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THE JUBILEE MEETING
ON CHRONIC MYELOID LEUKEMIA

3rd International Conference
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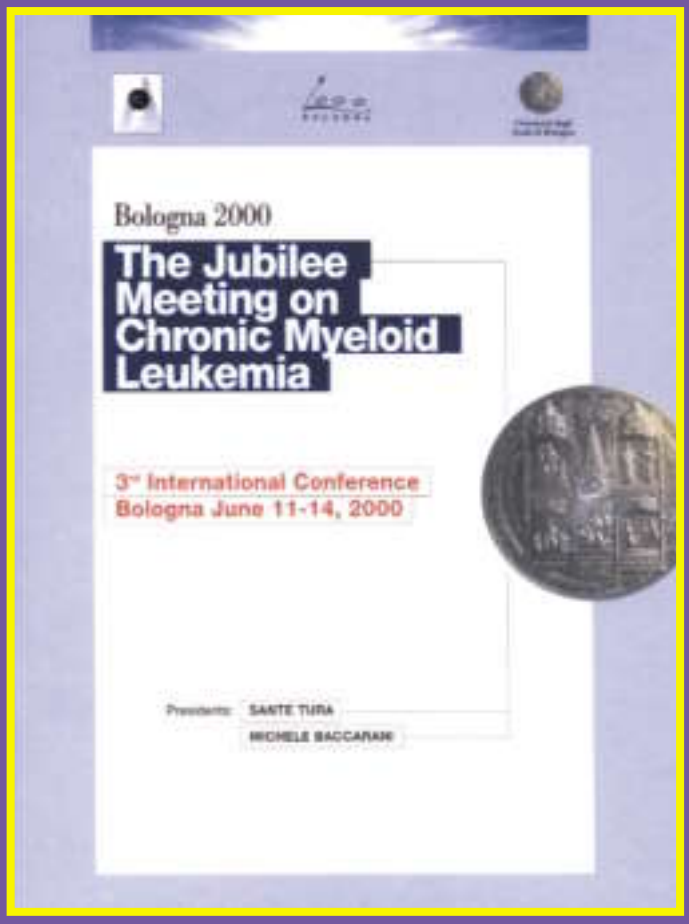

Haematologica

Journal of Hematology

founded in 1920 by Adolfo Ferrata

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Journals (standard journal article,[1,2] corporate author,[3] no author given,[4] journal supplement[5]):

1. Najfeld V, Zucker-Franklin D, Adamson J, Singer J, Troy K, Fialkow PJ. Evidence for clonal development and stem cell origin of M7 megakaryocytic leukemia. Leukemia 1988; 2:351-7.
2. Burgess AW, Begley CG, Johnson GR, et al. Purification and properties of bacterially synthesized human granulocyte-macrophage colony stimulating factor. Blood 1987; 69:43-51.
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Books and other monographs (personal authors,[6,7] chapter in a book,[8] published proceeding paper,[9] abstract book,[10] monograph in a series,[11] agency publication[12]):

6. Ferrata A, Storti E. Le malattie del sangue. 2nd ed. Milano: Vallardi; 1958.
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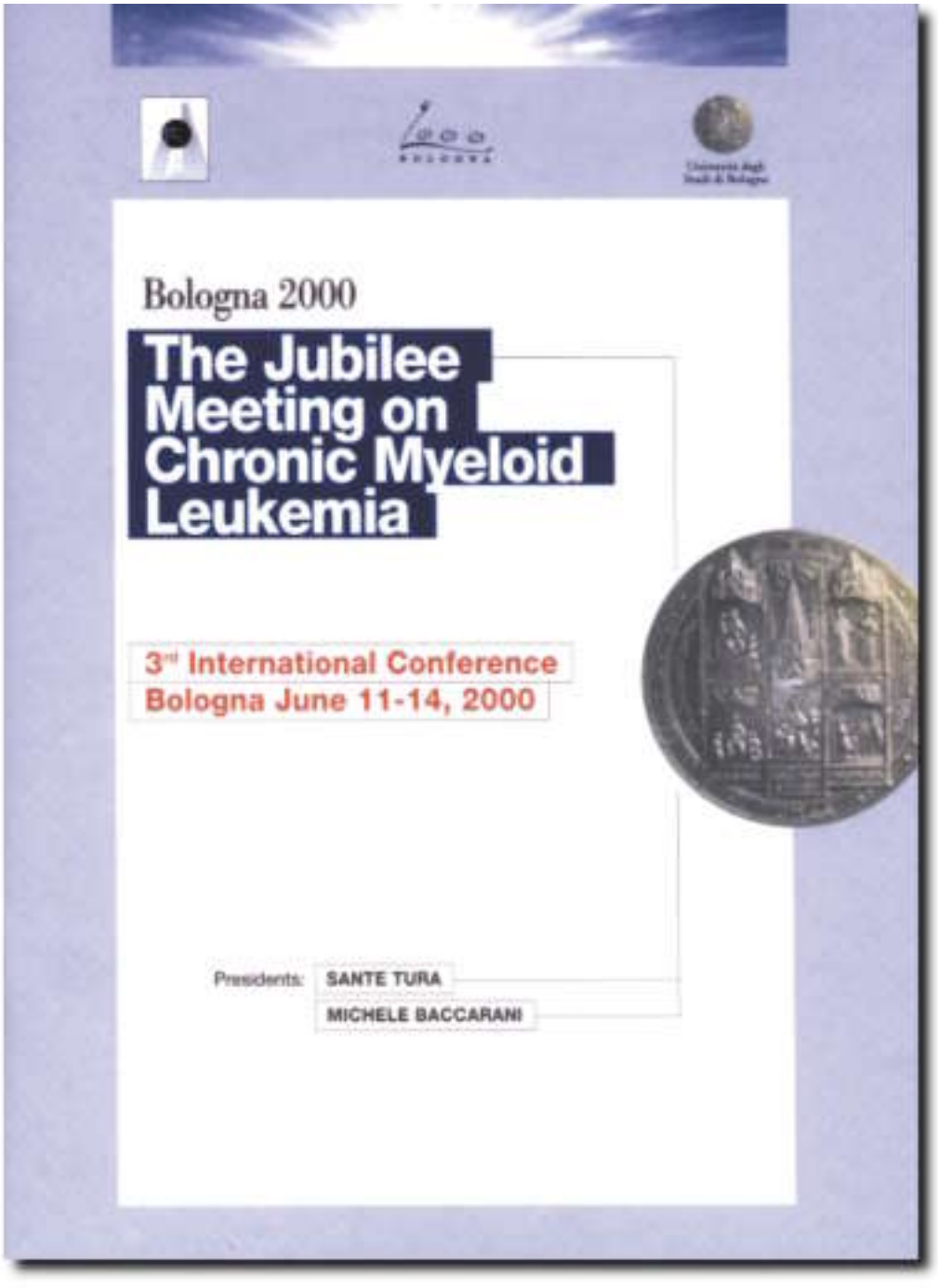

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Contents

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SELECTED PAPERS

POSTERS

MOLECULAR BASIS OF DISEASE

MECHANISMS OF TRANSFORMATION BY BCR/ABL.

Griffin JD

Dana-Farber Cancer Institute, Boston, MA, USA

The t(9;22) translocation associated with chronic myeloid leukemia (CML) fuses the c-ABL gene on chromosome 9 with the BCR gene on chromosome 22, resulting in the production of one or more of a family of chimeric oncoproteins, p190, p210, or p230 BCR/ABL. These proteins have activated ABL kinase activity and are located in the cytoplasm of CML cells, predominantly in the cytoskeleton. Recent studies have led to the identification of a large number of potential substrates for BCR/ABL, including many proteins which normally function in signal transduction pathways downstream of hematopoietic growth factor receptors. BCR/ABL is autophosphorylated on tyrosine residues and attracts a variety of adapter proteins and other signaling proteins, setting up large complexes of signaling complexes which ultimately result in growth, viability, and adhesion signals. Using new *in vitro* and animal model systems, it is now becoming possible to link specific signaling pathways to biological abnormalities of CML cells. Furthermore, the relative importance of some BCR/ABL-activated pathways is becoming clearer. *In vivo* studies in certain lines of transgenic mice suggest that the anti-apoptotic effect of Bcr/Abl is more important than previously thought. This is supported by preliminary studies in patients receiving a tyrosine kinase inhibitor, which induces rapid loss of viability of CML cells *in vivo*. There is likely to be more than one signaling pathway involved in viability signaling, and our current studies favor important roles for PI3K/Akt and for STAT molecules. As a result of these more detailed biochemical analyses of BCR/ABL function, new targets for future drug development have been identified.

PROGNOSIS

PROPOSAL FOR A STANDARD SET OF BASELINE, FOLLOW-UP, AND OUTCOME VARIABLES TO BE USED IN STUDIES ON CHRONIC MYELOID LEUKEMIA

Hasford J, Schink T, Pfirrmann M

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Currently, there is a considerable variability with regard to baseline, follow-up and outcome variables measured and recorded in different chronic myeloid leukemia (CML) trials. For example, in a data bank of about 1,400 patients of 14 studies from 11 countries, at baseline, only in 55% of cases had blasts in bone marrow been measured and recorded. Between studies, the evaluation of cytogenetic response was based on different definitions and a varying minimal number of metaphases required. These differences between the CML-trials impair comparative analyses and interpretation of trial results, meta-analyses, and identification of prognostic factors. To improve this rather unsatisfactory situation, we propose a standard set of baseline, follow-up, and outcome variables including relevant definitions which should be used in CML-trials. Comments and suggestions are welcome and will be collected so that a final consensus-proposal will be available soon.

PROGNOSTIC FACTORS FOR BONE MARROW TRANSPLANTATION

Gratwohl A

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Allogeneic hematopoetic stem cell transplant (HSCT) is the most potent antileu-kemic therapy but remains associated with high morbidity and substantial mortality. Assessment of risk is essential for making an appropriate comparison with alternative approaches. These considerations are not restricted to CML, and risk factors for outcome of transplants have been analysed in a variety of disease categories. In general, outcome is influenced by disease, subtype and disease stage at time of transplant; patient factors (age, sex, history, current status), transplant factors (donor type, sex, age; stem cell source; transplant product and cell composition), treatment and external factors. To reach a better quantitative estimate, the *Chronic Leukemia Working Party of the European Group for Blood and Marrow Transplantation* (CLWP EBMT) has analyZed data on 3,142 patients transplanted in Europe and reported to the EBMT. The published data show that transplant related mortality increases linearly with increasing score.

EBMT CML RISK SCORE

Disease stage	
chronic phase	0
accelarated phase	1
blastic phase	2
Age	
< 20 years	0
20 - 40 years	1
> 40 years	2
Donor recipient sex combination	
all other	0
female donor/male recipient	1
Histocompatibility	
HLA-identical sibling	0
unrelated matched donor	1
Time interval; diagnosis to transplant	
< 12 months	0
> 12 months	

A recent update confirms the predictive value and illustrates that a simple risk score, based on five major pre-transplant factors not influenced by the procedure, allows adequate risk assessment and provides a basis for patient counselling and decision making. Risk adapted therapy should be the future strategy.

ALLOGENEIC TRANSPLANTATION

RELATED DONOR TRANSPLANTS FOR CHRONIC MYELOGENOUS LEUKEMIA

Horowitz MM

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Allogeneic hematopoietic stem cell transplantation is effective therapy for chronic myeloid leukemia (CML), producing durable cytogenetic and molecular remissions in most patients. A survey done in the early 1990s suggested that 30-40% of patients with CML under the age of 55 years receive allografts, primarily from family donors. This study indicated that most young CML patients with an HLA-identical sibling receive a transplant. According to data reported to the IBMTR, there are about 4,000 allogeneic transplants for CML yearly, 70% of which are from related donors. The median age of transplant recipients is 36 years; 10% are < 20 years of age and 10% are older than 50 years. The strongest determinant of outcome after an HLA-identical sibling transplant is disease phase at transplant. Among 5,573 HLA-identical sibling transplants done between 1991 and 1997, registered with the IBMTR, three-year actuarial probabilities (95% confidence intervals) of survival were 64% (63-66%) for 4, 452 patients transplanted in first chronic phase, 42% (38-45%) for 847 transplanted in accelerated phase, and 20% (16-26%) for 274 transplanted in blast phase. The median interval from diagnosis to an HLA-identical sibling transplant is currently nine months. Timing of transplantation influences outcome; survival is significantly higher when transplants are done < 1 year after diagnosis. Among those receiving HLA-identical sibling transplants for chronic phase CML, 3-year survival was 68% (66-70%) for 2,837 who received their transplant within one year of diagnosis and 58% (55-60%) for 1,615 transplanted > 1 year after diagnosis. Other prognostic factors are recipient age, donor parity and prior treatment. Patients receiving busulfan for non-transplant therapy have higher risks of transplant-related mortality than those receiving hydroxyurea. Whether alpha-interferon affects transplant outcome is controversial. An IBMTR study of 209 patients receiving alpha-interferon before an HLA-identical sibling transplant suggests no adverse effect. The most common conditioning regimen used prior to HLA-identical sibling transplantation for CML is busulfan and cyclophosphamide (Cy), accounting for about 54% of transplants; Cy and total body irradiation are used for about 36%. Survival is similar with the two approaches. The high-dose therapy given for pre-transplant conditioning likely cures some persons with CML, accounting for long-term leukemia-free survival of 30-50% of patients receiving identical twin transplants. However, there is strong evidence for the importance of graft-versus-leukemia (GVL) effects in preventing CML relapse after allogeneic transplantation. Patients with graft-versus-host disease (GVHD), particularly chronic GVHD, after HLA-identical sibling transplants for CML have significantly lower risks of relapse than those without GVHD. GVL effects of allografts in CML are substantially reduced by removing T-lymphocytes from the graft. Long-term survivors of transplants for CML generally have good functional status, with 80% reporting Karnofsky scores of 90 or 100%. However, mortality remains higher than that of the general population for at least 10 years after transplantation.

MARROW VS. PERIPHERAL BLOOD STEM CELLS FROM HLA-IDENTICAL SIBLINGS OR UNRELATED DONORS

Anasetti C, Bensinger W

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A multicenter, prospective randomized study compared peripheral blood stem cells (PBSC) to bone marrow (BM) from HLA-identical siblings for patients with hematologic malignancies undergoing allogeneic transplant. The endpoints of the study were mortality, chronic graft-versus-host disease (GVHD), relapse and overall survival. A total of 172 patients with hematologic malignancies, ages 12-55 years were randomly assigned to receive BM or PBSC. Patients were stratified by age, disease status and transplant center. Allogeneic PBSC significantly improved the *tempo* of engraftment, compared to BM, without significant differences in acute or chronic GVHD. The use of PBSC was associated with reduced transplant-related mortality and relapse, primarily for patients with unfavorable diseases, and this contributed to markedly improved overall survival.

In a prospective study of unrelated donor marrow, we investigated whether a higher CD34 cell dose is associated with faster engraftment, lower transplant-related mortality and better survival after transplantation of T cell-replete marrow. A total of 121 patients were enrolled. A higher CD34 cell dose was associated with fewer episodes of delayed neutropenia after initial engraftment and with improved platelet recovery. Higher CD34 cell dose was associated with improved survival and lower non-relapse mortality accounted entirely for the difference in survival. There was no association between CD34 cell dose and the risk of acute or chronic GVHD. These studies support the model that marrow is a limiting source of hematopoietic progenitors. PBSC may be utilized to provide more hematopoietic progenitors to improve the quality of hematopoietic reconstitution and protect against transplant-related mortality. The safety of unrelated donor PBSC remains to be tested.

SURVIVAL OF PATIENTS WITH PH+CHRONIC MYELOID LEUKEMIA RELAPSING AFTER AN ALLOGENEIC STEM CELL TRANSPLANT

Arcese W, Guglielmi C

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Allogeneic blood or marrow stem cell transplantation (SCT) from an HLA-identical sibling is the treatment of choice for younger patients with chronic myeloid leukemia (CML). Using standard conditioning regimens and graft-versus-host disease (GvHD) prophylaxis, more than 50% of patients are alive and well with no sign of disease more than 10 years from transplant. Transplant-related mortality and relapse remain the major obstacles to success. Relapse occurs in about 20% of patients transplanted in first chronic phase (CP) with unmanipulated marrow cells; the risk increases to more than 50% for patients transplanted at a later stage of the disease or those transplanted in first CP with a T-cell depleted marrow. Not all patients who relapse will die as a consequence of disease recurrence. Immune modulation to achieve a graft-versus-leukemia effect, standard therapy for CML, or second allogeneic SCT have all been used with variable degrees of success. Thus some patients may regain complete remission of the disease following withdrawal of immunosuppression, donor lymphocyte infusions (DLI), treatment with α-interferon (IFN), or a second allogeneic SCT. Features of both the patient and the disease influence the efficacy of these salvage treatments.

Three consecutive studies of the *Chronic Leukemia Working Party* (CLWP) of the *European Group for Blood and Marrow Transplantation* (EBMT) showed that survival after relapse is related to risk factors.[1-3] In the first study, based mostly on patients who relapsed before 1990, survival after relapse was significantly affected by the disease phase at relapse, time from SCT to relapse and patient gender.[1] In addition, treatment with α-IFN was associated with better survival. None of such patients had been treated with donor lymphocyte infusion (DLI).

Over the last 20 years, there have been major changes in detection and treatment of relapse after an allogeneic transplant for Ph+ CML: cytogenetic techniques became available in the eighties and more sensitive molecular techniques in the nineties to detect disease before hematologic relapse; second transplants and α-IFN were already being used in the eighties, while DLI was introduced only in the nineties. Because of such changes in the approach to relapse, the CLWP re-evaluated the prognostic factors after relapse, not only by updating information on the 130 patients included in the previous study,[1] but also by adding 370 new cases with primary relapse between 1990 to 1994.[2] The actuarial survival from relapse was 34.2% (95% CI: 29.9-38.5%) at 5 years and 23.4% (95% CI: 18.9-27.9%) at 10 years. Survival after relapse was significantly related to 5 factors: time from diagnosis to transplant (< 2 years vs ≥ 2 years), disease phase at transplant (1st chronic phase vs other), disease stage at relapse (cytogenetic or chronic phase vs advanced phase), time from transplant to relapse (<1 year vs ≥1 year) and donor type (HLA-identical sibling vs volunteer unrelated donor [VUD]). The effects of individual adverse risk factors were cumulative: the probability of survival at 10 years decreased stepwise from 42% (0 factors), 32% (1 factor), 14% (2 factors), 3% (3 factors) to 0% (4 or 5 factors).[2] The number of patients relapsing after a VUD transplant was rather limited in this cohort: their prognostic features are worse compared to patients relapsing after transplants from an HLA identical sibling donor. For this reason, the multivariate analysis was also performed after exclusion of the patients relapsing after a VUD transplant.[2] This analysis showed all 4 remaining variables being significant with similar hazard rates and confidence intervals.[2] Moreover, patients at different risk may be easily identified by the cumulative number of adverse features at relapse in homogeneous treatment groups, suggesting that novel treatment strategies should be found to treat patients with 2 or more adverse features at relapse.[2] This group represented more than one third of the cases and less than 10% of them were alive at 10 years.[2] These prognostic factors should be reported in studies of salvage therapy of relapse after an allogeneic transplantation for Ph+CML.

In a third study, the CLWP aimed at showing a date effect on survival after relapse as a consequence of the major changes over the last few decades in detection and treatment of relapse after allogeneic bone marrow transplant for Ph+ chronic myeloid leukemia.[3] Data from more than 1,000 patients who relapsed after receiving a transplant of marrow cells from an HLA-identical sibling (86%) or VUD (14%) in the last 21 years at 148 EBMT centers were analyzed.[3] Univariate analysis showed a significant increase of survival with the year of relapse (p=0.01) suggesting an improvement in the management of such patients.[3] Throughout the period of recruitment survival was also related to other prognostic factors which were included in a Cox model together with the year of relapse. Multivariate analysis showed a 3% average decrease per year in the speed of dying after relapse. This study indicates that a significant improvement in survival has been reached for such patients. Prognostic factors are essential for stratification of patients entering prospective randomized trials comparing different salvage treatment modalities such as those started by the CLWP of the EBMT for molecular relapse, cytogenetic relapse and hematological relapse, respectively. Further progress may be foreseen if we improve the efficacy of salvage therapy of patients relapsing after allogeneic SCT for Ph+CML.

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AUTOLOGOUS TRANSPLANTATION

NEWS AND PERSPECTIVES FROM ROME

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Our experience of autografting in chronic myelogenous leukemia (CML) started in 1982, in patients in blastic phase. This first approach was soon abandoned due to the discouraging results, relapsing all patients before 6 months from the transplant. In 1988 we started to autograft patients in first chronic phase (CP). We treated a total of 63 cases, with an overall survival projected at more than 50% at 10 years (minimum follow up of 6 years for the last case treated). We will report results on 50 patients treated according to 3 different studies, performed between 1988 and 1993.

In the first study we treated a total of 26 patients. Autograft was performed with peripheral blood stem cells collected within 1 year from diagnosis without any mobilization procedure. All patients received a cytoreductive treatment with hydroxiurea and, subsequently, busulphan (4 mg/kg/die for 4 days p.o.) and melphalan (60 mg/sqm i.v. on the 5th day) (Bu-Mel) followed by unmanipulated stem cells reinfusion ($1x10^9$/kg mononucleated cells) after 24 hours of rest. α-IFN was given after a median of 6.5 months from transplant to all patients in continuous CP, at escalating doses starting from $0.5x10^6$ IU, 3 times a week, on the basis of the clinical and hematological tolerance. The 10-year projected probability of survival from diagnosis for this group of patients is 55%, with a median follow up of 9.5 years (ranging from 8 to 10.5) for the surviving cases.

A second group of 12 patients received the autograft after α-IFN treatment. All patients were in complete hematological response and have achieved a various degree of cytogenetic improvement. Bone marrow was harvested after a median interval of 5 months (range 2-8) from α-IFN discontinuation, at the time of peripheral blood and bone marrow reconstitution according to the result of CFU-GM assay. The same above reported conditioning regimen was utilized. All patients restarted α-IFN treatment at the time of complete hematological reconstitution. Eight patients died after a median time of 66 months from diagnosis, and 4 are alive after more then 14 years from diagnosis.

In the third group, autograft was performed in the framework of a national prospective trial promoted by the *Italian Cooperative Study Group on CML*. Untreated CML patients were assigned to receive α-IFN for at least one year. If they achieved a cytogenetic response consisting in a percentage of Ph negative metaphases of more than 25%, they were eligible for marrow harvesting and subsequent autografting after Bu-Mel regimen. We autografted a total of 12 cases. To now, 9 are alive after a median time of 9 years (ranging from 6 to 10) from transplant.

Our data confirm that autograft is feasible in CML in chronic phase, either early after diagnosis or after α-IFN treatment, and that α-IFN may be administered after the autograft without major toxicity. Clinical results obtained in the first group of cases are promising, and should be confirmed in a prospective randomized study to evaluate the role of autograft in treatment strategy for CML.

INTERFERON I

INTERFERON α-BASED DRUG TREATMENT VERSUS ALLOGRAFTING

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Mortality and morbidity associated with allogeneic bone marrow transplantation (BMT) pose a considerable problem to patients and their doctors in the search for the best treatment for chronic meyloid leukemia (CML). If there was not the aspect of cure by BMT, the decision for treatment with interferon-α (IFN) would be easy. Meanwhile, however, results with interferon alpha based drug treatment have improved considerably, and ten year survival of IFN-treated low-risk patients ranges around 40% and is even higher in cytogenetic IFN responders. In order to advise patients on the prospects of survival after the two treatment alternatives – BMT or IFN – a comparative quantification with regard to survival after various time intervals is useful. Two retrospective analyses have shown that survival is better after drug treatment during the first 3-4 years and better after BMT later on. The survival curves after drug treatment and after BMT cross after 4-6 years depending on the risk profile of drug-treated patients and on the time after diagnosis when a BMT was carried out. Because of the importance of this question, the German CML Study Group started a randomized study comparing outcomes after BMT and after IFN-based drug treatment 5 years ago. Drug treatment could also comprise intensive or high dose chemotherapy. For this study, the instrument of genetic randomization was used. After definition of the baseline sample by suitability for, and consent to BMT, the availability of a related donor was used for randomization. Analysis was according to the intention-to-treat principle. By May 2000, 1,010 patients had been recruit-

ed of whom 556 were allocated to the baseline sample. At the first evaluation of the study 5 years after its start, 367 patients had been randomized and were evaluable, i.e. had documentation from at least one follow-up. The results of this first evaluation indicate that, with regards to survival, drug treatment is better than BMT during the first 3-4 years, but that the superiority of drug treatment decreases starting in years 4-5. The advantage of drug treatment is more pronounced in low-risk patients. The study plan provides a second analysis seven years after the start of the study and the final analysis 2 years later. It is expected that the results of the study will provide a reliable basis for the estimation of survival after BMT vs. IFN-based drug treatment.

COMPLETE CYTOGENETIC RESPONDERS AND LONG TERM SURVIVORS

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The duration of survival of a CML patient has improved substantially in the last 20 years due to the application of allogeneic BMT (alloBMT), either from HLA identical sibling or unrelated volunteer donor and to the widespread treatment with interferon (IFN) based regimens. Among IFN treated patients those who obtain a cytogenetic response and, particularly, a complete cytogenetic response (CCR) are expected to survive longer than the others. However, the survival duration of these CCR is not yet defined and it is not clear if and when they will die of leukemia, or if some of them can be ever defined clinically cured. More information on more of these patients with CCR is required, but the overall probability of obtaining a CCR in published series ranges around 10% and consequently each serie includes a relatively small number of complete responders. A collaborative effort of the EICML allowed the creation of a database containing information on a large serie of 514 patients with CCRs. Eight national databases and single institutions provided demographic and hematologic information on the study cases, including an analytical survey of the IFN-based regimen before CCR, time to response and hematologic evolution. We report here on 317 cases of CCR who achieved CCR with IFN alone and were never submitted to any other procedure such as allo or auto BMT.

Clinical characteristics and risk profile

The diagnosis was made between 1983 and 1997 (roughly 50% before and 50% after 1993). The median age at diagnosis was 47±13 years, 180 (57%) were males and 137 (43%) females. As far as concerns risk distribution for Sokal score 179/317 (56.5 %) were in low Sokal risk at diagnosis while 111 (35%) intermediate + high risk (27 patients or 8.5% were not evaluable). For Euro score, 164/317 (52%) were low risk, and 117 (37%) were intermediate plus high risk; (36 patients or 11% were not evaluable). The whole patient population, looking at the distribution among risk categories, includes, not unexpectedly, patients with an overall better prognostic profile with respect to unselected series, in which the percentage of low risk patients is lower by 10%. The relatively high number of non low risk patients obtaining CCR is, however, noteworthy.

Time to response

The average dose of IFN/week from diagnosis to first demonstration of a CCR, calculated on 235/317 patients evaluable (74%), was 37.62±18.95 IMU, for an average daily dose of 5.3 IMU, while the mean minimum weekly dose and the mean maximum weekly dose were 24.4 and 46.6 IMU (or 3.4 and 6.6 mean daily doses), respectively. As far as time to reach the CCR, median time to "any cytogenetic response" was 7 months, to major cytogenetic response was 11 months and 19 months to CCR. No difference by Sokal and Euro risk in the times to reach the aforementioned cytogenetic responses was noted. Time to CCR was significantly shorter for patients treated with IFN doses higher than 35 IMU/weekly: in fact the actuarial median time to first CCR was 25 months (95% confidence intervals 21-29 months) for patients treated with a mean weekly dose below 35 IMU and 18 months (95% confidence interval 15-21 months) for patients treated with a mean weekly dose above 35 IMU.

CCR and survival

The median observation after the first dose of IFN is now 66 months (extremes 11-171 months): as already mentioned, half of the patients were diagnosed before 1993. The 10 years overall survival probability, calculated from the first dose of IFN to death or last contact, is 75% (95% confidence intervals 66-84%). After more than 10 years, the number of cases at risk is 34 and none has suffered of any adverse event as yet.

After stratification for Sokal score, the survival curves for low, intermediate and high risk patients are significantly different: to date, 7/179 (4%) low risk patients have died as compared with 9/76 (12%) intermediate risk and 10/35 (29%) high risk. The 10-year survival probability of a low Sokal risk CCR is 89% (95% confidence intervals 79-98%); that of an intermediate Sokal risk is 74% (95% confidence intervals 55-94%) and finally that of a high risk CCR is 42% (95% confidence intervals 15-68%). The overall *p* for comparison among the 3 Sokal risk categories is 0.00001. For Euro score, the 10-year survival of a low risk patient is 82% (95% confidence intervals 69-94%), that of an intermediate risk one is 82% (95% confidence intervals 71-94%) and that of a high risk 28% (95% confidence intervals 0-61%)(overall $p = 0.0001$). The same considerations and the same, statistically significant, differences of survival probability are valid measuring the survival after CCR (that is,

time from the first CCR to death or last contact).

The analysis of the survival probability of this large serie of patients with CCRs by Sokal and Euro risk allows some considerations: 1) disease categorization by risk maintains its strong predictive value even among this highly selected population and 2) the long-term survival of a low and even intermediate risk patient in CCR is very high. It is not possible to define these patients as being *cured* since molecular biology studies allow detection of signs of MRD in all cases but their fate is, however, dramatically different; 3) the survival probability of a high risk patient in CCR is significantly worse with respect to that of a low or intermediate risk CCR patient, however, 4) the long-term survival of a high risk patient in CCR is far longer with respect to a high risk patient not responsive or responsive to a lessere degree to IFN: the median survival probability of a high risk/CCR patient is around 102 months (95% confidence interval 74-130) whereas at this time, virtually all not responsive/lower degree responsive patients have already progressed to accelerated/blastic phase (AB/P) or died.

Evolution of patients losing the CCR

To date, 105/317 (33%) patients have lost their CCR and the overall median duration of CCR is 88 months. The risk of losing CCR is fairly constant during the first 48 months, after that it apparently declines. The risk of losing CCR correlates with the Sokal risk: in fact 53/178 low Sokal risk, 24/76 intermediate risk and 16/35 high risk patients lost the CCR. The overall *p* value is 0.0175. The clinical and hematologic evolution of the 105 patients losing CCR do not deteriorate rapidly: in fact, the median probability of survival after having lost CCR is still 72 months and the analysis of their status reveals that 36/105 (34%) are in major/partial CR, 20 (19%) are in complete hematologic response and 10 (9%) lost the hematological response and are in florid chronic phase.

IFN discontinuation

To date, information on treatment continuation or discontinuation is available in 292 patients of whom 215 (73%) are continuing IFN, 2 patients discontinued the treatment before CCR while 75 (27%) discontinued IFN after having obtained CCR. Twenty-three out of 75 (31%) discontinued IFN because it was no longer effective (disease progression to AB/P) while the remaining 52 (69%) abandoned IFN during CCR because of toxicity (27 patients) or other reasons (25 patients). The fate of the 52 patients who discontinued IFN after CCR not for progression to AB/P is of particular interest: 28/52 (54%) of them lost their CCR while 24 (45%) are still in CCR. The median duration of CCr after IFN discontinuation is actually 23 months (extremes 1-91 months). This suggests that a substantial proportion of CCR even maintain CCR also if IFN is discontinued for a long time.

In conclusion, although the preliminary analysis of this clinical database cannot provide an answer to the question of a cure, the data show quite clearly that most low risk patients who achieve a CCR become very long survivors, while most high risk patients who achieve a CCR eventually relapse and die, suggesting that the concept of a clinical cure with IFN is restricted to low risk patients. It should not be overlooked that low risk patients are about 50% of all cases and provide the majority of complete cytogenetic responders. A biological evaluation of these cases is required to understand the molecular basis of their disease and of their excellent response to IFN.

INTERFERON-α IN CHRONIC MYELOID LEUKEMIA. DOSE AND DURATION OF TREATMENT

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The dose of interferon-α (IFN) chosen by Talpaz, 5 MU/m^2×7d/week, was shown to induce complete hematologic remissions and complete cytotogenetic responses (CCR) at rates of 70-80% and 25% respectively, with variations according to risk categories. Polymerase chain reaction (PCR) demonstrates complete molecular remissions to occur in some cases. Survival prolongation is significant and related to prognosis score and degree of cytogenetic response. Similar results have been achieved in several large multicenter randomized studies The association of cytosine-arabinoside or homoharringtonine seems beneficial. However, because of the cost and toxicity of this 35 MU/m^2/week regimen, several groups used lower doses of IFN. In a series of 27 patients of unknown prognostic scores receiving 2 MU/m^2/×3 d/w, i.e. 6 times less IFN/w, 7% achieved CCR with a possible survival lenghthening (*Schofield, Robinson et al.*) and CCR rate was 9% in Benelux patients on 3 MU/d × 5d/w. Comparing the benefits of high doses and the expenses and toxicity they cause is difficult. Ongoing randomized protocols comparing high and low IFN doses may provide us with useful data and further evaluation of intermediate doses is warranted. Meanwhile, as pharmacokinetic data are scarce, one may wonder whether studies of serum concentrations [c] might be of value and, aside from intrinsic cell sensitivity, explain in part different individual responses to IFN. It is to be noted that in vitro experiments are often carried out at 500-1,000 U/mL IFN [c], far more than the mean and median <100 U/mL serum[c] obtained for a few hours in patients receiving 9 MU. As regards duration of treatment, difficult decisions have often to be made in cases of responses deemed insufficient, as well as in responding patients. From the MRC trial, it appears that IFN may be of benefit even in the absence of cytogenetic response. CCR may require up to 6 years to be achieved. Finally, it is generally recommended that IFN is continue until loss of cytogenetic response or for 2-3 more years in complete cytogenetic responders. The analysis of >300 cases of CCR collected by the European Group of Investigators on IFN in CML may help to solve some of the problems.

INTERFERON II

PREDICTIVE VALUE OF QUANTITATIVE REAL-TIME EVALUATION OF MOLECULAR RESPONSE IN CML PATIENTS TREATED WITH αIFN

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A substantial minority of patients with chronic myeloid leukemia (CML) achieve a complete cytogenetic response (CCR) to treatment with interferon-α (IFN), defined as the disappearance of Philadelphia chromo-some-positive metaphases. We used a competitive RT-PCR and Real-Time TaqMan to quantify BCR-ABL transcripts in 323 bone marrow and peripheral blood specimens collected from 44 patients who had achieved CCR with IFN. The median duration of observation was 2.4 yr. (range 1.2-12.2). Total ABL, GAPDH, β_2-microglobulin transcripts were quantified as internal control and expressed as BCR-ABL transcripts/*RNA and as BCR-ABL/ABL, BCR-ABL/GAPDH, BCR-ABL/β_2-microglobulin ratios. All 44 patients had evidence of residual disease. The actual level of minimal residual disease correlates with the probability of cytogenetic response. The median number BCR-ABL/mg RNA at the time of maximal response for each patients was 4 (range 3-4,600) and was significantly lower in patients who remained in CCR than in those who had a major karyotypic response (4,490 versus 4, p<0.0001). Our findings show that the level of residual disease falls with time in complete responders to α-IFN. We used the same analysis on 118 samples (17 bone marrow, and 101 peripheral blood) of 18 CML patients in accelerated or blastic phase, who received tyrosine kinase inhibitor therapy (STI 571). We found that BCR-ABL transcript levels fell with time in STI 571 responders.

Our competitive RT-PCR and Real-Time TaqMan assays both proved reliable and sensitive methods for monitoring CML patients receiving different types of treatment, and in particular predicting α-IFN.

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CHARACTERISTICS OF REAL-TIME AND COMPETITIVE RT-PCR QUANTIFICATION OF BCR-ABL TRANSCRIPTS IN CML PATIENTS

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Some chronic myeloid leukemia (CML) patients can achieve a complete cytogenetic response (CCR), defined as the disappearance of Philadelphia chromo-some-positive metaphases, which is the clinical goal of treatment with interferon-α (IFN). We used both competitive RT-PCR and Real-Time TaqMan to quantify BCR-ABL transcripts in 323 bone marrow and periph-eral blood specimens collected from 84 CML patients (40 at diagnosis and 44 after achieving CCR with IFN) (median age 47.5 yr, range 18-65; median Sokal score 0.9, range 0.53-2.78). Total ABL, GAPDH, β_2-microglobulin transcripts were quantified, as internal con-trols and expressed as BCR-ABL transcript/mRNA and as BCR-ABL/ABL, BCR-ABL/GAPDH and BCR-ABL/β_2-microglobulin ratios. All 44 CCR patients had evidence of residual disease. Wide variations in the amount of BCR-ABL transcript were found at diagnosis, ranging from 17,300 to 750,000 with competitive RT-PCR and 30,900 to 398,000 with Real-Time TaqMan (median values 78,000 and 102,000, respectively). Median value of BCR-ABL/ABL was 8.86 while BCR-ABL/β_2-microglobulin ratio was 0.10576 (β_2-microglobulin being the most stable internal standard RNA control gene). Amount of BCR-ABL transcript at diagnosis was associated with the number of blast cells and Sokal's score. The median BCR-ABL/mRNA at the time of maximal α-IFN response with Real-Time TaqMan was 4 (range, 3-4,600) and was significantly lower in patients who remained in CCR than in those who had a major karyotypic response (4,490 versus 4, p<0.0001). Our findings show that the level of residual disease falls with time in complete respond-ers to α-IFN. Our competitive RT-PCR and Real-Time TaqMan assay both provide highly sensitive and reliable methods for monitoring CML patients and predicting α-IFN therapy response. Our results confirm the greater resolution and enhanced sensitivity of Real-Time TaqMan analysis for the easy detection and quantification of BCR-ABL.

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DETECTION AND QUANTIFICATION OF RESIDUAL DISEASE IN CHRONIC MYELOGENOUS LEUKEMIA

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The degree of tumor load reduction after therapy is an important prognostic factor for patients with chronic myeloid leukemia (CML). Conventional metaphase analysis has been considered to be the *gold standard* for evaluating patient response to treatment but this technique normally requires bone mar-

row aspiration and is therefore invasive. The frequency of cytogenetic analyses can be considerably reduced if patients are also monitored by molecular methods, which can be performed on peripheral blood specimens. Of the various techniques available, most attention has been paid to reverse transcription polymerase chain reaction (RT-PCR) for BCR-ABL mRNA since this is by far the most sensitive. Simple, non-quantitative RT-PCR analysis gives only limited information on patients after treatment. Quantitative RT-PCR assays have been developed to monitor the kinetics of residual BCR-ABL transcripts over time. Variables in the quantitative PCR assay may be controlled for by quantification of transcripts of a normal gene (e.g. ABL or glucose-6-phosphate dehydrogenase, G6PD) as an internal standard. After allogeneic stem cell transplantation, most patients become RT-PCR negative, often after a period of low level positivity that may persist for several months. Those patients destined to relapse are characterized by the reappearance and/or rising levels of BCR-ABL transcripts. In contrast, in patients treated with interferon-α (IFN) residual disease is rarely, if ever, eliminated. The actual level of minimal residual disease in complete cytogenetic responders to IFN correlates with the probability of relapse. The level of residual disease falls in time in patients who maintain their cytogenetic response to IFN.

New real time procedures promise to simplify the protocols that have been used. We established a rapid and reliable RT-PCR approach using LightCycler technology. This device combines rapid thermocycling with online detection of PCR product formation and is based on the fluorescence resonance energy transfer between two adjacent hybridization probes carrying donor and acceptor fluorophores. A pair of probes was designed that was complementary to ABL exon 3, thus enabling detection of all known BCR-ABL variants and also normal ABL as an internal control. Conditions were established to amplify less than 10 target molecules/reaction and to detect one CML cell in 10^5 cells from healthy donors. To determine the utility of the assay, we quantified BCR-ABL and ABL transcripts in 254 samples from 120 patients with CML after therapy with IFN-α (n=219), allogeneic bone marrow transplantation (n=17), chemotherapy (n=11), or at diagnosis (n=7). A highly significant correlation was seen between the BCR-ABL/ABL ratios determined by the LightCycler and (i) the BCR-ABL/ABL ratios obtained by nested competitive RT-PCR (n=201, r=0.90, $p<0.0001$); (ii) the proportion of Philadelphia chromosome positive metaphases determined by cytogenetics (n=81, $p<0.0001$), and (iii) the BCR ratio determined by Southern blot analysis (n=122, $p<0.0001$). Standardization and the introduction of rigorous, internationally accepted controls are required to enable RT-PCR to become a robust and routine basis for therapeutic decisions.

MOLECULAR BASIS OF PROGRESSION

BCR AND BCR-ABL INTERACTION: BCR FIRST EXON SEQUENCES BLOCK GROWTH AND SURVIVAL OF BCR-ABL POSITIVE LEUKEMIA CELLS

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Our studies have demonstrated mutual cross regulation of the Bcr-Abl tyrosine kinase and Bcr serine/threonine kinase. Phosphorylation of tyrosines 328 and 360 of Bcr by Bcr-Abl is responsible for Bcr Ser/Thr kinase inhibition (*Wu et al., Oncogene 1998; 16:141; Liu et al., Mol Cell Biol 1996; 16:998*). However Bcr, when not phosphorylated on tyrosine residues, inhibits the Bcr-Abl tyrosine kinase (*Wu et al., Oncogene 18:4416, 1999*). Our hypothesis is that tyrosine phosphorylation of Bcr neutralizes Bcr's inhibitory activity towards Abl by preventing the autophosphorylation of Bcr on Ser residues. Bcr(64-413) is encoded by the first exon of BCR but lacks the oligomerization domain and yet retains Bcr's Ser/Thr protein kinase activity. It also contains the Ser-rich A and B boxes involved in Abl SH2 binding. We have shown that a mutant form of Bcr [Bcr(64-413)] is resistant to tyrosine phosphorylation, and Bcr(64-413) inhibits Bcr-Abl's tyrosine kinase activity and its oncogenic activity in hematopoietic cells (*Liu et al., Cancer Res 1996; 56:5120*). We now show that an adenovirus encoding Bcr(64-413) (AdDBcr) inhibited the growth and survival (by inducing apoptosis) of Bcr-Abl expressing hematopoietic cells (both primary cultures from active disease patients with chronic myeloid leukemia (CML) and CML cell lines) but not hematopoietic cells lacking Bcr-Abl expression. Normal marrow cultures were not affected by AdDBcr infection. Moreover, AdDBcr, but not the control adenovirus, induced cell-cell clumping in CML cells, which did not occur in normal marrow cultures or a Bcr-Abl negative pre-B cell line. We conclude that Bcr(64-413) expression actively inhibits cell growth and survival of CML patients' blood cells but has no detectable effects on normal hematopoietic cells.

Studies on the mechanism of Bcr's inhibition of Bcr-Abl are consistent with a hypothesis which proposes that the phosphoserine form of Bcr inhibits the Bcr-Abl tyrosine kinase by binding to the Abl SH2 domain. Our published studies showed that a phosphoserine 354 Bcr peptide (350-SSRVpSPSPTTYRMFRDK-366) inhibited Bcr-Abl and c-Abl kinase *in vitro* but did not inhibit the Src kinase (*Liu et al., ibid*). Further studies have shown that the S354E and S354D forms of this peptide were more inhibitory than the phosphoserine 354 peptides. These peptides strongly inhibited a commercial SH2SH1 Abl kinase lacking the SH3 domain, but these peptides were also strongly phosphorylated *in vitro* because they contain one of the targets of Bcr-Abl (Y360). To eliminate competition between the added protein substrate (casein or Crk sequence) and these inhibitory pep-

tides, we tested Bcr peptides lacking tyrosine 360, namely 346-SSGQSSRVpSPSPTT-359 (pSer 354 S14T). As expected, pSer 354 S14T was not tyrosine phosphorylated *in vitro* by the Abl kinase, but yet maintained its ability to inhibit phosphorylation of added substrates in these assays. Of interest, S354E S14T (IC50, 55 mM) had higher inhibitory activity whereas S354A S14T had weaker activity on a molar basis. In other studies we in collaboration with others have begun to measure the binding affinity of these peptides to the Abl SH2 domain. Given the published studies by Pendergast *et al.* (*Cell 66:161, 1991*), our findings are strongly suggestive that phosphoserine Bcr inhibits the Bcr-Abl kinase by high affinity binding to the SH2 domain of Bcr-Abl.

METHYLATION OF THE ABL1 PROMOTER IN PH-POSITIVE LEUKEMIAS

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We previously reported that the ABL1 promoter (Pa) undergoes *de novo* methylation in the course of chronic myeloid leukemia (CML). The methylation status of the ABL1 promoter was monitored by polymerase chain reaction (PCR) amplification of the Pa region, following digestion with several methylation-sensitive restriction enzymes. Some 74% of the DNA samples from blood and marrow drawn in the chronic phase were unmethylated, similar to the percentage in control samples from non-CML patients. The remaining 26% were partially methylated in the ABL1 Pa region. Methylated samples were mostly derived from patients clinically indistinguishable from others but known to have a disease of longer duration (26 months versus 7.5 months, p=0.01). Samples of 30 interferon-α (IFNα)-treated patients were sequentially analyzed in the course of treatment. Fifteen patients with no evidence of Pa methylation prior to treatment remained methylation-free. The remainder, who displayed Pa methylation before treatment, reverted to the methylation-free status. The outcome is attributed to IFNα therapy as the Pa methylation status was not reversed in any of the patients treated with hydroxyurea. More recently we have adapted the techniques of methylation-specific PCR and bisulfite sequencing to study the regulatory regions of ABL1 and other genes with a role in DNA repair or genotoxic stress response. In cell lines established from CML blast crisis which only carry a single ABL1 allele nested within the BCR-ABL fusion gene, only methylated ABL1 promoters were detected. By contrast, in clinical samples from patients in advanced stages of the disease both methylated and unmethylated alleles were detectable. To distinguish between allele-specific methylation and a mixed cell population pattern, we studied the methylation status of colonies derived from single hematopoietic progenitors. Our results show that both methylated and unmethylated alleles co-exist in the same colony. Furthermore, ABL1 methylation was detectable in the vast majority of colonies from blast crisis, and only in a small proportion of colonies derived from patients in the chronic phase. Topologically, in both cell lines and clinical samples from acute phase CML uniform hypermethylation of the promoter region was noted. Moreover, we showed that ABL1 methylation does not reflect a generalized process and may be unique among DNA repair/ genotoxic stress response genes. Our data suggest that specific methylation of the Ph-associated ABL1 allele accompanies clonal evolution in CML. We asked ourselves whether methylation of the ABL1 promoter is an epigenetic modification also associated with Ph-positive acute lymphoblastic leukemia (ALL) and whether it has any clinical significance. The methylation status of the ABL1 promoter in 18 Ph-positive ALL samples was studied. We found that gene-specific ABL1 promoter methylation is associated mainly with the P210 form of BCR-ABL and not the P190 form. While 6 out of the 7 P210-positive ALL samples had ABL1 promoter methylation, all 11 P190-positive ALL samples were methylation-free. In addition, we estimated the extent and relative abundance of ABL1 promoter methylation in several Ph-positive ALL samples and compared them to the methylation pattern in chronic and acute phase CML samples. We put forth a model that correlates the different types of leukemias with the different levels of ABL1 promoter methylation.

IN VIVO MODELS

STUDYING CELL SIGNALLING IN CML THROUGH MOUSE MODELS

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CML can be modeled in mice using retroviral transduction of the BCR/ABL gene into bone marrow, followed by transplantation into irradiated syngeneic recipients. When donors are pretreated with 5-fluorouracil, recipients of BCR/ABL-transduced marrow uniformly develop a fatal myeloproliferative illness that closely resembles the chronic phase of human CML. Mice with CML-like disease develop marked leukocytosis and infiltration of liver, spleen, and lungs with maturing myeloid cells. The disease is polyclonal and transplantable, and the target cell is a primitive hematopoietic progenitor/stem cell with multilineage repopulating ability. Thus, the disease induced in mice by BCR/ABL appears to be a very faithful and quantitative model of established chronic phase CML in humans. Although biochemical analysis is difficult, the model system can be employed in several different experimental strategies to genetically analyze the signaling pathways that are critical for BCR/ABL-induced leukemogenesis. One example is analysis of the effect of mutations in well-defined functional domains of the Bcr/Abl protein, and it is already very obvious that the results of *in vitro* transformation assays with

BCR/ABL do not necessarily correlate with leukemogenesis *in vivo*. Bcr/Abl is a constitutively active tyrosine kinase that stimulates several intracellular signaling pathways, including activation of Ras through direct binding of the SH2-containing adapter protein Grb2 to Bcr tyrosine 177. A tyrosine to phenylalanine mutation (Y177F) at this site blocks co-association of Bcr/Abl and Grb2 *in vivo*, and impairs focus formation by Bcr/Abl in fibroblasts. However, the Bcr/Abl Y177F mutant can transform hematopoietic cell lines and primary bone marrow cells *in vitro*, so the importance of the Bcr/Abl-Grb2 interaction to myeloid and lymphoid leukemogenesis *in vivo* is unclear. The Y177F mutation greatly attenuates the myeloproliferative disease induced in mice by Bcr/Abl, with animals developing B- and T-lymphoid leukemias of longer latency. In addition, the v-abl oncogene of Abelson murine leukemia virus, whose protein product lacks interaction with Grb2, is completely defective for induction of CML-like disease in mice. These results suggest that direct binding of Grb2 is required for efficient induction of CML-like myeloproliferative disease by oncogenic Abl proteins.

We have also tested the effect of mutations in the Src homology 2 (SH2) domain of the BCR/ABL oncogene on leukemogenesis. The SH2 domain was not required for induction of B-lymphoid leukemia in mice by BCR/ABL. Under conditions in which the p190 and p210 forms of BCR/ABL induce fatal CML-like myeloproliferative disease within 4 weeks, p210 SH2 mutants induced CML-like disease in some mice only after a significant delay, with other recipients succumbing to B-lymphoid leukemia instead. In contrast, p190 BCR/ABL SH2 point and deletion mutants rapidly induced CML-like disease. These results provide the first direct evidence of significant differences in cell signaling by the Bcr/Abl tyrosine kinase between these distinct leukemias. Contrary to previous observations, we found high levels of phosphatidylinositol 3-kinase (PI 3-kinase) activity in primary malignant lymphoblasts and myeloid cells from recipients of marrow transduced with the BCR/ABL SH2 mutants. Hence, the decreased induction of CML-like disease by the p210 BCR/ABL SH2 mutants is not due to impaired activation of PI 3-kinase. These studies illustrate both the promise and pitfalls of modeling CML in mice, and suggest that continued careful application of this system will further increase our knowledge about the molecular pathophysiology of CML.

NORMAL AND LEUKEMIC ENGRAFTMENT IN THE NOD/SCID MOUSE MODEL

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The NOD/SCID mouse model has been used to study chronic myeloid leukemia (CML) because it supports engraftment of both normal and leukemic human hematopoietic cells in the absence of exogenous cytokines. Intravenous infusion of >10 million chronic phase CML cells into irradiated (325 cGy) NOD/SCID mice resulted in engraftment levels of > 1% in the marrow of 84% of mice studied. FISH analysis of murine bone marrow revealed a median level of leukemic engraftment of 42%. Immunophenotyping showed BM engraftment was multilineage with myeloid, T- and B-cell involvement. No engraftment was seen even when large numbers of $CD34^-$ CML cells were infused but good levels of engraftment occurred when >3 million $CD34^+$ selected cells were given, demonstrating that NOD/SCID mouse engraftment potential resides in the $CD34^+$ fraction of CML cells. After 6 weeks, BM engraftment was detected at levels of 2-63%. The percent of human engraftment which was leukemic at this stage was between 23-64%. $CD34^+$ cells remained prominent at 6 weeks at levels between 4 and 11%. These findings support the use of the model to determine the fate of normal and leukemic stem cells which have been manipulated *in vitro* or *in vivo*. In order to track engrafting cells, the fluorescent dye CFSE (carboxyfluorescein diacetate, succinimidyl ester) was used to label CML cells before i.v. infusion. Surprisingly, CFSE labeled CML cells were undetectable in blood, marrow, spleen and lungs by 12 hours post-infusion. Given that CFSE labeled murine lymphocytes can be detected at frequencies as low as 1:10,000 cells post i.v. transfer, this suggests that only rare injected cells survive to contribute to engraftment. We estimate that this is less than 1 per 5,000 cells injected. When CFSE labeled CML cells are injected into the peritoneal cavity, significant numbers of labeled cells are seen in peritoneum, blood and spleen, but not bone marrow, at 6 and 21 hours after injection.

The tyrosine kinase inhibitor STI571 selectively inhibits cABL, and to a lesser extent c-KIT and the PDGF receptor, at concentrations which are attainable *in vivo*. Exposure of BCR/ABL positive cell lines, or fresh leukemia cells to STI571 *in vitro*, profoundly and selectively blocks proliferation, and commits cells to apoptosis. Furthermore, STI571 has been shown to inhibit the *in vivo* growth of human BCR/ABL cells lines transplanted into murine recipients. Currently, very little is known about the relative sensitivities to STI571 action against stem cells, committed progenitors and more differentiated elements forming the hematopoietic *hierarchy* within the BCR/ABL positive leukemic clone. This question is testable in the NOD/SCID model. As an adjunct to our *in-vivo* murine studies, we examined the activity of STI571 *in-vitro* on the growth of CML and normal $CD34^+$ progenitor cells in response to combinations of human growth factors. Our investigations suggested that at low doses of STI571 the inhibition of growth of normal progenitors is almost entirely mediated through c-kit/SCF. In the case of CML progenitors, the majority of the inhibition was not via the c-kit/SCF pathway, but presumably mediated through BCR/ABL. Thus far, our studies have shown that no growth factor combination tested protected CML progenitors from the inhibitory effects of STI571.

We are currently studying the effect of intraperitoneal injections of STI571 on CML engraftment in NOD/SCID mice. We hypothesized that engrafting leukemic stem cells may be uniquely sensitive to STI571 because they proliferate rapidly in the early phase of hematopoietic recovery. Our initial experiments have

been designed to determine the safety of STI571 given at various intervals post-irradiation. Systemic toxicity of STI571 is significantly enhanced by prior radiation exposure of 325 cGy. At STI571 doses that are well tolerated in non-irradiated mice, rapid wasting, hepatic and splenic atrophy and marrow aplasia were observed. The maximal tolerated dose of STI571 was 2-4 times lower than in non-irradiated hosts. The toxic effects of STI571 were apparent when given to mice in the first 4 days post-irradiation, however the toxicity was similar to not irradiated NOD/SCID mice when STI571 was administered beyond 10 days post-irradiation. We are currently investigating whether STI571-mediated inhibition of cABL and/or cKIT following radiation induced cell damage contributes to the toxicity. These studies may help to determine the critical repair mechanisms activated following sub-lethal irradiation and also define a dose and interval post-irradiation (and possibly chemotherapy) when STI571 can be safely administered.

NEW AGENTS

THERAPEUTIC STRATEGIES TARGETING BCR/ABL PATHWAYS

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Chronic myelogenous leukemia (CML) is caused by BCR/ABL, a constitutive tyrosine kinase oncoprotein that mimics cytokine receptor signaling, inhibits apoptosis, and alters integrin function in hematopoietic cells. We have systematically assessed the contributions of the AKT, Ras, and STAT signaling proteins in BCR/ABL transformation, and identified significant redundancy and modest specificity in their roles in mediating hematopoietic cell survival, proliferation, and resistance to apoptosis. To identify genes activated or repressed as a consequence of BCR/ABL signaling, we performed an expression analysis using high density oligonucleotide arrays. We identified the critical induction of cyclin D2 and have demonstrated that bone marrow cells from mice that are deficient in D2 resist transformation by BCR/ABL. We are investigating the importance of several other genes implicated by expression analysis with the goal of refining the mechanism of action of BCR/ABL and defining novel targets for inhibition. We have demonstrated the potent activity of a novel class of anticancer compounds, farnesyl transferase inhibitors (FTIs), in several cell culture assays of BCR/ABL-transformation and in vivo models of leukemia induction, and are studying the specific mechanism of action of these compounds and their synergy with the ABL kinase inhibitor STI-571. Finally, we have demonstrated that the *Interferon Consensus Sequence Binding Protein* (ICSBP) acts as a tumor suppressor for hematopoietic cells and sensitizes leukemia cells to immune rejection, and are investigating means by which ICSBP might be used in the immunotherapy of CML. Detailed knowledge of the signal transduction pathways emanating from BCR/ABL, as well as insights into the mechanism of action of interferons should facilitate novel therapeutic strategies.

TREATMENT STRATEGY

TREATMENT STRATEGY: BONE MARROW TRANSPLANTATION

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Chronic myeloid leukemia (CML) in first chronic phase is the most frequent single indication for an allogeneic hematopoetic stem cell transplant (HSCT). Near to one thousand of these transplants were performed in Europe in 1999; two thirds from an HLA-identical sibling, one third from an unrelated matched volunteer donor. Allogeneic HSCT remains the sole therapeutic approach with a potential cure for this disease. Eradication of the Ph+ clone and absence of the bcr-abl transcript by molecular methods over a prolonged period of time is only observed after this form of treatment. To find a compatible donor should be the primary goal for each patient with newly diagnosed CML. However, HSCT is associated with significant risks. A patient with newly diagnosed CML faces the dilemma of early morbidity and mortality but prospects of long term survival versus less invasive but palliative forms of treatment. The dilemma is further complicated by the fact that the delay between diagnosis and transplant increases the risk of subsequent TRM by about 10%, independent of any other factor. In addition, pre-transplant interferon can influence TRM, too. Now that the main pre-transplant risk factors for allogeneic HSCT in CML are known, the situation has improved. These risk factors are cumulative and give a direct estimate of the risk for transplant related mortality (TRM). It can be as low as less than 20% for a young patient transplanted early in the disease but exceed 50% for a patient with unfavourable risk profile. Treatment approach should be tailored today according to the risk profile. Patients with low disease risks (e.g. low Sokal/Hassford score; early disease) but high risk for TRM (e.g. age, disease, donor factors) require other forms of therapy than patients with high disease risk (e.g. high Sokal/Hassford score; advanced disease) and low TRM risks (e.g. age). Young patients with CML and a HLA identical donor (EBMT risk score 0 to 2) should be treated as soon as possible with HSCT using conventional conditioning regimens. In contrast, an initial trial with interferon alpha or STI 571 might be appropriate in an elderly patient with low risk disease. Patients with an EBMT risk score >2 represent the patient population where new low intensity conditioning approaches should be evaluated prospectively. Concepts for a prospective study will be presented.

TREATMENT STRATEGY: INTERFERON

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Twenty years ago the annual death rate in an unselected population of patients with Ph+ chronic myeloid leukemia (CML) was close to 10%, median survival was 4 years, and less than 10% of the patients were alive after 10 years.[1] The prognosis of individual patients could be predicted based on simple clinical and laboratory factors leading to the identification of at least three risk groups.[2] Low risk patients had a median survival of about 6 years. High risk patients had a median survival of less than 3 years. During the last 20 years an ever increasing proportion of patients are diagnosed fortuitously, so that the percentage of low risk cases, that was 30% twenty years ago, is now 50% or more, especially in the young or middle-aged.[3,4] The reasons for these changes in the risk pattern profile are not entirely clear, but the change is important and any approach to the treatment of Ph+ CML patients less than 55 years old should not overlook that 50% or more of them fall in a low risk category. The current treatment of Ph+ CML patients is based substantially on α-interferon (IFN) and allogeneic bone marrow transplantation (alloBMT), which can be now applied to almost all patients through the bone marrow donors worldwide.[5] The main differences between α-IFN and alloBMT are a) that only about 50% of patients are responsive to α-IFN while almost 90% of patients who are submitted to alloBMT in chronic phase respond, b) that IFN does not cure (though some long-term survivals are equivalent to a cure) while alloBMT cures almost 80% of cases, and c) that transplant-related mortality (TRM) ranges from 10 to more than 50% while IFN does not kill. Therefore, any treatment decision should take into consideration the risk of alloBMT, which is a risk of dying, and the risk of IFN, which is a risk of failing. A number of studies, including the treatment surveillance program of the Italian Cooperative Study Group on CML,[4,6] have shown that disease–related risk (such as Sokal's risk) is unrelated to the outcome of alloBMT, but has a strong relationship with the result of IFN. Low risk patients who are assigned to IFN have a median survival of 7 to 9 years and 30 to 40% of them are alive after 10 years. For the low risk patients who are responsive to IFN, hematologically and cytogenetically, the median survival cannot yet be calculated, since 70 to 80% of them are alive after 10 years. Also non-low risk patients can benefit from IFN, but they respond less frequently to IFN and, more important, the absolute benefit from IFN is substantially less than that obtained by low risk patients. In summary, a low risk patient has at least a 50% chance of responding to IFN and becoming a long-term survivor. A non-low risk patient has less than a 25% chance of responding to IFN and will rarely become a long-term survivor. While the probability of responding to IFN depends on the disease, the risk of TRM does not depend on the disease and can be assessed based on the factors that were identified by the *European Blood and Bone Marrow Transplantation Group*,[6] namely donor type, age and time lapsed from diagnosis. To give an example, a patient who is less than 40 years old and is transplanted from an HLA-identical, sex-matched sibling within one year from diagnosis, has about an 80% chance of success, while a patient who is more than 40 years old and is transplanted from a matched unrelated donor more than one year after diagnosis, has a predicted failure rate of more than 50%. In conclusion, any discussion of treatment allocation should be based on the evaluation of treatment-(alloBMT) related risk and of disease-related risk, such as Sokal's score or the most recent European risk score.[7] A low risk patient is a good candidate for IFN and would be a good candidate also for alloBMT only if his/her predicted TRM was low. A non-low risk patient is never a good candidate for IFN. But almost 40% of all patients with Ph+ CML are not good candidates for either treatment, because their predicted response rate to IFN is low and their predicted TRM is high. For these patients, and probably for all patients, progress is not entrusted to randomized studies but to the improvement of both treatments and to the application of new agents and treatment modalities.

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FLUORESCENCE *IN SITU* HYBRIDIZATION FOR BCR/ABL ON PERIPHERAL BLOOD AND BONE MARROW COMPARED TO CONVENTIONAL CYTOGENETICS ON BONE MARROW FOR MONITORING PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Conventional cytogenetic analysis (CCA) is the standard method for diagnosing and monitoring the Ph chromosome in chronic myeloid leukemia (CML). Interphase fluorescence *in situ* hybridization (FISH) on bone marrow (BM) samples (IBF) may replace CCA since it does not need metaphase preparations and large number of cells can be analyzed. Evaluation of peripheral blood (PB) for BCR/ABL fusion using interphase FISH (IPF) might be another approach to quantify the presence of Ph(+) cells, allowing more frequent and less invasive follow-up investigations. Previous studies comparing IPB with metaphase and interphase BM suggest a significant correlation between results of the methods. The aim of our study was to evaluate the influence of lymphocytes on the results of IPF analysis and to compare the results of IPF with those of CCA and IBF. IPF analysis for BCR/ABL fusion was combined with CD3 immunophenotyping on 21 smears from 19 patients with CML in chronic phase. IPF results were corrected for lymphocytes according to the leukocyte differential count (corrected IPF).

Table 1. Statistical comparison of results of CCA, IBF and IPF from 21 sets of PB and BM samples take from 21 patients with CML.

		IPF		
	Evaluated	*Corrected*	*CD 3(-) cells**	
Correlation (r, p)*	(0.6532, 0.001) (0.8822, 0.0001)	(0.6316, 0.002) (0.7135, 0.001)	(0.5465, 0.001) (0.7648, 0.0001)	CCA IBF
Differences** (mean, range)	(24, 3-46) (10.6, 3-27)	(9.0, 0-20) (8.9, 0-20)	(14.8, 1-29) (5.3, 1-17)	CCA IBF
Limits of Agreement ***	(49%) (25%)	(26%) (21%)	(35%) (14%)	CCA IBF

**Spearman's correlation; °mean and range of differences between results of methods; #this identifies a percentage (mean±2SD) of differences between results of two methods and defines a 95% interval of confidence that, for any new patient analyzed by one method, gives measurements that differ by less than this percentage from the other method.*

Furthermore, IPF was recorded in CD3(-) cells to exclude the effect of T-lymphocytes on evaluation. The incidence of BCR/ABL(+) fusion signals in CD3(+) T-cells of CML patients was 5.3% (SD±1.9) and did not exceed the normal cut-off value of 8%. A statistically significant correlation was found between results of IPF (evaluated, corrected and CD3(-) cells) and results of CCA or IBF analysis. The results of IPF analysis (corrected IPF and IPF on CD3(-) cells) reduced the mean of differences and improved the limits of agreements between results of IPF and CCA or IBF. The best agreement was noted between results of CCA and the corrected IPF, whereas, results of IBF agreed best with those of IPF on CD3(–) cells (Table 1). Adopting classical response criteria, CCA and corrected IPF analysis agreed on the classification of all cytogenetic responders but 6 out of 8 non-responders according to CCA were found to be minor-responders by corrected IPF. Our results imply that the corrected IPF from IPF analysis is reliable for monitoring CML patients and may replace BM analysis for the evaluation of response to conventional or myeloablative treatment.

LOW INCIDENCE OF GRAFT-VERSUS-HOST DISEASE AND RELAPSE AFTER VOLUNTEER UNRELATED DONOR TRANSPLANTS FOR CHRONIC MYELOID LEUKEMIA IN CHRONIC PHASE CONDITIONED WITH ANTITHYMOCYTE GLOBULIN

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From September 1995 to December 1999, 18 consecutive adult patients with chronic myeloid leukemia (CML) in chronic phase (CP) underwent volunteer unrelated donor (VUD) transplants. HLA typing required molecular identity at the HLA A, B, DRB1, DQB loci. One patient, however, had one class I mismatch. Mean interval between diagnosis and transplant was 32±26 months. Conditioning was based on single fraction total body irradiation (TBI), 120 mg/kg Edx and 3 mg/kg/die from days -6 to -2 (total dose 15 mg/kg) rabbit antithymocyte globulin (ATG) (Fresenius, Bad Homburg, Germany). Graft-versus-host disease (GVHD) prevention consisted of cyclosporin A and a short course of methotrexate. The patient population had a median age of 35 years (range 22-50); median donor age was 35 years (range 19-52). All patients engrafted, with a median time to 0.5×10^9/L polymorphonuclear leukocytes of 21 days and to 50×10^9/L platelets of 27 days. All patients were evaluable for acute GVHD; it occurred in 8 patients (grade I 3, grade II 2 and grade IV 3). Chronic GVHD (survival >100 days) has occurred in 2/15 patients at risk, always being limited. Fourteen patients are alive, with a median follow-up of 21 months (range 3-52 months). No hematologic relapses have occurred: one patient had a molecular relapse and received interferon-α, after which he became negative; two other patients had transient molecular positivity which disappeared without intervention. Caus-

es of death were acute GVHD/infections (3 patients) and multiorgan failure (1 patient). Actuarial disease-free survival at one year is 77% (95% CI, 59-97), with no additional events after that point to 4 years. These data show that a low incidence of severe GVHD, both acute and chronic, is achievable in most patients who receive low dose ATG pre-transplantation, without an increase in relapse. These results are equivalent, if not better, than those obtained in patients transplanted from HLA siblings.

ALLOGENEIC TRANSPLANTS WITH BONE MARROW OR PERIPHERAL BLOOD STEM CELLS IN CHRONIC PHASE CHRONIC MYELOID LEUKEMIA. EXPERIENCE FROM A SINGLE CENTER

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Peripheral blood stem cells (PBSC) have became, in the past two years, a major source of stem cells for allogeneic bone marrow transplantation (BMT). One expectation of the procedure is a lower incidence of relapse (related to the higher number of transfused T-lymphocytes) than following BMT, because of a graft-versus-leukemia effect. We examined the incidence of relapse (cytogenetic and hematologic) and chronic graft-versus-host disease (GVHD) in 13 consecutive patients with chronic myeloid leukemia (CML) in chronic phase (CP), transplanted from fully HLA-compatible siblings who received heavy busulfan/cyclophosphamide as conditioning and cyclosporine/methotrexate for GVHD prophylaxis. As a reference group, we analyzed our historic cohort of 26 patients treated in the same way. PBSC transplants were performed between October 1989 and July 1998. Twenty-six patients (mean age 37±9 years, mean diagnosis-treatment interval 24 ± 12 months) received BMT; 13 patients received PBSC (mean age 35±9 years, mean diagnosis-treatment interval 14±6 months). Chronic GVHD was defined as mild, moderate or severe, with a minimum follow-up of 80 days; cytogenetic relapse was defined as any Ph+ occurring >6 months after transplantation. Chronic GVHD occurred in 10/13 PBSC and 10/24 BMT (p=0.04, chi square test). In the PBSC group GVHD was mild (4 cases), moderate (1), severe (5); respective numbers for the BM group were 6, 2, 2 (p=0.04, Kruskal-Wallis test), indicating more severe chronic GVHD in the PBSC group. One cytogenetic and one hematologic relapse occurred in the 24 BMT and one hematologic relapse in the 10 PBSC (p=0.6). Actuarial disease-free survival of the BMT group is 79% (95% CI: 63-92) at 9 years; that of the PBSC is 78% (95% CI: 50-100) at 3 years. Despite the vastly increased incidence of severe chronic GVHD, PBSC transplants do not seem to offer any advantage over BMT in terms of relapse of the disease when conventional GVHD prophylaxis is applied in CML-CP.

e19a2 bcr/abl FUSION TRANSCRIPT WITH ADDITIONAL CHROMOSOME ABNORMALITIES IN A PATIENT WITH ACUTE MYELOID LEUKEMIA-M1

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The BCR/ABL rearrangement is detected in only 1% of patients with acute myeloid leukemia (AML). A breakpoint falling within the micro bcr region, giving origin to e19a2 transcripts and to a P230, has been associated with a mild form of chronic myeloid leukemia (CML). Up to now an e19a2 transcript has been observed in ten patients with CML in chronic phase, in two with CML in blastic phase and in only one with AML. We report on a 49-year old man, suffering from bone pains, intermittent fever and nocturnal sweating. On physical examination liver and spleen enlargement (4 and 1 cm) and a left arm nodular, round, hard and unpainful red lesion, adhering to the underlying structures were noted. Sixty percent agranulated blasts were observed in his peripheral blood. A bone marrow aspirate revealed the almost exclusive presence of two blast cell populations: one (40%), constituted of agranulated elements; the other (60%) of granulated elements. This blast cell population was CD34, CD33, DR, $CD4^{dim}$, CD38, CD25, CD7, CD11b and CD11c heavily positive on immunophenotyping. The karyotype was: 47,XY,+8, t(9;22)(q34;q11)/48, idem, iso(17q),+der(22) t(9;22)(q34;q11). A fluorescent *in situ* hybridization (FISH) analysis with the two-color minor-breakpoint BCR-ABL translocation DNA probe revealed the presence of one fused spot in all the metaphases and in 74% of the nuclei studied. Sixteen per cent of the interphase cells examined showed two fused spots. At first a PCR assay failed to discover either the transcripts b2a2 and b3a2 or the transcript e1a2; in contrast it identified an e19a2 transcript, corresponding to a P230 protein. A diagnosis of AML-M1, probably a myeloid blast crisis of CML with a preceeding silent chronic phase, was made. A bone marrow complete hematologic and cytogenetic remission with the persistence of an e19a2 transcript was achieved just after the first course of chemotherapy. Despite this, the patient's extramedullary disease progressed until radiotherapy was administered with partial success. Our patient is interesting for two reasons:

1. he showed an e19a2 rearrangement along with additional chromosome abnormalities typically seen in CML blast crisis. This datum suggests that our patient, like seven out of the ten P230 positive CML reported in the literature, had probably had a silent CML chronic phase, although we do not know whether classical or mild;
2. he, like two of the three cases presenting with an acute leukemia phenotype, showed an iso(17q). An additional chromosome abnormality might suggest that in P230 positive CML acute transformation occurs only after the development of mutational events very frequently involving gene(s) mapped on 17p.

VACCINATION OF CHRONIC MYELOID LEUKEMIA PATIENTS WITH A P210-DERIVED PEPTIDE VACCINE PLUS QS-21 AND GM-CSF: RATIONALE AND PRELIMINARY DATA

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Chronic myelogenous leukemia (CML) presents a unique opportunity to develop therapeutic approaches using vaccination against a truly tumor specific antigen that is also the oncogenic protein involved in neoplasia. CML is, in fact, always characterized by the t(9;22) that results in the bcr-abl fusion oncogene and expression of a chimeric protein product p210. Previously we found that peptides derived from amino acid sequences crossing the b3a2 breakpoint in p210 elicit class I restricted cytotoxic lymphocytes (CTLs) and class II restricted specific proliferation of CD4 T-lymphocytes *in vitro* (HLA A3, A11, B8 and DR11 respectively). Moreover, b3a2-derived peptide specific T-cells have been shown to kill and/or proliferate in the presence of CML blasts, thus suggesting that bcr-abl fusion sequences are naturally processed and expressed on CML cells. These *in vitro* data provided the rationale for developing peptide based vaccines for this disease. Scheinberg *et al.* have recently published the results of the first phase I dose escalation study of a multivalent breakpoint peptide vaccine plus the immunologic adjuvant QS-21. In this trial HLA restriction was not required and most of the patients had large tumor burdens. Nevertheless 3/12 patients treated (3/6 at the two highest dose levels of vaccine) generated peptide-specific T-cell proliferative responses *ex-vivo* (n=3) and/or delayed-type hypersensitivity (DTH) responses (n=2). Peptide-specific CTLs have not been identified. As it is more likely that effective vaccination strategies will target patients with minimal tumor burden, we recently started a phase II trial including patients with appropriate breakpoint and HLA types and major or complete cytogenetic response. Furthermore, in order to improve peptide immunogenicity, we added low doses of GM-CSF to a fixed medium-high dose of CML peptides plus QS-21. Two patients have so far entered the study. Both of them showed a prompt and consistent DTH response already evident after the first 3 vaccinations.

RELATIONSHIPS BETWEEN DOSE OF INTERFERON AND COMPLETE CYTOGENETIC RESPONSE

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A collaborative study of the EICML allowed the creation a database containing information on a large series of 514 patients with complete cytogenetic response (CCR). Eight national databases and single institutions provided data of the study cases, including an analytical survey on the interferon (IPN)-based regimen before CCR, time to response and hematologic evolution. The overall report on 317 cases of patients with CCR who achieved CCR with IFN alone and were never submitted to any other procedure such as allo or auto BMT is presented elsewhere during this meeting. This analysis is devoted to investigating the correlations which exists between the dose of interferon before CCR and response duration. The average dose of IFN/week from diagnosis to the first demonstration of a CCR, calculated on 235/317 patients evauable (74%), was 37.62±18.95 IMU, for an average daily dose of 5.3 IMU, while the mean minimum weekly dose and the mean maximum weekly dose were 24.4 and 46.6 IMU (or 3.4 IMU and 6.6 IMU mean daily doses), respectively. Stratifying the patients as a function of the Sokal risk, for low Sokal the mean weekly dose was 40.72±16.30; the mean of the minimum weekly dose was 25.73±15.69 IMU and the mean of the maximum weekly dose was 50.51±17.71 IMU. For Sokal non-low risk (intermediate plus high risk) the mean weekly dose was 34.96 ±22.18 IMU; the mean of the minimum weekly dose was 22.81±17.38 IMU and the mean of the maximum weekly dose was 41.75 ± 18.90 IMU. We looked for a possible relation between dose of IFN before CCR and CCR duration. To date, 105/317 (33%) patients have lost their CCR and the overall actuarial median duration of CCR is 88 months and risk of losing CCR was significantly different according to Sokal risk: in fact 53/178 low Sokal risk, 24/76 intermediate risk and 16/35 high risk patients lost CCR. The overall *p* value is 0.0175. Among low Sokal risk patients, the risk of CCR loss was similar if the patients achieved CCR with a mean weekly dose of IFN lower or higher than 35 IMU. On the contrary, among non-low Sokal risk patients, the risk of losing the CCR seems to be higher in patients who received more than 35 IMU (as mean weekly dose) before CCR. The above considerations are applicable either choosing a different cut-off of mean IFN weekly dose: i.e.; evaluating the risk of loosing the CCR as function of a dose lower or higher than 21 IMU/weekly, we reached the same conclusions. This is retrospective analysis of a selected group of patients and consequently, no conclusion can be drawn on the probability of achieving a CCR in function of dose. Conversely a relation apparently exists between the dose of IFN BEFORE CCR and CCR duration. In fact, there is a trend for a more stable CCR in patients who received lower doses of IFN, particulary for non-low Sokal risk patients.

COMPETITIVE POLYMERASE CHAIN REACTION OF GENOMIC DNA AS A METHOD TO DETECT THE AMPLIFICATION OF THE BCR-ABL GENE OF CHRONIC MYELOID LEUKEMIA

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The chimeric product of bcr-abl rearranged gene is critical in the pathogenesis of chronic myeloid leukemia (CML), yet its role in the progression of the disease remains unclear. So far, increased bcr-abl expression levels, possibly due to gene amplification, precede the clonal evolution of CML hematopoietic progenitors toward a fully transformed phenotype and might be, altogether, involved in their resistance to interferon-α or tyrosine kinase inhibitors.

The aim of the study presented here was that of developing a competitive polymerase chain reaction (PCR) strategy useful for monitoring the bcr-abl expression levels at different stages of CML. A competitive PCR technique is based upon the coamplification of the sample template (target) together with increasing amounts of a DNA fragment (competitor) sharing with the target the primer recognition sites, but differing in size. A competitor for the quantification of both a2b2 and a2b3 alternative splicing forms of the bcr-abl chimera was obtained by cloning within the a2b2 PCR product sequence a 37 bp DNA fragment present in a commercially available molecular weight marker preparation. The PCR reactions were performed both on genomic DNA and reverse transcription (RT) products in microcapillary tubes using a rapid cycle DNA amplification instrument (Idaho Technology). In preliminary experiments carried out on bcr-abl-transduced clones of the 32D hematopoietic cell line, we established, by means of titration assays, the accuracy and reproducibility of our competitive strategy carried out on both genomic and retrotranscribed DNA. Competitive PCR of genomic DNA had a sufficient sensitivity to detect a single copy of the bcr-abl rearranged gene, thus it enabled us to measure the bcr-abl gene amplification precisely, when present. Competitive PCR carried out on RT products was highly sensitive and reproducible. We are presently attempting to correlate the bcr-abl genomic copy number and its transcription level in clinical samples, and on this basis to build up a molecular prognostic classification of CML patients.

INTERFERON PLUS ORAL ARA-C IN CHRONIC MYELOID LEUKEMIA PRIMARILY RESISTANT OR WITH MINIMAL CYTOGENETIC RESPONSE TO INTERFERON

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Interferon (IFN) plus subcutaneous low-dose Ara-C produces higher cytogenetic response rates and longer survival than interferon (IFN) alone in chronic myeloid leukemia (CML). A phase II pilot study was designed to determine the efficacy of and tolerance to IFN plus oral Ara-C (YNK-01) in CML patients hematologically resistant or with minimal cytogenetic response (marrow Ph-negative metaphases < 10%) to IFN, after a minimum of one year of therapy. Treatment included the IFN dose the patients were receiving at time of protocol entry plus monthly 14-day cycles of oral Ara-C at a starting dose of 500 mg/day, with progressive dose escalation if tolerated. Patients were initially scheduled to receive 6 Ara-C cycles and to continue treatment in case of response. Of the 18 patients currently included in the protocol, 14 have completed the initial 6-month period. The results in the first 6 patients (chronic phase >2 years, n=5) were as follows: grade III-IV (WHO scale) hematologic toxicity, 5 cases (mainly thrombocytopenia, leading to treatment discontinuation in 2 patients), and grade I gastrointestinal toxicity, 3 patients; reduction in the IFN dose was required in all cases, and in the Ara-C dose in 5 cases. Hematologic (without cytogenetic) response was achieved in 1 of the 4 evaluable patients. In the following 4 patients (early chronic phase, n=3), in whom the starting Ara-C dose was 300 mg, the results were: grade III hematologic toxicity (thrombocytopenia), 2 patients, and grade II gastrointestinal toxicity, 2 patients; IFN dose reduction, 2 cases, and Ara-C reduction, 2 cases; Ara-C dose increased in 2 patients; treatment was discontinued in 2 patients due to disease progression or toxicity; minor cytogenetic response, 2 cases, one of which progressed to major cytogenetic response after 6 additional Ara-C cycles. In the next 4 patients (early chronic phase, n=4) the starting Ara-C dose was reduced to 200 mg, with the following results: grade I-II hematologic toxicity, 3 patients, and grade I gastrointestinal toxicity, 1 case; IFN dose reduction required in 2 patients and Ara-C dose reduction in 1; Ara-C dose increased in 3 patients; hematologic response, 2 patients, one with minor and the other one with minimal cytogenetic response. In CML patients with primary resistance to IFN, the addition of oral Ara-C at a starting dose >200 mg/day (14 days a month) is associated with substantial hematologic and, to a lesser degree, gastrointestinal toxicity. Use of oral Ara-C at lower starting dosages in patients with less advanced disease will allow us to identify the possible role of the above drug as an alternative to subcutaneous Ara-C in CML.

AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN PATIENTS WITH NEWLY DIAGNOSED CHRONIC MYELOID LEUKEMIA

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Autologous peripheral blood stem cell (PBSC) transplantation has been recently proposed as an alternative approach to standard treatments for chronic myeloid leukemia (CML) with the aims of obtaining major karyotypic responses and perhaps

prolongation of survival, although this has not yet been demonstrated to occur by the data available in the literature. We have used this strategy in a protocol which included treatment with α-interferon (IFN) at the maximum tolerated dose following PBSC transplantation. From November 1997 to July 1999, 7 patients were enrolled: they were aged between 53 and 62 years, mean 58; the disease status was chronic in 4 patients and accelerated phase in 3 patients. Five of the patients were male and 2 female. None had a compatible syngeneic donor and all of them were excluded from the MUD transplantation program because of their age. Mobilization treatment consisted of ICE (idarubicin 12 mg/m^2 over three days, VP-16 120 mg/m^2 over three days and aracytin 2,000 mg/m^2 over three days). The mean time required for mobilization of CD34$^+$ cells was 18.8 days (range 14-28); one patient never reached an adequate number of circulating CD34$^+$ cells for collection. The mean number of CD34$^+$ collected was 4.93×10^6/kg; the Ph1 of the collected products was negative in 5 out of 6 evaluable patients; bcr-abl rearrangement was still negative in the PBSC of Ph1- patients. Six patients underwent autologous transplantation of PBSC following a conditioning regimen with busulfan 16 mg/kg over four days. Marrow recovery was demonstrated at a mean of 12.5 days (range 11-16) following the reinfusion of PBSC. The peritransplant course was uneventful. One patient developed blastic crisis three months following the procedure and died 5 months later. Five patients started IFN one to three months after transplantation; the tolerated dose was no more than 3 MU/day. Three patients are in hematologic remission 12, 15 and 25 months following the procedure, two of them with a major karyotypic response; the other two patients are in chronic and accelerated phase, 14 and 28 months after the transplant, respectively. The patient who failed the collection of PBSC is now in blastic transformation. None of the patients achieved molecular remission and remained bcr-abl positive. In conclusion, the procedure of PBSC transplantation in CML is feasible in most of patients although mobilization of CD34 cells can be delayed with some failures; major karyotypic responses are obtained and maintained over time; that this approach to the treatment of CML is superior to standard therapies remains to be demonstrated.

INTERNET AND CLINICAL TRIALS

de Vivo A, Fiacchini M, Bassi S, Bonifazi F, Trabacchi E and Rosti G on behalf of the Italian Cooperative Study Group on Chronic Myeloid Leukemia

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Internet and the World Wide Web (WWW) provide new opportunities for the implementation and management of clinical trials. Often a multicenter trial or a long sampling period is necessary to obtain a large sample size. The larger the trial the larger the unit cost is. Internet and the WWW can provide global access, fast interaction, automation and therefore lower costs. Internet and WWW have been exploited in the management of clinical trials for tasks such as data entry and distribution of information on trial progress. The *Italian Cooperative Study Group on Chronic Myeloid Leukemia* (ICSG on CML) has developed a computerized network system for data management of studies on CML. Through a personal computer each participating center can connect by Internet to a website and download reports on the progress of the trial; moreover, the investigator can choose from a list of standard forms to upgrade the database in real time. The advantages of this system are: a) the real time updating of the clinical trial, b) reduction in number of mistakes in data completion, c) reduction in the human and economic resources required. Electronic mail and the website are used to communicate between people involved in the clinical trial. This system has been adopted in the CML94 trial of the ICSG on CML (77 Care Units participating, 827 patients recruited).

SECONDARY CUTANEOUS EFFECTS OF HYDROXYUREA IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA.

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Hydroxyurea (HU) is a cytoreductive agent commonly used in the conventional treatment of chronic myeloproliferative disorders.Long-term HU therapy has been associated with cutaneous side effects, these include:xerosis, diffuse hyperpigmentation, nail changes, dermatomyositis-like eruptions and, rarely, leg ulcers. We report the cases of four patients who developed cutaneous lesions during treatment with HU for chronic myeloid leukemia (CML). From 1986 to 1999, 32 patients suffering CML had received HU, because they could not undergo interferon (IFN) therapy. Four patients, 2 males, 2 females, median age 58 years (range 52-73), developed cutaneous lesions after mean period of treatment with HU of 26.5 months (range 15-52).The median daily dose of HU was 2 g.

The first of these 4 patients, developed a cutaneous leg ulcer adjacent to the malleoli without a coexisting varicose disease and with Doppler flowmetry in the normal range.The discontinuation of HU led to the resolution of the leg ulcer after 2 months.

The second patient, too, developed perimalleolar skin ulcer. He refused the advice to discontinue therapy with HU. After 72 months a carcinoma of the right cornea appeared and this was treated with radiotherapy. At that time the administration of HU was stopped with an improvement of leg ulcers.

Papulo-erythematous lesions of the face appeared in the third patient.The cutaneous biopsy showed a perivascular inflammatory infiltrate compatible with the diagnosis of acne rosacea.The discontinuation of HU led to the healing of the skin lesions. The fourth patient had dark spots on the oral mucosa.Histological examination showed the presence of "melanophages" in the oral mucosa, compatible with melanosis. HU was withdrawn.

The appearance of HU-related skin lesions may,

sometimes, represent a serious clinical problem for CML patients in long-term continuous treatment. The incidence of skin side effects could be underestimated. We observed skin lesions in 4 out of 32 patients (12.5%). The improvement of the lesions required interruption of the therapy, suggesting a close correlation with HU cumulative dose. More medical information would be useful to understand the pathophysiological process of the skin side effects of HU, which are currently unknown and may be multifactorial.

SURVIVAL OF LEUKEMIC CFU-GM TO GROWTH FACTOR DEPRIVATION: EFFECTS OF CELL CONCENTRATION AND EXPOSURE TO RETINOIDS ± α INTERFERON

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In cell line models, the BCR/ABL chimeric protein confers resistance to apoptosis induced by growth factor deprivation. However, conflicting results were reported about survival of chronic myeloid leukemia (CML) myeloid progenitors (CFU-GM) in growth factor devoid cultures (*Bedi et al, Blood 1994; 83:2038; Amos et al, Br J Haematol 1995; 91:387*).

In this study, normal and CML hematopoietic progenitors (CD34+ cells) were highly enriched, from low density bone marrow or peripheral blood cells, using immunomagnetic methods, either by negative (CD2, CD19, CD11b, CD16, CD9, anti-glycophorin monoclonal antibodies (MoAb) and Dynabeads) or positive selection (CD34 MoAb and Miltenyi Mini Macs). $CD34^+$ cells were incubated at 5×10^4/mL (*mass cultures*) for 11 days in IMDM medium containing either 10% fetal bovine serum (FBS) or a mixture of human saturated transferrin, insulin, bovine serum albumin and lipids (serum-free medium), without growth factor addition. In some experiments, either 13cis- or all-trans retinoic acid (both at 5×10^{-7} M) ± α interferon (IFN) 300 U/mL were added to the medium. CFU-GM concentration was evaluated at day 0 and after 4, 7, and 11 days of culture, by replating 50-100 µL of cell suspension onto agar medium with 20% FBS and 10% supernatant of 5637 cell line (5637 SN) as a source of CSFs. Highly purified $CD34^+$ cells were also cultured at limiting dilution in 96 microwell plates (10-1 cells/well), both in 10% FBS and in serum-free medium, for 4 and 7 days. A mixture of FBS and 5637 SN was then added to all microwells (to final 20% FBS and 10% 5637 SN concentrations) to allow CFU-GM growth for 14 further days. Growth-positive wells were then scored. The cloning efficiency, calculated by Poisson's statistics, was compared to that of cells seeded from day 0 in the presence of FBS and 5637 SN, this allowing the evaluation of CFU-GM loss during the 4-7 days of growth factor deprivation. Highly enriched CFU-GM survived well in *mass cultures* for 7-11 days, both in 10% FBS and in serum-free medium (70-100% recovery, compared to day 0 concentration), whereas normal CFU-GM declined steadily, day 7 recovery reaching only 18±10% in FBS and 4±6% in serum-free medium. Conversely, at limiting dilution CML CFU-GM declined in 4-7 days in a similar way to normal ones. The addition to *mass cultures* of either isoform of retinoic acid reduced CFU-GM recovery by more than 50% in 11/17 cases. The suppressive effect of retinoids was even more evident in the presence of IFN, with a CFU-GM recovery comparable, in most of cases, to values observed in normal cultures of progenitors. Therefore, the resistance of CML CFU-GM to growth factor deprivation appears to depend on a relatively high cell concentration, in agreement with the recent report of autocrine CSF production by CML $CD34^+$ cells (*Jiang et al, Proc Natl Acad Sci USA 1999; 96:12804*). The combination of retinoic acid and IFN, at therapeutic concentrations, can overcome the survival advantage of CML progenitors to growth factor deprivation *in vitro.*

OCULAR BLAST METAMORPHOSIS IN A PATIENT WITH CHRONIC GRANULOCYTIC LEUKEMIA

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A 56-year old patient was hospitalized in the ophthalmology department because of right-sided, unilateral loss of vision of gradual onset, atrocious pain in the right eyeball, ache in half of the head, dizziness, and anxiety. The objective clinical examination revealed swelling of the right orbit, exophthalmos, conjunctival edema and hemorrhages.

The echography OD and the computerized tomography of OD showed a right-sided intraocular tumor formation, localized to the anterior chamber of the right orbit with posterior expansion. Concomitantly the patient also had splenomegaly, hepatomegaly, and nose bleeding. Paraclinical examinations: hemoglobin 9.2 g/dL, number of leukocytes 56,000/mm^3, Mb 8%, Pr 18%, Mc 20%, Mtc 20%, Ba 6%, E 6%, Ly 10%, Mo 2%, PMN 9%, FAL 38 IU. The cytogenetic analysis showed t(9;22). The platelet count was 75,000/mm^3. The bone marrow examination confirmed the diagnosis of chronic granulocytic leukemia, with a possible blast metamorphosis localized to the right orbit. Therapy was started with hydroxyurea 2 g/day, cytosar 20 mg at 12 hours, s.c., and interferon alpha 2b $3x10^6$ IU three times a week, s.c. After 10 days, the general state of the patient improved, the number of platelets reached 100,000/mm^3 and surgical excision of the right orbit was decided. The histopathology examination, immunohistochemical and immunophenotypic tests confirmed the diagnosis of chronic granulocytic leukemia (CML) with blast metamorphosis localized (anterior chamber of the right orbit being infiltrated by blast cells $CD11^+$, $CD13^+$, $CD33^+$, $HLA\text{-}DR^+$). Although cases of blindness caused by ocular hemorrhage as a result of the low platelet count in CML have been reported, cases of acute metamorphosis with an intraocular localization are rare in the literature.

INVERTED INSERTION INS(22;9)(Q11;34Q21) IN A PATIENT WITH CHRONIC MYELOID LEUKEMIA CHARACTERIZED BY FLUORESCENCE *IN SITU* HIBRIDIZATION

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Purpose. To describe an unusual cytogenetic rearrangement in a patient suffering from chronic myeloid leukemia (CML). *Case report.* A 49-year old man was diagnosed as having CML in January, 1999. Clinical manifestations of his disease were abdominal pain, fatigue and weight loss. Physical examination showed a greatly enlarged spleen (12cm). Analytical data: hemoglobin 104 g/L, WBC 630×10^9/L; peripheral blood MG staining: neutrophils 25%, bands 17%, metamyelocytes 13%, myelocytes 32%, promyelocytes 4%, blasts 2%, eosinophils 1%, basophils 4%, lymphocytes 2%. Platelets 432 $\times10^9$/L; leukocyte alkaline phosphatase score 4; LDH 1151 U/L (N < 460 U/L); B12 vitamin levels 7329 pg/mL (N=180-900). *Bone marrow aspirate.* Hypercellularity with myeloid hyperplasia (94.2%). Decreased erythropoiesis 2%, and increased number of hypolobulated and small size megakaryocytes. Bone marrow biopsy: mild reticular fibrosis of irregular distribution. *Methods.* Chromosomal analysis was performed on the bone marrow. Samples were processed using standard short-term unstimulated cultures. Metaphases were G-banded. FISH was performed using locus-specific and whole painting probes. *Cytogenetic results:* a total of 50 metaphases were evaluated. Karyotype was 46,XY, der(9)t(9;22)(q13; q11), der(22) ins(22;9) (q11; q3421). RT-PCR revealed a b3a2 fusion transcript. FISH was reported as: ish der(9)t(9;22) (wcp22$^+$, BCR-ARSA$^+$), der(22) ins(22;9) (wcp9$^+$, BCR$^+$, ABL^{++}, TUPLE1$^+$). Therapy with hydroxyurea was started to induce cytoreduction (2g *qd p.o.*); α-interferon therapy was started one month later with escalating doses (up to 9MU/d) plus cytosine arabinoside 10 mg/m^2 s.c. for 10 days every 4 weeks. After one year of therapy the patient shows partial hematologic remission, with normal blood peripheral counts and differential leukocyte distribution and more than 60% of initial spleen size reduction. Nevertheless no cytogenetic changes have been observed. *Comments.* About 5% of CML cases have cytogenetic abnormalities other than Ph'. This is the first report of this unusal cytogenetic rearrangement. Clinical follow-up will determine the prognostic significance of this genetic abnormality.

BCR-ABL VARIANTS (B3A2,B2A2) IN PATIENTS WITH CHRONIC MYELOGENOUS LEUKEMIA. IS THERE A PROGNOSTIC SIGNIFICANCE?

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Chronic myelogenous leukemia (CML) is a myeloproliferative disorder that is characterized by a reciprocal translocation between chromosomes 9 and 22 resulting in the formation of the Philadelphia (Ph) chromosome. This abnormality is found in 90% of patients with CML and leads to a fusion gene bcr-abl. The majority of patients are diagnosed in the chronic phase of the disease and nearly all of them will transform to a blastic crisis through an accelerated phase. For the time being the only curative procedure for the disease is bone marrow transplantation, either autologous or from a compatible unrelated donor.

The aim of our study was to investigate whether there is a correlation between the most common Ph chromosome rearrangements b3a2 and b2a2 and the clinical outcome of the patients.

We evaluated 11 patients, 5 men with a median age of 54 years and 6 women with a median age of 62 years. The bcr-abl rearrangement was detected by reverse transcription polymerase chain reaction using samples of whole blood and bone marrow collected during the chronic or the accelerated phase of the disease. The b2a2 rearrangement was detected in 5 patients. Four were in the chronic phase of the disease and were treated with interferon and hydroxy urea and the fifth died after entering the accelerated phase. The b3a2 rearrangement was found in 6 patients. Two remain in chronic phase, two died from blastic crisis and two are in accelerated phase. It seems that patients with b2a2 rearrangement have a more benign course, sustain a longer chronic phase and are more easily managed with hydroxyurea and interferon. Whether the bcr-abl rearrangements should be considered as prognostic factors remains to be evaluated by prospective studies including larger numbers of patients.

EFFECTS OF TYPE OF CHEMOTHERAPY AND INTERFERON ON SURVIVAL OF CHRONIC MYELOID LEUKEMIA PATIENTS

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We conducted a retrospective study of 248 patients with chronic myelogenous leukemia (CML) who were treated at Ramathibodi Hospital from 1990 to 1999. There were 136 male and 112 female patients with a mean age of 39.0±15.7 years. The mean initial hemoglobin, white blood cell count, and platelet count were 9.90 g/dL, 213,317/mm^3, and 499,324/mm^3 respectively. The patients were treated with busulfan, hydroxyurea, both busulfan and hydroxyurea, and combination chemotherapy, with or without interferon alpha. We found that overall survival differed between groups of patients who received different kinds of chemotherapy, with the shortest being in the group that received combination chemotherapy (p < 0.001). Patients who also received interferon had a longer survival (p = 0.001). By using multivariable analysis, the factors that had a significant influence on patients' survival were percentage of blasts at diag-

nosis, using of interferon and age. In conclusion, the characteristics and prognostic factors of our patients are similar to those reported in the literature, including the benefit of using interferon-α.

A PROCLIVITY TO COMMIT SUICIDE DURING TREATMENT FOR CHRONIC MYELOID LEUKEMIA

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Background. The announcement to a patient of a diagnosis of chronic myeloid leukemia (CML) and the initial therapeutic proceedings are followed, in most cases, by an intense psychological reaction, particularly in young patients, who had not had any experience of others diseases before the onset of CML.

Case report. A 17-year old boy who had an intense social life and performed many physical activities started treatment for CML immediately after diagnosis without having been given adequate explanations or psychological support. The initial refusal to co-operate and undertake examinations and treatment, was followed by a total resignation accompanied by continuous self-destructive and even suicidal attempts. After a year`s intense psychological sessions and administration of antidepressant drugs onco-operative behavior was reduced. However, the patient's lack of co-operation persisted and periodically he displayed self-destructive behavior which resulted in his death.

Conclusions. In CML, psychological support and preparation of the patient is essential and should precede any other therapeutic approach.

DETECTION OF THE BCR/ABL FUSION GENE IN INTERPHASE AND METAPHASE CELLS OF PATIENTS WITH CHRONIC MYELOID LEUKEMIA AND OTHER MYELOPROLIFERATIVE DISORDERS

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BCR/ABL transcripts have been reported in patients with myeloproliferative disorders (MPD) other than chronic myeloid leukemia (CML), such as essential thrombocythemia (ET) and polycythemia vera (PV). In order to explore this interesting finding further we studied bone marrow samples from 63 patients with MPD (30 with Ph-positive CML, 15 with ET, 13 with PV and 5 with unclassified MPD) and 15 hematologically healthy individuals using fluorescence *in situ* hybridization (FISH) and nested RT-PCR. Until recently, the usefulness of FISH in interphase cells was limited by the relatively high false positive (FPR) and false negative rates (FNR). A new commercial kit (LSI bcr/abl ES, Vysis, IL), employing a dual color probe, offers the possibility of overcoming this problem. In this kit, the spanning abl probe is approximately 650 kb and the bcr probe 300 kb, thus producing four signals instead of three, the additional one coming from the residual abl on chromosome 9. Standard bcr/abl probes cover only 200 kb. Using the ES-LSI kit we found in the CML group a FNR in interphase cells of only 0.52% (range 0.1-1.2%) compared to 8.9% (5.7-12.5%) when the standard bcr-abl probe was used. The FPR in the control group was 0.33% instead of 6.2% (5-8%). Three patients with ET had slightly elevated bcr-abl signals in 6%, 9.8% and 14.8% of interphase cells. However, none of the metaphases examined from these pts was bcr/abl positive and PCR was also negative for bcr-abl transcripts. Two patients with a diagnosis of PV had very high numbers of bcr-abl signals (88% and 85%) and the fusion gene was present in metaphases too. Three patients with unclassified MPD had bcr-abl fusion gene signals in 8%, 14.2% and 62% of cells and the third was also PCR positive. Metaphases were not available from these patients. In conclusion: (1) in patients with MPD an increased number of bcr-abl signals can be found in interphase cells but not necessarily in metaphases too. We believe that high percentages of bcr-abl signals in interphase cells and/or presence in metaphases are consistent with CML while small percentages may represent occasional low expression of bcr-abl in fast proliferating hematopoietic cells; (2) the use of a probe spanning 650 kb of the breakpoint area in the abl gene impressively reduces the FPR and FNR in patients with MPD and could be useful in the diagnosis and monitoring of these disorders.

TIME TO MAJOR KARYOTYPIC RESPONSE TO IFN-A BASED REGIMENS IN CHRONIC MYELOID LEUKEMIA

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The aim of this study was to evaluate the time to achieve a partial (P< 35% of Ph'+ metaphases) or complete (C=0% of Ph' + metaphases) karyotypic response (KR) in 64 Ph' positive patients with chronic myeloid leukemia (CML) treated with IFN-alfa based regimens (IFN-α alone or in combination with s.c. or oral ARA-C). Fifty-six patients, evaluable for KR, were analyzed. Thirty-nine patients received IFN-α treatment early (<6 months) and 8 late (> 12 months) after diagnosis. The other 9 patients were treated shortly after diagnosis with IFN-α/ARA-C combination therapy. Seventeen major KRs were documented. Two, 12 and 3 patients treated with late or early IFN-α alone or IFN-α/ARA-C, respectively obtained PKR (7 patients) or CKR (10 patients). The median time to the best KR was 19 (4-72) months. Eight KRs were observed after more than (late KR) and 9 less than (early KR) one year of treatment. The 8 late KRs (4 P and 4 C) were documented 35 (18-72) months post-therapy. Three of 4 CKRs documented 19, 49 and 66 months post-therapy were preceded by a PKR. The 9 early KRs (3P and 6 C) were observed after a median of 9 (3-12) months of therapy. Four patients, who initially had a PKR, obtained

a CKR at month +6, +12, +21 and +24 post-therapy. Less than PKRs were documented after a median of 7 (4-12) months of therapy in 10 patients who later showed an improvement to major KRs before (5 patients) or after (5 patients) the first year of treatment. In the other 7 cases, the first KR documented was P in 5 patients and C in 2 patients; 4 responding before and 3 after the first year of therapy. In conclusion, late P or C KRs accounted for 47% of the overall major KRs observed in this series. Forty per cent of the major KRs observed, were obtained after more than one year of therapy, even in the subgrup of patients treated early with IFN-α based regimens.

MECHANISMS OF RESISTANCE TO THE TYROSINE KINASE INHIBITOR STI571 IN BCR-ABL POSITIVE CELL LINES

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Targeting the tyrosine kinase activity of Bcr-Abl with STI571 is an attractive therapeutic strategy in chronic myeloid leukemia (CML). A minority of CML cell lines and primary progenitors are, however, resistant to this compound. We have investigated the mechanism of this resistance in clones of the murine BaF/3 cells transfected with BCR-ABL (Baf/BCR-ABL) and in 4 human cell lines (K562, LAMA84, AR230 and KCL22) from which sensitive (s) and resistant (r) clones were generated by either methylcellulose plating or long-term (3 months) gradual dose-escalation exposure to the inhibitor. Although the resistant cells were able to survive in the presence of STI571, their proliferation was approximately 30% lower than that of their sensitive counterparts in the absence of the compound. The concentration of STI571 needed for a 50% reduction in the number of viable cells after a 3-day exposure was on average 10 times higher in the resistant (2-3 mM) as compared to the sensitive (0.2-0.25 mM) clones. The mechanism of resistance to STI571 varied among the cell lines. Thus, in Baf/BCR-ABL-r, LAMA84-r and AR230-r there was upregulation of the Bcr-Abl protein, associated with amplification of the BCR-ABL gene. In K562-r, there was no Bcr-Abl overexpression, but the STI571 IC_{50} for inhibition of Bcr-Abl autophosphorylation was increased from 0.5mM in the sensitive to 2.0 mM in the resistant clones. Sequencing of the Abl kinase domain in the latter revealed no mutations. The multidrug resistance P-glycoprotein (Pgp) was overexpressed in LAMA84-r indicating that at least two mechanisms of resistance operate in this cell line. KCL22-r showed neither Bcr-Abl upregulation nor a higher threshold for tyrosine kinase inhibition by STI571. We conclude that BCR-ABL-positive cells can evade the inhibitory effect of STI571 by different mechanisms such as Bcr-Abl overexpression, by reduced intake mediated by Pgp, and possibly by acquisition of compensatory mutations in genes other than BCR-ABL. These findings may have some bearing on the *in vivo* resistance to STI571 developed by some patients treated in the blast crisis of CML.

A COMPLETE HEMATOLOGIC AND MAJOR CYTOGENETIC LONG-LASTING RESPONSE IN CHRONIC MYELOGENOUS LEUKEMIA FOLLOWING TREATMENT BY INTERFERON

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There have been rare reports of complete hematologic and cytogenetic remissions of chronic myelogenous leukemia (CML) occurring after non-intensive chemotherapy or spontaneously. We report the case of a patient with Philadelphia-positive CML associated with typical bcr/abl molecular features and prolonged survival *off interferon-therapy*. A complete hematologic and major cytogenetic response was achieved after 10 months of IFN-α (IFN) therapy. Interestingly, this response was long-lasting, despite the withdrawal of IFN for over 6 years, while residual clonal hematopoiesis persisted, as detected by the PCR technique. The patient is still living a normal life, 15 years after the initial diagnosis of CML. Modulation of the immune response in patients who are *interferon-responders*, has been reported in literature. In our patient we, too, found an increase in absolute numbers of $CD3^+$ (1.7×10^9/L), $CD4^+$ (0.98×10^9/L), $CD8^+$ (0.74×10^9/L) and $CD16^+$ lymphocytes (0.34×10^9/L). These immunologic alterations, induced by the IFN-α therapy, which persisted even after the interruption of the treatment, could have a biological significance in the control of the disease and might be responsible for this atypical prolonged survival.

AUTOIMMUNE HEMOLYTIC ANEMIA DURING TREATMENT WITH α-INTERFERON FOR CHRONIC MYELOID LEUKEMIA: A REPORT OF FIVE CASES

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Among 80 patients with chronic myeloid leukemia (CML) treated at our Institution with alpha-interferon (IFN), we have observed 5 cases of autoimmune hemolytic anemia (AHA) (1 male and 4 females; mean age 51.1 years, range 22-59). At the onset of AHA, the patients had been receiving IFN therapy (27 to 63 MU per week) for 1 to 24 months (mean 11.4). Hemoglobin (Hb) levels ranged from 4.4 to 7.7 g/dL (mean 6.3). All patients showed a positive direct antiglobulin test (DAT), with panagglutinating IgG antibodies, low haptoglobulin levels and high reticulocyte count, LDH and indirect bilirubin values. Bone marrow examination showed marked erythroid hyperplasia dissecting myeloid cells. Interestingly, in 2 studied patients, FISH analysis did not show the Bcr-Abl fusion signal in erythroblasts, thus suggesting a non-

clonal erythroid response. In all patients IFN was immediately discontinued and substituted with hydroxyurea. Methylprednisolone (0.8 mg/kg) or equivalents were administered at full dose for 1 month and then progressively tapered, until suspension, within 3 months. Only 2 subjects required red cell transfusions. All patients stably recovered from their increased hemolysis, and the DAT test became negative. Four patients reached normal Hb levels; in the remaining patient Hb values never exceeded 10.5 g/dL. Four patients went into blastic crisis or accelerated phase 8 to 36 months after diagnosis of the AHA. Autoimmune phenomena are well known possible complications of IFN treatment. In our experience, AHA occurred in 6.2% of CML patients receiving IFN, a percentage unusually higher than that reported in other CML series. Such a complication, however, was never life-threatening and resolved after adequate treatment. AHA should be immediately considered as a possible cause of sudden, unexplained anemia in CML patients treated with IFN.

NOVEL TYPES OF BCR-ABL TRANSCRIPT IN CML PATIENTS

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We have identified two novel BCR-ABL fusion transcripts in CML patients.The first patient was diagnosed with chronic phase Ph+ CML, underwent allogenic BMT from an HLA-identical sibling donor, relapsed at month 12 post-BMT and showed no response to two escalating doses of DLI. RT-PCR analysis was performed at hematological relapse, as no sample from diagnosis were available. No amplification products were seen when primers for the P210 proteins were used, whereas using the primers for the e1a2 (P190) transcript, a band of higher molecular weight (195 Kd) with respect to the classical e1a2 control was observed. Sequencing revealed the presence of an in-frame fusion consisting of part of BCR exon 3, 44 nucleotides derived from ABL intron 1b and ABL exon 2. This rearrangement has therefore generated an abnormal BCR exon e3 in which the 3' sequence has been substituted by 44 nucleotides derived from the ABL intron 1b, creating a new BCR exon 3 that can be spliced to ABL exon 2 maintaining the correct frame of translation of the ABL sequences. The second patient was a chronic phase CML at diagnosis. Molecular analysis performed using primer set for P210 protein revealed, at the first step of RT-PCR, a band of 540 bp, 123 bp higher than the classical b3a2 rearrangement. Sequencing confirmed this finding, as a 123 nucleotides stretch was interposed between BCR exon 14 and ABL exon 2. This fragment was not derived from the BCR gene, neither from ABL gene; the search on the Gene-Bank database did not show any homology with known human genes. Anyway, since the fragment encode for a 41 AA polypeptide without stop codons, the ABL reading frame is maintained.

IMPACT OF TIME-DEPENDENT RESPONSE VARIABLES ON THE PROGNOSIS OF CHRONIC MYELOID LEUKEMIA

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The New Score for patients with chronic myeloid leukemia (CML) developed by the C.P.F.P. Group (*J Natl Cancer Inst 1998; 90:850*) could be validated as a reliable discriminator of three risk strata with clearly differing survival times. Based on the qualities of the New Score, the question was raised to what extent hematologic and cytogenetic response can provide additional and independent prognostic information on survival at time points of primary medical relevance in the course of treatment. The New Score was evaluable in a learning sample of 1,105 patients with early chronic phase CML, treated with interferon-alpha (IFN-α). Median survival time was 71 months. Stratified for risk groups, the 456 low risk patients had a median survival time of 98 months (5-year survival rate: 75%), the 497 intermediate risk patients of 66 months (5-year survival rate: 58%), and the 152 high risk patients of 42 months (5-year survival rate: 28%). Hematologic data were available for 899 cases and cytogenetic data for 775 cases. A complete hematologic response (CHR) (*Talpaz et al., Blood* 1987; 69:1280) was observed in 540 patients (60%), a major cytogenetic response (MCR) ($\leq$ 35% Ph-positive cells) in 220 patients (28%). Results of cytogenetic evaluations were only accepted if based on at least 20 metaphases. Of those responding fully, 434 patients (80%) had had their CHR within 9 months after starting therapy (CHR9) and were still alive at this medically and statistically most informative cut-point for hematologic response in the given sample. At the optimal cut-point for MCR, 21 months after starting therapy, 169 patients (77%) had had their MCR (MCR21) and were still alive. Continuous New Score values had a statistically significant prognostic association with CHR and with MCR. Based on landmark analyses at the respective time points, 9 and 21 months, neither CHR9 nor MCR21 provided additional prognostic information for patients with high risk according to the New Score. However, within the intermediate and low risk groups, both time-dependent variables provided four pairs of statistically significantly different survival curves when stratified according to response.

All median survival times are given from diagnosis; death and censoring times up to 9 and 12 months were taken into account for each risk group separately. Not all patients with hematologic data had data on cytogenetics and *vice versa*. Within group A, of 33 patients observed for > 8 years, only two died. The last 25 patients of group E were all censored alive after > 8 years and at a survival rate of 72%. These first analyses of time-dependent prognostic variables showed encouraging results. Further analyses, also

using different approaches, e.g. Cox models, will be performed.

Table 1.

Risk group	*Median survival*	*5-year survival rate*	*n*	*n died*
A Low, CHR9	not reached	82%	215	51
B Low, no CHR9	81 months	66%	146	51
C Intermediate, CHR9	76 months	68%	183	78
D Intermediate, no CHR9	61 months	52%	201	111
E Low, MCR21	not reached	84%	86	14
F Low, no MCR21	78 months	72%	176	66
G Intermediate, MCR21	not reached	79%	75	16
H Intermediate, no MCR21	63 months	56%	240	136

LYMPHOID BLAST CRISIS OF CHRONIC MYELOID LEUKEMIA: REPORT OF FIVE CASES TREATED WITH HYPER-CVAD / MTX-ARA-C REGIMEN

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Chronic myeloid leukemia (CML) is a biphasic disease with an initial chronic phase and a terminal blastic phase. To control the chronic phase there are several options which give good results. However, when the blastic phase appears, the options to control the disease are very few.

We describe five cases of CML patients with lymphoid blast crisis, diagnosed between April 1999 and April 2000, who were treated with Hyper-CVAD/MTX-ARA-C regimen. Treatment consisted of 8 courses of alternating intensive chemotherapy; courses 1, 3, 5 and 7 consist of cyclophosphamide, 300 mg/m^2, i.v., q 12h, given on days 1-3; vincristine 2 mg, i.v., given on days 4 and 11; doxorubicin 50 mg/m^2, i.v., on day 4; dexamethasone 40 mg/day, i.v. or orally on days 1-4 and 11-14. Courses 2, 4, 6 and 8 consist of methotrexate 1g/m2, i.v., given on day 1, with folinic acid rescue; and cytarabine 3g/m^2, i.v., q 12 h, for 4 doses, given on days 2 and 3. There were four males and one female with a median age of 46 years (28-54), and a median duration of 40 months of chronic phase (23-63). The treatment used in the chronic phase was hydroxyurea (HU) and interferon-α (IFN) in four patients and HU alone in one patient. At the diagnosis of blast crisis the median number of leukocytes was 18,700/μL (14,000-84,000) and in two patients splenomegaly was present. All the patients had Philadelphia chromosome (Ph$^+$) and in three, additional abnormalities were present. During induction phase all patients had fever: a microbiological agent without focus was identified in 2 of them and 1 patient had pneumonia without an agent being identified. The median number of days of neutropenia (<500/μL) was 20 days (20-28) and that of thrombocytopenia (< 20,000/μL) was 19 (17-28). One patient had hepatic toxicity that resolved after stopping itraconazol. All the patients had mucositis grade I/II.

After induction two patients reached complete remission (CR), one patient partial remission (PR) and two patients were resistant. The patients who entered complete remission are alive. One of them has 9 months of follow-up and was submitted recently, after 6 cycles of chemotherapy, to an allogeneic bone marrow transplantation with a mismatched graft from his mother; the second patient has been in complete remission for 4 months and is still receiving treatment with Hyper-CVAD/MTX-Ara-C. The patient in PR entered CR after the second cycle and continued the treatment until the sixth cycle, when progression of disease was documented; at this time a lineage switch to a myeloid phenotypic leukemia was detected. Salvage treatment with idarubicin, VP-16 and high doses of cytarabine was attempted without response. The patient is alive with progressive disease. One of the two resistant patients died at day +42 of a respiratory infection, after a second cycle. The second resistant patient is still alive with a follow-up of 7 months.

In conclusion, the Hyper-CVAD/MTX-Ara-C is a well tolerated regimen, with some activity in lymphoid blast crisis of CML. It seems that it can be used in some patients with success, especially those who could have consolidation with bone marrow transplantation.

QUANTIFICATION OF BCR/ABL EXPRESSION BY REAL-TIME RT-PCR IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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Chronic myeloid leukemia (CML) is characterized cytogenetically by t(9;22), corresponding to the bcr/abl rearrangement. The detection of bcr/abl hybrid transcript by RT-PCR is not predictive of disease progression. By contrast, the kinetics of bcr/abl mRNA expression might provide information for monitoring minimal residual disease or assessing response to treatment. A real-time PCR assay was developed and used to quantify bcr/abl mRNA in peripheral blood or bone marrow samples positive for bcr/abl rearrangement by RT-PCR. Total RNA extracted from the K562 cell line, serially diluted in total RNA from HeLa cells, was used as a standard for quantification. cDNA synthesis was performed using random hexamers with 1 µg of RNA, and 1/4 of cDNA was used for each amplification. PCR primers and fluorogenic probes were chosen in order to allow detection of both b3a2 and b2a2 transcripts. The mRNA encoding for the housekeeping gene GAPDH was used as a reference to normalize RNA quantity and quality and to monitor the efficiency of cDNA synthesis. Real-time PCR was carried out in duplicate using the GeneAmp 5700 Sequence Detection System (PE Biosystems). RNA derived from K562 cells was positive until 10^{-4} dilution, corre-

sponding to 1 K562 cell diluted in 10^4 HeLa cells (5 log dynamic range), with 7% intra-assay CV% and 12% inter-assay CV%. The real-time PCR assay was used to analyze 19 RNA samples from 10 patients. Results were expressed as ng of K562 total RNA with the same level of bcr/abl mRNA expression and were normalized to GAPDH mRNA expression (bcr/abl normalized Dose, nD). The bcr/abl nD ranged from 23.2 to 1,880 in samples collected at diagnosis and from 0.214 to 186 in follow-up samples. A significant correlation was observed between the % of Ph' chromosome and the level of bcr/abl expression (p<0.001, Spearman coefficient). *Conclusions:* 1. real-time PCR is a rapid and reliable method for quantifying bcr/abl mRNA expression; 2. the correlation between cytogenetic and quantitative PCR data indicates that it may represent a clinically useful method for monitoring residual disease.

RESULTS OF A NATIONAL PROSPECTIVE RANDOMIZED TRIAL COMPARING INTERFERON AND INTERFERON + LOW DOSE CYTARABINE IN EARLY CHRONIC PHASE CHRONIC MYELOGENOUS LEUKEMIA: RATES OF HEMATOLOGIC AND CYTOGENETIC RESPONSES AND EFFECTS ON SURVIVAL

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Between February 1994 and March 1997 the ICSG on CML recruited 837 patients with newly diagnosed chronic myeloid leukemia (CML): 542 eligible patients with Ph+ and/or bcr/abl+ CML were randomized to receive interferon (IFN) alone, target dose 5 IMU/m^2/day (266 pts) or IFN associated with 10-day monthly courses of low dose cytarabine (LDAC), 40 mg/day/sc (276 pts). The endpoints were: hematologic response (HR) at 6 months, karyotypic response (KR) and overall survival. There were no significant differences between the 2 groups as far as regards mean age, sex, hematologic features at diagnosis and Sokal risk distribution. The analyses were performed in March, 2000. At 6 months, 61% of patients in the IFN arm obtained a complete HR as compared with 71% in the IFN+LDAC arm (p=0.028). A major plus complete KR (>66% to 100% Ph-neg) was obtained in 54 patients (21%) of the IFN arm and in 81 patients (30%) of the IFN+LDAC arm (p=0.01). As far as concerns effects on survival as a function of assigned treatment, with a median observation period of alive patients of 44 months (range 12-72 months), and bone marrow transplants (BMT) in chronic phase (CP) censored observations, there was a trend in favor of IFN+LDAC but this difference was not significant (p=0.1). Data did not change if the BMT in CP patients were not censored or if the BMT patients were completely excluded. A previous interim report, with a shorter follow-up (median observation of alive patients: 24 months) showed a significantly better survival for those receiving IFN+LDAC.[1] After stratification of the patients in function of Sokal and Euro prognostic scores (low, intermediate and high risk categories) and evaluating the survival probability by treatment arm, there were no significant differences for Sokal score (any risk category) or for Euro intermediate and high risk patients. Among patients with a Euro low score, the survival curve of IFN+LDAC patients was significantly better than that of the IFN-treated patients (p<0.04).

1. *G. Rosti et al, 41st ASH meeting, New Orleans3-7/December 1999, abstract 2669.*

INTERFERON THERAPY IN CHRONIC MYELOID LEUKEMIA: COMPLIANCE AND SIDE EFFECTS IN A RANDOMIZED TRIAL, MRC CML 3

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A group of 298 patients were randomized to receive interferon (IFN) therapy as compared to chemotherapy for therapy of chronic myeloid leukemia (CML) in chronic phase. This demonstrated a survival advantage for IFN over chemotherapy: 5-year survival, 54% vs 37%. In fact, 261 patients were actually started on IFN therapy. The remainder did not start IFN therapy because of a decision to proceed to bone marrow transplantation (BMT) or because the disease rapidly transformed. The table gives the cumulative proportion abandoning IFN over time for a variety of reasons. By 3 years only 40% of patients continued to receive IFN therapy.

No. started IFN = 261	*S/E*	*BMT*	*Failure to control disease*	*Other **	*Total*
By 1 yr	28(11%)	13(5%)	12(5%)	7(3%)	60(23%)
By 2 yrs	51(20%)	27(10%)	24(9%)	10(4%)	112(43%)
By 3 yrs	67(26%)	38(15%)	32(12%)	19(7%)	156(60%)
By 4 yrs	78(30%)	42(16%)	44(17%)	26(10%)	190(73%)
By 5 yrs	84(32%)	44(17%)	51(20%)	31(12%)	209(80%)
> 5 yrs	90(34%)	45(17%)	54(21%)	36(14%)	225(86%)

**Includes 4NK, 14 deaths while on IFN, 18 other reasons*

Overall compliance, defined by the percentage of patients remaining in chronic phase(CP) who could be taking IFN and were, in fact, receiving it was 51% at 5 years.

Years since started IFN	*1*	*2*	*3*	*4*	*5*	*6*	*7*	*8*
Still in CP	207	159	129	92	63	36	21	12
% taking IFN	84%	74%	68%	59%	51%	47%	38%	58%

Overall 90 patients abandoned IFN primarily because of side-effects (34%); the type of side-effect was malaise/fatigue (13%), neurologic problems

(7%), cytopenias (5%), skin allergies (3%), bone/joint pain (2%), others (5%). Only one third of these patients had good control of the white cell count ($<10\times10^9$/L) on IFN alone when the drug was discontinued.

The median duration of IFN therapy was 75 months for durable (>1 year) complete cytogenetic responders (dCCR), 57 months for complete cytogenetic responders (CCR), 54 months for partial cytogenetic responders (PCR), 26 months for minor cytogenetic responders (MiCR) and 17 months for non-cytogenetic responders (NoCR) The dose of IFN received/week after 2 years was CCR 18MU, PCR 22MU, MiCR 20MU, NoCR 27MU.

These data can be used for better assessment of the cost-effectiveness of IFN therapy in the treatment of CML patients.

FEATURES OF Ph-POSITIVE CHRONIC MYELOID LEUKEMIA BLAST CRISIS: SINGLE INSTITUTION ANALYSIS OF 97 CASES

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Background. Chronic myeloid leukemia (CML), a stem cell disorder characterized by the Philadelphia chromosome, invariably terminates in blast crisis (BC) and the prognosis of patients in this phase of CML is extremely poor. On the basis of morphologic and immunologic analysis, the majority of the BC blasts are recognized as myeloid (My-BC), whereas 15-20% shows lymphoid characteristics (Ly-BC).

Patients and Methods. A total of 97 adult patients referred from 1980 to 1999 with a diagnosis of BC-CML were included in this analysis; 79 (81%) patients had My-BC and 18 (29%) a Ly-BC immunophenotypical pattern. To verify whether there are clinical and biological differences between My-BC and Ly-BC groups, we considered hematologic parameters (WBC, Hb, Plt counts), the molecular rearrangement of BCR-ABL type, extramedullary (EMD) BC, response to treatment and overall survival (OS).

Results. The Plt count at the onset of BC was 169 (range 6-1975) $x10^9$/L vs 55 (range 12-548) $x10^9$/L in Ly-BC and My-BC, respectively (p=0.006). The median Hb value was 10.8 g/dl in the Ly-BC group and 8.8 g/dL in the My-BC group (p=0.001). There was a statistical association between BC-EMD and immunophenotype: we found that 7 (39%) were Ly-EMD and 12 (15%) My-EMD (p = 0.04). There was an association between response to treatment and immunophenotypical pattern: 4 (5%) patients achieved complete remission in My-BC and 11 (61%) in Ly-BC (p < 0.0001). No differences were observed in the two groups as regards WBC count, the type of chimeric mRNA (b2a2 vs b3a2) and OS (Ly-BC 44 months vs My-BC 44 months).

Conclusions. In our series of CML-BC patients, the My-BC group had poorer prognosis in terms of response to treatment, although extramedullary BC was more frequent in Ly-BC. On the basis of our data, the BC immunophenotype seems to identify a disease with different clinical and biological characteristics. Future studies may help to define the biological and prognostic significance of the immunophenotypic pattern in CML- BC.

EXTRAMEDULLARY BLAST CRISIS IN CHRONIC MYELOID LEUKEMIA

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Background. The few studies on the incidence, evolution and prognostic significance of extramedullary blast crisis (EMD-BC) made in a large series of chronic myeloid leukemia (CML) patients suggested that CML with EMD-BC has poor prognosis in comparison with other types of BC.

Patients and Methods. Among 274 patients with Ph+ CML we reviewed 97 (33%) patients with blast crisis diagnosed between 1980 and 1999; 19 of the 97 (20%) had developed extramedullary disease (EMD).

Results. The sites involved were the lymph nodes in 12 (63%) cases, central nervous system (CNS) in 4 (21%) cases, suborbital zone in 1(5%) and CNS together with lymph nodes in 2 (11%); 12 (63%) were classified as myeloid (My-EMD) and 7 (37%) as lymphoid-type (Ly-EMD). There was a statistical association between BC-EMD and immunophenotype: we found that 7/18 (39%) and 12/79 (15%) were Ly-EMD and My-EMD, respectively (p = 0.04). Moreover, we observed a significant difference between the site of BC and the immunophenotypic pattern: 10 My-BC had lymph nodes involvement while 5 Ly-EMD showed a CNS localization, 3 having CNS EMD and 2 cases CNS plus lymph nodes involvement (p= 0.04). In terms of response to treatment of EMD-CB, we found that 11 (92%) My-EMD patients had failure to respond to treatment while 7 (100%) Ly-EMD patients achieved complete remission (p = 0.0002). The median overall survival (OS) in the BC group was not significantly different compared with that of BC-EMD patients (BC 46 months vs BC-EMD 32 months). In terms of OS, the immunophenotype in EMD-BC patients was not a prognostic factor (My-EMD 34.5 months vs Ly-EMD 28 months).

Conclusions. Our study suggests that the type (lymphoid or myeloid) of BC is an important feature in terms of extramedullary involvement and BC site. In fact, we found that Ly-EMD during BC is more frequently associated with a CNS localization than My-EMD. Furthermore, patients with Ly-EMD were more likely to respond to treatment than My-EMD patients. The significance of this difference is unknown, and further biological and clinical aspects need to be investigated.

AUTOIMMUNE ALTERATIONS ARE UNDERESTIMATED IN CHRONIC MYELOID LEUKEMIA PATIENTS TREATED WITH INTERFERON-α

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Background and rationale. Autoimmune alterations in patients with chronic myeloid leukemia treated with interferon (IFN)-α have been described to be rare (5%), but the published studies have been retrospective. *Purpose.* To study the incidence and severity of autoimmune phenomena in CML patients in its chronic phase (CP) treated with IFN-α.

Patients and methods. Forty-six consecutive patients with CML in 1st CP treated in a single institution. Routine tests included DAT and eluate, free thyroxine levels, thyroid stimulating hormone (TSH) and antithyroid antibodies. Other autoimmune tests were done according to clinical indications. The target IFN dose ranged between 4.5 and 9 MU/d.

Results. Out of 46 patients, 13 (28%) had autoimmune alterations, and 8 (17%) had two or more. The most frequent were anti-erythrocyte autoantibodies (8 patients), ANA (5), thyroiditis (4), Raynaud's syndrome (2), sarcoidosis-like dermatitis (2), autoimmune hepatitis (1), cryoagglutinins (1), Sjögren's syndrome (1). Autoimmune thyroiditis was the most frequent clinical complication, with hypothyroidism in 3 out of 4 affected patients. One patient with anti-erythrocyte antibodies developed autoimmune hemolytic anemia after a subsequent bone marrow transplantation. In one patient IFN-α was stopped because of severe autoimmune active chronic hepatitis. Patients with autoimmune alterations had a more prolonged exposure to IFN (4 vs 1,6 years; p=0.02) and the association with female sex was strong and significant (69.2% vs 33,3%, X^2:4.8; p=0.02) There were no significant differences between the two groups in age, stage of disease, hematologic toxicity or IFN dose. *Association with response.* Cytogenetic response was more frequently seen in patients who developed autoimmune phenomena (76.9% vs 42.4%, X^2=4.6, p=0.031). In 7 out of 13 patients the autoimmune alteration was detected after achieving the cytogenetic response. The Kaplan-Meier estimated probability of obtaining a cytogenetic response was significantly higher in patients with autoimmune alterations (p=0.02), but there was no difference in terms of major cytogenetic response.

Survival. With a median follow-up of 5.6 years (1-10.4), the estimated overall survival at 8 years was not significantly different (0.51±0.15 vs 0.58±0.12; p=0.5). *Conclusions.* 1) Autoimmune alterations are seen in more than a quarter of IFN-treated CML patients, and sometimes are clinically relevant. Anti-erythroid and anti-thyroid antibodies are the most frequent abnormalities. 2) Patients with autoimmune complications obtained more, and earlier, cytogenetic responses. Given the immunomodulating effects of IFN-α, these facts merit further consideration.

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INTERMEDIATE VERSUS STANDARD DOSES OF INTERFERON-α IN CHRONIC MYELOGENOUS LEUKEMIA PH+: NO SIGNIFICANT DIFFERENCES IN CYTOGENETICIC RESPONSE OR TOXICITY

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Spanish Group on CML and IFN alpha2a.

Purpose. To study the efficacy and tolerability of two different doses of interferon-α (IFN-α) in the treatment of chronic myeloid leukemia (CML) in its chronic phase (CP).

Patients and methods. Consecutive patients with CML in CP were entered in a multicenter randomized trial of intermediate (group I) versus standard dose (group S) of IFN-α. The target dose was 4.5 MU/day in group I and 9 MU/day in group S. Hydroxyurea was administered prior to randomization to lower leukocyte counts, and was subsequently allowed whenever necessary. Hematologic response, cytogenetic response, toxicity, probability of transformation, survival and treatment costs were analyzed.

Results: Out of 123 enrolled patients, 109 were suitable for analysis (53 in group I and 56 in group S). There were no significant differences between the two groups in age, stage of disease, time from diagnosis to IFN, duration of treatment or follow-up. The patients were distributed in stages (MDACC) as follows: 57% stage I, 31% stage II and 11% stage III.The mean treatment duration was 460.1±288.9 days in group I and 496.8±381.7 days in group S. Median follow-up of the series: 2.1 years. The mean dose of IFN-α was 3.88±1.07 MU/day (group I) and 6.7± 2.01 MU/day (group S) (p<0.00001). Patients in group I received more hydroxyurea: 0.48±0.5 vs. 0.30±0.32 g/d (p=0.05). Hematologic response: this was complete in 84.6% and 83.9% and partial in 7.7% and 12.5% in groups I and S respectively. Cytogenetic response in the first year: this was evaluable in 41 and 43 patients of groups I and S, respectively. In these patients, it was complete in 14.6 and 11.6%, partial in 19.5% and 14.5% and minimal in 22% and 28% in groups I and S, respectively (p=0.9). Time to reach a complete cytogenetic response was 279.7±113.9 days in group I and 444.5±243.4 in group S (p=0.09). The estimated probability of obtaining any cytogenetic response or a major one was not significantly different. Toxicity: no significant differences in toxicity (general status, GI, skin, liver, hematologic) were found. IFN-α was stopped in 9 patients in group I and 10 in group S because of tox-

icity. Survival: the estimated overall survival at 3 years was 0.70 and 0.84 respectively (p=0.21). Costs: the median treatment cost per day was 32 ¢ in group I and 67¢ in group S (p<0.00001).

Conclusions. Intermediate doses of IFN-α for the treatment of CML appear to produce no significant differences in hematologic response, cytogenetic response or toxicity when compared to standard doses, and allow significant cost savings.

P-GLYCOPROTEIN FUNCTIONAL ACTIVITY IN CHRONIC MYELOID LEUKEMIA

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Multidrug resistance (MDR) is the protection of the tumor cell population against numerous drugs differing in chemical structure and mechanisms of influence on the cell. P-glycoprotein (Pgp) mediated MDR is believed to be one of the major causes of failures of chemotherapy of human malignancies. Prognostic value of Pgp functional activity in patients with chronic myeloid leukemia (CML) has not been established. The main aim of this study was to understand whether elevation of Pgp functional activity is one of the causes of patient's resistance to chemotherapy. Fifty patients in chronic phase (CP) and blast crisis (BC) of CML were studied. Some patients were investigated several times during the blast crisis of CML. Pgp expression and Pgp function were analyzed by flow cytometric analysis of UIC2 binding and of Rhodamine 123 retention by the cells. We found that Pgp was expressed by peripheral blood cells (PB) more often in the BC CML than in CP. In some CP patients PB cells demonstrated increased Pgp functional activity (enchanced Rh123 efflux) while Pgp expression was not found. Sequential studies of Pgp activity in 11 patients showed that Rh123-effluxing cells did not disappear from PB during the course of therapy, while our previous examinations of more then 40 patients demonstrated that Pgp expressing cells (UIC2+) disappeared during therapy both in CP and BC. The prognostic value of Pgp functional activity will be discussed.

CIRCULATING FISH+ CELLS EXPRESSING THE BCR-ABL GENE AND TRANSPLANTABLE INTO NOD/SCID MICE IN A PATIENT WITH CHRONIC MYELOID LEUKEMIA IN STANDARD COMPLETE CYTOGENETIC REMISSION FOR OVER 40 YEARS FOLLOWING BUSULFAN-INDUCED HYPOPLASIA

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Prior to bone marrow transplantation (BMT), chronic myeloid leukemia (CML) was regarded as an inevitably fatal disease. However, RT- PCR was negative in one patient 30 yrs after busulfan induced hypoplasia (BuIH) (*AM Sproul, Leukemia, 1990*). This is why we were surprised by our recent findings in B.Pe, whom we have been following since 1956. At the age of 3, he presented with a fully developed adult-type CML. After an episode of severe BuIH lasting one year, he recovered and has been perfectly well since, with serial normal blood counts and bone marrow karyotypes. But RT-PCR, done in 1996 for the first time, revealed the presence of small amounts of b2/a2 mRNA (ratio of BCR-ABL/ABL transcripts < 0.01%), and this was repeatedly confirmed. Moreover, D-FISH revealed around 2% Ph^+ circulating cells on several occasions. Such results are similar to those of other patients of ours who had BuIH and compare with those reported by Chomel *et al.* in cases successfully treated by interferon or BMT (*Blood, 2000; 95:404*). Determining the nature and characteristics of the cells capable of maintaining the Ph^+ clone is of obvious interest and B.Pe's mononuclear cells were inoculated into NOD/SCID mice as a possible approach to investigating this. Indeed, RT-PCR showed a striking result: 3/6 animals had b2/a2 positive circulating cells at 4 months. Studies are underway to determine the proportions of BCR-ABL^+ elements among B.Pe's clonogenic cells and in different tissues of the sacrificed mice. We are also attempting to establish whether the limited proliferation of these cells is due to intrinsic and/or extrinsic factors, among which immune reactions may be suspected of playing a role. Junctional b2-a2 peptides or other fragments of p210, are known to bind to some of the few HLA molecules studied thus far by different groups and some of the MHC proteins of our A28, A31, B7, B51, DRB1 0301/DRB3 0101, DRB1 0701 DRB4 01 /patient were reported to react with Bcr/Abl. Therefore his reactivity to CML antigens is being explored in several ways. Whatever the lineage and level of differentiation of these cells, a risk of expansion, transformation or therapy-induced disorder may still exist and his stem cells should perhaps be harvested. One may also wonder whether the tyrosine-kinase inhibitor STI 571 could eradicate B.Pe's Ph^+ cells. Finally we think this is far more than an anecdotal case: it demonstrates that the persistence of stable minimal residual CML cells may be associated with a very durable remission, in relation to factors remaining to be determined. It also raises the question: what is the definition of cure in CML?

PRESENCE OF BCR-ABL GENOMIC REARRANGEMENT DETECTED BY FISH IN CHRONIC MYELOID LEUKEMIA PATIENTS IN COMPLETE CYTOGENETIC REMISSION

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Conventional and molecular cytogenetic studies are performed in clinical practice to monitor the effectiveness of treatment in patients with chronic myeloid leukemia (CML). In the majority of preliminary studies of FISH, the use of BCR-ABL DNA probes that

detected only a single BCR-ABL fusion signal hampered the precision of the percentage of false-positive nuclei. New FISH probes (D-FISH probes) detect BCR-ABL fusion in interphase nuclei with a false-positive signal rate close to zero. Such probes have been employed in the current study. To date we have performed FISH in 13 patients (9 men and 4 women) in complete cytogenetic but not molecular remission, either after interferon (IFN-α) therapy (7 cases) or allogeneic bone marrow transplantation (BMT) (6 cases). At diagnosis, 12 cases presented the classical t(9;22) (q34;q11) translocation, without additional abnormalities and 1 patient had a complex chromosomal rearrangement: t(6;9;22) (p21;q34;q11) and an additional add(20)(p11). The type of chimeric transcript was assessed by qualitative RT-PCR experiments; all patients had the typical b2a2 or b3a2 transcript. After treatment with IFN- α or allogeneic BMT, the monitoring of the disease mainly involved cytogenetic analyses. When the patient showed at least 50 Ph- metaphases and a positive RT-PCR assay, FISH was performed. A minimum of 500 cells in interphase were scored for each sample by FISH. The final results were expressed as percentages of nuclei with fusion signals. Negative FISH control studies were performed in bone marrow from 7 patients with hematologic disorders other than CML. The cut-off limit for BCR-ABL positivity was calculated at 0.5% of positive nuclei. Persistence of the BCR-ABL genomic rearrangement was shown in all patients: FISH detected 0.8 to 4.4% nuclei with a BCR-ABL fusion gene. Recent studies showed that FISH can also be positive in patients in complete cytogenetic remission, when RT-PCR were negative or weakly positive. These findings, together with the molecular data, suggest that a low number of non-proliferative neoplastic cells persists in patients in complete cytogenetic remission. Therefore, these patients need to be monitored by FISH and RT-PCR methods in order to evaluate minimal residual disease and their risk of relapse.

REARRANGEMENT OF ABR, AN ACTIVE BCR-RELATED GENE ON CHROMOSOME 17P, WITH ABL IN A CML PATIENT WITH THE CLASSICAL B3A2 TRANSCRIPT

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While we were testing by nested RT-PCR a series of P210-positive CML patients at diagnosis for the presence of alternative splicing sites of BCR gene localised downstream of M-bcr, we found a case in which ABL exon 2 (a2) is rearranged also with ABR gene. ABR is an active BCR-related gene located on chromosome 17p; ABR displays a great homology with BCR (68% identity), interacts with members of the Rho family in cellular signalling and is particularly expressed in the brain. In our case the junction was between ABL exon 2 and a region of ABR corresponding to BCR exon 16, therefore outside M-bcr.

Conventional cytogenetic performed on bone marrow and FISH analysis on interphase nuclei with a BCR-ABL translocation DNA probe showed a classical t(9;22). Metaphase FISH was done with a 17 painting probe, an ABL cosmide probe and a centromere probe for chromosome 9; this analysis demonstrated that chromosome 17 doesn't rearrange with any other chromosome.

We therefore tested the patient's sample with a set of primers ABR-specific and located 3' than the region homologous with M-bcr, to avoid the possible amplification of the classical BCR-ABL rearrangement. RT-PCR pointed out a specific band, detectable only at the second step, indicating a small amount of ABR-ABL transcript. It is plausible that there is a small population of cells of unknown Ph status (i.e. Ph+ or Ph-) that bear an atypical fusion between ABL and the BCR-related gene, ABR. Further studies are required to investigate the prevalence of ABR-ABL expression in patients with BCR-ABL rearrangements and its biological and clinical significance; however, this finding shows the tendency of ABL and BCR (or related genes) to join.

ALTERNATIVE SPLICING OF BCR GENE OUTSIDE M-BCR IN PATIENTS WITH P210-POSITIVE CML

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Philadelphia-positive (Ph+) CML patients are usually characterised by BCR-ABL rearrangements involving BCR exons 13 or 14 (e13 and e14, or b2 and b3), and ABL exon 2 (a2) resulting in the hybrid protein P210: on chromosome 22 the breakpoint is located in the so called "major breakpoint cluster region" (M-bcr). In rare cases the breakpoint on chromosome 22 is located downstream of M-bcr, in a region called m-BCR, and involves BCR exon 19 (e19) and ABL exon 2, which maintain its reading frame and gives origin to protein P230.

As we found that a P230-positive CML patient was also expressing some amount of e14a2 (P210) transcript, we decided to investigate if other CML cases positive for the e13/e14a2 (i.e. P210) transcripts were indeed P230 (e19a2 cases) expressing also the classical P210 hybrid protein. Therefore we used a set of primers located downstream to M-bcr, in BCR exon 15 (e15) and we tested 100 classical CML patients at diagnosis.

Using a nested RT-PCR we found three cases with bands shorter than the P230-positive control. The sequence of one of these bands showed a fusion between BCR exon 18 (e18) and ABL exon 2, a rearrangement that does not maintain ABL correct reading frame.

Our data suggest that approximately 2-3% of the CML cases with the e13/e14a2 (P210) transcripts have a breakpoint 3' to the classical M-bcr region and express also small amount of alternative BCR-ABL rearrangement involving various BCR genes.

IFN AND YNK01: RESULTS OF A ICSG ON CML PROTOCOL

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Rationale: Currently the golden standard treatment of CML in chronic phase (CP) is based on Interferon-α (IFN) associated with low dose Ara-C (LDAC). A phase II, single arm, clinical protocol based on the combination of IFN with the oral formulation of LDAC (YNK01) has been performed by the ICSG on CML to evaluate the efficacy (hematological and cytogenetic responses) and toxicity of the association. In a 12 months period (1/98-1/99), 92 patients with a newly diagnosed Ph+ and/or bcr-abl+ CML in CP, not previously treated, have been enrolled: they received IFN (3 IMU/sm/day for the first week up to 5 IMU/sm/day from the second week onward) and YNK01 (600 mg/day/14 days a month for 12 months; dosage was adjusted monthly according to hematological tolerance and toxicity ±300 mg, maximum dose 1200 mg/day/14 days a month). Hydrea was allowed before study drugs for high counts, not permitted during the first three months and then allowed to control disease expansion. Patients (pts): 90 pts are evaluable; gender distribution is 44/46 males and females respectively; the median age at diagnosis was 51 years (range: 22-71) and the Sokal risk distribution (87/90 evaluable) was 33 (38%) for low, 40 (46%) for intermediate and 14 (16%) for high risk group respectively. Results: All the analysis has been done on an intention to treat basis. 63/90 (68%) pts obtained a complete hematological response (HR) and 11/90 (12%) a partial HR, 11/90 (12%) were refractory, 5/90 (8%) are not evaluable (early treatment discontinuation); 55/63 (87%) complete responders got the result within 3 months from starting therapy. As for the cytogenetic response (CR), at 1 year, 13/90 (14%) pts showed a minimal CR (Ph neg: 1-32%), 13/90 (14%) pts a minor CR (Ph neg: 33-65%), 12/90 (13%) pts a major CR (Ph neg: 66-99%) and 9/90 (10%) pts a complete CR. The HR and the CR rates at 1 year are in the range of equivalence with respect to a previous experience of ICSG on CML (Protocol CML 94 - arm A) with IFN and s.c. LDAC (40 mg/day/10 days a month). We got disappointing results with the compliance to this intermittent schedule of YNK01; particularly the gastro-intestinal toxicity was high. During the first 3 months, 40 to 49% of the pts had nausea/vomiting (mostly grade I and II, only 4/93 recorded toxic episodes were grade III), 25 to 29% had diarrhea (mostly grade I and II, only 3/56 episodes grade III) and 20 to 28% had anorexia/weight loss (mostly grade I and II, only 1/69 episodes grade III). For comparison, in Protocol CML 94 - arm A (IFN + LDAC) during the first 3 months 24% of the pts had nausea/vomiting (mostly grade I and II, only 3/66 episodes were grade III and 2/66 grade IV), 3% had diarrhea (grade I or II) and 9% had anorexia/weight loss (mostly grade I and II, only 2/24 episodes grade III and 1/24 grade IV). *Present status (median observation: 18 months):* 70 pts are alive in CP, 8 alive in accelerated /blastic phase (ABP), 6 alive after BMT, 1dead in CP, 2 dead in ABP, 3 dead after BMT. Conclusions: The combination is active as for HR and for CR but compliance at this schedule is low. *Future directions:* Based on these results, the ICSG on CML planned a new clinical trial (open to accrual in April 2000) where IFN is scheduled at the same dosage whereas YNK01 at 200 mg/day continuously starting from the 3rd month onward.

SECOND CHRONIC PHASE BEFORE TRANSPLANTATION IS CRUCIAL FOR IMPROVING SURVIVAL OF BLASTIC PHASE CHRONIC MYELOID LEUKEMIA

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Prognosis of patients with blastic phase chronic myeloid leukemia (BP-CML) is extremely poor, even when allogeneic stem cell transplantation (allo-SCT) is performed in this phase. Fludarabine plus high-dose cytarabine has shown valuable activity in acute myeloid leukemia (AML). Since successful outcome after transplantation seems to depend, in AML and in chronic phase CML, on disease status at the time of transplantation, we tested whether use of FLAN induction before allo-SCT may be useful in BP-CML. From January 1988 to June 1998, 20 patients with BP-CML were studied: 10 patients received FLAN induction chemotherapy before proceeding, if a suitable HLA donor was found and clinical conditions were adequate, to early allo-SCT, whereas 10 patients were submitted to BMT without remission induction. Overall 8/10 (80%) patients achieved second chronic phase after 1 course of therapy with FLAN; 1/10 (10%) showed a partial response and 1 (10%) was refractory. The refractory patient did not proceed to allo-SCT, nor did one patient who obtained second chronic phase but lacked a suitable HLA donor. A further patient who achieved second chronic phase but experienced early relapse with rapid regrowth of the disease was not submitted to allo-SCT. Thus 7 patients (6 in second chronic phase and 1 with partial response) were submitted to allo-SCT within the 3 months following FLAN. Of these, the patient who had shown only partial morphologic response to FLAN obtained karyotypic, morphologic and molecular second chronic phase after allo-SCT, but relapsed within 3 months of the transplant. Of the other 6 patients transplanted in second chronic phase, all obtained molecular remission, four are still in second chronic phase, at intervals ranging from 10 to 54 months, one died of infection having relapsed 14 months after SCT, and one died of transplant-related complications in second chronic phase. Mean duration of second chronic phase and survival (analyzed on intention-to treat basis) after allo-SCT are both significantly longer than in the group of 10 BP-CML patients submitted to allo-SCT without FLAN remission induction treatment [22.4 (range 1-61) vs 3.5 months (range 1-10) and 22.7 (range 2-61) vs

6.4 (range 1-16) months respectively], even though 9/10 of the patients not submitted to FLAN obtained morphologic second chronic phase after BMT, and 4 and 3 obtained cytogenetic and molecular second chronic phase, respectively.

We conclude that FLAN induction therapy followed by early allo-SCT appears effective in the treatment of BP-CML and could provide a possibility of cure for BP-CML patients, deserving wider study in the context of a multicenter trial.

UPDATING THE PROTOCOL ROFERON/CML: A LONG-TERM FOLLOW-UP

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We compared recombinant interferon α-2A with conventional chemotherapy (hydroxyurea or busulfan) in a prospective trial that enrolled 322 patients with chronic myeloid leukemia (CML), between July 1986 and July 1988. The patients were randomly assigned to be treated either with α-interferon (218 patients) or with conventional chemotherapy (104 patients). Serial statistical analyses and several papers published in the last few years showed the advantage of α-IFN treatment in term of survival duration and karyotypic response rate.

Updating the study to March 2000, 14 years after the start of the trial, the median follow-up of living patients was 142 months (ranging from 121 and 158 months), and the survival analysis confirmed that survival was longer in the α-IFN arm than in chemotherapy arm (median 76 months vs 52, p=0.001). The difference was strongly associated with karyotypic response (KR): no statistically difference in survival was detected between chemotherapy patients and α-IFN patients who had no karyotypic response. Sixty-six patients are still alive; 53 in the α-IFN arm and 13 in the chemotherapy arm.

In the α-IFN group, 17/53 alive patients were submitted to allogeneic BMT, while 36 are in first chronic phase; 29/36 had a KR at least once and 14 patients are at present in major or complete KR and receiving low-dose interferon. BCR/ABL transcript was not detected by molecular methods in 4/14 patients with a complete KR.

In the chemotherapy arm 4 out of 13 alive patients were submitted to allogeneic BMT, 2 are in accelerated/blastic phase and 7 are in chronic phase (6 of them had some degree of karyotypic conversion, even if minimal, during the follow-up).

Late α-IFN treatment related toxicity was neither more frequent nor different from early toxicity, but grade 3-4 side effects were unusual; the main causes of treament discontinuation were chronic fatigue and arthro-myalgias. We observed a secondary tumor in 4 patients in the α-IFN arm (2%) and in 3 patients in the chemotherapy arm (3%); so the development of secondary neoplasia appeared to be unrelated to the type of treatment.

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