines and in plasma cells obtained from myeloma patients. In addition, ATO produced significant responses in SCID mice transplanted with malignant myeloma cells. In two phase II trials, 9 out of 21 and 3 out of 14 patients with relapsed or refractory MM achieved > 25% M-protein reduction when treated with arsenic trioxide.52,53 In a recent report 3 of 10 patients with advanced myeloma showed only a minimal response.54 Of note, the responses achieved with ATO used as a single agent were of short duration. It has been shown in preclinical studies that the apoptotic activity of arsenic trioxide is enhanced by ascorbic acid, probably through changes in the redox balance of the cell resulting in glutathione depletion.55 The preliminary results of a tri-

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**Table 1. Thalidomide and bortezomib in multiple myeloma. Future directions.**

<table>
<thead>
<tr>
<th>Front-line therapy</th>
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<th>Cytotoxic agents</th>
<th>Novel agents</th>
<th>Maintenance therapy</th>
<th>Pre and post-transplant</th>
<th>Different schedules and dosing regimens</th>
</tr>
</thead>
</table>

**Table 2. Novel therapeutic targets in multiple myeloma.**

- Bcl-2
- Farnesyltransferase
- Vascular endothelial growth factor
- p38 kinase
- Interleukin-6
- Heat shock proteins
al combining ATO with ascorbic acid and low-dose melphalan indicated that the combination was well tolerated in patients with relapsed/refractory myeloma and resulted in some degree of M-protein reduction in all the first 14 patients included, with a PR rate of 54%. The described side effects include leukopenia, anemia, abdominal pain and diarrhea, fever, fatigue, increase in liver enzymes, neuropathy, encephalitis and fluid retention. Clinical trials on the use of arsenic trioxide in combination with ascorbic acid and other anti-myeloma agents are in progress.

**Novel targets**

As shown in Table 2, a number of novel agents targeting cell circuits of myeloma cell growth and survival are being investigated.

**Future prospects**

The definitive results of ongoing trials comparing HDT/ASCT versus conventional chemotherapy and single versus tandem transplants, as well as meta-analyses based on individual patients’ data from these trials should establish the definitive role of HDT as part of up-front therapy in MM. In addition, the long-term results of early non-myeloablative allografts after autologous transplant are eagerly awaited. In any event, it seems difficult to improve the results that we are currently achieving using only combinations of classic cytotoxic agents and high-dose therapy. The introduction of thalidomide in the treatment of multiple myeloma has been a major step forward, not only because of its recognized activity, but also because it has opened the door to the development of more innovative therapies targeting not only the plasma cell but also the bone marrow microenvironment. I am certain that the incorporation of new drugs with different mechanisms of action into the traditional anti-myeloma armamentarium will result in long-lasting disease control for patients with multiple myeloma.

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Multiple myeloma: the therapeutic approach to younger patients

For more than 50 years conventional-dose chemotherapy has been the cornerstone of the therapeutic approach to patients with multiple myeloma, with several different strategies based on the age. Several alkylating agent combinations have been evaluated in the last 30 years and in 1998 a metaanalysis, performed on 27 randomized studies, showed definitely that there are no statistical differences in terms of overall survival between treatment with melphalan alone or various combination of chemotherapy.

By contrast, the use of high-dose melphalan followed by autologous transplantation has significantly improved the outcome of patients with newly diagnosed symptomatic myeloma. The Intergroupe Francais du Myelome (IFM) was the first to conduct a randomized trial comparing conventional chemotherapy with high-dose therapy. Attal et al. demonstrated that high-dose therapy increased the percentage of good responses, prolonging both the event-free and the overall survival. Similarly, the MRC VII trial showed that progression-free and overall survival were superior among patients receiving high-dose therapy than in those receiving conventional chemotherapy. The data regarding the use of high-dose therapy in older patients are more controversial. Siegel et al. compared the outcome of 49 patients older than 65 years with that of 49 pair mates younger than 65 years. The transplant-related mortality was higher (8% vs 2%) and both event-free survival and overall survival were shorter in the older group. The complete response (CR) rate was significantly lower in the elderly patients. These data were confirmed in a subsequent study performed by Badros et al.; thus autologous transplantation cannot be unequivocally recommended for elderly myeloma patients. Most of the high-dose programs reported in the literature include at least two phases before transplantation: an induction or debulking therapy and mobilizing treatment. The majority of the protocols include the VAD or a VAD-like regimen as front-line therapy. This regimen does, in fact, produce quick and high responses rates, is generally well tolerated and can be safely administered even to patients with renal failure, and does not damage stem cells, thus not jeopardizing subsequent CD34+ stem cell mobilization. Other possible options for initial therapy are high-dose dexamethasone alone or in combination with thalidomide. Dexamethasone alone produces lower response rates than those achieved by VAD and causes a concerning amount of toxicity (susceptibility to infections, gastric/esophageal problems, fluid retention and weight increase, visual impairments including cataract, and so on). The combination thalidomide plus dexamethasone produces a higher percentage of good responses in myeloma patients but at the same time gives more side effects: neurological disturbances, and higher risk of deep vein thrombosis (12–16%). So, at the moment VAD or a VAD-like regimen seems to be the best option for the induction of untreated patients. High-dose cyclophosphamide (HDCTX) is considered the standard mobilizing therapy, even though it is burdened by several toxic effects which often require hospitalization. Alternative mobilising regimens have shown a higher mobilizing capacity than HDCTX, but often with higher toxicity. We previously reported that the DCEP protocol is better tolerated than HDCTX and also has better mobilizing properties. Other studies have demonstrated that this regimen also has good anti-myeloma activity in refractory MM patients. As far as concerns autologous transplant, there are some overall recommendations: the standard conditioning regimen is melphalan 200 mg/m², total body irradiation as well as stem cell purging are not recommended because they add toxicity and expense but no clinical benefit; peripheral blood stem cells are recommended over...
bone marrow because of their ease of collection and more rapid engraftment. In contrast, some issues remain open, in particular the adoption of double transplants or the timing of a second transplant (upfront or at first relapse).\(^{9,10}\)

Another option for younger patients is allogeneic transplantation. The main advantages of allotransplantation are the absence of contaminating tumor cells in the graft and the presence of a graft-versus-myeloma (GVM) effect exerted by alloreactive T donor lymphocytes. The toxicity related to this procedure remains its main problem. In fact, transplant-related mortality, mainly due to graft-versus-host disease and infectious complications, ranges from 30 to 50%. The EBMT study reported a decrease in transplant-related mortality from 46% in patients submitted to allograft before 1994 to 33% in those receiving allografts from 1994 to 1998. However, only 10% to 20% of patients receiving an allograft, were in CR 5 or more years after transplantation.\(^{11}\) So the curative potential of allogeneic transplantation is mitigated by a shortened of survival due to the high transplant-related mortality. There is also some interest in dose-reduced intensity allotransplants, the so-called mini-allogeneic transplants. Studies have been published showing the feasibility of the procedure with a good CR rate and an acceptable 20% of transplant-related mortality, but the results are still too preliminary.\(^{12}\)

Thus, at the moment, the first option to offer to myeloma patients younger than 65 years is HDT with a single or double transplant.

**Design and Methods**

On the basis of these data in 2000 we started a high-dose program designed as follows:

<table>
<thead>
<tr>
<th>1st phase (Debulking)</th>
<th>2nd phase (Mobilization)</th>
<th>3rd phase</th>
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<tbody>
<tr>
<td>pulsed-VAD×2</td>
<td>DCEP×2</td>
<td>Double ASCT with Melphalan 200 mg/m²</td>
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</table>

Patients aged less than 65 years with Durie and Salmon stage II and III multiple myeloma or stage I in progression from previous monoclonal gammopathy of undetermined significance were eligible. The criteria for exclusion were prior treatment for myeloma, another cancer, abnormal cardiac function (indicated by a systolic ejection fraction < 50%), chronic respiratory disease (indicated by a vital capacity or carbon monoxide diffusing capacity less than 50% of predicted), abnormal liver function (indicated by a serum bilirubin level more than 2 mg/dL or an alanine aminotransferase or aspartate aminotransferase level more than four times the upper limit of normal), psychiatric disease and evidence of previous infection by hepatitis C or human immunodeficiency virus. The study was approved by the institutional ethics committees, and the patients gave written informed consent.

From 2000 to May 2004, 123 consecutive untreated MM patients (62 males, 61 females) with a median age of 55 years (range 35-65), were enrolled in this high dose program. Patients received 2 courses of pulsed-VAD (vincristine 2 mg i.v. on day 1, doxorubicin 50 mg/m² on day 1, dexamethasone 40 mg/die i.v. days 1-4, 14-17), followed by 2 courses of DCEP plus granulocyte colony-stimulating factor (G-CSF). Peripheral blood stem cells were collected after each cycle in order to obtain an adequate number of CD34+cells for two transplants. DCEP was as follows: dexamethasone 40 mg/die for 4 days, and 4-day continuous infusion of cyclophosphamide 400 mg/m²/die, etoposide 40 mg/m²/die and cisplatin 10 mg/m²/die. G-CSF 5 µg/Kg was started 48 hours after the end of chemotherapy until leukaphereses were concluded. Peripheral blood stem cells were collected after each DCEP cycle.

Patients were assessed for response after each step of the protocol. The response criteria were defined as follows: complete response (CR): absence of M component in serum and urine by immunofixation and <5% plasma cells in bone marrow aspirate; very good partial response (VGPR): 90% decrease of serum and urine paraprotein level; partial response (PR): at least 50% decrease of serum paraprotein level and 90% decrease of Bence Jones protein; stable disease (SD): less than 25% decrease of serum paraprotein level and Bence Jones protein; no response (NR): no variation or increase of serum or urine paraprotein level.

**Statistical analysis**

Continuous variables were summarized as medians and ranges, and categorical variables were calculated from the date of first VAD until the time of disease progression or death; overall survival was defined as the time from the start of chemotherapy to the last observation. The progression-free and overall survival curves were calculated with the Kaplan–Meier method and compared by means of the log–rank test. Stata 8 software (StataCorp, College Station, TX, USA) was used for computations. A 2-sided \( p \) value < 0.05 was considered as statistically significant.
Results

Characteristics of 123 patients registered at the time of enrollment are detailed in Table 1. At the time of this preliminary analysis 106/123 patients (86%) have undergone the first transplant, 14 patients dropped out before transplant, and 55/106 (52%) completed the second transplant. Toxicity was very low after the VAD and DCEP cycles. In particular, after 246 VAD cycles we had registered 25 cases of grade III WHO neutropenia (10%) and 10 severe infections (4%). After 227 DCEP cycles we had observed 32 cases of grade III WHO neutropenia (14%) and 9 severe infections (4%).

Transplant procedures were similarly well tolerated. In detail, the median time to neutrophil and platelet
engraftment was similar after the first and the second transplants, being respectively 8 and 10 days. The incidence of extra-hematologic toxicity (infections and mucositis) was not statistically different in the two transplants. The median time of hospitalization was 18 days for both procedures. At the time of the evaluation 4 patients had died, 2 of transplant-related causes (1 after the first transplant and 1 after the second) and 2 of progression. The response rates after every phase of the protocol are listed in Table 2. As shown, there is a progressive increase of good responses (CR+VGPR) from the debulking phase to the second transplantation, after which 85% of good responses were reached. The high percentage of patients who had progression after DCEP regimens is related to a group of patients who did not maintain the timetable for the transplants.

After 227 mobilizations, the median number of CD34+ stem cells collected per mobilization was 5.34×10^6/kg (range 0-25.7), and the median number of CD34 cells per patient, considering the two cycles of mobilization, was 11×10^6/kg (range 4.02-32.8). Only 7 patients failed to mobilize peripheral stem cells. With a median follow-up of 20.5 months from entry to the study, the median OS has not been reached and the median progression-free survival was 31.8 months. Figure 1 reports the curves relative to the progression and the overall survival of all patients. The analysis of progression-free and overall survival, performed also on the basis of the type of response to chemotherapy before transplant, did not show any statistical difference. Furthermore, no statistically significant differences were observed between the progression-free survival of patients who obtained a CR after second transplant with respect to the others. Even when considering patients with a CR or VGPR together, the median did not differ significantly (15.9 vs 15.3) but the curves suggest a better trend for good responders after the first years following transplant. Thus, the lack of difference could be due to the still short follow-up.

### Discussion

Autologous transplant is currently offered as part of front-line therapy to patients with newly diagnosed MM aged less than 65 years. What is known about autologous transplant so far? Autologous transplant increases the CR rate, EFS and OS, this last ranging from 4 to 5 years in the majority of centers; no drugs toxic to stem cells must be used before transplant. Mobilization should be performed in order to harvest as many stem cells as possible and for each transplant at least 4×10^6 CD34+ peripheral stem cells per Kg of body weight should be used. The better the response achieved before transplant, the greater the benefit that can be obtained from the transplant procedure; high-dose melphalan alone at a dose of 200 mg/m^2 is the standard conditioning regimen; total body irradiation is not recommended.

The high-dose program reported here was designed in order to follow these recommendations. Although the analysis is quite preliminary, due to the short follow-up, some information can be drawn from this study. DCEP is a safe and effective mobilizing regimen. Actually, 74% of patients yielded an adequate number of peripheral stem cells for two transplant procedures with a very low incidence of severe hematologic or extrahematologic side effects. Consequently, apart from the days of DCEP infusion most of the patients were managed in an outpatient setting. So, as previously reported, DCEP is as effective as other mobilizing regimens but with a significantly lower toxicity. At the same time, a high percentage of good responders can be obtained with the VAD-DCEP sequence.

We recently reported a comparison between two different high-dose protocols adopted in a single center. The first program included a sequence of VAD-high dose chemotherapy and the second VAD-DCEP. We showed that the VAD-DCEP sequence is more effective and better tolerated than the VAD-high dose chemotherapy regimen.

Although this protocol was well tolerated by the majority of patients, there was a number of patients who were reluctant to proceed to the second transplant. Seventeen patients (16%), in fact, decided not to undergo the second transplant. The more frequent reason for refusal was the length of the treatment, which was usually correlated with good quality of life. This is in line with other studies which report from 10 to 20% of patients withdrawing between the first and second transplant.

### Conclusions

In conclusion, although data reported here are very preliminary, this high-dose program seems to be feasible and effective in myeloma patients aged less than 65 years. In fact, the percentage of good responders increases at each step up to the 85% of patients who achieve a CR or a VGPR after the second transplant. This could represent the basis of a lasting progression-free survival, as also seems to suggested by Figure 2 which shows a trend to a longer duration of response after the first year of follow-up.
References

Multiple myeloma: new therapeutic options for elderly patients

In recent years, new therapeutic options have been introduced for the treatment of multiple myeloma (MM). Autologous peripheral blood stem cell supported high-dose therapy has been introduced for elderly patients. New active drugs include immunomodulatory agents such as thalidomide, CC-5013 (revlimid) and the proteasome inhibitor, bortezomib (velcade). All together these new approaches have significantly changed the therapeutic strategy for elderly patients with MM. Conventional chemotherapy has been the treatment of choice for MM since 1960. High-dose therapy followed by stem cell rescue has significantly improved clinical outcome, but it is reserved to patients younger than 65 years. Unfortunately, only 27% of myeloma patients are younger than 65 years, 33% are 65–74 years old and 40% are over 75 years old. Alternative approaches that could safely be administered to patients 65–75 years old have been tested. To address this issue the Gruppo Italiano Studio Mieloma Multiplo conducted a multicenter, randomized trial on the efficacy of tandem melphalan 100 mg/m² (MEL100) versus oral melphalan and prednisone (MP) in elderly MM patients. Two hundred patients were enrolled from 18 Italian centers. In the oral MP group, 15% did not complete all 6 cycles of MP. In the MEL100 group, 20% did not complete tandem MEL100. In the MP arm, the frequency of partial responses (PR) and complete responses (CR) were 38% and 7%, respectively. In the MEL100 group, the frequency of PR and CR were 61% and 10%, respectively, after the first MEL100 and 48% (26%) after the second. MEL100 significantly improved the CR rate (p<0.0001). The median event–free survival (EFS) was 16 months after MP and 28 months after MEL100 (p<0.0001). The probability of EFS for 3 years after diagnosis was 16% after MP and 38% after MEL100. The median overall survival (OS) was 43.3 months for MP and has not been reached (58+ months) for MEL100 (p=0.0008). In the multivariate analysis, EFS was only related to the administration of MEL100 and serum b2–microglobulin level. The goal of treatment in MM patients is to obtain the best response rate with minimum toxicity. In the present study we investigated whether MEL100, which is half the dose of the typical MEL200, might still improve clinical outcome in comparison with oral MP. Despite the dose reduction, MEL100 still produced a survival benefit. After MEL100, the incidence of toxicities was not age-related, indicating that this approach is particularly suitable for elderly patients. Both MEL100 and tandem melphalan 200 mg/m² (MEL200) are clearly superior to standard oral–dose melphalan. Their comparative toxicities and outcomes, however, were unclear. In another study, patients with similar disease characteristics were treated with tandem MEL100 or tandem MEL200 and their toxicities and outcomes compared. In this case–matched controlled analysis, the clinical characteristics of both groups were similar, except their age. The median age was around 50 years for the MEL200 group and 60 years for the MEL100 group. On an intention–to–treat basis, 92% of patients completed the entire MEL100 program, 88% of patients completed the MEL200 regimen. The rates of CR (35% vs 48% p=0.08) and very good partial response (15% vs 30% p=0.01) were slightly increased after 2 courses of MEL200. The CR rates were also increased by administration of both the second MEL100 (19% vs 35% p=0.016) and second MEL200 course (32% vs 48% p=0.03). The median EFS was 31.6 months after MEL100 and 42.1 months after MEL200 (p<0.0005). The median OS was similar for both groups: 67 months for MEL100 and 75 months for MEL200 (p=0.4). A multiple regression model, including all major clinical prognostic factors affecting outcome, was used to estimate the effect of treatment. MEL200 was the only factor that retained independent significance on EFS, but not on OS. In this retrospective case–matched investigation of the clinical effect of MEL100 and MEL200, MEL200 was superior to MEL100 in terms of EFS while both regimens were equally effective in terms of OS. When...
oral melphalan was compared with MEL100 and/or MEL200 the CR rate increased from 1–5% to 30–50%. This tremendous response increase may explain a significant outcome improvement. When we compared MEL100 with MEL200, the CR rate increased from 35% to 48%. This slight increase prolonged EFS but was not enough to induce a significant improvement in survival. Our results clearly show a lower toxicity for the MEL100 regimen. Severe mucositis, duration of neutropenia and thrombocytopenia, and red blood cell and platelet transfusions were drastically reduced. The median duration of hospitalization dropped from 15 to 7 days. Age per se, is not a contraindication to the use of MEL200. Age increases the incidence of poor clinical conditions and concomitant diseases, and those were the major causes of the increase in treatment-related toxicity. In conclusion, the MEL100 conditioning regimen is less toxic and slightly less effective than MEL200. MEL200 should be considered the standard for younger myeloma patients in good clinical condition. MEL100 must be the first alternative in elderly patients or in those in a poor clinical condition. Thalidomide blocks the ability of vascular endothelial growth factor (VEGF) and basic fibroblast growth factor (b-FGF) to stimulate neovascularization of bone marrow, directly inhibits the growth and survival of myeloma cells, modulates some adhesion molecules, and inhibits tumor necrosis factor-α (TNF-α) and interleukin-6 (IL-6) secretion. This drug is effective in refractory and recurrent myeloma. Low-dose thalidomide in combination with dexamethasone has proved effective in advanced myeloma. The survival advantage induced by thalidomide has not been determined or compared with that of standard chemotherapy strategies. To address this issue, low-dose thalidomide and dexamethasone (TD) have been used as a salvage regimen for poor prognosis myeloma patients. The outcome of patients treated with this combination was compared with that of matched patients treated with conventional chemotherapy (CC).

One hundred and twenty patients (median age 63 years) who had relapsed or were refractory to chemotherapy were treated with thalidomide 100 mg/day (continuous) and dexamethasone 40 mg (days 1–4 of each month). Their clinical outcome was compared to that of a control group of 120 pair mates (median age 60) selected from relapsed or refractory patients treated with conventional chemotherapy and matched for serum β2-microglobulin levels and Durie and Salmon clinical stage. On an intention-to-treat basis, 84% of patients completed the entire TD program. All patients were considered: 52% showed a myeloma protein decline >50%, in 24% this decline was 75–100%, in the other 28% it was 50–75%. The median time to the maximum response to TD was 4.2 months. Forty-five percent of CC patients showed a myeloma protein decline >50%, in 19% this decline was 75–100%, in 26% it was 50–75%. No differences in response rate were observed between the TD and the CC groups. The median PFS was 12 months compared with 11 months for the CC group. The progression-free survival in both the TD and CC groups were similar. The median OS from the beginning of TD and CC was 27 and 19 months, respectively. Differences were statistically significant (p<0.05). Constipation was relatively frequent but well controlled. Sedation was recorded in 13% of patients, and 8% showed confusion. Tingling and numbness were frequently observed. Drug reduction and/or discontinuation were mainly due to neurotoxicity. Drug reduction and/or discontinuation seem to be dose and time-dependent.

Unexpectedly, TD improved survival; the mechanisms of this improvement are unclear. After salvage chemotherapy, severe hematologic toxicity, hampering the use of other cytotoxic drugs, or drug resistance followed by uncontrolled progressive disease are often observed. These are the major causes of death. TD was effective and without any hematologic toxicity. It postponed the delivery of chemotherapy and hence the establishment of severe hematologic toxicity or progressive disease. This may explain its survival advantage. An increased risk of deep-vein thrombosis has been reported after thalidomide administration. In our series, the incidence of deep-vein thrombosis was identical in both TD and CC groups. Neuropathy was the main complaint. It was dose- and time-dependent, and the major cause of drug reduction/discontinuation. Unfortunately, prevention is impossible and the only option is withdrawal of the thalidomide. This study provided further evidence of thalidomide’s effectiveness in myeloma. It was also the first demonstration that TD is an effective salvage treatment and induced a survival benefit. We first demonstrated that TD was superior to CC. We then investigated whether TD was as effective as autologous stem cell transplant (ASCT) for relapsed or refractory patients. In the salvage setting, the best treatment option for patients relapsing after autologous transplant has not been defined. CC is widely employed. AT can be effective used for relapse, but cryopreserved stem cells are not always available for salvage treatment. TD has proved effective in advanced myeloma. In this study, the efficacy of salvage TD, ASCT and CC was evaluated and compared. We conducted a retrospective analysis on 90 patients who were treated at diagnosis with ASCT. At first relapse, 3 patients received TD as their salvage regimen, 28 underwent a repeat ASCT and 19 received CC. The major prognostic factors were similar among the three different groups. The median times between diagnosis and start of salvage treatment were similar. EFS from diagnosis were also similar in the 3 subgroups: 32.3 months for the TD subgroup, 28.9 for the ASCT subgroup and 32 for the group receiving CC (p=0.16). After the sal-
The addition of dexamethasone increased this PR rate to nearly 30%; the addition of dexamethasone increased the PR rate to 50% and the combination of thalidomide with chemotherapy further increased the response rate to 70%. In untreated patients, thalidomide produced PR in 25% of patients and CR 14.3%. Myeloma protein was reduced by 90-99% in CR patients. Their median age was 71 years and their median serum β2 microglobulin concentration was 3.7 mg/L. After 6 MPT courses, the frequency of immunofixation negative CR was 26.5% and that of immunofixation positive near CR 14.3%. Myeloma protein was reduced by 90-99% in 8.2% of the patients and by 50-89% in 30.6%; no response occurred in 8.2% of the patients and 2% had progressive disease. The daily dose of thalidomide was reduced from 100 mg to 50 mg in few patients. Patients suspended thalidomide because of deep vein thrombosis (DVT), infections, constipation, neurologic and hematologic toxicity. Neurologic toxicity occurred in 39% of patients. Acute infections and DVT were the most acute adverse events. Infections were observed in 29% of patients: most related to pneumonia. Thromboembolism was observed in 20% of patients. One patient died of acute pulmonary thromboembolism and one of pneumonia. No conventional treatment produces such response rates, these results can only be achieved with high-dose therapy followed by autologous stem cell support. In a polychemotherapy regimen, the response rate of 18% was doubled to 36% with the addition of thalidomide alone. Marked cyto reduction does not guarantee survival benefit, but it is the first step toward a sustained remission. The absence of myelosuppression makes thalidomide an ideal drug for combination with cytotoxic agents. We observed a small increase in hematologic toxicity in comparison with that caused by MP and infections were a clinical problem. The immunosuppressive activity of thalidomide seems to play an important role and antibiotic prophylaxis should be instituted in elderly patients. The major adverse event was DVT; all but one thromboembolic events occurred within the first 4 months of treatment. These events obliged us to amend the protocol, adding enoxaparin prophylaxis.

We thank Miss Federica Leotta for her technical assistance in the preparation of manuscript. We also thank the many medical and nursing colleagues who have participated in the treatment of patients. This work was supported in part by the Associazione Italiana Ricerca e Cura Concorso (AIRC), Associazione Italiana Leucemia (AIL), Ministero Università e Ricerca Scientifica e Tecnologica (MURST), Consiglio Nazionale Ricerca (CNR), Compagnia di S. Paolo, and Associazione per lo Studio e la Cura delle Malattie del Sangue.

**References**

Salvage therapy for refractory and relapsed multiple myeloma

Standard chemotherapy for multiple myeloma with prednisone and melphalan produces responses in 40 to 60% of newly diagnosed patients; complete responses are rare and the median survival rate is approximately 3 to 4 years. High-dose therapy followed by autologous stem cell transplantation can extend survival to 4 to 5 years. This leaves a large portion of patients in need of additional treatment in order to increase the response rate, to achieve longer survival and, possibly, a better quality of life. Several therapeutic approaches are available for patients in whom first-line therapy fails and who are diagnosed with refractory or relapsed multiple myeloma (Appendix 1). These approaches comprise both standard chemotherapy regimens and, more interestingly, novel agents with different antitumor mechanisms of action. Here we give a brief overview of available options.

Conventional therapeutic approach to refractory/relapsed multiple myeloma

Standard therapy for patients with refractory and relapsed multiple myeloma relies on a number of salvage chemotherapy courses and includes the option of transplantation of autologous peripheral blood stem cells (PBSC). Chemotherapy schemes are summarized in Table 1. For patients with a poor performance status, single agent therapy with a glucocorticoid (e.g.: dexamethasone 40 mg p.o. day 1-4; 9-12; 17-20 every 4 to 5 weeks) may be an option; response rates in refractory patients and in relapsed patients are 27% and 21%, respectively. The VAD scheme yields a 65% response rate in relapsed patients, being less effective in refractory patients (31% response rate). Similarly low response rates in both subsets of patients are reported using combination chemotherapy with high dose dexamethasone and cyclophosphamide or other poorly myelosuppressive schedules of chemotherapy. Good response rates, especially in patients with high proliferative index disease, have been achieved with the DCEP scheme. In primary chemotherapy resistant disease, the response rate to autologous PBSC transplant varies between 65 and 88% with a progression-free survival (PFS) between 8 and 26 months. A second procedure can be performed in patients who relapse after the first autologous PBSC transplant; however, transplant-related mortality, which increases to > 10%, must be taken into consideration when selecting candidates. Patients who relapse after a double autologous PBSC transplant may benefit from a third procedure: the reported 2-year overall survival is 19%. In this subset of patients, if younger than 55 years old and with an HLA compatible donor, the option of allogeneic PBSC transplant can be considered. Patients who have already undergone an HLA compatible donor PBSC transplant can be candidates for donor lymphocyte infusion (DLI) with the aim of re-inducing remission.

Innovative therapeutic strategies in refractory/relapsed multiple myeloma

In view of the unsatisfactory results of standard chemotherapy courses, novel agents with alternative antitumor mechanisms of action have been employed in refractory/resistant myeloma patients, either alone, as single agent therapy, or in combination with high dose dexamethasone and/or standard chemotherapy.

Anti-angiogenic and immunomodulatory drugs: thalidomide

Thalidomide is a glutamic acid derivative first commercialized in the 1950s as a tranquilizer and later withdrawn because of its teratogenic effect. It has been employed in the treatment of multiple myeloma since 1999 because of its antiangiogenic action. Since then it has been recognized to have a variety of antitumor effects and, although its mechanism of action is still not fully understood, its immunomodulating properties seem to be essential for its therapeutic effect. Several cytokines are modulated by...
thalidomide including endothelial growth factor, fibroblastic growth factor, hepatocytic growth factor, interleukin-6 and tumor necrosis factor-α, this last being of particular importance also in clinical practice.\textsuperscript{19-21}

Response to thalidomide administration seems to be highest in patients with a high tumor burden (expressed as serum and/or urinary M component levels and bone marrow plasma cell percentage) and without significant bone lesions of extramedullary plasmacytoma.\textsuperscript{21-23} Homing of plasma cells within the bone marrow seems to be crucial for response to thalidomide, as demonstrated by data presented by Rosiñol and Bladé.\textsuperscript{21} In their experience, 42% of 38 patients with refractory/relapsed myeloma responded to thalidomide; however, no response was observed among patients with extramedullary involvement, while 59% of patients without extramedullary involvement improved (\(p = 0.0006\)). The effectiveness of thalidomide therapy increases with increasing dosages, but in the majority of patients satisfactory results are achieved with low doses (100-200 mg/day). Longer overall and event-free survivals have been reported for primary refractory myeloma patients responding to thalidomide.\textsuperscript{23} According to the literature review published by Weber,\textsuperscript{28} approximately 30% of refractory/relapsed patients respond to thalidomide as single agent therapy.

According to retrospective analyses, very good partial responses and complete responses are achieved in approximately 13% of refractory/relapsed patients, with a median survival of 13 months; progression-free survival and overall survival at one year range between 23 and 50% and 56 and 86%, respectively.\textsuperscript{21,29} The presence of cytogenetic abnormalities is associated with a poor outcome: according to UAMS data, only 7% of patients with such abnormalities are alive at 5 years compared to 43% of patients with normal karyotype.\textsuperscript{29}

### Table 1. Conventional therapeutic approach to refractory/relapsed multiple myeloma.

<table>
<thead>
<tr>
<th>Type of treatment</th>
<th>Regimen</th>
<th>Response</th>
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<tr>
<td>Glucocorticoid single agent\textsuperscript{11}</td>
<td>Dexamethasone 40 mg/d (days 1-4, 9-12, 17-20 every 4 or 5 weeks)</td>
<td>27% response rate in refractory patients</td>
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<td></td>
<td>21% response rate in relapsed patients</td>
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<td>VAD\textsuperscript{6,7} (Vincristine, adriamycin, dexamethasone)</td>
<td>24 h i.v. infusion Vincristine 0.4 mg/day + Adriamycin 9 mg/m²/day (days 1-4); Dexamethasone 40 mg/d i.v. (days 1-4, 9-12, 17-20) every 28 days</td>
<td>31% response rate in refractory patients; 65% response rate in relapsed patients</td>
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<tr>
<td>Prednisone and Cyclophosphamide\textsuperscript{8,43}</td>
<td>Cyclophosphamide 1 g/m² i.v. every 21 days + Prednisone 100 mg p.o. (days 1-5)</td>
<td>Low response rate in both subsets of patients</td>
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<tr>
<td>DCEP\textsuperscript{10,11} (Dexamethasone, cyclophosphamide, etoposide, Cis-platinum)</td>
<td>Dexamethasone 40 mg/day p.o. (days 1-4) + 24 h i.v. infusion Cyclophosphamide 400 mg/m²/day (days 1-4) + 24 h i.v. infusion Etoposide 40 mg/m²/day (days 1-4) + 24 h i.v. infusion Cis-platinum 10 mg/m²/day (days 1-4)</td>
<td>Good response rate, especially in high proliferative index disease</td>
</tr>
<tr>
<td>Autologous peripheral blood stem cells transplant\textsuperscript{12,17}</td>
<td>1, 2, or 3 procedures</td>
<td>Response rate 65-88% with PFS 8-26 moths. A second procedure is possible in patients who relapse after the first autologous PBSC transplant; however, transplant-related mortality (up to &gt; 10%), must be taken into consideration. Patients who relapse after a double autologous PBSC transplant may benefit from a third procedure (2-year overall survival is 19%). In this subset of patients, if younger than 55 years old and with an HLA compatible donor, the option of allogenic PBSC transplant can be considered.</td>
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\textit{DEX: dexamethasone, VINC: vincristine, Adria. Adryamicin, Cycl: cyclophosphamide, Etop: etoposide, Cis: cisplatin; Predn: Prednisone.}
Several studies have outlined the importance of TNF-α levels in disease prognosis during treatment with thalidomide. Brenne et al. reported that low levels of soluble TNF-α receptor (TNFR p55) correlate with a significant higher response rate and with longer survival in patients with advanced multiple myeloma treated with thalidomide. Bladé et al. observed higher TNF-α plasma levels in patients with soft tissue plasmacytoma not responding to thalidomide than in patients without extramedullary involvement.

Side effects (Table 2) often occur during thalidomide administration; they are usually dose-dependent and reversible upon discontinuation of the drug. WHO grade 3 and 4 toxicity in about 58% of patients receiving thalidomide was reported by Barlogie: 25% of patients experienced central nervous system toxicity (drowsiness, sedation, confusion, depression, tremors); 16% complained of constipation which progressed to paralytic ileus in 2% of cases; 9% developed sensory neuropathy most frequently involving the lower limbs; deep venous thrombosis was observed in 8% of patients; skin rash were rare (4%) but epidermolysis bullosa, which may be fatal, may develop.

**Thalidomide plus dexamethasone**

Resistant patients who do not respond to high dose dexamethasone or thalidomide as single drug therapy often respond to combination therapy with the two agents (Table 3). Clinical and in vitro data point to a synergistic anti-myeloma effect of these two molecules. According to Anagnostopoulos et al., approximately 50% of patients respond to combination therapy and very good partial responses are obtained in 30% of patients. Median time to response is about 2

### Table 2. Thalidomide in refractory/relapsed multiple myeloma.

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<tr>
<th>Author</th>
<th>No.</th>
<th>Regimen</th>
<th>Paraprotein Reduction</th>
<th>WHO Grade 3-4 Toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kumar, 2003</td>
<td>32</td>
<td>Thalidomide, 200 to 800 mg/day</td>
<td>NR 10 17 (31%) (53%)</td>
<td>10 neutropenia, 5 neuropathy, 4 sedation 2 neuromotor effects, 2 constipation, 2 sinus bradycardia, 2 febrile neutropenia, 1 dyspnea, 1 fatigue, 1 raised aspartate transaminase, 1 cerebral ischemia, 1 thrombosis, 1 rash, 1 vertigo</td>
</tr>
<tr>
<td>UAMS Barlogie</td>
<td>169</td>
<td>Thalidomide, 200 to 800 mg/day</td>
<td>24 51 NR (14%) (30%)</td>
<td>58% of patients had toxic side effects (25% sedation, somnolence, confusion, depression, tremors, 16% constipation, 9% neuropathy) no myelosuppression</td>
</tr>
<tr>
<td>Waage 2004</td>
<td>65</td>
<td>Thalidomide, 200 to 800 mg/day</td>
<td>4 9 9 (6%) (14%) (14%)</td>
<td>13% constipation 5% heart failure, bradycardia 3% tingling or numbness 3% mood changes 3% rash or dry skin 3% altered hearing or vision 3% headache 2% incoordination 2% mouth dryness</td>
</tr>
</tbody>
</table>

NR: not reported.
months; at 5 months, 95% of patients respond. Responsive patients are candidates for PBSC harvest and autografting. Initial concern about an unfavorable action of thalidomide on PBSC harvest was not confirmed by other studies which showed that stem cells of patients pre-treated with this drug can be successfully mobilized to the periphery.

**Thalidomide plus chemotherapy**

Due to its unique mechanism of action, thalidomide has also been included in standard chemotherapy courses (Table 4) with the aim of increasing response rates without increasing toxicity. Of note, combined therapy with antracyclines is not recommended because of the reported higher incidence of deep venous thrombosis (16%) among patients receiving thalidomide and doxorubicin.

Table 4 summarizes some of the proposed schemes combining thalidomide and standard chemotherapy.

Recently, Dimopoulos et al. reported encouraging results in pre-treated multiple myeloma patients using a regimen based on oral pulsed administration of cyclophosphamide, thalidomide and dexamethasone (pulsed CTD). This regimen consists of cyclophosphamide 150 mg/m² p.o. every 12 hours on days 1-5, thalidomide 400 mg p.o. on days 1-5 and 14-18, and dexamethasone 20 mg/m² p.o. on days 1-5 and 14-18. The pulses of CTD were repeated every 28 days for three courses; responding patients were scheduled to receive maintenance treatment with CTD administered only for the first 5 days of each month. Partial responses were obtained in 60% of the 53 patients treated, with a median time-to-response of 1.5 months; this regimen also proved to be effective also in 30% of patients pre-treated with thalidomide and in some patients (3 of 6) with extramedullary disease, thus offering an additional treatment option for this subset of poor prognosis patients. Toxicities are reported to be mild to moderate, in contrast with the known high incidence of side effects, especially deep venous thrombosis and peripheral neuropathy, associated with long-term administration of thalidomide. However, myelotoxicity may be a relevant side effect: WHO grade 3 and 4 neutropenia was recorded in 18% and 8% of patients, respectively.

**Immunomodulatory drugs: revimid**

In view of the high frequency of untoward effects — with special regard to peripheral neuropathy — and the low rate of complete responses observed in patients on thalidomide, agents with a chemical structure similar to that of thalidomide have been synthesized with the aim of increasing the response rate and reducing the side effects. Compared to thalidomide, these immunomodulatory drugs (ImiD) inhibit TNF-α production by a factor of 50,000. According to data from the Dana Faber Cancer Institute on patients with refractory/relapsed myeloma who received revimid, the maximal tolerated dose (MTD) is 25 mg p.o./day for 21 days/month. Of the 24 evaluable patients treated according to this schedule, 29% had a M component reduction of > 50%; in 71% of patients, the M component decreased to at least 25% of base-line values. In this series, 46% of revimid-responsive patients had been pre-treated with thalidomide. Hematologic toxicity was the dose-limiting tox-

<table>
<thead>
<tr>
<th>Author</th>
<th>n.</th>
<th>Regimen</th>
<th>Efficacy</th>
<th>Grade 3-4 Toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Dimopoulos 2001</td>
<td>44</td>
<td>Thalidomide 200-400 mg/day</td>
<td>OR: 24 (55%)</td>
<td>Constipation, somnolence, fatigue</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dexamethasone 20 mg/m²/day p.o.</td>
<td>VGPR: 13 (30%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(days 1-4, 9-12, 17-20,</td>
<td>PR: 11 (25%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>followed by days 1-4 monthly)</td>
<td>MR: 1 (2%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MTTP: 4.2 months</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>MS: 12.6 months</td>
<td></td>
</tr>
<tr>
<td>Palumbo 2001</td>
<td>120</td>
<td>Thalidomide 100 mg/day</td>
<td>OR: 62 (52%)</td>
<td>Constipation, sedation, neuropathy</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dexamethasone 40 mg/day p.o.</td>
<td>MPFS: 12 months</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(days 1-4 monthly)</td>
<td>MS: 27 months</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td>2-year OS: 64 (53%)*</td>
<td></td>
</tr>
<tr>
<td>Alexanian 2003</td>
<td>47</td>
<td>Thalidomide 200 mg/day</td>
<td>OR: 22 (47%)</td>
<td>8% Deep vein thrombosis</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(max 600 mg/day)</td>
<td>CR: 6 (13%)</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>Dexamethasone 20 mg/m²/day p.o.</td>
<td>MS: 30 months</td>
<td></td>
</tr>
<tr>
<td></td>
<td></td>
<td>(days 1-5 fortnightly)</td>
<td></td>
<td>8% paresthesias</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>4% rash</td>
</tr>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td>2% ileum</td>
</tr>
</tbody>
</table>

OR: overall response; VGPR: very good partial response; PR: partial response; MR: minor response; MTTP: median time to progression; MS: median survival; MPFS: median progression-free survival; OS: overall survival. *Probability calculated after a median follow-up of 9 months.
icity in this study. Encouraging results have also been reported by Zangari et al.\(^4\) In their study population of heavily pre-treated patients, treatment with revimid halved M component serum levels in 20% of cases.

A European-Australian multicenter phase III study comparing dexamethasone plus placebo vs dexamethasone plus revimid in refractory/relapsed myeloma patients is currently underway. The side effects found in dose escalation studies were WHO grade 3 thrombocytopenia in 20% of treated patients and WHO grade 3 and 4 neutropenia in 60% and 16% of patients, respectively. Rare cases of thromboembolic complications and syncope have been reported. Peripheral neuropathy, drowsiness and constipation, while frequent on thalidomide treatment, are only very seldom reported with revimid.

**Inhibitors of the 26S proteasome: velcade (bortezomib - PS341)**

Bortezomib is a potent and reversible inhibitor of the 26S proteasome, a multi-subunit protein complex which is present in both the cytoplasm and nucleus of eukaryotic cells and whose function is to degrade a variety of proteins with critical functions such as regulation of the cell cycle, transcription, apoptosis, angiogenesis, and cell adhesion. Bortezomib binds with high affinity to the proteasome, but its inhibitory effects are reversible thus allowing the return of most proteasome activity by 72 hours after administration; moreover, while inducing apoptosis in a number of cancer cell lines (multiple myeloma, lymphoma, chronic lymphocytic leukemia, head and neck cancer, prostate cancer), toxic effects on normal cells are reported to be mild. These effects are mediated through inhibition of translocation to the nucleus of NF-κB, a transcription factor which induces the expression of genes which inhibit cell death, promote proliferation, and regulate expression of adhesion molecules. NF-κB is segregated to the cytoplasm by binding to its inhibitor, IκB. Degradation of IκB by the proteasome is prevented by bortezomib, thus keeping NF-κB inactive within the cell cytoplasm.

It follows that exposing neoplastic cells to bortezomib induces a number of antitumor effects, including induction of apoptosis, interference with IL-6 production and the intracellular IL-6-mediated signalling pathway in myeloma cells, interference with synthesis and expression of pro-angiogenic factors. *In vitro* studies have also shown that bortezomib enhances the pro-apoptotic action of a number of antitumor drugs, including doxorubicin.\(^4\)

---

**Table 4. Thalidomide + chemotherapy in refractory/relapsed multiple myeloma.**

<table>
<thead>
<tr>
<th>Author</th>
<th>n</th>
<th>Regimen</th>
<th>Efficacy</th>
<th>Grade 3-4 Toxicities</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moehler 2001(^3)</td>
<td>56</td>
<td>Thal + CED(^a) <em>(Thalidomide + cyclophosphamide, etoposide, dexamethasone)</em></td>
<td>OR: 34 (68%)</td>
<td>Thalidomide: somnolence, constipation, neuropathy. Other: myelosuppression, infection, cardiotoxicity.</td>
</tr>
<tr>
<td>GARCIA-SANZ 2002(^2)</td>
<td>22(^b)</td>
<td>ThaCyDex <em>(Thalidomide + cyclophosphamide + dexamethasone)</em></td>
<td>PR: 9 (53%)</td>
<td>Thalidomide: constipation, somnolence, neuropathy. Other: neutropenia, infection.</td>
</tr>
<tr>
<td>Kropff 2002(^4)</td>
<td>60</td>
<td>HyperCDT <em>(Hyperfractionated cyclophosphamide, dexamethasone + thalidomide)</em></td>
<td>OR: 72%</td>
<td>Thalidomide: neuropathy, constipation, deep vein thrombosis. Other: neutropenia, infection, thrombocytopenia</td>
</tr>
<tr>
<td>DIMOPOULOS 2004(^5)</td>
<td>43</td>
<td>Pulsed CTD <em>(Cyclophosphamide, thalidomide + dexamethasone)</em></td>
<td>PR: 67%</td>
<td>Deep vein thrombosis (2%), neuropathy (2%)</td>
</tr>
</tbody>
</table>

OR: overall response; CR: complete response; PR: partial response; MR: minor response; MPFS: median progression-free survival; PFS: progression-free survival; OS: overall survival; EFS: event-free survival; MS: median survival; HyperCDT: hyperfractionated cyclophosphamide and dexamethasone, and thalidomide; MEFS: median event-free survival; Pulsed CTD: pulsed cyclophosphamide, thalidomide, and dexamethasone. *Fifty patients were evaluable for response; °granulocyte colony-stimulating factor was recommended; $estimated; ^seventeen patients were evaluable for response; #projected.
The largest phase II study published up to now enrolled 202 heavily pre-treated patients with myeloma on a single arm schedule of bortezomib 1.3 mg/m² twice weekly for 2 weeks followed by 1 week without treatment for up to eight cycles. All patients had progressive disease after their most recent treatment. The response rate to bortezomib was 35%, with 27% of patients reaching a complete or partial response and 7% showing a minimal response.48

On the basis of results from phase II study showing good overall response rates, including some complete responses, the U.S. Food and Drug Administration has recently approved the use of bortezomib for patients with refractory multiple myeloma in whom at least two lines of therapy have failed and who have progressive disease.

**Velcade, thalidomide and dexamethasone (VTD)**

Among the 73 patients treated by Zangari et al.49 over 50% obtained partial responses and 20% achieved either a complete response or a very good partial response. These data refer to a population of heavily pre-treated patients (including some who had been treated autologous PBSC transplant and radiotherapy). The presence of cytogenetic abnormalities does not interfere with response to therapy; however, patients with abnormal karyotypes have a worse event-free survival. VTD combination therapy is well tolerated and toxicity is reported to be mild: WHO grade 3-4 fatigue and peripheral neuropathy developed in 30 and 20% of patients, respectively.

**Appendix I**

**Refractory multiple myeloma**

Refractory multiple myeloma is defined as disease not responding to at least two courses of induction chemotherapy or responding only transiently and recurring during chemotherapy. It is characterized by one or more of the following features:
- minimal decrease (<25%) or increase in the concentration of serum and/or urinary M component;
- bone lytic lesion increase and/or development of new lytic lesions;
- development of soft tissue plasmacytomas and/or increase of pre-existing soft tissue lesions;
- development of hypercalcemia not attributable to any other co-existing condition.

**Progressive multiple myeloma**

Progressive disease in a patient in complete remission after induction chemotherapy is defined by one or more of the following features:
- reappearance of M component on electrophoresis or immunofixation;
- bone marrow plasma cell increase >5%;
- development of at least one new lytic bone lesion or definite increase in the size of residual bone lesions;
- development of a compression fracture involving a pre-existing lytic lesion is not a feature and does not indicate progression by itself;
- development of soft tissue plasmacytoma;
- development of hypercalcemia not attributable to any other co-existing condition.

Progressive disease in a patient who never achieved complete remission after induction chemotherapy is defined by one or more of the following features:
- >25% increase in serum M component compared to nadir values corresponding to an absolute increase > 500 mg/dL;
- > 25% increase in urinary light chains;
- >25% of bone marrow plasma cells, corresponding to a > 10% absolute increase of marrow cellularity;
- development of at least one new lytic bone lesion and/or of soft tissue plasmacytoma;
- definite increase of pre-existing lytic bone lesions;
- 25% increase of soft tissue plasmacytoma compared to nadir;
- development of hypercalcemia not attributable to any other co-existing condition.

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Recombinant human erythropoietin in the treatment of anemic patients with multiple myeloma

The issue of treatment of anemia in multiple myeloma has been recently examined in the preparation of the guidelines from the Italian Society of Hematology (SIE), Italian Society of Experimental Hematology (SIES) and Italian Group for Bone Marrow Transplantation (GITMO) for management of multiple myeloma and related-disorders.

Anemia in patients with multiple myeloma

Kyle and co-workers have reviewed the records of all patients in whom multiple myeloma was initially diagnosed at the Mayo Clinic in Rochester from January 1, 1985, to December 31, 1998. Of the 1027 study patients, 2% were younger than 40 years, and 38% were 70 years or older: anemia was present initially in 73% of patients. As summarized in the above guidelines, anemia reflects the course of the disease since it worsens during resistant or progressive disease, but it ameliorates when the disease is controlled by treatment.

Beguin and co-workers have shown that most myeloma patients have defective red cell production even in the absence of massive marrow infiltration and that defective endogenous erythropoietin production represent a major factor in the pathogenesis of their anemia.

Erythropoietin treatment

Barosi and co-workers have done a comprehensive systematic review of studies on the use of recombinant human erythropoietin (rHuEpo) in multiple myeloma. Various preparations of rHuEpo have been extensively been used and a total number of 630 MM patients were randomized in 8 trials examining anemia and erythropoietin use. Response rates ranged from 31% to 78% depending on the criteria for defining response. Two randomized studies reached the conclusion that 5000 UI per day is the optimal dose (e.g., from 30,000 to 40,000 UI per week). Österborg and co-workers investigated the effect of recombinant human erythropoietin (epoetin beta) on anemia, transfusion need, and quality of life (QOL) in severely anemic patients with low-grade non-Hodgkin’s lymphoma, chronic lymphocytic leukemia, or multiple myeloma. Findings of this randomized, double-blind, placebo-controlled trial showed that many severely anemic and transfusion-dependent patients with advanced multiple myeloma and a low performance status benefited from epoetin therapy, with elimination of severe anemia and transfusion need, and improvement in QOL.

Recommendations of the Italian Society of Hematology (SIE) with respect to treatment of anemia in patients with multiple myeloma

The recommendations of the Italian Society of Hematology (SIE) with respect to treatment of anemia in multiple myeloma patients are as follows:

1) MM patients with a hemoglobin level below 10 g/dL should receive rHuEpo (grade A);
2) the initial dose should not be lower than 30,000 UI/week (grade B);
3) rHuEpo should not be continued in MM patients who have not experienced an increase of hemoglobin concentration of at least 1 g/dL after 4 weeks of treatment (grade D);
4) full blood count, reticulocyte count, and iron status (serum ferritin, serum transferrin saturation) should be assessed before starting therapy and monitored during the treatment (grade D).

Once weekly erythropoietin treatment

Epoetin three times weekly (tiw) is generally used in the treatment of anemia in patients with hematological malignancies including multiple myeloma. However, our recent study has suggested that a more convenient and cost-effective once weekly (qw) regimen is equally as effective. The NOW study was an open-label, randomized,
parallel group, phase III trial, conducted at 51 centers in 12 countries. All MM patients were adults (≥18 years), had a histologically confirmed diagnosis of multiple myeloma, a Hb level of 9–11 g/dl and a serum Epo level of 100 mU/ml. Patients had a World Health Organization (WHO) performance status grade of 0–2 and a life expectancy of >6 months. If systemic anti-cancer therapy was given, it was maintained for at least 4 months following the time of first study treatment. Patients were randomized (1:1) to receive epoetin beta qw (30 000 IU per dose) or tiw (10 000 IU per dose) subcutaneously (SC) for 16 weeks. Failure to respond in the first 4 weeks (blood transfusion requirement in the previous week or a Hb increase of <0.5 g/dl) resulted in a doubling of the dose; conversely, if Hb increased by >2 g/dl, the dose was halved. Iron supplementation was permitted in patients with transferrin saturation of <20%. The primary efficacy variable was the time-adjusted Hb area under the curve from week 5–16 (Hb AUC5-16) in the per-protocol population.

A total of 161 patients with multiple myeloma were enrolled into the NOW study; of these, 158 received treatment (qw = 78; tiw = 80). Over half of these patients had advanced stage disease (Durie-Salmon stage IIIA/B = 52%). The per-protocol population comprised 140 patients (qw = 69; tiw = 71); baseline parameters were similar in both groups.

Overall, qw treatment with epoetin beta was equally as effective as tiw; the difference in Hb AUC5-16 between the qw and the tiw dosing groups was only -0.14 g/dl (Table 1). In addition, the proportions of patients with corrected anemia (Hb nadir ≥11 g/dl) during the last 4 weeks of the study were similar in the qw and tiw groups; 76% and 82%, respectively. Likewise, the percentage of patients with a Hb nadir of ≥12 g/dl were also similar in qw and tiw groups (59% and 63%, respectively).

The issue of deep vein thrombosis in MM patients receiving thalidomide

Thalidomide is increasingly used for treatment of MM patients, and an adverse effect of this treatment is deep vein thrombosis. The estimated risk of deep vein thrombosis in MM patients receiving thalidomide ranges from less than 5% to approximately 30% according to whether the drug is administered alone or in combination with anthracycline-based chemotherapy. In MM patients less than 65 years old, first-line therapy with thalidomide and desamethasone in preparation for autologous stem cell transplantation involves a risk of deep vein thrombosis of approximately 16%. Recent reports suggest an increased incidence of symptomatic venous thrombosis in cancer patients treated with recombinant human erythropoietin. Thus the combined use of thalidomide and recombinant human erythropoietin might increase the risk of thrombosis in the individual patient with MM receiving both drugs.

This issue has been recently examined by Galli and co-workers. Among 199 patients treated with thalidomide for multiple myeloma, four thromboses occurred in 49 cases during erythropoietin therapy (prevalence 8.1%, annual rate 7.25%); and another 14 events occurred in patients not on erythropoietin (prevalence 9.3%, annual rate 7.56%). The authors conclude that the administration of erythropoietin does not seem to increase the thrombotic risk of patients treated with thalidomide for multiple myeloma.

Conclusions and practical recommendations

Anemic patients with multiple myeloma can greatly benefit from erythropoietin treatment, and those with Hb levels below 10 g/dL should be given rHuEpo.

Our practice guidelines are as follows.
1) Anemic patients with multiple myeloma who have renal failure and are not receiving chemotherapy. Start with a weekly dose of about 100 U/kg; if optimal Hb is not reached after 4–6 weeks, the dose should be escalated to 200 U/kg/week;
2) Anemic patients with multiple myeloma who do not have renal failure and are not receiving chemotherapy. Start with a weekly dose of about 200 U/kg; if optimal Hb is not reached after 4–6 weeks, the dose should be escalated to 400–500 U/kg/week;
3) Anemic patients with multiple myeloma who are receiving chemotherapy. Start with a weekly dose of about 450–500 U/kg/week.

The target Hb should be from 10 to 12 g/dL without transfusion in most subjects. Young active

<table>
<thead>
<tr>
<th>Hb curve from week 5-16 (Hb AUC5-16)</th>
<th>qw (n=69)</th>
<th>tiw (n=71)</th>
<th>Difference (qw vs tiw)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mean</td>
<td>12.11</td>
<td>12.25</td>
<td>-0.14</td>
</tr>
<tr>
<td>95% CI (lower, upper)</td>
<td>11.81, 12.41</td>
<td>11.95, 12.55</td>
<td>-0.56, 0.28</td>
</tr>
</tbody>
</table>

Hb: hemoglobin; qw: once weekly; tiw: three times weekly.
patients, however, may require higher Hb levels: these should be decided on an individual basis.

Consider iron supplementation in all patients but those with parenchymal iron overload (as indicated by high serum iron and transferrin saturation): a high serum ferritin level does mean per se parenchymal iron overload, in particular in individuals with normal to low serum iron and transferrin saturation.

Anemic patients without evidence of parenchymal iron overload should be routinely given oral iron during the first 4 of rHuEpo treatment in order to improve response to rHuEpo.

Patients showing evidence of functional iron deficiency (serum iron < 60 µg/dL, transferrin saturation < 20%, hypochromic red cells > 10%) should be given IV iron supplementation. We recommend dosages of 62.5–125 mg of iron gluconate given by intravenous infusion (100 ml of saline) once weekly for 4 weeks.

References


Allogeneic transplantation: a risk-adapted tailored program

For many years allogeneic transplantation (allo-tx) has not been widely used in patients with multiple myeloma (MM) because of the relatively high median age at disease onset, lack of availability of an HLA identical sibling donor and, as reported in early studies, no survival advantage in comparison with autologous transplantation (auto-tx). Randomized and retrospective trials showed better remission and survival rates following auto-tx than with standard multi-agent chemotherapy, making auto-tx the gold standard treatment in multiple myeloma patients aged < 65 years. Unfortunately the majority of patients relapse within 3-5 years, and only a few patients, with favorable prognostic factors at diagnosis (absence of cytogenetic abnormalities, low β2microglobulin), are alive and in clinical complete remission (CR) after 7 years. By contrast, allogeneic transplantation can eradicate myeloma at a molecular level (ASO probes) in half of patients. To understand how allo-tx can modify the prognosis of patients with MM we review how clinical results are improved by reducing transplant-related mortality, better knowledge of risk factors and evidence of a graft-versus-myeloma effect which is the rationale for using reduced intensity conditioning regimens (RIC) for allo-tx also in myeloma patients.

Clinical trial of allo-transplantation in MM

Eighty MM patients were allo-transplanted after standard myeloablative conditioning with busulphan (BU) + cyclophosphamide (CY) with or without total body irradiation (TBI). The CR rate was 36%. Fifteen patients developed acute GVHD (aGVHD) grade III–IV and 23 patients developed extensive chronic GVHD. Overall survival (OS) and disease-free survival (DFS) at 4.5 years were, respectively, 24% and 20%. In another series of patients, 62% obtained a CR. Grade II–IV acute GVHD occurred in 20 of the patients, and was the cause of death in three chronic GVHD (which develops in 12 of the 21 patients at risk) also caused three deaths. The two-year OS and progression-free survival (PFS) were 47% and 40%, respectively.

The Bologna University group carried out a clinical trial to investigate toxicity and myeloma activity of BU (16 mg/Kg) plus CY (200 mg/Kg) as the conditioning regimen for allo-tx. Eight out of 19 patients (42%) achieved a CR. The incidence of grade II–IV acute GVHD and chronic GVHD was 33% and 15%, respectively. PFS and OS at 4 years were 33% and 21%.

More recently Majolino et al., in a series of 30 patients transplanted with allogeneic peripheral blood stem cells, reported a CR rate of 81% with OS and EFS at 73 months reaching 60% and 67%, respectively. Twenty-six patients experienced acute GVHD, which was grade II–IV in 15 (53%). Seventeen patients (71%) developed chronic GVHD.

The CR rate of about 50% for MM allografted patients was confirmed by the recent European Bone Marrow Transplantation Group (EBMT) update: 690 MM patients transplanted from related sibling donors have been reported to EBMT registry; 334 of the transplants were performed during the period 1983–1993 (group 1) and 356 during 1994–1998 (group 2). The CR rate remained quite stable over time and, in fact, the probability of having achieved a CR at 6 months after transplantation was 53% for group 1 and 54% for group 2, and reached 60% for both groups at 2 years.

The incidence of GVHD did not differ in the different transplantation periods. PFS was significantly better ($p$$<$$0.0001$) for patients transplanted after 1994 than for those transplanted before 1994, with the median being 19 and 7 months, respectively. The 4-year survival rate increased from 32% to 50% ($p$$<$$0.0001$) for patients transplanted before and after 1994.

In order to assess the quality of response, Corradini et al. recently performed a molecular evaluation of minimal residual disease (MRD) in patients with myeloma in CR after
transplantation. Analysis of 29 patients (15 auto-tx and 14 allo-tx) showed that molecular remissions were rarely achieved (7%) after autotransplantation (single or double); whereas a higher proportion (50%) of allografts recipients achieved molecular remission.8

The clinical impact of molecular CR was evaluated in 48 patients. Sixteen (33%) obtained durable polymerase chain reaction (PCR) negativity during a median follow-up of 36 months (range 6–120), whereas 13 (27%) remained persistently positive over a median follow-up of 23 months (range 6–47), and 19 patients showed a mixed pattern during a median follow-up of 46 months (range 4–113). The cumulative risk of relapse at 5 years was 0% for PCR-negative patients, 33% for patients with mixed PCR results and 100% for PCR-positive patients. The authors suggested that a prospective study with a larger number of patients could clarify the impact of molecular MRD monitoring and identify any clinical features predictive of durable PCR negativity.12

**Transplant-related mortality**

For many years the major problem of allotransplantation in MM was the high transplant related mortality (TRM). A retrospective study by the EBMT examined survival and freedom from progression in 189 allo-tx and auto-tx recipients. The higher TRM rate observed in the allo-tx group (41%) was not compensated by the lower relapse rate and there was no survival advantage from either of the two procedures.1 When comparing TRM among the various series, patients’ characteristics must be considered. In one study of a cohort of 80 patients the high proportion of patients with resistant disease (71%) could explain why TRM exceede 50%, whereas in two series including patients with less than 10% of plasma cell in bone marrow or a high proportion of chemosensitive disease (21 out 26 patients) the TRM rate varied from 15% to 31%. The principal cause of TRM was infection.12,13

A more recent report on allo-tx for MM in Europe demonstrated that early and late TRM rates recorded before 1994 (38% at 6 months and 46% at 2 years) were significantly higher than TRM rates recorded from 1994 onwards (21% and 30% at 6 months and 2 years, respectively). The main reason for the lower TRM appears to be fewer deaths due to infection.8 Other authors have suggested that the use of PBSC has a favorable impact on TRM (16% at 100 days and 30% overall).8 In conclusion, better and earlier selection of patients, more effective infection control and probably the use of PBSC have improved the outcome of allograft recipients, reducing TRM in these patients.

**Risk factors**

It is very difficult to define characteristics having unequivocal prognostic significance on outcome for MM patients who have undergone allo-tx. In fact there are data from series with limited numbers of patients, even though with similar characteristics (usually pilot studies or single center experiences), or from series with larger numbers of patients but with different conditioning regimens, GVHD prophylaxis, and clinical management. Furthermore the principal series on risk factors take into consideration patients allografted between 1983–1994.

We emphasize that when physicians evaluate a patient’s risk, this should not be limited to only the biological risk, but the patient’s personal priorities and expectations should also be known, understood and shared; as a matter of fact, risks considered acceptable for one patient could be excessive for another.

There is general agreement that disease status at and response to transplant influence outcome. In a series of 26 patients, all 5 patients with chemotherapy-resistant disease at the time of allo-tx died.8 Even in the Bologna experience patients with chemosensitive resistant disease had a longer survival (p=0.004) and EFS (p=0.01) than those in whom previous therapy had failed.3

In a retrospective case-matched analysis comparing auto and allo-tx, when only patients in CR were considered, there was no significant difference in OS and PFS, although the curves showed trend in favor of allo-tx with there being a shorter median relapse time in auto-tx (23 versus 56 months, p=0.02).1

Patients in CR at the time of conditioning had better survival (p=0.05), and patients who entered CR following allo-tx had a longer survival than those who engrafted but did not enter CR (p=0.001).4

A low tumor burden has a positive impact on outcome: patients in stage I at diagnosis had a better survival than those in stage II or III (p=0.05) and the risk of relapse, progression or death due to any cause was 1.9-fold greater for patients in stage III.7,14

The intensity of treatment before allo-tx also has an influence on outcome. The lower 100-day mortality (p= 0.005) and better survival (p=0.03) observed for patients allografted within 1 year from diagnosis reflects the impact of previous treatment on allo-tx. Patients who had received only one line of treatment had a better survival than those treated with three or more chemotherapy lines (p=0.02). Not only lines of chemotherapy but also number of cycles (more than eight) had an impact on relapse or progression (p=0.01). Therefore, in order to reduce TRM, allo-tx should be considered as part of the initial treatment rather than salvage therapy. Patients with a high β2-microglobulin have a worse prognosis (p=0.009).7,14 The presence of acute GVHD grade III or IV also adversely influences survival (p= 0.0006).11 The effect of gender on risk of relapse or progression remains unclear. One study concluded that female patients transplanted from male donors had a
greater risk of relapse progression \((p=0.02)\) as occurs in chronic myeloid leukemia, while data from the EBMT registry showed that female patients had a better survival than males \((p=0.04)\) and no significant differences were reported for the donor-recipient sex match.

The response rate of IgA myeloma seems to be higher than that for IgG myeloma or light-chain myeloma.\(^7,14\)

Deletions of chromosomes 13 and 11 identify a subset of patients who have a worse prognosis even following auto-tx;\(^15\) the impact of cytogenetic abnormalities on allo-tx has not been studied yet.

**Graft versus myeloma**

Early experience suggested that the lower relapse rate observed after allo-tx compared than after auto-tx was related to the absence of tumor cell contamination in the graft.\(^16\) The long-lasting (over 14 months) CR, achieved after donor lymphocyte infusion DLI) in a 40-year old woman with MM who had relapsed after an allogeneic transplant, proved the existence, *in vivo*, of a graft-versus-myeloma (GVM) effect mediated by donor lymphocytes as observed in chronic myeloid leukemia.\(^17,18\)

A multicenter report on DLI in 25 myeloma patients who had relapsed after allo-tx, confirm the existence of the GVM effect. Nevertheless in a large number of patients DLI has no response or produces only a short-lasting response.\(^19\)

The largest experience in MM and DLI concerns 95 courses of DLI 54 patients who had relapsed after allogeneic transplantation. Reinduction therapy was given to 40 of them before DLI was administered. The overall response rate was 54% (28 patients), with 19 patients (35%) achieving PR and 9 CR (17%). Three patients died from toxicity. Following DLI, the acute and chronic GVHD observed in 57% and 47% of cases were the

### Table 1. Result of allotransplants (standard dose conditioning) in MM.

<table>
<thead>
<tr>
<th>Author</th>
<th>N. of Pts. (MUD)</th>
<th>GVHD Acute</th>
<th>GVHD Chronic</th>
<th>CR</th>
<th>TRM</th>
<th>OS</th>
<th>EFS</th>
<th>Comment</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bensinger(^9) 1996</td>
<td>80</td>
<td>15 pts</td>
<td>23 pts</td>
<td>36%</td>
<td>57%</td>
<td>24%$</td>
<td>20%$</td>
<td>Transplant within 1 year of diagnosis, low β2microglobulin, stage at tx, number of chemotherapy cycles, gender result associated with outcome.</td>
</tr>
<tr>
<td>Reece(^9) 1994</td>
<td>26</td>
<td>20 pts</td>
<td>12 pts</td>
<td>50%</td>
<td>26%</td>
<td>46%*</td>
<td>40%*</td>
<td>Chemosensitive disease and early transplantation better outcome.</td>
</tr>
<tr>
<td>Cavo(^9) 1998</td>
<td>19</td>
<td>6 pts</td>
<td>11 pts</td>
<td>42%</td>
<td>0%</td>
<td>26%$§§</td>
<td>21%$(^5)</td>
<td>Chemosensitive disease had a better outcome than resistant disease.</td>
</tr>
<tr>
<td>Majolino(^10) 2003</td>
<td>30</td>
<td>26 pts</td>
<td>17 pts</td>
<td>81%</td>
<td>30%</td>
<td>60% o</td>
<td>67% o</td>
<td>aGVHD had a negative influence on TRM and OS; cGVHD positive influenced EFS.</td>
</tr>
<tr>
<td>Anderson(^11) 1993</td>
<td>13</td>
<td>6 pts</td>
<td>1 pts</td>
<td>50%</td>
<td>15%</td>
<td>64% §</td>
<td>57%(^1)</td>
<td>All pts had chemosensitive disease with less than 10% of plasma cell in bone marrow.</td>
</tr>
<tr>
<td>Russel(^11) 1997</td>
<td>13</td>
<td>NR</td>
<td>NR</td>
<td>77%</td>
<td>23%</td>
<td>69%$§§</td>
<td>NR</td>
<td>Transplant was performed in early disease, in not heavily treated patients.</td>
</tr>
<tr>
<td>Gahrton(^14) 1995</td>
<td>162</td>
<td>41 pts</td>
<td>NR</td>
<td>44%</td>
<td>NR</td>
<td>28%§&amp;</td>
<td>34%(^9)</td>
<td>Female, disease stage at diagnosis, number of treatment line, status at Tx, time from Dx to Tx, presence of aGVHD, response to Tx, associated with outcome.</td>
</tr>
<tr>
<td>Gahrton(^11) 2001 before 1994</td>
<td>334</td>
<td>46%</td>
<td>27%</td>
<td>60%</td>
<td>46%</td>
<td>32%$§§</td>
<td>NR</td>
<td>See text</td>
</tr>
<tr>
<td>Gahrton(^11) 2001 after 1994</td>
<td>356</td>
<td>40%</td>
<td>17%</td>
<td>60%</td>
<td>30%</td>
<td>50%$§§</td>
<td>NR</td>
<td></td>
</tr>
</tbody>
</table>

*MUD: matched unrelated donor; GVHD acute: only grade > II are considered; NR: not reported. *at 4.5 years, *at seven years, *at six years; *at three years; §: at two years, §§: at four years.*
strongest predictors of response; 80% of patients who developed grades II-IV acute GVHD responded to DLI, including the 20% who achieved a CR, whereas only 33% of patients with GVHD grades 0-I responded, with CR rate of 12% (p<0.0001); 73% of patients with chronic GVHD responded to DLI, including 9% with a CR, whereas only 37% of patients without chronic GVHD responded to DLI (p<0.0001). A high T cell dose (>1×10^8 T cell/kg) was also shown to correlate with response. Nevertheless, the duration of response was limited in this study: in fact median PFS and OS were 19 (range 3-116) and 23 (range 2-118) months, respectively. It has been suggested that a better long-term outcome could be achieved by administering immune-modulating drugs, such as interferon and thalidomide, after the DLI as maintenance treatment. It is interesting to note that the presence of chromosome 13 abnormalities had no influence on response or outcome of DLI.20-22

**Reduced-intensity conditioning**

The recent demonstration of engraftment and development of graft-versus-malignancy effects following reduced-intensity conditioning (RIC) regimens has extended the use of allo-tx even to older patients and to patients with co-morbid conditions that preclude high dose chemoradiotherapy.23-25 At Arkansas University, 34 high risk MM patients were transplanted (11 patients received a transplant from a matched unrelated donor) using an immunosuppressive conditioning regimen with intermediate dose melphalan (100 mg/m^2). In this series of patients even though GVHD prophylaxis with cyclosporine A was maintained for 120 days after transplantation, the incidence of severe acute GVHD and chronic GVHD resulted high (42% and 58, respectively) also because a large number of patients required DLI for incomplete chimerism (8 patients) or disease progression (14 patients). Overall, 61% of patients achieved CR or near-CR (defined as normal bone marrow, with positive immunofixation of serum or urine) with a TRM rate of 38%. The authors have published an update of this study including 45 patients, analyzing factors with an influence on outcome. DFS and OS at three years were significantly higher in patients with chemosensitive disease (DFS 64% vs 12% p=0.002 and OS 72% vs 18% p=0.03), good performance status (DFS 34% vs 7% p=0.01, and OS 47% vs 10% p=0.0004), in those who showed a response to transplantation (DFS 33% vs 0% p=0.004, and OS 46% vs 19%, p=0.06), and in patients who developed cGVHD (DFS 30% vs 0% p=0.01, and OS 42% vs 30% p=NS); acute GVHD had a little impact on survival.26,27

An immunosuppressive approach alone is not indicated for patients with MM (at least high risk patients)

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**Table 2. Result of allotransplants (reduced intensity conditioning) in MM.**

<table>
<thead>
<tr>
<th>Author</th>
<th>No. of Pts. (MUD)</th>
<th>Conditioning regimen</th>
<th>GVHD acute %</th>
<th>GVHD chronic %</th>
<th>CR %</th>
<th>TRM %</th>
<th>OS %</th>
<th>EFS %</th>
</tr>
</thead>
<tbody>
<tr>
<td>Badros27</td>
<td>45</td>
<td>L-PAM 100 mg/m^2</td>
<td>78</td>
<td>58</td>
<td>64</td>
<td>38</td>
<td>36*</td>
<td>13*</td>
</tr>
<tr>
<td>Perez-Simon28</td>
<td>29</td>
<td>Fluda 150 mg/m^2+</td>
<td>52</td>
<td>21</td>
<td>28</td>
<td>21</td>
<td>60i</td>
<td>33i</td>
</tr>
<tr>
<td>Kroger29</td>
<td>17</td>
<td>Fluda 180 mg/m^2+</td>
<td>38</td>
<td>40</td>
<td>73</td>
<td>11</td>
<td>74i</td>
<td>56i</td>
</tr>
<tr>
<td>Kroger30</td>
<td>22</td>
<td>Fluda 180 mg/m^2+</td>
<td>38</td>
<td>37</td>
<td>40</td>
<td>26</td>
<td>74i</td>
<td>53i</td>
</tr>
<tr>
<td>Maloney31</td>
<td>54</td>
<td>TBI 2Gy</td>
<td>38.5</td>
<td>46</td>
<td>57</td>
<td>15</td>
<td>78**</td>
<td>55**</td>
</tr>
<tr>
<td>Einselvide32</td>
<td>22</td>
<td>Fluda 150+CTX 40mg/kg</td>
<td>5</td>
<td>6</td>
<td>27</td>
<td>23</td>
<td>25.5i</td>
<td>22i</td>
</tr>
<tr>
<td>Giralt33</td>
<td>22</td>
<td>Fluda 120 mg/m^2</td>
<td>46</td>
<td>30</td>
<td>32</td>
<td>40</td>
<td>30i</td>
<td>19i</td>
</tr>
<tr>
<td>Peggs34</td>
<td>20</td>
<td>Fluda+L-PAM+Campath1</td>
<td>35.7</td>
<td>NR</td>
<td>2</td>
<td>NR</td>
<td>80**</td>
<td>53**</td>
</tr>
</tbody>
</table>

*: tandem transplant protocol; *: at 36 months; **: at 18 months; §: at 24 months.
because of the relatively high percentage of patients with mixed chimerism (7%) and the inadequate disease control. The impact of GVHD and chemosensitive disease on outcome was observed even when a more myelotoxic conditioning (fludarabine 150 mg/m² and melphalan 140 mg/m²) was used. OS and EFS were influenced by the presence of cGVHD (presence vs absence EFS 51% vs 0% p=0.02; OS 72% vs 42% p=0.0013) and by disease status at transplantation (chemosensitive or stable disease vs refractory or progressive disease EFS 43% vs 0%, OS 63% vs 41% p=0.013); the presence of acute GVHD had a negative impact (presence vs absence 37% vs 13%).

The combination of high dose chemotherapy and autologous transplantation to reduce tumor burden, followed by allogeneic transplantation after RIC treatment (tandem protocol) to take advantage of a graft-versus-myeloma effect, seems very interesting. A multicenter study employed 200cGy TBI as the conditioning regimen for allogeneic PBSC transplantation (HLA identical sibling in all cases) within 4 months after melphalan 200 mg/m² and autologous rescue. GVHD prophylaxis was cyclosporine A and mycophenolate mofetil. None of the 52 patients treated were hospitalized within the first 60 days; all patients engrafted. The incidence of acute GVHD (generally grade II) was 38%, and 46% of assessable patients developed chronic GVHD which required therapy. At a median follow-up of 6 months, considering patients not in CR at enrollment, 52% and 29% had obtained a CR and PR, respectively. The TRM rate was 17%. At a median follow-up of 18 months 79% of patients were alive, with a PFS of 55% at two years.

Kroger et al. used a more cytotoxic conditioning regimen (fludarabine 180 mg/m² and melphalan 100 mg/m² and antithymocyte globulin) in 17 patients who underwent a single auto-tx (tandem tx). The GVHD prophylaxis was methotrexate-cyclosporin A. Full donor chimerism was obtained between days +30 and +40 in all patients. The incidence of acute and chronic GVHD was 38% and 40%, respectively while TRM at 100 days was 11%. After a median follow up of 13 months, 12 of the 13 patients were alive without evidence of relapse or disease progression. OS and DFS at 2 years were 74% and 56%. In a subsequent study including 22 patients receiving a transplant from a matched unrelated donor, the importance of a tandem tx approach emerged as the TRM rate increased from 18% after auto-tx to 73% after allo-tx. From these preliminary experiences it seems that chronic GVHD rather than acute GVHD plays the principal role as an anti-tumor effect.

Overall acut GVHD for unmanipulated allo-tx ranges from 38.5% to 52% without any clear advantage derived from different prophylaxis schemes (cyclosporine A + methotrexate or mycophenolate mofetil or FK506). Different strategies have been used in order to reduce TRM related to acute GVHD. Antithymocyte globulin in association with an intensive RIC is able to minimize acuteGVHD and TRM rates, but has a negative effect on the prognosis of disease as OS and EFS at 2 years are only 25.5% and 22%, respectively. These data and experience from Germany seem to indicate that a profound T-cell depletion will not negatively influence engraftment, chimerism status or disease control when performed in a tandem tx protocol including RIC regimen, while less encouraging results are obtained when antithymocyte globulin is employed in other therapeutic strategies.

Peggs et al. added campath-1H to a conditioning regimen containing fludarabine and melphalan as part of front-line treatment in 17 patients with chemosensitive myeloma, none of whom had undergone a previous auto-tx. Acute GVHD developed in 35.7% of patients; of the assessable patients 4 were in PR, 1 had CR, 3 a minimal response, and 2 remained in stable disease. Ten patients received DLI (escalating doses from 1×10⁶/Kg up to 3×10⁶/Kg) for residual disease from 6 months post transplantation. After DLI, 4 out of 10 patients developed acute GVHD and a clinical response (1 patient achieved CR); 2 patients who did not experience GVHD after DLI showed a rapid disease progression. Actuarial 18-month OS and PFS were 80% and 53%, respectively. The low anti-myeloma activity and graft-versus-myeloma effect of post-campath 1H DLI were disappointing. In vitro T-cell depletion offers the advan-
tage of defining the amount of CD34, T and B cells infused in order to balance the risk of toxicity, rejection and to maintain the GVM effect. Data on this strategy, although extremely exciting, are still preliminary and inconsistent; so it is not yet known whether it is better to perform unmanipulated or T-cell-depleted RIC transplantation, or what is the best strategy for T-cell depletion.

In conclusion from these limited series it seems that:

1) RIC allo-tx are able to produce stable chimerism even in MM patients.
2) better results are achieved in patients with one of these characteristics: chemosensitive disease, previous auto-tx, development of chronic GVHD.
3) TRM and GVHD after unmanipulated RIC allo-tx are quite similar to those observed after conventional dose conditioning. Therefore RIC should be considered an extension rather than an alternative to conventional dose regimens.
4) T-cell depleted RIC, because of inadequate antitymoma activity, often requires the use of DLI as an integral part of the treatment strategy. The optimal dose, timing and eventual escalating strategy for DLI still have to be defined.

**The experience of the Bone Marrow Transplantation Unit of Niguarda Hospital**

In our Department all patients under 65 years of age are evaluated for the availability of a donor. When a donor is not available a tandem auto-Tx is scheduled.

---

**Table 3. Patients’ characteristics at allo-tx.**

<table>
<thead>
<tr>
<th>UPN</th>
<th>Sex</th>
<th>Age at Tx</th>
<th>Type</th>
<th>Stage at DX</th>
<th>Therapy pre allo-Tx</th>
<th>Status at allo-TX</th>
<th>Δt dx to Tx (months)</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>F</td>
<td>38</td>
<td>IgA/λ</td>
<td>IIIA</td>
<td>Standard dose</td>
<td>SD</td>
<td>7</td>
</tr>
<tr>
<td>53</td>
<td>F</td>
<td>42</td>
<td>IgG/κ</td>
<td>IIIA</td>
<td>Standard dose</td>
<td>PR</td>
<td>24</td>
</tr>
<tr>
<td>61</td>
<td>F</td>
<td>33</td>
<td>IgG/κ</td>
<td>IA</td>
<td>Standard dose</td>
<td>CR</td>
<td>22</td>
</tr>
<tr>
<td>76</td>
<td>M</td>
<td>34</td>
<td>IgG/κ</td>
<td>IIIA</td>
<td>Standard dose</td>
<td>PR</td>
<td>14</td>
</tr>
<tr>
<td>142</td>
<td>F</td>
<td>49</td>
<td>IgG/κ</td>
<td>IA</td>
<td>Standard dose</td>
<td>CR</td>
<td>20</td>
</tr>
<tr>
<td>166</td>
<td>M</td>
<td>39</td>
<td>κ</td>
<td>IIIB</td>
<td>Auto-Tx</td>
<td>PR</td>
<td>22</td>
</tr>
<tr>
<td>192</td>
<td>M</td>
<td>50</td>
<td>λ</td>
<td>IIIA</td>
<td>Auto-Tx</td>
<td>CR</td>
<td>40</td>
</tr>
<tr>
<td>238</td>
<td>F</td>
<td>32</td>
<td>IgA/κ</td>
<td>IA</td>
<td>Standard dose</td>
<td>PR</td>
<td>20</td>
</tr>
<tr>
<td>244</td>
<td>F</td>
<td>51</td>
<td>IgG/κ</td>
<td>IIIA</td>
<td>Standard dose</td>
<td>PR</td>
<td>12</td>
</tr>
<tr>
<td>254</td>
<td>M</td>
<td>38</td>
<td>IgG/κ</td>
<td>IIIA</td>
<td>Auto-Tx+ tal</td>
<td>PR</td>
<td>29</td>
</tr>
<tr>
<td>318</td>
<td>F</td>
<td>53</td>
<td>IgG/κ</td>
<td>IIIA</td>
<td>Standard dose</td>
<td>PR</td>
<td>10</td>
</tr>
<tr>
<td>366</td>
<td>M</td>
<td>42</td>
<td>IgA/λ</td>
<td>IIIA</td>
<td>Standard dose</td>
<td>PR</td>
<td>12</td>
</tr>
<tr>
<td>372</td>
<td>F</td>
<td>56</td>
<td>IgG/κ</td>
<td>IA</td>
<td>Auto-Tx</td>
<td>PR</td>
<td>19</td>
</tr>
<tr>
<td>416</td>
<td>M</td>
<td>64</td>
<td>IgG/κ</td>
<td>IIIA</td>
<td>Auto-Tx</td>
<td>PR</td>
<td>17</td>
</tr>
</tbody>
</table>

Δt dx to Tx: time from diagnosis to allo transplant.

**Table 4. Response to allo-tx.**

<table>
<thead>
<tr>
<th>UPN</th>
<th>Source</th>
<th>Engraftment Days</th>
<th>GVHD ac Grade</th>
<th>GVHD cr</th>
<th>Response</th>
<th>Status</th>
<th>Follow-up</th>
</tr>
</thead>
<tbody>
<tr>
<td>37</td>
<td>BM</td>
<td>23</td>
<td>–</td>
<td>–</td>
<td>SD</td>
<td>Dead</td>
<td>10</td>
</tr>
<tr>
<td>53</td>
<td>BM</td>
<td>22</td>
<td>II</td>
<td>–</td>
<td>CR</td>
<td>Alive</td>
<td>112</td>
</tr>
<tr>
<td>61</td>
<td>PBSC</td>
<td>19</td>
<td>–</td>
<td>–</td>
<td>CR</td>
<td>Alive</td>
<td>107</td>
</tr>
<tr>
<td>76</td>
<td>BM</td>
<td>25</td>
<td>II</td>
<td>EXT</td>
<td>CR</td>
<td>Alive</td>
<td>96</td>
</tr>
<tr>
<td>142</td>
<td>BM</td>
<td>18</td>
<td>–</td>
<td>–</td>
<td>CR</td>
<td>Alive</td>
<td>70</td>
</tr>
<tr>
<td>166</td>
<td>BM</td>
<td>21</td>
<td>II</td>
<td>EXT</td>
<td>CR</td>
<td>Alive</td>
<td>56</td>
</tr>
<tr>
<td>192</td>
<td>PBSC</td>
<td>23</td>
<td>II</td>
<td>–</td>
<td>CR</td>
<td>Alive</td>
<td>43</td>
</tr>
<tr>
<td>238</td>
<td>PBSC</td>
<td>19</td>
<td>–</td>
<td>–</td>
<td>CR</td>
<td>Alive</td>
<td>42</td>
</tr>
<tr>
<td>244</td>
<td>PBSC</td>
<td>15</td>
<td>II</td>
<td>–</td>
<td>PR</td>
<td>Alive</td>
<td>40</td>
</tr>
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BM: bone marrow; PBSC: peripheral blood stem cells; EXT: extended; SD: stable disease; CR: complete response; PR: partial response; follow-up months from allo-Tx; NE: non evaluable.
If a donor is available a tailored program is started; in fact, the personal priorities of a well-informed patient must be taken into consideration.

In particular we modulate the intensity of pre-transplant therapy in order to perform allo-tx in the presence of the lowest tumor burden (Figure 1).

1) patients under 55 years of age in PR or CR are offered allo-TX with standard myeloablative conditioning (related or unrelated donor).

2) patients not in CR or PR are offered an auto-tx (conditioning L-PAM 140-200 mg/m²) before proceeding to allo-tx.

3) more recently patients with advanced disease, successfully rescued by thalidomide, are also accepted for standard dose allo-tx.

4) patients over 55 years old or not eligible for standard dose conditioning are offered a RIC allo-tx.

From 1994 up to now 12 patients have undergone conventional myeloablative allo-tx and 2 patients a RIC allo-tx (11 related and 1 unrelated donor). The median time from diagnosis to allo-tx was 19.5 months (range 7–40); the source of stem cells was bone marrow in half of the patients. After a median of 19 days (range 12–25) all patients engrafted (Table 3).

Six patients (42%) experienced acute GVHD (grade II in all cases), which evolved into extensive chronic GVHD in two patients. One patient transplanted with RIC developed acute GVHD two months after the scheduled discontinuation of cyclosporine A. Of the 13 assessable patients after allo-tx we recorded 8 CR (57%), 4 PR (31%) and one case of stable disease that subsequently had progression after 8 months and died 2 months later. Only one of the 12 responding patients developed recurrent disease 20 months after the allo-tx; he is still alive after a follow up of 42 months. The patient transplanted with a graft from an unrelated donor experienced severe veno-occlusive disease after discharge, and Pneumocystis carinii pneumonia later; she is still alive in CR. After a median follow-up of 32 months (range 3–112) all patients but one are alive with a TRM of 0%, EFS of 85.7% and OS 93%; the Karnofsky score is > 90% in all patients. A Table 4 summarises characteristics at transplant and response. Figure 2 shows OS and EFS of 14 patients allografted.

**Conclusions**

In conclusion, about one third of patients who undergo allo-tx obtain prolonged DFS; for patients presenting at transplant with a low tumor burden and chemosensitive disease or those who enter CR following allo-tx the probability of cure is significantly increased. This opportunity of cure has recently also been offered to patients over 55 years or medically ineligible for standard dose conditioning regimen by using RIC allo-tx. Better results and longer survival may be obtained by optimizing the use of DLI or administering new agents after allo-tx.

All these considerations have changed allo-tx from an experimental approach to a therapeutic choice. It is a physician’s duty not only to inform patients about scientific details but also to understand the patient’s wishes. Patients, being emotionally involved in the decisions, need help to understand all aspects of the problem and to bring out all personal priorities which have some weight the decisions. This alliance between patient (and his/her family) and physician should be a guarantee for an appropriate therapeutic program tailored to each single patient. The outcome of such an alliance may be better than that resulting from automatic application of guidelines.
References


