Autologous Bone Marrow Transplantation

Proceedings of the Sixth International Symposium

Edited by
Karel A. Dicke
Armand Keating

and Guest Editors
N.C. Gorin
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The organizers wish to thank Mrs. Kathryn White who was instrumental in the organization of the meeting as well as in putting the proceedings together. Also, her contribution to the next meeting will be significant.

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Without the generous support of our industrial colleagues, Caremark, Bristol-Myers, Glaxo, Immunex, Amgen, and Lederle, this meeting could not have been held. Hopefully in the future this teamwork will be continued.

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Preface

The Sixth International Symposium on Autologous Bone Marrow Transplantation was a great success. New avenues were discussed such as the use of immune modulation after transplantation, the use of radiolabelled antibodies incorporated into the transplantation conditioning regimens, the use of peripheral stem cells harvested after mobilization, and the use of stem cell concentrates for transplantation. It is amazing and exciting that so many new developments have surfaced in this symposium. The field is maturing since randomized studies in leukemia and lymphoma to compare high dose therapy with standard treatment have been initiated. The first results are very interesting and are very promising in favor of the concept, "more is better". The quality of this meeting certainly motivates us to organize the 7th symposium in 1994. The organizers are looking forward to the continuous new flow of information as a follow-up of what has been published in these proceedings.

Karel A. Dicke
Armand Keating

December 2nd and 3rd, 1992
Houston, Texas

Session I: Leukemia

UK MRC10: EVALUATION OF AUTO-BMT IN ACUTE MYELOID LEUKAEMIA

A.K. Burnett, A.H. Goldstone, R.F. Stevens, l. Hann, J. Rees, R. Gray and K.Wheatley, on behalf of the Adult and Childhood Working Parties.

Plentiful anecdotal data has suggested that autologous BMT will reduce relapse risk for a substantial proportion of patients with AML. In previous MRC Trials (AML8 or 9) which ran through most of the decade to 1988, investigators were given the option to perform autologous BMT in first remission if they wished. While this non-randomized assessment is not free from sources of bias, at least prior therapy and time censoring effects were known. Patients who received auto BMT have a survival of 67% at 5 years compared with 55% for trial recipients of allografts, and 45% for those who received chemotherapy alone. All such data persuaded the MRC Working Parties to commence a new trial to better define the role of auto BMT in patients with AML under 55 years old.

The major questions were: [1] does transplant (auto or allograft) confer any additional benefit to patients who have already received intensive chemotherapy? [2] Is it as effective to collect marrow in first remission but reserve the autograft for those who relapse and enter second remission? The protocol design is shown in figure 1. All patients who fulfill the criteria for AML - including those who may have had an antecedent hematological disorder - are randomized to receive either DAT (3 + 10) and (3 + 8) or ADE (10 + 3 + 5) and (8 + 3 + 5) as the first two courses. Remission status is checked after each course. When CR is not achieved after the second course, participants are free to remove the patient from protocol for alternative therapy. All patients will then receive a further 2 courses (MACE and MidAC) of intensive treatment. During this time the availability of a sibling donor will be established. Those patients for whom allograft is not available have bone marrow harvested immediately before the MidAC course, and are randomized to have Auto BMT using cyclo+TBI with unpurged marrow or to STOP treatment. Those randomized to STOP would receive an autograft if they relapse, and enter a second remission. Patients who relapse within 6 months of the harvest are recommended not to receive an autograft using that marrow because the risk of occult contamination is considered high. The second remission autograft will use Busulphan/Cyclophosphamide myeloablation because the data available at the time using TBI in CR2 was not encouraging. It was recognized that the four chemotherapy courses were of an induction level of intensity and that this may prevent some patients progressing through to BMT, and that the toxicity of BMT might be greater than anticipated. On the other hand it was regarded as important to minimize the early relapse risk (censoring effect) and ensure that the best possible chemotherapy was available for comparison.

TRIAL PROGRESS

The trial opened in late summer 1988 and involves physicians from over 100 treatment centers in the UK, with a very important collaboration from New Zealand. By October 1992, 1148 patients had entered. Of the 20% of trial entrants who were children (<15 years), 93% entered remission, whereas 79% of adults achieved CR (overall CR 82%). Of remitters, 70% achieved the CR after course 1.

Within the older subgroups the remission rate did reduce slightly with age (15-25, 85%; 26-35, 86%; 36-45, 79%; 46-55, 75%). As previously reported there was significant delay in haemopoietic recovery after courses 3 and 4, introducing logistic difficulties in progressing patients through the schedule. There was little difference between DAT or ADE with respect to clinical toxicity recorded; haematological toxicity was not markedly age-related and in the first 2 courses was marginally less for ADE. The actuarial relapse in the first 6 months was less than 10%, thus minimizing time censoring effects due to relapse.

PROGRESS TO TRANSPLANTATION

Of 760 patients eligible for the second randomization or allograft option, 196 had an HLA matched sibling donor, and would normally be expected to proceed to allogeneic BMT. At present the statistical center is aware that 128 transplants have been completed. Of the remainder, several will be scheduled or underway - at present it is not possible to determine how many will never be transplanted because of prior death or relapse, or other reasons. Anxiety that the prior intensity of chemotherapy would increase transplant-related mortality was not confirmed, with an observed procedural death rate of 19%.

Sixty-two patients (i.e. 8%) relapsed or died in CR before they could be randomized, or in some cases receive an allo BMT. Physicians, or the patients themselves, elected to have an auto BMT for various reasons, including in some cases in which there was anxiety about an increased relapse risk because of delay to enter CR or CNS disease. In a substantial number of patients (n=174, i.e. 25% of those available) either the physician or the patient elected for no further treatment and they elected for the STOP arm. There was a range of reasons for this election being made - in several cases haematological recovery was very slow, or incomplete following chemotherapy. Most, but not all these patients completed the scheduled chemotherapy.

Two hundred and sixty patients, i.e. 52% of available patients, have undergone the main randomization to Autograft or STOP arms. We are aware of around 90 of these autografts being completed, some are pending or in progress, but a small number died or relapsed between the time of randomization and getting to transplant. Final analysis will be on an "intention to treat" basis. The procedural related mortality is 8-9% which is perhaps slightly higher than that reported from previous single center studies. As with previous experience protracted thrombocytopenia is a feature of the autograft recipients compared with the allografts (Platelets to 50 x 109/1 median = 75 days auto: 20 days allo).

OUTCOME FOR RELAPSED PATIENTS

A major question addressed by this trial is whether it is feasible to reserve the autograft for those who relapse and enter a further remission. The option to use the autograft as the primary treatment of the relapse was not considered fea-

sible in an environment where access to a transplant unit usually involved some delay. In the event, 9 patients who relapsed were autografted in relapse - only one survives, with relatively short follow-up.

One of the early concerns was whether re-induction would be more difficult given that the first line treatment was much more intensive than in previous trials. Our previous trials had found a dominant effect of the length of first CR on the chances of entering remission. In fact this pattern of reinduction in AMLI0 has been at least as good (duration of CR <6mo 40%; 6-12mo 45%; >12mo 78%). Within these subdivisions DAT or ADE was at least as successful as alternative, usually more aggressive or exotic, combinations (table 1). It should be pointed out that these data are not randomized and there may well be selection biases in what re-induction schedule was given.

Of 170 patients who relapsed, of whom the statistical center is aware, 48 achieved a second CR. It should be noted that not all patients had an attempt at reinduction. Of the 48 CR2 patients, only 18 so far have received an auto BMT with a survival of around 30% on relatively short follow-up. This mimics the single center and registry experience. However, this was a selected patient group because 30 eligible patients did not receive the second CR autograft because of relapse (n=4) or early death (n=16) or were not considered fit. Ten of this group remain in CCR. This preliminary experience suggests that only a small number of patients who relapse in practice get to autograft. It therefore remains to be seen whether the adoption of a delayed transplant strategy will make any significant impact on survival in AML.

OVERALL TRIAL OUTCOME

No analysis relating to the major trial questions has so far been undertaken but is intended by late 1994. However, entrants to this trial are enjoying a significantly better outlook than MRC Trial entrants in the previous decade, even when the influence of the pediatric sub-group is allowed for, Such benefits over AML9 are apparent even if the transplanted groups are excluded from both trials. This suggests that the recipients of chemotherapy alone are enjoying an important benefit but until complete analysis is performed we are not in a position to know whether or not any of the BMT options bring additional benefit on top of that.

Preliminary inspection has suggested certain subgroups of patients who are enjoying a better than average survival. These include those with an 8:21 or 15:17 translocation and patients who enter remission with the first course. Whether these are the same patient group is not yet clear. Nor does this infer that they do or do not derive benefit from a transplant option. But these could clearly be used as landmarks for more individually oriented risk-directed therapy in the future. Interestingly, the experience of the EBMT Group suggests that purging the autograft will not benefit patients who enter remission quickly, presumably because they already are a good risk group. Our 4 year accrual of 1150 patients has produced 950 patients in remission, of whom a subset of around 250 fail to enter remission promptly, and might, as one option for the future, benefit from a purging approach. This is approximately the patient number which would need to be recruited to a randomization to confirm, with sufficient statistical confidence, the apparent benefit of purging in this subgroup.

Table 1.
Achievement of Second Remission

Reinduction therapy	Duration of Ist CR			
	< 6 months	6-12 months	> 12 months	Total
ADE or DAT	40% (4/10)	45% (9/20)	78% (14/18)	56% (27/48)
Other multiple agent	22% (6/27)	25% (5/20)	14% (1/7)	22% (12/54)

This is not a randomised comparison, so patient selection could be a factor (e.g. poor-risk patients may be more likely to get alternative therapy).

Figure 1

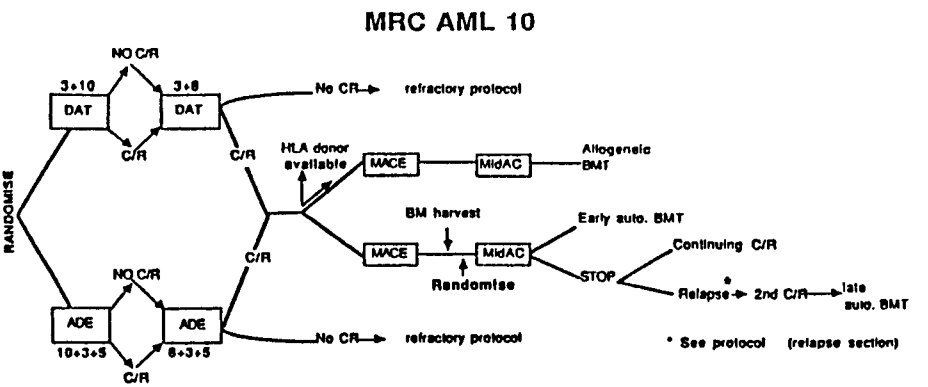


Figure 2
Progress to Second Randomisatoin

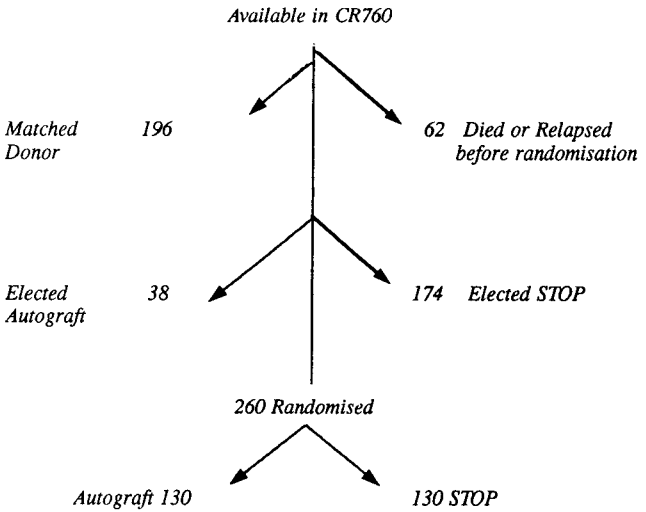
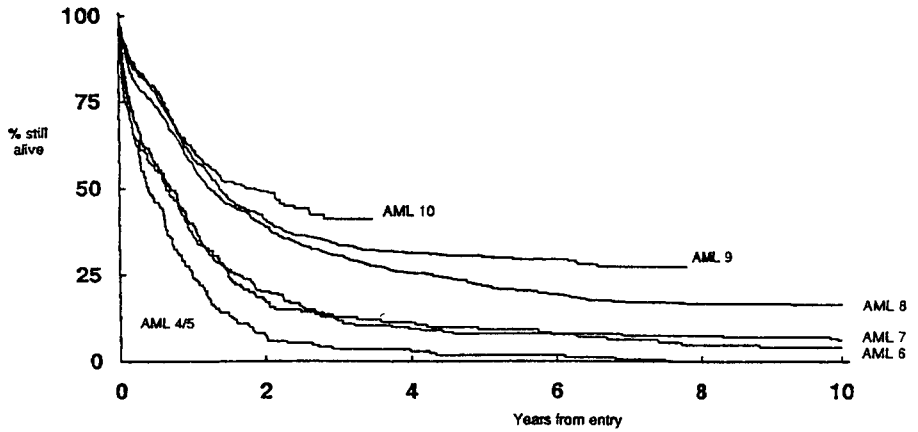


Figure 3
Survival of adults aged 15-55 in MRC AML trials has improved dramatically



ALLOGENEIC VERSUS AUTOLOGOUS BONE MARROW TRANSPLANTATION (BMT) VERSUS INTENSIVE CONSOLIDATION IN ACUTE MYELOGENOUS LEUKEMIA (AML) IN FIRST REMISSION. AN EORTC-GIMEMA PHASE III TRIAL (AML 8 A) A PRELIMINARY ANALYSIS

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Allogeneic BMT (allo-BMT) is considered presently as the best therapeutic choice in AML patients less than 45 years old when they achieve a complete remission (CR), providing a HLA-identical sibling is available. However, the exact value of allo-BMT during 1st CR of AML is still a matter of debate: most comparisons with post-CR chemotherapy are based on data of transplant registries, or on single center results, where allo-BMT is actually performed. A major cause of bias stems from the fact that allo-BMT is performed in a selected population of patients (especially patients transplanted being younger than those treated with chemotherapy), at various times following achievement of CR, and is compared to chemotherapy consolidation on the basis of treatments actually received instead of on intention to treat [(1)]. In addition, the chemotherapy regimen to be compared with is frequently suboptimal instead of being intensive [(2)].

More recently, autologous BMT (ABMT) has been proposed as one of the best therapeutic options in AML patients achieving a CR. The results originating from the European BMT registry are encouraging [(3)], and the value of ex vivo purging is still controversial, good results being obtained as well after reinjection of unpurged bone marrow [(4)]. The EORTC and GIMEMA cooperative groups decided therefore to study prospectively the relative value of allo-BMT, ABMT and intensive consolidation (IC) during 1st CR of AML. The IC was based on a limited number of courses combining intermediate/high dose Ara-C to intercalating agents, following the results of pilot studies in first CR [(5,6)]. The trial was activated in late 1986; it is kept open, but since the expected number of patients has been recruited, preliminary results have been analyzed and disclosed and will be presented here.

PATIENTS AND METHODS

All patients from 10 to 45 years old are considered as eligible, providing they have a recently diagnosed and untreated AML and give an informed consent. Patients with an AML secondary to an antineoplastic treatment are eligible, providing the first cancer is cured, but patients with a transformation of a myeloproliferative syndrome, or a myelodysplastic syndrome of more than 6 months are excluded,. In some centers, patients from 45 to 60 years were also

included in the ABMT/intensive chemotherapy arms. Blood and BM smears are centrally reviewed. For the induction treatment patients receive a combination of Daunorubicin (DNR), 45 mg/sq m/d days 1-3 and Cytosine-arabinoside (Ara-C), 200 mg/sq m/d by IV continuous infusion days 1-7. A second course of induction is administered in case the blood and BM at day 21 following the end of the first induction course show a partial remission (PR). In case of resistance, a salvage treatment is now proposed, combining intermediate dose Ara-C and Idarubicin. Patients achieving a CR according to the usual criteria after one or two induction courses receive one intensive consolidation course combining intermediate dose Ara-C (500 mg/sq m over 2 hrs IV infusion every 12 hrs days 1-6) and m-AMSA, 120 mg/sq m days 5-7. Then an allo-BMT is proposed to all pts with a HLA identical sibling, using Cytoxan, 60 mg/kg/d for 2 days combined with TBI as conditioning regimen. On the other hand for pts without a donor and who recovered a normocellular BM, randomization is centrally performed at the EORTC Data Center between ABMT and a second intensive consolidation chemotherapy (IC) course. The BM is collected after recovery of normal cellularity following the 1st IC course, and is cryopreserved without purging. In most centers, the harvest is performed even in pts randomized for the IC arm, for possible use in case of relapse after achievement of a 2nd CR, the main end-point of the study being disease-free survival (DFS). Two different conditioning regimens were used for ABMT, Cytoxan-TBI for EORTC, and Busulfan-cytoxan for GIMEMA.

For pts randomized in the CT arm, the 2nd IC combined high-dose Ara-C, 2g/sq m every 12 hrs day 1-4 and DNR: 45 mg/sq m day 5-7. Between November 1986 and November 1992, 988 pts from 57 institutions were registered and presently, 871 are evaluable.

RESULTS

Results of induction treatment.

A CR was achieved in 581 pts (67 %), mostly after a single induction course (81% of CRs). In addition, a PR was observed after the 2nd induction course in 2.3 % of cases, and 0.4 % had persistent extra-medullary leukemia. Resistance, whether absolute or corresponding to leukemia regrowth, was observed in 24% of pts, while 6.9% died during the induction period. Among non-responding and surviving pts, 106 reached a CR after a salvage treatment, giving a total CR rate of 78.9%. The evaluation of the post-remission will be based however, on the 581 pts, who entered into CR following a standard induction

Post-remission treatment.

The 1st IC course was administered to 526 pts. This IC was relatively toxic, mainly because of the severity of the resulting hypoplasia. This toxicity explains the high number of pts who did not complete their treatment protocol at the time of present evaluation. 119 pts (20.5 % of the remitters) underwent an allo-BMT. A randomization was performed for 239 pts (41.1% of the remitters), 119 for ABMT and 120 for the 2nd IC course. On the basis of the summary forms received so far, 79 pts completed the ABMT and 92 the second intensive chemotherapy course, corresponding respectively to 13.5 % and 15.8 % of the remitters. The major causes of non-completion were early relapse (47 pts), excess toxicity (96 pts), treatment refusal (72 pts), protocol violation (12 pts), lost to follow-up (5

pts). The other 56 pts are too early/no summary form sent so far. The median duration of follow-up is 2.5 years. It is important to underline that the time from CR to start of the last step of treatment was shorter for the IC arm (median: 10 wks, range: 2-32) than for ABMT (median: 13 wks, range 7-49), or allo-BMT (median: 15 wks, range 4-48).

Out of the 581 pts who achieved a CR, 283 are alive in ist CR. The major cause of failure was relapse, observed in 217 pts (191 BM relapse, 5 CNS relapse, 11 BM + CNS and 10 other types of relapse). In addition 81 pts died in 1st CR, 27 of the 119 pts who completed an allo-BMT, 9 in the ABMT arm, 7 in the second intensive consolidation arm, and 38 among the 209 patients not allografted and not randomized for toxicity or refusal.

Presently, the actuarial DFS for allo-BMT is 51% $\pm$10.4, and for pts randomized 42% $\pm$9 % (p= 0.78). There is a trend for a better DFS of pts randomized for ABMT (50 % $\pm$11.2) than for IC (31% $\pm$11.4) but the difference is yet not significant (log rank p = 0.08) (Fig. 1). However, the overall survival following CR of the 2 randomized arms is practically identical (59 % $\pm$11.6 for ABMT and 54 % $\pm$ 14 % for IC) (Fig. 2). The overall survival of pts allografted is also identical at 3 yrs (56 %), despite a higher early mortality for allo-BMT. The overall survival of 871 registered and evaluable pts is 37 % $\pm$ 4.4 at 4 yrs, and the survival following CR 51% $\pm$5.8.

DISCUSSION

These preliminary results confirm that intensive post-CR treatment for adult AML of relatively young age groups (the median age of our pts is 33 yrs, range 14-59 yrs) provides a better DFS and overall survival than those previously achieved with more conventional post-CR chemotherapy regimens. In the previous AML6 EORTC protocol for pts under 65 yrs old, who received one consolidation then 6 courses of semi-intensive maintenance following CR, the DFS was 23 % at 4 yrs [7]; it is 40 % in the present one.

The results obtained with allo-BMT correspond to those published by transplant registries or achieved in prospective studies comparing this treatment with post-CR chemotherapy [8]. Our study was intended to compare the 3 post-CR therapeutic options according to genetic chance (allo-BMT) or randomization (ABMT or IC), and on the basis of intention to treat. Presently, the DFS of pts randomized to ABMT confirm the results of pilot studies or the EBMT registry [3, 4]; they are equivalent to allo-ABMT, and could be considered as having a better therapeutic index since there is less related morbidity and treatment-related mortality with ABMT than with allo-BMT. Our results contradict those from recent prospective studies that showed a superiority of allo-BMT over ABMT but were based on smaller number of pts [9, 10]. ABMT seems superior to our IC arm, but the difference for DFS is not yet significant. However, the difference between the two randomized arms is no longer evident when overall survival is considered. This observation is explained in our series by the fact that pts relapsing in the IC arm are more easily reinduced in 2nd CR and had more salvage ABMT afterwards than pts randomized to ABMT during 1st CR. A salvage ABMT at relapse or 2nd CR following intensive consolidation could be considered as a suitable therapeutic option. Other observations could influence the choice of treatment in AML: there is no difference for DFS in the ABMT arm between the

EORTC and the GIMEMA groups. despite two different conditioning regimens (CTX-TBI or BU-CY). Further comparative studies of the 3 treatment options intended to evaluate the respective quality of life and cost-effectiveness could help in making the optimal medical decision. In addition. further progress can be expected to increase the efficacy and reduce the toxicity of each of these 3 options.

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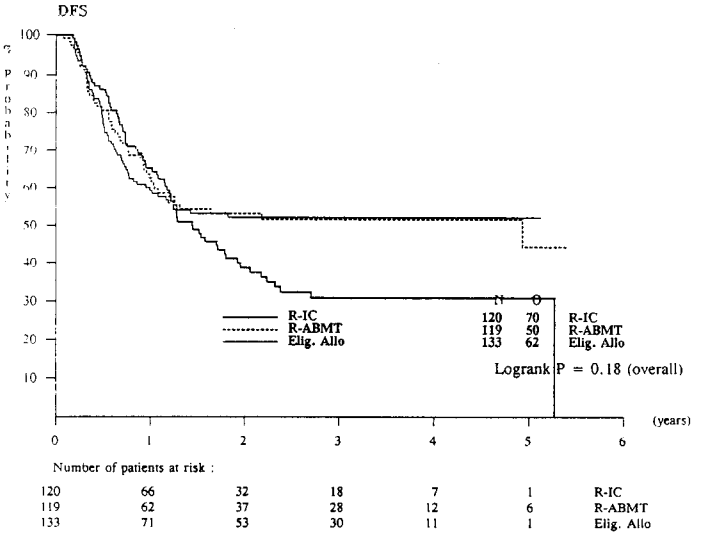
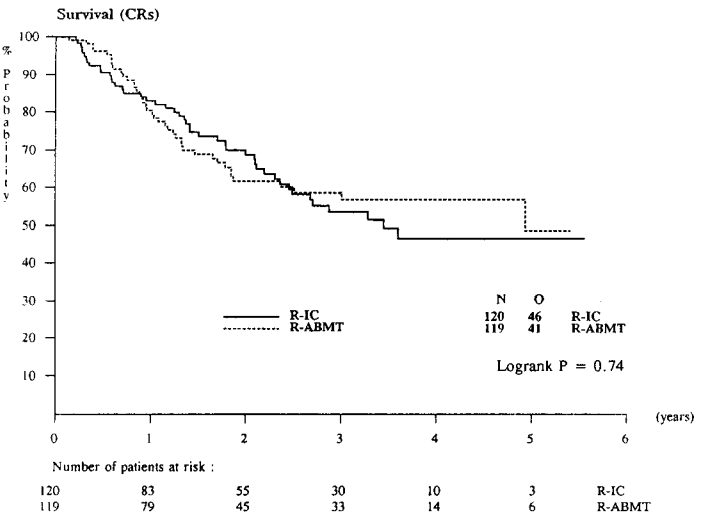
Figure 1



EORTC-GIMEMA AML 8 A

Figure 2



EORTC-GIMEMA AML 8 A

RANDOMIZED TRIAL COMPARING AUTOLOGOUS STEM CELL TRANSPLANTATION AND CHEMOTHERAPY IN ADULT PATIENTS WITH ACUTE MYELOID LEUKEMIA IN FIRST REMISSION : THE BGMT GROUP EXPERIENCE

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INTRODUCTION

Using standard induction chemotherapy consisting of Cytosine arabinoside (Ara-C) combined with an anthracycline complete remission is achieved in 70-80% patients with "de novo" acute myeloid leukemia (AML) under the age of 50-60 years.[1] However, once a complete remission has been achieved controversy still persists as to the choice of the optimal subsequent treatment designed to prevent leukemic relapse. Three different approaches are available: allogeneic bone marrow transplantation (AlloBMT) which is only suitable for young patients with an HLA identical sibling donor, intensification with high-dose chemotherapy (CT) with or without maintenance CT and ablative treatment followed by autologous marrow or blood stem cell rescue. The choice between CT and autologous stem cell transplantation (ASCT) is very difficult because the results of these different therapeutic strategies are overlapping.

In 1984, we started a prospective cooperative study (BGM 84 Study) comparing AlloBMT AutoBMT and intensive CT. We found that the results of ASCT were slightly better (but not significantly) than those for the group of patients who were treated with CT.[2] In 1987, we designed a new protocol (BGMT 87 study) to confirm the results of the BGM 84 Study. The aims of this BGMT 87 Study were: 1) to compare AlloBMT with other therapeutic approaches of ASCT + CT; 2) to compare ASCT and CT; 3) to compare ASCT using either unpurged bone marrow (AutoBMT) or peripheral blood stem cells (ABSCT).

We report herein the results of the comparison of ASCT versus CT and in order to avoid some biases in patient selection, the results will be reported according to "intention to treat".

PATIENTS AND METHODS

Patients

The BGMT 87 Study was conducted in four French hematology departments (Hopital Haut-Leveque, CHU Bordeaux; Hopital La Tronche, CHU Grenoble; Institut Paoli-Calmettes, CHU Marseille; Hopital Purpan, CHU Toulouse). Eligible patients (age between 15 and 55 years) had to have a diagnosis of AML according to the FAB classification.

Induction and Consolidation Treatment

For induction chemotherapy the patients received Ara-C (100 mg/m^2/day continuous infusion) over a 10 day period and DNR (60 mg/m^2/day intravenously for three days). When a complete remission was achieved (after one or two courses of induction treatment), the patients received consolidation therapy 28-35 days after completion of induction treatment consisting of Ara-C (50 mg/m^2/12 hours subcutaneously, 7 days) and DNR (60 mg/m^2/day intravenous bolus, 2 days). One month after consolidation (about 60-70 days after diagnosis), the patients who were still in CR were designated to receive either AlloBMT or to be given another form of treatment (ASCT or CT).

Study Design

All patients who were still in CR but who did not fulfill the eligibility criteria for AlloBMT received intensification CT (high-dose Ara-C: 3g/m^2/12h x 8 doses and DNR: 45 mg/m^2/day x 3 days). Thirty to 40 days after intensification (about 100 days after diagnosis), these latter patients were randomly designed to receive either ASCT or maintenance chemotherapy (CT).

Autologous Stem Cell Transplantation (ASCT)

After intensification chemotherapy the patients who were randomized for ASCT underwent either a bone marrow harvest (in Grenoble, Marseille and Toulouse), or peripheral blood stem cell collection (Bordeaux). The patients were subsequently given a conditioning regimen consisting of Busulfan (4mg/kg/day x 4 days) and Melphalan (140mg/m^2) before the reinfusion of bone marrow or PBSC. No further treatment was administered after ASCT.

CHEMOTHERAPY (CT)

The patients who were randomly assigned to receive CT after intensification treatment were given maintenance chemotherapy for two years. This treatment consisted of five cycles of Ara-C (50 mg/m^2/12 hours subcutaneously x 5 days) and DNR (1 mg/kg/day one day) which were administered 1, 3, 6, 9 and 12 months after intensification treatment (or as soon as the hematopoietic recovery following intensification was obtained). Between these five courses of chemotherapy and after the fifth cycle the patients received continuous treatment with 6-Mercaptopurine and Methotrexate for a total period of two years.

The results were analyzed after June 1, 1992, with a minimum follow-up of 12 months for each patient.

RESULTS

From January 1987 to December 1990, a total number of 204 adult patients were entered into the study. The median age of these patients (male/female = 113/91) was 39.8 years (15-55). 162 patients (79.5 %) achieved complete remission (CR) after one (n = 148) or two courses (n = 14) of induction chemotherapy and were given consolidation treatment. After consolidation 26 patients were excluded for various reasons and 136 patients were still alive in CR.

Ninety-nine patients who could not undergo AlloBMT were given intensification chemotherapy. A substantial proportion of patients (n = 6) died during this chemotherapy and 16 other patients could not be randomized for ASCT or CT.

Finally, 77 patients were randomized for either maintenance CT (n=38) or ASCT (n=39). The main maintenance CT (n=38) or ASCT (n characteristics of these two groups were similar (sex ratio age initial WBC count FAB distributing number of courses for CR). Of the 39 patients allocated to the ASCT arm 33 patients did undergo ASCT using either bone marrow (n=16) or blood stem cells (n=17) and six did not because of relapse (n=3), refusal (n=2), or poor hematopoietic progenitor content in the transplant product (n=1).

In the ASCT group there were two transplant-related deaths (ABSCT=1; ABMT=1), 18 relapses and finally 19 patients are still alive in first CR 6 to 48 months after ASCT. Among the 38 patients in the CT group 22 patients had leukemic relapse and 16 patients are still alive in CR 7 to 51 months after obtaining CR. The actuarial risk of relapse was 48.7 +/- 8.8 % (95% CI) in the ASCT group and 61.1 +/- 8.4 % (95% CI) in the CT Group (p=N.S). The estimated chance of surviving without disease at three years was similar in both groups (48.3 +/- 8.5 % and 38.9 +/- 8.4 % respectively) (p=N.S) (Fig 1). Using multivariate analysis we found that the outcome of the 77 patients who were randomized to receive either ASCT or CT was not influenced by the type of treatment given after intensification. No significant difference could be found between ABMT and ABSCT for the disease free survival and the risk of relapse. However, as previously reported, the hematopoietic recovery was significantly shorter after ABSCT than after ABMT.

DISCUSSION

The results we report in this study for ASCT or CT do not differ significantly from those published elsewhere. In patients undergoing ASCT, we found that the estimated chance of surviving without disease at three years was 48.3 +/- 8.5%. This is similar to that reported in three other studies which compared ABMT and AlloBMT.[3,4,5] For patients treated with CT alone, the actuarial proportion of patients who were still alive in remission at three years was 38.9 +/- 8.4%. This latter result does not differ significantly from that reported elsewhere.[1]

Although AlloBMT has been compared extensively with CT,[1] there is no published prospective study comparing AutoBMT (or ASCT) and CT. This prospective study shows that there is no major difference between ASCT and maintenance CT in adult patients with AML in first CR and that the patients who received the more intensive treatment do not survive longer than other patients.[6]

Thus, as the results of some ongoing studies are not yet available, the best treatment for AML patients is still debatable when an HLA-identical sibling donor is not available.

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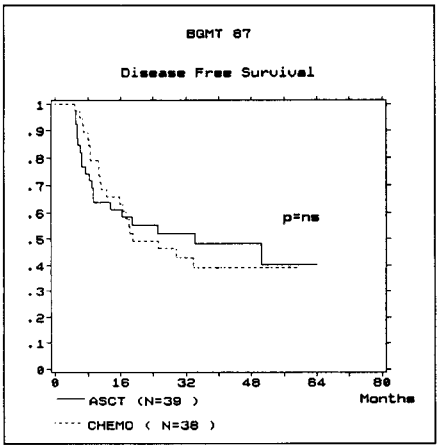
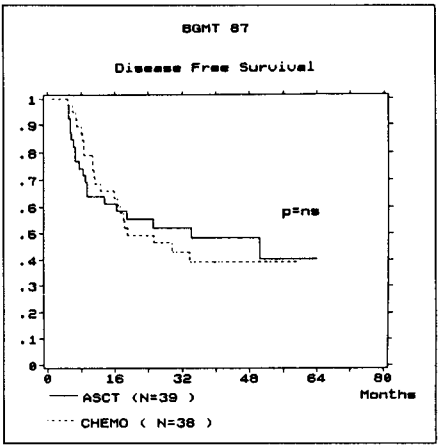

BGMT 87
Disease Free Survival
p=ns
ASCT (N=39)
CHEMO (N=38)
Months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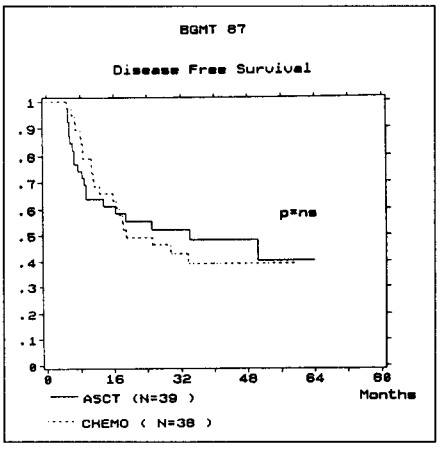
BGMT 87
Disease Free Survival
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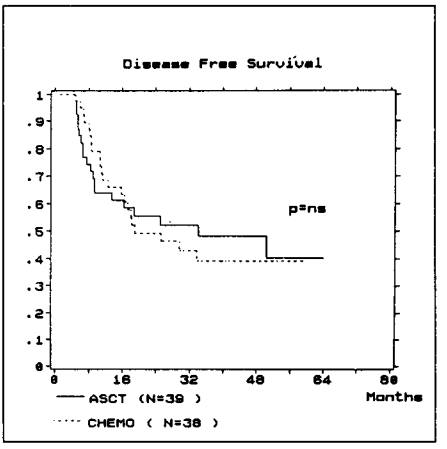
ASCT (N=39)
CHEMO (N=38)
Months


Disease Free Survival
p=ns
ASCT (N=39)
CHEMO (N=38)
Months

COMPARISON OF INTENSIVE CONSOLIDATION CHEMOTHERAPY (ICC) AND UNPURGED AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) AS POST REMISSION THERAPY IN ADULT ACUTE MYELOID LEUKEMIA (AML).

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INTRODUCTION

Recently published series have shown that a disease free survival of 30 to 50% could be achieved after short term Intensive Consolidation Chemotherapy (ICC) without maintenance treatment [1-5]. Autologous Bone Marrow Transplantation (ABMT) after myeloablative treatment is another attractive approach. In pilot single center studies or in the annual survey of the European registry, disease free survival rates around 50% were obtained [6-8]. However in all these studies, the issue of patient selection was raised. Thus, randomized studies comparing ABMT and ICC were mandatory. In 1987, the French group GOELAM initiated such a study.

PATIENTS AND METHODS

Patients (pts) aged 15 to 50 years with de novo AML were included in a multicenter study involving 16 centers of the GOELAM group. Pts with preexisting myelodysplastic syndromes or with blastic transformation of chronic myeloproliferative disorders were not included. Chemotherapy/radiation-induced leukemias were also excluded. From November, 1987 to December 1991, 318 pts were enrolled and 308 pts are evaluable.

INDUCTION TREATMENT

Patients were randomized to induction treatment between Cytarabine (Ara-C) 200 mg/m^2/d (continuous infusion) for 7 days plus Idarubicin (IDR) 8 mg/m^2/d IV for 5 days and ARA-C at the same dosage plus Zorubicine (ZRB) 200 mg/m^2 for 4 days. A bone marrow aspiration was performed at day 17: if the marrow remained blastic (< 50% blasts) a second course was administered with 3 days of Ara-C plus 2 days of either IDR 10 mg/m^2/d or ZRB 200 m g /m^2/d .

POST REMISSION TREATMENT

Pts in complete remission (CR) were allografted if they were under the age of 40 years and had an HLA-identical sibling. The conditioning regimen and the graft versus host disease prophylaxis and treatment varied according to protocols used in the different transplant centers. Pts over 40 years or without a suitable donor received a first course of ICC (ICC1) Ara-C (3 g/m^2 in 3 hours infusion every 12 hours for eight doses, d1 to d4) and either IDR 10 mg/m^2/d d5-6

or ZRB 200 mg/m^2/d d5-6. After hemopoietic recovery, marrow was collected. No in vitro manipulation was done. Pts were then randomized between a second course of ICC (ICC2) and ABMT. The second course was m AMSA 150 mg/m^2/d dl to d5 and VP16 100 mg/m^2/d dl to d5. The preparative regimen for ABMT was the Baltimore protocol of Busulfan 4mg/kg/d for 4 days and Cyclophosphamide 50 mg/kg/d for 4 days.

RESULTS

The median age of the 308 evaluable pts was 36.5 years. There was no significant difference between the IDR arm (154 pts) and the ZRB arm (154 pts) regarding the following initial characteristics: sex, age, performance status, fever, DIC, WBC count, FAB classification, karyotypic abnormalities.

INDUCTION TREATMENT

Of the 308 pts, 241 (78%) achieved CR with no significant difference between the IDR arm (76.5%) and the ZRB arm (80%). CR was achieved in one course in 92% of the cases. There were 5 early deaths, 10 deaths in aplasia and 52 (17%) failures, with no significant difference between the two groups.

FIRST INTENSIFICATION

Of the 241 pts in CR, 56 (23%) underwent an allogeneic BMT 40 to 220 days (median 64) after CR achievement. Twenty-nine pts who should have received ICC1 were excluded for the following reasons: protocol violation 13, infection 8, other visceral complication 5, refusal 2, relapse 1. In 5 cases data are not yet available and l pt is lost to follow up. Thus 150 pts are evaluable for ICC1. The median time between CR achievement and ICC1 was 20 days (1-112). The median duration of neutropenia after ICC1 was 29 days and 5 toxic deaths (3%) were recorded.

SECOND INTENSIFICATION

Of the 145 pts still in CR after ICC1, only 106 have been randomized (52 ICC2, 54 ABMT). Nine pts in the ABMT arm and 2 pts in ICC2 arm did not receive the assigned treatment. Thus 50 pts are nonevaluable for the second intensification (poor or slow hemopoietic reconstitution 20, refusal 12, protocol violation 7, relapse 6, toxicity of ICC1 5). Only 95 pts (50 ICC2, 45 ABMT) actually underwent the randomized treatment. Overall, 151 pts (49% of the 308 pts and 63% of the 251 pts in CR) did receive the assigned intensive post remission therapy.

SURVIVAL

The median follow up is 34 months (10-60). By November 1992, there were 13 relapses and 9 procedure related deaths in the BMT group, 23 relapses in the ICC group, 20 relapses and 1 toxic death in the ABMT group. The actuarial risk of relapse at 4 years is 51% in the ICC group, 49% in the ABMT group and 30% in the BMT group Fig 1. The event free survival (EFS) curves for pts in CR are shown in Fig 2. The ICC and ABMT actuarial curves are strictly superposable. The comparison with BMT is statistically invalid since all BMT pts did not receive the same post remission therapy and the same conditioning regimen. However, the EFS after BMT appears to be identical (52.4% at 4 years). More-

over, 13 pts who had received ICC1 and were not able to undergo ABMT were treated with ICC2 (off protocol). When adding these pts to the 50 protocol ICC2 pts the EFS curve remains identical with a 54% probability of 4 year survival.

The overall survival for the 308 pts is 40.5% at 4 years (median survival 23 months). There is a non significant trend in favour of the IDR arm (48.5% versus 33.5% at 4 years p=0.45).

DISCUSSION

The trial is still ongoing and definitive conclusions cannot be drawn. However several points can be made.

1) The CR rate is high (78%) especially when considering that it is a multicenter study and that all pts having received the first day of treatment have been evaluated. This confirms the results we previously achieved with ZRB (5) and the good antileukemic activity of IDR recently shown by other groups [9-10].

2) Regarding the post remission therapy, up to now, there is no significant difference between ICC2 and unpurged ABMT. To demonstrate a hypothetical advantage of one arm would require a very large cohort of pts. It should be noted that 13 pts who could not undergo ABMT because of poor or slow hemopoietic recovery after ICC1 were treated with ICC2 and they share the same prognosis as randomized pts. Thus after ICC1, ICC2 appears to be as effective as ABMT and can be offered to a larger number of pts. These results differ from those of the EORTC study in which ABMT appeared to be superior to chemotherapy in terms of EFS [11]. As in other studies on intensive post remission therapy, the issue of tolerability must be raised. Only 63% of the pts in CR could complete the whole protocol treatment.

3) The overall results of this short term intensive treatment are encouraging. With a median follow up of 34 months, the median survival for the entire group of 308 pts is 23 months and the actuarial probability of being alive at 4 years is 40.5%.

CONCLUSION

These results confirm that a significant improvement of survival can be obtained in AML for pts up to 50 years of age due to different modalities of intensive therapy. However this study fails to demonstrate the superiority of any of the 3 approaches tested.

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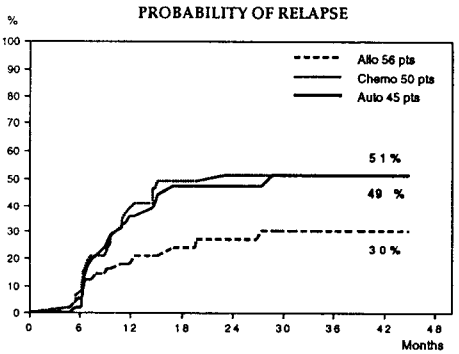

Figure 1

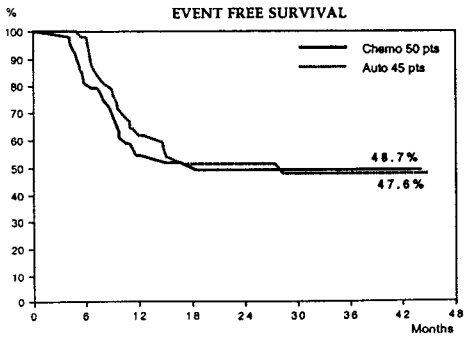

Figure 2

Session II: Future Directions

IMPROVED OUTCOME FOR HIGH-RISK AML USING AUTOLOGOUS BONE MARROW TRANSPLANTATION AND MONOCLONAL ANTIBODY PURGED BONE MARROW

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University of Pittsburgh Medical Center, Dartmouth-Hitchcock Medical Center, Bowman Gray School of Medicine, Scripps Clinic and Hospitals and the University of Iowa

Allogeneic bone marrow transplantation (BMT) has been shown to reduce relapse rates in patients with AML in first or later remissions. However, due to significant treatment-related mortality, overall disease-free survival is approximately 50% for first remission patients and 20 - 30% for second remission patients[1]. A major limitation of allogeneic BMT is that it can be applied to only a minority of patients with AML. Only about 40% of patients with AML have an HLA matched donor, and patients over 50-55 years are considered too old to tolerate this procedure. Studies using allogeneic BMT with matched unrelated donors have demonstrated that advanced disease, older age and higher degrees of HLA disparity are associated with a poor outcome due to excessive morbidity and mortality of GVHD 2. Therefore, other treatment strategies are necessary for the majority of patients with AML.

Autologous BMT (ABMT) is a promising therapy for the treatment of AML. The lack of a bone marrow donor does not preclude treatment, it can be applied to patients as old as 65 and, due to the lack of graft versus host disease, morbidity and mortality are greatly reduced. There is concern, however, that the relapse rate with autologous BMT will be higher than that seen with allogeneic BMT possibly due to the potential reinfusion of marrow contaminated with clonogenic leukemia cells and because of the absence of the graft verses leukemia effect. To attempt to increase the efficacy of this treatment, methods of purging autologous marrow using monoclonal antibodies (MAbs) or cytotoxic drugs are being evaluated [2-6].

A panel of cytotoxic MAbs have been described by one of us (E.D.B.) that react specifically with myeloid cells and recognize antigens expressed on AML blast cells [7-9]. Of these, monoclonal antibodies PM-81 (anti-CD15) and AML-2-23 (anti-CD14) are the most reactive, binding with leukemia cells from greater than 95% of AML patients [7-9]. These MAbs are cytotoxic with complement to cells bearing the respective cell surface antigens, so they can lyse leukemia cells from almost all patients with AML, including their progenitor cells [7]. However, studies have shown that PM-81 and AML-2-23 do not recognize antigens on multilineage progenitor cells and thus probably not on the pluripotent stem cells necessary for successful engraftment of bone marrow [7].

From August, 1984 until April, 1, 1992, we harvested, MAb-purged, and performed 63 autologous bone marrow transplants on patients who were in CR or first relapse at the time of transplant. Thirty of these patients were described

in a previous report [3]. Analysis as of August 1, 1992, suggests that long term disease free survival and overall survival can be achieved in patients with advanced remissions and relapsed AML.

Materials and Methods

A. Patients

Patients between the ages 11-57 years with a Karnovsky performance status of 80-100% and an expected survival time of greater than 2 months were eligible for this protocol.

Patients had the diagnosis of AML in second or third CR, AML in first relapse or AML in first CR. Leukemia blast cells obtained at diagnosis or at relapse, when available, were required to express the antigens reactive with PM-81 and/or AML-2-23 on > 20% of cells. The study was approved by the Institutional Review Board of the respective institutions and a signed informed consent was obtained from each patient prior to study entry.

Marrow Harvesting and Purging

Bone marrow ($6x10^8$ cells/kg) was harvested from the posterior and anterior iliac crests under general anesthesia and passed through a series of filters. A mean of 6.56×10^8/kg were actually harvested. Postpheresis, there was a mean recovery of 16.5% of the cells which were then treated with MAb + C'. A mean of 8.08×10^7 cells/kg were treated with MAb as previously described [10], and from that there was a mean recovery of 55.6%. An average of 4.77×10^7 cells/kg was used for the transplant.

Preparation Regimen

Thirty six patients were treated with the following preparative regimen: Cyclophosphamide (CY) (60 mg/kg i.v. for 2 days) and fractionated TBI (fTBI) (200cGy twice daily for 3 days, total dose of 1,200 cGy). In 1988, the preparative regimen changed from CY/fTBI to Busulfan (Bu) and CY.

Twenty-seven patients were treated with the following regimen: Bu (4mg/kg/day p.o. for 4 days and CY (60mg/kg/day i.v. for 2 days).

RESULTS

Patients

Sixty-three AML patients ranging in age from 11 to 57 (median 36) who were in CR or first relapse were transplanted between August, 1984, and April 1, 1992. All but three patients had de novo AML at the time of initial diagnosis. The FAB subclasses of the cases were as follows: M1/M2, 29; M3, 8; M4/M5, 23; M6/M7, 1; Biphenotypic, 1; Unknown, 1. The median time between the current remission or relapse and ABMT was 45 days.

Data on cell surface antigen expression were available on 38 patients. All cases were greater than 20% positive for PM-81. The median PM - 81 binding was 82%. On average, 25% of leukemia cells were positive for binding to MAb AML-2-23.

TOXICITY

Preparation Regimen

The preparative regimens were generally well-tolerated. Most patients experienced mild to moderate nausea and vomiting during the administration of chemotherapy and fTBI. Mucositis was moderate to severe. Diarrhea was experienced by the majority of patients in the first two weeks after TBI. Almost all patients became febrile during the period of marrow hypoplasia and leukopenia and required multiple parenteral antibiotics including amphotericin B.

Eight patients died within two months of ABMT while in the recovery phase. Six patients died from overwhelming sepsis despite aggressive antimicrobial therapy, one from hemorrhagic complications due to refractoriness to platelet transfusions, and one from pulmonary and hepatic failure.

Marrow Infusion

The infusion of bone marrow was well-tolerated. Patients were premedicated with acetaminophen, diphenhydramine and hydrocortisone. Hydration at 1.5 - 2 times maintenance was maintained for 24 hours with marrow infusion. Blood pressure and cardiac monitoring were carried out during bone marrow infusion.

Five patients required a second infusion of MAb-treated marrow when engraftment appeared delayed. In each case, a moderately severe reaction occurred. In one patient this was manifest as hypotension associated with syncope. In the other patients, respiratory distress associated with pulmonary infiltrates developed several hours after the infusion. Each patient was treated with aggressive fluid and corticosteroid therapy, and all reactions were reversed without sequellae. No patient required intubation and mechanical ventilation. In each case engraftment followed the infusion of the treated "back-up" bone marrow. None of the patients with prolonged thrombopenia received "back-up" marrow.

CFUS

The effect of the MAb and C' treatment on CFUS was determined by culture of cells in methylcellulose. The median recovery of CFU-GM progenitor cells was 38% (range 22-150) for the first CR group, 48% (range 1-190) for the second/third CR group and 87% (range 58-248) for the relapse group. Median recovery of BFU-E was 49% (range 29-136) for the first CR group, 69% (range 1-2500) for the second/third CR group and 100% (range 0-392) for the relapse group. Median recovery of CFU-MIX was 50% (range 17-60) for the first CR group, 37% (range 0-381) for the second/third CR group and 13% (range 0-249) for the relapse group.

Engraftment

A median number of 4.0×10^7 cells/kg body weight (range 2.30×10^7 to 8.23×10^7) were infused into each first CR patient. The median number of cells transfused into the second/third CR group was 2.80 x 107 (range 7.50×10^6 to 1.16×10^8). A median number of 4.10×10^7 cells/kg body weight (range 2.38×10^7 to 5.96×10^8) were infused into each first relapse patient.

Engraftment of granulocyte, monocyte and erythrocyte precursors was prompt in most patients. Median observed recovery times for neutrophils to 500 cells/uL were 33, 37 and 41 days for the first CR, second/third CR and first relapse patients respectively. Median times to reach platelet counts of greater than 20,000 and greater than 50,000 uL were 50 and 90 days (first CR), 42.0 and 69 (second/third CR) and 49 and 79 days (first relapse). Median times in days to reach a hemoglobin concentration of greater than 10g% were 71, 46, and 67 for first CR, second and third CR and first relapse patients, respectively.

Relapse

Three first CR patients relapsed at 11, 17 and 43 months post-ABMT. At the time of ABMT the median CR1 duration of the patients that relapsed was 12.3 months. The median pre-ABMT CR durations of the other four first CR patients now surviving relapse-free after the transplant was 7.3 months (range 6-11). Fourteen second/third CR patients relapsed at times ranging from 1.5 to 30 months post-ABMT, (median 4.2 months), and their median time in first complete remission was 9.8 months. Two patients transplanted in first relapse relapsed at 6 and 12 months post-ABMT.

Survival

Relapse-free survival from transplant of all patients as of August 1, 1992 is shown in Figure 1, by CR group. The median relapse-free survival of the CR1 group is 1762 days (59 months) after ABMT. Four of the seven patients remain in CR at 666 (22 months), 1723 (57 months), 1800 (60 months) and 2100 (70 months) days post ABMT respectively. Actuarial two and three year disease free survival is 71% and actuarial two and three year relapse rate or death is 29%.

The patients transplanted in CR2/3 have an actuarial two and three year disease-free survival of 36% and 32% respectively. The median relapse-free survival of this group post-ABMT is 32 months. The two and three relapse year rates are estimated to be 42% and 49%. For those patients transplanted in first relapse, the two and three year actuarial disease-free survival is 36% while the estimated two and three year relapse rate is 43%. The median relapse-free survival of this qroup post-ABMT is 10 months.

Data analysis by treatment regimen (Bu/CY vs. CY/TBI) yields encouraging results (Figure 2). The two and three year disease-free survival of patients who received CY/TBI as the conditioning regimen are 33% and 30% respectively, while those patients who received Bu/CY have two and three year DFS of 53% (P- 0.08).

Further analysis by regimen of the high risk patients only (patients transplanted in CR 2/3 and R1) reveal statistically significant benefit for those patients conditioned with BU/CY (Fig. 3). Twenty-seven patients in CR 2/3 and R1 received Bu/CY. They have actuarial two and three year DFS of 53% while 29 CR2/3, R1 patients conditioned with CY/TBI have 24% and 21% two and three year DFS ($p = .02$). In addition, analysis of the 9 R1 patients conditioned with Bu/CY reveal a two and three year DFS of 56%.

DISCUSSION

BMT after high-dose chemotherapy and/or radiation therapy offers the potential for complete elimination of occult leukemia cells during complete remission, and BMT is probably the only curative treatment for patients with AML after first relapse [11]. Encouraging results have been reported with allogeneic BMT, but the majority of patients with AML cannot undergo this therapy due to lack of an HLA-matched donor and/or age greater that 55.[12,13] These people can potentially be treated with autologous BMT.

Although no randomized studies directly comparing autologous BMT with and without marrow purging have been reported from any center, long term survival for AML patients after autologous BMT using various methods for removing occult leukemia cells has been reported.[6,10] Our results using monoclonal antibody purging in this study are very encouraging, especially for those patients transplanted in R1 and CR2/3 who were conditioned with BU/CY. Thus far, the median relapse-free survival of 32 months for those patients transplanted in CR2/3 is very promising. Despite the small number of R1 patients transplanted to date, a three year DFS of 56% warrants continuation of clinical trials with monoclonal antibody purging. This data compares well with alternative approaches to autologous BMT in AML, such as the use of 4-hydroperoxycyclophosphamide and to allogeneic BMT for patients at similar risk for relapse. Since most remissions in AML induced by standard chemotherapy continue to be limited in duration, we think that this combined immunologic and chemotherapeutic approach to eradicating leukemia cells will continue to prove to be efficacious in a substantial number of patients.

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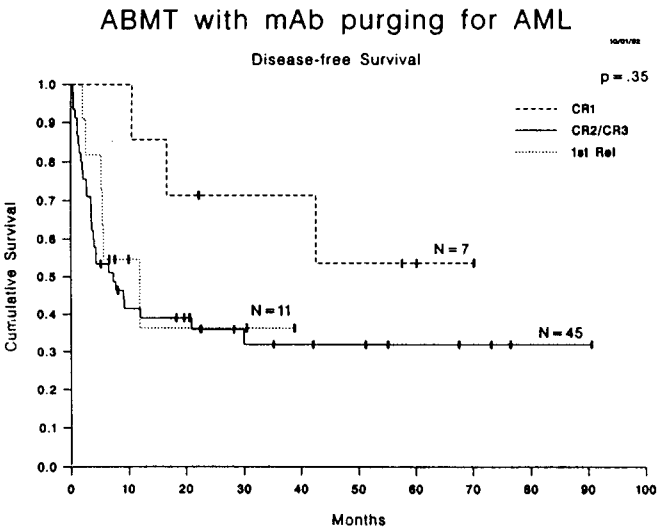

Figure 1

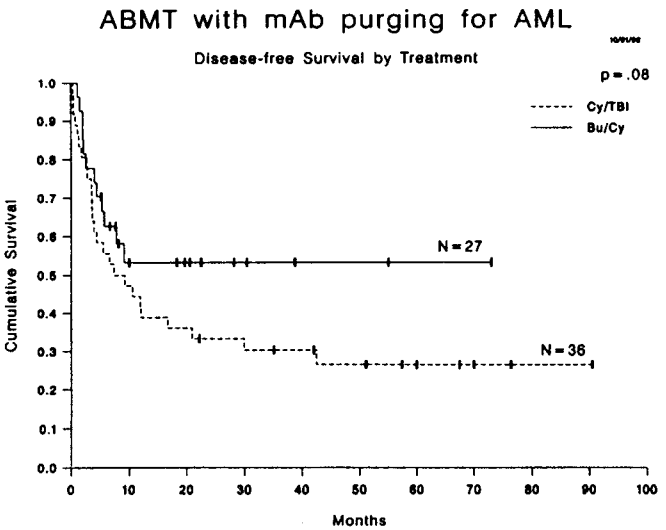

Figure 2

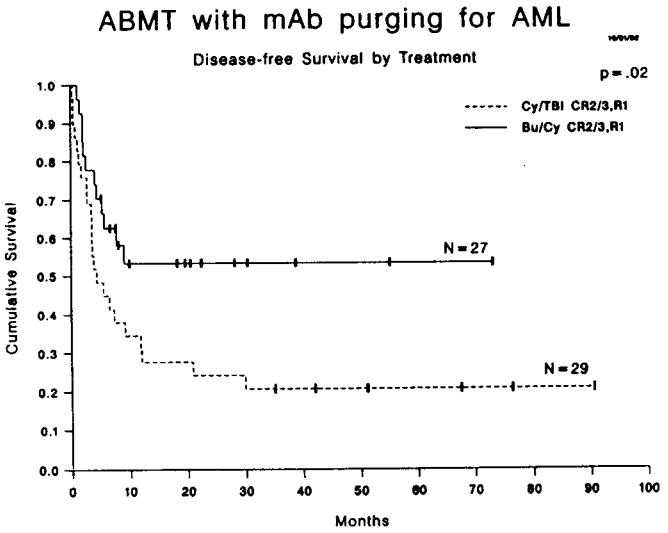
ABMT with mAb purging for AML
Disease-free Survival by Treatment
p = .02
Cy/TBI CR2/3,R1
Bu/Cy CR2/3,R1
N = 27
N = 29
Cumulative Survival
1.0
0.9
0.8
0.7
0.6
0.5
0.4
0.3
0.2
0.1
0.0
0
10
20
30
40
50
60
70
80
90
100
Months

Figure 3

ENHANCED REGENERATION OF NATURAL KILLER CELLS IN PATIENTS UNDERGOING MAFOSFAMIDE PURGED AUTOGRAFTS

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INTRODUCTION

Autologous bone marrow transplantation (ABMT) after high-dose chemotherapy and/or total body irradiation is increasingly used as consolidation for patients with acute myeloblastic leukemia (AML), acute lymphoblastic leukemia (ALL) and non-Hodgkin's lymphoma (NHL) [1,2]. Because of the theoretical possibility that the reinfused marrow may be contaminated with residual malignant cells [3], in vitro marrow purging with pharmacological agents, such as 4-Hydroperoxycyclophosphamide (4-HC) or mafosfamide (Asta-Z 7557), has been proposed in order to achieve elimination of residual neoplastic cells from the graft [4]. Various in vitro purging methods available today result in a 4-6 log cell kill in vitro and in 1 log reduction in tumor burden [5]. The impact of purging techniques on clinical outcome appears to be disease-dependent. For AML patients, relapse rates of 30% and 50% have been reported for adjusted-dose purged and unpurged grafts, respectively [6]. For ALL patients, the corresponding figures are 58% and 50%, respectively [6]. These findings suggest that chemical purging acts through different mechanism(s).

A preferential leukemia cell killing effect is commonly considered the main mechanism of action of mafosfamide [5]. By using clonogenic assays, it has been demonstrated that mafosfamide inhibits in a dose-dependent manner the in vitro growth of normal adherent CFU-Blast, multipotential CFU-GEMM, erythroid BFU-E, and granulocyte-macrophage CFU-GM, as well as leukemic CFU-L [7,8]. Normal CFU-Blast as well as CFU-GM express an individual wide variation in sensitivity to mafosfamide [9,10]. However, tumor cell killing may not be the only mechanism of action of chemical purging. In fact, in mice mafosfamide purging results in the activation of immune effectors cells [11]. Natural killer (NK) cells constitute an immunocompetent cell subpopulation with a regulatory function on hematopoiesis and the capability to exert an immune surveillance on neoplastic cell growth. For this reason, NK cells have been considered as effectors of an immune response that could play a role in preventing relapse following marrow transplantation [12]. Studies aimed at evaluating the recovery of immunocompetent cells, demonstrated a rapid recovery in number and function of NK cells after allogeneic BMT, whereas controversial data are reported after ABMT [13].

It was therefore the aim of the present study to investigate the possible modifications of the immunologic recovery in ALL, AML, and NHL patients.

MATERIALS AND METHODS

Patients. Fifteen consecutive patients undergoing ABMT were included in this study: 6 patients with acute myeloid leukemia (AML), 3 with acute lymphoid leukemia (ALL) and 6 non-Hodgkin's lymphoma (NHL). Table 1 shows the main clinical and hematologic data of the patients at the time of autograft. The median age was 42 years (range, 20 to 56). The median follow-up time post-ABMT was 12 months (range, 2 to 28). All patients were transplanted in complete remission. AML and ALL patients as well as 3 out of 6 patients with NHL were autografted with mafosfamide purged marrow. The method of adjusted dose marrow purging [10] was performed on an individual basis aimed at evaluating mafosfamide-induced growth inhibition of primitive adherent blast colony-forming unit (CFU-Blast). Marrow was purged with the concentration of mafosfamide resulting in 50% inhibition of CFU-Blast growth (ID_{50}). With this technique the value of CFU-Blast ID50 has been shown to be significantly higher ($P \leq 0.05$) than CFU-GM ID95 value [10]. Two patients with AML and two with ALL relapsed at 5, 7, 2, and 14 months after ABMT. The other patients are well and disease-free.

Cell separation procedures. After informed consent, light-density mononuclear cells (MNCs) were separated by centrifugation (400 g, 30 min, 4° C) on a Ficoll-Hypaque gradient (density 1.077 g/ml). Interface cells were washed three times, suspended in RPMI-1640 medium (GIBCO, Grand Island, NY, U.S.A.) supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, Utah, U.S.A.) and 2 mM glutamine (GIBCO, Grand Island, NY, U.S.A.), and then plastic adherent cells were removed by incubating (60 min, 37° C, 5% CO_2) MNCs (5 x 10^6/ml) in 75-cm^2 tissue culture flasks.

Immunofluorescence analysis. The surface antigen analysis was performed by two-color immunofluorescence. Cells were incubated for 30 minutes on ice with fluorescein-conjugated antibody and for 30 more minutes with rhodamine-conjugated antibody. Each two-color fluorescence analysis included a double negative control (MsIgG-FITC/MsIgG-PE). The following monoclonal antibodies were used: CD16 (Leu-llb), CD56 (NKH-1), CD25 (IL2-R1). Phenotypic analysis was performed with a Coulter EPICS-Profile II flow cytometer (Coulter Electronics Inc., Hialeah, FL, USA).

Cytotoxicity assay. Cytotoxic activity was measured in standard 4-hour ^{51}Cr release assays at multiple effector (E) to target (T) cells ratios. The K562 cell line, was used as target for fresh NK cell activity. K562 targets were labeled in a total volume of 0.5 ml RPMI supplemented with FBS (10%) for 45 minutes at 37° C with 100 uCi of Sodium ^{51}Chromate (specific activity 5 mCi/ml) (Medgenix, Brussels, Belgium). The percentage of specific lysis at a given E:T ratio was calculated from the formula: (experimental release - spontaneous release)/(maximum release spontaneous release) x 100%, where the maximum release was that obtained from target cells exposed to 0.5% Triton X. Spontaneous release from K562 was <10% of maximum.

Statistical analysis. Statistical analysis was performed with the statistical package Statview (BrainPower Inc., Calabasas, CA, USA) run on a Macintosh II personal computer.

RESULTS

The in vivo immune effects triggered by in vitro mafosfamide purging were evaluated in 6 patients with AML, 3 with ALL, and 3 with NHL (NHL-Pur) grafted with marrow purged at adjusted dose. In addition, 3 NHL patients grafted with unpurged marrow (NHL-Unpur) were studied. The mean (±SD) pre-transplant values of spontaneous NK activity were 32 ± 22%, 9 ± 7%, 21 ± 8%, and 33 ± 25% for AML, ALL, NHL-Pur, and NHL-Unpur, respectively. The post-transplant behavior of NK activity was not homogeneous for the different subgroups of patients. In AML patients, NK activity reached pre-transplant value (41 ± 15%, $P \leq .375$) two months after ABMT. The values detected at 6 and 12 months post-transplant were 50 ± 7% ($P \leq .05$) and 52 ± 8% ($P \leq .05$), respectively. The behavior of NK functional activity was paralleled by an increase of the percentage of CD16- and CD25-positive cells. In NHL-Pur patients, the values of NK activity detected at 2, 6, and 12 months post-transplant were 40 ± 9% ($P \leq .05$), 22 ± 6% ($P \geq .05$), and 57 ± 5% ($P \leq .05$), respectively. Again, this functional activity was paralleled by changes in the percentages of peripheral blood MNCs expressing the NK cell marker CD16 as well as the IL-2 receptor. In contrast, ALL patients as well as NHL-Unpur patients failed to show any significant post-transplant increase in NK cell number and function.

DISCUSSION

Clinical data reported by several single centers as well as the European registry demonstrate an overall beneficial effect of chemical marrow purging on the outcome of patients with hematological malignancies [1, 6, 14-16]. However, despite marrow purging the percentage of long-term disease-free survivors is different when the impact of marrow purging on different diseases is evaluated. In particular, a different clinical outcome is observed for AML and ALL. In AML patients mafosfamide purging at adjusted-dose significantly increases disease-free survival and decreases the probability of relapse as compared to patients grafted with unpurged marrow, whereas ALL patients fail to show any difference in terms of disease-free survival and probability of relapse when purged or unpurged autografts are compared [6, 14, 15]. This observation may have several explanations but at least suggests that mafosfamide marrow purging acts not only through a preferential leukemia cell killing effect but also by triggering immune effects, such as NK cell regeneration, as has been shown in mice [11]. Similar immune mechanisms have been demonstrated in humans following allogeneic BMT [13] but not after chemotherapy [13 and our unpublished data]. Data reported herein demonstrate that in AML grafted with purged marrow as well as NHL-Pur patients, a long-lasting increase in NK function and number is observed post-transplant. Such findings are not observed in ALL and NHL-Unpur patients. Although a larger number of patients is required to reach definitive conclusions on the immune activation after mafosfamide purging, data so far collected consistently demonstrate a different reactivity of the immune system in AML and ALL patients in response to marrow purging. Such a different reactivity in response to rnafosfamide treatment can be explained by a direct involvement of the lymphoid stem cell in the neoplastic process underlying ALL. The increase in NK activity and percentage of NK cells could probably be due to the lack of feedback inhibition of hematopoiesis, normally mediated by mature hematopoietic

cells and their precursors that are destroyed by mafosfamide treatment [11].

In conclusion, our data demonstrate that: (a) mafosfamide acts not only through a potent killing effect on clonogenic cells but also by enhancing NK regeneration; (b) this immune activation is observed in AML grafted with purged marrow as well as NHL-Pur but not in ALL grafted with purged marrow and NHL-Unpur. The clinical relevance of the immune effects of mafosfamide needs to be confirmed in randomized clinical trials.

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Table 1
CHARACTERISTICS OF THE PATIENTS AT THE TIME OF ABMT

	AML	ALL	NHL PURGED	NHL UNPURGED
NO. OF SUBJECTS	6	3	3	3
SEX				
MALE	5	1	3	3
FEMALE	1	2	0	0
AGE (YR)				
MEDIAN	43	26	36	43
RANGE	28-56	20-40	35-36	42-44
DISEASE PHASE				
REMISSION	6	3	3	3
REGIMENS				
BU/CY	6	-	-	-
VP16/BU/CY	-	3	-	-
BEAM	-	-	3	3
ASTA-Z (mG/ML)				
MEDIAN	117	107	97	0
RANGE	95-148	100-140	95-100	
CELL DOSE (X108/KG)				
MEDIAN	1.0	0.8	1.0	1.2
RANGE	0.9-1.0	0.7-1.0	0.9-1.1	1.1-1.4

CYCLOSPORINE-INDUCED GRAFT-VERSUS-HOST DISEASE AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA

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INTRODUCTION

Although autologous bone marrow transplantation (ABMT) may be curative in patients with acute myeloid leukemia (AML) [1,2], the post-ABMT relapse rate is high (50-60%). The risk of leukemic relapse after ABMT or syngeneic BMT [3] is similar and is substantially greater than that seen after allogeneic BMT, reflecting the absence of an allogeneic graft-versus-leukemia effect [4,5].

After clinical ABMT, induction of sufficient immunoregulatory perturbation to provide a self-limited syndrome mimicking graft-versus-host disease (GVHD) might be associated with a complementary graft-versus-tumor effect and lead to a lower leukemic relapse rate. Preclinical studies have shown that rodents given short-course cyclosporine (CSP) after autologous or syngeneic BMT develop a GVHD-like syndrome [6]. This syndrome is associated with appearance of auto- and allo-reactive cytotoxic T lymphocytes directed against the public determinants of Ia antigens [7], and at least one study has shown an inhibitory effect of autologous GVHD on the growth of Ia-positive myeloma in rats [8].

Induction of self-limited acute GVHD-like reactions with short-course CSP in clinical autologous BMT with unpurged marrow has been demonstrated in patients with refractory lymphomas [9] and AML [10]. However, the optimal dose of CSP to induce clinical autologous GVHD has not been established, nor is it known whether GVHD can be induced in recipients of chemopurged autologous marrow or after pre-ABMT regimens that do not use total body irradiation. In this study we determined the incidence and extent of autologous GVHD induced by graded doses of CSP in patients with AML given ABMT with 4-

hydroperoxycyclophosphamide (4HC)-treated marrow and a preparative regimen of busulfan and cyclophosphamide.

MATERIALS AND METHODS

Twenty-one adults (median age, 34 years; range, 18-50) with AML in remission (13 CRl, 7 CR2, 1 CR3) were enrolled in this study. The median duration of CR1 was 10 months (range, 9-22) for patients receiving ABMT in CR2 or CR3, and the median duration of current CR (1, 2, or 3) at the time of ABMT was 4+ months (range, 2.2+ to 10+). Autologous marrow was collected in the current remission, and the mononuclear cell fraction obtained by centrifugation on Ficoll-diatrizoate was incubated with 60 ug/ml of 4HC, washed, and cryopreserved in liquid nitrogen [11]. All patients received pre-ABMT conditioning with oral busulfan (16mg/kg) and intravenous cyclophosphamide (200 mg/kg) [1]. Autologous marrow was thawed and infused 48 hr after the last dose of cyclophosphamide. All blood products were irradiated to at least 1500 rad before administration to patients.

Patients received daily intravenous CSP for 28 days, beginning on the day of ABMT. In this dose-escalation trial, the starting dose of CSP was 1.0 mg/kg/day and was increased in subsequent groups to 2.5 mg/kg/day and then to 3.75 mg/kg/day, based on satisfactory engraftment and acceptable toxicity in the previous dosage group(s). Patients were examined daily for cutaneous manifestations, jaundice, diarrhea, and other potential clinical indicators of GVHD. Skin biopsies were obtained weekly for 7 weeks after ABMT and if patients developed rashes during or after CSP administration and were independently graded by a dermatopathologist for histopathological alterations of acute GVHD, according to published criteria [12].

RESULTS

Seven patients received 1.0 mg/kg/day of CSP, 8 received 2.5 mg/kg/day and 6 received 3.75 mg/kg/day. Sixteen of 21 patients (76%) developed positive skin biopsies for grade 2 acute GVHD: 6 of 7 patients given 1.0 mg/kg/day of CSP, 6 of 8 given 2.5 mg/kg/day, and 4 of 6 given 3.75 mg/kg/day. The median time to appearance of positive skin biopsies for GVH was 34 days after ABMT (range, 14-49) and was similar among the 3 CSP dosage groups (P=NS; Wilcoxon rank-sum test). Eleven of the 16 patients had macular and/or papular rashes involving less than 25% of the body surface at the time of positive biopsies, but none developed confluent erythroderma or bullae. Five patients had no rashes or other manifestations of GVHD but had grade 2 acute GVH alterations on routine "blind" skin biopsies. No patients had gastrointestinal or hepatic GVHD, and all cutaneous manifestations resolved without treatment in 5-10 days. Neither lymphocyte nor neutrophil recovery was required for the development of positive skin biopsies: at the time of positive biopsy, 6 of the 16 patients had peripheral blood lymphocyte counts $\leq 0.2 \times 10^9/1$ and 11 had absolute neutrophil counts $\leq 0.5 \times 10^9/1$.

Three patients, 1 of whom had a positive biopsy, died in remission with ABMT-related complications (1 each from sepsis during aplasia, hepatic veno-occlusive disease, and cytomegalovirus interstitial pneumonitis). Nine patients (5 CRl, 4 CR2) relapsed with AML at a median of 411 days (range, 115 to 633)

after ABMT; 8 had positive skin biopsies for GVHD. The actuarial relapse rate in this series is 50%. Nine patients (7 CR1, 1 CR2, and 1 CR3) are alive in remission at a median of 833+ days (range, 476+ to 1143+) after ABMT; 7 had evidence of GVHD in the post-transplant period. The actuarial disease-free survival in this group is 42%.

DISCUSSION

Modulation of host cellular immunity to mimic graft-versus-host and graft-versus-leukemia reactions after ABMT for AML is an attractive approach to improve the disease-free survival by reduction of the relapse rate. This approach is supported by observations that immunological graft-versus-tumor effects may be operative against neoplastic cells that bear Ia (Class II) antigens on the cell surface [8; 13,14]; autologous GVHD after short-course CSP is also associated with the appearance of anti-Ia cytotoxic T lymphocytes in rodent [7] and in clinical [9] autologous BMT settings and with antitumor effects in a rodent Ia-positive myeloma model [8]. It is not known, however, whether the disrupted immunoregulatory process of CSP-induced autologous GVHD in clinical ABMT is associated with an autologous graft-versus-tumor effect as well. Although 7 of the 9 patients in this series who are alive without recurrent AML had histopathologic evidence of cutaneous GVHD, 6 of the 7 patients who relapsed after ABMT also had positive skin biopsies.

Our observations suggest that as little as 1.0 mg/kg/day of CSP for 21-28 days is sufficient to induce autologous GVHD in recipients of chemopurged ABMT for AML; higher doses of CSP did not induce the syndrome more rapidly or with any greater frequency or severity, unlike in rodent systems in which increasing CSP dosage directly correlated with both frequency and intensity of autologous GVHD [6,7]. Additionally, *ex vivo* chemopurging of the marrow did not prevent the development of autologous GVHD, consistent with preclinical observations indicating that autologous GVHD can be induced in rodents given CSP after BMT with 4HC-treated [15] or lymphocyte-depleted [16] syngeneic marrow. Finally, even though preclinical studies have suggested that thymic irradiation plays an important role in the development of autologous GVHD [17], our findings demonstrate that total body irradiation is not a prerequisite for the development of clinical autologous GVHD.

The antitumor effects of CSP-induced autologous GVHD may be enhanced by administration of other agents such as interferon, which increases cellular expression of Ia antigen [8,18,19]. Preliminary reports from our center and others [19] suggest the feasibility of addition of a-interferon to CSP after ABMT in patients with high-risk hematologic malignancies without increasing the risks of prolonged marrow hypoplasia. Furthermore, in patients undergoing ABMT for metastatic breast cancer, the addition of a-interferon to CSP has resulted in increased extent of cutaneous GVHD when compared with CSP alone [20].

This pilot study does not allow one to evaluate the antitumor effectiveness of CSP-induced autologous GVHD in patients receiving ABMT for AML. This issue may be addressed by randomized prospective controlled trials, probably conducted on a national or international collaborative basis, to determine whether autologous GVHD induced by CSP, alone or with other immunologic modulators such as interferon, can favorably affect relapse rate and disease-free

survival in patients undergoing ABMT for leukemias, lymphomas, and potentially other neoplastic diseases such as breast cancer.

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IMMUNOTHERAPY OF AML AFTER ABMT - SCIENTIFIC RATIONALE AND EARLY EXPERIENCES WITH LINOMIDE

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Abstract

Relapse of leukemia is the most important cause of treatment failure in acute myeloid leukemia. Complete remission can be achieved in most patients, but the majority of patients treated with chemotherapy or ABMT will eventually relapse. Experiences from allogeneic bone marrow transplantation as well as other information suggest that immune function is related to the relapse rate.

Linomide is an orally active novel immunomodulatory agent. Eleven patients with AML or CML were treated with Linomide intermittently in 3 week courses with 3 weeks treatment-free intervals for a maximum of 24 weeks. Twenty-six such treatment cycles were evaluable and prominent increases of NK cell number and activity could be demonstrated. In individual patients increases in T cell and monocyte numbers were also shown. Release of IL-6 during treatment periods was prominent.

Since the immunologic activity of Linomide was confirmed in the initial study, prospective randomized trials comparing Linomide and placebo are underway in Europe, North America and Australia. The aim of these studies is to determine if Linomide can reduce the risk of relapse of AML after ABMT. Toxicity has so far been manageable and dominated by musculoskeletal discomfort, nausea, vomiting, edema, skin rash and diarrhea. Results from ongoing trials have to be awaited before the role of Linomide in AML can be defined.

Introduction

Relapse of leukemia is the most important cause of treatment failure in acute myeloid leukemia. Complete remission can be achieved in a majority of patients [1,2], but if treated with chemotherapy only, most of the patient will eventually relapse [3,4].

In spite of intensive induction, consolidation, and maintenance treatment, at present the best prospects for cure of AML are for patients who undergo allogeneic bone marrow transplantation (BMT) from a HLA-identical sibling donor with 45 to 50% of such patients becoming long term survivors [5,6].

BMT is however, burdened by limited availability of donors as well as by significant procedure related morbidity and mortality [5,8]. Even if graft versus host disease (GVHD) is a major cause of procedure related mortality after allogeneic BMT this condition reduces the risk of relapse of leukemia 7,[9-11]. GVHD may be prevented or treated but the risk of relapse has been shown to be correspondingly higher with increasing success of such treatments [12-14].

T lymphocytes seem to be particularly active in eradication of minimal residual disease (MRD) in leukemia 14. Therefore it may be conceived that an increased T-cell activity may lead to a decreased risk of leukemia relapse. NK and LAK cells are capable of mediating graft versus leukemia effects and may lyse allogeneic and autologous leukemic blasts *in vitro* [15,16].

NK activity and LAK cell inducibility are more prominent in complete remission than in blastic AML [17,18]. It has also been shown that the NK cell activity in complete remission of AML is inversely related to the risk of relapse 19. Activated macrophages may be non-specifically cytotoxic for tumor cells independent of the MHC, but they may also be phagocytic or mediate antibody dependent cellular cytotoxicity. Macrophages may also release a number of cytotoxic mediators such as TNF alpha, interferon alpha or gamma, and others [20-22].

All in all, a body of evidence suggests that immunotherapy studies would be worthwhile in order to determine if relapse rate of AML may be decreased, and thus an overall increase in cure rate might be achieved.

Linomide (roquinimex) is an orally active novel immunomodulator that enhances T cell, NK cell, and monocyte-macrophage activity [23-26]. In the first clinical experiences it appeared that Linomide AHD manageable toxicity at doses that elicited activation of immune parameters [26].

Linomide after ABMT - immunologic effects

Eleven patients with acute or chronic myeloid leukemia were treated intermittently in 3 week periods with 3 week treatment-free intervals for up to 24 weeks with Linomide as has been previously reported for five of these patients[27].

Informed consent was obtained from all patients, and the study was approved by the Institutional review board at the University Hospital of Uppsala, as well as by the National Board of Health and Welfare in Sweden. During treatment periods the first five patients received Linomide 0.3 mg/kg orally once a week starting the day of marrow infusion. The following six patients received 0.2 mg/kg orally twice weekly also starting immediately after marrow infusion. In some of the latter patients the dose was gradually increased. These eleven patients received Linomide during a total of 26 treatment periods.

Peripheral blood samples (40-60 ml) were obtained before start of the conditioning therapy and from once a week to every 3 weeks for up to 6 months. Samples during treatment periods were always drawn 48 hours after drug administration. Preparation of mononuclear cells, immunoflourescence staining, flow cytometry, lymhocyte proliferative responses, and cytotoxicity assays were as described previously [27]. Cytokines were measured using commercially available radioimmunoassays (Medgenix, Fleurus, Belgium).

Thus patients were treated immediately after ABMT with Linomide for 3 weeks followed by a 3 week rest period. Such cycles were repeated up to a maximum number of 4 cycles per patient. Six patients had AML and five had CML. One AML patient was excluded since she received on 1 incomplete cycle of treatment and was withdrawn due to a periocular edema. Another AML patient was withdrawn after 2 complete cycles due to the same reason. Two CML patients received 1 cycle only and were withdrawn because of pancytopenia. Two patients were withdrawn after 5 and 13 weeks respectively due to intracranial hemorrhage. One AML patient was withdrawn after 3 cycles because of relapse.

Otherwise toxicity was mild to moderate and dominated by musculoskeletal discomfort, fatigue, fever, skin rashes and nausea.

An increase in NK cell (CD 16+, and CD 56 + CD3-) numbers was observed during the treatment periods with lower numbers recorded before and after the treatment (Figure 1 and 2). NK cell activity measured as killing of K562 cells as well as LAK cell activity measured as killing of Daudi cells were increased in individual patients (Figure 3). In individual cycles increases in monocyte and T cell numbers were also observed.

Increased production in individual treatment cycles of TNF alpha, IL-1 and interferon gamma has been reported previously [27]. To these observations have now been added the measurements of IL-6 in serum before, during and after treatment. Figure 4 shows that increases of IL-6 could be demonstrated during the treatment.

Comparative studies underway

The knowledge of the importance of the immune function for the risk of relapse of AML together with the data on the immunomodulatory properties of Linomide as well as the safety of this compound formed the rationale for two different prospective randomized studies aiming to determine if Linomide can reduce the risk of relapse of AML after ABMT. One study is active in ten European countries and one is in the US, Canada and Australia. Both of the studies include patients with AML in complete remission eligible for autologous bone marrow transplantation, regardless of prior antileukemic therapy.

Taken together the two studies have recruited almost 200 patients who are treated with Linomide 0.2 mg/kg twice weekly or placebo. Time to relapse, relapse rate, overall survival will be recorded as well as safety and immune parameters. Toxicity has so far been manageable and dominated by musculoskeletal discomfort, nausea, vomiting, edema, skin rash and diarrhea. No efficacy data is available since the studies are blinded.

Conclusion

The high rates of remission as well as of relapse in acute myeloid leukemia indicate that the most important opportunities for improvement of the cure rate of this disorder lies in successful prevention of relapse. The experiences from allogeneic bone marrow transplantation indicate that the immune function is of importance for the relapse rate in this disorder. A variety of agents including Linomide modulate immune functions in such a way that clinical studies are warranted, where the aim is to determine if the relapse rate of AML can be decreased by use of such agents. Results from currently ongoing clinical studies will have to be awaited before the role of immunomodulatory agents in acute myeloid leukemia can be defined.

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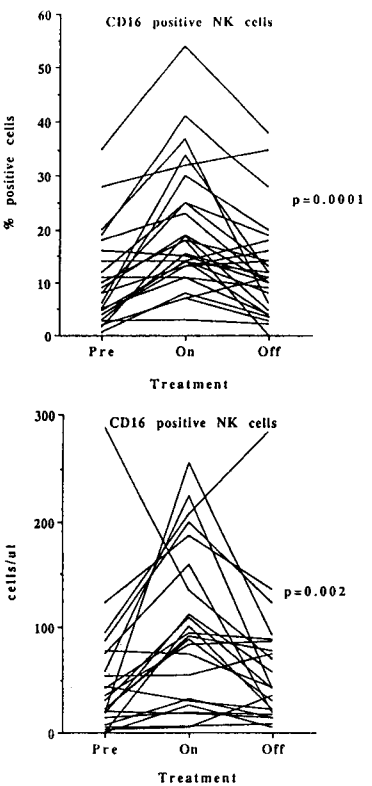

Figure 1.
CD16+ NK cells increased in relative as well as absolute numbers in peripheral blood during Linomide treatment.

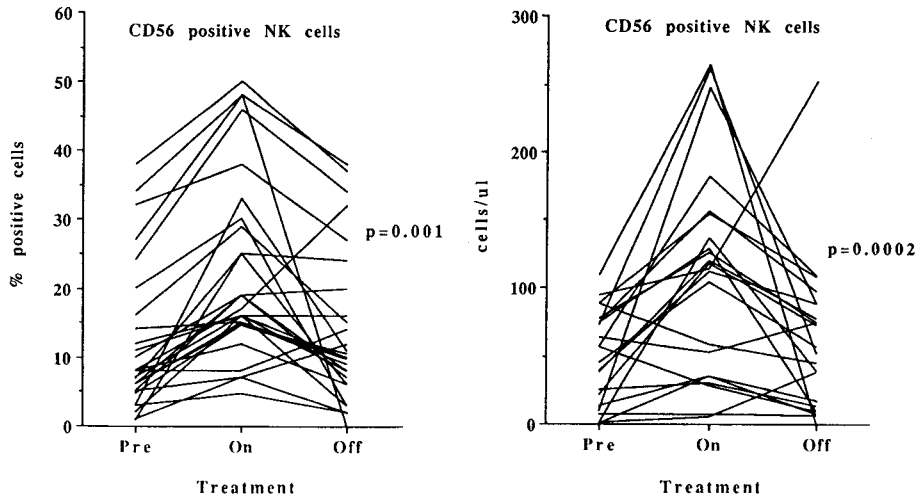

Figure 2.
CD56+CD3- NK cells increased in relative as well as absolute numbers in peripheral blood during Linomide treatment.

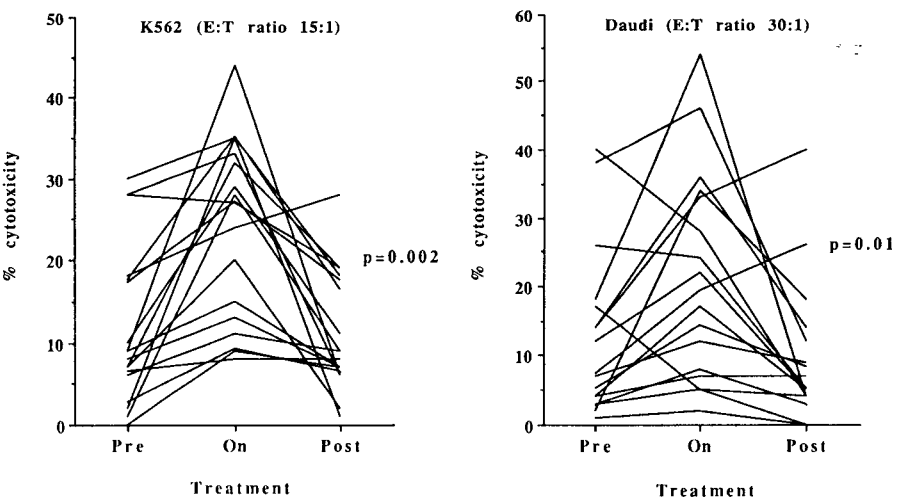

Figure 3.
NK and LAK cell activity increased during Linomide treatment.

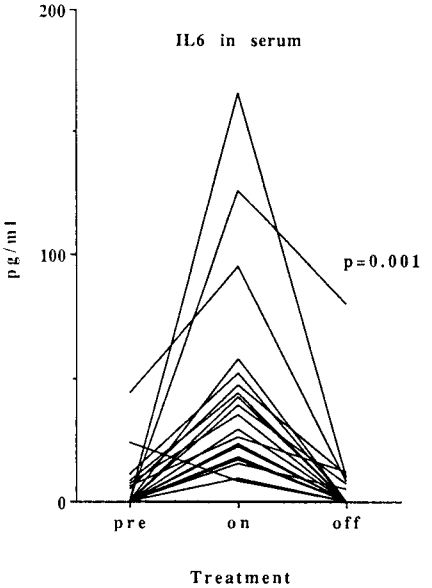

Figure 4.

THE USE OF GRANULOCYTE COLONY-STIMULATING FACTOR (G-CSF)FOLLOWING AUTOLOGOUS BMT IN LYMPHOMAS.

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Since hemopoietic growth factors have become available in clinical practice, a number of trials of both G-CSF and GM-CSF, Phase I, II and III, have taken place in many centers and have involved patients with various neoplastic diseases treated with chemotherapeutic regimens or myeloablative therapies followed by BMT. The purpose of combining hemopoietic growth factors with chemotherapy is to accelerate hematopoietic recovery and to decrease adverse effects associated with aggressive protocols. This approach may also enable clinicians to avoid dose reduction or delay in therapy, and potentially to escalate dose. G-CSF, a lineage specific hemopoietic growth factor, and GM-CSF, an agent that affects a broader range of cell lineages, have both been studied in neutropenic patients following cancer chemotherapy and autologous bone marrow transplantation. A question has been raised as to whether the use of a lineage restricted growth factor such as G-CSF immediately following ABMT is of any advantage. In fact if the target of this factor were solely the late maturational stages of myeloid precursors, then an acceleration of neutrophil recovery would hardly be explained, following myeloablative chemotherapy and G-CSF administration. However a body of literature exists [1,2,3,] indicating that the target of G-CSF may include multipotential hemopoietic stem cells. Thus, the expansion of the myeloid compartment seems to be matched by an expanded earlier pluripotential compartment, probably with an increased fraction of the stem cell pool entering the proliferative pool. This recruitment and expansion of the more primitive precursors may be accomplished by acting in synergy with other cytokines, specifically interleukine-l (IL-1), IL-6 or the c-kit ligand/stem cell factor to stimulate proliferation of primitive precursors with functional or phenotypic features of stem cells [4].

Results of Phase I/II studies in patients receiving BMT suggested that the optimal dose was 250 mg/m^2, and that this achieved an absolute neutrophil count (ANC) of >0.5 x 10^9/L in 17 days, compared with 25 days in historical controls and was well tolerated.

A multicenter, randomized, placebo-controlled trial was conducted in patients with lymphoid malignancies (65 in the GM-CSF arm and 63 in the placebo arm)[5]. Active treatment group received GM-CSF (250 mg/m^2/day) by i.v. infusion for 21 days, starting within four hours of the infusion of autologous marrow. No toxic effects specifically ascribed to GM-CSF were noted. The active treatment group recovered to a neutrophil count of 0. x 10^9/L seven days earlier than the patients who received placebo (19 days vs 26 days, p<O.001). Administration of GM-CSF was associated with fewer severe infections (2 vs 12 epi-

sodes), fewer days of i.v. antibiotics (24 vs 27 days) and shortened initial hospitalization (27 vs 33 days), but it did not decrease the number of days with fever.

In all the studies performed in the BMT setting, G-CSF was well tolerated and accelerated neutrophil recovery. Moreover it has been shown that in standard cancer chemotherapy G-CSF not only enhanced neutrophil recovery, but, as compared with the placebo group, also clearly reduced days with fever >38° C.

We analyzed data on 8 patients with high-grade non-Hodgkin's lymphoma (NHL) and 17 patients with Hodgkin's disease (HD), who were treated with G-CSF following autologous bone marrow transplantation (Tab I). Patients were conditioned with BEAM (NHL) or CVB (HD 15 patients); two patients with HD and bone marrow involvement had peripheral blood stem cells collected and were then conditioned with BEAM.

G-CSF was started at the dose of 5 mg/kg body weight, i.v. by 1 hr daily infusion, from day +1 to day +20, following infusion of the autologous bone marrow at day 0. Overall the mean time to ANC >500 x 10^9/L was 9 days (range 5-15) for patients with HD and 14 days (range 10-21) for NHL, and it took a mean time of 14 days to achieve an ANC of 1.0 x 10^9/L (Tab. II).

Patients had fewer and shorter febrile episodes (average 3 days with fever>38°C) and fewer days of i.v. antibiotics compared with historical controls. (Table III).

According to our results and from experimental work, the early use of G-CSF is fully justified. As to infection, there is a response, both through activation of the immune system, and by increasing the production of neutrophils. Perhaps it is not coincidental that G-CSF, together, perhaps with IL-6 that increases Ig secretion by B cell, are also capable of stem cell interaction. At a dose of 5 mg/kg, G-CSF was very well tolerated and appeared to have activity when compared with a comparable retrospective control group, by reducing the neutropenia which follows cytotoxic chemotherapy.

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Table 1. G-CSF in ABMT: Patient Characteristics

Disease	HD	NHL
No. Patients	17*	8
M/F	9/8	3/5
Age (mean)	25	28
Status		
RC	3	4
RP	-	2
AR	14	2
*2 PBSC		

Table 2. G-CSF in ABMT: ANC Recovery

Disease	No. Patients	ANC >100	ANC >500	ANC >1000
HD	17*	5(3-12)	9(5-15-)	10(7-21)
NHL	8	11(9-16)	14(10-21)	16(12-23)
CONTROLS				
Nemanaitis	86	16(8-41)	25(10-60)	29(17-64)
Sheridan	16	-	20	29
Taylor	58	18(7-34)	22(10-51)	30(14-61)
*2 PBSCT				

Table 3. G-CSF in ABMT: Incidence of Infection

Disease	No. Patients	No. of days fever >38°C	Bacteremic episodes	Days of IV antibiotic
HD	17	15	4	14(5-32)
NHL	8	4	2	14(9-21)
CONTROLS				
Nemunaitis	86	12(0-30)	-	-
Sheridan	18	9	-	18
Taylor	58	7	19	-

Session III: Myeloma

PERIPHERAL BLOOD STEM CELL TRANSPLANTATION: POTENTIAL BENEFIT AND COST EFFECTIVENESS IN MYELOMA

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The few groups which first experimented autologous blood stem cell transplantation (ABSCT) into the middle of the eighties, at the same time expected a lower likelihood of graft contamination by residual tumor cells and also worried about the actual capacity of blood-derived hematopoietic progenitor cells to restore permanently a normal hematopoiesis. If the first point remains disputable so far, it rapidly appeared that ABSCT resulted in not only a satisfactory but also a more rapid hematopoietic recovery as compared to bone marrow transplant, what represented an unexpected advantage of the procedure ([1]). This hastened hematopoietic recovery following ABSCT leads to briefer hospitalization time, lower antibiotic and transfusional needs, and consequently to a probable lower cost effectiveness as compared to other transplant procedures ([2,3]), what is nevertheless the subject of still impassioned controversies. Starting from an initially homogeneous group of poor-risk myeloma patients, we have tried to retrospectively value the actual cost of ABSCT in this indication, related with the overall survival and the quality of life.

PATIENTS AND METHODS

Patients

Twenty consecutive patients (11 males, 9 females) have been enrolled in a prospective study, the aims of which being: 1) a prime reduction of the tumor cell load and a simultaneous mobilization of peripheral blood stem cells (PBSC) with infra-lethal chemotherapy, 2) a secondary complete tumor eradication by reinforcement of the treatment with supra-lethal high-dose chemo-radiotherapy immediately followed by reinfusion of PBSC, when available ([4]). The age of the patients at diagnosis ranged from 35 to 65 years (average: 60y). At the time of inclusion in the study, eighteen patients presented a Stage III disease in the classification of Durie-Salmon and had not received any prior treatment, while two other patients were refractory to prior chemotherapy. All presented various painful bone fractures, eight being even bedridden.

Therapeutic protocol

The therapeutic protocol, which has been previously described ([4]), is summarized in Fig. 1. Briefly, it comprised two successive phases corresponding with the aims of the study. When adequate collection of PBSC was not available, patients underwent a further conventional chemotherapy (Group 1). On the con-

trary, in case of adequate collection of PBSC after the first phase, the patients were transplanted within the 4 following months after a conditioning regimen combining total body irradiation (TBI) and high-dose melphalan (HDM) (Group 2).

Evaluation of the cost of therapy

All expenses related to the first therapeutic phase, to the second therapeutic phase (either conventional chemotherapy or ABSCT) and to the post-transplant survey were wholly taken into account for estimation of the cost. Three methods of calculation have been used: a) Calculation of the Cost Price, adding up all effective expenses: daily hostelry package, drugs, transfusions, single usage material, laboratory tests, X-Ray examinations, kits for PBSC collection, TBI, medical and non-medical staff time. b) Calculation of the Global Cost Price, adding provisions for material depreciation to the former c) Cox Model, taking in account five pre-definite parameters (table 1) ([5]).

Appreciation of the quality of life from therapeutic Phase 1

A staging scale starting from score 1 (normal quality of life) down to score 0.025 (bedridden patients) as proposed by Bulpitt et al ([6]), was related to the number of weeks that each patient spent in different scored stages since therapeutic Phase 1. It allowed to determine a coefficient of quality of life.

Appreciation of the potential professional or housekeeping productivity

This appreciation was done for each patient according to the scale related to age established by Dolan et al ([7])

Statistical analysis

The correlative parametric test was used for determination of positive or negative correlations between cost calculation methods. The significance of difference in overall survival and quality of life coefficient was assessed by Mann-Whitney analysis.

RESULTS

1) Therapeutic results

Three patients died during Phase 1, one by aplasia-related sepsis, two by uncontrolled progressive disease. The rebound phase following the chemotherapy-induced aplasia was unsufficient to allow adequate PBSC mobilization and collection in 7 patients, who consequently were not transplanted (Group 1). Two of these achieved a complete remission (CR) after HDM, for 2 and 3 years respectively, receiving only an oral maintenance chemotherapy during this period. Nevertheless, both have relapsed, and are now difficultly controlled with conventional chemotherapy. Five other patients remained evolutive after HDM, of whom 3 died within 6 months; the remaining two are still alive, although almost bedridden and uncontrolled despite conventional chemotherapy.

Ten patients were transplanted with PBSC (Group 2). All achieved CR, except one who only partially responded; he relapsed and died 18 and 28 months from diagnosis respectively. Four other patients died within months or years following ABSCT: one of procedure-related complications 45 days post-trans-

plant; two others died of intercurrent events while they were still in CR, one of a sepsis shock related to a renal lithiasis 10 months post transplant, the other in a car crash! Eighteen months post-transplant; the fourth biologically relapsed 24 months post-transplant, remained well and controlled with only oral chemotherapy for two additional years, but finally died of evolutive disease at 58 months from diagnosis. Five patients are unmaintained, alive and well, still in CR 58, 54, 28, 24 and 14 months from diagnosis respectively.

The median overall survival from diagnosis of the two sub-groups of patients is expressed in table 2.

Cost effectiveness

The average costs of therapy in the 2 groups of patients are expressed in table 3, according to each calculation method. Into each group, there was a strong correlation between the Cox Model and the Absolute Cost Price (p-value < 0.001), a slighter one, while still significant, between those both and the Cost Price ($p< 0.01$). There was no significant difference between Groups 1 and 2 except when the Cost-Price was considered ($p< 0.01$). Furthermore, when the potential patient's productivity, largely in favour of Group 2, was substracted from Cost Price, the difference between the 2 Groups increased still more significantly ($p< 0.001$) (table 4)

Quality of life

The relationship between average scores of quality of life and the overall survival is expressed in table 5. The coefficient of quality of life is significantly different between Group 1 and Group 2 (0.35 vs 0.80, $p < 0.001$).

DISCUSSION AND CONCLUSIONS

Even treated with the most intensive conventional chemotherapies, high risk myeloma remains a very poor risk disease, the average overall survival being lower than 12 months. Several patients receiving high-dose chemotherapy may respond favourably ([8]), but they relapse most of the time within few months or years, needing further treatments, which imply numerous stays at hospital but finally do not avoid extreme bone pains and death in difficult conditions. When high-risk myeloma patients can be transplanted early in the course of the disease, the average overall survival increases considerably, as we report here similarly to other groups ([9, 10]). Long complete remissions can be achieved, and perhaps some patients could even be cured. Curiously in our study, the median overall survival appeared significantly longer in female vs male subgroups, so as male sex could be an additional risk factor.

Furthermore, except within a period comprised between the beginning of the first therapeutic phase and the end of the first quarter following ABSCT, the quality of life of most

of the transplanted patients returns to normal progressively, allowing them to perform again all their previous activities, as well demonstrated in this study.

Unlike some people proclaimed, the cost of the whole therapeutic procedure ending at ABSCT survey was not higher in our study than that of patients who were not transplanted and consequently needed a frequent survey and further chemotherapy courses. It appeared even cheaper when the Cost Price calcula-

tion is considered, and all the more when the potential productivity and the younger age of the patients is related to this Cost Price.

Additionally, the Cox Model proves in this study to be an easy and reliable method of cost calculation, fully applicable to hematopoietic transplant procedures.

In conclusion, high-dose therapy combined with ABSCT in high-risk myeloma allows a significantly prolonged overall survival in better conditions of life and at lower cost than do conventional chemotherapies. Addition of hematopoietic growth factors to the mobilization procedure and also eventually to ABSCT, could both achieve a lower mortality rate of the whole procedure and a higher proportion of adequate collections of PBSC, even in case of disease-related inhibition of hematopoiesis frequently observed in high-risk myeloma patients [11]).

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Table 1: Cox Model

Parameters	1983 Cost (USD)
I.C.U.*	871.25 x nb of days
Non I.C.U.**	244.55 x nb of days
Lab.	36.83 x nb of tests
X-rays	344.91 x nb
Op. Proc.***	3283.08 x nb of acts

* Intensive Care Unit
** Non-Intensive Care Unit
*** Setting up of intravenous catheter

Table 2: Median Overall survival from diagnosis

Group	Sex	Median Age	Overall Survival	p-values	
1	M(3)	58	8 (3-20)	)	
	F(4)	56	20 (4-35)	) <0.01	
	M+F(7)	57	14	)	)
					) <0.01
2	M(5)	62.8	21 (8-30)	)	)
	F(5)	60	33 (8-58)	) <0.01	
	M+F(10)	61	27	)	

p-values were determined using the Mann-Whitney analysis. Significant differences in overall survival are observed in each group between males and females. The overall survival is twice as long in the transplanted as in the non-transplanted group.

Table 3: Cost effectiveness of high dose therapy in myeloma

	Method of Calc. of cost	Phase 1	Phase 2	Survey	T Cost
Group 1	1) Cost price (US$)	34,864	42,425		77,289
7 pts.	2) Cox model (US$)	43,096	49,976		93,072
	3) Absolute Cost Price (US$)	44,730	51,744		96,072
Group 2	4) Cost Price (US$)	26,475	21,737	13,619	61,831
10 pts.	5) Cox model (US$)	37,087	29,951	22,468	89,506
	6) Absolute Cost Price (US$)	39,668	30,740	22,573	92,981

Correlative p-values between:

1) and 3) < 0.01 l) and 4) < 0.02
2) and 3) < 0.001 2) and 5):0.08 (NS)
4) and 6) < 0.02 3) and 6):0.8 (NS)
5) and 6) < 0.001

In Group 2, "Survey" corresponds to the post-transplant period: most of the patients needed iterative irradiated platelets and red blood cell transfusions for several weeks after early hospital discharge (average: 18 days). Bone marrow and blood controls with progenitor cultures were also systematically performed at day 30, 60, 120, 180 and 365 post-transplant. Acyclovir was prophylactically given for 6 months after transplantation.

Table 4: Relationship between average cost price and patients productivity

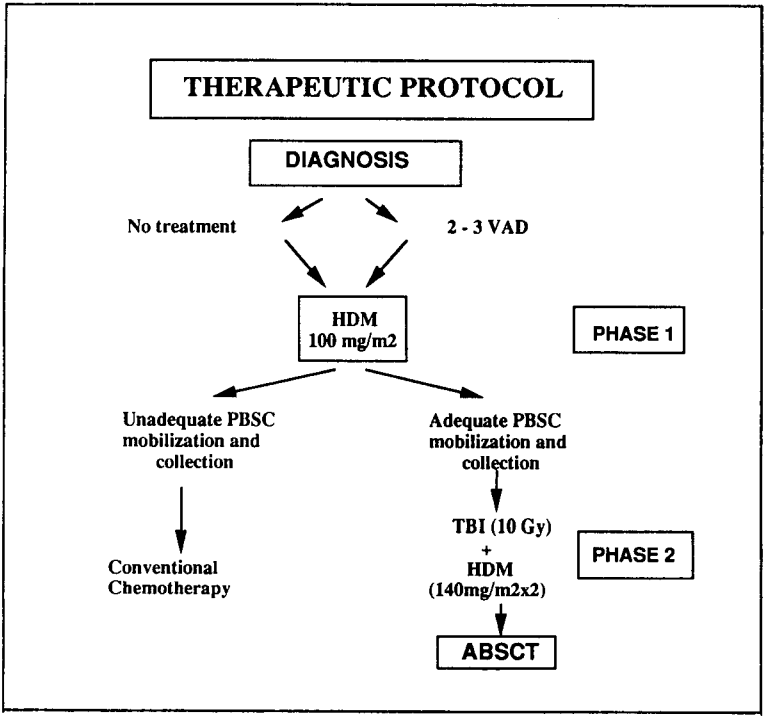
	Group 1	Group 2	p-values
Cost Price (US $)	77,289	61,831	<0.02
Productivity (US $)	504	9,996	<0.0001
Difference	76,785	51,835	<0.001

The average productivity favourably balances the average cost price in Group 2 as compared to Group 1.

Table 5: Patient's quality of life

Score	Median Overall Survival (weeks) Group 1	Group 2	p-value
1	0.0	42.3	
0.975	0.2	7.0	
0.875	1.6	2.6	
0.8	0.8	30.0	
0.75	10.3	10.9	
0.675	0.8	0.4	
0.375	22.0	9.3	
0.125	9.2	1.5	
0.025	13.0	0.2	
TOTAL (T)	58.0	104.2	
*Corrected Total (CT)	20.19	84.1	
Coefficient (CT/T)	0.35	0.80	<0.001

The quality of life is scored from index 1 (normal) down to index 0.025 (bedridden patient). Numbers facing each score index express the average number of weeks spent by patients of each group at this level of quality of life. Total (T): overall median survival expressed in weeks. Corrected Total (CT): Score index x corresponding number of weeks. Coefficient of quality of life: CT/T ratio.



Session IV: Lymphoma

AUTOLOGOUS BONE MARROW TRANSPLANTATION IN LOW GRADE B CELL NON-HODGKIN'S LYMPHOMAS: ANALYSIS OF PROGNOSTIC FACTORS FOR IMPROVED DISEASE-FREE SURVIVAL

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INTRODUCTION

The overwhelming majority of patients with low grade non-Hodgkin's lymphomas (NHL) are not curable with currently employed treatment approaches. [1,2] The rationale for the use of high dose ablative therapy in low grade NHL is based upon the fact that relapsed patients can continue to respond to further conventional treatment and salvage regimens. However, a continuous rate of relapse continues to be observed with salvage regimens. As had been previously demonstrated for relapsed/refractory NHLs, resistant disease could be overcome with dose intensification with high dose therapy, followed by allogeneic and autologous bone marrow transplantation (ABMT)[3]. However, in contrast to patients with relapsed intermediate/high grade NHL, this approach has been used in a relatively limited number of patients with low grade NHL [4-9]. These studies suggest that a subset of patients with low grade NHL may benefit from high dose therapy and bone marrow transplantation. A major problem with studies in low grade NHL is that long follow-up is required before the effectiveness of this modality can be confirmed due to the natural history of these diseases.

The major factor which identifies patients with relapsed NHL who have a favorable prognosis following ABMT is sensitivity of disease to conventional and salvage therapy. However within the good prognostic group of patients with sensitive relapses there are likely to be factors which identify patients with a favorable prognosis. Since the majority of relapses are observed in the first two years following ABMT, examination of patients with at least 2 years of potential follow-up may provide insight into additional prognostic variables which impact on outcome. In this study we report an analysis of 54 patients with relapsed low grade B cell NHL in sensitive relapse who underwent ABMT before 1/5/90. This study may provide further insight into which patients with low grade NHL may in fact benefit from high dose therapy and ABMT.

METHODS

Selection of patients and treatment protocol. Patients were eligible for this study if they were less than 65 years of age; had relapsed low grade NHL as defined by the International Working Formulation, after standard chemotherapeutic regimens; and had lymphoma cells that expressed the CD20 (B1) antigen as previously described ([8]). In addition, patients with sensitive low grade NHL but who had failed to enter complete remission after one or more standard chemotherapeutic regimens were eligible. For all patients, a minimal disease status had to be attained through chemotherapy, radiotherapy, or both prior to entry. This status was defined as lymph nodal mass less than 2 cm in its greatest diameter and histologic evidence of bone marrow involvement of 20% or less of the intratrabecular space as determined by iliac crest biopsy. Additional criteria for entry included the absence of comorbid disease of the heart, kidney, lung, and liver and a Karnofsky score above 80 percent. Informed consent was obtained from all patients.

Preparative therapy consisted of cyclophosphamide, 60 mg/kg of body weight, infused on each of two consecutive days before radiotherapy. TBI, was administered in fractionated doses (200 cGy) twice daily on three consecutive days (total of 1200 cGy) in all patients. Supportive care was provided as previously described ([8]).

Collection, processing, and infusion of marrow. Bone marrow was obtained, treated in vitro as previously described ([8]).

Evaluation and statistical analysis. Before treatment, all patients were evaluated by physical examination, blood-chemistry profile, complete blood count, chest x-ray, abdominal-pelvic CT scanning (chest CT if indicated), bone marrow aspirate and biopsy, as well as cell surface phenotypic studies of peripheral blood and bone marrow mononuclear cells. Other studies such as gallium scanning were done as needed to determine the extent of disease. Follow up restaging was carried out every 6 months after transplantation or as clinically indicated.

Complete remission (CR) was defined as the disappearance of all measurable and evaluable disease. Failure were defined as relapse of disease or toxic death. Disease-free survival (DFS) was calculated from the day of marrow transplantation (day 0). Multiple pre-ABMT factors were identified and a Cox proportional hazards regression of the entire patient group was performed.

RESULTS

Fifty-four patients with low grade B cell NHL in sensitive relapse or incomplete first remission who attained a minimal disease state underwent ABMT between 3/84 and 1/90. The median age of these patients was 42 (17 female, 37 male). The histologies of these patients included 38 with follicular small cleaved cell, 14 with follicular mixed small cleaved and large cell, and 2 with diffuse intermediate lymphocytic lymphoma. The majority of patients had a history of stage IV disease (43 patients) and marrow involvement (43 patients), with 15 patients having extramedullary extranodal disease, and 14 had a history of B symptoms. Twenty-nine patients had a history of never achieving a CR prior to consideration for ABMT. At BM harvest only 21 patients were in complete clini-

cal remission, 28 patients had no residual histologic evidence of BM infiltration.

As of 6/1/92, 23 of these patients have relapsed, and 3 died without relapse. The overall DFS for the 54 patients is 58% at 36 months (Figure 1). The median follow-up for the 28 patients who are disease-free is 36 months. A number of pre-ABMT variables were examined in an attempt to predict which patients are at increased risk for relapse. These variables included remission status at ABMT, bone marrow involvement, the presence of 13 symptoms, extranodal disease, stage, splenomegaly, age, gender, history of CR, interval from diagnosis to ABMT and interval from first treatment to ABMT. Using Cox proportional hazards regression of the 54 patients, we found the lack of a prior CR was associated with significantly longer DFS (76% vs 43% at 36 months) ($p<0.005$). The other factors examined were not associated with statistically significant ($p<0.05$) differences in DFS. We performed a stepwise logistic regression to identify the characteristics of the patients with no prior history of CR using the above pre-ABMT clinical variables. To date we have not yet identified factors which explain the two-fold reduction in risk among these patients. We have previously reported that patients in CR at harvest have a significantly improved DFS than patients in PR ([8]). With longer follow-up we now find that the DFS survival is not significantly different between patients in CR or PR (Figure 3). Moreover, with longer follow-up, later failures were seen in the patients in CR at ABMT. This further supports the notion that long follow-up is critical in evaluating the role of ABMT in low grade NHL.

SUMMARY

In the present study we have examined 54 patients with low grade B cell NHL who underwent ABMT prior to 1/90. Using this patient group with longer follow-up we have attempted to identify factors which would predict which patients might have the greatest benefit from this treatment modality. As of 6/92, 23 of these patients have relapsed, and 3 died without relapse with a DFS for this population of 58% at 36 months. Of a number of clinical variables examined only a lack of CR prior to consideration for ABMT was associated with a significantly improved DFS. We were unable to further identify the clinical characteristics of this group to explain this finding. It is possible that these patients had false positive restaging studies and therefore were treated more aggressively prior to harvest in order to achieve a CR. These preliminary observations will require further study, however, the identification of patients with an increased risk of relapse may be candidates in the future for alternative treatment strategies to improve the DFS for relapsed low grade NHL.

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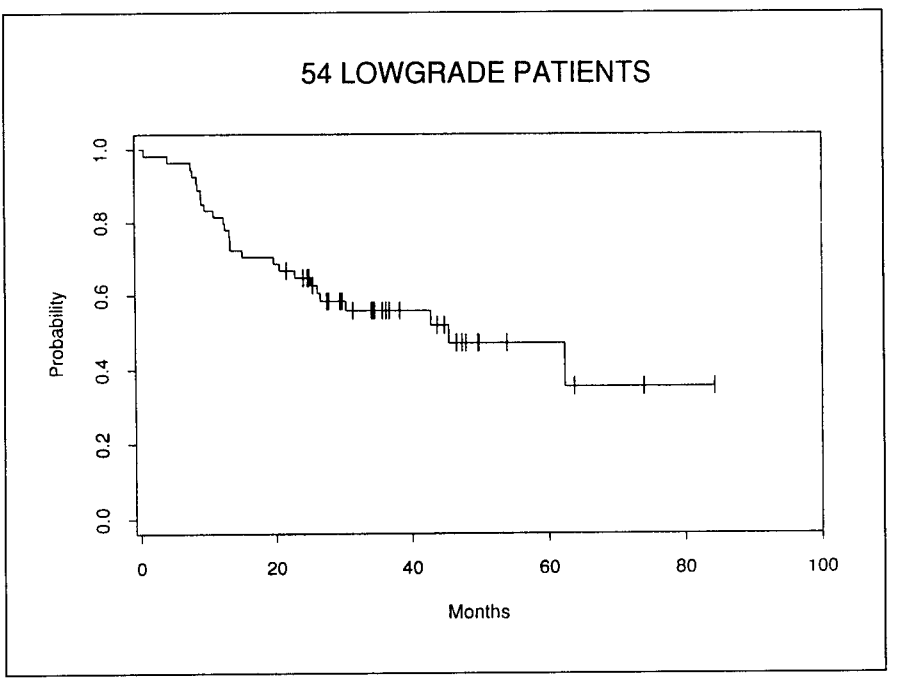

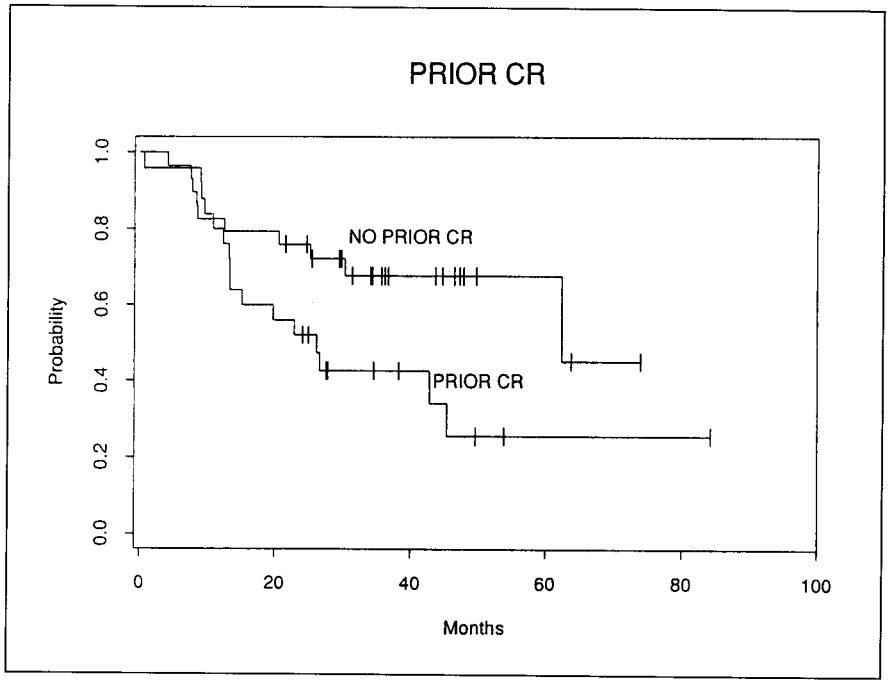
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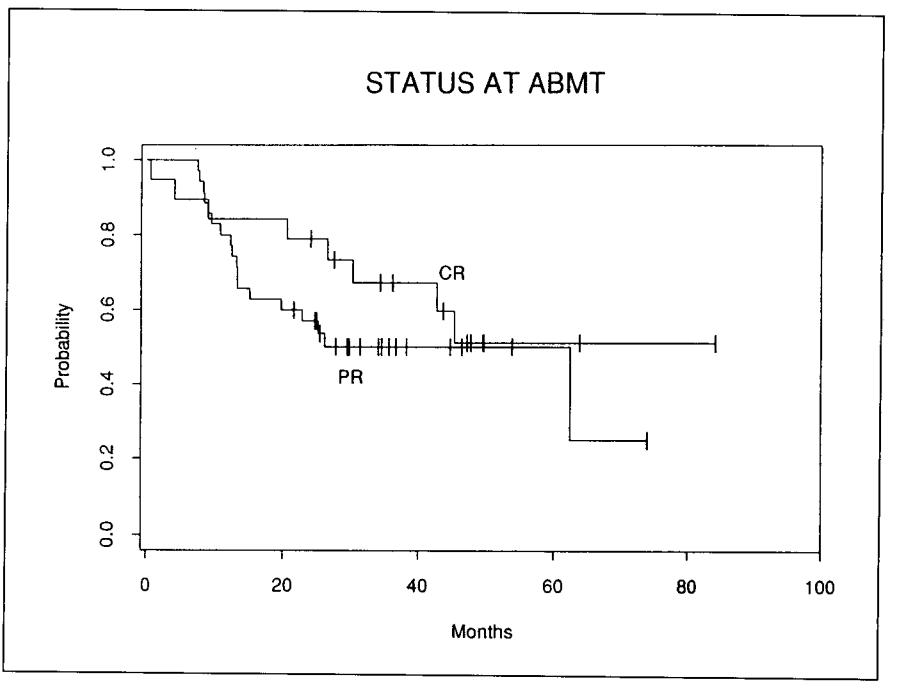
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HIGH DOSE THERAPY WITH HEMATOPOIETIC STEM CELL RESCUE IN FOLLICULAR LYMPHOMA: A FRANCE AUTOGREFFE STUDY

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ABSTRACT

The value of high dose chemotherapy with haematological stem cell rescue is not yet determined in the treatment of low grade Non Hodgkin's lymphoma. We report herein, results of a "France Autogreffe" retrospective study about 42 patients grafted in first partial remission (n=13) or chemosensitive relapse (n=29) for follicular lymphoma before January 1990. The median age was 38 years (range 26-61). Preparative regimen was pure chemotherapy in 22 patients and TBI-containing regimen in 20 patients. Thirty-seven patients received marrow hematopoietic stem cells. Bone marrow purging was performed in 15 patients. Five patients received peripheral blood stem cells.

Three patients died from transplantation toxicity and two others died in CR ten months after Autologous Bone Marrow Transplantation (ABMT). With a median follow up of 43 months, relapse free survival is 60 %, event free survival 58 % and overall survival 83 %.

Follicular lymphoma is relatively common non Hodgkin's lymphoma. Median survival of advanced stage subgroup ranges from 5 to 10 years [1-3]. However, if complete remission (CR)is regularly obtained, a pattern of continued relapse is observed and the 5-year disease free survival is less than 25 % [2]. Although low grade lymphomas (L-NHL) are often still responsive to chemotherapy after relapse, the duration of subsequent responses progressively decreases and the percentage of histologic progression increases [2].

Few data exist about the value of ABMT in low grade non Hodgkin's lymphomas [10-15]. In the present retrospective study, we report data from 42 patients with non transformed follicular lymphomas who were treated in France before January 1990 with ablative therapy and hemopoietic rescue in first partial remissions (PRl) or in chemosensitive relapse. Influence of prognostic factors (age, histology, tumour burden at diagnosis, length of CR, status at ABMT, conditioning regimen, purging) is presented.

PATIENTS AND METHODS

PATIENTS AND TREATMENT PROTOCOL

Patients with non transformed follicular lymphoma according to the working formulation [10] either in first partial remission after first line chemotherapy, or in chemosensitive relapse were included in this retrospective study. The data of all patients grafted before January 1990 in France were collected. Forty two case reports were collected for the study. Characteristics of patients are summa-

rized in table 1. There were 21 males and 21 females with a median age 38 years (range 26-61 years). At diagnosis and at ABMT, ten patients had follicular small cleaved, 30 follicular mixed and 2 follicular large cell lymphoma.

For each patient, staging including physical examination, thoraco-abdominal CT scan and trephine biopsy was performed at diagnosis, at relapse, and after transplant at 3, 6, 12 months and subsequently every 12 months. Bone marrow involvement was detected only by trephine biopsy examination. Prognostic factors were determined at diagnosis: Ann Arbor stage, constitutional B-symptoms, bulky disease defined as peripheral lymph nodes > 5 cm or abdominal mass > 10 cm and number of extra nodal sites involved. All patients had previously received polychemotherapy. At the time of ABMT, 13 patients were in first partial remission (PRI) and 29 in chemosensitive relapse (second complete remission (CR2) = 16, PR2 = 5; CR3 = 4; PR3 = 4).

Preparative therapy was a combined chemotherapy in 22 patients and a TBI containing regimen in 20 patients. Chemotherapy regimens were BEAM in 17 patients (carmustine 300 mg/m^2 on day 1, cytosine arabinoside 200 mg/m^2/day on days 2 to 5, etoposide 200 mg/m^2/day on days 2 to 5 and melphalan 140 mg/m^2 on day 6), BEAC (cyclophosphamide 35 mg/kg/day on days 2 to 5 instead of melphalan)in one patient, CBV (BCNU 300 mg/m^2 on day 1, cyclophosphamide 35 mg/kg/day on days 2 to 5 and etoposide 200 mg/m^2/day on days 2 to 5) in 3 patients and TACC (CCNU 200 mg/m^2 on day 1, cytosine arabinoside 200 mg/m^2/day and 6-thioguanine 200 mg/m^2/day on days 2 to 5, cyclophosphamide 45 mg/kg/day on days 2 to 5) in one patient. Among these patients, 8 received radiotherapy in nodal involved sites after ABMT. Twenty patients were conditioned with TBI and cyclophosphamide (60 mg/kg/day) for two days; four patients received additive drugs: VP 16 (one patient), VP 16 and ARA C (two patients), BCNU and VP 16 (one patient).

COLLECTION, PROCESSING AND INFUSION OF HEMATOPOIETIC STEM CELLS

Thirty seven patients were grafted with marrow hematopoietic stem cells. In some institutions, if marrow involvement was documented at diagnosis or at relapse, bone marrow purging was performed. Two techniques were used: chemical purging with adapted doses of Asta Z (10 cases) and immunological purging with two pan B monoclonal antibodies (5 cases). No assessment of bone marrow purging efficiency was performed after purging. Five patients received peripheral blood stem cells.

Peripheral Blood Stem Cell (PBSC) collection

In all patients PBSC were collected with a Haemonetics Model V50 apheresis device (Haemonetics, Braintree, MA). Stem cells were collected during the recovery phase following aplasia induced by a conventional chemotherapy.

PBSC were collected using the lymphosurge program of the Haemonetics V50 blood cell processor. Three or four cytapheresis were performed after each course of chemotherapy, as soon as leukocyte count had reached 1 x 10^9/1.

Chemical Purging [11]

Two weeks before harvesting an individual pre-test was performed to determine the dose of ASTA Z corresponding to 95 % destruction of CFU-GM (LD95). Purging was performed after adjustment to 20 x 10^6 nuclear cells par ml

in TC 19 medium (Flobio), with an haematocrit of about5 %. Adapted ASTA Z doses corresponded to the LD95. Treatment lasted for 30 min at 37°C and bone marrow was washed twice before freezing.

Immunological Purging [12]

Samples were treated with cocktails of two pan B monoclonal antibodies (MoAb) either RFB_7 (IgM isotype, 1/250 final dilution from G. JANOSSY, CD20) and Y2955 (IgG isotype, 1/200 final dilution from Hoffman Laroche, CD19) or RFB_7 + SB_4 from Sanofi (IgM isotype, 1/50 final dilution, CD19). Samples were incubated 20 minutes with MoAbs and then twice with baby rabbit complement (from Institut Pasteur), with one wash between complement treatments. Complement was used at 1/3 final dilution and D Nase l (pancreatic DNase, sterile, non pyrogenic, from Sigma CC, Saint Louis, USA) 5 units/ml was added to each complement treatment.

STUDY DEFINITIONS AND STATISTICS

Complete remission (CR) was defined as the tumour disappearance according to all indexes. PR was defined as reduction of measurable lesions of at least 50 % without the appearance of new lesions. Event free survival was calculated from the day of transplantation, using the Kaplan and Meier method [13].

RESULTS

ENGRAFTMENT AND TOXICITY

Median time to reach 0.5 x 10^9 PMN/L, 1 x 10^9 WBC/l and 50 x 10^9 platelets/l were respectively 17 days (range 8-38), 17 days (range 7-35) and 21 days (range 10-240). Three patients died of treatment-related toxicity at days 12, 15 and 26, one from gram negative rod septicemia, one from CMV pneumonitis and one from veno-occlusive disease. Two late deaths from non lymphomatous causes were observed, ten months after ABMT: one sudden death and one progressive encephalitis of unknown origin.

TUMOR RESPONSE AND SURVIVAL

All the patients in PR before ABMT went to CR. With a median follow up of 43 months, the relapse free survival (RFS), the event free survival (EFS) and the overall survival (OS) are respectively 66%, 58%, and 83% with a plateau from 3 to 6.5 years (figure 1). Thirteen patients relapsed between 3 and 36 months after ABMT. One patient who relapsed 9 years after transplantation. Only two of the 13 relapsed patients died seven and ten months after ABMT.

We looked at some prognostic factors that might exert an adverse effect on RFS: age, histology, adverse prognostic factors at diagnosis (stage, peripheral lymph node size, bulky abdominal mass, B symptoms and number of extra nodal sites), duration of first CR, status at ABMT (PR1 versus PR2/CR2 versus PR3/CR3 and PR/CR), previous bone marrow involvement. At this time, none of these factors were shown to be of prognostic value. We found only a trend but no statistical difference in favor of CR versus PR before ABMT: 9 of 22 patients in PR before ABMT and 4 of 20 patients in CR relapsed.

According to the conditioning regimens, six patients relapsed after BEAM, 3 after CBV and TACC and four after TBI containing regimen. However, all patients who died from toxicity or in CR received a TBI preparative regimen. No

significant difference was observed in terms of RFS. Relapses were observed in three of 15 patients with bone marrow purging, eight of 22 patients without purging and one of the 5 patients grafted with PBSC.

DISCUSSION

The median survival time of low grade non Hodgkin's lymphoma is between 5 and 10 years according to the studies. Adequate treatment of L-NHL is not yet defined and single alkylating agent chemotherapy remains the most current treatment. Intensive chemotherapy with autologous bone marrow rescue has been shown to be of value in the treatment of intermediate or high grade non Hodgkin's lymphoma in first partial remission or in chemosensitive relapse, but few data have been already published about ABMT in follicular lymphomas. In two studies ABMT has been performed in first line therapy; FOUILLARD et al [6] published a series of 9 patients grafted with bone marrow purged with adapted dose of mafosfamide. With a median follow up of 34 months, all the patients are alive, two are in relapse. HORNING et al [8] recently published a series of 17 patients in first partial or complete remission; conditioning regimen was TBI and cyclophosphamide and bone marrow was purged with a panel of anti-B monoclonal antibodies and complement; two toxic deaths occurred. With a median follow up of 42 months, 14 of the 15 patients are alive without progression.

Four studies have been published about ABMT in the treatment of mainly relapsed patients. At the Nebraska Medical Center, 33 patients with refractory disease or in relapse were conditioned with TBI-cyclophosphamide. Most patients received peripheral blood stem cells. With a median follow up of 21 months, expected disease free survival is 42% [4]. At the Dana Farber Cancer Institute in Boston and at St Bartholomew's Hospital in London, patients received cyclophosphamide-fractionated TBI and bone marrow was purged with monoclonal antibodies and complement [7, 9]. Sixty nine patients in sensitive relapse or PR1 in Boston and 35 patients in sensitive relapse in London have been included in these two studies. With a median follow up of 18 and 22 months respectively, expected DFS are 52% and 55%. In the Dana Farber study, patients in complete remission before ABMT experienced longer DFS than those in partial remission. We have already published a series of 26 patients from Tours grafted in PR1 or sensitive relapse. Conditioning regimens and bone marrow purging was heterogeneous [5]. With a median follow up of 30 months, expected relapse free survival was 65%. The present study confirms with a longer follow up that more than 50% of patients can be alive and free of disease for a long time after ABMT.

The propensity of follicular lymphoma to infiltrate the bone marrow raises the question of the necessity of in vitro treatment for depletion of potential residual lymphoma cells. While the significance of bcl2 in patients in long remission from follicular remains unclear [14], GRIBBEN et al showed the prognostic value of bcl2 disappearance after purging as indicator of relapse [15]. Bcl2 assessment was not performed in this study; however 57% of stage IV NHL remain disease free with a median follow up of 42 months.

In conclusion, this retrospective study shows with a long follow up that about 50% of selected patients with follicular non transformed lymphoma grafted in first partial remission or chemosensitive relapse can remain free from

progression for a long time after ABMT. It is too early to conclude that some of these patients are cured, but it appears urgent to initiate randomized studies to evaluate this approach in terms of disease free survival and overall survival.

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TABLE 1. PATIENT CHARACTERISTICS

No. of patients	42
Sex M/F	12/21
Mean age (range)	38 (26-61)
Histological type	
Follicular small cleaved	10
Follicular mixed	30
Follicular large cell	2
STATUS AT DIAGNOSIS	
Ann Arbor stage	
I	4
II	7
III	10
IV	21
Peripheral lymph nodes size	
< 5cm	17
5-10 cm	21
>10 cm	4
Bulky abdominal tumour	14
B symptoms	10
Number of extra nodal sites (stage IV)	
1 (bone marrow)	14
≥2	7
leukemic phase	2
STATUS AT GRAFT	
Remission status at transplant	
PR1	13
CR2	16
PR2	5
CR3	4
PR3	4
Sensitive relapse	29
CR2	16
PR2	5
CR3	4
PR3	4
Median duration of first CR (# 29 pts)	18 mo (4-70) (5 CR1 > 30 mo)

Table 2.

STATUS AT GRAFT	
Remission status at transplant	
PR1	13
CR2	16
PR2	5
CR3	4
PR3	4
Sensitive relapse	29
CR2	16
PR2	5
CR3	4
PR3	4
Median duration of first CR (# 29 pts)	18 mo (4-70)
(5 CR1 > 30 mo)	
CONDITIONING REGIMENS	
CHEMOTHERAPY ALONE	
BEAM	17
BEAC	1
TACC	1
CBV	3
INCLUDING TBI	20
HEMOPOIETIC RESCUE	
Bone marrow	37
PBSC	5
Purged marrow	15
with anti-B MoAb	5
with Asta-Z	10

Figure 1

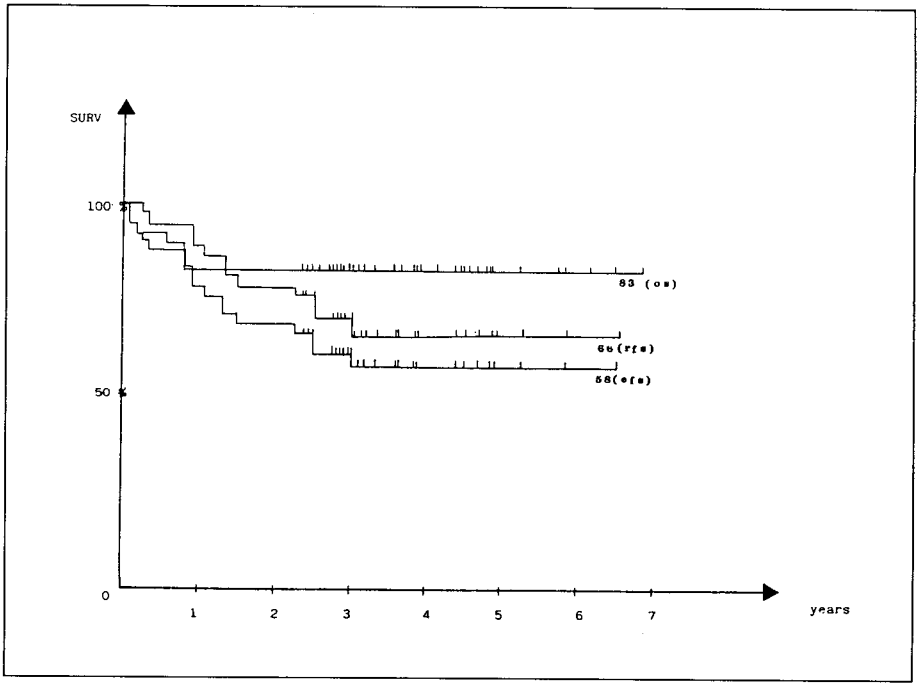


Figure 2

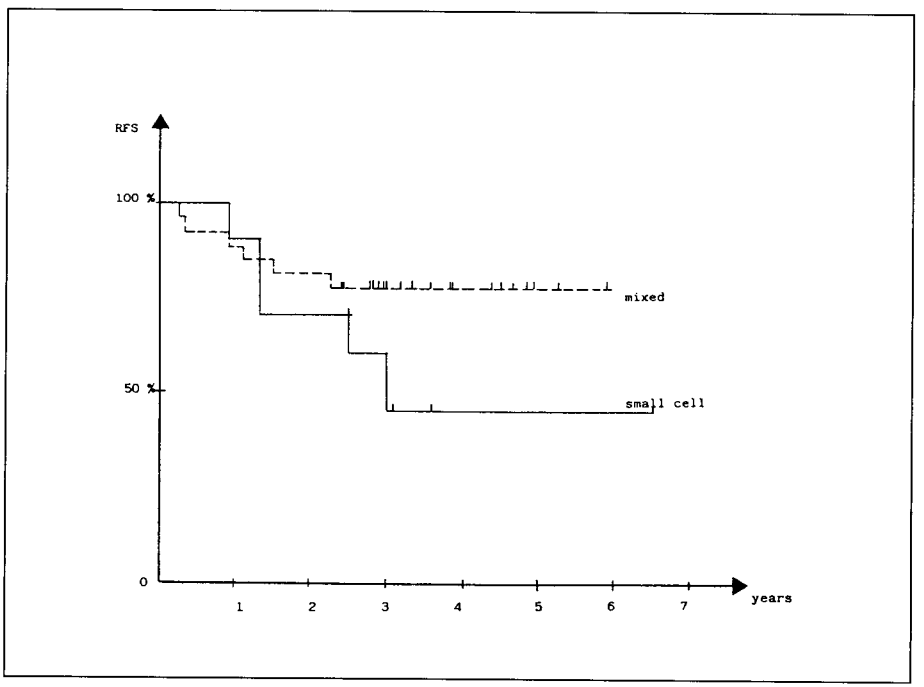
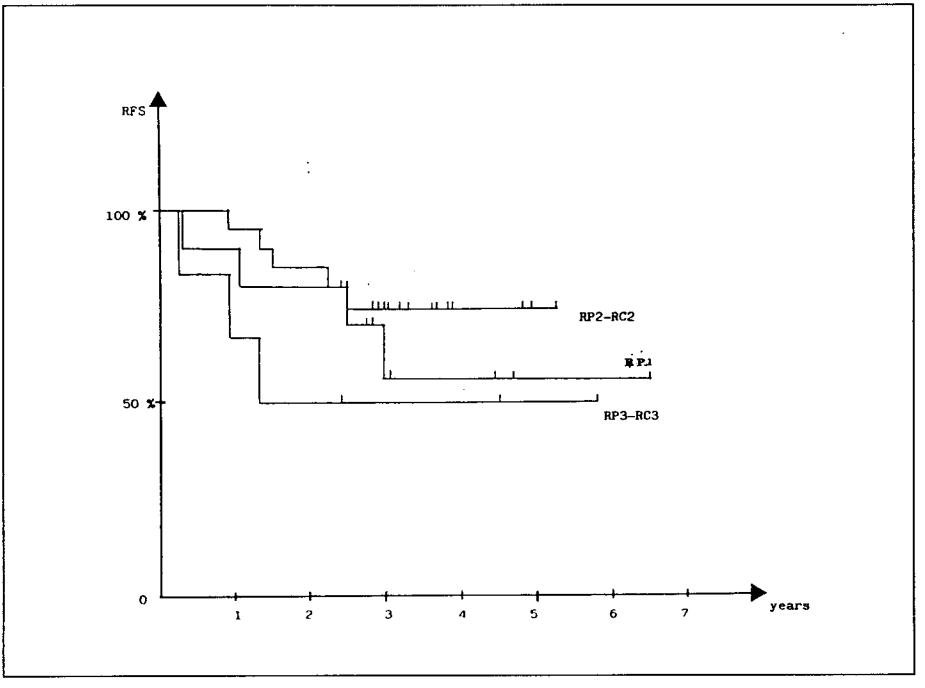


Figure 3



AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR LOW GRADE NONHODGKIN'S LYMPHOMA: THE EUROPEAN BONE MARROW TRANSPLANT GROUP (EBMT) EXPERIENCE.

H.C. Schouten, Ph. Colombat, L.F. Verdonck, N.C. Gorin, B. Bjorkstrand, G. Taghipour, A.H. Goldstone on behalf of the EBMT Working Party for Lymphoma.

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INTRODUCTION

Non-localized low-grade non-Hodgkin's Lymphoma (NHL) is not a curable disease. Influenced by the successful data of high-dose therapy in intermediate and high-grade NHL, this treatment modality is increasingly applied in low-grade NHL . Only a few studies including limited numbers of patients have been published [1-4].

The Lymphoma Working Party of the European Bone Marrow Transplant Group (EBMT) collects data on autologous bone marrow transplantation (ABMT) for malignant lymphoma. In this registry 92 patients with low-grade NHL could be identified who had been treated with high-dose therapy followed by ABMT. Here, we present the results with a median follow-up of 19 months.

PATIENTS

The characteristics of the transplanted patients are listed in Table 1. The majority of patients had chemo-sensitive disease in first or subsequent remission (37%) or diasease with a good response to chemotherapy (49%). The median time from diagnosis to ABMT was 20 months. At time of transplant 19 patients had marrow involvement and 27 patients received a purged autograft. The majority of patients (53/92) received a chemo-only pretransplant conditioning regimen.

RESULTS

Only 3 toxic deaths were reported. The results for progression free survival are shown in figure 1. CR or responding relapse at the time of ABMT was related with a better progression free survival (see figure 2). The application of TBI in the pre-transplant conditioning or the use of purged autografts did not have any impact on outcome (figures 3 and 4). Age of the patients at ABMT was not of any influence on progression free survival (data not shown).

CONCLUSIONS

This is the largest series reported of patients with low-grade NHL treated with high-dose therapy followed by ABMT. Progression free survival after ABMT is very promising. The data support the observations done in other types of NHL that chemosensitive disease reponds better to high-dose therapy fol-

lowed by ABMT than refractory disease at the time of ABMT [5]. Surprisingly the use of TBI and/or purged autografts did not have any impact on outcome. Because this is a registry analysis, this may also be explained by selection of patients transplanted.

Because of the natural behaviour of this disease longer follow-up is necessary. However, we conclude that the data are very promising and warrant a randomized trial comparing purged vs. unpurged BMT with standard chemotherapy.

The EBMT lymphoma working party has developed this randomized study in cooperation with transplant centers in Scandinavia, England, France, Germany, Italy and the Netherlands.

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Figure 1

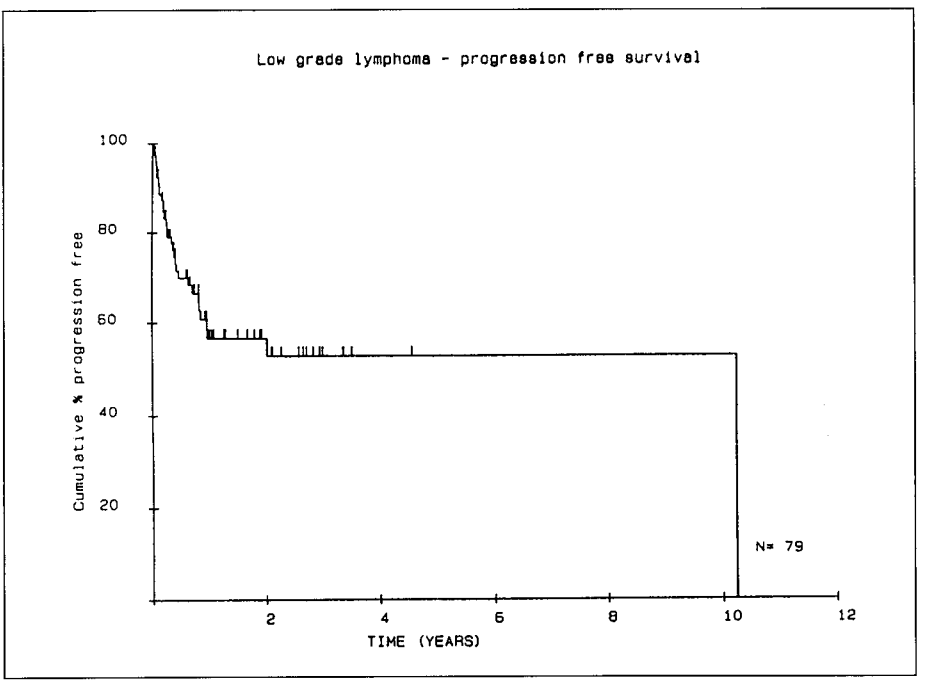


Figure 2

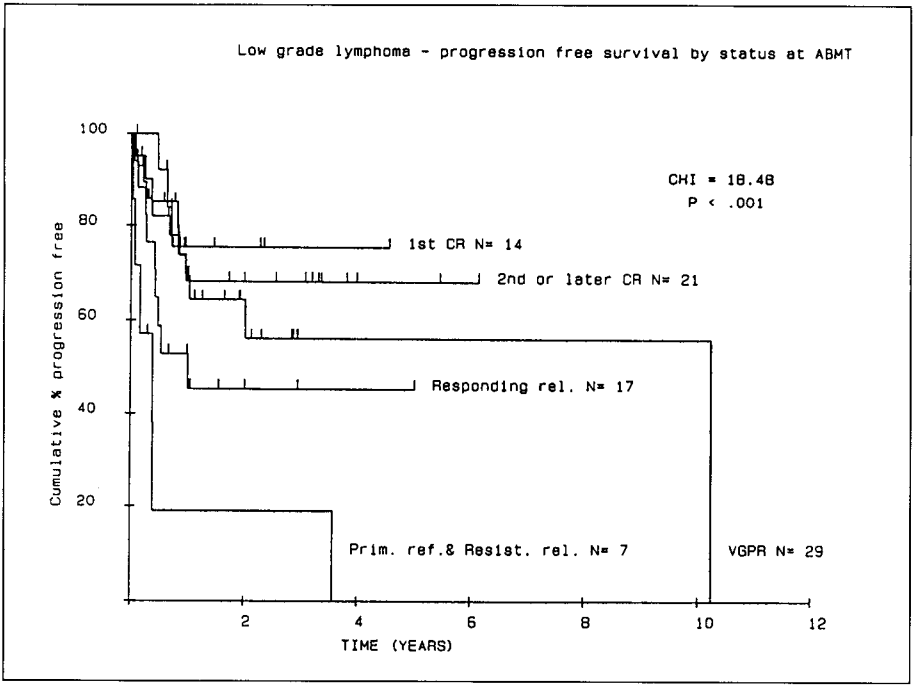


Figure 3

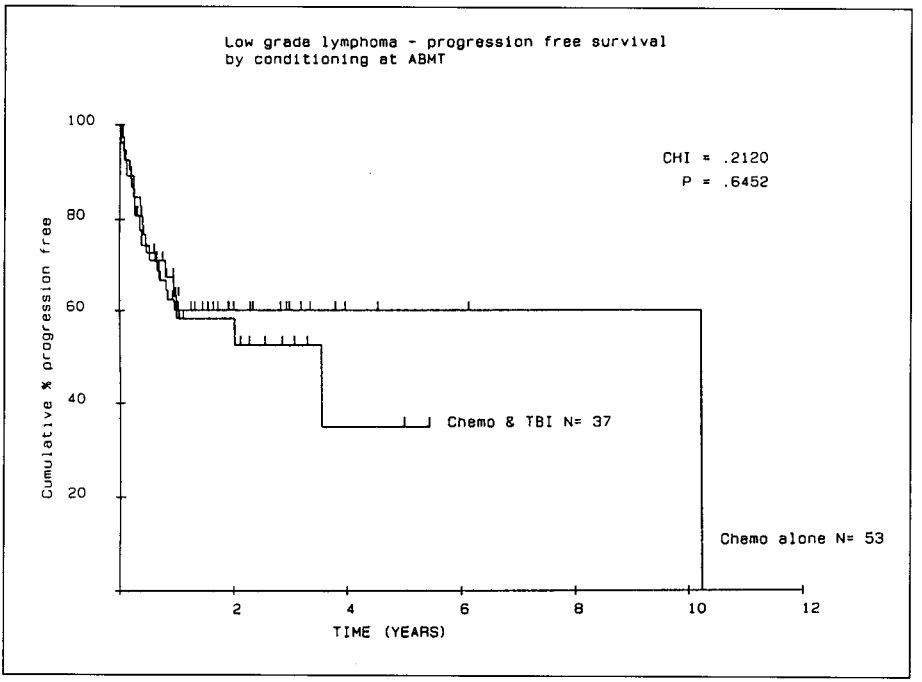
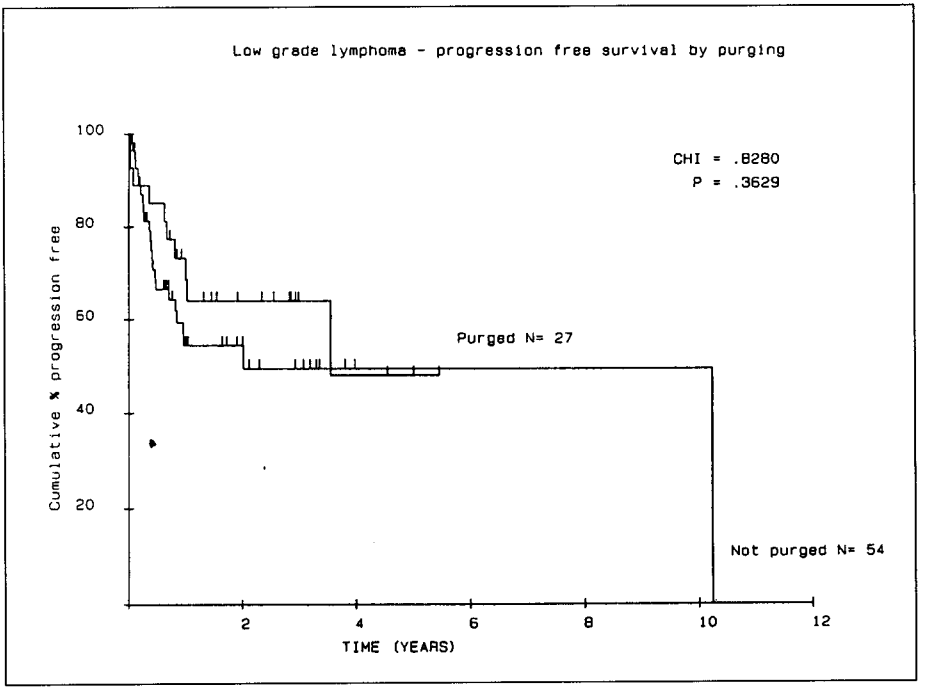


Figure 4


Low grade lymphoma - progression free survival by purging
CHI = .8280
P = .3629
Purged N= 27
Not purged N= 54
Cumulative % progression free
100
80
60
40
20
2
4
6
8
10
12
TIME (YEARS)

AUTOLOGOUS BONE MARROW TRANSPLANTATION IN 100 CASES OF POOR-PROGNOSIS NON-HODGKIN'S LYMPHOMA. A REPORT OF THE NON-HODGKIN'S LYMPHOMA CO-OPERATIVE STUDY GROUP (NELCSG).

G. Santini, A.M. Congiu, P. Coser, T. Chisesi, R. Sertoli, A. Porcellini, L. Miglio, A. Contu, A.M. Carella, D. Pierluigi, S. Nati, E. Rossi, M. Spriano, R. Vimercati, E. Pungolino, D. Occhini, F. Chimirri, V. Vitale, E. Damasio and V. Rizzoli.

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INTRODUCTION

Over the last ten years, in spite of the claiming that second and third generation chemotherapy regimens [1-4] have improved survival of advanced stage, intermediate or high-grade malignancy non-Hodgkin's lymphoma (NHL)(Groups F-G-H-J/Working Formulation), the percentage of complete remissions obtained (CR) is between 50 to 70%, and about 50% of these patients later relapse. Consequently, the percentage of real cure is about 40% [5-7]. Negative prognostic factors, together with histologic subtype and advanced stage, on the one hand, affect both the possibility of obtaining CR and survival, and, on the other, CR maintenance and disease-free survival(DFS) [4,8-13].

Lymphoblastic lymphoma deserves particular attention. Here, the advanced stage of the lymphoma and adult age of patient play an essential role in the probability of CR maintanance, with a three-year probability of survival and a DFS of about 20% [14].

Failure to obtain CR, or subsequent relapse, has serious consequences in the course of these lymphomas, because second-line therapies offer poor possibility of salvage[15,16].

Autologous Bone Marrow Transplantation (ABMT) has been seen to overcome resistance, allowing an increase in the dose of available drugs and radiotherapy. Stem cell rescue can shorten the hypoplastic period decreasing life-threatening risks. Initially used after first-line chemotherapy for relapsed or refractory lymphomas[17-19], ABMT has since been used in more favourable clinical conditions[20-25] and finally in first CR [26-32].

This study reports our experience with poor prognosis NHL patients treated with high dose chemotherapy and/or radiochemotherapy (HDT) followed by ABMT.

Results analysis will be evaluated for difference of response and procedure related toxicity in the various groups of treated patients.

PATIENTS AND METHODS

Up to January 1992, 100 adult intermediate or high-grade malignancy NHL were treated with HDT and ABMT rescue. Patients had a median age of 32 years

(range 15-55); 68 males and 32 females. At diagnosis, 11 patients were in stage II (bulky > 10 cm.), 14 in stage III, and 75 in stage IV. Other characteristics present at diagnosis are described in Table 1.

Histologic subtypes according to the Kiel Classification and the Working Formulation, and the status at ABMT are reported in Table 2. Performance status at transplant was O-2(ECOG). All patients had received at least one regimen containing Doxorubicin (CHOP, ProMACE-MOPP, MACOP-B). The Lymphoblastic Lymphoma had been pretreated with a sequential chemotherapy [31].

Forty-five patients underwent ABMT in 1st CR. Twenty patients (Groups F-G-H-J/WF) fulfilled the following criteria for ABMT procedure: 1) diffuse, intermediate or high-grade malignancy NHL, in advanced stage at diagnosis (II bulky > 10 cm, III or IV); 2) two or more negative prognostic factors at diagnosis: CR > 3 courses, LDH >500 IU/L, bone marrow involvement, bulky disease >10 cm (for stage III and IV), B symptoms, two or more extranodal localizations. Lymphoblastic lymphoma in 1st CR (25 patients) was admitted in stage II bulky, III and IV stage, with an age > 15 years, with less than 25% of blasts in bone marrow and less than 10% of circulating blasts.

No additional negative factors were required in 2nd CR (16 patients) and in 1st partial response (PR 1) (21 patients).

The last group of 18 patients included, 6 refractory to front-line therapy, 5 relapsed and directly admitted to ABMT (untreated relapse), and 7 relapsed and responding to a second-line therapy (sensitive relapse).

In the 35 patients who presented with partially infiltrated marrow at diagnosis, the harvested marrows were purged with a dose of mafosfamide (ASTA Z 7557) able to spare 5% of normal CFU-GM, as reported by Gorin et al [33]. In our cases, a median dose of 90 mcg/2 x 10^7 cells was used (range 50-100). The marrows were cryopreserved and stored in the vapour phase of a liquid nitrogen freezer.

The conditioning regimens and the number of patients were as follows: 1) Cytoxan plus Total Body Irradiation (10 Gy in a single dose) - 74 patients; BEAM regimen - 13 patients; BEAC regimen - 5 patients; BU-CY regimen - 5 patients; CVB regimen - 3 patients.

In all cases, the harvested marrows were reinfused at day 0. The patients were adequately hydrated and received Mesna, Allopurinol, Furosemide and anti-emetic drugs. They also received parenteral hyperalimentation. Platelet transfusions from individual donors were given for platelet counts under 20 x 10^9/1. Leucocyte-free erythrocyte concentrates were administered when hemoglobin values fell below 10 gr/dl. All allogeneic cells were irradiated. Patient's response was evaluated one month after ABMT, and a complete restaging after three months. Continuous complete remission (CCR) was defined as CR maintenance after transplantation. Complete remission (CR) refers to the complete disappearance of the tumor after therapy. The incidence and severity of adverse events were recorded and graded according to WHO criteria. Overall survival and overall DFS were estimated by the Kaplan and Meyer method and compared by log-rank test. The stastical analysis was performed in September 1992.

RESULTS AND TOXICITY

In all cases the response was evaluated by computerized tomography three months after ABMT, together with clinical and radiological review. Results of the ABMT procedure are summarized in Table 3. Patients treated in 1st CR, 2nd CR and 1st PR are best suited to analysis. The other groups consist of 18 patients, divided into 3 categories. To facilitate analysis, these are presented together on the actuarial curves to evaluate overall survival and overall DFS as progression disease (PD).

Twenty-five 1st CR patients out of 45 are currently in continuous complete remission (CCR). Median follow-up time is 70 months (range 8-89), with an actuarial 7-year overall probability of survival and DFS of 57% and 55% respectively (Fig. 1 and 2). Fifteen out of 25 (60%) lymphoblastic lymphoma which underwent ABMT in 1st CR are now in CCR, with a probability of survival and DFS of 62% and 59% respectively.

Nine 2nd CR patients out of 16 are in CCR. Median follow-up time is 17 months (range 8-71), with an actuarial 6-year probability of survival and DFS of 53% and 54% respectively.

Twelve out of 21 1st PR patient obtained a CR (57%). At the moment 9 out of 21 (43%) are in CCR. Median follow-up time is 17 months, with an actuarial 4-year overall probability of survival and DFS of 56% and 36% respectively. The last group, PD, shows a 5-year probability of survival and DFS of 16% and 11%.

All patients had pancytopenia (leucocyte nadir < 0.1 x 10^9/L). Marrow engraftment occurred in all. Median time to self-sustaining granulocyte recovery (>0.5 x 10^9/L) was 13 days (range 10-83), while median time for platelet recovery (>20 x 10^9/L) was 20 days (range 12-105). Recovery was generally delayed in patients whose marrow had been purged. All patients suffered from nausea and vomiting (grade 1-3), and most had diarrhea. Grade 1-4 mucositis was observed in all patients. Severe liver, kidney, pulmonary and cardiac toxicity (grade 4) occurred in 4 patients. Grade 4 infection was observed in two patients. Procedure related deaths occurred in 11 patients (11%), distribution being similar in 1st CR, 2nd CR and in 1st PR patients (13.3%, 12.5%, and 9.5% respectively). Six patients (54,5%) died because of cerebral hemorrage, 2 of cardiac failure (18.1%). Three other patients died, one each from isolated sepsis, broncopneumonia and VOD.

CONCLUSIONS

The NHLCSG experience suggests that ABMT is useful in 1st CR, 2nd CR and in 1st PR patients. It must be remembered that patients treated in 1st CR were penalized by various negative prognostic factors at diagnosis. These were expressed as "tumor burden", and indicated possible relapse once complete remission was obtained. Survival and DFS results (57% and 55%) are undoubtedly significant and agree with those of the European Group[26,28]. Experiences reported in literature concerning patients with aggressive lymphoma and negative prognostic factors were compared with our results. Such a comparison leads us to agree that ABMT could be useful for these groups. However, the lack of randomized clinical studies means that this cannot be confirmed. Only retrospective indication are available [27].

This is also the case for adult advanced stage lymphoblastic lymphoma. In

our experience, this has a seven-year projection in terms of survival and DFS of 62% and 59% respectively. The relationships between survival and prognostic factors are unclear in this particular lymphoma. A randomized study was set up in October 1992 by the EBMT/UK Lymphoma Groups to investigate these questions. A similar study carried out on other poor-prognosis lymphomas would undoubtely prove useful.

The decision to undertake transplantation is influenced by procedure related mortality which in our experience is 11%. In 1988, Goldstone reported similar mortality figures (13%). The use of hematopoietic growth factors or peripheral stem cells would reduce this problem [34].

The use of ABMT in 2nd CR is interesting. In our experience this has a survival and DFS similar to 1st CR. The usefulness of transplantation in these patients is beyond discussion. The difficulties are met in obtaining CR with traditional therapy in poor prognosis patients relapsed after front-line therapy.

The fact that transplantation in 1st PR offers possible long term DFS of 40% or more [23] causes some surprise. But it must be remembered that the Parma Protocol has not shown statistical differences in sensitive-relapse patients randomized for traditional therapy or ABMT[35].

In conclusion, as declared by Philip in 1988: "Randomized studies are necessary and welcome. They should all be considered as high priorities".

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Table 1. Patient Characteristics at Diagnosis

Stage:	**II b>10 cm (11); III (14); IV (75)**	
Bulky	Mediastinal	>10 cm = 43
	Nodal	>10 cm = 18
LDH >500 U/L	74	
B Symptoms	39	
BM Involvement	35	
Two or More Extr. Involv.	29 (9 with BM inv.)	

Table 2. Histology According to the Status at ABMT

Lymphoblastic (LBL) (I)		37
Centroblastic-Centrocytic (CB/CC) (F)		18
Centroblastic (CB) (G)		15
Immunoblastic (IBL) (H)		26
Burkitt (BURK) (J)		4
1st CR (45)	LBL 25; CB/CC 5; CBL 7; IBL 6; BURK 2.	
2nd CR (16)	LBL 4; CB/CC 3; CBL 5; IBL 3; BURK 1.	
1st PR (21)	LBL 5; CB/CC 6; CBL 2; IBL 7; BURK 1.	
Refractory (6)	LBL 1; CB/CC 1; IBL 4.	
Untr/Rel (5)	LBL 1; CBL/CC 1; CBL 1; IBL 2.	
Sens/Rel (7)	CB/CC 2; CBL 2; IBL 3.	

Table 3. ABMT in NHL: Overall Response.

	Procedure rel. deaths	Response (CR)	Relapses (overall)	CCR
1st CR (n. 45)	6/45 (13.3%)	— (35.8%)	14/39 (55.5%)	25/45
2nd CR (n. 16)	2/16 (12.5%)	— (35.7%)	5/14 (56.2%)	9/16
1st PR (n. 21)	2/21 (9.5%)	12/21 (57.1%)	3/12 (25%)	9/21 (42.8%)
Refr. (n. 6)	1/5 (16%)	1/5 (20%)	— —	1/5 (20%)
Untr/R (n. 5)	0/5 (0%)	5/5 (100%)	4/5 (80%)	1/5 (20%)
Sens/R (n. 7)	0/7 (0%)	2/7 (28.5%)	1/2 (50%)	1/7 (14.2%)
(100 pts.)				

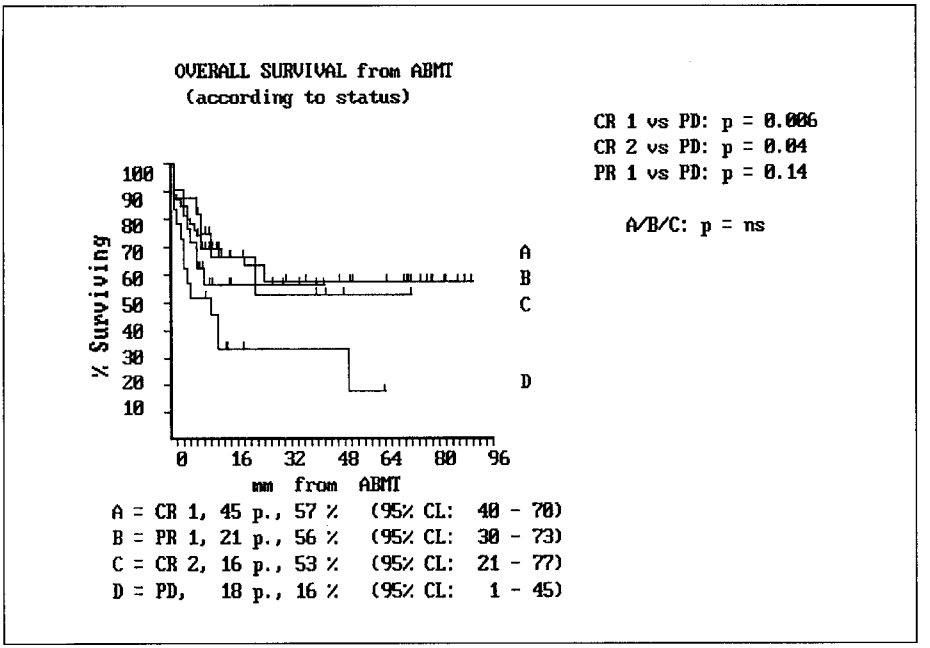

Figure 1

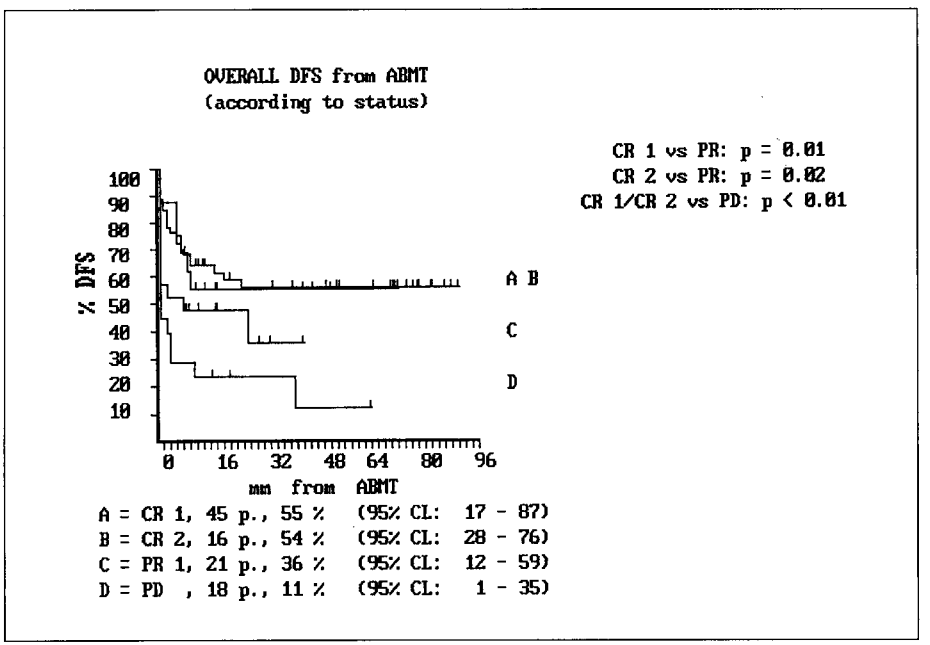

Figure 2

Session V: Hodgkin's

PROGNOSTIC FACTORS IN ADVANCED STAGE HODGKIN'S DISEASE. DEFINITION OF PATIENTS WHO CAN BENEFIT FROM ABMT.

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Over these last two decades, modern therapy has resulted in approximately 70% of patients cured. If a patient fails to achieve remission or precociously relapses, the chances of being cured by conventional "salvage" therapy is small. Such poor risk patients have now been investigated worldwide with high-dose therapy and ABMT. Review of the worldwide experience enrolled ABMT protocols in Hodgkin's disease shows that the patients normally selected for such procedures will fall into the following categories:

1. patients with advanced disease who do not achieve remission upon ABVD-containing regimen, who have nodular sclerosis (grade II), mixed cellularity or lymphoid depletion histology, combined with ESR > 50 at presentation.
2. patients who relapse within a short time (usually < 12 months) after ABVD - containing regimen - inducing remission.
3. patients who relapse after receiving two or more lines of treatment.

Factors that have been suggested to have a negative prognostic impact in advanced Hodgkin's disease are many but these analyses have been done retrospectively and on the basis of the results of single institutions rather than of comprehensive results obtained by several cooperative groups. Recently in UK, patients who were nonresponders to first-line therapy, precociously relapsed or failing two or more lines of treatment, were given high-dose therapy with ABMT and 67% of them had a remission (CR+PR). The actuarial survival of this group at 5 years is 55% with a progression free survival of 50%. These results seem apparently better than the previously reported data from conventional salvage chemotherapy but, unfortunately, this study was not a prospective randomized trial ([1]).

The only prospective randomized trial for this category of patients has been done from British National Lymphoma Investigation ([2]). The patients were randomized to receive high-dose therapy (BEAM) and ABMT or conventional therapy (mini-BEAM). The conclusion of this study were: 1) BEAM has a greater overall survival over mini-BEAM; 2) Overall event-free survival (event is death from any cause or progression of disease) is in favor of BEAM (p=0.025); 3) at 3 years, 53% of patients randomized to receive BEAM were alive and well compared to only 10% of the mini-BEAM arm. Progression free survival is also in favor of BEAM and it is highly superior (p=O.005). The results achieved with BEAM seem to confirm the previous single center studies.

HIGH-DOSE THERAPY AND ABMT IN FIRST REMISSION

The utilization of ABMT as a consolidation therapy in first remission at the time of maximal response is yet merely experimental; however, two recent analyses effected in USA and in Italy, in which the patients received seven or eight drugs in induction, demonstrated that age > 40 years, mediastinal bulk, extranodal disease, LDH > 400 U/L, anemia and B symptoms, were all bad characteristics and the patients were destined to do poorly ([3,4]). According to the good results in terms of survival and tolerance achieved in leukemias and non-Hodgkin's Lymphomas when appropriate timed aggressive chemoradiotherapy is followed by ABMT in first CR or PR, the same strategy has been applied by us in very poor prognosis HD patients ([5]). In a previous report patients with HD were selected on the basis of the most unfavorable prognostic features currently considered. The Genoa preliminary study has involved patients with many bad prognostic factors such as more than two extranodal sites of disease combined with mediastinal mass greater than 0.45 of the thoracic diameter at the level of the carina, high level of LDH and B symptoms.

Eighteen out of 20 (90%) patients receiving this complex program in Genoa, remain alive and well in unmaintained remission at a median time of 52 months (range, 16-87mo.).

The excellent results of this preliminary study, recently confired by EBMTG on 23 patients (6), should be viewed as preliminary. More patients and longer follow-up are needed to define accurately the curability of very poor prognosis Hodgkin's disease patients.

CONCLUSIONS

Using a recent review by J.Armitage, this year a lot of patients will receive autotransplant for lymphoma worldwide (7). However this represents a small proportion of the patients who will need this procedure. As recently referred, in USA alone, patients not cured with conventional therapies and aged <60 years number >9000 (7). Clearly, the new clinical applications of hematopoietic growth factors may allow an increased dose of chemotherapy and probably obviate the need of BMT in some patients. More likely, these growth factors will make BMT safer and studies now in progress will answer many of these questions.

Most patients with advanced-stage Hodgkin's disease younger than 60 years should be treated initially with MOPP/ABV(D); those who progress should receive ABMT as their primary salvage therapy. For reasons not discussed herein, we prefer the routine use of autologous (rather than allogeneic) BMT in Hodgkin's disease.

For patients at very high risk of relapse (i.e. have > one "E" site of disease combined with B Symptoms, Bulkies and high levels of ERS, CD30 and IL-2 receptor), who enter an initial CR after MOPP/ABV(D), a Phase III trial between conventional chemotherapy vs. ABMT during first remission should be feasible, and in this way we are organizing an international cooperative group. Accordingly, attention to identification of prognostic factors, as well as efforts to find new methods of pre-BMT cytoreduction and of reducing post-BMT toxicity, should be directed toward patients who fail MOPP/ABV(D) - at the earliest sign of failure.

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HODGKIN'S DISEASE: AUTOLOGOUS BMT FOLLOWING RELAPSE AFTER PRIMARY CHEMOTHERAPY

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INTRODUCTION

Myeloablative therapy and autologous bone marrow transplant (AuBMT) regimens are widely used for patients with Hodgkin's disease.1 However, the optimal time in the course of a patient's disease to employ such therapy is unclear.[2] While this uncertainty is chiefly due to a lack of randomized trials,[3] this statement is simplistic, as it is arguable whether AuBMT regimens have been developed to the extent that such trials would be appropriate to test this question.[4] Alternatively, a meta-analysis that addresses this issue has been reported;[5] while of interest, the meta-analysis is based on a series of assumptions and is therefore sensitive to the accuracy of those assumptions.

In any case, we believe that the optimal time to employ AuBMT is at the first sign of failure of optimal primary chemotherapy - either failure to achieve an initial complete remission ("primary induction failure") or first relapse - before any salvage chemotherapy has been given. (Since there is no reasonable alternative to the use of AuBMT for primary induction failure patients, this situation will not be discussed further herein.)

More controversy exists regarding subsequent treatment in those patients who achieve a documented complete remission before relapsing. Conventional salvage chemotherapy has a degree of efficacy6 which, although limited, may produce very satisfactory results in patients with an initial lymphoma-free interval of > 12 months.[7] Moreover, it is at least possible that the apparently superior results of AuBMT regimens are due to patient selection[4]; if so, it is possible that AuBMT should be reserved until after conventional salvage chemotherapy fails. Alternatively, conventional salvage therapy could be used to produce a state of "minimal residual disease" ("sensitive relapse") before the use of AuBMT.

However, if one accepts that use of AuBMT regimens *at some point* after relapse is optimal, utilization of AuBMT at the time of *first relapse* is an attractive option as it exposes relatively few patients who could be cured by safer, more conventional therapies to the intrinsic risks of AuBMT regimens[7]; its use only in patients whose lymphoma-free interval is < 12 months emphasizes this strategy, which permits treatment of a relatively healthy patient who is neither heavily pretreated (with associated severe organ dysfunction) nor has an extensive population of highly resistant tumor stem cells. However, we admit that this position is controversial,4 and rather than contrive various comparisons of pub-

lished data to attempt to assess this point further, the results of 58 consecutive patients transplanted in Vancouver at the time of untreated first relapse will be reported.

PATIENT SELECTION

Of the 58 autotransplanted in Vancouver, only those 42 patients from British Columbia (B.C.) are traceable from diagnosis. These patients were part of a larger group of 53 B.C. patients with Hodgkin's disease who failed primary chemotherapy (mostly with MOPP/ABV[D]) and were 15-65 years of age; this number represents virtually all such patients residing in B.C. who were seen during that time. The other 11 B.C. patients were not transplanted: 3 refused any subsequent therapy, 7 were preferentially given local irradiation due to an anticipated good prognosis and 1 patient who was previously misdiagnosed received induction chemotherapy alone. No patient was excluded for disease- or treatment-related reasons. Even if these 11 patients were included in the analyses described herein, the results would differ only slightly from those detailed below. In brief, we did not exclude patients from AuBMT due to anticipated adverse prognostic factors and thus do not believe that patient selection (as defined) spuriously "improves" our results.

PATIENT CHARACTERISTICS (TABLE 1)

All 58 patients had definite progression; patients with mere persistence of radiographic abnormalities were not treated. Patient median age was 31 years; 49 patients had nodular sclerosing histology. Previous treatment included MOPP/ABV(D) in 51 patients; radiotherapy had been given in 20. Thirty-five of these patients had a median time between initial remission and progression of < 12 months and 16 had "B" symptoms at the time of AuBMT. Six had a history (i.e., at any time since diagnosis) of bone marrow positivity; at the time of marrow harvest, however, all save one had normal (or near-normal) marrow cellularity and histology. This patient underwent unstimulated peripheral blood harvest and subsequent reinfusion.

TREATMENT SCHEMA (FIGURE 1)

Patients who had a progression-free interval of > 3 months were harvested, and then received MVPP x 1-4 courses, either with (n=25) or without (n=24) local radiotherapy; 3 received radiotherapy alone. Those few who had shorter intervals were harvested and transplanted without receiving MVPP; some received radiotherapy. It is important to emphasize that the MVPP cycles were not used as a selection method and such patients were not meticulously restaged after MVPP; all patients who were harvested were eventually transplanted.

TREATMENT DETAILS

Intensive conditioning was given with "CBV" as previously reported8 and, more recently, with "CBViP"9 (Figure 2). Our own toxicity data, as well as the Phase I study of Wheeler et al,10 have led us to conclude that these represent near-maximal doses of the agents utilized - especially BCNU.

Fifty-seven patients received unpurged autologous marrow and 1 received

"unstimulated"[11] peripheral blood cells. No post-transplant therapy was given until definite progression occurred. Eighteen patients received planned qrowth factor support post-AuBMT.12

RESULTS

All but 2 patients had recovery of the absolute neutrophil count (ANC) > 0.5 x 10^9/L on a median of day +16 (range +9 to +33) post-AuBMT. Fifty-three patients became independent from platelet transfusions on a median of day +19 (range +6 to +103). (As expected, patients receiving hematopoietic growth factors generally had the more rapid ANC recoveries.12)

At present, 42 of these 58 patients are alive and disease-free, almost all with Karnofsky scores of 100%. Conversely, 13 patients relapsed and 3 suffered non-relapse mortality. Actuarial progression-free survival is 64% at 6 years (95% CI 47% to 80%) (Figure 3). Of analyzed factors, the presence of "B" symptoms at the time of AuBMT ($p < 0.001$) and an initial progression-free interval of < 12 months following primary chemotherapy ($p = 0.025$) were significant adverse prognostic factors in multivariate testing. (As noted above, this result was similar - althouqh not identical - to one obtained after analyzing all 53 B.C. patients in first untested relapse, regardless of treatment, between 1970 and 1988.[13]) The results in patients classified by a lymphoma-free interval of > versus < 12 months is of interest, as such is often used to determine whether or not patients should proceed to AuBMT; the former had ~30% and the latter ~85% subsequent progression-free survival post-AuBMT.

CONCLUSIONS

The most definitive way to evaluate whether first untested relapse (as compared to any tested relapse) is indeed the proper timing for HD is to perform an appropriate randomized clinical trial. However, for a number of reasons, such a trial is unlikely to be performed - at least in the near future. Our results encourage us to consider first untested relapse as the current optimal timing for AuBMT in most patients. Of course, many patients transplanted in first untested relapse are not cured, especially those with the adverse prognostic factors noted above, and it is important to consider new ways of treating such "high-risk" patients which will reduce relapses post-AuBMT without producing an excessive number of deaths due to toxicity. Various methods to upgrade conditioning regimens are being evaluated;14 an alternative approach would be the development and verification of post-AuBMT immunomodulation.[15]

Although perhaps more controversial, the reliable identification of patients at extremely high risk of relapse during their initial remission would permit the use of the AuBMT procedure as "consolidation" after primary therapy. Such an approach has been pursued by Carella, et al[16] and has produced favorable preliminary results, but these require confirmation. Each approach has certain advantages and disadvantages and should be tested further.

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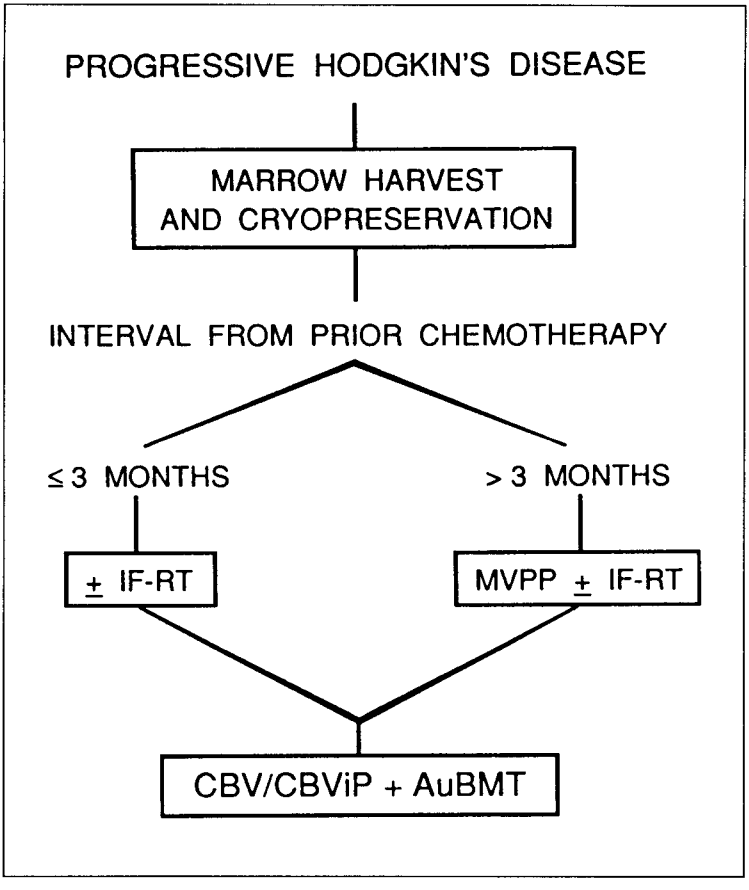

Figure 1: Treatment schema.
Abbreviations: IF-RT = involved-field radiotherapy; MVPP = mustine, vinblastine, procarbazine, prednisone; CBV = cyclophosphamide, BCNU, VP16-213; CBViP = cyclophosphamide, BCNU, VP16-213 by infusion, platinum

A.

AGENT	DAILY DOSE	DAY -7	-6	-5	-4	-3	-2	-1	0
Cyclophosphamide	1.8 g/m^2	•	•	•	•				B
VP16-213	0.8 g/m^2	•/•	•/•	•/•					M
BCNU	0.6 g/m^2					•			T

B.

AGENT	DAILY DOSE	DAY -7	-6	-5	-4	-3	-2	-1	0
VP 16-213	2.4 g/m^2/34hr	•——							B
Cisplatin	50 mg/m^2	•	•	•					M
Cyclophosphamide	1.8 g/m^2		•	•	•	•			T
BCNU	0.5 g/m^2						•		

Figure 2:
Intensive combination chemotherapy regimens given
A. CBV conditioning regimen
B. CBViP conditioning regimen

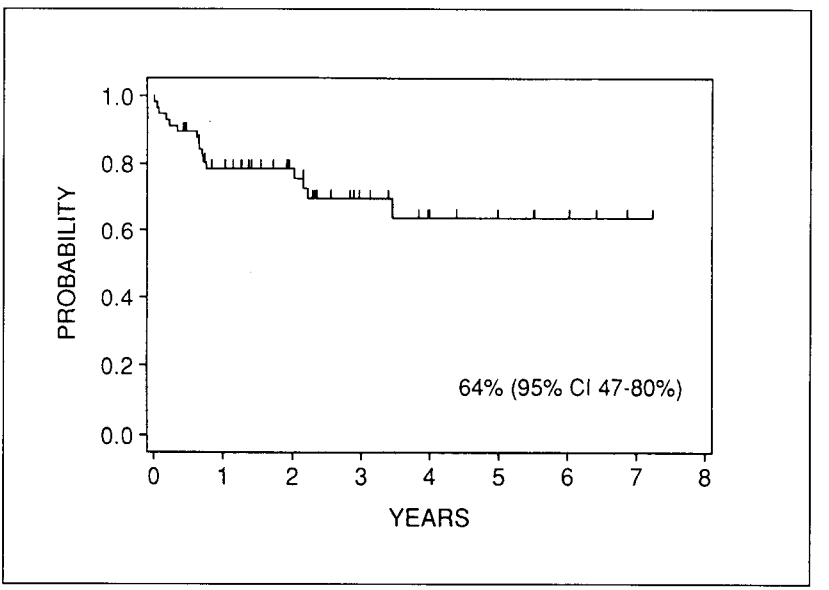

Figure 3
Progression-free survival in 58 Hodgkin's disease patients transplanted in untreated first relapse

MARROW TRANSPLANTATION AFTER MARROW TRANSPLANTATION FOR HODGKIN' S DISEASE

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High dose chemotherapy with autologous marrow rescue is useful in achieving long term disease free survival in patients with recurrent or relapsed Hodgkin's disease. However, high dose chemotherapy with autologous bone marrow transplantation (ABMT) is not uniformly curative and 50-70% of patients undergoing such therapy relapse. In fact, in patients with disease refractory or unresponsive to initial or subsequent chemotherapy, high dose chemotherapy is often deferred as a therapeutic option.

MATERIALS AND METHODS

Since 1984 a series of dose intensive chemotherapy (DIT) regimens were prospectively evaluated in 110 patients with Hodgkin's disease failing previous chemotherapy and form the basis of this report. Twenty three of these patients served as historical controls. The other 87 were stratified into high and low risk groups: Patients with disease sensitive (SR) to standard dose salvage therapy received one cycle of DIT and ABMT whereas those with disease refractory to initial or subsequent standard dose therapy (HR) were scheduled to receive two separate cycles of potentially non-cross resistant DIT and ABMT. Patients with long disease free intervals (>3 years), or nodal relapse were treated with standard dose chemotherapy and/or radiotherapy and not offered DIT and ABMT.

All patients were followed for at least one year. Progression free survival was calculated by using the method of Kaplan and Meier. All patients progressing or dying after signing written informed consent were included in calculations.

The treatment protocols are summarized in Table 1. Initially patients were treated with BEC-1 chemotherapy. Once data from the initial cohort were mature, patients were treated with the BEC-2 regimen. Patients with SR were treated with BEC-2 only. Patients with HR were eligible to receive BEC-2 and if possible a second course of DIT with either TAVe or TMJ and BMT. The final cohort of patients were treated with TMJ only for SR and TMJ followed by EC for refractory disease. Patient characteristics are outlined in Table 2.

Of the 23 patients treated with BEC-1, the vast majority had HR. One patient continues in continuous complete remission more than 60 months after BEC-1 (Figure 1). Of 67 patients treated in the second cohort with BEC-2, 21 had SR: Their progression free survival is shown in Figure 2. Of 46 patients in the second

cohort with HR treated with BEC-2 initially with a planned second cycle of DIT, 2 patients with resistant relapse died prior to the first cycle of DIT, 5 experienced toxic death after BEC-2, 2 refused retransplantation, 6 had unacceptable toxicity after BEC-2 and 3 progressed prior to retransplantation. Of 28 patients retransplanted, ten are in continuous complete remission after TAVe or TMJ therapy following BEC-2. The third and final group of 20 patients with Hodgkin's disease was treated with TMJ first. Six patients had SR and 14 had HR including 8 who had primary refractory Hodgkin's disease. Four patients had evidence of bone/marrow involvement. Patients with HR were retreated with EC if they responded to TMJ. Six patients with sensitive relapse were treated with TMJ alone and are in continuous complete remission for a median of 15 months (range 12 to 23). For fourteen patients with high risk disease sequential bone marrow transplantation was planned, however, one died after TMJ alone, four progressed, and one developed myelodysplastic syndrome after TMJ. Four of eight patients retransplanted on TMJ/EC2 are in continuous complete remission with a median follow up of 15 months (range 12 to 25). Figure 1 shows the progression free survival of patients with sensitive relapse transplanted once compared to patients with high risk disease who underwent sequential transplantation. Patients with standard risk relapse who were treated with BEC-2 alone had a higher rate of relapse than patients with high risk relapse transplanted twice (Figure 2). The risk of relapse in sensitive relapse patients was 10/21 vs. 6/28 in high risk patients. This risk was more magnified in the patients with refractory relapse undergoing sequential BMT (10/21 vs. 2/21; p=O.03).

TOXICITY

The major toxicities of these regimens are listed in Table 3. Pulmonary toxicity was prominent in the BEC treated patients and was directly related to the BCNU dose. Patients requiring ventilatory support were often able to recover completely with steroids. However, long term steroid therapy was often necessary and contributed to fatal pulmonary super infections including aspergillosis, tuberculosis and pneumocystis carinii pneumonia in one case each of patients who were clinically free of Hodgkin's disease.

Pulmonary toxicity was not a major problem with the non-BCNU containing regimen. The incidence of pneumonitis with those regimens was statistically significantly lower than the BCNU containing regimens (p=O.05, Fisher exact test).

The median time to white blood count >1000 cells/ml or ANC>500 was 27 days (r 14-36) on the BEC-1 regimen. The median time to WBC recovery was 21 days (r 11-36) on the BEC 2 regimen. The median time to platelet transfusion independence was shortened in the cohort of patients treated with BEC 2 who received peripheral progenitor cells compared to those who received marrow alone for hematopoietic support. Growth factors were not given following BEC therapy. With TMJ as initial therapy, the median time to neutrophil recovery was 15 days (range 9-21). GM-CSF and peripheral stem cells were given to all 20 patients.

Toxicity for patients receiving ThioTEPA containing regimens as second transplants was greater than that for those receiving TMJ as the first transplant

(Table 2). Patients receiving ThioTEPA 900 mg/m^2 exhibited evidence of CNS toxicity generally manifested by confusion. Only 1 of 36 patients receiving ThioTEPA 750 mg/m^2 exhibited confusion. CNS toxicity was not seen on the BEC regimens. The addition of one or two doses of high dose Ara-C resulted in prohibitive cerebellar toxicity. When this was seen consistently in 4 successive patients Ara-C was deleted from the TAVe regimen and no further episodes of cerebellar toxicity were noted.

Dose intensive therapies have found their niche among the therapeutic options for recurrent Hodgkin's disease. Our data suggest that survival of patients with refractory Hodgkin's disease can be improved if repetitive cycles of dose intensive therapy are administered. The progression free survival of patients with Hodgkin's disease refractory to standard dose therapy, i.e. the subset least likely to do well with one cycle of dose intensive therapy and ABMT, can be improved if an initial response is obtained with one cycle of DIT and then a second consolidative ABMT is performed. The toxicity of regimens used for ABMT is dependent in part on prior therapy. High dose carmustine is associated with a dose related pneumonitis. The elimination of carmustine from the conditioning regimen reduces the risk of interstitial pneumonitis. Patients with refractory relapse of Hodgkin's disease should not be excluded from ABMT regimens.

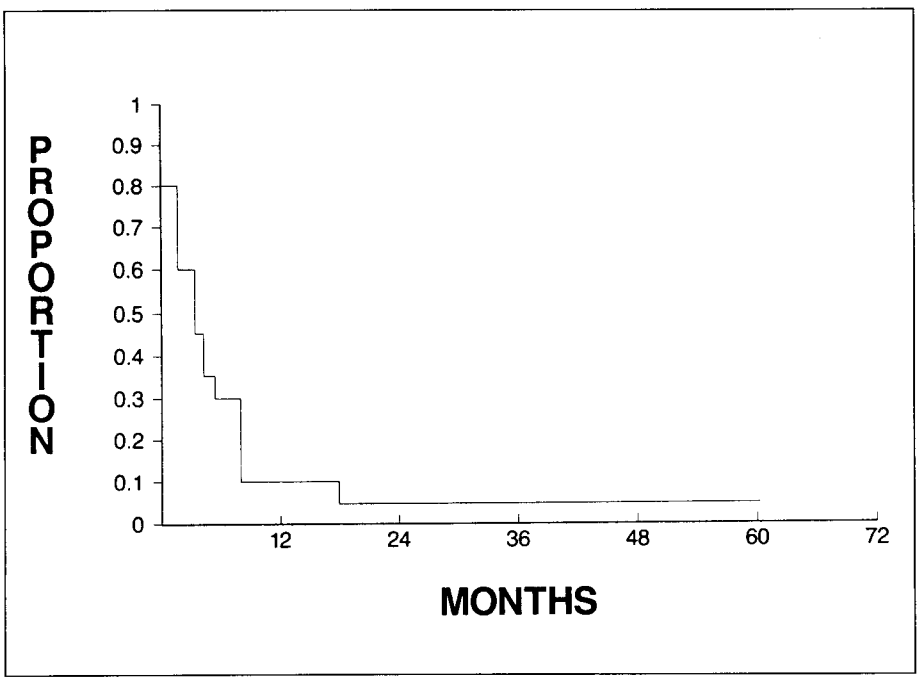

FIGURE 1:
PROGRESSION FREE SURVIVAL OF 23 PATIENTS TREATED WITH BEC-1

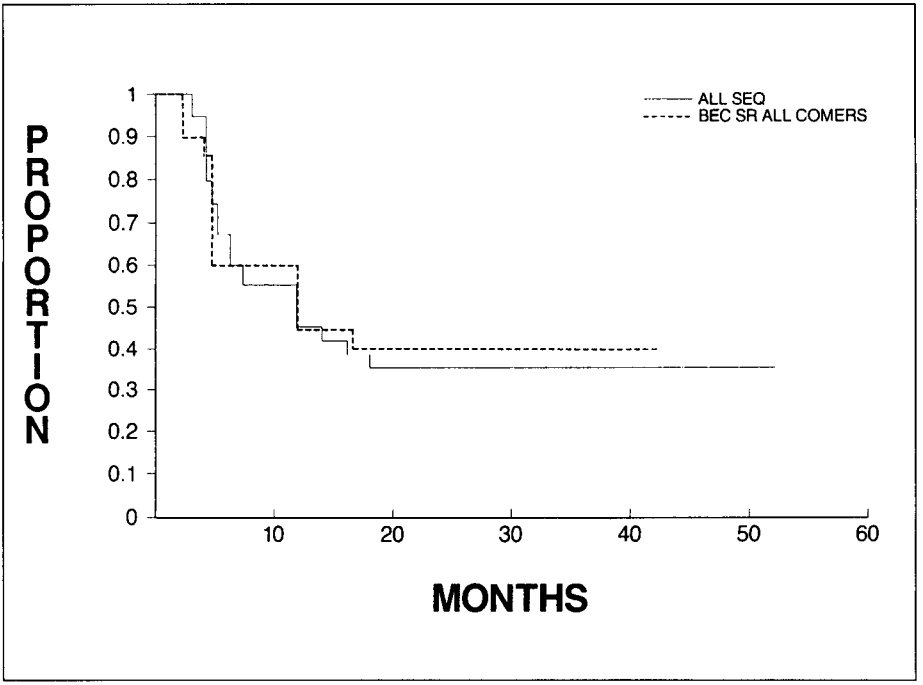

FIGURE 2:
PROGRESSION FREE SURVIVAL OF PATIENTS WITH SENSITIVE RELAPSE UNDERGOING ONE BMT COMPARED WITH PATIENTS WITH RESISTANT/REFRACTORY DISEASE UNDERGOING SEQUENTIAL BMT.

TABLE 1: REGIMENS

BEC 1: BCNU 450-600 MG/M2, ETOPOSIDE 2200 MG/M2, CYTOXAN 5G/M^2

BEC 2: BCNU 400 MG/M2, ETOPOSIDE 1800 MG/M2, CYTOXAN 5G/M2.

TAVe: ThioTEPA 900 MG/M^2, ARA-C 3-6 G/M^2, VELBAN 0.4-0.6 MG/KG

TMJ: ThioTEPA 750 MG/M^2, MITOXANTRONE 40 MG/M2, CARBOPLATINUM (JM-8) lOOO MG/M^2

TABLE 2. PATIENT CHARACTERISTICS

# ENTERED	110
# MALES	58
KPS	80
	(30-100)
PRIOR DRUGS	8
	(14-15)
PRIOR REGIMENS	2
	(1-5)
SENSITIVE RELAPSE	32
PRIMARY REFRACTORY	30
REFRACTORY RELAPSE	48
INITIAL STAGE:	
STAGE I	2
STAGE II	43
STAGE III	42
STAGE IV	24
HISTOLOGY:	
LP	3
NS	96
MC	11
PRIOR DISEASE INVOLVEMENT:	
NODES	67
LUNG	38
MARROW/BONE	16
LIVER	10
CNS/CORD	4
CHEST WALL	4

TABLE 3. TOXICITY

	BEC	TAVe	TMJ as 2nd BMT	TMJ as 1st BMT
# TREATED	90	12	16	20
MUCOSITIS	80	12	11	17
HEMORRHAGIC CYSTITIS	8	4	1	0
COLITIS	6	5	2	4
ILEUS	0	7	0	0
HEPATITIS	45	8	3	7
PNEUMONITIS	31	4	2	1
MYALGIAS	0	9	0	0
CNS DYSFUNCTION	0	4	0	0
TOXIC DEATH	12	7	1	1
PRE BMT DEATH	2			

RADIOIMMUNOTHERAPY FOR BONE MARROW TRANSPLANTATION PATIENTS

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GENERAL PERSPECTIVE

Radioimmunoglobulin therapy (RIT) is considered feasible and promising since the late 1950's but remains an investigational new cancer treatment modality till today. The relatively slow progress in RIT is caused by its complexity requiring participation of many different scientific disciplines and allocation of considerable resources. Continued interest in RIT appears justified for several reasons. RIT is an active single agent in patients with relapsed Hodgkin's disease or lymphoma.[1-3] Bone marrow damage is the only side effect of RIT regularly observed in human patients so far, which can be corrected by a bone marrow transplant or hemopoietic growth factors [1,3-5]. The most important remaining limitation to RIT is the relatively low uptake of radiolabeled antibody by tumor tissues in human patients. Currently, the best RIT reagents deliver approximately 20 Gy to tumor in one week in human patients 6. Promising results have been obtained in preclinical animal models, indicating the possibility of tumor dose escalations in human patients by a factor 5 [6-8].

IMMUNOGLOBULIN PERMUTATIONS

Theoretical considerations indicate that radiolabeled monoclonal antibodies deliver higher tumor doses than similarly labeled polyclonal antibodies. However, non-specific binding of the murine monoclonal antibodies or immune complexes to normal human liver prevents the realization of this principle *in vivo*.3 Enzymatic removal of the CF fragment will decrease liver uptake. The resulting F(ab')2 fragment will target the tumor, but rapidly split in two F(ab') fragments. This fragment is quickly released by tumor and taken up by kidney or excreted in urine [9]. F(ab')2 fragments are good diagnostic reagents, but have tumor dwell times that are too short for the delivery of significant doses of radiation. Removal of the Fc fragment decreases the immunogenicity of the radioimmunoconjugate and increases the possibilities for repeated RIT cycles. Stabilized F(ab')2 fragments, in which the labile interchain disulfide bridges are replaced by more stable thioether linkages, appear to be more promising RIT reagents in experimental animal models and need further analysis in human patients [9, and Quadri et al., submitted for publication].

Another method to circumvent the application of the full immunogenic, Fc bearing mouse immunoglobulin is the use of "humanized" monoclonal antibod-

ies.[10] Longer blood half-lives have been observed for such antibodies, which will lead to increased bone marrow toxicity in comparison to RIT with murine antibodies labeled with similar activity.[11] The selective removal of radiolabel from humanized immunoconjugate in normal tissues might be possible by the introduction of a labile linker between the chelated-radioisotope and the immunoglobulin. Enzymes present in normal tissues, but absent in tumor could cleave the low molecular weight linker chelate isotope complex from the radioimmunoconjugate. This would lead to rapid urinary elimination of radioactivity from normal tissues without significantly decreasing radioactivity in tumor. This principle was applied successfully in experimental animal models.[7]

RADIOISOTOPE SELECTIONS

The most appropriate isotopes for RIT are listed in Table 1. For diagnostic and targeting applications, gamma emitters with energies in the 100-200 KeV range are optimal. For therapeutic purposes localized energy deposition is required. Radionuclides with high energy beta emissions (> 1 MeV) are desirable for clinically detectable human cancer, (i.e.tumor over 1 cm in diameter). For smaller tumors lower energy beta emissions, alpha emissions, or Auger electrons might be more suitable. Such patients will have clinically undetectable tumors, obviating possibilities for tumor targeting and tumor dosimetry studies. This decreases the interest in the application of RIT in an adjuvant setting, as RIT requires further optimization, which can only be achieved in studies of patients with measurable disease.

The half-life of the radioisotopes should be long enough to allow substantial accumulation of radioimmunoconjugate in the tumor, i.e. > 24 hours for intact immunoglobulins. Shorter half-lives of radioisotope are appropriate for diagnostic radiolabeled immunoglobulin fragments. Isotopes with a long half-life (>5 days) will cause more normal tissue damage if they are not eliminated early and decay in circulation and normal organs.

Rhenium-186 is a good example of an isotope with an acceptable half-life (3.8 days) and acceptable emissions for diagnostic purposes (0.137 MeV gamma) and therapeutic purposes (1.07 MeV beta). Yttrium 90 (t 1/2 2.7 days) has a more powerful beta emission (2.3 MeV), but no scannable gamma emission. Indium 111 (t 1/2 2.8 days, gamma emissions 0.173 - 0.247 MeV) has been utilized in low activity prior to Yttrium injections in an effort to predict the behavior of the Yttrium labeled immunoconjugate. This can only be of value if the biodistribution and pharmacokinetics of the immunoconjugate are independent of the radioactive label used. Stable Yttrium complexation requires 8 ligands while for Indium 7 ligands suffice. Cyclic dianhydride DTPA and site specific GYK DTPA are examples of chelate-immunoconjugates with insufficient ligands for Yttrium chelation and are ineffective therapeutic agents.[8] Iodine-131 is becoming a less attractive radionuclide for RIT. Its half-life is long (8 days); its gamma emissions contain high energy photons that decrease the accuracy of diagnostic scans; its beta emissions are weak (0.61 MeV) and will deliver lower and more inhomogeneous doses to larger tumor masses than isotopes with higher energy beta emissions. In addition the volatile nature of Iodine can cause hazardous vapors in radiopharmacy and patient rooms.

PATIENT SELECTION

RIT is in a developmental phase. Patient studies need to be designed carefully to obtain answers on questions of RIT optimization. RIT itself should be simple in the initial stages of analysis, i.e. consist of a single injection per treatment cycle and not be combined with other treatment modalities (surgery, chemotherapy, external beam irradiation). The most prominent theoretical advantage of RIT is its selectivity. Therefore, the high therapeutic ratio of RIT needs to be defined and maintained in initial clinical studies. Normal tissue damage can be evaluated in most patients. However, therapeutic ratio can only be determined if tumor responses are observed in the same patients. Tumor responses can only be expected in patients whose malignancies have sufficient radiosensitivity to respond to the doses of radiation that can be delivered by present day RIT. Relapsed Hodgkin's disease, lymphoma, ovarian cancer, and breast cancer patients are probably the best candidates for single agent RIT studies at this time. Other malignancies will become candidates for study when improved RIT delivers higher tumor doses.

BONE MARROW TRANSPLANTATION

Bone marrow transplantation can be utilized after high radioisotope activity RIT to decrease the severe hematological side effects of such treatment.[1,3,5] Theoretically RIT can be used to supplement or replace total body irradiation (TBI) in the conditioning of bone marrow transplant patients. Previously we have shown that dose homogeneity is not necessary for effective TBI schedules. Indeed, selectively non-homogeneous TBI is expected to have a better therapeutic ratio.[12] RIT could deliver non-homogeneous TBI, with the highest doses delivered to volumes containing the highest concentrations of the radiolabeled immunoglobulin. Such volumes would be dependent on the specificity of the antibody used and adapted to the problems presented by the patient.

Radioactivity in blood and bone marrow can interfere with the success of a bone marrow transplant by inactivating the transplanted stem cells. Current recommendations are to delay the transplant till the dose rate in bone marrow and blood has decreased below 1 cGy per hour.[5,13] One study reports an improved outlook for patients treated with poor prognosis relapsed Hodgkin's disease after treatment with Yttrium 90 labeled antiferritin, high dose cyclophosphamide, etoposide and carmustine followed by an autologous bone marrow transplant.[14] In general, activity escalation studies for RIT have shown a positive correlation between intensity of treatment and severity of side effects in normal tissues. Unfortunately dose-effect relationships for tumor responses have been less clear cut.[1,3] We anticipate finding a positive correlation between tumor dose and response when high tumor doses after RIT can be achieved on a regular basis. It is our opinion that activity escalation with RIT reagents up to the level requiring bone marrow transplant supportive care should be delayed until a good therapeutic ratio has been obtained with the reagents in low activity studies.

NEW SEQUENTIAL PHASES FOR THE STUDY OF RIT REAGENTS

Chemotherapy and RIT are similar in several aspects. Both offer systemic cancer treatment. Both will be tested in cancer patients for which other "older" curative treatments are not available or have failed. Cancer chemotherapeutic

agents have been developed cautiously in specifically designed controlled clinical studies. Sequential study "phases" were introduced and number 0 to 4. Phase 0 is the preclinical analysis; Phase 1 studies define toxicity in human patients (dose escalation); Phase 2 studies determine activity of agent in human cancer (patients with measurable disease); Phase 3 studies compare in a randomized trial "old" treatment and new agent; Phase 4 studies attempt to incorporate the new agent into a multimodality treatment regimen. RIT is sufficiently different from chemotherapy to require different study phases. The major difference is the "accountability" of RIT agents *in vivo*. External body scans by gamma camera and counting of tissue samples can demonstrate the presence of the isotope. Dosimetry calculations are possible for normal tissue and tumor. It would appear to be unwise to do an old fashioned Phase 1 study (dose escalation) with an RIT agent, unless it is known to target tumor and to be stable *in vivo*. Our present preference for the type of sequential studies for an RIT reagent is shown in Table 2. The proposed phases 1, 2A and 2B for RIT are different from the classical chemotherapy study phases of the same number.

RIT is a promising systemic cancer treatment modality with a high potential for an excellent therapeutic ratio. Recent developments indicate that future explorations of this modality can be more focused than previously possible. We recommend single agent studies with high energy beta emitters in patients with radiosensitive and measurable disease. Study phases different from the one currently used in man for chemotherapeutic agents should be used for RIT agents to emphasize the selectivity and favorable therapeutic ratio of RIT. Changes in size of the Ig, i.e. F(ab')2 stabilized fragments, changes in chelation chemistry, e.g. labile linkers, can enhance the effectiveness of RIT and will require further testing.

Simple scientific principles can be applied to the introduction of RIT in the management of bone marrow transplant patients. It appears that the most successful RIT agents can be added to bone marrow transplantation conditioning protocols without an increase in normal tissue toxicity, if the bone marrow transplant is delayed until the blood and bone marrow dose rate has decreased to less than 1 cGy per hour. RIT has the potential to increase the efficacy of bone marrow transplantation (tumor control), while decreasing the side effects of high dose treatment due to its selectivity. It would appear inadvisable to test RIT in the setting of bone marrow transplantation prior to full realization of its "selective" potential in low activity studies.

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Table 1. Radioisotopes for RIT.

Purpose	Isotope	T 1/2 (days)	Beta max (MeV)	Gamma (MeV)
diagnosis	technetium-99 ml	0.25	-	0.140
dosimetry	Indium-lll	2.8	-	0.173-0.247
	Iodine-123	0.54	-	0.159
diagnosis	Copper-67	2.4	0.57	0.184
dosimetry	Rhenium-186	3.8	1.07	0.137
therapy	Rhenium-188	0.7	2.12	0.155
	*Nickel-66	2.9	0.20	—
therapy	Yttrium-90	2.7	2.27	—

* for adjuvant RIT onlv.

Table 2. Sequential Study Phases for Radioimmunoglobulin Therapy Agents.

Phase	Study Aim
0	Preclinical analysis
1	In vivo stability in human patients
	tumor targeting in human patients
2A	Optimize therapeutic ratio in human patients
	Tumor dosimetry
	Human Toxicology
2B	Radioactivity escalation in human patients
3	Randomized Trial
	RIT vs "old" therapy
4	Incorporation into multimodality treatment

Note: Delay of toxicology and escalation studies until therapeutic radio of RIT has been optimized. Biodistribution and pharmacokinetic studies are much easier to perform for RIT than for chemotherapy. They allow for the conservation of selectivity of RIT and decrease the side effects experienced by patients in "classical" chemotherapy phase 1 and 2 studies.

HIGH DOSE INTERCALATOR BASED THERAPY WITH MARROW SUPPORT IS EFFECTIVE FOR REFRACTORY HODGKIN'S DISEASE (HD).

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INTRODUCTION

Hodgkin's disease is usually regarded as a curable malignancy, even in advanced stages. However, as many as one third of the patients with advanced stage disease may fail to achieve a complete remission (CR) with initial combination therapy and up to 40% of those who initially respond will eventually suffer a relapse ([1]). A significant number of patients with relapsed Hodgkin's disease who had a long initial CR (lasting at least 1 year) can achieve a durable second remission with salvage chemotherapy. In contrast, patients with a short initial CR (less than 1 year) and those who never achieved a CR only rarely achieve a durable response to salvage therapy ([2]).

Several hundred patients with recurrent advanced Hodgkin's disease havebeen treated with high-dose chemotherapy or chemoradiotherapy, followed by autologous bone marrow (ABMT) or peripheral stem cell support. The results show convincingly that this is a safe treatment modality that offers long-term progression-free survival to a large fraction of the patients. Thus, Jagannath et al demonstrated that patients responding to conventional salvage chemotherapy prior to receiving high-dose cyclophosphamide, BCNU, and etoposide (CBV) with ABMT had a major survival advantage over patients resistant to conventional salvage therapy prior to receiving CBV with ABMT ([3]). Based on this notion, we evaluated a novel high-dose chemotherapy program designed for patients with recurrent Hodgkin's disease resistant to conventional salvage therapy or for patients relapsing after having received a previous high-dose (i.e. autologous transplant) regimen. This program consists of a combination of mitoxantrone, etoposide and thiotepa (MVT). None of our patients had previously been exposed to mitoxantrone or thiotepa. Mitoxantrone has shown activity in Hodgkin's disease both when used alone and in combination with other cytotoxic agents, and response rates as high as 50% have been reported ([4-6]). Mitoxantrone causes less mucositis and less cardiac toxicity than doxorubicin ([7-11]). Etoposide has been used extensively in high-dose regimens and is very active in HD, the dose-limiting side effect being mucositis, even at doses above 1,000 mg/m^2 ([12]). When high dose thiotepa with ABMT was evaluated in clinical phase I-II studies, it was very active against lymphoma, and mucositis was the only serious side effect at total doses up to about 1,100 mg/m^2 ([13]).

The treatment criterion in this study was the presence of advanced, refractory Hodgkin's disease. This included:

1.) patients who were initially refractory to treatment with MOPP or ABVD, or alternating MOPP/ABVD, or a similar regimen such as CVPP-ABDIC ([14]).

2.) patients in relapse who failed to achieve at least a partial remission with conventional (platinum-based) salvage chemotherapy.

3.) patients who had previously undergone an autologous marrow transplantation for HD but subsequently experienced progressive disease.

Here we report the treatment results from 38 patients in the above groups of treatment-refractory Hodgkin's disease who were treated with MVT followed by autologous hematopoietic stem cell support.

PATIENTS AND METHODS

The eligibility criteria were: histologically proven Hodgkin's disease, no therapy during the previous 3 weeks, age >15 and <61 years, performance status 2 or less on the Zubrod scale, life expectancy at least 12 weeks, adequate hepatic, renal, cardiac and pulmonary function, an absolute granulocyte count of at least 1,500/ul, and a platelet count of at least 100,000/ul to allow for storage of an adequate volume of hematopoietic stem cells to support the intended chemotherapy regimen. For each patient, adequate autologous bone marrow (at least $2xl0^8$ mononuclear cells/kg body weight) or peripheral blood stem cells (at least $4xl0^8$ mononuclear cells/kg body weight) had been cryopreserved. Written informed consent was obtained from all patients.

Patients:

The patient characteristics are listed in Table 1. All thirty-eight patients were treated between January 1988 and March 1992. There were 25 men and 13 women with a median age of 34 years (range 18-61). The majority of the patients had nodular sclerosing histology, and 27 had extranodal involvement. The number of previous chemotherapy regimens ranged from 2-7 with a median of 3; 28 patients had received prior radiation therapy, and 17/38 had induction-refractory disease.

Treatment Regimen

Patients were hospitalized in a private room and received high-dose chemotherapy consisting of mitoxantrone 30 mg/m^2 i.v. over 30 min on day 1, etoposide 400-500 mg/m^2 i.v. over 4-6 h on days 1-3, thiotepa 250 mg/m^2 i.v. over 2 h on days 1-3 (MVT) followed by autologous marrow or peripheral blood cell support on the sixth or ninth day. We suspected that a protracted clearance of thiotepa and mitoxantrone, with undue toxicity to the infused hemopoietic stem cells, could be the explanation for slower than expected hemopoietic recovery in the first 10 patients, and in subsequent patients the hemopoietic stem cells were instead infused on day 9 from the start of chemotherapy. Subsequent hematopoietic reconstitution was as expected in relation to our historical experience with the CBV regimen in similar patients. All patients received continuous i.v. hydration while receiving chemotherapy. Blood component therapy was administered when indicated. All patients received prophylactic antibiotics

throughout their hospital stay and therapeutic antibiotic therapy as necessary. Patients were discharged from the hospital when their absolute granulocyte count was more than 500/ml for 2 consecutive days. Growth factor support was not utilized in the first few patients, but when subsequently available, recombinant human granulocyte colony stimulating factor (G-CSF) was routinely dispensed either as an i.v. infusion over 4-6 h or as a subcutaneous injection once daily in a dose of 5-10mg/kg body weight.

Response Criteria

Patients were seen and reevaluated at least monthly for 3 months after BMT. A complete clinical and radiological restaging was performed immediately upon hematologic reconstitution and again at 3 months after transplant and then every 3 months for 1 year; this was repeated every 4 months during year 2 and then every 6 months or as clinically indicated during years 3 and 4. In patients who remained progression-free, subsequent restaging procedures were performed annually. CR was defined as disappearance of all clinical and radiological evidence of disease for a minimum of 8 weeks. PR was defined as a 50% or more decrease in the sum of the products of the diameters of all measurable lesions persisting for at least 8 weeks. Anything less was considered a treatment failure. Early death was defined as death within the first 30 days after transplant and precluded assessment of disease response.

RESULTS

Thirteen of the 38 treated patients achieved a CR remission and 5 patients had a PR for an overall response rate of 47% (Table 2). Four of the 17 patients who had induction-therapy-resistant disease obtained a CR with MVT and 3 additional patients in this group realized a PR. With a minimum follow-up of 6 months, the median time to progression for the 18 responding patients was 14 months (Fig. 1). Six patients remained in CR at 8+, 15+, 18+, 21+, 46+, and 46+ months. Three of the 5 patients who remained in CR after 12 months were initial induction failures. The median survival for all patients receiving MVT was 16 months (Fig. 2).

Seventeen patients failed to respond to MVT and 3 patients who died from treatment-related complications were regarded as inevaluable. One patient died of respiratory failure 37 days after the transplant. She had no evidence of Hodgkin's disease at autopsy. This patient had received CBV with ABMT less than 15 months prior to MVT and had also completed a course of palliative radiation therapy to the chest (total dose 46 Gy) less than 6 months prior to receiving the MVT regimen. One patient died in CR at 10 months after the transplant from complications of a (possibly secondary) myelodysplastic syndrome. Three patients died within the first 30 days of the transplant of alpha-streptococcal septicemia (day 7), pulmonary hemorrhage (day 17) and aspergillus pneumonia (day 27). These 3 early deaths and the patient who died on day 37 from progressive pulmonary failure were considered therapy-related. Thus, the risk of treatment-related mortality is about 10% with the MVT regimen.

Severe mucositis, necessitating continuous i.v. opiate analgesia and parenteral nutrition for 7-14 days, was the dose-limiting toxicity and it occurred in

more than 80% of the cases. Patients receiving the higher etoposide dose on average developed grade 3-4 mucositis, and those receiving the lower dose developed grade 2 mucositis. No patient developed clinically apparent cardiac toxicity.

DISCUSSION

High dose chemotherapy with hematopoietic stem cell support has changed the outlook for patients with progressive HD. However, the prognosis remains bleak for patients whose disease never responded to conventional therapy or who suffer a recurrence that is chemotherapy resistant ([3]). Likewise, patients with recurrent disease after a previous autologous transplant have mostly been subjected to palliative measures. The MVT regimen was introduced to explore the possibility of disease control in this "poor-prognosis" patient population at the potential price of higher treatment-related morbidity/mortality. We consider the response rate of 47% very encouraging, comparing favorably to results with CBV at our institution ([3]), and to that obtained with more intensive autologous transplant regimens at other centers ([15,16]). Although 2 CR patients died from complications, another 6 were still progression-free, five of whom had been followed 15+ months. One additional patient who achieved a clinical CR lasting 9 months subsequently had recurrence in abdominal lymph nodes. He was given local consolidation radiotherapy and remained in an extended clinical CR for 9+ months following radiation.

The overall survival and the progression-free survival of our patients compare favorably to a historical group that had induction-refractory HD, or disease that recurred after a short (<1 year) clinical CR ([2]). A treatment-related mortality of about 10% is higher than in our experience with the CBV regimen although not higher than that seen with more intensive regimens at other centers ([15,16]). We find this risk acceptable. The death of a patient from respiratory failure was very frustrating and points out the possible increased risk of using high-dose intercalator/alkylator based therapy in patients who have received recent extensive chest radiation.

The MVT regimen showed a high therapeutic potential that warrants further trial in a patient population with better prognosis.

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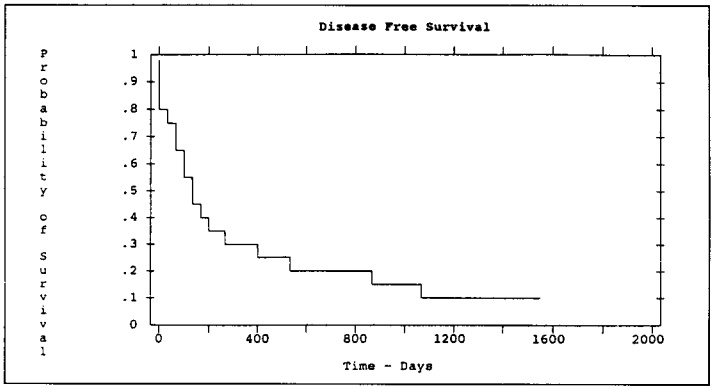

FIGURE 1.
Progression-free survival of responding patients after MVT. Eighteen patients achieved a complete (clinical) or partial remission. These patients had a median progression free survival of 14 months.

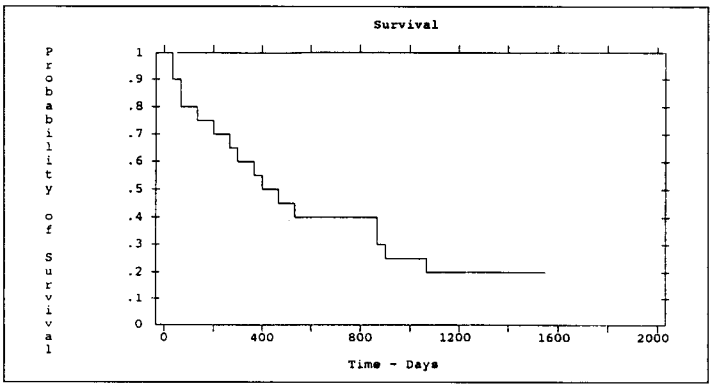

FIGURE 2.
Duration of survival of all patients receiving MVT. The median survival was 16 months.

Table 1. Patient Characteristics

	No. Patients
Total	38
Female	13
Male	25
Histology	
Nodular sclerosing	36
Mixed cellularity	1
Lymphocyte predominant	1
Median Age (range)	34(16-61)
Disease extent	
Nodal only	11
Extranodal	27
Previous chemotherapy regimens	
Median	3(2-7)
Previous radiation therapy	28
Response to initial therapy	
PD	5
PR	12
CR <12 mos.	8
CR >12 mos.	13

Table 2. Response to MVT

Duration of Initial Response	No.	Clin. CR	PR	<PR	Not eval.
Initial Failure	17	4	3	9	1
Initial CR < 1 year	8	2	—	5	1
Initial CR > 1 year	13	7	2	3	1
Total	38	13	5	17	3

Session VI: Breast Cancer

RESULTS WITH CONVENTIONAL DOSE CHEMOTHERAPY IN HIGH-RISK PRIMARY METASTATIC BREAST CANCER.

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Breast cancer (BC) is moderately sensitive to multiple cytotoxic agents. Combination chemotherapy (CT) has become the established treatment for most patients (pts) with metastatic and high-risk primary BC. Over the last 8-10 years, there has been increasing interest in intensifying the dose and the dose/schedule of administration of cytotoxic agents whose dose limiting toxicity is myelosuppression. This has been facilitated by the use of autologous stem cell support (ASCS) and hematopoietic growth factors (G-CSF, GM-CSF). Overall, the results of dose intensification with hematopoietic support have been encouraging for both metastatic BC and high-risk primary BC.

For pts with metastatic BC, very high overall response rates (RR) and complete RR in the range of 50-60% have been obtained with high-dose CT. However, these high overall and complete RR have not translated into prolongation of median survival, and the information available to date precludes making firm conclusions about the relative usefulness of these dose intensive approaches to metastatic disease. Several investigators have succumbed to the temptation of using the poorest available results with standard dose CT to support their hypothesis that high-dose CT results in improved outcome. This paper will present results with standard dose CT in both metastatic and high-risk primary BC to try to establish an acceptable baseline to which to compare the results of high-dose CT regimens.

METASTATIC BREAST CANCER

Hormone therapy is a good palliative tool for 25-30% of pts with metastatic BC. The rest rely on combination CT. Even pts who have the hormonal option eventually develop resistant metastatic disease that requires cytotoxic therapy. Regimens incorporating cyclophosphamide, doxorubicin, and fluorouracil are considered to be the most effective standard regimens, while other two, three, or four drug combinations are also used with some degree of success. For metastatic BC, standard dose CT produces objective RR between 40-80%, and complete RR between 5-30%; median response durations vary from 8-15 months, depending on patient eligibility, and to some extent, the treatment regimen utilized. Table 1 shows several prospective randomized trials comparing a doxorubicin-containing regimen vs. a non-doxorubicin-containing regimen.[1-8] In most, but not all of these trials, the doxorubicin-containing regimen was shown to be superior in overall RR, complete RR, the response duration and median survival. Median duration of survival from the institution of front-line CT varied from 15-25 months in most studies. The duration of survival depends in many of these studies from the objective response achieved. On average, pts who

achieved a complete remission survived from 24-36 months; conversely, those whose disease progressed during induction front-line therapy had a very poor prognosis with median survival durations well under six months.[8] We recently reviewed the long-term results of a large group of pts with metastatic BC treated on various doxorubicin-containing protocols at this institution between 1972 and 1983. The results of each protocol have been published previously, and the data of pts included in these protocols were pooled for analysis of prognostic factors.[9] The overall RR for the entire group of pts was 65%. Two hundred and forty-five (245) of these 1545 pts achieved a complete remission (17%). The updated results of the group of complete responders showed that 30 of them remained in complete remission five years after the initiation of front-line CT. Six of these 30 died with progressive metastatic BC between 5-12 years after initiation of front-line CT. Eighteen (18) of the 30 remain in first complete remission, and two additional pts died of intercurrent disease with no evidence of recurrent or metastatic BC. The last four of these 30 pts developed progressive disease within five years of achieving a complete remission, were retreated, and are now in a second remission which in each case exceeds five years and is longer than the first complete remission.[10] Thus, slightly under 10% of pts who achieved a complete remission with a doxorubicin-containing regimen achieved a long-term complete remission, the longest of which now reaches 19 years. While this represents less than 2% of all pts entered on these various protocols, it demonstrates the biological feasibility of achieving a long-term progression-free survival for pts with metastatic BC. This finding would suggest that strategies of remission consolidation (such as dose intensive therapy) might increase the fraction of long-term disease-free survivors, and provides added encouragement for clinical research with dose intensive therapies.

HIGH-DOSE PRIMARY BREAST CANCER

The rate of treatment failure after optimal therapy of primary BC is inversely proportional to the initial stage at diagnosis. Thus, pts with stage I BC have an 80% chance of remaining alive for 10 years, those with stage II a 50% probability, while those with stage III BC have a 70% chance of dying during the first 10 years of diagnosis, despite appropriate treatment. Numerous clinical trials with radical mastectomy and more recently, with combined modality therapy, have identified a host of prognostic factors that help us to identify high-risk and low-risk subgroups.[11] The number of positive axillary lymph nodes, the size of the primary tumor, oncogene expression, and various other factors are commonly used to make these determinations. I will concentrate on three subgroups of pts with high-risk primary BC, where I believe the role of high-dose CT with hematopoietic support needs to be investigated.

A) Stage II-Breast Cancer with More than 10 Positive Axillary Lymph Nodes

This group has a very high risk of relapse, metastases, and death from metastatic disease. However, not all pts develop metastases or die of their disease. Even pts treated with surgery alone have a 15% probability of surviving 5-10 years after primary therapy, as shown by several cancer centers and cooperative groups.[12,13] With the addition of standard dose adjuvant CT, 25-53% of pts in this high-risk group remain relapse-free at five years. Our own data with com-

bined modality therapy that included a total mastectomy and a doxorubicin-containing adjuvant CT suggested that at 10 years 35% of these pts remained alive, most of them disease-free.[13] Therefore, the high-dose CT programs should demonstrate disease-free and overall survival rates superior to 30-35% at 10 years (preferably in a randomized clinical trial) to determine the relative value of conventional-dose and high-dose regiments, and their role in the standard therapy of primary BC.

B) Locally Advanced Breast Cancer

This is a heterogenous group of pts that vary in tumor sizes and regional lymph node involvement. Consequently, although the long-term prognosis of most of these pts is poor, the 5-year survival rate may vary from 40-77% with the appropriate use of combined modality therapies.[14] The survival rates for this group of pts continue to drop between 5-10 years of follow-up. Nevertheless, the efficacy of high-dose CT programs in the context of combined modality treatment have to measure up against the best results obtained today with standard dose CT. Table 2 shows the results of a number of recently published Phase II trials with combined modality regimens of locally advanced BC.

C) Inflammatory Breast Cancer

This subtype of BC represents 1-3% of all BCs. However, this group of pts has the single most aggressive and rapidly lethal type of BC, with fairly uniform biological behavior, and a very poor prognosis after local/regional therapy alone.[14] Thus, after either surgery alone or radiotherapy alone, or a combination of the two, less than 5% of pts with inflammatory BC are expected to survive 5 years; furthermore, most of them will be dead after the first two years of follow-up. Table 3 shows a number of recently published results after combined modality therapy with primary CT and radiation therapy with or without surgery for inflammatory BC. This table also shows that combined modality therapy that includes standard-dose CT has modified the natural history of this disease substantially with 3-year survival rates that vary from 35-75%.[15] The few publications with long-term follow-up show that 10 years after initiation of combined modality therapy 30-40% of pts remain disease-free and alive.

It is clear that progress has been made with standard-dose CT both in metastatic and high-risk primary BC. The results of high-dose CT programs are encouraging, and the aggressive evaluation of these therapeutic strategies should be pursued. However, the benefits of high-dose CT should be demonstrated against the best available standard dose CT regimens and not against the poorest results one can find by a selective review of the literature. Prospective randomized trials (many of them ongoing) will be the most appropriate way to detect the relative risks and benefits of high-dose CT programs. Enthusiastic participation in these trials on both sides of the Atlantic is encouraged.

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Table 1. Randomized trials of doxorubicin-containing vs. non-doxorubicin-containing combination chemotherapy in pts with metastatic breast cancer

Drug Regimen	Author	No. of Pts	Percent Responses	P	Median Duration (Month) Response	P	Survival	P
CMF	Bull	40	62	0.01	8	NS	17	0.13
CAF		38	82		10		27	
CMFVP	Smalley	54	37	0.007	5	0.02		
CAF		59	64		7			
CMFVP	Muss	72	57	0.47	13	NS	20	0.07
CAFVP		76	58		15		33	
CMFVP	Tormey	109	50	0.003	7	<0.01	13	0.01
CAFVP		107	71		14		19	
CMF	Aisner	99	37	≤0.01	6	0.01	14	0.04
CAF		82	55		9		24	
CAFVP		79	58		7		16	
CMFT	Brincker	154	45	0.01	13	0.03	--	--
CAFT		153	58		20			
CMFVP	Kolaric	36	55	NS	7	NS	--	--
CMFAP		38	65		9			

Table 2. Stage III breast cancer treated with combined modalty programs

Reference	Year	Treatment	Pts	% CR	Median survival (months)	Survival (%) 3 Year	5 Year
DeLena [97]	1978	CT + RT + CT	110	83	36	50	NR
Rubens [98]	1980	CT + RT + CT	12	67	36	50	NR
		RT + CT	12	75	36	50	NR
DeLena [99]	1981	CT + RT	67	64	NR	62	NR
		CT + S	65	78	47	55	NR
Hortobagyi [100]	1983	CT + RT + S + CT	52	94	65	65	55
Pawlicki [102]	1983	CT	40	NA	NA	13	NR
		CT + RT + CT	34	NA	NA	32	NR
		CT + S + RT + CT	13	NA	NA	62	NR
Valagussa [103]	1983	CT + RT	72	64	30	43	20
		CT + RT + CT	126	75	42	60	36
		CT + S + CT	79	82	58	64	49
Balawajder [104]	1983	CT + RT	23	NA	NA	NA	46
		CT + RT + S	30	NA	NA	NA	38
Schaake-Koning [105]	1985	RT	45	75	42	59	37
		RT + CT	34	71	45	59	37
		CT + RT + CT	39	71	50	61	37
Conte [106]	1987	H + CT + S + H + CT ± RT	39	92	NR	60	NR
Olson [107]	1986	CT + RT + CT	51	34	NA	33	
	119	32					
		RT + CT	42	24	NA		
Lesnick [108]	1986	CT + S + CT	100	NR	65	NR	
	105						
		CT + RT + CT	52	34	47	NR	
Pouillart [109]	1986	CT + S + RT					
	82	100	NR	85	NR		
		CT + RT + CT					
Jacquillat [111]	1987	CT + RT + CT	98	100	NR	77	NR
Hortobagyi [92]	1987	CT + RT + S + CT	174	96	66	65	55
Swain [112]	1987	CT + RT + S + CT	75	100	39	42	NR

NR-not reached; NA-not available; S-surgery; RT-radiotherapy; CT-chemotherapy; H-hormone therapy

Table 3. Combined modality treatment of inflammatory breast carcinoma

Reference	Year	Treatment	Pts	% CR	Median survival (months)	Survival 3 Year	5 Year
DeLena [97]	1978	CT + RT + CT	36	73	25	24	NA
Chu [67]	1980	RT + H	14	NA	15	NA	NA
		RT + CT	16	NA	> 2 6	NA	NA
Pouillart [114]	1981	CT + RT + CT	77	51	34	45	NA
Zylberberg [115]	1982	CT + S + CT + RT	15	100	NR	75	70
Pawlicki [102]	1983	CT + S + RT	72	NA	NA	28	NA
Loprinzi [94]	1984	S + CT + RT + CT	9	100	>25	60	55
Fastenberg [116]	1985	CT + RT + S + CT	63	92	43	58	34
Keiling [117]	1985	CT + S + CT	41	100	NR	75	63
Jacquillat [118]	1986	CT + RT +CT	34	100	NR	77	NA
Alberto [119]	1986	CT + S + CT + RT	22	95	26	47	10
Ferriere [120]	1986	CT + RT + S + CT	75	93	NR	68	54
Pourny [121]	1986	CT + S + RT + CT	33	82	70	70	60
Chevallier [122]	1986	CT + RT + CT + S	56	83	30	NA	23
Rouesse [123]	1986	CT + RT + CT	91	41	36	50	40
		CT + RT + CT	79	54	NR	80	66
Israel [124]	1986	CT + S + CT	25	96	NR	68	62
Buzdar [125]	1987	CT + S + CT + RT	43	95	NR	55	NA

NR-not reached; NA-not available; S-surgery; RT-radiotherapy; CT-chemotherapy; H-hormone therapy

LONG TERM FOLLOW UP OF POOR PROGNOSIS STAGE IV BREAST CANCER PATIENTS TREATED WITH TWO COURSES OF HIGH-DOSE CHEMOTHERAPY AND BONE MARROW SUPPORT

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INTRODUCTION

Stage IV breast cancer patients who are estrogen receptor-negative (ER-) or estrogen receptor-positive (ER+) "refractory" to hormonal manipulation represent a poor prognosis subgroup which behaves in an aggressive manner with a short survival and few protracted disease-free survivors. With conventional chemotherapy the median survival of this subgroup is 15-18 months, with a 10% three-year progression-free and overall survival.[1-6]

We describe long term follow up data in this poor-risk subgroup of breast cancer patients. They were initially treated with conventional induction chemotherapy followed by intensificationwith two courses of high-dose cyclophosphamide 4.5-6g/m^2/course; etoposide 750-1500mg/m^2/course; cisplatin 120-180mg/m^2/course. We include data of response rates, duration of response, survival, and toxicity.

Patient Population and Treatment Plan

Eighty stage IV ER- or ER+ "primary hormonal refractory" breast cancer patients were treated between May, 1985 and August, 1990. All patients were in first relapse. Patients were initially treated for stage IV disease with conventional combination chemotherapy (Induction Phase) to the point of maximal response. Patients with stable or responding disease then received high-dose therapy (Intensive Phase)(Fig.l). Forty-four patients were randomized to receive marrow infusion. Thirty-six were randomized to the no-marrow arm.

Intensive hydration to maintain high urine output was started 24 hours prior to intensification chemotherapy and continued for one week. Cyclophosphamide was infused intravenously over one hour on days 1, 2, and 3. Etoposide was infused over four hours every 12 hours on days 1, 2, and 3. Cisplatinwas administered over 2 hours on days 1, 2, and 3.

RESULTS

Response

Seventy-seven patients had measurable or assessable disease at the time of intensification. Three patients had no evidence of disease (NED) after surgical resection, these were not assessable for response to induction.

Seventy-nine patients were treated with median 4 cycles of induction chemotherapy (77 patients with measurable disease and two NED patients). Seventy-nine percent (79%) received Adriamycin-based induction chemotherapy. Twenty-one percent (21%) were treated with Methotrexate chemotherapy combinations.

Seven patients suffering early death during the intensive phase were considered treatment failures and scored as treatment related early deaths. Median duration of follow-up for the entire group of 80 patients is 83+ weeks (range, 13-283+). Median duration of follow-up for the 24 surviving patients is 172+ weeks(range 74+-283+).

Response to induction chemotherapy was 77%. Thirty-one percent (31%) were complete responders and 46% were partial responders. Twenty-three percent (23%) had nonresponsive stable disease.

The "overall response" was defined as response in those patients with measurable disease who completed both induction and intensive phases. The three NED patients were censored from response evaluation. The "overall response" was 79%. Complete remission in 55% and partial response in 25%. Thirty-seven percent 37% of partial responders and 33% of stable disease patients to induction chemotherapy were converted to complete remission after the intensive phase.

Median progression-free survival measured from beginning induction therapy is 50 weeks (95% confidence interval, 44 to 61 weeks)(Fig. 2). Median survival is 83 weeks (95% confidence interval, 68 to 110 weeks) (Fig. 3). Patients followed long term show that the progression-free interval and overall survival curves have reached a plateau after 142 weeks (2.7 years) and 180 weeks (3.5 years) respectively, persisting at greater than 5 year follow-up. Characteristics of patients constituting the tail of the progression-free interval and survival curves reveal predominately one or two sites of parenchymal lung or regional nodal relapse at the time they started induction chemotherapy.

Using univariate analysis, four features predicted for improved progression-free survival or overall survival. These include: (1) disease site distributed to lung or regional lymph nodes (P=.093 and .007); (2) one disease site (P=.030 and .006); (3) disease-free interval (DFI) from diagnosis to detection of metastasis of greater or equal to 1 year (P=.038 and .042); (4) response to induction chemotherapy with complete response or no evidence of disease (P=.011 and .037).

By multivariate analysis five variables were significant "negative" predic-

tors both for progression-free survival and for overall survival. Probability values for these five variables are respectively: (1) soft-tissue site (P=.047 and .013); (2) liver site (P=.007 and .007); (3) prior chemotherapy (P=.004 and .008); (4) disease-free interval from mastectomy to metastasis < 1 year (P=.027 and .047); (5) non-caucasian race (P=.0001 and .0002).

DISCUSSION

The observed overall complete remission rate of 55% is twice the (1%-21%) range observed using conventional chemotherapy for this unfavorable subgroup of patients.[1,2,3,4,5,6]

This report identifies a subset of patients who benefit the most as having 1-2 sites of disease primarily distributed to parenchymal lung or regional draining lymph nodes. Prognostic Factors predicting for poor survival by this analysis include liver site, soft-tissue site, >1 disease site, prior chemotherapy exposure, short DFI, and non-caucasian race.

In this report progression-free survival and overall survival curves reveal a plateau that persists at 5 year follow-up. The encouraging observed "overall complete response" rate in this report (55%) is equivalent to most other reported high-dose protocols (range 20-64%).[7,8,9,10,11,12,13,14,15] Other high-dose protocols exclude patients over age 55, patients with stable disease, and report an early toxic death rate of range 3%-40%.[8,9,14,16,17,18,19] Our low mortality of 8% while including patients up to age 62 (in the pre-growth factor era), supports a double high-dose approach.

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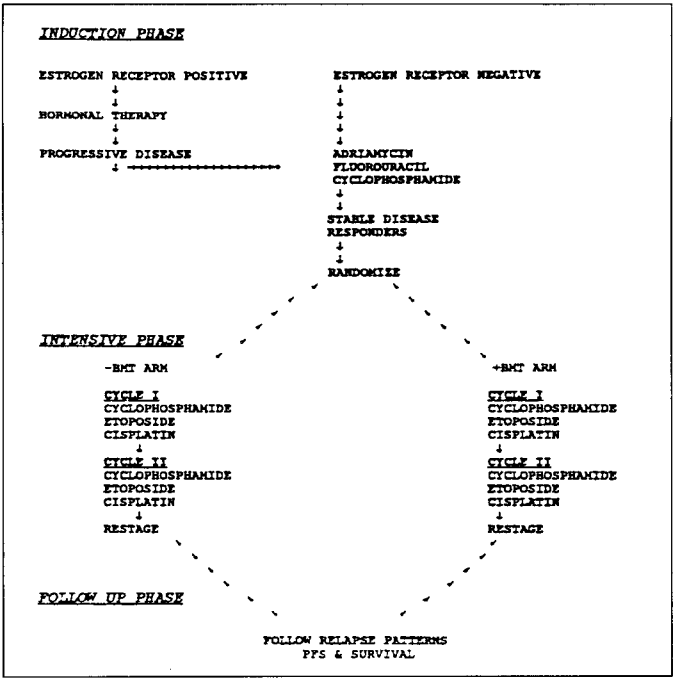

Figure 1.
Flow diagram of overall treatment plan for Stage IV Breast Cancer; ER- or ER+ Hormone Refractory. Abbreviations: PFS, progression-free survival.

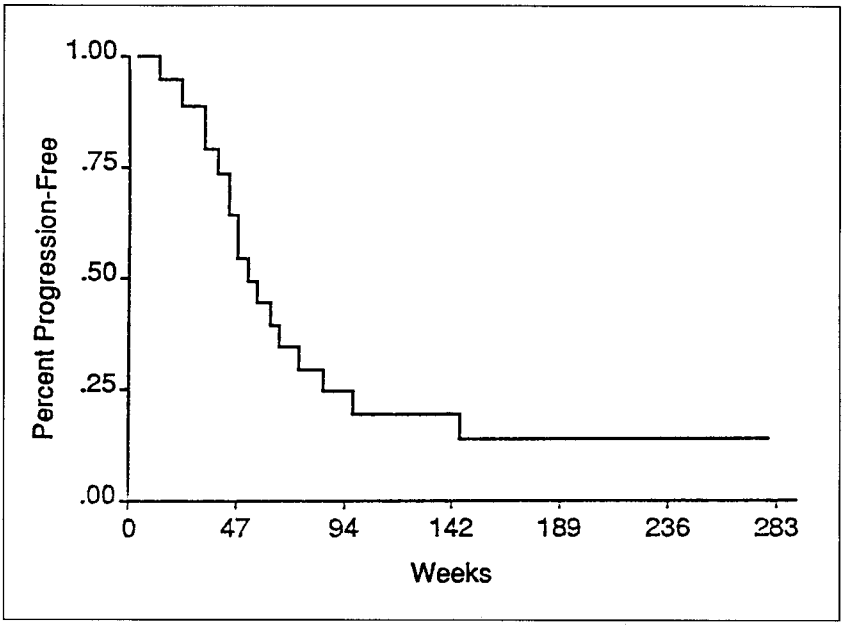

Figure 2.
Progression free survival post-induction (80 patients): Median 50 weeks (95% confidence interval, 44-61 weeks).

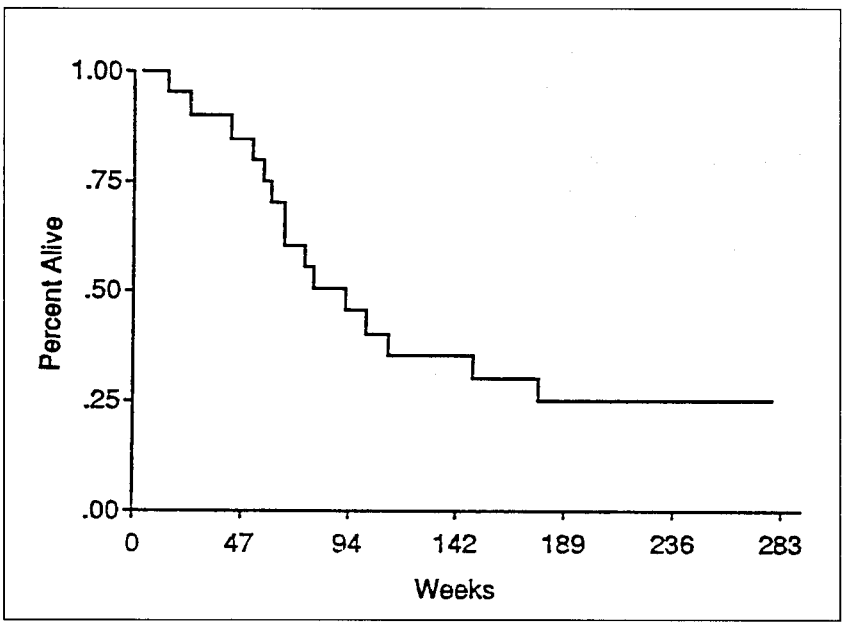

Figure 3.
Survival post-induction (80 patients): median survival from beginning induction therapy; 83 weeks (95% confidence interval, 68-110 weeks).

THE PHARMACOLOGY OF INTENSIVE CYCLOPHOSPHAMIDE, CISPLATIN AND BCNU (CPA/CDDP/BCNU) IN PATIENTS WITH BREAST CANCER.

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Combinations of alkylating agents in intensive doses with autologous bone marrow support (ABMS) are commonly used to treat advanced adult solid tumors, particularly breast and ovarian cancer. These drugs are usually administered in the maximally tolerated doses adjusted for weight or body surface area. The specific drug combinations and schedule of administration often lack firm scientific or clinical rationale, and, with rare exception, no further individualization of dose is ever done. In an attempt to lend further rationale to these treatments, we embarked on a program of comprehensive pharmacokinetic (PK) monitoring of the CPA/cDDP/BCNU regimen. The major goals of this program were:

1) Description of the PK variability of the regimen
2) Definition of pharmacodynamic (PD) correlations of this PK variability
3) Development of predictive methods for the PK of each drug in each patient to allow individualized drug dosing.
4) Testing the impact of individualized dosing on patient outcomes.

BACKGROUND AND SIGNIFICANCE

The use of PK measurements to direct drug dosing is common in medicine. Antibiotics, neurotropic agents, and immunosuppressants exemplify classes of drugs where therapeutic drug monitoring is routinely performed. The goal of this activity is to optimize the therapeutic index of the drug by optimally balancing toxicity and therapeutic effect through dosage individualization. Several hypotheses underlie these programs:

1) Important variability in drug PK exists between patients in spite of standardized drug dosing

2) This variability should correlate with toxic or therapeutic outcome, preferably both.

3) Correction of this variability should be possible using therapeutic drug monitoring, and should improve the therapeutic index of the drug (regimen).

It is ironic that therapeutic drug monitoring is almost never used in anticancer therapy. Antineoplastic agents have the narrowest therapeutic index of any class of drugs and are the most common class of drugs to produce fatal toxicity in routine use. The only antineoplastic agent frequently monitored is methotrexate, ironically a drug where a "rescue" compound (leucovorin) is available.

There are important deterrents to antineoplastic drug monitoring. Most

agents are given intermittently in multiple drug regimens, thus preventing repetitive refinement of dose by multiple dose monitoring over time as done with many oral agents. More accurate modeling of PK is thus required than the usual "random blood level" or "peak and trough" strategy used in most instances to obtain adequate accuracy of dose adjustment. For drugs other than methotrexate, validated drug assays for monitoring (having standardized precision, reproducibility, biologic relevance, and rapid result availability) are often unavailable. These topics are discussed in greater detail elsewhere[1].

In the field of high-dose chemotherapy with autologous hematopoietic cell support (HDC/AHCS), antineoplastic drug monitoring might have maximal justification. Sporadic life-threatening and fatal toxicities are seen, and conventional prognostic factor analysis poorly defines patients likely to achieve multiyear tumor-free survival. It is reasonable to hypothesize that variability in drug exposure might affect toxic and therapeutic outcome in this setting.

Only limited investigations in this area have been reported. Ayash et al. treated patients with advanced breast cancer with high-dose cyclophosphamide, carboplatin, and thiotepa (CPA/CBDCA/TT) and demonstrated that CPA PK correlated with both risk of cardiotoxicity and probability of progression-free survival[2]. Jones, et al treated similar patients with CPA/cDDP/BCNU and correlated the risk of acute pulmonary injury with BCNU PK[3]. Groshow et al. treated patients with high-dose busulfan and cyclophosphamide (Bu/Cy) and correlated the risk of hepatic veno-occlusive disease (VOD) with BU PK[4]. Importantly, in subsequent studies she demonstrated that PK-directed dose adjustment of BU could be used to reduce the incidence of VOD[5].

With this background, we proposed to perform a comprehensive PK/PD study of the CPA/cDDP/BCNU regimen. This high-dose regimen is frequently used in patients with breast cancer and its use is the subject of an NCI high-priority intergroup trial in patients with St II breast cancer involving 10 or more axillary lymph nodes. Preliminary data justifying this trial was generated at Duke University, and showed that 72% of treated patients are projected to remain progression-free survivors 5 years following treatment[6]. Comparable patients treated with conventional chemotherapy have a progression-free survival of 30% in many series. The potency of this regimen and the likelihood of its expanded use in the future added justification to this investigation.

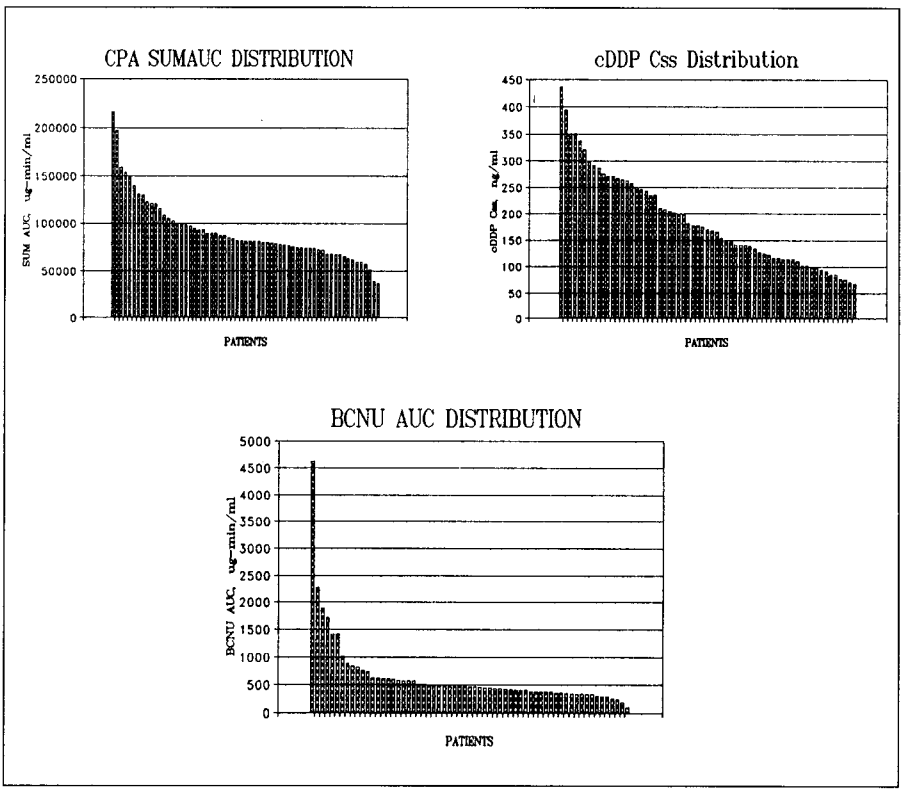
The doses and schedule of CPA/cDDP/BCNU are shown below:

	Day							
	-6	-5	-4	-3	-2	-1	0	+1
CPA 1875 mg/m^2/day	+	+	+					
cDDP 55 mg/m^2/day	<———	———	———	———	———	———	———	——>
BCNU 600 mg/m^2					+			
Marrow								+

PK VARIABILITY OF CPA/CDDP/BCNU

When the variability in exposure to drugs is described, the most common measure is the AUC, or area under the plasma drug elimination curve. This area represents the aggregate drug exposure at the capillary-cell membrane. For drugs given by continuous infusion over a period much longer than their elimination half-life (tl/2 elim), the steady-state plasma concentration (Css) represents a similar measure. In the case of the CPA/cDDP/BCNU regimen, CPA (tl/2 elito. = 2-5 hr) and BCNU (tl/2 = 15-45 min), are administered by one and two hr infusions, respectively and the AUC measure is used. cDDP (tl/2 = 30 min) is administered as a 72 hr continuous infusion and the Css is used.

Studies in 63 patients (for whom complete PK data sets were available) treated with the CPA/cDDP/BCNU regimen demonstrated wide interpatient variability in the AUC of CPA/BCNU, and Css cDDP, as shown below.



The variability of BCNU AUC was most striking, suggesting that might be most likely to produce differences in patient outcome.

Importantly, during this data accrual period, we demonstrated that assays for these drugs could be reproducibly performed, and allowed PK data to be delivered to the bedside within 18 hr of dosing so that PK-directed dose changes were feasible.

PD CORRELATES OF PK MEASUREMENTS.

Because of the wide PK variability of BCNU, and the known pulmonary toxicity of BCNU when low chronic doses are given, we hypothesized that BCNU PK might correlate with the risk of acute pulmonary injury. We evaluated 44 consecutive patients treated with CPA/cDDP/BCNU for the development of acute pulmonary injury and covariates of this injury. Six patients in this group had either incomplete PK data, short followup, or comorbid pulmonary conditions making interpretation of their pulmonary function impossible.

Of the 38 evaluable patients, 20 (53%) developed acute pulmonary injury. Twelve of the 20 patients had an elevated exposure to BCNU (>600 ug-min/ml AUC). Only two of the 18 unaffected patients had a similar elevated BCNU exposure, a difference which was statistically significant. There was no association between CPA or cDDP PK and the acute pulmonary injury. Other known pretreatment covariates of the risk of pulmonary injury (PFT's with DLCO, smoking, cardiac, hepatic or renal function, chest irradiation) were not different between the two groups. We thus concluded that elevated BCNU AUC is a covariate of acute pulmonary injury following CPA/cDDP/BCNU, and might be of causative importance in the development of this injury.

After a further followup of a larger cohort of breast cancer patients, we will assess the association between CPA and cDDP PK and the risk of toxicities such as cardiac injury or renal insufficiency, and evaluate all PK for relationship to 2-year relapse-free survival.

FUTURE DIRECTIONS

Given the growing body of observations which show correlations between antineoplastic agent PK and patient outcome, we plan to develop mechanisms to perform PK-directed drug dosing for CPA/cDDP/BCNU, and compare the outcome of patients treated on the basis of therapeutic drug monitoring with patients treated with standard doses of the regimen. Measurable outcomes will include the effectiveness of the PK-directed method itself to reduce the variability of CPA/cDDP/BCNU PK and whether or not clinical outcomes are affected by this maneuver. If clinical outcomes were favorably affected by therapeutic drug monitoring, this investigation would suggest an important role for the clinical laboratory in monitoring and directing high-dose chemotherapy regimens.

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Evening Session

THE BIOLOGY OF CD34.

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INTRODUCTION

The CD34 antigen is of interest because it is the only molecule identified to date whose expression within the blood system, is restricted to a small number of primitive progenitor cells in the bone marrow (BM). The availability of CD34 antibodies has greatly aided the development of techniques for the enrichment of primitive progenitor cells for a variety of studies of hematopoiesis *in vitro*. Additionally, the use of CD34 antibodies for the 'positive selection' of hematopoietic stem/progenitor cells represents an alternative strategy to 'purging' for the large-scale manipulation of cells prior to transplantation. The availability of pure populations of the most primitive hematopoietic progenitor cells may also facilitate the development of genetic techniques for the repair of specific blood cell disorders. Though its precise function remains unknown, the pattern of expression of the the CD34 structure suggests that it plays an important role in early hematopoiesis.

SEROLOGY AND EPITOPE MAPPING STUDIES

All seven antibodies, MY10, B1.3C5, 12.8, 115.2, ICH3, TUK3, and QBEND 10, assigned to the CD34 cluster [1], identify an antigen which is expressed on 1-3% of normal BM cells. This population has been shown to include virtually all unipotent, and multipotent myeloid progenitors as well as pre-CFU. Primitive T and B lymphocyte precursors and leukemias of primitive myeloid and lymphoid lineages also express CD34. Significantly, CD34+ BM cells can reconstitute all lineages of the hematopoietic system in lethally-irradiated baboons and rhesus monkeys, suggesting, that the 'stem cells' responsible for long-term reconstitution of hematopoiesis, express the CD34 antigen (see [2] and refs therein). This population is also capable of reconstituting hematopoiesis in humans [3]. Outside of the blood system, CD34 is found on vascular endothelial cells. As reviewed in detail elsewhere [4], CD34 strikingly is located on the luminal surfaces of ECs and on the loose interdigitating surfaces between adjacent cells, rather than in 'tight junctional' areas.

From early studies, it was apparent that the CD34 antibodies MY10, B1.3C5 and ICH3 were detecting distinct, non-overlapping epitopes and that the carbohydrate moieties in general, and the terminal sialic acid residues in particular, were important for antibody binding. Recently, we demonstrated that a novel proteolytic enzyme from *Pasteurella haemolytica* (P.h.glycoprotease), which is highly specific for glycoproteins rich in O-linked glycans, cleaves the CD34 antigen. We subsequently showed that the epitopes detected by five of the seven

CD34 antibodies are removed by glycoprotease treatment [5]. All epitopes previously shown to be variably susceptible to neuraminidase treatment (class I epitopes), i.e. MY10, B1.3C5, 12.8 and ICH3, are efficiently removed by the P.h.glycoprotease. The enzyme also removed the sialic acid-independent epitope detected by QBEND 10 (class II). The epitopes (class III) detected by TUK3 and 115.2 which were not cleaved by either enzyme, are therefore closer to the extracellular membrane surface than the others. In other studies reviewed elsewhere [2], there is some cell-type variation in the glycosylation of the CD34 antigen. Furthermore, at least with respect to class I and class II antibodies, there is also some discordance of CD34 epitope expression in normal BM progenitor cells [2, 4], suggesting that for purposes such as immunophenotyping or stem cell purification, it may be advantageous to use CD34 antibodies that detect sialic acid independent, (ie class II or class III) epitopes.

STRUCTURAL AND MOLECULAR CONSIDERATIONS.

All seven workshop CD34 antibodies immunoprecipitate a monomeric structure of about 110 kD from lysates of CD34+ cell-lines, albeit with different efficiencies. Similar bands can be isolated from fresh CD34+ acute leukemias of primitive myeloid, B-lymphoid and T-lymphoid phenotypes. These antibodies also react with COS cells transfected with a CD34 cDNA providing conclusive evidence that they recognize the same gene product [2, 4]. Several CD34 antibodies identify denaturation-resistant epitopes in western blots, though with widely different efficiencies. MY10 and ICH3 are partially dependent and B1.3C5, 12.8 are totally dependent on the presence of sialic acid residues for their binding, in agreement with the epitope mapping studies described above. Strikingly, the de-sialylated form of this structure exhibits an increased molecular weight of about 150 kD. Such aberrant mobilities in one-dimensional SDS-PAGE reflect the influence that multiple negatively-charged sialic acid residues (particularly in O-linked configuration) can have on glycoprotein mobility in SDS gels.

Metabolic labeling, using a range of radiolabeled precursors, together with tunicamycin experiments, indicated that the CD34 antigen 'turns-over' very slowly *in vivo*. These observations, coupled with the fact that CD34 antibodies used in early studies would only detect the fully processed or sialylated cell-surface form of the CD34 antigen, precluded an accurate estimate of the size of the core polypeptide. However, a combination of lectin binding studies and endoglycosidase cleavage experiments demonstrated the presence of several complex-type N-linked glycans. Alkaline hydrolysis followed by gel filtration of released glycans indicated the presence of O-linked carbohydates. Binding of the desialylated CD34 structure to Peanut lectin confirmed the presence of O-linked glycans of the sialylated Galb1-3Gal NAc-R type. Partial amino acid sequence analysis of N-terminal, and internal peptides of purified CD34, did not indicate similarity of these with other previously documented proteins [6].

The CD34 antigen is a substrate for, and can be phosphorylated to high stoichiometry by activated protein kinases C both in CD34+ cell-lines and fresh leukemic lymphoblasts. Recently, it has also been demonstrated that other protein kinases including glycogen synthase kinase and casein kinase II can phosphorylate the CD34 antigen. Furthermore, activated PKC can stimulate a rapid up-regulation of CD34 expression from intracellular stores of pre-formed CD34 in

normal CD34+ progenitor cells [see refs in 2, 4].

The CD34 cDNA has recently been cloned using a mammalian expression system and MY10 [7]. The predicted amino acid sequence of this cDNA contains the partial amino acid sequences previously determined from the purified CD34 protein. The full length cDNA (which does not exhibit sequence similarities with previously described structures) predicts a type I integral membrane protein of about 40Kd with a maximum of 9 potential N-glycosylation-sites. Since the de-N-glycosylated and desialylated forms are 90Kd and 150Kd respectively [2, 4], the native molecule is anticipated to contain a considerable number of O-linked glycans. In fact, over 35% of the 145 amino acids in the N-terminal domain of this molecule are serine or threonine residues [7]. The clusters of O-linked glycans in this domain, some of which based on size fractionation studies may be large and/or complex [6], may induce the polypeptide to take on an extended conformation. Thus the NH2-terminus of the CD34 antigen can be anticipated to protrude a considerable distance above the cell membrane. Between this N-terminal domain and the proximal extracellular domain is a cysteine rich segment of 66 amino acids which probably exhibits a globular conformation. The cytoplasmic domain of 73 amino acids contains several potential phosphorylation sites for a variety of protein kinases (reviewed in 2, 4). Overall, the data from the immunochemical and cDNA analyses of the CD34 antigen suggest a general model structure illustrated schematically in figure 1.

CD34 GENE.

Using somatic cell hybrids, and in situ hybridization, the gene was localized to chromosome 1, band q32. Using the human cDNA probe under conditions of lower stringency, the murine CD34 cDNA has been cloned, and contains 8 coding exons. The human gene, which covers about 26 Kb in length, is very similarly organized [4, and refs therein). The murine and human cDNAs are highly homologous: the cytoplasmic domains (encoded by exon 8) are>90% identical. The transmembrane and proximal extracellular regions (encoded by exons 6 and 7) and the cysteine-rich domain (encoded by exons 4 and 5) are also very similar (>75% and >70% identical respectively). The N-terminal domains of about 145 amino acids (encoded by exons 2 and 3) are only 45% sequence-identical. However, the presence of high levels of serine and threonine residues in this domain is conserved between the species, suggesting that the O-linked carbohydrate moiety may determine the functional capabilities of this part of the CD34 structure.

Previous studies have demonstrated that there is a close correlation between CD34 mRNA and CD34 antigen expression in a variety of cell-lines and tumor tissues. Together with the observations that all CD34 antibodies bind to COS cells transfected with the CD34 cDNA, these data indicate that in most situations, control of CD34 gene expression is primarily at the level of transcription. However, as reviewed elsewhere (2), there may cell-type variations in the complex patterns of glycosylation that this structure exhibits, which may give rise to occasional discrepancies between gene transcription and epitope-restricted surface antigen expression. It is probable that post-translational modifications (N-, O-glycosylation, terminal sialylation, phosphorylation by a variety of kinases,

glycoprotein half-life, etc) of the CD34 polypeptide may be important modulators of this structure's functional capabilities.

TRANSPLANTATION.

Several studies of autologous BM transplantation using autografts purged of malignant cells are suggestive of clinical benefit. Such trials have employed 'purging' to eliminate neoplastic cells from the autografts of patients with hematologic malignancies. As indicated earlier, 'positive selection' of hematopoietic stem/progenitor cells represents an alternative strategy for 'processing' BM cells prior to transplantation. We have recently described a simple, rapid and flexible method to isolate primitive hematopoietic progenitor cells from normal BM. The CD34+ cells were selected using the high-affinity, 'class II' antibody QBEND 10 (see epitope mapping studies), and immunomagnetic beads. Prior depletion of naturally adherent cells was not required. After magnetic selection of CD34+ cells/magnetic beads, the cells were detached from the beads by incubation with the *Pasteurella haemolytica* glycoprotease which selectively cleaves glycoproteins rich in O-linked glycans, like CD34 [5]. The purity of the released cells was assessed by immuno-fluorescence using either of the class III CD34 antibodies, TUK3 or 115.2, whose epitopes are not removed by the glycoprotease. The purity (up to 95%) and yield (up to 80%) of the enzyme-released cells was high. The purified cells, which generated normal numbers of hematopoietic colonies in semi-solid media, were enriched 81-fold for multi-lineage progenitors and reconstituted hematopoiesis in long-term culture, indicating that the functional competence of CD34+ progenitor cells *in vitro*, was unaffected by glycoprotease treatment [8]. This procedure could additionally serve as an initial step in the selection of normal, Ph-negative hematopoietic stem cells from CML marrows. Glycoproteins such as CD7, CD19, CD33, CD38 and HLA-DR, that are expressed on lineage-committed progenitors or their activated precursors are not cleaved by the glycoprotease. Thus, by employing secondary cell-sorting techniques, it may be possible to separate the leukemic progenitor cells from the residual, normal stem cells that express only CD34. Recent data indeed suggests that small numbers of primitive normal hematopoietic stem cells with the CD34+/lin-/HLA-DR- phenotype can be isolated from some CML marrow aspirates [9].

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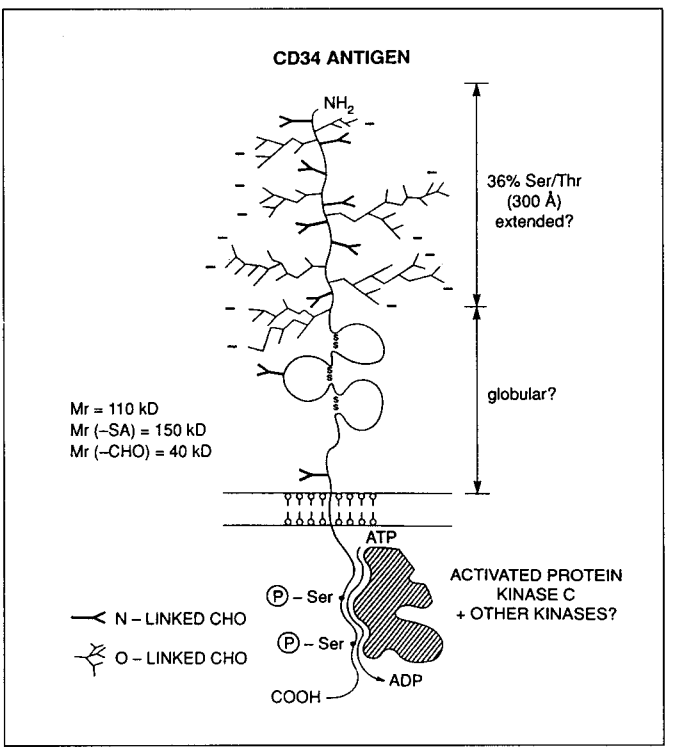

Schematic representation of the structural characteristics of the human CD34 antigen. The number and approximate location of the cysteine residues and N-linked glycosylation sites are based upon the full-length cDNA clones (7). Though the presence of multiple, negatively charged (heavily sialylated), Olinked glycans is well documented (6), the precise number, location (-) and complexity of them is not known. The precise locations of the serine residues phosphorylated by activated protein kinases C or other kinases (refs in 2) are not currently known. The estimate of the length of the extended NH2-terminal domain of about 300 Angstroms is based upon observations of the extended extracellular domain of the similarly heavily O-glycosylated CD43 molecule (see 2).

SELECTION OF PH-NEGATIVE PROGENITORS BY STROMA ADHEBENCE

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INTRODUCTION

Chronic myelogenous leukemia (CML) is a clonal disorder arising at the level of the pluripotent hematopoietic stem cell ([1]). The hallmark of the disease is the Philadelphia (Ph) chromosome, which results from the rearrangement of the b*cr* and *abl* genes ([2]). Conventional single-agent chemotherapy is not able to cure CML patients (3). Allogeneic bone marrow transplantation (BMT), although curative, is restricted to <20% CML patients (4). Therefore, based on the persistence of normal stem cells in the majority of CML cases ([5-9]), autologous (A)BMT has been proposed as an alternative therapeutic option ([10]). ABMT might be undertaken with curative intent provided that malignant stem cells could be eliminated from the autograft and Ph-negative stem cells could be efficiently and selectively isolated ([11]). In a subset of CML patients the cyclophosphamide derivative, mafosfamide, exerts a preferential cytotoxic effect on Ph-positive CML cells ([12]). In addition, it has been shown that CML progenitor cells are defective in their ability to attach to normal marrow-derived stromal layers ([13]) and that this defective attachment is due to a deficiency in a phosphatidylinositol-linked cell adhesion molecule ([14]).

It was the aim of the present study to investigate the possibility to enrich Ph-negative clonogenic cells by combining mafosfamide incubation and fractionation of CML cells on the basis of their ability to adhere to preformed, allogeneic, normal marrow-derived stromal layers.

MATERIALS AND METHODS

Patients. Fifteen patients with Ph-positive CML were included in this study, for a total number of 19 experiments. One patient was studied at diagnosis and prior to any treatment, the others had been diagnosed from 1 months to 5 years prior to the time of the study, and had received prior treatment with hydroxyurea and/or interferon-alpha. At the time of the study one patient was in blast crisis, two were in accelerated phase, whereas the remaining patients were in chronic phase.

Cell separation procedures. After informed consent, marrow was obtained by aspiration from the posterior iliac crest and mononuclear, light density marrow cells (MNCs) were separated by centrifugation on a Ficoll-Hypaque gradient (density = 1.077 g/ml). Plastic non adherent MNCs were obtained by incubating marrow cells for 120 min in 75-cm2 tissue culture flasks.

In vitro purging. Treatment with mafosfamide (100 mg/ml, Asta Pharma,

Bielefeld, FRG) or control medium was performed by incubating the cells (2xlO7/ml) for 30 min at 37°C in a water bath with frequent agitation. The cells were subsequently incubated for 5 min on ice to stop the reaction.

Preparation of stromal layers. Stromal layers were prepared according to the technique described by Gordon et al. (15). Normal bone marrow was obtained from consenting donors by aspiration from the posterior iliac crest. MNCs were isolated as above, washed and resuspended (5 x 105/ml) in alpha-medium supplemented with 15% FBS and 2 x 10^{-6} M Methylprednisolone. One ml aliquots were plated in 35 mm Petri dishes and incubated at 37°C in a humidified atmosphere supplemented with 5% C02. The cultures were fed weekly by complete replacement of medium and serum until confluent.

Clonogenic assay. The assay for pluripotent colony-forming units (CFU-Mix), erythroid bursts (BFU-E), and granulocyte-macrophage colony-forming units (CFU-GM) was carried out as described in detail elsewhere (16). Briefly, 5xlO4 were plated in 35-mm Petri dishes in l-ml aliquots of Iscove's modified Dulbecco's medium (IMDM, Miles Laboratories, Naperville, IL, U.S.A.) supplemented with FBS (30%), 2-mercaptoethanol (5 x 10^{-5} M), and methylcellulose (1.1%, w/v). Cultures were stimulated with a mixture of human recombinant colony-stimulating factors (CSFs) including: interleukin-3 (IL-3, 10 ng/ml), granulocyte-CSF (G-CSF, 10 ng/ml), granulocyte-macrophage-CSF (GM-CSF, 10 ng/ml) and erythropoietin (Epo, 1 U/ml). Recombinant IL-3, GM-CSF and Epo were generously provided by Behringwerke AG (Marburg, FRG); G-CSF was kindly provided by Amgen Inc. (Thousand Oaks, CA, U.S.A.). Progenitor cell growth were scored according to previously described criteria (16) after incubation of the dishes for 14 days. Four dishes were set up for each individual data point per experiment.

Cytogenetic analysis. Cytogenetic analysis and standard GTG- or QFQ-banding techniques were performed according to standard methods (17). Single colony karyotyping was performed according to Dube et al (1^{8}).

Experimental design. Following incubation with mafosfamide (100 mg/ml) or control medium, MNCs were seeded onto normal marrow-derived, allogeneic stromal layers to allow attachment of stroma-adherent cells. After 3 hrs incubation at 37°C, stroma non-adherent cells were harvested. Both adherent and non-adherent cell fractions were then cultured in suspension (3 days, 37oC, 5% $C0^{2}$). At the end of the incubation period, stroma adherent cells were harvested by repeatedly washing each dish. Stroma adherent and stroma non-adherent cells were then cultured in methylcellulose to allow the growth of CFU-GM to be analyzed by single colony karyotyping. Stromal layers were neither irradiated nor fixed, and to exclude the possibility that Ph-negative metaphases were derived from the stromal layers, the sex of the patient and the stroma donor were mismatched.

RESULTS

Both the multilineage (CFU-Mix) as well as the granulocyte-macrophage (CFU-GM) and erythroid (BFU-E) lineage-restricted progenitor cells were significantly inhibited following exposure to 100 mg/ml of mafosfamide. The percentages of surviving CFU-Mix, BFU-E, and CFU-GM were 0%, 3.3%, and 13%, respectively. However, following stroma adherence and short-term suspension

culture, CFU-GM growth was significantly enhanced, thus allowing the growth of a sufficient number of CFU-GM to be analyzed at the cytogenetic level.

On direct cytogenetic analysis, the overall mean (+SD) percentage of Ph-negative metaphases was 7 ± 20%. The mean (±SD) percentages of Ph-negative CFU-GM grown from untreated and mafosfamide-treated marrow samples were 19 ± 29% and 31 ± 37%, respectively. As compared to direct cytogenetic analysis, a statistically significant increase in the percentage of Ph-negative CFU-GM was observed both from untreated ($P < 0.025$) as well as mafosfamide-treated marrow samples ($P < 0.025$).

Following stroma adherence (3 hrs.) and short-term suspension culture (3 days), four different CFU-GM populations, i.e., adherent CFU-GM, adherent mafosfamide-treated CFU-GM, non-adherent CFU-GM, and non-adherent-mafosfamide-treated CFU-GM, were available for cytogenetic analysis. The mean (+SD) percentages of Ph-negative clones were 33 ± 25% for adherent CFU-GM, 59 ± 40% for adherent mafosfamide-treated CFU-GM, 12 ± 16% for non-adherent CFU-GM, and 32 ± 26% for non-adherent mafosfamide-treated CFU-GM.

Overall, these data show an enrichment of Ph-negative clonogenic cells grown within the stroma adherent either untreated or mafosfamide-treated fractions, associated with an enrichment of Ph-positive CFU-GM within the stroma non-adherent fraction.

If patients showing a percentage of Ph-negative clones ≤ 20% were considered "unresponsive" to the treatment and excluded from this analysis then, the mean (±SD) percentages of Ph-negative clones were 47 ± 19% for adherent CFU-GM, 81 ± 21% for adherent mafosfamide-treated CFU-GM, 21 ± 17% for non-adherent CFU-GM, and 39 ± 23% for non-adherent mafosfamide-treated CFU-GM.

DISCUSSION

The aim of this report was to investigate, at the clonogenic cell level, the distribution of Ph-positive and Ph-negative CFU-GM between stroma adherent and stroma non-adherent CML marrow cells.

It is demonstrated that: (a) a significant proportion of stroma adherent CFU-GM is Ph-negative; and (b) mafosfamide incubation significantly increases the percentage of Ph-negative CFU-GM within the stroma adherent fraction.

Based on the results presented herein three major conclusions can be reached: (1) Ph-positive CML progenitor cells have a reduced binding capacity to normal marrow stroma; (2) mafosfamide has a selective effect on the Ph-positive clones and, at least in our experimental system, seems to spare Ph-negative clones with maintained stroma adherence activity; (3) the altered interactions of CML progenitors with marrow stroma might represent a new purging approach in view of autologous marrow transplantation.

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TRANSDUCTION AND EXPRESSION OF THE HUMAN GLUCOCEREBROSIDASE GENE IN THE LONG TERM MURINE MODEL, RHESUS MONKEY BONE MARROW AND HUMAN CD34+ CELLS.

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Experiments conducted in mice indicate that retrovirus mediated gene therapy may represent a viable treatment for Gaucher disease. Prior to initiation of human trials, however, there should be clear indication that retroviral transduction can lead to clinically relevant expression of the transferred glucocerebrosidase (GC) gene in mature macrophages, since pathological storage of glucosylceramide is restricted to these cells. As an indication that the treatment can provide long term benefit, transduction of primitive marrow repopulating stem cells should be obtained. In addition, the risks associated with retroviral infection, i.e. proto-oncogene activation or insertional mutagenesis leading to neoplastic transformation, must be low.

Recently we reported use of a retroviral vector, MFG-GC, in a murine model of bone marrow transplantation.(1) This vector resulted in high levels of expression in long-term reconstituted animals; such expression was commonly not observed in previous studies.([2,3]) Expression of human GC was demonstrated in mature bone-marrow-derived macrophages cultured from primary (1°) recipients 4-7 months after transplant, and virtually 100% of the secondary (2°) spleen colonies (produced from 1° bone marrow in 2° irradiated mice) contained transduced vector sequences at this time. We report here additional preliminary findings for 2° long-term reconstituted animals.

The murine model offers advantages not present for human studies, including induction of stem cell cycling in the donor with 5-fluorouracil (5-FU) and the option to use coculture of bone marrow cells with viral producer cells. The former lacks effectiveness in primates (4), although other means of stem cell stimulation may substitute. Coculture causes problems with FDA requirements for human trials. We report here preliminary results obtained using filtered viral supernatants for infection of Rhesus monkey bone marrow cells and of human CD34 peripheral blood (PB) cells from G-CSF primed patients. The latter cells may be appropriate targets for treatment of Gaucher patients.

MATERIALS AND METHODS

Viral vector and producer cells: The MFG retroviral vector was originally constructed by Drs. Robbins and Guild in Richard C. Mulligan's laboratory (Whitehead Institute, MA) and was modified in our laboratory by insertion of the cDNA for human GC as described (1). The ecotropic producer, y-cre, was used for transduction of murine cells. An amphotropic producer, y-crip, multi-

ply infected with supernatants from the y-cre producer of MFG-GC, produced supernatants containing the amphotropic vector for transduction of monkey and human cells. Producer cells were maintained in 10% calf serum (CS) in high glucose DMEM containing pen/strep and L-glutamine at 37oC in 5% CO_2 in air. Amphotropic supernatants were prepared using 10% FBS in IMDM; no difference was evident in transduction efficiency for 3T3 targets using supernatants prepared in 10% CS/DMEM or 10% FBS/IMDM. For control infections, supernatants from an amphotropic MFG-lacZ producer were used.

Transduction of murine bone marrow: Bone marrow was prepared and transduced as previously described (1). Briefly, donor mice were injected with 5-FU five days prior to extraction of bone marrow. Marrow was prestimulated with cytokines IL-3 (Genzyme), IL-6, and stem cell factor (SCF) (generous gifts of Dr. K. Zsebo, Amgen, CA) for two days, then was infected by coculture with irradiated ecotropic producer cells for two days. Recipient mice were lethally irradiated prior to tail vein injection of transduced bone marrow. Conversion to donor phenotype was assessed using GPI electrophoresis; C57B1/6J (Gpi-1b) and B6-Gpi-1^a congenic mice were used ([5]) (Jackson Laboratories, Bar Harbor, ME). Secondary irradiated recipients were given 1 x 107 nucleated bone marrow cells from the femurs of 1° recipients at 4-7 months post transplantation.

Immunohistochemistry: Immunochemical detection of the human GC protein was performed according to manufacturers directions using the Vectastain ABC Kit (Vector Laboratories). This kit contains a biotinylated antimouse IgG and produces an avidin-biotin-peroxidase complex. Monoclonal antibody 8E4 was used as the primary antibody. Cells were identified by staining with the horseradish peroxidase substrate 3-amino-9-ethyl-carbazole (Sigma, St. Louis, MO) and by counterstaining with hematoxylin.

Transduction of Rhesus bone marrow: Rhesus bone marrow was obtained from the femur of an adult monkey. The cells were stored cryogenically in 10% DMSO, 40% FBS/DMEM prior to transduction. Thawed cells were cultured in 30% FBS/IMDM containing 10 ng/ml human recombinant (hr) IL-3 (Genzyme, MA), hrIL-6, and rrSCF for two days prior to infection with viral supernatant. For infection, cells were pelleted and resuspended in supernatant containing 8 ug/ml polybrene. After about 2 hours at 37oC, culture medium with cytokines was added. Infection was repeated daily for three days, after which time the cells were expanded in the IL-3, IL-6, SCF mixture or in 30%FBS/IMDM containing human M-CSF (100 U/ml) and GM-CSF (10 U/ml). After 2 weeks in culture, cells were harvested for assay of GC enzyme activity.

Transduction of human CD 34+ cells: PB cells from G-CSF primed patients were enriched for CD34+ cells using an avidin biotin immunoadsorption technique (CellPro™). Pre- and post-enrichment samples were analyzed for CD34+ lineage$^-$ (i.e. $CD3^-/CD19^-$) cells using a FACScan flow cytometer. Enriched cells were cultured in 30% FBS/IMDM containing various cytokine mixtures: A) hrIL-3, hrIL-6 and hrSCF, B) IL-3, IL-6, SCF, and hrGM-CSF, or C) SCF and PIXY, a fusion protein of hIL-3 and hGM-CSF. Viral supernatant and polybrene (2 ug/ml) were added to the cultures a total of six times over six days beginning two days after enrichment. Cells were analyzed for enzyme activity at this time, and some cells were further expanded over a total period of 3 weeks. In a second experiment, enriched cells were cultured in a mixture of hrIL-6, rrSCF, and PIXY

and were infected five times over a four day period beginning two days after enrichment. Cells were counted using eosin exclusion and a hemacytometer.

Glucocerebrosidase enzymatic activity: Cells to be assayed were washed twice in PBS, pelleted and stored at -80° C prior to analysis. Pellets were extracted in cold 50 mM potassium phosphate buffer (pH 6.5) containing 2.5 mg/ml Triton X-l00. Ultrasonication (Branson Sonifier 450, power setting 8, 5-10 seconds) was used to disperse and lyse the cells, followed by 15 minute centrifugation in a microfuge at 4° C to yield a clear supernatant for analysis. Enzyme activity was determined by addition of one part lysate to three parts of synthetic substrate (4-methyl-umbelliferyl glucopyranoside, Sigma Chemical Co, MO) at 10 mM in citric acid-sodium phosphate (0.12 M) buffer (pH 5.4) containing 2.5 mg/ml sodium taurocholate, 2 mg/ml Triton X-100, and l0 mg/ml bovine serum albumin. The reaction was terminated after 30 minutes at 37° C by addition of 0.17 M glycine-carbonate buffer (pH 10.4), and the fluorescence of the 4-methylumbelliferone product was measured with a fluorometer. Protein concentration was determined using bicinchoninic acid (Pierce, Rockford, IL). Specific GC enzymatic activity is expressed as nmoles per hour per mg of protein (U/mg).

Southern hybridization: Southern hybridization was performed as previously described (1), except that for DNA extraction the salt precipitation method of Miller et al(6) was used without phenol extraction. Digestion of transduced genomic DNA with SstI yields a 4.3 Kb diagnostic fragment which hybridizes to the human GC cDNA probe.

RESULTS AND DISCUSSION

We have shown that significant glucocerebrosidase (GC) expression is maintained in the tissues (bone marrow, spleen, thymus, and liver) of mice up to seven months after transplantation of transduced bone marrow (1); the levels of expression were consistent with high specific activity in the hematopoietic cells in these tissues. Southern and enzyme analyses showed that virtually all the individual spleen colonies from 2° animals transplanted with bone marrow from 4 to 7 month old 1° recipients contained and expressed the viral vector (Figure IA). Now more than one year after the initial transduction, cytospins of peripheral blood (PB) from 4/4 donor-positive 2° long-term recipients stain positive for the human GC enzyme by immunohistochemistry (see Figure lB), indicating that transduction initially occurred among some of the most primitive stem cells testable in the murine model. Based upon these murine experiments, it appears that certain requirements for clinical testing have been satisfied: long term expression has been maintained in PB and in mature macrophages, very primitive stem cells were initially transduced, and transduced cells have successfully reconstituted hematopoiesis in many animals (37 2o recipients are now surviving) without indication of pathology.

Infection of autologous bone marrow from Rhesus monkeys was contemplated as the next step in preparation for human trials. Bone marrow from a Rhesus monkey was infected by multiple exposures to high titer supernatant from an amphotrophic producer line (y-crip) (Figure 2). It was evident from this experiment that endogenous expression was higher in cells cultured in the presence of M- and GM-CSF in comparison to IL-3, IL-6 and SCF, indicating that

controls must be carefully matched with respect to culture conditions. Expression was consistently elevated in the MFG-GC infected groups of cells, but improvement in efficiency of transduction/expression is clearly necessary.

It has been reported that PB cells from patients receiving hematopoietic growth factors during recovery from high-dose cyclophosphamide therapy make good targets for retroviral transduction (7). Our preliminary results show that exposure of CD34+ enriched PB cells from G-CSF primed patients to supernatant containing MFG-GC resulted in nearly 85% increase in GC activity in comparison to noninfected and MFG-*lacZ* infected controls (Figure 3A). There was no significant difference between expression levels in cells grown in different cytokine mixtures (see Materials and Methods), and elevated expression continued throughout a two week in *vitro* expansion period following transduction. The high degree of expansion possible with CD34+ enriched cells (Figure 3B) enabled isolation of sufficient DNA for Southern analysis, which suggested a transduction efficiency of between 5 to 15%.

In a second experiment with $CD34^+$ cells, only a 20% increase in GC activity was obtained following exposure to MFG-GC. In this case a shorter transduction period, 4 days versus 6 days, may explain the difference between the two experiments.

The requirement of cell cycling for retroviral transduction (8) probably accounts for much of the difficulty in transducing hematopoietic stem cells from higher mammals. The treatment of patients with hematopoietic growth factors should assist in inducing stem cells into cycle. Moreover, the use of peripheral blood as a source of stem cells may provide a significant advantage over the use of autologous bone marrow, since the marrow is often difficult to aspirate from Gaucher patients.

Further experiments will address the following: Are transduced CD34+ cells capable of long term reconstitution or are they more committed progenitors? What is the best time window for transduction? What are optimal transduction conditions? Is transduction occurring in high copy numbers among a few cells or low copy among many? Will expression continue in mature human macrophages with this vector, as it did in mice? Can transduction of CD34+ cells from Gaucher patients yield therapeutic levels of GC expression? The answers to these questions will provide a basis for planning human trials.

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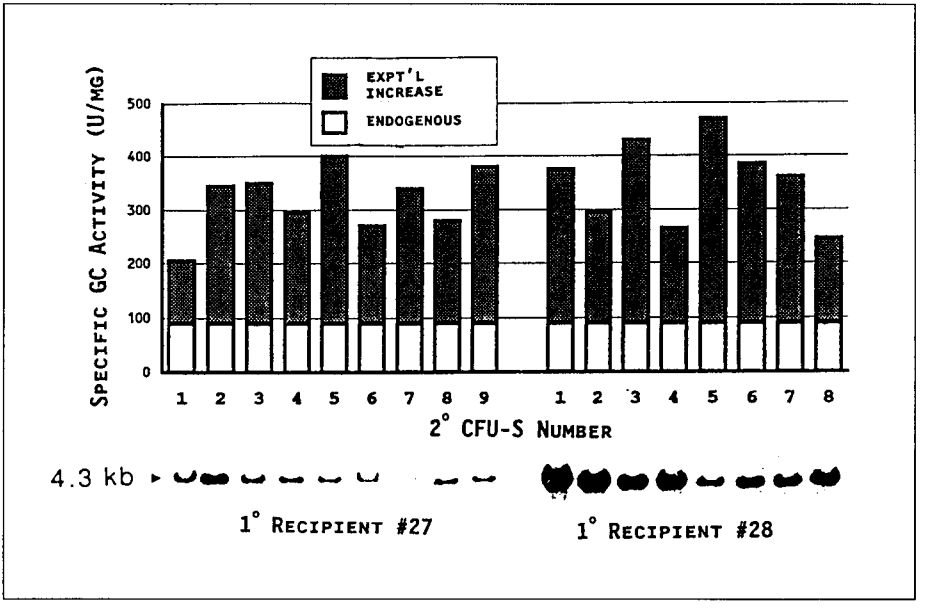

Fig. 1A: Southern analysis and enzyme activity of secondary (2o) spleen colonies. Individual spleen colonies were bisected and analyzed for the presence of vector sequence and for GC activity. Shown here are 9 and 8 colonies from the spleens of mice which received bone marrow from primary (1o) recipient numbers 27 and 28, respectively.

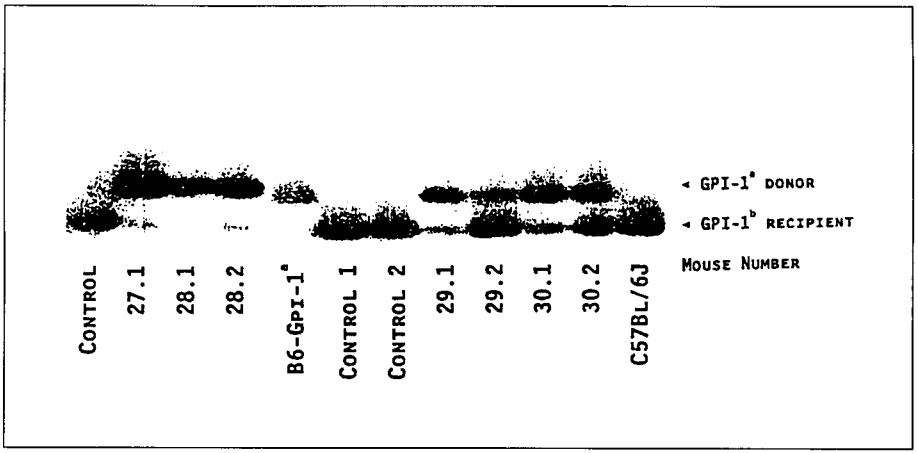

Fig. 1B: GPI isozyme analysis of 2° long-term reconstituted mice. Peripheral blood (PB) samples were obtained 6-8 months following serial transplantation of bone marrow from 1o recipients into 2° recipients. Primary recipients were sacrificed 6 months after retroviral transduction. Each 1o mouse was used to transplant two 2° mice, e.g. 28.1 and 28.2 GPI-1a donor and GPI-1ᵇ recipient isotype electrophoretic bands are indicated. Peripheral blood leukocytes were lysed in deionized water prior to electrophoresis as previously described (1). Immunohistochemical staining (color photos not shown) indicated that a majority of the PB leukocytes from mice #'s 27.1, 28.2 and 30.1 were positive for expression of the transferred human GC.

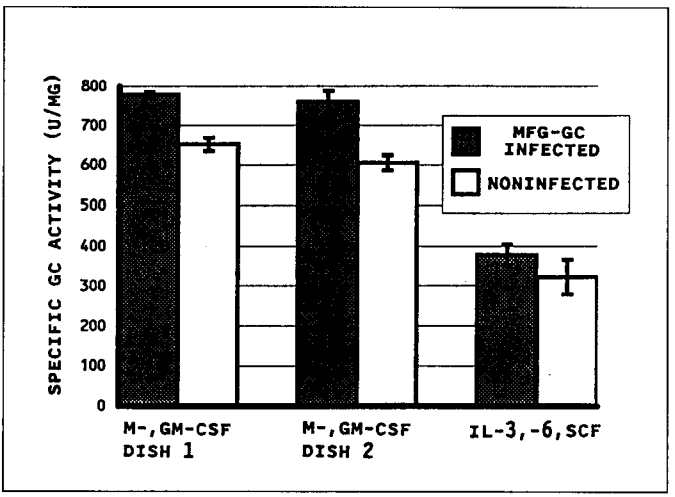

Fig. 2: Increased GC activity in Rhesus monkey bone marrow following exposure to filtered supernatant containing amphotropic MFG-GC. Cells were transduced and analyzed as described (Materials and Methods). Following transduction, cells were expanded in medium containing IL-3, -6, and SCF (present during transduction) or in medium containing M-CSF and GM-CSF. The latter cells were split into a second dish (Dish 2) one week prior to harvest. Error bars indicate the range between duplicate pellets assayed on separate days.

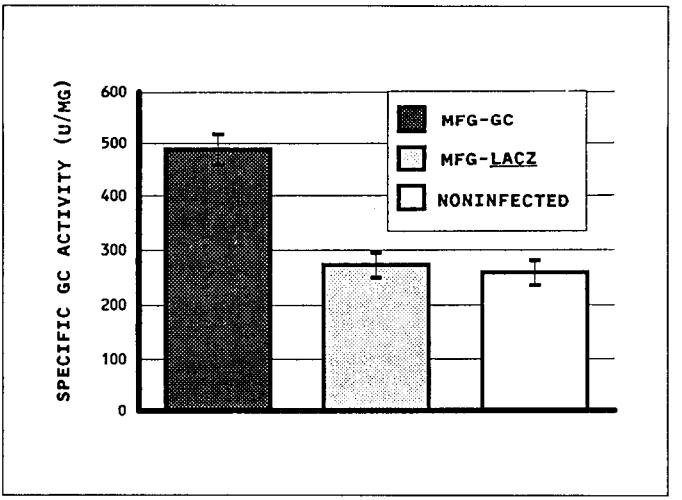

Fig. 3A: Transduction of CD34+ enriched PB cells from a G-CSF primed lymphoma patients. Cells were transduced and analyzed as described (Materials and Methods). Three separate cytokine mixtrues were tested during transduction; subsequently cells were expanded in a single mixture. Analysis of cells at vrious time points up to 3 weeks after enrichment showed no significant difference between the mixtures or between times of harvest, therefore results were pooled for all samples in each infection group. Error bars indicate + the standard error for n=10 pellets.

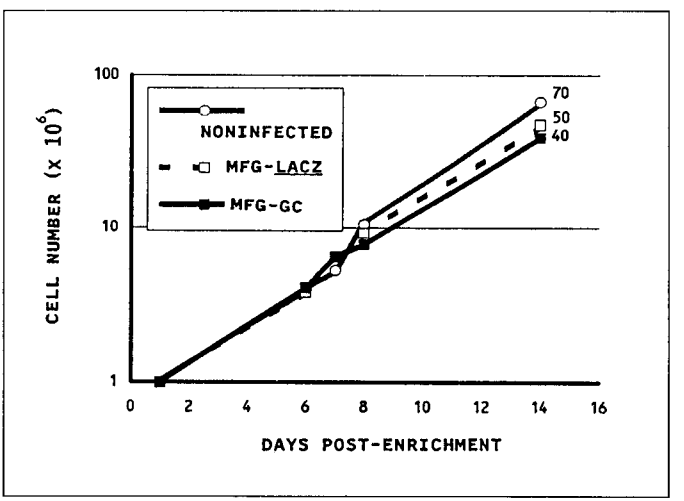

Fig. 3B: Growth of cells in vitro following CD34+ enrichment. PB cells were collected on day 0 and enriched on day 1; transduction occurred on days 3 through 8. Cells exposed to supernatant and polybrene typically show reduced growth in comparison to noninfected controls. Total cell number is extrapolated based upon quantitative splittings into fresh medium during harvest at various time points.

PROCESSING OF STEM CELLS FOR TRANSPLANTATION

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ABSTRACT

The success of autologous stem cell transplantation using bone marrow or peripheral blood stem cells depends not only on *in vivo* irradication of the disease by cytotoxic therapy but also on complete hematopoietic engraftment with no risk of relapse from the infused cells. Various methods are available for removing (purging) such contaminants. Purging techniques were developed by use of various *in vitro* models utilizing cell lines and fresh cancer cells. Once effective conditions were developed, they were advanced onto clinical trials. Various approaches for purging and subsequently freezing stem cells have been used. Subgroups of leukemia and lymphoma patients have now been shown to clinically benefit by the use of purged, cryopreserved stem cells. Emphasis is now being placed on utilizing the information already obtained by the pre-clinical investigators on designing better clinical trials.

INTRODUCTION

High dose cytotoxic therapy with hematopoietic stem cell (HSC) rescue is now the treatment of choice for various malignancies. It has improved the long term disease free survival (LT-DFS) of patients with lymphoma, Hodgkin's disease and acute myeloblastic leukemia [1-3]. Many factors (Table 1) affect the quality and quantity of hematopoietic stem cells obtained from the bone marrow or peripheral blood stem cells (PBSC). Improvements in the methods of HSC harvest and purging will result in better hematopoietic engraftment and lower relapse rate.

IS HEMATOPOIETIC STEM CELL RESCUE REQUIRED AFTER CYTOTOXIC THERAPY?

We now have substantial understanding about how much dose escalation is possible without any hematopoietic stem cell support, especially with the use of hematopoietic growth factor(s). If good therapeutic benefit can be obtained with conventional or intermediate dose therapy, the patients need not be subjected to the risks associated with more intensive treatment followed by stem cell rescue (relapse from infused cancer cells, poor hematopoietic engraftment, cost, etc.). For drug(s) under phase I evaluation, emphasis can be placed on deciding this threshold (maximum tolerable dose with growth factors [no HSC rescue] and if needed, with HSC rescue) rather early in clinical trials [2].

AUTOLOGOUS STEM CELL HARVEST AND EVALUATION OF TUMOR INVOLVEMENT

Our ability to detect small numbers of cancer cells in HSC harvest is limited. Routine morphological stains are heavily relied upon. Often diseases with cytogenetic markers can benefit from karyotypic analysis, but the cells have to be able to undergo mitosis, and screening large numbers (over 100) of chromosome preparation is very tedious. The polymerase chain reaction (PCR) offers the possibility of extremely high sensitivity (>1 in 1 million), where specific markers (e.g., translocations) are available to identify the tumor cells [4]. The technique is particularly prone to technical variability, and there have been problems of standardization in multicenter studies. Fluorescence *in situ* hybridization (FISH) offers similar potential sensitivity, and these may become the methods of choice as the technology improves. Tumor-directed monoclonal antibodies can pick up one tumor cell in up to 100,000 normal cells in diseases such as neuroblastoma and small cell lung cancer. Flow cytometry is of similar sensitivity and can detect rare aneuploid cancer cells [4].

PURGING TECHNIQUES

The aim of purging is to remove clonogenic tumor cells from the marrow or PBSC preparation without adversely affecting the engraftment potential of the normal stem cells. The most important method to decrease the risk of relapse with AuBMT is to reduce the tumor burden in the patient prior to bone marrow harvest (BMH)[2]. With this approach, the need to depend on *ex vivo* purging will be less. *Ex vivo* purging can be achieved by negative selection in which the tumor cells are eliminated, or by positive selection, in which the pluripotent hematopoietic stem cells are selectively enriched. Animal models have shown that injection of as few as 25 promyelocytic leukemia cells could establish a lethal tumor in 50% of the recipients. Extrapolation to humans, indicated that this dose would translate up to 3,500 leukemic cells are needed for causing leukemia [6]. Based on this model, transplant recipients receiving 15-150 tumor cells in the graft would have a 1-5% risk of disease relapse originating from these cells. Depending upon the sensitivity of the method used to detect tumor involvement, the number of cancer cells likely to be reinfused may be as high as 10^6-10^7 (0.01-0.1% infiltration of a harvest of 10^{10} nucleated cells) in a remission marrow with a correspondingly increased risk of contributing to relapse.

In our experimental model, we utilize cell lines alone and in combination with irradiated and un-irradiated human bone marrow cells to simulate the *ex vivo* purging conditions. Our investigations showed that a combination of 4-Hydroperoxycyclophosphamide (4-HC) and VP-16 was very effective in eradicating lymphoma and AML cells. Therefore, 4-HC and VP-16 combination was chosen for clinical use [7,8]. Definitive proof of the clinical value of purging remains to be established; however, preliminary results have suggested improved outcome in patients receiving purged grafts[4,10].

Disease relapse following HSC transplantation may be from residual tumor in the recipient and/or from cancer cells that were reinfused with the graft. To date it has not been possible to determine which source is predominant. Recurrences in sites where reinfused stem cells transiently persist, e.g., the liver and lung, suggest that reinfused cells may contribute to relapse. Studies are currently

in progress using marker genes to label the graft and track the source of relapse. Supporting evidence for the role of reinfused tumor cells in recurrence comes from a recent study in non-Hodgkin's Lymphoma. PCR with bcl-2 probe was used to document tumor infiltration of the marrow after purging in 114 patients with known presence of bcl-2 before purging. Following antibody and complement-mediated purging (capable of removing 3-6 logs of tumor cells), 57 marrows were found to be free of bcl-2 signal. Patients transplanted with "tumor-free" marrow (no evidence of bcl-2 by PCR and absence of lymphoma involvement by morphology) had only three relapses out of 55 patients treated. On the other hand, among the 57 patients who had presence of bcl-2 by PCR, 26 patients had already relapsed with a short follow-up [4]. Several other issues need to be resolved. Three out of 55 patients who were negative for bcl-2 gene have relapsed (Table 2). This suggests that in approximately 5% of the patients, the technique is either not sensitive enough or that other mechanisms for relapse (inadequate treatment of patient, drug resistance mechanisms, alteration in other gene, etc.) may be involved.

At our institution, we observed that patients with relapsed/refractory lymphoma do poorly on most conventional salvage therapy regimens and with AuBMT when total body irradiation (TBI) and cyclophosphamide are used for conditioning. Improvement is obtained when VP-16 is added to the conditioning regimen of TBI and cyclophosphamide (Figure 1). In this study, we made the following assumption regarding purging of the patient's bone marrow (BM): All patients with refractory/relapsed lymphoma were evaluated with BM aspirate and biopsy, history, physical examination and extent of disease work-up. Patients with no history of BM involvement throughout their clinical course received untreated BM. Patients with previous history of BM involvement, but no involvement at the time of BM harvest were considered to have minimal disease and were felt to benefit from 4-HC, VP-16 purged AuBMT. Patients with BM involvement at the time of consideration for harvest, were felt to have too much lymphoma and were determined to be unsuitable for purging. These patients received further therapy to see if the BM would clear so that they could qualify for BM purging. From the work of various investigators, it appears that patients with BM involvement at anytime during their clinical course do poorly. Figure II describes the results of purged and unpurged transplants. The results appear identical and support the concept that patients receiving purged BMT (these patients usually have poorer prognosis) did just as well as patients whose BM was not involved, and received unpurged bone marrow transplantation (BMT).

In patients with AML, several publications with small numbers of patients suggest that patients had minimal benefit from BM purging [1,9]. European investigators have now compiled the results of 1483 AuBMT using BM and/or PBSCT and have retrospectively analyzed the data for evaluating the benefit of purging with mafosfamide [10]. Some interesting conclusions can be drawn from this study and are summarized in Table 3. The most likely explanation for the data is also suggested.

CRYOPRESERVING STEM CELLS

Stem cells can be cryopreserved in 5 to 10% DMSO with various concentrations of albumin [1-3,11]. We find the technique of Stiff et al [11] utilizing final concen-

tration of 5% DMSO, 4% albumin and 6% HES to be better (less agglutination on thawing) and there is no need to use control rate freezers. We usually freeze our bone marrow in electric freezers at -110 to -135°C. The cells retain biological activity for 3 to 4 years.

From the data synopsis presented, we feel that properly planned bone marrow harvest with the best use of purging conditions has helped patients with lymphoma and AML. Further investigations are needed to optimize the purging conditions [12-15], develop better methods for detecting minimal disease, and extend these studies to clinical trials.

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Table 1. Factors Affecting Quality and Quantity of Hematopoietic Stem Cells.

1. Cumulative toxicity of previous therapy.
2. Acute toxicity of patient's recent treatment or infection.
3. Dose, timing of hematopoietic growth factors(s) prior to harvest.
4. Sites of bone marrow harvest and quantity removed.
5. Duration and technique of PBSC collection.
6. Freezing, thawing methods and duration of storage.
7. Cancer cell contamination. If purging methods used, toxicity of purging agents.

Table 2. Clinical Course of 114 Patients with bcl-2 Positive Lymphoma, Purged with Antibodies, evaluated again. Modified from Gribben et al. NEJM 325:1525, 1991.

	bcl-2 by PCR	(Subsequent Relapse)	Total
BM involved	Negative	Positive	# Pts.
None	35 (1)	30 (10)	65
<5%	20 (2)	18 (10)	38
>5%	2 (1)	9 (6)	11
Total # of Pts.	57 (4)	57 (26)	114

"True negative" bcl-2 negative, normal morphology; correlation with no subsequent relapse (short follow-up): 52/55 = 95%
bcl-2 positive, BM involvement; with subsequent relapse: 6/9 = 67%

Table 3. Prognostic factors in predicting clinical benefit of mafosfamide purging in AML.

Group	Prognostic Variable AML first remission	% Disease Free Survival Purged	Unpurged	Benefit	Most Likely Explanation
1A	Remission to AUBMT <6 mo.	65	45	20	Leukemic burden is treatable (partial?)
1B	Remission to AUBMT >6 mo.	58	58	0	Inadequate purge (mafosfamide not fully effective, drug resistance)
	AML First Remission				
2A	Dx to remission <40 days	62	65	-3	Low leukemia burden after induction
2B	Dx to remission >40 days	63	41	22	Residual leukemia, but partially treatable

Data abstracted from figures published by Labopin and Gorin, Leukemia 6:(Suppl 4)95-99, 1992, with permission.

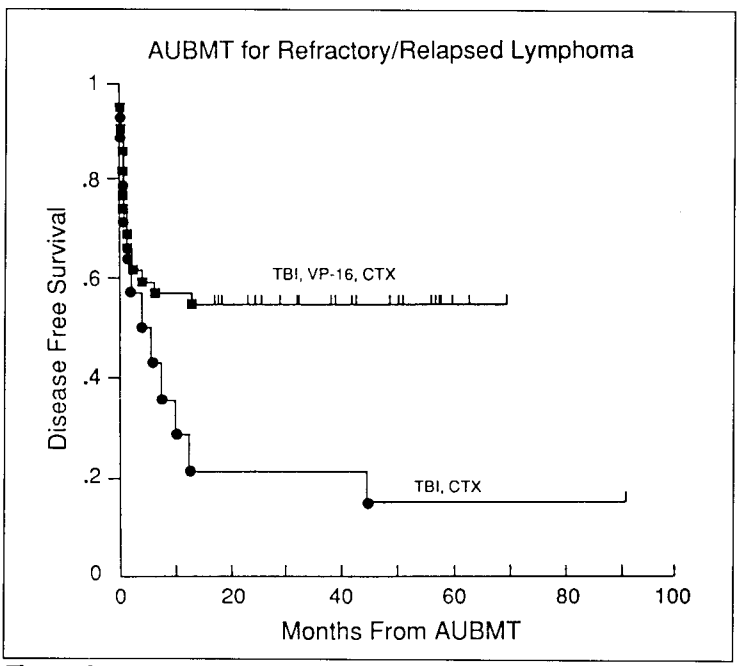

Figure 1

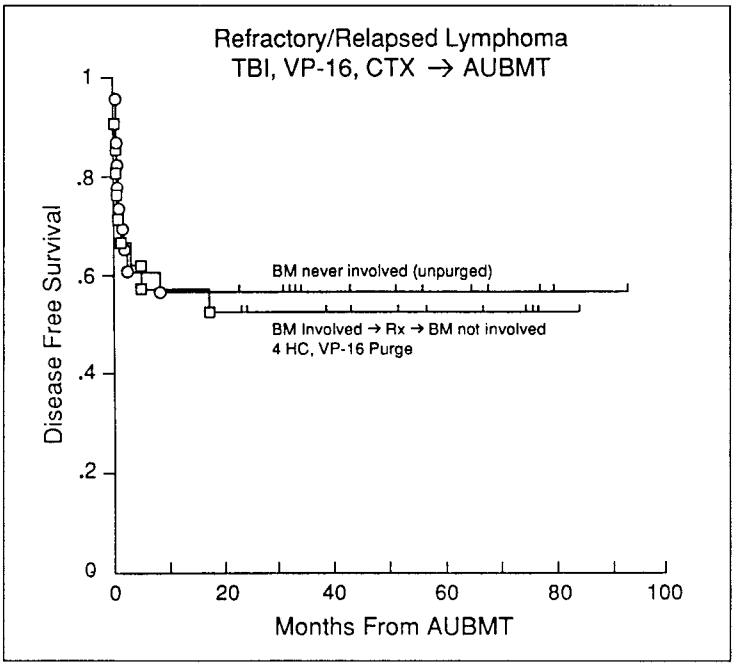
Refractory/Relapsed Lymphoma
TBI, VP-16, CTX → AUBMT
Disease Free Survival
1
.8
.6
.4
.2
0
BM never involved (unpurged)
BM Involved → Rx → BM not involved
4 HC, VP-16 Purge
0
20
40
60
80
100
Months From AUBMT

Figure 2

Session VII: Testicular

HIGH DOSE CHEMOTHERAPY IN GERM CELL CANCER

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Clinical trials in testis cancer have contributed to the development of highly effective therapy in this rare neoplasm. Twenty years ago patients with disseminated germ cell cancer would invariably die of their illness and currently 80% of such patients are cured with brief courses of combination chemotherapy. As such, testis cancer has been dubbed "a model of a curable neoplasm". Of interest, many of the straightforward randomized comparisons of chemotherapy dose intensity have been performed in patients with testicular cancer. Review of these trials provides an opportunity to explore the impact of dose intensity on treatment outcome in this rare but important tumor.

In the last decade, investigators have begun to explore the extreme limits chemotherapy dose in efforts to overcome resistance to conventional treatments. Such large doses usually require extraordinary support measures such as autologous bone marrow or peripheral blood stem cell infusions to hasten hematopoietic recovery. In testicular cancer, a number of phase II trials have been performed utilizing this approach. These trials are summarized in Table I. Taken as a whole, several conclusions can be drawn. First, high dose chemotherapy can cure 15-20% of patients experiencing multiple relapses of germ cell cancer. Patients who are overtly cisplatin-refractory or patients with recurrent mediastinal nonseminomatous germ cell cancer have a significantly diminished chance of permanent disease control (<5%). Second, therapy-related mortality in this heavily treated patient population is substantial (10-20% therapy-related death). Third, there is no "standard" high dose salvage regimen and similar results have been achieved with high dose carboplatin/etoposide with double autologous transplant, cisplatin (or carboplatin) etoposide and cyclophosphamide with single transplant or combinations including ifosfamide.

HIGH DOSE CHEMOTHERAPY AS INITIAL SALVAGE TREATMENT OF GERM CELL CANCER

Since the overall cure rate for recurrent testis cancer with ifosfamide-cisplatin based therapy is 20-25%, the proper next investigation seems to be incorporation of high dose chemotherapy as a component of initial salvage therapy. One of the first reports of such an approach is from Barnett, Coppin and colleagues at the Cancer Control Agency of British Columbia [1]. These investigators report the results of using high dose chemotherapy as part of initial salvage chemotherapy. In this trial, 18 patients with recurrent or persistent germ cell cancer after cisplatin-based primary therapy were given conventional induction chemotherapy with cisplatin, etoposide, vincristine, and bleomycin given on a weekly schedule or vinblastine, ifosfamide, cisplatin combinations. At the

completion of conventional salvage chemotherapy, consolidation with high dose chemotherapy was given with autologous bone marrow support. Patients received high dose carboplatin, etoposide and either high dose cyclophosphamide or ifosfamide. There were two toxic deaths, two patients were too early to evaluate and 8/14 remained free of progression from germ cell cancer.

Siegert and colleagues in Germany report the results of high dose carboplatin, etoposide and ifosfamide in the treatment of recurrent testicular cancer[2]. Patients had received a median of six cycles of cisplatin-based chemotherapy. Patients were given two induction courses of conventional dose cisplatin, etoposide and ifosfamide prior to receiving escalated therapy. Fifty-five patients received treatment with conventional therapy followed by carboplatin 1500-2000 mg/M^2, etoposide 1200-2400 mg/M2 and ifosfamide 0-10 gm/M^2. Two patients died related to treatment. Responses included 12 patients (21%) with complete remission and 16 patients (28%) with marker negative partial remission. Twenty-one of these patients (38%) have maintained their response from 3+ to 26+ months. While the precise degree of chemotherapy-resistance in this patient population is not given, it is encouraging that a high percentage of patients with recurrent disease after conventional therapy remain progression-free.

A recent pilot study at Indiana University enrolled 25 patients with first recurrence of germ cell cancer after BEP. None of these patients were gauged to be cisplatin-resistant. Patients were treated with conventional salvage therapy (usually vinblastine, ifosfamide and cisplatin (VeIP)) for two courses followed by a single course of high dose carboplatin and etoposide. Several preliminary results of this trial merit emphasis. First, only one patient had a therapy-related death and this patient died due to acute renal failure associated with sepsis after his first course of VeIP. Thus, there were no transplant-related deaths in this series. Only seven of the 25 patients enrolled did not enter the transplantation portion of the protocol: one VeIP death, two refusal, two progressive disease, two patients in whom insurance coverage was denied. Of the 18 patients completing the protocol, ten patients (56%) obtained a complete remission, one obtained disease-free status by resection of residual cancer, five patients (28%) obtained partial remission for an overall response rate of 88%. Two of the 18 patients going to transplantation actually required two transplantations for sluggish decline in serum biomarkers. Of the seven patients not going to transplantation, one patient obtained a complete remission and one patient a partial remission for an overall response rate of 28%. The response rate for all patients entering the protocol was 16/25 (72%). Twelve of the 25 patients remain without disease at a median follow-up of 19 months. It is unclear that these results are superior to conventional salvage approaches as these patients are highly selected, but the excellent tolerance of therapy and the high response rate are encouraging.

HIGH DOSE CHEMOTHERAPY AS PRIMARY TREATMENT OF GERM CELL CANCER

Memorial Sloan Kettering has begun to use high dose chemotherapy as a portion of initial treatment in selected patients[3]. Patients are given conventional chemotherapy (VAB-6) and those patients in whom there is a suboptimal decline in serum HCG or AFP after 2-3 cycles of treatment are given high dose

carboplatin and etoposide with autologous marrow support To date, the majority of patients entered on the protocol have required transplantation and there is early evidence of improved outcome relative to a comparable group of patients from earlier trials. Sixteen patients have been treated with high dose carboplatin and etoposide after suboptimal response to VAB-6. Nine patients (56%) have obtained a complete remission and eight remain free of disease ranging from 8+ to 27+ months. These reports compare favorably to a similar prognostic group with suboptimal serologic response treated with VAB-6 alone. Only 14% of these patients have had durable response to treatment. It is not clear that these results are superior to results of other modern series of conventional dose etoposide-containing regimens in poor risk patients.

Investigators at Institut Gustave-Roussy (IGR) have recently completed a phase III trial testing the addition of high dose chemotherapy to conventional dose induction therapy for patients with untreated poor risk germ cell cancer[4]. Patients with poor risk features as assigned by the IGR prognostic system were randomly allocated to receive PVeBV as described by Ozols and colleagues or a modified PVeBV X 2 cycles followed by high dose intensification with PEC[5]. Preliminary results suggest no benefit for patients receiving high dose intensification. Of 49 patients randomized to receive PVeBV X 4, there were two early deaths and one refusal. Complete response was obtained in 30/49 (61%) and 82% of patients were 2 year survivors. Of 53 patients randomized to two cycles of modified PVeBV plus consolidation, there were eight early deaths and two refusals. Complete response was obtained in 21/53 (41%) and 61% of patients were 2 year survivors. A statistically significant improvement in complete remissions (p=O.01) and a trend toward improved survival (p=0.1) were seen in the standard arm relative to the "dose intense" arm.

Whether high dose chemotherapy will become a component of standard initial therapy in poor risk patients with germ cell cancer remains to be seen. The difficulty in reliably identifying a subgroup of patients with very poor outcome and the relative rarity of these malignancies will complicate these clinical investigations. The growing concerns regarding the role of high dose etoposide in the development of unique therapy-related leukemias may further diminish the enthusiasm for moving high dose therapy into primary treatment for a group of patients who generally do well with conventional treatment[6,7]. However, the poor results of currently available salvage treatment certainly justifies the incorporation of newer higher risk approaches for this patient population.

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Table 1. Results of High Dose Chemotherapy in Patients with Refractory Testicular Cancer

Treatment	# of Pts	%CR	%PR	Toxic Deaths	Continouosly NED	Author
1. CBDCA/VP-16	33	25%	19%	21%	12%	Nichols
2. CBDCA/VP-16	39	24%	21%	13%	13%	Nichols
3. PEC	15	33%	Pico			
4. CBDCA/VP-16/lFX	21	38%	—	—	24%	Rosti
5. CBDCA/VP-16/CTX	41	29%	24%	7%	22%	Linkesch
6. CBDCA/VP-16	13	46%	—	8%	15%	Motzer

CBDCA = Carboplatin, VP-16 = Etoposide, PEC - Cisplatin, Etoposide, Cyclophosphamide, IFX = Ifosfamide, CTX = Cyclophosphamide

HIGH DOSE THERAPY IN GERM CELL TUMORS: THE ITALIAN EXPERIENCE

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INTRODUCTION

Combination chemotherapy of advanced germ cell tumors is one of the few true results in terms of cure in the last ecade in solid tumors. Seventy to Eighty percent of the patients with metastatic disease have a durable complete response to cisplatin-based chemotherapy [1]. Nevertheless patients refractory to platinum treatment (i.e. relapse within one month from the last dose of cisplatin) or relapsing after previous CR still have a dismal prognosis.[2]

In the last few years the use of high dose chemotherapy rescued with ABMT became an interesting approach to try to circumvent drug resistance and some studies had been carried out in the USA and Europe. Until now, no randomized phase III trial had been started in relapsing patients.

THE ITALIAN EXPERIENCE

ABMT in Europe is an increasing treatment option for leukemias, lymphomas and solid tumors. In 1991, 171 teams in 21 European countries performed a total of 4,961 bone marrow transplants (2,786 autologous). The number of registered cases of ABMT in germ cell tumors is 120, that accounts for one fifth of all solid tumors grafting procedures in 1991 [3]. In Italy, from July 1986, until November 1990, we activated a trial including 28 patients (26 male and 2 female with yolk sac tumors). Mean age was 31 years (range 18-50). All patients received previous cisplatin-based combinations (mean number of 2.3 schedules).

At the time of transplantation, 17 patients (61%) had disease progression after at least two courses of reinduction chemotherapy, 2 had stable disease, and 8 obtained an unresectable PR before graft. One case was transplanted in CR1 following PEB plus surgery and VIP at relapse (table 1). Three cases had extragonadal retroperitoneal germ cell tumors.

High dose chemotherapy schedule consisted of: Carboplatin 1,350 mg/sqm and VP 16 1,800 mg/sqm in three days; the last 11 patients received Ifosfamide at a dose of 12 gr/sqm, reducing the dose of Etoposide to 1,200 mg/sqm. In the triple drug combination, Ifosfamide was delivered in the first three days, while Carboplatin and Etoposide were administered thereafter to avoid possible interactions on renal function.

A total of 40 courses were administered. Bone marrow was reinfused at least 72 hours from the last Etoposide dose. Carboplatin was always given at a fixed dose and not according to the Calvert formula.[4]

TOXICITY

Mean time to PMN count >500 u1 was 16.2 days after ABMT, and 15.4 days to reach a platelet count of 50,000 u1 (range 7-22 and 6-33 respectively). In 33/40 courses granulocytopenic fever occurred, in 12 cases with positive blood cultures (11 Gram positive and 1 Gram negative). All patients received Ketoconazole as fungal prophylaxis. Two patients died within 30 days from ABMT, one with VOD and 1 with rapidly growing tumor.

Interestingly, no case of renal failure occurred and in the triple drug combination mean creatinine level was 1.3 mg/dl (range 0.9-1.6). Eleven patients underwent a neurophysiologic evaluation to assess peripheral toxicity; neurological symptoms score, neurological disability score, electroneurography and computed stabilography were performed before and after transplantation. A shift from mild to moderate grades of peripheral neurological toxicity was detected in 3 cases, but at a subsequent control all scores returned to baseline.

RESULTS

Twenty-four patients were evaluated for response on markers (one case was in CR at ABMT and 1 in PR with normal values) and 24 on lesions (one CR and 1 seric only disease). Sixteen patients (66%) had normalization on markers for at least one month, while 38% had PR or CR on masses.

Among the 17 patients in PD at graft, 6 CR on markers were achieved lasting from 1 to 8 months. The results on the 9 patients treated in a sensitive phase of the disease were: 7 CR and 1 SD, while the CR1 case relapsed after 10 months. Five patients are alive with no disease at 23+, 24+, 27+, 28+ and 61+ months, all being in sensitive relapse at transplantation (figure 1).

ONGOING TRIAL

In 1991 a new trial was activated in sensitive relapses with the same schedule but lowering Ifosfamide dose to 10 gr/sqm. GM-CSF (Schering-Plough) was used at the dose of 5 ug/Kg. This trial is ongoing and up to now 10 patients have been treated; reinduction chemotherapy was classic VIP combination; eight patients received 1 single course of high dose chemotherapy while 2 received double graft. Six patients are alive cancer free with a short follow up (2+ to 9+ months). One patient died at day 11 due to multiple organ failure and three others progressed a few months after ABMT; in the same time 5 patients were treated with the same combination in progression, and again no patient experienced long lasting CR.

Due to the lack of GM-CSF toxicity, we increased its dose to 10 ug/Kg. Mean time to PMN >500 ul was 14.9 days and to Plts >50,000 18.6 days (range 9-23 and 5-53 respectively) (mixed data on the whole GM-CSF population).

DISCUSSION AND THE EUROPEAN PROPOSAL

There is a large amount of data on ABMT in germ cell tumors to set up some considerations: there is a marginal role for high dose programs in refractory patients. The proportion of long term CR is between 0 and 20% [5,6]. Our trial does not confirm the possibility of achieving cure in this subset, but of course our number may be too small to demonstrate it.

Confusing data come out from early intensification ABMT programs in poor risk patients [7,8] and certainly in the future an adequate randomized trial should be planned. The exact role of high dose chemotherapy in refractory patients sensitive to salvage chemotherapy may be established only in a phase III trial.

In Europe a randomized trial is planned from spring 1993. After two courses of reinduction chemotherapy (VeIP or VIP according to single centre's choice) only patients with sensitive tumors will be randomized to two more courses of the same regimen versus two courses plus one single CarboPEC shot (Carboplatin 1,600 mg/sqm, VP16 1,750 mg/sqm, Cyclophosphamide 6.4 gr/sqm). A total of 200 patients are expected to be registered, and assuming a non response rate of 20%, 80 patients per armwill be randomized.

ACKNOWLEDGEMENTS.

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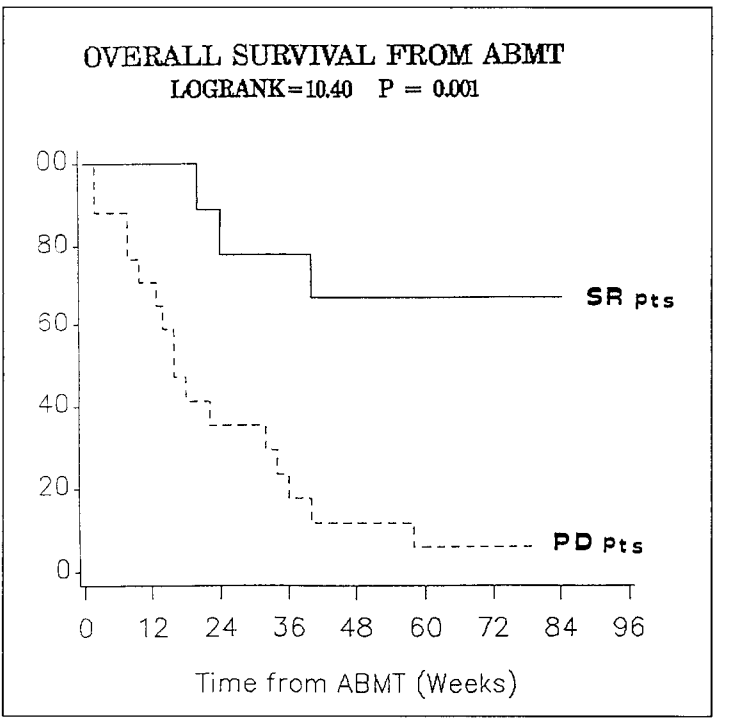
TABLE 1: PATIENTS' CHARACTERISTICS

	Refractory pts 17	CR-PR-SD pts 11
Male/female	15/2	11/0
No. of chemotherapy lines (mean)	2.5	2.2
No. of total courses (mean)	8.7	5.2
Total Cisplatin dose (mg/sqm)	753	540
Cisplatin refractory	17	0
Primary site (gonadal/extra)	14/3	11/0



Session VIII: Lung Cancer

INTENSIVE COMBINED MODALITY THERAPY FOR RESPONDING SMALL CELL LUNG CANCER

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ABSTRACT

Standard dose chemoradiotherapy for SCLC has achieved high response rates but rare long term survivors. Dose intensification using autologous bone marrow support (ABMT) has increased the complete responses without clearly documenting an overall survival benefit. These trials generally used single alkylating agents with inconsistent application of chest irradiation leading to high local relapse rates.

Unique to this trial is the use of high dose combined alkylating agents, cyclophosphamide, carmustine and cisplatin (CBP), followed by CTI 50-56 Gy, prophylactic cranial & involved field radiotherapy in patients responding to conventional dose chemotherapy. Patients with histologically documented limited or extensive stage in response to first line chemotherapy were eligible.

As of April 1992, 30 patients have been treated: 20 with stage III limited disease (7 complete (CR), 13 partial (PR) responders prior to ABMT), and 10 with extensive stage disease (2CR, 8PR prior to ABMT). Two patients died from Candida. Of 16 patients evaluable for response, 11 (69%) converted from partial to complete response.

For limited disease, post ABMT, unmaintained median event-free survival and survival were 16 and 17 months. For extensive disease, post ABMT, unmaintained median event-free survival and survival were 10 and 13 months. Actuarial 2-year survival was 47% & 14% for limited and extensive disease, respectively, with 3 patients, two with limited and one with extensive disease followed for over 5 years.

Of the 14 limited stage patients in or near CR, 9 (64%) remain disease free at a median 24 (1276+) months followup. Relapse was primarily in sites of prior involvement and within the first year, but one patient died of adenosquamous lung cancer. Of the 7 ED pts in or near CR, 2 (29%) remain disease free at a median 10 months followup and one patient is alive with disease at 65 months.

The unmaintain remission and survival post ABMT appears favorable, particularly in those patients with an excellent preABMT response, compared with that expected from conventional chemoradiotherapy. Additional patients and further followup are needed to evaluate its utility in extensive disease in excellent response. For limited stage patients in or near complete response, a randomized comparison between this approach and conventional dose therapy is being developed.

INTRODUCTION

SCLC is a tantalizingly resistant solid tumor to chemoradiotherapy. Chemoradiotherapy at conventional doses has resulted in high response rates: 80-100% (50-70% complete) for disease limited to the chest, and 60-80% (20-40% complete) for metastatic disease 1. However, cure is elusive. Median survival of 14-18 months for limited disease and 8-12 months for extensive disease are achieved. About 20% of limited stage and 5% of extensive stage patients will survive two years. Survival beyond 5 years occurs in 3-8% of patients with SCLC 1.

The inability to destroy the residual cells despite "chemosensitivity" suggests the existence of a tumor stem cell resistant to cytotoxic therapy. Maximizing dose and dose intensity to the limits of acceptable toxicity might overcome resistance 2, 3. Hematopoietic stem cell support, using marrow or peripheral blood progenitor cells, provides the opportunity to evaluate dose-response to the limits of organ tolerance.

This phase II trial was designed to determine the disease-free & overall survival of patients with SCLC responding to conventional dose chemotherapy following treatment with high dose combined alkylators (cyclophosphamide, cisplatin and carmustine) and radiotherapy, to ascertain the subset of patients most likely to benefit and to evaluate the toxicity associated with this therapy. The first 19 patients with limited stage disease have been previously described 4.

METHODS

Adult patients aged 18 to 59 with histologically documented small cell lung cancer, responding to first line conventional dose induction chemotherapy, were eligible for this study. At the time of high dose chemotherapy, patients were required to have a performance status of 0-1, with leukocytes >3000/ul, platelets $\geq$100,000/ul, creatinine clearance $\geq$ 60 cc/min and SGOT and bilirubin $\leq$ 1.5 x normal and no active tumor involvement of the brain or marrow. Pulmonary function had to be > 60% of predicted (FVC, DLCO corrected for hemoglobin) were required. Written informed consent was obtained.

The treatment schema is outlined in Figure 1. As patients were usually referred after initiation of induction therapy, no specific induction chemotherapy was required. After maximal response from induction chemotherapy (generally ~4 cycles), eligibility was determined with restaging studies including head,

chest and upper abdominal computerized tomography (CT) scans, bone scan, bilateral marrow aspirates and biopsies.

High dose "intensification" chemotherapy contained cyclophosphamide (1875 mg/m2 as a two hour infusion daily for 3 days), cisplatin (55 mg/m2 per day by continuous infusion over 72 hours) and carmustine (BCNU) (60 mg/m2/ dose given as a one hour infusion twice daily for 4 days). The total doses were 5625, 165 and 480 mg/m2, respectively. Initially, bladder irrigation was employed, but for the past two years, mesna at 375 mg/m2 q 3h for 3 days was given for uroprotection. Hematopoietic support, reinfused 3 days after completion of chemotherapy (day 0), consisted of marrow alone in 17, or marrow augmented by GM-CSF-mobilized peripheral blood progenitor cells (PBPC) in 13 patients.

Consolidative chest (50-60 Gy/5-6 weeks) and prophylactic cranial radiotherapy (30 Gy in 15 fractions) were administered after complete recovery from the acute morbidity of intensification. Involved field radiotherapy to tissue tolerance was given to isolated bulk areas of metastatic disease in the setting of oligometastatic disease. Patients may have had chest radiotherapy prior to intensification concurrent with induction therapy.

Statistical Methods: Standard response criteria were used for complete and partial response, stable disease and disease progression. Near complete response was defined as ~ 90% reduction with persistent radiographic abnormalities such as residual parenchymal scarring without nodular characteristic, residual pleural or pericardial thickening, residual mediastinal adenopathy less than 1.5 cm in greatest diameter for at least four weeks. Event-free survival was calculated from day 0 of intensification (unmaintained by further systemic therapy) to the documentation of progression or death from any cause. Survival was calculated from day 0 of intensification to the documentation of death from any cause. Event-free and overall survival were estimated by the Kaplan-Meier method 5. The logrank test 7 and proportional hazards regression 8 were used for univariate and multivariate analysis. However, due to small sample sizes, a lack of significance should not be interpreted as no association. A generalized Wilcoxon statistic 6 was used to compare the time to granulocytes > 5OO/ul, to platelets > 20,000/ul, and to RBC transfusion independence between patients treated with and without PBPC.

RESULTS

From December 1985 to April 1992, 30 patients were enrolled and have completed high dose intensification (Table 1). All patients who were entered on the protocol were treated and considered evaluable for toxicity and survival. Median age was 49 (range 25-61) years; 60% had performance status 0 at initiation of high dose therapy; and 67% were male. Except for chronic bronchitis or emphysema, most patients had no active comorbid disease. One patient had sequelae from childhood polio and three had previous vascular angioplasty. All limited stage patients had stage III disease. Patients with extensive disease had a median of 2 (1-7) metastatic sites, including three with marrow involvement.

A median of four cycles of various induction chemotherapy regimens was given (range 2-6). Fourteen limited patients and seven of the ten extensive patients achieved complete or near complete response. The remainder had partial

responses with persistent nodular disease.

Sixteen of 20 (80%) limited stage patients received chest radiotherapy; three before and 13 after high dose therapy. Toxicity precluded chest radiotherapy in four patients: congestive heart failure, interstitial pneumonitis, persistent thrombocytopenia and early infectious death in one patient each. The median doses to the extended (primary lesion, hilum, mediastinum and bilateral supraclavicular areas) and involved fields were 40.0 (16-45) Gy and 50 (16-59.4) Gy, respectively. Four extensive stage patients received chest with or without additional involved field sites. The other patients had disease too widespread to administer radiotherapy. Prophylactic cranial irradiation (PCI) (median dose 30 Gy in 2 Gy fractions) was given to 16 limited and four extensive stage patients. One patient had therapeutic cranial radiation prior to high dose therapy.

Toxicity to the high dose therapy is summarized in Table 2. Two patients died (7%) of Candida sepsis. Aside from the expected fever during neutropenia, interstitial pneumonitis was the principal morbidity. The median decline in the % predicted FEV1 and FVC was 4-6%, but DLCO decreased by 22%. Seven patients (23%) developed dyspnea, non-productive cough, intermittent fever, and a chest x-ray pattern compatible with interstitial pneumonitis within three months of high dose chemotherapy. All responded promptly to two weeks of 1 mg/kg prednisone followed by a gradual taper over three to four weeks. Three had recurrent symptoms (one following an abbreviated course of chest radiotherapy) and required prolonged steroid treatment with slow taper over 2-3 months. One patient each developed pneumocystis carinii and cerebral toxoplasmosis following steroid therapy.

Four patients developed severe but reversible congestive heart failure. Reversible elevations in creatinine were observed in sixteen patients, appearing immediately after completion of chemotherapy administration in four. The remainder occurred after prolonged aminoglycoside and amphotericin B exposure. One patient developed reversible hemolytic uremic syndrome seven months after high dose therapy. The single patient who had cranial radiotherapy six weeks prior to high dose therapy developed an acute, prolonged (2 months), but eventually reversible encephalopathy, a toxicity that had not been observed in any other patients.

Response to High Dose Chemotherapy: Fourteen patients were not evaluable for response to high dose chemotherapy (nine were in complete response, two had persistent but unchanged radiologic abnormalities and were found to be free of disease at subsequent surgical staging, one had bone scan findings with sclerosis on plain radiograph, and two died of toxicity). Of the 16 patients evaluable for response, 11 achieved complete response after high dose chemotherapy for an overall partial to complete response conversion rate of 69%.

Event-free survival and relapse (Table 3; Figure 2): The median event-free survival from high dose therapy for all patients, limited stage, and extensive stage patients was 11, 16, and 10 months, respectively. The 2-year event-free survival for the subset of limited stage patients in or near complete response prior to high-dose chemotherapy was 60%. Of the seventeen patients who relapsed with SCLC, 15 occurred within the first year after high dose therapy with a median time from relapse to death of 5 (2-27) months. Relapse was most commonly in sites of prior involvement (summarized in Table 3). One patient, originally diag-

nosed by fine needle aspirate, presented with adenosquamous carcinoma at nine months. One patient who presented with widespread lytic bone metastases, relapsed at 25 months with an isolated nerve root nodule, was treated and is currently alive with indolent disease at 66 months.

Survival (Figure 2): The median survival for all patients, those with limited and those with extensive disease was 15, 17, and 13 months, respectively. In addition to the stage of disease (limited vs extensive), the degree of response to induction chemotherapy (CR/near CR vs PR) was the most important prognostic factor ($p = .008$) for survival after high dose chemotherapy. Nine of 14 limited stage patients in or near CR remain alive and event-free with a median followup of 24 (12-76+) months after high dose therapy. While limited by patient numbers, no association between age, sex, performance status, weight loss, or induction chemotherapy (alkylators vs no alkylators) and relapse or survival was found by univariate analysis.

DISCUSSION

Historically, high dose therapy with marrow support in solid tumors was developed, in part, for patients with responding SCLC, based on its sensitivity to multiple chemotherapeutic agents at conventional doses 1. The high dose regimens consisted of either single chemotherapeutic agents (5 series; 3 with chest radiotherapy) 9-15, a single alkylating agent with etoposide (5 series; 2 with chest radiotherapy) 16-20, or combination alkylating agents (4 series; one with chest radiotherapy) 21-24. These trials have, in general, demonstrated enhanced complete response rates without an overall survival benefit. Within the most favorable subset, patients with limited disease in complete response prior to high dose therapy, 24 of 71 (34%) patients remained disease-free with a median follow-up >3 years 9-24. Since physiologically robust patients were selected, but high treatmentrelated morbidity and mortality was encountered, investigators concluded that potential benefits did not justify the risks of the procedure. The one randomized trial compared conventional therapy to high dose intensification with marrow support in patients with responding SCLC, but without chest radiotherapy 23. Despite high local-regional relapse rates, this study demonstrated greater responses and disease-free survival with a trend toward improved survival for the high dose arm. The investigators, however, concluded that the results were not sufficiently promising to recommend high dose therapy as the standard of care because of its greater toxicity.

Chest radiotherapy, in combination with systemic chemotherapy, is now routinely administered for the management of limited stage disease. Not only is the benefit small 25, primarily due to systemic relapses, but local control remains a challenge despite conventional dose chemoradiotherapy 26. In one study reported by Perry et al, the addition of radiotherapy to chemotherapy reduced the chest relapse rate from 90% in the chemotherapy alone group to 60% in the chemoradiotherapy arms, a finding corroborated by other randomized trials 28, 29. Multiple factors contribute to a high rate of including the presence of bulk disease (109-10ll tumor cells), possible presence of chemotherapy-resistant non-small or small cell elements, poor drug delivery and tumor hypoxia contributing to a variety of resistance mechanisms. Moreover, as systemic control is en-

hanced, detection of initial failure in local-regional sites may increase.

Enhanced survival by intensifying chest radiotherapy without significantly increasing the intensity of systemic therapy has recently been reported 30-33. Turrisi et al reported an 80% local control and a 56% actuarial survival at 2 years in 32 limited stage patients with cisplatin, etoposide and concurrent twice daily chest radiotherapy to 45 Gy 31. Preliminary analysis of an Eastern Cooperative Oncology Group trial appears to corroborate these findings and is the basis for an ongoing randomized trial comparing conventional daily vs the hyperfractionated chest radiotherapy 32.

This study was designed to administer a dose-intensive multimodality approach utilizing maximum systemic therapy (combinations of alkylating agents) 33, 34 with the addition of intensive local-regional radiotherapy to attempt to achieve local control. The therapy is associated with low morbidity and an acceptable mortality of 7%. Most patients were able to return to full-time work as few late complications were observed. Morbidity and mortality from high-dose chemotherapy is likely to decline further with the application of hematopoietic cytokines 35 and peripheral blood progenitor cells 36.

For patients with responding extensive stage disease, a high rate of conversion to complete response was achieved. Our ability to consolidate sites of disease involvement with an additional modality (radiotherapy) is limited and its role remains to be defined. Despite this, two patients remain disease-free and the overall survival is not inferior to that expected from conventional therapy. More patients and longer followup is required to determine whether this approach provides meaningful long-term disease-free survival and whether certain subsets (eg, those with fewer sites of metastatic involvement or those with disease amenable to consolidative radiotherapy) have more favorable prognosis.

For patients with responding limited stage disease, 45% (9 of 20), all in or near complete response prior to intensification, are enjoying prolonged and unmaintained disease-free survival. The results of this trial are sufficiently promising to spur the development of a randomized comparison between high dose and conventional dose therapy for limited stage patients in or near complete response to induction therapy.

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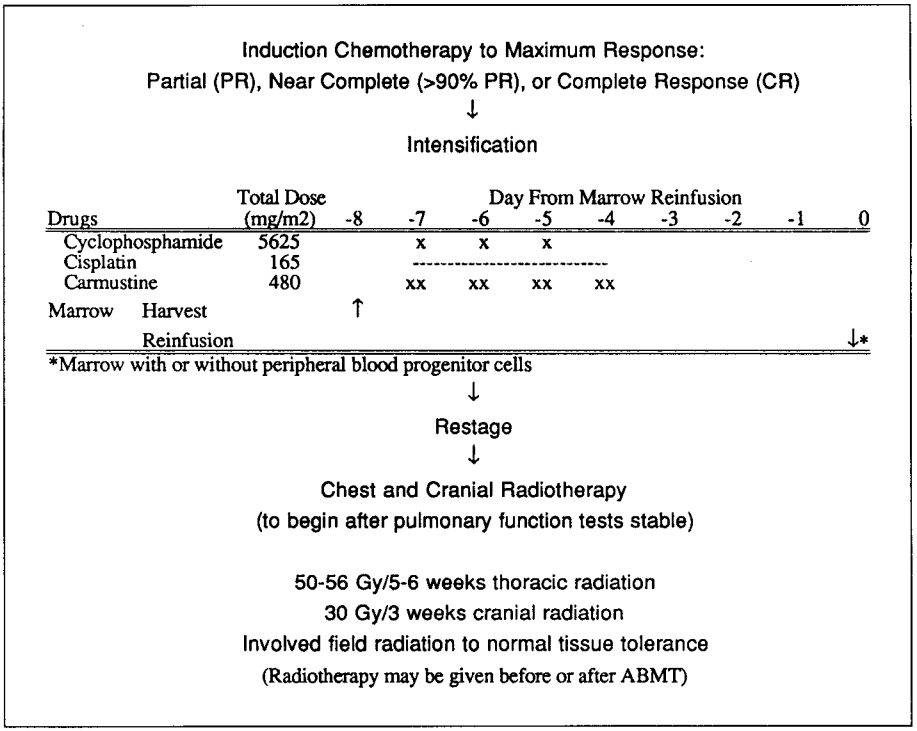
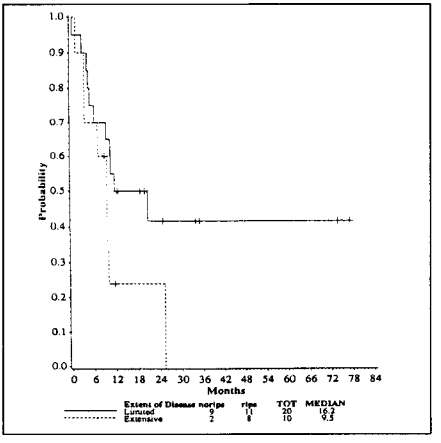


Figure 1:
Schema of High dose therapy for patients with limited or extensive SCLC



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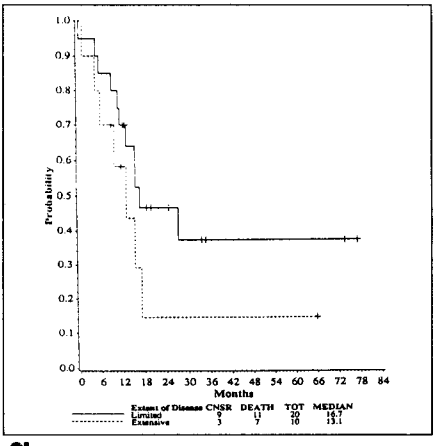

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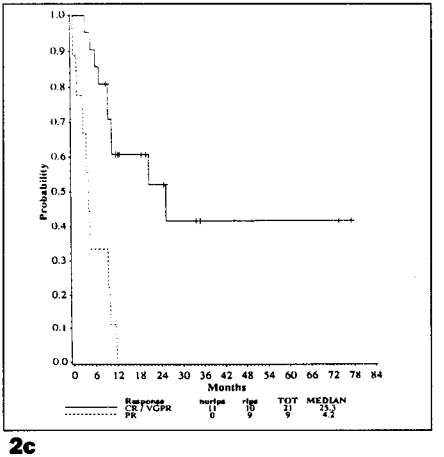

2c

Figure 2: Kaplan-Meier plots for event-free and overall survival:
a) Event-free survival: Time from initiation of high dose chemotherapy to first failure (progression of disease, or death from other causes) for those with limited (n=20) or extensive (n=10) disease. / = followup time for patients.
b) Survival: Time from initiation of high dose chemotherapy to death from any cause for those with limited (n=20) or extensive (n=10) disease. One patient died of adenosquamous carcinoma at 11 months and two died of Candida sepsis at 3 weeks. / = followup time for patients.
c) Event-free survival: Time from initiation of high dose chemotherapy to first failure (progression of disease, or death from other causes) for those in or near complete response prior to intensification (n=21), or those in partial response prior to intensification (n=9). / = followup time for patients.

Table 1: Characteristics of 30 Patients with Responding SCLC

Characteristic	N	(range)%
Patients	30	
Median Age (range)	49	(25-61)
Male	20	67
Median pack years smoking	55	(20-100)
Limited Disease (LD)	20	67
Stage IIIA (T1-3N2)	9	45
Stage IIIB (T4 and/or N3)	11	55
Extensive Disease (ED)	10	33
Median # metastatic sites	2	(1-7)
Median #induction cycles	4	(2-6)
Months from diagnosis to high dose therapy	4	(2-8)
Best response to induction:		
Complete response (6 LD,2 ED)	8	27
>90% response response (8 LD,5 ED)	13	43
Partial response (6 LD,3 ED)	9	30

Table 2:
Toxicity of CBP Regimen for 30 Patients with Responding Small Cell Lung Cancer

	# Patients (n = 30)	%
Death within 90 days (Candida Sepsis)	2	7
Infectious Complications:		
Aspergillus fungal ball	1	3
Bacterial Sepsis (all central venous line related)	7	23
Clostridia difficile induced diarrhea	4	13
Herpes zoster (within 4- 12 months after high dose therapy)	6	20
Pneumocystis carinii (after steroids)	1	3
Cerebral toxoplasmosis (after steroids)	1	3
Non-hematologic toxicity:		
Grade 3 GI bleed during thrombocytopenia	2	7
Interstitial pneumonitis requiring steroids	7	23
Reversible Hemolytic uremic syndrome	1	3
Reversible congestive heart failure	4	13
Reversible Encephalopathy*	1	3
Reversible creatinine 1.5-3.0 mg/dl	8	27
Creatinine > 3.0 mg/dl**	3	10
Ototoxicitv grade 2-3	6	20
Hematologic support:		
Marrow given:		
Alone	14	47
With Peripheral Blood Progenitor Cells	13	43
With Erythropoietin	3	10
Hematologic toxicity:		
Median days duration from marrow reinfusion to:	Median days	Range
Granulocytes ≥ 500/μl	21	10-46
Platelets ≥2 20,000/μl	27	9-49
Platelets ≥ 50,000/μl	35	10-119
Red cell transfusion independence	18	3-43

*received preABMT therapeutic cranial radiotherapy
**reversible in two; the other died of candida sepsis without recovery

Table 3:
SITES OF RELAPSE

		N	Relapse	Chest	Both	Distant	ED
LD	CTI postABMT	13	6	1	*3	^2	
	CTI preABMT	3	1		1		
	no CTI	4	3	2	1		1
ED	CTI	4	4	—	2	**2	
	no CTI	6	3		***3		1

2 died without relapse (toxic deaths); *one relapsed with adenosquamous carcinoma outside XRT port;
**one with isolated nerve root nodule at 25 months; one isolated to brain and chest (no radiotherapy given); ^ one brain only (no prophylactic cranial irradiation).

TREATMENT OF NONSMALL CELL LUNG CANCER (NSCLC) WITH AGGRESSIVE COMBINATION CHEMOTHERAPY

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Estimates for new cases of lung cancer in the Untied States in 1992, suggest that there will be 157,000 new cases, 102,000 occurring in men and 55,000 in women. In 1990, lung cancer was the most frequent new cancer in men and among the most frequent in women. It was also the most frequent cause of cancer deaths in men (33%) and in women (21%) even more frequent than breast cancer (18%).1 Of the lung cancers, non-small cell carcinoma is more frequent, 80%, than small cell carcinoma, 20%. Of the NSCLC, squamous cell is regarded as the most common, followed by adeno carcinoma and large cell undifferentiated carcinoma.2

The overall cure rate for NSCLC is only 10%. Cures are obtained mainly in patients with very early disease by surgery. The majority of patients presents with later stage disease, too extensive for surgical resection and therefore with bad prognosis. Recent treatment efforts are directed toward increasing surgical resectability by introducing neoadjuvant chemotherapy and radiotherapy as well as toward increasing survival of patients with unresectable tumors.

Until recently the role of chemotherapy for NSCLC has been questionable. Recent studies demonstrate that current chemotherapy regimens can improve survival in patients with NSCLC. The Canadian multi-center randomized trial showed a statistically significant advantage with combination chemotherapy for both response and survival.3

Current evidence suggest that cisplatinum or Platinol is the most effective single agent. In an effort to obtain greater therapeutic effect, investigations have focused on dose and schedule of Platinol administration. It appeared that a pulse dose schedule was more effective than bolus administration 4 and that higher doses of Platinol were more effective than lower dose [5]. This letter observation suggests a dose-response relationship.

Platinol is useful in various combination regimens. Especially in combination with etoposide or VP-16 higher response rates were obtained than the original CAMP regimen.6 This is the reason that we have embarked on a VP-16/Platinol regimen which has been schematically outlined in Table 1. Since dose relationship may exist, we plan six courses of VP-16/Platinol with a short interval between each course. In order to achieve this, G-CSF was administered after each course for 10 days to ameliorate myelosuppression. If the observations of dose response relationship by Gralla et al are representative for NSCLC, intensification after VP-16/Platinol with higher dose chemotherapy would be of benefit. This is the reason why after six courses of VP-16/Platinol two courses of cytoxan/Platinol (CP) and one course of cytoxan/VP-16/Platinol (CVP) are administered. The program has been outlined in Table 2. It can be noted that bone

marrow support with or without peripheral stem cells has been included in the CVP protocol. Eleven patients have been entered in the study. Patient characteristics are outlined in Table 3. It can be noted that the median age is 64, all patients are smokers and 3 patients suffer from severe COPD. All patients were diagnosed with stage III or IV disease. In Table 4, the overall results of the VP-16/Platinol program have been listed. The response rate is 72% and the median survival time is 7+ months. The median progression-free survival is 7+ months. Three patients achieved complete remission on the basis of radiographic and CT scan analysis, 6 patients achieved objective response, in 3 patients disease was stable, and in 1 patient no tumor response was noted. At the moment, no difference in survival between the various groups is evident except the patient with the nonresponding tumor who died four months after onset of treatment.

Hematologic toxicity was acceptable as has been outlined in Tables 4 and 5. The WBC nadir was not lower than 1500, whereas the median platelet nadir was not less than 40,000/mm3. It is remarkable that the WBC nadir after the second, third and fourth course was higher than after the first course. In one patient, a catheter-related bacterial infection was noted, responding to antibiotic treatment. Several patients needed red blood cell transfusions and the need for platelet transfusions was minimal, see Table 5. Of the patients who had objective response to the first three courses of VP-16/Platinol, marrow and peripheral stem cells were harvested. Since most patients had compromised pulmonary function, and also for economic reasons, marrow was harvested under local anesthesia on an outpatient basis. Eight hundred cc of marrow was harvested in two different sessions. The results of marrow harvest have been documented in Table 6. It appears that the yield in terms of nucleated cells, GM-CFC and CD34-positive cells is not significantly different from the marrow cell suspension harvested under general anesthesia. In the outpatient setting, 30-60 cc per puncture site was collected when no more than 30 cc in the inpatient setting was collected. The procedure is very well tolerated with minimal discomfort for the patient. The total cost per outpatient marrow collection is $800 (one dollar per cc marrow), whereas the costs for the inpatient procedure is over $5000. Several patients have been enrolled in the intensification part of the program, the results are too early to evaluate. So far the program is very well tolerated.

In conclusion, the intensive VP-16/Platinol program has promising results and will form the basis of treatment of NSCLC. The role of intensification with three additional courses of Platinol-based chemotherapy of which the last course is in conjunction with hematopoietic stem cell support is under evaluation. The next step is to evaluate the feasibility of radiotherapy added to VP-16/Platinol in a fractionated fashion as has been outlined by Dr. Woo in this session.

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Table 1. Combination Chemotherapy Regimen VP-16/Platinol in NSCLC

Day	1	2	3	4	5	6-16
VP-16 200 mg/m^2	+	+	+			
Platinol 30 mg/m^2	+	+	+			
Neupogen						+-+
Cycle q 3 weeks						

Table 2. Intensification Program in NSCLC

		Support
Step 1:	Cytoxan 2 gm/m^2	
	Platinol 90 mg/m^2	G-CSF
Step 2:	Cytoxan 2 gm/m^2	G-CSF
	VP-16 600 mg/m^2	
Step 3:	Cytoxan 4-5 gm/m^2	BMT
	VP-16 750 mg/m^2	G-CSF
	Platinol 90 mg/m^2	GM-CSF

Table 3. NSCLC Patient Characteristics

Age	:	64 (49-72)
Male/Female	:	5/6 pts.
COPD	:	3/11 pts.
Smokers	:	11/11 pts.
Adeno Ca	:	5 pts.
Large cell Ca	:	4 pts.
Squamous cell Ca	:	2pts.
Stage III	:	3 pts.
Stage IV	:	8 pts.

Table 4. Results of VP-16/Platinol Program in NSCLC

Number of patients:	11
Duration response:	*7+ (5+-13+)
Duration survival:	*7+ (4-13+)
Alive:	8
Dead:	3
Response Rate:	72% (8 out of 11)

*Median

Table 5. Hematological Data of the VP-16/Platinol Regimen in NSCLC (1)
White Blood Cells

Course	Onset $X 10^3$	Nadir Count	Nadir Day $X 10^3$	Peak Count	Peak Day
1	6(5-10)	1.5(1.5-2.9)	12	12	15
2	9(5-27)	3.1(2.6-6.8)	11	53	15
3	10(5-25)	4.6(2.2-6.9)	11	48	15
4	14(4-20)	2.7(0.9-3.4)	13	50	16
5	5(4-13)	2.0(1-3.1)	10	51	16
6	12(5-52)	1.6(1.0-5.2)	12	54	16

Table 6. Hematological Data of the VP-16/Platinol Regimen in NSCLC (2) Platelets

		Nadir		Transfusion	
Course	Onset	Count	Day	RBC	Plat.
1	283(240-389)	93(62-111)	13	1	0
2	201(182-365)	82(37-97)	12	2	0
3	363(119-617)	47(36-134)	13	1	0
4	244(119-283)	33(15-131)	13	1	0
5	228(182-561)	43(31-202)	2	2	0
6	149(100-263)	45(24-139)	2	2	1

*1 transfusion = 2 U of RBC or 10 U of Platelets.

Table 7. Comparison of Marrow Harvest Methods: Outpatient/Inpatient

Method	Number of Harvests	WBC/ml $X10^6$	GM-CFC/ml $X10^3$	CD34 %
Outpatient	22	13.9 (6.6-40.5)	4.7 (0.5-10)	.72 (0.19-2.4)
Inpatient	16	12.8 (6.1-24.8)	3.1 (0.82-6.5)	.92 (0.80-0.98)

HIGH DOSE RADIOTHERAPY FOR NON-SMALL-CELL CANCER OF THE LUNG

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It is established that for early stage resectable non-small-cell lung cancer, surgery is more effective than radiotherapy; however, radiotherapy alone has cured a small number of patients with potentially resectable but medically inoperable cancer.[1] Postoperatively, radiotherapy has been used to treat the surgical bed in cases of close or positive margins, and to treat the regional lymph nodes in cases of positive mediastinal node metastasis. At least one randomized study showed an improved disease-free survival for radiotherapy in the latter situation.[2] The most common application of radiotherapy in the therapy of non-small-cell lung cancer is to palliate symptomatic metastases or to treat the primary unresectable disease when there is no obvious distant metastasis. The long-term outcome of most patients with Stage III non-small-cell lung cancer is poor despite the comprehensive radiotherapy. Several radiotherapeutic regimens have been studied as attempts to improve the prognosis.

In the 70's the Radiation Therapy Oncology Group (RTOG) performed a four-arm trial to establish a dose response relationship in the treatment of non-small-cell lung cancer.[3] The patients were randomized to 4000 cGy split course (2000 cGy in 5 fractions, two weeks rest and then repeat 2000 cGy in 5 fractions), 4000 cGy continuous, 5000 cGy continuous, or 6000 cGy continuous treatment at 200 cGy per fraction. The two-year survival rate for 6000 cGy was better than that for 4000 cGy (19% versus 10%). Local control was 61% for 6000 cGy and 48% for 4000 cGy. This trial established the standard total radiation dose for conventional radiotherapy of non-small-cell lung cancer.

Since clinical failure patterns[4] and autopsy studies[5] suggested that local tumor progression was an important element leading to death in these patients, it appeared logical to explore higher total dose of radiation to improve loco-regional control. Based on radiobiological principles, if one wants to increase the total radiation dose but not to increase late toxicities, one needs to reduce the radiation dose-per-fraction. If the radiation dose-per-fraction is reduced, more than one treatment per day can be delivered so that the overall treatment time is not unduly delayed. Such a radiotherapy schedule is called hyperfractionated radiotherapy. Hyperfractionated regimens using 120 cGy twice a day for the treatment of Stage III non-small-cell lung cancer were initiated in 1983 by the RTOG. Patients were randomized to receive minimal total doses of 6000, 6480, 6960, 7440, and 7920 cGy.[6] For patients with favorable stage III disease (Karnofsky performance status 70-100, less than 6% weight loss) a dose response was found for survival: survival with 6960 cGy (median 13 months 2 year 29%) was significantly (P=0.02) better than the lower total doses. There was no differ-

ence in survival among the three highest total dose arms. Comparison with results in similar patients treated with 6000 cGy in 30 fractions of 200 cGy, 5 days per week for six weeks suggests a benefit from hyperfractionated radiation therapy with 6960 cGy. There was no significant increase in toxicity at total dose of 6960 cGy. This encouraging result has lead to a three-arm phase III study comparing hyperfractionated radiotherapy, conventional fractionation radiotherapy alone and conventional radiotherapy combined with systemic chemotherapy.

As attempts to control microscopic distant metastases and to compliment radiotherapy so as to improve loco-regional control, several trials have been performed testing the addition of chemotherapy before, during or after radiotherapy in Stage III non-small-cell lung cancer. The most provocative results came from the Cancer and Leukemia Group B (CALGB) study[7] and from the Radiotherapy and Lung Cancer Cooperative Groups of the European Organization for Research and Treatment of Cancer (EORTC)[8]. The CALGB study randomized the patient with Stage III disease and with excellent performance status and minimal weight loss between pre-radiotherapy chemotherapy using cisplatin and vinblastine followed by standard radiotherapy and standard radiotherapy to 6000 cGy over six weeks. Median survival, two-year survival and three-year survival were all significantly better in the chemotherapy treated arm. Of note, is that the one-year and two-year survival figures for combined chemo/radiotherapy were similar to those of the similar patients in the RTOG trial using 6960 cGy hyperfractionated radiotherapy. The EORTC trial randomized patients to one of three arms: radiotherapy for two weeks (300 cGy x 10, 5 fractions/week, followed by three-week rest, and then radiotherapy of 250 cGy x 10 five times per week), radiotherapy on the same schedule combined with 30 mg of cisplatin per square meter of body surface given on the first day of each treatment week, or radiotherapy of the same schedule combined with 6 mg of cisplatin per square meter given daily before radiotherapy. Survival was found to be significantly better in the radiotherapy-daily cisplatin group as compared to the radiotherapy group (P=0.009). The one-year and two-year survival rates for the radiotherapy-daily cisplatin group were 54% and 26% respectively, again similar to the figures in the RTOG and CALGB trials. One logical study based on all of these observations is to test the combination of radiosensitizing chemotherapy concurrent with high dose hyperfractionated radiotherapy. We have initiated such a trial of high dose cisplatin, VP-16 chemotherapy concurrent with hyperfractionated radiotherapy in a split-course fashion (in order to reduce acute toxicity). The availability of growth factors will most probably allow us to deliver the treatment cycles on schedule and not to reduce the intensity of therapy.

Recently there have been significant technologic advances in radiotherapy, particularly in the availability of three-dimensional conformal radiotherapy planning and delivery systems. These systems will allow better delivery of high dose radiotherapy to the tumor and will at the same time improve sparing of normal tissues. These technologies are being actively integrated into the treatment of lung cancer.

Although radiotherapy is not the most optimal treatment modality for lung cancer, it continues to play a significant role. It remains to be seen if new regi-

mens that incorporate biological principles and technical advances would truly lead to improved prognosis for the majority of patients with unresectable non-small-cell lung cancer.

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Session IX: Ovarian Cancer

CONVENTIONAL TREATMENT RESULTS IN EPITHELIAL OVARIAN CANCER

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CONVENTIONAL TREATMENT RESULTS IN EPITHELIAL OVARIAN CANCER

Ovarian cancer is the most frequent cause of death due to a gynecologic malignancy. The overall survival of all patients with ovarian cancer is 38% [1]. Approximately 80% of these patients present in stages III and IV. Moreover, only 20% of the patients who present in stages III and IV survive five years with the best currently available therapy.

Standard treatment of epithelial ovarian cancer (EOC), the most common type of ovarian malignancy, includes surgical removal of the primary and metastatic tumors, followed by chemotherapy. The separate roles of surgery in chemotherapy are discussed below.

SURGERY

Abdominal surgery is performed initially to establish a histopathologic diagnosis of ovarian cancer and to establish the stage (Table 1). At exploration the feasibility of tumor reductive surgery is determined and what can reasonably be accomplished without subjecting the patient to undue morbidity. Although there are exceptions, typically the operation includes bilateral salpingo-oophorectomy with or without a hysterectomy and a total or subtotal omentectomy. In certain situations portions of the small or large intestine or of other organ systems may be removed to accomplish an optimum tumor reduction. The main objective of the operation is to be able to obtain residual tumor masses in which the diameters of individual metastases are less than 2 cm. This approach is adopted by surgical oncologists since a number of studies have shown longer survival patterns amongst those patients whose tumor residual has been optimally reduced [2,3]. If this objective cannot be reasonably accomplished because of extensive tumor infiltration or plaque formation, or multiple

lymph node involvement or parenchymal liver metastases, then as minimum, an attempt is made to remove both ovaries and any large masses that may be contributing to symptoms. Removal of the primary tumors is helpful in establishing the diagnosis of EOC. This is important for subsequent chemotherapy decisions since ovarian carcinomatosis is more sensitive to chemotherapy as compared to abdominal carcinomatosis which has originated from other sites such as the gastrointestinal tract. If a mucinous tumor of the ovary is discovered (it is presumed that a patient has had appropriate radiocontrast studies to rule out intrinsic G.I. tract lesions), then appendectomy should also be performed to exclude a primary carcinoma of the appendix. Where the disease appears to be localized to one or both ovaries surgical treatment usually includes a total hysterectomy and bilateral salpingo-oophorectomy. However, in younger patients and particularly in those where preservation of fertility is desired, it is important to establish the histologic diagnosis of the affected ovary and to examine carefully the opposite ovary and the remainder of the pelvic and abdominal organs in case a more conservative approach is indicated. A more conservative approach may be appropriate in patients with borderline or grade 1 unilateral disease or patients with unilateral nonepithelial germ cell tumors. The omentum is frequently infiltrated with carcinoma and since involvement of this organ contributes to a patient's symptoms, the involved omentum should be resected whenever possible. There are few patients indeed that are considered "inoperable". Despite the aggressive surgical approaches that are utilized for treating ovarian cancer the impact of surgical reduction on survival remains unknown. This is so because it is difficult to determine to what extent surgical resectability is a consequence of the biological behavior of the tumor. The role of "delayed" or "interim" tumor reduction following chemotherapy is also undetermined. With our current knowledge, therefore, it is appropriate that every patient who has suspected ovarian carcinomatosis and whose medical condition is suitable for major surgery, should undergo an exploration prior to receiving chemotherapy.

Other surgical interventions for ovarian carcinoma: A second look laparotomy (SLL) is frequently performed after a series of courses of chemotherapy. This operation is somewhat controversial today since it is not clear that the operation itself contributes to improved survival of individual patients [4,5]. SLL, however, can provide very useful information to the patient and to her physician on the status of the tumor which may be unavailable from clinical or radiologic examinations. Moreover, SLL may provide additional options for the patient in regard to alternative treatments, some of which may include experimental drugs. Recent improvements in laparoscopy equipment and the laparoscopy procedure itself may result in a broader application of laparoscopic "second look" operations in patients who have been treated with one or more lines of chemotherapy.

CHEMOTHERAPY

Standard first-line chemotherapy includes cisplatin (cDDP) or carboplatin combined with cyclophosphamide. There is little doubt that platinum-based chemotherapy has contributed both to improvements in the survival and in the quality of life of patients with ovarian carcinoma. However, the long term re-

sults of treatment remain unsatisfactory and several important questions are pertinent to the currently available first-line chemotherapy. These questions have included: (1) Whether there is an advantage of combinations of drugs over single agents; (2) cDDP versus carboplatin; (3) The importance of dosing; and (4) The contributions of other agents to an improvement in survival.

The results of more recent randomized trials (summarized in Table 2) have provided some answers to these questions [6-10]. These trials have included large numbers of patients and are comparable for the high risk factors of stage and large tumor residual post surgery. These studies also included pathologic complete response data obtained at SLL. The median survival of EOC patients treated with chemotherapy is markedly reduced in patients who have large residual stage IIIc or IV tumors after surgery, as compared to those patients who have microscopic or small visible residual disease after primary surgery. The median survival in the first category of high risk patients is approximately 20 months. Prospective trials should be able to show a balance for the high risk factors in the randomized treatment arms or may restrict their patients to those with more uniform risk patterns.

Several trials have compared chemotherapy combinations that included cDDP against single agent therapy. Although overall response rates and pathologic complete responses appeared to be higher, median survivals were not statistically different. The Italian cooperative group study [8] showed no difference in the median survival between cDDP alone, cDDP plus cyclophosphamide and cDDP, doxorubicin and cyclophosphamide. Moreover, the median survival from single dose cDDP even at the moderate dose of 50 mg/m^2 appeared to be as good as other chemotherapy combinations where comparable numbers of patients with high risk disease were included. However, a recent meta-analysis conducted on patients from multiple studies has revealed an improvement in survival that favors the combination with cDDP versus cDDP alone [11]. In yet another meta-analysis [12] it appeared that there could be an advantage from the addition of doxorubicin to the standard cDDP and cyclophosphamide.

With the introduction of carboplatin and the discovery that the spectrum of toxicity from carboplatin was quite different from that of cDDP, a number of trials have been conducted to determine comparisons in terms of response and survival [9-11]. In comparison to the studies with cDDP, the dosing effect of carboplatin seems to be more critical in relation to survival [13]. In two recent trials conducted respectively by SWOG [8] and the Canadian group [9], carboplatin at a dose of 300 mg/m^2 with cyclophosphamide at 600 mg/m^2 produced median survival times and response rates that were equivalent to the cDDP combination arm in each trial [8,9]. The SWOG study employed cDDP at 100 mg/m^2 whereas the Canadian trial employed cDDP at a dose of 75 mg/m^2. In patients at higher risk (large post-surgical residual disease and stage IV) there are few pathologic complete responses and the anticipated rate currently is less than 10% of all patients treated.

It is possible to conclude from the recent studies that for first-line treatment, cDDP at doses of either 75 mg/m^2 or 100 mg/m^2 plus cyclophosphamide is equivalent to carboplatin 300 mg/m^2 and cyclophosphamide in terms of response and survival. However, patients who received cDDP in both studies had

higher frequencies of neurotoxicity and elevated creatinine values as might be expected. Those patients who received carboplatin had overall higher frequencies of anemia that required transfusion and thrombocytopenia. Both cDDP and carboplatin have a role as first-line agents in the treatment of ovarian cancer. The choice of either cDDP or carboplatin may be determined by the physical condition of the patient including age, and susceptibility to a particular agent's toxic effects. Use of the Calvert formula [14] may also help to accomplish more optimum carboplatin dosing in certain patients. There is no published information available on the efficacy of taxol as a first-line agent in combination with cDDP or its analogue carboplatin. Recent studies suggest that there is a critical dose response relationship for taxol. It therefore remains unknown whether and how taxol will be optimally combined with cDDP or carboplatin in first-line combination therapy.

Results obtained from certain studies that used cDDP alone or in combination at a dose of 50 mg/m^2 [6,7] suggested similar response and survival data to other studies with similar frequency of high risk factors. We are currently studying cDDP at 50 mg/m^2 in combination with thiotepa. Thiotepa is an old drug with known activity against EOC. The MTD for thiotepa has only recently been defined at 60-65 mg/m^2 [15]. Thiotepa has interesting properties which may be pertinent to its application in EOC. The limiting toxicity of thiotepa is myelosuppression yet high doses of thiotepa with bone marrow rescue have been tolerated and produced responses in patients with refractory EOC [16]. There may also be some non-cross resistance between cDDP and thiotepa. We previously conducted a phase I trial in patients with gynecologic malignancies that included patients with ovarian carcinoma [16]. An optimum dose for this combination was determined at 50 mg/m^2 for cDDP and 40 mg/m^2 for thiotepa. Fifty percent of patients with EOC who had previously been treated with a platin combination, responded to the cDDP thiotepa combination in the phase I trial. A subsequent phase II study was initiated for patients with primary untreated EOC who had stage IIIc or IV high residual disease. The study has accrued 27 eligible patients with 25 evaluable at this time for response and toxicity. Seventy-two percent of the patients have had evaluable responses, including two PCRs (Table 3). Myelosuppression was the main dose limiting toxicity and was primarily due to the thiotepa. The protocol permitted dose reductions of the thiotepa without concurrent reduction in the dose of cDDP myelosuppressive events (Table 4). Forty-five percent of evaluable patients achieved full dose thiotepa over four courses and 45% required only one dose reduction at four courses. Twenty-five percent of the patients who received six courses of treatment did not require a thiotepa dose reduction. Thirty-one percent required but one dose reduction by the sixth course. The remaining 59% required more than one dose reduction. None of the patients experienced significant neurotoxicity or nausea and emesis. Myelosuppressive toxicity affected both platelets and granulocytes. Two patients received platelet transfusions. Our preliminary conclusions are that a combination of cisplatin and thiotepa is well tolerated and produces a response rate that is at least comparable to that of other reported studies for this group of patients. The main dose limiting toxicity was related to the myelosuppressive effects of thiotepa. It is clear that prolonged maintenance or escalation of thiotepa dose will not be possible without both granulocyte and

platelet support. This drug combination however may be considered in combination with bone marrow rescue or growth factor support for high risk patients with ovarian carcinoma.

In summary, the standard treatment of EOC involves surgery followed by chemotherapy with cDDP or carboplatin plus cyclophosphamide. Surgery is important to initial staging and diagnosis and to achieve a maximum effort at tumor debulking. Both cDDP and carboplatin are useful drugs in the first-line treatment of ovarian carcinoma. In deciding which of these two drugs to use, attention needs to be given both to the patient profile and to the anticipated toxicities for each drug given at particular dosages. With cDDP doses in the range of 50-75 mg/m^2 the neuro and renal toxicity may be more acceptable whereas higher doses are associated with an increasing incidence of both neuro and renal toxicity. The preferred starting dose of carboplatin appears to be at 300 mg/m^2, although it may be possible to increase the dose of carboplatin with the Calvert formula which is based upon area under the curve (AUC) utilizing an AUC value that reflects the patient's profile and anticipated toxicity response. The addition of other chemotherapy agents such as doxorubicin [12] will need to be balanced against unwarranted toxicities especially those related to the heart.

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Table 1. Operative Staging of Ovarian Cancer (FIGO)

Stage	I	Growth limited to ovaries
Stage	Ia	Growth limited to 1 ovary; no ascites; no tumor on external surface; capsule intact
	Ib	Growth limited to both ovaries; no ascites; no tumor on external surfaces; capsule intact
	Ic*	Tumor stage Ia or Ib, on surface of 1 or both ovaries; or with capsule ruptures; or with ascites present containing malignant cells or positive peritoneal washings
Stage	II	Growth involving 1 or both ovaries with pelvic extension
	IIa	Extension and/or metastases to uterus and/or tubes
	IIb	Extension to other pelvic tissues
	IIc*	Tumor stage IIa or IIb, on surface of 1 or both ovaries; or with capsule(s) ruptured; or with ascites present containing malignant cells or positive peritoneal washings
Stage	III	Tumor involving 1 or both ovaries with peritoneal implants outside pelvis and/or positive retroperitoneal or inguinal nodes; superficial liver metastases - stage III; tumor limited to true pelvis but with histologically proven malignant extension to small bowel or omentum
	IIIa	Tumor grossly limited to true pelvis with negative nodes but with histologically confirmed microscopic seeding of abdominal peritoneal surfaces
	IIIb	Tumor in 1 or both ovaries with histologically confirmed implants of abdominal peritoneal surfaces, none >2 cm in diameter; negative nodes
	IIIc	Abdominal implants >2 cm in diameter and/or positive retroperitoneal or inguinal nodes
Stage	IV	Growth involving 1 or both ovaries with distant metastases; if pleural effusion is present, cytology must be positive to allot case to stage IV; parenchymal liver metastases - stage

*To evaluate prognostic impact of findings and assign case to Ic or IIc, it would be helpful to know if rupture of capsule was spontaneous or caused by surgeon and if source of malignant cells was peritoneal washings or ascites

Table 2. Randomized Trials Employing Combination Chemotherapy in Ovarian Cancer

	Chemo	N	RR,	Resid* PCR*	>.2 cm	MST STIV*	alive*	(mos)
Williams '85	P 80 mg x 1 A 40 mg x 1 C 1 gm x 1 R	42	*68*	26	74	30	19	13
CB	10mg POx14	43	26	15	76	30	12	11
Conte '86	P 50 mg x 1 A 45 mg x 1 C 600 mg x 1 R	62	56	37	52	19	53	28
	P 50 mg C 600 mg	63	54	24	52	14	40	24
Neijt '87	C 100 mg x 14 H 150 mg x 14 A 35 mg x 14 P 20 mg x 5 R	94	80	35	51	22	40	31
	P 75 mg C 750 mg	97	74	36	50	38	35	24
	P 50 mg R	174	51.4	20	69	18	ND	19.4
GI-COG '87	P 50 mg C 650 mg R	182	61.5	20	71	19		21.4
	P 50 mg A 50 mg C 650 mg	175	71	26	65	20	ND	23.8
Alberts et al (SWOG '92)	P 100 C 600 R	143	52	7 **	100	31	29	17.4
	Carb 300 C 600	148	61	8 **	100	28	27	19
	P 75 C 600	210	57	24 +	58	18	~20	25
Swenerton (Can. Trials)	R Carb 300 C 600	207	42	19 +	60	16	~20	27 *

*percentage **all pts; +suitable for relaparotomy

Table 3. Response

Complete response	7
Partial response	11
NR	1
Stable (minimum 8 weeks)	6
Total response rate	18/25 (72%)

Table 4
Number of Treatment Cycles per Dose Level of Thiotepa

	1	2	3	4	5	6
40	27 (100)	18 (75)	12 (50)	10 (45)	5 (26)	4 (25)
30	-	6 (25)	12 (50)	10 (45)	7 (37)	5 (31)
20	-	-	-	1 (5)	5 (26)	5 (31)
0	-	-	-	1 (5)	1 (5)	2 (12)
Total patient courses	27	24	24	22	18	16

BONE MARROW TRANSPLANTATION FOR OVARIAN CARCINOMA IN THE UNITED STATES: A SURVEY OF ACTIVE PROGRAMS

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INTRODUCTION

Ovarian carcinoma is the the fourth most common cause of cancer death in women in the United States. Almost 80% present with advanced disease and despite initial responses to platinum-based chemotherapy, in approximately 70-80%, only 20% are long term survivors[1]. Only 30% respond to conventional salvage therapy, with response durations usually lasting only a few months. While new agents such as taxol[2] might improve the prognosis of patients with advanced ovarian carcinoma when used early, the incurability of relapsed and all but minimal disease at the time of second look surgery have led many groups to explore new approaches for these patients. These include dose-intensive therapy given as either intraperitoneal chemotherapy and or systemic chemotherapy with either myeloid growth factors or autologous bone marrow transplantation (ABMT).

While intraperitoneal therapy appears promising for the 20% of patients with < 0.5 cm of disease at the time of second look surgery, still 80% of this highly select group of patients ultimately die of their disease[3]. However these responses and the *in vitro* and *in vivo* evidence of a favorable dose-response curve for many of the active agents used to treat patients with ovarian carcinoma has led to investigation of dose-intensive systemic chemotherapy, with growth factors or with autologous bone marrow transplantation (ABMT). Evidence to support the concept of dose escalation in ovarian carcinoma exists for platinum compounds, alkylating agents and mitoxantrone. Bruchner and Ozols administered cisplatin at the upper limit of the conventional range of 200 mg/m^2 to patients not responsive to lower doses of this agent and observed a response rate of 20-32% [4,5]. Cisplatin doses can not be escalated further however because of neurotoxicity. The dose limiting toxicity for carboplatin on the other hand is hematopoietic. Carboplatin doses up to 800 mg/m^2 have in fact been administered with GM-CSF and without bone marrow rescue. Responses of 35% have been seen which are approximately twice that for more conventional doses of the drug[6]. However while the response rates are higher and infections are controlled with GM-CSF, the patients are severely thrombocytopenic and thus full treatment courses can not be routinely given at the full dose.

Higher dose carboplatin with marrow rescue has been even more effective and the thrombocytopenia seen with lower doses is eliminated by the re-infusion of cryopreserved hematopoietic stem cells. Shea et al have shown that carboplatin can be escalated to 2000 mg/m^2 with marrow rescue. He described in this single agent study, a response rate in patients with refractory ovarian carcinoma of 55%[7].

Alkylating agents including melphalan, cyclophosphamide and thio-TEPA are active in ovarian carcinoma and these agents have also been successfully dose escalated with marrow rescue[8,9]. Stoppa reported a 75% response rate for patients treated with high dose melphalan who had failed cisplatin induction therapy. In this study 15/35 patients were alive and disease-free at a median follow-up of 23 months[10]. Along the same lines Dauplat found a 36% 2 year disease-free survival for similarly treated patients failing standard induction therapy[18]. This compares to a median survival of 6-9 months for other salvage therapies available to patients and a 2 year survival of less than 15%. Based on *in vitro* studies demonstrating synergism between cisplatin and either cyclophosphamide or thio-TEPA in ovarian carcinoma, investigators at Duke University performed and completed a pilot study of high dose cisplatin, cyclophosphamide and thio-TEPA with bone marrow rescue in ovarian carcinoma. Of the first 9 patients who had failed at least 2 prior regimens, there was a 78% response rate, documented surgically even though all had progressed after receiving cisplatin[12].

Similarly, mitoxantrone has been shown by Alberts et al to be the most effective anti-tumor agent at high doses against resistant ovarian carcinoma from patients *in vitro*[13]. Because of this and the fact that the drug is nearly devoid of non-hematopoietic toxicity at conventional doses, several groups have completed studies of high dose mitoxantrone in patients with refractory ovarian carcinoma[14,15]. Mulder combined either cyclophosphamide or melphalan with high dose mitoxantrone. Of their initial 6 patients there was a 66% complete remission rate of up to and beyond 36 months post-therapy. A trial conducted at Loyola University treated 25 patients with primarily breast and ovarian carcinoma and demonstrated an overall response rate for ovarian carcinoma of 83% with 80% of the responses complete remissions[16]. The complete remissions have lasted up to, and in fact longer than, 30 months, with the median survival for this heavily pre-treated group of 12 months.

SURVEY RESULTS

Based on enhanced responses to high dose chemotherapy with ABMT, many U.S. groups are now performing transplants for advanced ovarian carcinoma. Because of the small numbers treated to date at each center, very little has been published. Yet as in the pilot studies as above, it is the feeling of many investigators that this form of therapy is effective in treating end stage disease, and that this therapy should be used earlier in the course of the disease to improve results, as has been done for patients with leukemia and lymphoma. To provide additional support for this therapy in this country, a survey of the U.S. programs known to be active was conducted in November, 1992. The survey was designed to determine the number of patients treated, the regimens used, the remission status, several prognostic factors, as well as response rates and durations (Table

1). Data from the survey was combined with that of several published U.S. studies that reported data on patients not covered in the survey. The goal was to determine if data from a pooled group of patients could be used to make the case for the consideration of this therapy earlier in the course of the disease.

The survey evaluated data from 11 centers on 153 patients (Table 1). Of these, 146 or 95% had either relapsed after initial therapy was completed, or did not have an initial response to therapy. The remaining 7 were transplanted as part of initial therapy, i.e. after a chemotherapy-induced remission. The median number of prior regimens that the relapsed/refractory group received was 2. There were 20 different regimens used and the median number of patients treated with each regimen was 7 (range = 1-20). Of the entire group, 11 were treated with single agent carboplatin, 8 with a TBI-based regimen, 9 with an alkylating agent only combination, 15 with mitoxantrone and an alkylating agent, and the remaining 110 with a platinum-based regimen with at least one alkylating agent.

Because of the large number of different regimens and the small number of patients treated with any one regimen, the response results and outcome data were pooled. Of the 146 recurrent/refractory disease patients, 82 were evaluable for response rate reporting, having measurable disease at the time of ABMT. The remainder had either evaluable disease only (elevated CA 125), or a surgical debulking procedure prior to the transplant. Of these 82, 58 or 71% had either a partial or complete remission (PR + CR) to the therapy, and 35 or 43% a clinical CR (Table 2). Only one center reported verifying responses by a post-transplant laporatomy, thus the clinical CR rate likely overestimates those who would have had a surgically documented CR.

Of the 82 patients evaluable for a response, data on platinum sensitivity or resistance was available in 56 or 68%. Platinum resistance was defined using the standard criteria of either progression on, or relapse within 6 months of a platinum-based regimen. Of the 56 evaluable patients 73% were platinum resistant at the time of transplant. The overall PR + CR for the platinum sensitive and resistant groups was similar at 87 and 85% respectively. This response rate is higher than that for the entire group (71%), as it does not include the 26 patients for whom platinum sensitivity or resistance was not obtainable. The number of patients entering a CR was approximately double for platinum sensitive patients (73% versus 34%).

Time to progression was reported as a median for each group's patients. Data on 98 patients from 9 centers was available and the median time to progression for the 9 centers was 6 months with a range of 3-12 months. All 11 centers reported data on the percent of patients disease-free at one year. Of the 121 patients evaluable, 17 or 14% were disease-free at one year.

Of the 7 patients transplanted in first remission 5 or 71% are alive and disease-free at 9-20+ months. Two progressed at 5 and 9 months.

The major non-hematopoietic toxicities varied based on the regimen used. For the entire group there was a 10.6% early death rate.

DISCUSSION

High dose chemotherapy with ABMT has over the past few years become an effective way to manage patients with many malignancies including acute

leukemia, Hodgkin's and non-Hodgkin's lymphoma, testicular carcinoma, neuroblastoma, and other solid tumors such as carcinoma of the breast. In some of these patients it is the only curative option for an otherwise incurable malignancy. Ovarian carcinoma shares many of the clinical features of these diseases: a high response rate to multi-agent chemotherapy, with some cures even in patients with advanced disease; rapid emergence of drug resistance; response to salvage therapy with even some complete remissions which are usally brief; and death usually due to drug resistant disease. The number of patients who have undergone this therapy to date is substantially less than breast carcinoma, however ovarian carcinoma is a relatively uncommon tumor. In fact this year in the U.S. less that 15,000 patients will be diagnosed with advanced stage ovarian carcinoma.

As expected, the vast majority of patients with ovarian cancer transplanted to date in the U.S have had advanced and predominately platinum resistant disease. Despite these poor prognostic features, a high proportion have had a significant response to the therapy. The high response rates yet brief durations for this group is not unexpected. These results are in fact similar to that of refractory leukemia and lymphoma, and breast cancer and provided the impetus to explore transplantation prior to the development of chemotherapy-resistant relapse for those tumors. Based on the large numbers of patients treated both in the U.S and Europe we believe that it is time to consider using this therapy earlier in ovarian carcinoma as well. Legros et al has recently reported on the use of high dose therapy as part of the initial management of patients with advanced ovarian carcinoma[16]. The overall disease-free survival at 3 years was 35% which is almost double that produced by conventional chemotherapy regimens alone.

The major groups that deserve consideration in large scale studies include those with sensitive yet minimal disease at second look laporatomy, and those with stage III and IV disease who have undergone suboptimal surgery at the time of diagnosis. These patients have responsive yet incurable disease, and would be similar to those patients with lymphoma or leukemia in second remission. If pilot data in these two groups subsequently indicated an improvement in progression-free survival as compared to historical controls, then a randomized trial that compared high dose chemotherapy with ABMT to the best standard therapy would be appropriate.

With the promising data from several pilot studies, two national Cooperative Group Trials have begun. The first, study #9106 of the Southwest Oncology Group, will examine two regimens in a randomized Phase II fashion for patients with >0.5 but less than 3.0 cm disease at second look laporatomy and those with recurrent disease after a 6 month or longer remission after a platinum based induction regimen (platinum sensitive relapse). The 2 regimens are high dose carboplatin, mitoxantrone and cyclophosphamide[15], and cisplatin, thio-TEPA, and cyclophosphamide[12]. Time to progression will be analysed, as well as toxicity for the two regimens, with the intent to take one of the two to a randomized trial of high dose therapy versus the best conventional therapy for early unfavorable disease.

The second study is Study #124 from the Gynecologic Oncology Group. Patients who have had an incomplete response or have relapsed following platinum-based chemotherapy will receive two transplants consisting of high dose

carboplatinum and ifosfamide. The second transplant will follow the first one by only a very brief period.

In summary, high dose chemotherapy with ABMT appears to be the most effective available therapy for patients with refractory/relapsed ovarian carcinoma, with a response rate approximately double that of any other salvage therapy for patients who have failed two prior regimens. In addition the 14% 1 year progression-free survival suggests that some of these remissions may be long term, as has been the case for patients with other refractory hematologic malignancies and solid tumors. While this therapy has not yet influenced the clinical course in the majority of patients treated thus far, there is the potential that a significant impact on this disease will come with the treatment of patients earlier in their course while the disease is still sensitive to conventional drug doses.

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Table 1

Clinical characteristics of U.S. ovarian cancer ABMT patients

--Centers or studies surveyed	11
--Total number of patients	153
--Number (%) with relapsed/refractory disease	146 (95%)
--Number transplanted in first remission	7 (5%)
--Median number (range) of prior regimens	2 (1-3)
--Number of ABMT preparative regimens	20
--Median number (range) of patients/regimen	7 (1-20)

Table 2

Clinical results of ABMT for relapsed/refractory ovarian cancer: Survey of U.S. programs

--Number of patients	146
--Number (%) early deaths	16 (10.5%)
--Number (%) evaluable for response*	82 (56%)
--Number (%) responding*	58 (71%)
--Number (%) entering a CR	35 (43%)
--Number (%) platinum sensitive	37/98 (38%)
--%CR+PR/CR with platinum sensitive disease	87%/73%
--% CR+PR/CR with platinum resistant disease	85%/34%
--Median time (months; range) to progression	6 (3-12)
--% disease-free at 1 year	14%

*Patients with measurable disease who have a PR or CR

Session X: Sarcoma

HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS BONE MARROW RESCUE IN POOR RISK SARCOMA PATIENTS. REPORT OF PILOT STUDY IN PEDIATRIC SARCOMAS.

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Primary solid tumors account for approximately 30% of all childhood malignancies. Sarcomas comprise approximately 1/4 to 1/3 of pediatric solid tumors and 1% of adult malignancies. Despite many advances in the understanding of the molecular biology and tumor cellular genetics, therapeutic options for these tumors remain limited. Treatment of localized disease utilizing the combined modalities of surgery, chemotherapy and radiotherapy results in excellent long term disease free survival in selected tumors. This is especially true for childhood rhabdomyosarcoma, osteogenic sarcoma and soft tissue sarcomas 1. However, results for treatment of advanced or relapsed sarcomas remain poor and new therapeutic modalities must be pursued.

The use of more intensive chemotherapy regimens with or without cytokine support have been demonstrated to improve prognosis in poor risk patients. The use of ifosfamide has improved response in combination therapies for patients with Ewing's sarcoma, rhabdomyosarcoma and soft tissue sarcoma 2. Additionally, intensification of regimens utilizing cyclophosphamide, cisplatin and adriamycin have resulted in improved response rates in poor risk patients 3,4. These data are strongly suggestive of a dose response effect for certain chemotherapeutic agents in sarcomas.

The use of high dose chemotherapy with autologous bone marrow rescue (ABMR) has been well studied in the lymphohematopoietic malignancies. However, fewer studies have been done in solid tumors for a variety of reasons including poor response rates with leukemia transplant regimens, development of multidrug resistance in the solid tumor and a less committed approach to transplant for these malignancies. More recently, studies using high dose chemotherapy and ABMR in Ewing's sarcoma, rhabdomyosarcoma, testicular carcinoma and a variety of other tumor types have demonstrated promise for this approach 5-7. A number of phase I-II trials in adult solid tumors have demonstrated the effectiveness of high dose combination treatments utilizing cyclophosphamide (CY) or ifosfamide (IFOS), etoposide (VP-16), thiotepa, melphalan (L-PAM), or carboplatin (CBDCA) 8-10. In many of these regimens dose intensity of 5-10 fold for the combinations has been achieved.

The prognosis for refractory or metastatic pediatric solid tumors is very poor with truly resistant patients having <10% 1 year progression free survival (PFS). Phase II studies of IFOS(I), VP-16(E),+/-CBDCA(C) have demonstrated promise in these patients with response rates of 32% (IE) and 39% (ICE) respectively 11,12. For the ICE (IFOS/VP-16/CBDCA) regimen the maximally toler-

ated doses in a recent Pediatric Oncology Group trial were IFOS 6gm/M^2, VP-16 300mg/M^2 and CBDCA 635mg/M^2 12. Due to the excellent response rates in refractory pediatric solid tumors with ICE we designed a phase I-II chemotherapy combination with a backbone of CY and VP-16 and escalated CBDCA in children with refractory CNS and solid tumors. The results of the toxicities of this regimen and specific response rates for sarcomas are presented here.

METHODS

Beginning January 1989, 58 children with refractory CNS or solid tumors were treated with increasing doses of CY/VP-16/CBDCA with ABMR according to an institutional protocol, JH#8831. All patients (ages 3-25 yrs) had a pathologically proven solid tumor and had either relapsed (n=15), progressed (n=36) or had persistent measurable disease (biopsy proven n=7) after frontline or salvage therapy. All patients had satisfactory performance status (creatinine <2.0mg/dl, bilirubin <2mg/dl, platelet >100,000/mm , cardiac ejection fraction >45%, pulmonary function >50% of predicted) and had complete tumor workup prior to BMT.

Patients who were eligible went on to have autologous bone marrow obtained by standard methods. Bone marrow was buffy coated, suspended in media with human plasma and 10% DSMO prior to cryopreservation by standard methods. No *ex vivo* treatment of the marrow was performed. Patients were treated in single bed isolation rooms with HEPA filtered air and all had double lumen Hickman catheters. Patients received irradiated matched blood products as indicated. All patients who were HSV IgG+ received acyclovir at 125mg/M^2 every 6 hours beginning day -2 until discharge and most (n=55) received fluconazole orally at 5mg/kg/day over the same time period. Fevers and infection were treated with broad spectrum antibiotics as indicated. Patients who demonstrated a sustained rise in absolute neutrophil counts (ANC) of greater than 500/mm and had resolved all toxicities were discharged and followed as outpatients.

Responses were measured with serial radiographic studies and scored by standard methods. Complete response (CR) was defined as disappearance of all sites of disease, partial response (PR) as a greater than 50% decrease in all sites of disease. Progression (PD) was defined as growth of >25% of any lesion or appearance of new lesions. All protocols were approved by our local institutional review board.

RESULTS

The transplant (BMT) preparative regimen is given in figure 1. Dose escalations of CBDCA and VP-16 were made according to the schedule outlined in Table 2. Each dose escalation was made after at least 3-6 patients had completed the preceding dose level with <50% having dose limiting toxicity. Overall, the therapy was well tolerated with elevated transaminases (grade 4 20x normal) and stomatitis (ulcerative mucositis requiring narcotics) being the dose limiting toxicities. Significant nephrotoxicity (n=3; grade 2 NCI criteria) or ototoxicity (n=4 > grade 2; NCI criteria) were unusual. All patients surviving past day =14 demonstrated autologous engraftment. Median time to achieve ANC>500/mm and platelet > 50,000/mm was 27 days and 28 days respectively. Patient demo-

graphics are given in Table 1. In all 58 patients were treated. One patient developed spinal cord compression during therapy and had his preparative regimen interrupted. This patient is not included in the analysis. Most had multiple prior chemotherapy regimens (median 2) and had received radiotherapy to axial (craniospinal, whole abdomen or pelvis; n=32) or non axial (n=17) sites.

Response data is given in Table 2. The overall response rate in the 50 patients with evaluable disease was 70% (19CR, 16PR). The data for sarcomas is given independently in Table 2. Overall 33 sarcomas were treated. In 28 patients evaluable for response there were 13CR and 6PR for a response rate of 68% (19/28). Median time to progression was 4 months. For the patients who achieved CR and survived to day 60 however, 9/11 remain progression free (Kaplan Meier estimate 80% at 1 year) with a median follow-up of 13 months.

Patients who received a CBDCA dose of > 2000mg/M^2 had a significantly higher CR rate than patients receiving less than this dose (11/20 vs. 2/13; $P<0.01$). At the time of this publication nine patients remain free of disease at 2+, 4+, 4+, 5+, 8+, 12+, 13+, 14+ and 26+ months after BMT. There has only been one relapse after 8 months. Four patients received this therapy as a second transplant and all survived. Three achieved a second CR including one sarcoma patient. There have been renal electrolyte abnormalities that were self limited. Most patients developed renal tubular wasting of calcium, phosphate, and magnesium within three days of starting the preparative regimen and resolved this syndrome by day +20. There were no instances of Fanconi's syndrome and no patient is receiving long term electrolyte replacement therapy.

SUMMARY

Our experience with CBDCA/VP-16/CY has been promising in patients with refractory solid tumors. This regimen has been reasonably well tolerated in heavily pretreated patients. The MTD of the combination includes a dose of CBDCA which is higher than the adult studies with single agent CBDCA (2000mg/M^2) and ABMR and includes a dose of CY that has been used in BMT regimens 5,10. With higher doses the response data have become more dramatic with a greater percentage of complete responses and longer event free survival. Exploration of other combinations of agents, methods to reverse multidrug resistance and use of biological response modifiers should provide additional benefit for these patients. The role of hematopoietic growth factors has been well studied but further studies need to be done to define their appropriate roles in these patients. The question of bone marrow manipulation for solid tumors receiving ABMR has not been clearly addressed but use of stem cell selection may provide a useful approach.

The results of this study and other trials provide evidence that the use of high dose chemotherapy has a definite role in solid tumors. Careful understanding of the biology of the underlying malignancy and use of prognostic factors should enable us to use autologous BMT earlier in the treatment of these patients. The higher response rates (not compared in randomized fashion) of this approach compared to standard chemotherapy regimens and the long remissions that may be achieved justify the application of this approach. Future studies should focus on the relationships between laboratory discoveries and clinical outcome as well as refining the techniques for stem cell selection, use of

cytokines to reduce hospitalization, and pharmacokinetics to assess efficacy and toxicity. The feasibility of multiple course treatments with hematopoietic rescue will also need to be assessed as this approach has already demonstrated promise in certain situations such as testicular carcinoma 13. Finally, well planned studies comparing these therapies as intensification in first remission need to be done. The future role of high dose chemotherapy and ABMR in solid tumors will continue to expand and, hopefully, result in higher cure rates in these patients.

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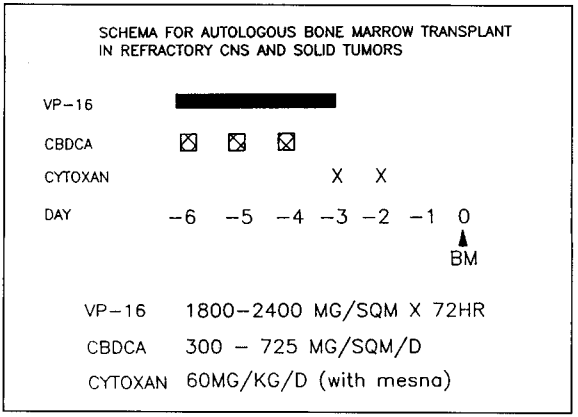

Figure 1
Schema for autologous bone marrow transplant in refractory CNS and solid tumors

TABLE 1
Solid Tumor ABMT Demographic Data

Age (yrs)	
Median	12
Range	2-25
Diagnosis	
Ewings	12
Neuroblastoma	7
Rhabdomyosarcoma	11
Misc Sarcomas	6
Osteosarcoma	4
CNS	12
Wilms	3
Other	3
Prior Chemotherapy (#regimens)	
1	12
2	30
3	14
Prior Radiotherapy	
Axial sites	32
Non-axial sites	17

Table 1: Characteristics of all 57 evaluable solid tumor/CNS tumor patients treated with CBDCA/VP-16/CY. Axial radiotherapy refers to treatment of pelvic girdle, whole abdomen, craniospinal, or Total Body Irradiation (TBI).

TABLE 2
Dose Escalation Schedule

Dose Level	CBDCA1	VP-161	#DLT2/# Treated
1	600	1800	0/3
2	900	1800	1/6
3	1200	1800	0/6
4	1600	1800	4/12
5	2000	1800	2/6
6	2000	2400	2/10
7	2400	2400	3/4 (1 fatality)
8	2175	2400	4/10 (2 fatalities)

1 Dose in mg/M^2
2 DLT = Dose limiting toxicity

Table 2: Schema of dose escalation for the entire group of pediatric solid tumors and the number of patients with dose limiting toxicity at each level. Dose limiting toxicity was determined using the NCI clinical toxicity scale.

TABLE 3
SARCOMA RESPONSE DATA

	CR	PR	NR	INEV	Total
Ewing's	4	4	4	0	8/12
Rhabdo	3	1	2	5	4/6
Misc Sarcomas	3	1	2	0	4/6
Osteosarcoma	3	0	1	0	3/4
	13	6	9	5	19/28 (68%)

Table 3: Response data given for sarcoma patients only. INEV refers to patients without measurable disease at the time of transplant.

Session XI:

CML

CHRONIC MYELOGENOUS LEUKEMIA WITH BAD PROGNOSTIC FACTORS: AUTOLOGOUS BLOOD STEM CELL TRANSPLANTATION FOLLOWED BY RECOMBINANT ALPHA INTERFERON

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INTRODUCTION

Chronic Myelogenous Leukemia (CML) is a clonal myeloproliferative disorder that progresses from a stable chronic phase lasting 3 to 4 years to a biastic transformation which usually marks the terminal stage of the disease. Despite encouraging results recently obtained with recombinant alpha interferon [(1)], allogeneic bone marrow transplantation (AlloBMT) remains the best means of eradicating the malignant clone. However AlloBMT is only applicable to patients under the age of 50 who have an HLA identical sibling donor, thus alternative therapeutic strategies have to be proposed.

High-dose chemotherapy and/or radiotherapy followed by autologous bone marrow transplantation (ABMT) was first used in 1974 by Buckner and al. to treatment for patients with CML in transformation [(2)]. The results of either ABMT or autologous blood stem cell transplantation (ABSCT) for CML in transformation reported in the 1980's can be summarized briefly as follows: a second chronic phase can be achieved in most cases but its duration is usually very short lasting approximately six months, and the benefit for survival is uncertain or marginal [(for review, see Ref 3)].

We report herein our results concerning 46 patients who underwent ABSCT for CML in transformation. They were treated with a double autologous transplantation of blood stem cells which was followed by alpha interferon treatment (IFN) in order to prolong the duration of the second chronic phase. The encouraging results observed in this series of patients prompted us to propose ABSCT followed by IFN in patients with CML in chronic phase with poor prognostic factors. The preliminary results are reported.

MATERIAL AND METHODS

CML in transformation (Group I)

Twenty-four adult patients (median age = 43 years; 27-64) were entered in the study. Seventeen patients were in accelerated phase and 7 patients in blast crisis. The interval time between diagnosis and transplantation was 40 months (median: 4-159). The conditioning regimen used for the first transplantation was:

Busulfan (4mg/kg/day 4 days) and high dose Melphalan (HDM) (140 mg/m^2, 1 day). Three months later, these patients underwent a second transplant with HDM alone as preparative regimen. Once hematopoietic reconstitution was observed after the second transplant (WBC count more than 5×10^9/l and platelets more than 100×10^9/l), subcutaneous IFN was given at a dose of 5×10^6 U/day/three times a week.

CML in chronic phase (Group II)

Twenty-two patients (median age: 44 years; 22-54) were transplanted in chronic phase. The reasons for ABSCT were either the presence of high risk prognostic factors according to Sokal's classification (n=15) or no response to IFN (n=7). This group underwent a single transplant after Busulfan and HDM as described above. Secondarily, the patients received IFN according to the same schedule as the patients transplanted in transformation.

Cytogenetic studies on at least 20 metaphases, were performed every three months. The cytogenetic response was classified as minimal, minor, partial or complete, according to criteria defined by Talpaz [1]. In patients achieving complete cytogenetic response, molecular studies were performed.

RESULTS

Group I

No patient died from transplant-related complications and a second chronic phase was achieved in all cases. Only 17 of the 24 patients received the second transplant. Seven patients did not undergo the second transplant for the following reasons: three patients had a recurrent transformation phase after the first transplant (median 2.5 months), two patients refused the second transplant, one patient suffered from veno-occlusive disease and one patient had a prolonged fever of unspecified origin. All the 17 patients who underwent the second transplant received IFN. This treatment was well tolerated and the doses were adjusted in accordance to hematological and cytogenetic response.

Twenty three of the 24 patients achieved a complete hematological response (CHR). Seven of the 20 evaluable patients achieved a cytogenetic response (complete = 1, partial = 2, minor = 4). Fifteen patients had recurrent transformation 3 to 23 months after ABSCT. Two patients died without transformation from infection. Seven patients are still alive in chronic phase or CHR 3 to 73 months after ABSCT. The complete cytogenetic response observed in the one patient occurred 12 months after the initiation of IFN and is still complete 48 months later. The PCR analysis of blood samples from this patient after four years of IFN, are negative [4].

Group II

One patient died from interstitial pneumonitis. Another patient had a graft failure. All other patients achieved a partial or complete hematological response following either ABSCT or IFN (or both). Only six patients had a transformation, so 14 patients are still alive 16 to 48 months in CHR or chronic phase after ABSCT. Only one patient achieved a complete cytogenetic response.

DISCUSSION

In this study, the use of intensive conditioning chemotherapy followed by autologous blood stem cell transplantation was associated with a high response rate in 95.8% Group I patients with a median survival of 20 months (1-73 months) and in 90% Group II patients with a median survival of 25.7 months (16-48 months). As few toxic deaths were observed in these groups and a complete hematopoietic reconstitution was achieved, we conclude that ABSCT is feasible in high risk CML.

The major problem of autografting for CML in transformation is the short duration of the second chronic phase. Most of the studies reported results observed in patients who underwent a single transplantation after many different conditioning regimens which may or may not include total body irradiation. The median second chronic phase duration was generally very short (4-6 months) due to the rapid recurrence of clonogenic blast cells implicated in the initial transformation [3]. The results in our present study seem to be encouraging as the median survival was 20 months with 7 patients still alive 3 to 73 months after ABSCT (median 28 months). It is difficult to know if these good results observed in group I patients were due to the double autograft of the addition of IFN after transplant. In a series of 51 patients. Haines et al. reported that the median survival was significantly longer for patients who received a double transplantation (52 weeks versus 13 weeks) [5]. We reported similar results in a series of 47 patients as the second chronic phase was noticeably longer in the 17 patients who underwent a double transplant followed by IFN than that we observed for 30 other patients who received a single or a double autograft without IFN [6].

For the Group II patients, the results are also encouraging since most patients achieved partial or CHR. These patients presented bad prognostic factors suggesting that alpha-IFN would not be efficient if administered without ABSCT. The annual transformation rate seems to be lower than that usually encountered in high risk CML patients [7]. However in terms of cytogenetic response, the results are not so appreciable than in Group I (difference not statistically significant) suggesting that the double autograft could play a critical role. A longer follow-up is needed to better evaluate the results of this therapeutic strategy for high-risk chronic phase CML.

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AUTOGRAFTING IN CHRONIC MYELOID LEUKEMIA WITH CULTURED MARROW: RESULTS OF A PILOT STUDY.

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INTRODUCTION I ON

In 1987, we initiated a study to evaluate the feasibility of using 10-day cultured marrow autografts to allow intensive treatment of patients with chronic myeloid leukemia (CML).[1] The rationale for this approach is based on evidence that leukemic stem cells (operationally defined as Philadelphia chromosome [ph[1]]-positive long-term culture initiating cells [LTC-IC]) are, on average, present in CML marrow at levels 10-fold lower than their normal counterparts and can be selectively "purged" (by a factor of 30-fold) following incubation for 10 days in LTC.[2-4] Patients were selected for this pilot study on the basis of a previous laboratory assessment of the frequencies of normal and leukemic LTC-IC remaining in their marrow at the end of 10 days of incubation in vitro under LTC conditions. The purpose of this was to establish whether a subsequently harvested and similarly treated autograft would be likely to have sufficient normal stem cells to allow engraftment and no detectable leukemic stem cells.[1]

METHODS

Between April 1987 and February 1992, 88 candidate patients (aged < 60 years, in morphological chronic phase of CML and ineligible for allogeneic bone marrow transplantation [BMT]) had their marrow assessed after 10 days of LTC for normal and leukemic LTC-IC numbers using previously described procedures.[1,5] In 36 patients (41%), normal LTC-IC in these cultured samples were present at > 2% of normal marrow values and leukemic LTC-IC were not detectable. Only patients meeting these criteria were considered eligible for autografting.

Twenty-two patients (aged 22 to 59 years, median 43), 15 in first chronic phase (Group 1) and 7 in accelerated or > first chronic phase (Group 2), were autografted with 10-day cultured marrow after either a total body irradiation-based (3 patients) or a busulfan-based (19 patients) regimen. The methods employed for manipulating the autograft were as previously described.[1] Briefly, ~2 x 10^{10} nucleated cells were set up in culture, and 10 days later all cells present (range 1.0 to 4.4 x 108/kg, median 1.7) were collected and infused.

RESULTS

Hematological recovery to > 0.5 x 109/L neutrophils and > 20 x 109/L platelets occurred in 16 of 21 evaluable patients at a median of 28 and 50 days post-autograft, respectively. During the initial phase of hematopoietic regeneration in these 16 patients, only Phl-negative cells were detected in 13, with predominantly phl-negative cells (76% to 94%) in the other 3. Graft failure occurred in 5 patients, 3 of whom were rescued by re-establishing chronic phase disease following infusion of unmanipulated reserve autologous cells (Table 1).

Subsequent to the initial recovery with Phl-negative cells, some Ph1-positive cells reappeared between 4 and 36 months post-autograft in all but one of the 13 patients in whom complete (morphological and cytogenetic) remission had been achieved (the remaining patient died in remission). Nine of these 12 patients were then treated with a-interferon 1-3 x 10^6 units/m^2, 3-7 days/week. Three subsequently returned to complete remission, 3 developed increasing numbers of Phl-positive cells and 3 are still too early to evaluate.

Sixteen patients remain alive and well, 9 in hematological remission (8 in Group 1), 6 to 62 months (median 26) post-autograft. Three patients (all in Group 1) continue in complete remission after treatment with a-interferon.

COMMENT

Cultured autografts from some patients with CML can result in a consistent and sustained restoration of Phl-negative hematopoiesis after intensive therapy. Strategies for improving the utility of this approach are being developed.

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Table 1

	Remission		Deaths		
	Hematological	Cytogenetic	Graft failure	BMT	CML
Group 1 (n= 15)	11	9	3	2	
Group 2 (n= 7)	5	4	2	2	2

AUTOLOGOUS PHILADELPHIA (PH) CHROMOSOME-NEGATIVE AND PCR-NEGATIVE BLOOD STEM CELLS CAN BE HARVESTED AND TRANSPLANTED IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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SUMMARY

In patients with first chronic phase (CP) CML, Blood Stem Cells (BSC) have been found to result Ph-negative when harvested during early recovery after intensive conventional chemotherapy.

INTRODUCTION

Allogeneic bone marrow transplantation (Allo BMT) is the only treatment option capable of being curative ([1,2]). Alpha interferon (a-IFN) may result in prolonged survival, but it is not yet clear if this approach could be curative (3). Intensive chemotherapy and autologous bone marrow transplantation with marrow incubation for 10 days, has been found to reduce the percentage of Ph-positive cells, but also this procedure does not seem to prolong survival (4). Double autotransplants followed by interferon therapy is a new interesting perspective and could be used for chronic-phase disease (5).

We have previously reported that in 5/8 patients in biastic phase - chronic myeloid leukemia, during very early recovery from marrow aplasia induced by intensive conventional chemotherapy, BSC resulted Ph-negative at cytogenetic analysis and in two patients also the PCR analysis was negative, further confirming that this approach may provide a possible source of normal progenitors ([6]). This paper summarizes the results with this procedure on 20 patients in first chronic phase (CP) and accelerated phase (AP).

MATERIALS AND METHODS

From July 1989 until November 1992, 20 patients who were refractory to a-IFN and ineligible for Allo BMT, were enrolled in this study. Fourteen patients were in first CP and 4 patients in AP-CML. Mobilizing chemotherapy consisted of idarubicin (6-8 mg/m2 per day for five days intravenously), citarabine (600-800 mg/m^2 per day for five days infused intravenously over two hours), and VP-16 (150 mg/m^2 for three days infused intravenously over a period of two hours). The drugs were given concurrently. BSC were collected during early recovery from initial chemotherapy. Leukapheresis was initiated when the WBC reached 0.5-2x109/1. Patients were judged to be suitable for autografting if Ph-positive cells were undetectable after this procedure. These subjects received VP-

16 (800 mg/m2 per day for two consecutive days, intravenously), cyclophosphamide (60mg/kg per day for each of two consecutive days) followed by 10 Gy of Total body irradiation at a dose rate of 5-9 cGy/min. Within 24 hours following radiation, the cryopreserved Ph-negative BSC were defrosted and reinfused intravenously. This is designated Day O.

RESULTS

Twenty patients in CP-CML or AP-CML have so far been treated with this intensive inductive regimen. Eleven (55%) out of 20 patients (CP:9/16 patients; AP:2/4 patients) had suppression on BSC of Ph-positive metaphases to 100% and 3 other CP-CML patients had a major Ph-suppression to less than 50% of Ph-positive metaphases. An interesting result derived from the PCR analysis performed on the BSC of 7 patients (AP:2 patients; CP:5 patients). The results of this analysis failed to show in the patients with CP-CML the presence of an amplification fragment of 273 bp, corresponding to the presence of BCR-ABL hybrid transcripts with a junction between the "BCR" region exon 3 and the ABL exon 2, which was expected on the basis of a PCR performed on the patient cells before treatment.

Five patients (AP:1; CP:4) have been so far transplanted with Ph-negative BSC. Hematological recovery after transplant to >l.Ox109/L neutrophils and >25xlO^9/L platelets occurred in all patients, at a median of 29 and 40 days post-ABMT, respectively. All the patients in CP-CML received rh-G-CSF at the dosage of 5ug/Kg/die. Graft failure occurred in 2 patients (CP:1 patient; AP:1 patient) and both died of fungal and Gram negative septicemias, despite that we had infused both patients with autologous marrow cells, previously harvested and cryopreserved. During the initial phase of hematopoietic regeneration post-ABMT in the other 3 patients, only Ph-negative marrow cells were detected.

Currently, 3/4 patients with CP-CML maintain a clinical and cytogenetic remission at 3, 7 and 14 months; of these, one is in biological and citogenetic remission (Ph-/PCR$^-$).

DISCUSSION

The preliminary results of this trial seem encouraging for a number of reasons. First of all, they provide strong support for the view that Ph-negative hematopoietic stem cells can be harvested after an intensive conventional chemotherapy; besides, these cells can result in a consistent and sustained restoration of Ph-negative (and PCR-negative) hematopoiesis. The best condition to achieve these results is avoiding the treatment with a-IFN in non-responding patients or at least, leaving a period of time of at least 2 months between the last day of a-IFN administration and the beginning of mobilizing therapy. In our series, we have observed an important delay of hematopoietic recovery after intensive conventional chemotherapy in patients pre-treated with a-IFN versus patients pre-treated with chemotherapy.

At the end, caution, however, must be used about the conclusive significance of these results. In fact, BSCs may have a very low mitogenic activity and this, of course, could bias the results of the cytogenetic analysis. Moreover, the negative results of the PCR analysis could also be due to the lack or to a very low degree of expression of the bcr/abl transcripts at the BSC differentiation

stage. However, the clinical results obtained after reinfusion of these BSCs in CP patients Ph-negative at 3, 7 and 14 months after ABMT, support the view that, even if it is not so absolute as it seems, at least a very good recovery of normal BSCs has been obtained.

The proposed mechanism of the normal hemopoietic rebound during early recovery phase following intensive conventional chemotherapy is difficult to be explained. A possible mechanism could be that the most primitive pluripotent stem cells are metabolically and mitotically quiescent and less sensitive to most cytotoxic agents than the more mature progenitor cells. Acute depopulation of the mature cells by intensive chemotherapy presumably sends signals which stimulate the pluripotent stem cells to repopulate the committed and maturing compartments (6). The combination of myeloblastolitic drugs such as Idarubicin, intermediate-dose ARAC and VP-16, produce severe but reversible marrow hypoplasia followed by vigorous hematological recovery which starts about two weeks after the chemotherapy.

In conclusion, though further work is needed to evaluate the durability of the results so far obtained and further refinements such as the use of synergistic combinations of CSFs with myeloblative chemotherapy for BSC mobilization still needs to be explored, we believe that this kind of approach may lead to mobilization of a higher percentage of normal BSC and, therefore, to a more effective autotransplant.

RESULTS OF IDARUBICIN-COINTAINING REGIMEN MOBILIZATION IN 20 PATIENTS WITH CML.

Patients	20
Median Total MNC (108/Kg)	3.1 (range 1.3-7.6)
Median Total CFU-GM harvested (104/Kg)	0.6 (range 0-13.3)
Median $CD34^+/CD33^-/Dr^-$ (106/Kg)	0.4 (range 0.1-1.13)

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Session XII:

CNS

HIGH DOSE NITROSUREA FOLLOWED BY ABMT AND RADIOTHERAPY IN HIGH GRADE ASTROCYTOMAS.

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INTRODUCTION

Phase II trials of high dose BCNU in high grade gliomas have been reported in the literature with response rates close to 40% ([1,2]). However, these responses were not long lasting in most of the patients ([1,2]). The use of adjuvant high dose BCNU followed by radiotherapy after surgery for high grade gliomas has been proposed by several authors ([3,4,5]).

Here, we present the results of two trials of high-dose nitrosurea followed by ABMT and radiotherapy in high grade gliomas.

We will first present an update (6) of the trial of high dose BCNU followed by ABMT and radiotherapy as post operative treatment, in which 103 patients have been included between March 1986 and January 1990 in three Centers in France. We also present the results of a phase I/II trial of high dose fotemustin in high grade gliomas conducted between April 1988 to May 1991 in 28 patients.

HIGH DOSE BCNU IN 103 PATIENTS WITH HIGH GRADE ASTROCYTOMAS

1. Patients and methods

1.1 Description of the patients

One hundred and three patients entered the program. Eighty were treated in Centre Leon Berard in Lyon, 18 in the regional hospital of TOURS, 3 in Centre Rene Huguenin in St Cloud and 1 in St Quentin Clinical. Patient age ranged from 17 to 64 years with a median of 47. Fifty-eight patients were males and 45 females. Before BCNU, 27 patients had a performance status (PS) grade 0 according to the ECOG scale, 50 patients had a PS 1 and 10 had a PS 2. Eleven had a bad performance status (9 grade 3, 2 grade 4). According to the Kernohan staging, 21 patients had grade III astrocytomas (20%) and 82 grade 1V astrocytomas or glioblastomas (80%).

1.2 Description of the study

The treatment plan was the following:

- Cyto-reductive surgery as wide as possible on day 0.
- Bone marrow harvest on d21:1 x 108 mononuclear cells/kg were harvested. A buffy coat was performed to reduce the volume to 100-150 ml. The marrow was then stored at 4° C.
- On the afternoon of marrow harvest, high dose BCNU (800 mg/m2) was administered over a 2-hour infusion.
- The patient was discharged from the hospital on the following day.
- Monitoring was organized on an out-patient basis with one weekly blood count, hepatic and renal biology, physical staging and chest X-ray.
- Radiation therapy was scheduled approximately on day 45. The total delivered dose was 45 Gy in 19 days, using the 18 MV energy of a linear accelerator: 24 Gy in 8 fractions to the whole subtentorial brain, followed by a localized boost of 21 Gy in 7 fractions to the tumor or tumor bed. Every radiotherapy field was designed using the dosimetry CT scan planning system.
- Overall survival was chosen as the main evaluation criteria.
- The first 16 treated patients also received an early post-operative chemotherapy given on day 3 after histology confirmation, combining. VM 26, 150 mg/m^2, BCNU, 100 mg/m^2 in a 5 mn IV short infusion, through a peripheral venous access. PROCARBAZINE (day 3 to 10), 100 mg/m2/day, per os. This part of the chemotherapy program was deleted in March 1987 after the 16 first patients because of the difficulty of feasibility.

2. Results

2.1 Evaluation of the toxicity

The toxicity observed in the 103 patients is similar to the first observations we presented and much lower in the most recently treated patients.

2.1.1 Bone marrow harvest

Following general anesthesia for bone marrow harvest, 3 of the first patients experienced a decrease of consciousness and enhancement of neurologic impairment or cranial hyperpressure which was suspected to be related to hydration administered during general anesthesia. The next patients received furosemide at the end of anesthesia and did not experienced these symptoms.

2.1.2 Hematological toxicity

Hematologic toxicity remained mild: 9 % of the patients experienced a grade 4 aplasia for neutrophil (PMNN) and 6% for WBC. Grade 3 toxicity was observed in 16% of the patients for PMNN and in 21% for WBC. Thrombopenia grade 4 was observed in 10% of patients, grade 3 in 18% and grade 0, 1 or 2 in 72 %. For patients with grade 3 or 4, the mean duration of thrombopenia is 10 days. Nadir for WBC, neutrophils is on the second week while nadir for platelets on the third week.

2.1.3 Extrahematological toxicity

Immediate tolerance of BCNU was good. Nausea and vomiting were mild: 31 grade 0, 58 grade 1 and 2, 9 grade 3.

Lung complications were observed 22 times in 18 patients: 3 embolisms of which 1 was fatal and 19 pneumonitis (18%). Fifteen occurred in the first two months. Among these 15 patients, 9 had partial pneumonitis while 2 had interstitial pneumonitis, life-threatening, requiring assisted ventilation (1 case fatal). Four patients experienced diffuse with 1 requiring assisted ventilation and fatal (surinfection with pneumocystis carinii). in 4 patients, pneumonitis occurred after 2 months in a context of tumor progression with neurologic degradation and corticosteroid treatment.

Hepatic toxicity was mild. 20 % of patients had a minor cytolysis with grade 1 and 2 increase of AST-ALT. 70 % of patients had GGT increased before BCNU probably due to the anticomital treatment and only 15% with normal GGT before BCNU, experienced GGT increased after treatment. Two patients presented with jaundice: 1 HBV hepatitis and 1 CMV.

Twenty-nine infectious episodes including pneumonitis and hepatitis already described were observed: 19 (66 %) in the first two months after BCNU. The others occurred later and are not related to the treatment program, intricated with the disease progression. Other complications were observed: phlebitis (n=6), toxidermia (n=4, in these patients, symptoms disappeared when phenytoin anticomital treatment was discontinued). Renal function remained normal except for 5 patients with a grade I toxicity.

Irradiation was performed as scheduled in 86 patients. The tolerance was good and the classical cerebral edema, often present at the initiation of the treatment could readily be controlled with appropriate medications. However, one patient died of a cerebral hemorrhage 3 days after the end of radiotherapy, with normal platelet count.

Finally, 32 critical events are recorded in the first month after ABMT and the total toxic death rate is 9% (8 patients): 1 septic shock, 1 hemorrhage, 1 pneumonitis, 1 brain toxoplasmosis infection, 3 septicemias (1 candida albicans, 1 staphylococcus, 1 unknown). One patient died from embolism that is not considered as a complication of treatment but of the disease itself.

2.2 Survival

Survival is the major evaluation criteria in this study as tumor response evaluation is difficult due to the surgical reduction before BCNU treatment.

The median follow-up of this study is now 44 months. Overall survival is 11% at 36 months with a median survival at 11 months after ABMT and 12 months after surgery for the whole group (103 patients). These results are comparable to those previously published in the literature ([3-5]).

Three prognostic factors were identified, histological grading, performance status and age. The median survival of patients with grade III tumors is 18 months compared to 10 months for those with grade IV. At 3 years 36 % grade III are surviving versus 4% for grade IV. Patients under 50 years with a good performance status (0, 1 or 2) have a median survival of 16 months versus 10 months for older patients or those with a poor PS, and versus 6 months for older patients with a PS>2. Complete resection was performed in 39 % of patients and was not associated with a better survival.

3. Conclusion

In conclusion, treatment with HD BCNU can be ethically considered in patients with high grade glioma since the toxicity is mild and the toxic death rate of the whole program is under 10%. After BCNU, patients can be monitored as out patients. Results remain poor for the whole group but quality of life is acceptable during the first year. Results are encouraging for patients with grade III astrocytomas. This procedure is still proposed for patients with a macroscopically complete resection of grade III astrocytomas. For other patients with residual tumor after surgery (grade III and IV), we designed a phase I-II trial of high dose fotemustine, a new nitrosurea compound with significant efficacy on high grade glial tumors in phase II trial at conventional doses ([7]).

PHASE I-I1 TRIAL OF HIGH DOSE FOTEMUSTIN (HDF) FOLLOWED BY ABMT IN HIGH GRADE GLIOMAS.

Fotemustin is a new nitrosurea with a significant antitumor activity in glioma. A response rate of 36% has recently been reported in patients with high grade gliomas at conventional doses (100mg/m^2/week, 3 weeks) ([7]).

This phase I-II trial was designed to determine the feasibility and tolerance of high (supraconventional) doses of fotemustin given with autologous marrow support in patients with high grade glioma. The second objective was to evaluate the antitumor activity of fotemustin in this setting.

1- Patients and methods:

1.1- Description of the patients.

Inclusion criteria were: age under 65 years, high grade astrocytoma with an evaluable residual tumor mass.

Twenty-five patients were included in this trial between April 1988 and May 1991 in the Centre Leon Berard. The median age was 43 with a range of 21 to 62. 18 were males, 7 were females.

13 had glioblastoma, 3 had grade IV astrocytoma, 5 had grade 3 astrocytoma, 3 had a high grade oligodendroglioma, 1 had a sarcomatous glioblastoma.

Primary surgery was a macroscopically complete excision in 2 patients, wide excision in 14, partial excision in 2 and stereotaxic biopsy in 7. Performance status according to the ECOG scale were as follows: ECOG 0: 7, ECOG 1: 10, ECOG 2: 5, ECOG 3: 3.

1.2- Description of the study

Treatment plan was comparable to that of HD-BCNU concerning cytoreductive surgery, monitoring after ABMT and radiation therapy. Bone marrow harvested was stored in liquid nitrogen until the date of ABMT.

Fotemustin was begun 48 hours after bone marrow harvest. Fotemustin was given in one hour infusion in two consecutive days. Half of the total dose was given day 1 and 2. The first dose level was twice the conventional dose: 600mg/m^2. Dose increase was 100 mg/m^2 for each level. The upper level was reached only if less than 2 grade 3 or 4 extrahematological toxicities were observed at the previous level. The hematological toxicity was not limitative for dose increment.5 patients were included at the 600mg/m^2 and 700mg/m^2 levels.4 patients

received 800mg/m^2. 7 patients received 900mg/m^2. 4 patients received 1000mg/m^2. Bone marrow was reinjected 72 hours after the second injection of fotemustin.

2- Results and discussion:

2.1. Extrahematological toxicity

The extrahematological toxicity of high dose fotemustin is depicted in table 1. 4 patients died during the first 2 months after the treatment with HDF. In three cases, the death is due to intracranial hyperpressure at least in part due to tumor progression. One of the patients had an associated systemic mycotic infection. The fourth patient died because of a leukoencephalopathy and radionecrosis.

Nine of the 15 patients who received HDF over 700mg/m2 experienced epigastric pain after HDF infusion. The characteristics of epigastric pain observed after HDF were as follows: pain began 4 to 6 hours after HDF infusion; it was isolated, brutal, severe and started in some patients immediately after the meal. All patients had normal gastroduodenal fiberoptic and liver ultrasound examinations. Anti spasmodic medications efficiently relieved this pain. We consider that this epigastric pain is an equivalent of vomiting or a gastric spasm.

2.2. Hematologic toxicity

Hematological toxicity was severe. 23 of the 24 (96%) evaluable patients had grade 3 (29%) or 4 (67%) toxicity on leukocytes. 23 of the 24 (96%) evaluable patients had grade 4 toxicity on PMNN. The median nadir for PMNN was on day 18. The median duration of WBC count under 1000/mL was 6 days (range 3 to 25). The median duration of PMNN count under 500/mL was 6 days (range 2 to 25).

Twenty-three of the 24 (96%) evaluable patients had grade 3 or 4 toxicity on platelets. The median nadir was 16000/mL and occurred on day 21. The median duration of platelet counts under 50000/mL was 21 days (range 2 to 53). Thrombopenia is clearly the major toxicity of this protocol.

We observed no significant correlation between the duration of neutropenia under 500/ml or thrombopenia under 50000/mL and the dose of fotemustin given. This indicates that the autologous marrow rescue was efficient in these patients.

2.3. Supportive care

The median stay in the hospital due to ABMT was 14 days with a range of 6 to 80. The median number of days with antibiotics was 14 days with a range from 5 to 40. The median number of platelets and red blood cell transfusions were 2 and 1 respectively.

2.4. Antitumor efficacy

Twenty-two of the 25 patients are evaluable for response. None of the patients achieved complete response to HDF. Partial responses to HDF were seen in respectively 8 (36%) of evaluable patients. Eight patients achieved stable disease (37%) and 6 (27%) progressed after HDF. The median survival of these patients is 10 months (Figure).

3. Conclusion

Hematological toxicity, in particular thrombopenia, is the major toxicity of HDF in these patients. The hematological toxicity is greater than that observed after high dose BCNU in the same patients. Extra hematological toxicity is mild, especially the liver toxicity. No lung toxicity was observed. Patients receiving more than 700mg/m2 in 2 days (>350mg/m2/d) experienced in more than 50% of the cases a severe gastric pain. The duration of hospitalization after HDF is higher than after HD BCNU in our experience but remains acceptable. The dose of 900mg/m2 of fotemustin in 2 days followed by ABMT is the maximum tolerable dose of this agent in single drug high dose therapy. In this phase I-II trial, the response rate to HDF in high grade glioma was not found different to that reported in a previous report with conventional dose (7). A phase II trial of HDF at the dose of 900mg/m2 in high grade glioma is ongoing in our institution.

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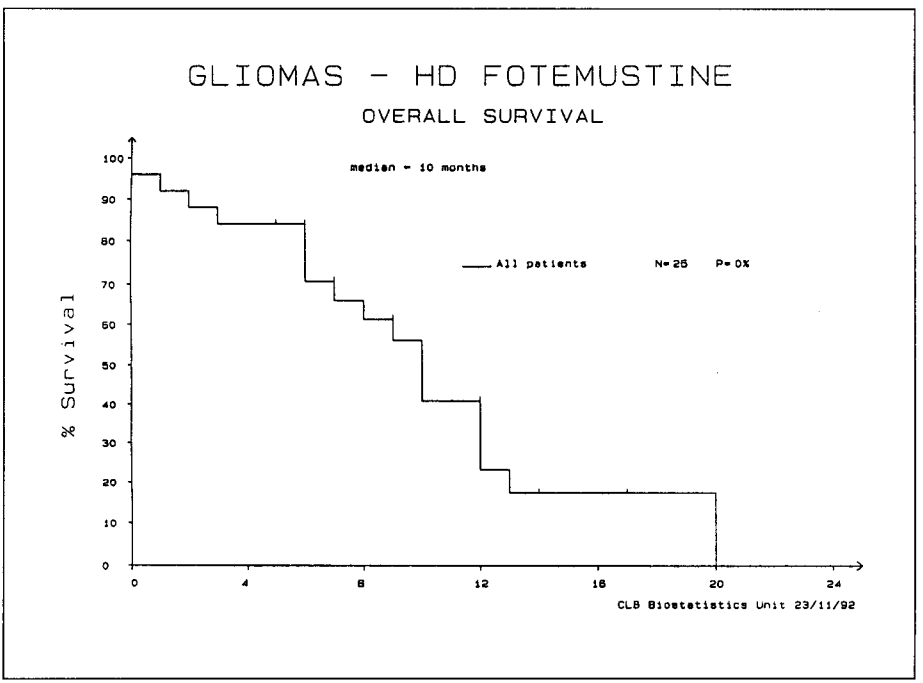
Table 1: Extrahematological toxicity of high dose fotemustin in patients with high grade gliomas.

WHOSCORE	NE	0	1	2	3	4
Nausea/vomiting	0	1	8	14	2	0
Venous	0	23	1	1	0	0
Fever	0	19	1	4	1	0
Hepatic						
gamma GT	1	0	6	9	7	2
ALT	1	11	10	0	2	1
Renal	1	21	3	0	0	0
Conciousness deterioration	0	23	1	0	0	1



Session XIII: Peripheral Stem Cells

INFLUENCE OF MINIMAL TUMOR CONTAMINATION OF HEMATOPOIETIC HARVESTS ON CLINICAL OUTCOME OF PATIENTS UNDERGOING HIGH DOSE THERAPY AND TRANSPLANTATION.

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INTRODUCTION

In high dose therapy protocols which employ reinfusion of harvested, cryopreserved autologous bone marrow or blood cells obtained by leukapheresis, as hematopoietic support, there is always a concern that contaminating tumor cells might be reinfused along with the hematopoietic cells (Sharp and Crouse, 1992; Sharp and Ressinger, 1992). Gribben and colleagues (1991) have shown that patients with low grade lymphoma who received a harvest purged of tumor cells so as to be judged negative using the polymerase chain reaction (PCR) for detection of rearrangements of the bc1-2 gene had a much better disease free survival than patients whose infused harvests were judged to still contain tumor cells. We have employed a sensitive culture technique which has the ability to amplify lymphoma cells and thus permit their detection in histologically normal appearing bone marrow and cytologically normal appearing apheresis products (Sharp et al. 1991; Sharp and Crouse, 1992). Using this technique we have shown that patients with intermediate or high grade lymphomas who receive a culture positive bone marrow harvest have a much poorer outcome than recipients of culture negative harvests (Sharp et al., 1992).

Similar culture methods performed using different media formulations can be employed to amplify and detect breast and epithelial gynecological cancer cells metastatic to bone marrow or circulating in blood harvested by apheresis. The detection system employed uses immunocytochemical staining for epithelial cell associated antigens such as epithelial membrane antigen (EMA) or cytokeratins that do not cross react with other cells found in blood or bone marrow (Sharp and Crouse, 1992). Culture of the harvest serves to provide for relative amplification of the tumor cells compared to the fresh population probably by promoting tumor cell growth but largely by facilitating loss by differentiation of the majority of normal nucleated hematopoietic cells. The culture period is not required provided one is willing to screen sufficient numbers of cells (often many hundreds of thousands) to establish an estimated frequency of tumor cell contamination in the harvest. Using these types of approaches several groups have now observed that histologically normal appearing marrow of patients with breast cancer is frequently contaminated with minimal disease. The frequency of contamination is generally greater with more advanced disease and minimal marrow contamination is clinically significant in nontransplanted breast cancer patients since it predicts for relapse (Diel et al., 1992; Sharp and

Crouse, 1992).

In the present report we update the clinical outcome of 65 patients with non-Hodgkin's lymphoma 20 of whom received autologous peripheral blood derived stem cell transplantation (PSCT) largely because their marrow was histologically and culture positive for lymphoma and 45 who received autologous bone marrow transplantation (ABMT). The ABMT patients received histologically negative marrows, however in 17 instances these marrows were culture positive for lymphoma. In addition, we present the outcome for 29 patients with breast cancer or other gynecological epithelial tumors whose marrow was histologically and/or culture positive for tumor cells and therefore, with the exception of one patient who died before transplant, they underwent PSCT. Culture studies evaluated retrospectively showed that 6 patients had apheresis harvests that grew abnormal, suspected epithelial tumor cells, 23 did not. The results overall demonstrate that minimal contamination of hematopoietic harvests is an important determinant of outcome of high dose therapy and hematopoietic cell transplantation.

MATERIALS AND METHODS

Bone marrow and/or peripheral blood stem cell harvests obtained by leukapheresis were examined histologically or cytologically, respectively, for the presence of tumor cells. Aliquots of these harvests were then placed into culture as described previously for non-Hodgkin's lymphoma patient samples (Sharp et al., 1991; Sharp et al., 1992) or breast cancer patient harvests (Sharp and Crouse, 1992) and aliquots obtained at various times subsequently and evaluated for the presence of tumor cells as described previously.

The frequency of tumor cell detection from both histologically and cytologically positive and negative harvests was noted. In addition clinical outcome in terms of whether or not patients achieved a complete remission (CR) when restaged at 100 days was noted and for those patients receiving a CR at 100 days, the time to subsequent disease progression was recorded. These data were then analyzed to determine the influence of tumor cells detected histologically or cytologically or by culture techniques, or both, on clinical outcome.

All of the protocols employed were approved by the University of Nebraska Institutional Review Board and patients voluntarily signed informed consent for these studies.

RESULTS AND DISCUSSION

Non-Hodgkin's Lymphoma: A total of 65 patients have now been followed for a median of 3 years with actuarial follow-up predicted to 5 years. Of these 65 patients 45 who had histologically negative marrows underwent ABMT. However 17 of these patients had culture positive marrows, 27 culture negative marrows and one was unevaluable. The remaining 20 patients had either histologically positive (19) or hypocellular (1) marrows and underwent PSCT. Two had tumor culture and/or molecular biology positive apheresis harvests, 18 were negative.

All grades of lymphoma were included since our experience suggests that primary refractory or relapsed low grade lymphoma patients have a poor outcome similar to the same categories of intermediate and high grade lymphoma

patients. Not surprisingly the PSCT group had a higher proportion of low grade patients (37%) compared to the ABMT group (9%) however this proportion was maintained among patients achieving a CR (33% versus 14%) and patients surviving disease free to the time of analysis (29% versus 14%). Consequently, the histological grade of the lymphomas was not a significant factor in differences in outcomes between the groups.

At 100 days post-transplant, 12 of 20 (60%) PSCT patients were in CR and all originally had histologically positive marrows but were recipients of culture negative apheresis harvests. Twenty-six of 45 (58%) evaluable ABMT patients achieved a CR at 100 days and 14 received culture negative marrow harvests, whereas 12 received culture positive harvests. Therefore there were no differences in the proportion of PSCT or ABMT patients achieving a CR at 100 days. There was no difference in the grade of lymphoma between patients with culture positive and negative harvests or between the patients achieving a CR and the entire group of patients.

There were however subsequent differences in outcome of these groups of patients. For patients achieving a CR at 100 days, the actuarial disease free survival projected to 5 years of the histologically marrow positive patients receiving PSCT with culture negative apheresis harvests was 58%, of the ABMT patients receiving culture negative bone marrow, 50% and the ABMT patients who received a culture positive marrow, 17%. These results indicate that tumor contamination of the infused harvest is associated with a significantly poorer outcome. This further suggests that the most likely cause of progression of disease in patients who achieve a CR at 100 days is re-infusion of tumor although we cannot exclude that culture detection of tumor is a surrogate which identifies more aggressive tumors. A very significant proportion of patients experience progression despite receiving culture negative harvests therefore tumor which survives high dose therapy also remains a major problem.

These results also suggest that unless investigators are going to undertake rigorous screening of marrow harvests for the presence of minimal disease, they would be more likely to achieve a superior clinical result by employing PSCT rather than ABMT.

Breast Cancer and Gynecological Epithelial Tumors: This series comprises 29 patients who had breast cancer (23) or cancer of the ovary or cervix (6). They are part of a larger series of patients of whom those with histologically negative and culture negative bone marrow underwent, or are candidates for, ABMT. However, there are too few ABMT recipients to date to make a meaningful comparison of ABMT versus PSCT for these patients. The 29 patients above all were scheduled to undergo PSCT because of a marrow abnormality, largely histological evidence or otherwise culture positivity for tumor or hypocellularity. The apheresis harvests were also subjected to evaluation by culture for the presence of suspected malignant epithelial cells. Note that we have not been able to confirm that these are malignant tumor cells, for example, by growth in immunodeficient mice, potentially because too few cells (by several logs) are obtained for evaluation. Of the 29 patients, 6 had suspicious epithelial cells in their apheresis harvest, one patient died before transplant and three others shortly after transplant. Only 1 of 5 patients transplanted with a suspicious harvest is currently alive. Of the 23 patients receiving culture negative harvests, 11 (48%) are alive

with no evidence of systemic disease on a curve projected actuarially to three years with a median follow-up of 2 years. This result suggests that the detection of suspected tumor cells in the circulation or apheresis harvests of patients with epithelial tumors is a marker of advanced disease and is associated with a poor outcome following transplantation. The survival of all transplanted patients falls off rapidly to about 60% in the first 6 months post-transplant suggesting better preparative regimens are needed. Despite these reservations, the outcome of the poor prognosis marrow positive patients in this series undergoing PSCT with culture negative harvests is encouraging and suggests that PSCT is a very viable alternative to ABMT for the significant proportion of patients with minimal marrow involvement. Patients whose marrow and apheresis harvest are both positive for tumor clearly are candidates for transplantation with purified CD34 positive hematopoietic cells (Bensinger et al., 1992).

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THE RECOMBINANT HUMAN LIGAND FOR *C-KIT* ENHANCES THE IN VIVO BIOLOGICAL EFFECTS OF RECOMBINANT HUMAN GRANULOCYTE COLONY-STIMULATING FACTOR FOR STIMULATING LEUKOCYTOSIS AND CIRCULATION OF HEMATOPOIETIC COLONY-FORMING PROGENITOR CELLS IN PRIMATES.

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The Fred Hutchinson Cancer Research Center, Seattle, Washington, USA; the University of Washington Regional Primate Research Center, Seattle, Washington, USA; Amgen, Inc, Thousand Oaks, California, USA. Supported by Grants and Contracts NO1-AI-85003, NIHRROO166, and CA39492 from the National Institutes of Health, and Amgen, Inc.

The ligand for *c-kit*, called stem cell factor (SCF), mast cell growth factor (MGF), kit ligand (KL), and Steel factor or Steel locus factor (SIF) [1-4], is a growth factor with pleiotropic effects that enhances the proliferation of hematopoietic colony-forming cells (CFC)in response to specific hematopoietic growth factors[5-7]. By itself, SCF stimulates little proliferation of CFC but prolongs the survival of CFC in vitro. In mice, SCF administration corrects many of the hematopoietic defects in SI/SId mice that have mutant SCF genes [1]. SCF administered to normal mice produces a spectrum of biological effects within the hematopoietic system, including the expansion of cells that reconstitute hematopoiesis after transplantation into W/Wv mice [8].

We have investigated the biological effects of the recombinant human ligand for *c-kit* (rhSCF) on in vivo hematopoiesis in baboons, with particular interest in the trafficking of hematopoietic progenitor cells. We showed previously that rhSCF is biologically active on baboon hematopoietic progenitor cells in culture, as it acts synergistically with G-CSF, GM-CSF, IL-3, Epo, and IL-6 to enhance the in vitro proliferation of individual CFC as well as increasing the number of CFC that proliferate in vitro in the presence of these factors (9). Monoclonal antibodies to the human c-*kit* molecule, that competitively block rhSCF binding, also bind to the subpopulation of baboon marrow cells that express CD34 which includes virtually all progenitor cells as well as transplantable hematopoietic cells. This suggests that baboons may provide a model for studying the in vivo biological effects of rhSCF on hematopoiesis.

RhSCF administered to baboons by either continuous intravenous infusion or subcutaneous injection stimulates a dose dependent leukocytosis, with increases in white blood cells of multiple types in the circulation, including neutrophils, monocytes, eosinophils, basophils, and both T and B lymphocytes (9,10). RhSCF also stimulates a reticulocytosis and increases the number of circulating red blood cells and the hematocrit. Platelet counts initially decrease slightly, after which they return to pretreatment levels, and then increase slightly above pretreatment values after administration of rhSCF is stopped.

Administration of rhSCF at doses of 200 mg/Kg/day results in an approximate doubling of marrow cellularity, as determined by sequential marrow biopsies (9,[10]). The marrow cellularity doubles after 7 days of SCF and remains hypercellular throughout the 28 day period of SCF treatment. At doses of 50 mg/Kg/day or less marrow cellularity is not detectably altered. The frequency of megakaryocytes in the marrows of treated animals also increases proportionately for the overall increase in marrow cellularity. Of interest, the frequencies of morphologically immature cells, erythroblasts, myeloblasts and promyelocytes, increase by 3 to 5 fold in marrows of animals after 7 days of SCF at 200 mg/Kg/day. This is accompanied by a change in the ratio of myeloid to erythroid cells (M:E ratio) in marrow aspirates from a pretreatment value of approximately 5:1 to almost 1:1 as well as the doubling of marrow cellularity. However, with continued administration of rhSCF the frequency of morphologically immature cells in marrow aspirates and the M:E ratio return to pretreatment values in the face of persistently hypercellular marrows.

Given the in vitro findings that rhSCF enhances the proliferation of CFC we then examined the effects of rhSCF administration on hematopoietic progenitor cells in marrow and the circulation. In marrow, the frequencies of the different CFC types, CFU-GM, BFU-E, CFU-MIX, and HPP-CFC (including colonies with diameter of > 1.0 mm), remain unchanged or increase modestly, during periods of rhSCF administration (10). Given the finding that the marrow cellularity doubles, this strongly suggests that the total number of CFC of these different types increases in marrow.

In blood, hematopoietic CFC can be quantified more readily. Normally, CFC can be detected in the circulation only at very low frequencies. Administration of rhSCF to baboons stimulates a dose dependent increase in both the relative frequencies (per 10^5 cells) and the absolute number (per ml of blood) of CFU-GM, BFU-E, CFU-MIX, and HPP-CFC in the circulation ([10]). In SCF treated animals there is no change in numbers of circulating CFC during the first 4 days of treatment following which there is a rapid rise on the fifth day that is maintained throughout treatment.

Studies in mice have provided evidence that SCF administered concomitantly with G-CSF may have synergistic effects on stimulating leukocytosis ([11,12]). In splenectomized mice, McNiece and colleagues found that SCF and G-CSF administered together induce higher leukocyte counts than either G-CSF or SCF alone ([11]). Also, the number of progenitor cells present in circulation is increased by the combination of factors over that observed with either factor alone. These cells are capable of engrafting lethally irradiated mice. Whether these progenitor cell populations also include increased numbers of pluripotent stem cells capable of serial transplantation remains to be determined.

We now have evidence that administration of low doses of rhSCF to baboons can result in synergistic interactions with G-CSF in vivo when both factors are administered at the same time. We studied 4 groups of baboons (3 animals per group) that were treated for 14 days with either SCF (25 mg/Kg/day) alone, G-CSF (100 mg/Kg/day)alone, the combination of G-CSF and SCF, or SCF alone for 7 days followed by G-CSF alone for 7 days.

Animals administered SCF alone at a dose of 25 mg/Kg/day did not develop a leukocytosis (Table 1). In 2 of these 3 animals there was no evidence for

a significant increase in the number of circulating progenitor cells, similar to previously published observations [10].

In contrast, all 3 baboons treated with G-CSF alone at 100 mg/Kg/day had a rapid rise in peripheral blood leukocyte counts, consisting primarily of increased numbers of mature neutrophils, that was evident after 2 days and reached a peak (Table 1) by day 12 of treatment. The total number of detectable CFC in the circulation increased significantly in 2 of the 3 animals treated with G-CSF alone. The maximum number of CFC in the circulation of animals treated with G-CSF alone was greater than that for animals treated with SCF alone.

Animals administered G-CSF (100 mg/Kg/day)and SCF (25 mg/Kg/day) together for 14 days had leukocyte counts increase at a significantly more rapid rate than did animals treated with G-CSF alone, and the maximum white blood cell counts achieved at days 12 to 14 of treatment were approximately 50% higher than in animals treated with G-CSF alone. Importantly, CFC per ml of blood in the circulation of these animals were increased more than 5 fold when compared to animals treated with either G-CSF or SCF alone. The kinetics of CFC trafficking were different than those seen in animals treated with G-CSF alone. Prior to treatment the total of all CFC in the blood were 2.1 ± 1.9 per 10^5 leukocytes (184 ± 216 per ml of blood). After 2 days of treatment with both G-CSF and SCF the total of all CFC was still only 0.5 ± 0.4 per 10^5 leukocytes (259 ± 224 per ml of blood). However, by day 5 of treatment the number of CFC had risen to 46 ± 6.7 per 10^5 leukocytes ($32{,}394 \pm 5{,}328$ per ml of blood) after which time the number of CFC in the circulation remained elevated and continued to increase through day 12 of treatment.

Animals given rhSCF for 7 days followed by G-CSF showed no significant changes in leukocytes or circulating CFC during the first 7 days. During the second week, the increases in leukocytes and CFC observed were similar to those observed during the first 7 days in animals administered G-CSF alone (data not shown). Thus, administration of rhSCF alone prior to G-CSF did not enhance the biological response to subsequent G-CSF administration, in contrast to administration of the two factors together. In these studies none of the animals had evidence for adverse reactions to the treatment.

What is not evident from these studies is if the cells induced to circulate by treatment with these factors include progenitors and/or stem cells capable of reconstituting lymphohematopoiesis in vivo. In humans [13] and mice [11,14,15] treated with G-CSF, peripheral blood cells contain increased numbers of cells that are capable of engrafting and rescuing lethally irradiated animals. Recently, we have shown that treatment of baboons with rhSCF also stimulates the circulation of cells capable of engrafting in vivo [16]. These were, however, autologous transplants. Therefore, there were no markers to distinguish transplanted from residual host cells and the long term repopulating abilities of these cells thus remains in question. In splenectomized mice, SCF also stimulates the circulation of transplantable progenitor and/or stem cells that reconstitute stable in vivo hematopoiesis [11]. Blood cells from splenectomized mice treated with both SCF and G-CSF also appear capable of rescuing lethally irradiated mice. Whether there are qualitative as well as quantitative differences between cells isolated from mice treated with both G-CSF and SCF as compared with those treated with G-CSF alone remains to be determined.

In summary, the ligand for c-kit has biological synergy both in vivo and in vitro with G-CSF. In vivo, the effects of G-CSF on the absolute leukocyte count and on the numbers of circulating progenitor cells are markedly enhanced by the simultaneous administration of rhSCF, but not by prior administration of rhSCF. In vitro, rhSCF enhances the proliferation of CFC and precursors of CFC that are stimulated by G-CSF. It is possible that such synergistic interactions may have clinical importance if circulation of transplantable "stem" cells also is enhanced or if stem cells in marrow are increased. Further studies to examine the in vivo synergy of rhSCF with other hematopoietic growth factors may provide novel therapeutic approaches.

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Table 1. In Vivo Synergy in Baboons: Administration of SCF (25 mg/Kg) and G-CSF (100 mg/Kg) Together Stimulate Greater Increases in the Numbers of White Blood Cells and Colony-Forming Progenitor Cells in Peripheral Blood Than Are Stimulated by Either G-CSF or SCF Alone

	DAY OF FACTOR ADMINISTRATION		
	DAY 0	**DAY 2**	**DAY 12**
G-CSF + SCF			
WBC/mL	9,067 + 1,721* (7,100 -10,300)@	47,500+13,952 (31,600 - 57,700)	125,900+29,978 (92,200 - 149,600)
CFC/10^5 PBL	1.8+0.7 (1.0 - 2.3)	0.5+0.4 (0 - 0.7)	72.2+32.4 (36.6 - 100.0)
CFC/ml Blood	157+62 (103 - 225)	259+224 (0 - 404)	91,033+52,123 (49,739 - 149,600)
G-CSF ALONE			
WBC/mL	7,330+950 (6,300 - 9,000)	32,433+6,747 (27,400 - 40, 100)	84,967+22,872 (75,800 - 111,000)
CFC/10^5 PBL	1.2+1.1 (0 - 2)	12.9+14.0 (1.0 - 28.3)	18.9+13.7 (4.6 - 32.0)
CFC/ml Blood	84+72.8 (0 - 128)	3,642+3,753 (401 - 7,754)	14,327+9,595 (5,106 - 24,256)
SCF ALONE			
WBC/mL	7, 100+2,900 (4,200 - 10,000)	6, 133+1,955 (4,500 - 8,300)	9,733+3,917 (6,300 - 14,000)
CFC/10^5 PBL	2.1+1.9 (0.7 - 4.3)	4.7+0.4 (4.3 - 5.0)	44.7+62.1 (2.6 - 116.0)
CFC/ml Blood	184+216 (430 - 29)	288+25 (225 - 390)	5,842+9,085 (231 - 16,324)

*data are mean $\pm$ 1 standard deviation for three animals in each group, prior to treatment (DAY 0), and after 2 and 12 days of administration of G-CSF alone at 100 mg/Kg/day, rhSCF alone at 25 mg/Kg/day, or both G-CSF at 100 mg/Kg/day and rhSCF at 25 mg/Kg/day together.
@data in parentheses represent the range of data from which the mean and standard deviations were derived. All factors were administered as single daily subcutaneous injections. CFC include CFU-GM and BFU-E grown in culture as previously described (10,16)

AUTOGRAFTING WITH G-CSF-MOBILIZED BLOOD STEM CELLS IN PATIENTS WITH CHEMOSENSITIVE MALIGNANCIES

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SUMMARY

Thirty-four patients (pt) with chemosensitive malignancies were treated with G-CSF either during steady-state hematopoiesis or starting 24 hours after cytotoxic chemotherapy. Blood stem cell collection was performed and monitored by assessment of CD34+ cells in the peripheral blood (PB) ($R = 0.84$, $p < 0.001$ for CD34+ cells/ul of PB and the number of CD34+ cells/kg harvested per leukapheresis). The yield of hematopoietic progenitor cells (CFU-GM and BFU-E) varied substantially between individuals and was, mainly dependent on the amount of previous chemotherapy. The 15 patients with $> 5 \times 10^6$/kg CD34+ cells in their autografts had significantly less cycles of previous chemotherapy (3 versus 11, $p < 0.001$). However, patients receiving G-CSF post-chemotherapy had a higher collection efficiency compared with the administration during steady-state hematopoiesis. Analyzing the antigenic profile of the CD34+ cells, the proportion of CD34+/HLA-DR- or CD34+/CD38- cells representing noncommitted hematopoietic stem cells was consistently < 5%. In contrast, the vast majority of CD34+ cells was found to coexpress the lineage associated markers CD33 or CD71. A distinct population of CD34+ cells expressing CD19 above the control level was not detectable suggesting that early lymphoid progenitor cells are not induced by G-CSF to enter the circulation. Following high-dose conditioning therapy 22 patients were autografted with the G-CSF-exposed blood stem cells. Except for two patients (one toxic death, one early relapse), complete engraftment was achieved after a median time of 15 days for 0.5×10^9/l neutrophils and 13 days for 20×10^9/l platelets. The number of CD34 positive cells transplanted proved to be highly predictive for platelet recovery ($R = -0.74$, $p < 0.001$). Patients transplanted with more than 5×10^6/kg CD34+ cells reached an unsubstituted platelet count $> 20 \times 10^9$/l with a median time of only 10 days. Our data demonstrate that complete and sustained engraftment can be achieved following myeloablative pretransplant conditioning therapy with G-CSF-exposed blood stem cells without additional bone marrow support or growth factor administration.

INTRODUCTION

Peripheral blood stem cells (PBSC) are being increasingly used for autografting in patients with malignant diseases to circumvent the myelotoxic effects of high-dose therapy. During the past few years, different cytokine-based mobilization regimens have been explored to improve blood stem cell collection. Recently, G-CSF has emerged as a potent mobilizing agent.[2,3]

It was the objective of our study to evaluate the efficacy of G-CSF to mobilize PBSC when administered during "steady-state" hematopoiesis or "post-chemotherapy". Furthermore, we analyzed parameters predictive for the yield of hematopoietic progenitor cells and factors influencing the individual PBSC harvest. Finally, the kinetics of hematological reconstitution following high-dose conditioning therapy and autografting with G-CSF-exposed PBSC were studied.

PATIENTS AND METHODS

Patients

We studied 34 patients with chemosensitive malignancies who received G-CSF for the mobilization of PBSC either during steady-state hematopoiesis or following cytotoxic chemotherapy. The patient characteristics are summarized in Table 1 and the treatment plan is shown in Figure 1. The chemotherapeutic regimens used were: Dexa-BEAM, HAM (high-dose ara-C/mitoxantrone); CHOP, VIP (etoposide/ifosfamide/cisplatin); IE (ifosfamide/epirubicine); high-dose cyclophosphamide.

Blood Stem cell collection and cryopreservation procedure

Leukaphereses were started when white blood counts (WBC) > 1.0×10^9/l (post-chemotherapy) or > 10.0×10^9/l (steady-state) were reached and completed when at least 0.4×10^9/kg total nucleated cells (TNC) had been obtained. Blood stem cell collection and cryopreservation were performed as previously described.[4]

Pretransplant conditioning regimen

The following high-dose conditioning regimens were used: TBI/CY, BEAM, and the CBV protocol.

Clonogenic assay for hematopoietic progenitor cells

The concentration of hematopoietic progenitor cells in each single leukapheresis product and in the peripheral blood was assessed using a semi-solid clonogenic culture assay (Terry Fox Laboratories, Vancouver, Canada).

Immunofluorescence staining and flow cytometry

For dual color immunofluorescence analysis 20 ul of whole blood or 1×10^6 mononuclear cells of the leukapheresis products were incubated for 30 min at 4°C with the fluorescein (FITC)-conjugated monoclonal antibody (moAb) HPCA2 (CD34).

We used a forward scatter versus CD45 (FITC-HLel) fluorescence dot plot to discriminate between the smallest lymphohematopoietic cell population and erythrocytes or debris. The CD34+ cell population was analyzed in a FL versus SSC scatter plot. Only cells with lymphoid or lymphoblastoid characteristics (low SSC) were counted as CD34+ cells and further assessed for their FSC characteristics.

RESULTS

Circulating hematopoietic progenitor cells

The G-CSF-related mobilization of circulating hematopoietic progenitor

cells and CD34+ cells observed in the "steady-state" and "post-chemotherapy" patient group is summarized in Fig. 2. On average, patients receiving G-CSF following cytotoxic chemotherapy tended to have a higher increase of circulating CFU-GM and BFU-E. Moreover, a typical rebound of committed hematopoietic progenitor cells only occurred in patients receiving G-CSF post-chemotherapy with maximal values of 11,375 CFU-GM/ml and 14,250 BFU-E/ml, respectively. The expansion of colony-forming units was paralleled by an increase of CD34+ cells. On average, G-CSF treatment during steady-state hematopoiesis resulted in a peak of 13.2 $\pm$ 5.6/ul of PB (mean $\pm$ SEM), while the mean peak level in the post-chemotherapy group was approximately 5-fold higher (63.1 $\pm$ 17.1/ul; $p < 0.05$). Concentrations of circulating CD34+ cells exceeding 100/ul were only found during G-CSF-enhanced marrow recovery following cytotoxic chemotherapy.

Blood stem cell collection

A minimum of 0.4×10^9/kg TNC was the target quantity for blood stem cell collection. To compare the collection efficiency within the two patient groups, we determined the number of CFU-GM, BFU-E and CD34+ cells harvested per kg bodyweight in each leukapheresis product (41 for "steady-state" and 167 for "post-chemotherapy" patients). Statistical analysis revealed that the number of CD34+ cells per leukapheresis was significantly higher in patients harvested following G-CSF-supported cytotoxic chemotherapy (Figure 3), while the number of CFU-GM and BFU-E collected tended to be higher without reaching the level of statistical significance.

We further analyzed peripheral blood parameters indicating the content of hematopoietic progenitor cells in the respective leukapheresis product. As expected, the quantity of CFU-GM/kg or BFU-E/kg harvested per leukapheresis was closely related to the number of circulating CFU-GM and BFU-E. However, this finding could not affect the immediate decision to start or continue with the leukapheresis procedures. Far more important is the strong relationship between the number of CD34+ cells in the peripheral blood and the respective leukapheresis product which is reflected by a correlation coefficient of $R = 0.84$ ($p < 0.001$). In contrast, neither the WBC nor MNC count proved to be useful in predicting the yield of hematopoetic progenitor cells.

Another significant finding was that 15 patients with autografts containing more than 5×10^6 CD34+ cells/kg had less cycles of previous chemotherapy (median 3 versus 11, $p < 0.001$). This finding should be considered in future therapeutic strategies incorporating blood stem cell collection for high-dose therapy.

Hematological reconstitution and toxicity after autografting with G-CSF-exposed blood stem cells

A total of 22 patients were autografted following high-dose conditioning therapy. Two patients are not evaluable for hematological reconstitution: one patient with NHL died of a respiratory distress syndrome 32 days post-transplantation, and another patient with ALL relapsed on day 66 without having reconstituted. The remaining 20 patients achieved stable trilineage engraftment. The kinetics of hematological reconstitution are summarized in Fig. 4.

Interestingly, patients autografted with > 5 x 10^6/kg CD34+ cells reached an unsubstituted platelet count of >20 x 10^9/l within a median time of only 10 days (6-13). The treatment-related toxicity was moderate, reflected by a median duration of fever > 38.5°C of only 3 days (1-14). The hospital stay following autografting was 24.5 days (17-47). At the time of writing, 4 patients had relapsed after a median time of 64.5 days (51 -141). Seventeen patients are alive with a median event-free survival of 3 months (1-12).

DISCUSSION AND CONCLUSION

Blood-derived hemopoietic progenitor cells can be successfully mobilized by G-CSF either administered during steady-state hematopoiesis or following cytotoxic chemotherapy.[2-4] We found that the G-CSF-related mobilization capacity was higher during chemotherapy induced marrow recovery resulting in substantial rebound levels of CFU-GM and BFU-E as well as CD34+ cells.

In order to harvest sufficient quantities of hematopoietic progenitor cells as soon as a distinct population of CD34+ cells is detectable in the PB, monitoring of blood stem cell collection was performed according to the level of circulating CD34+ cells. Analyzing factors associated with a low increase of circulating CD34+ cells, the amount of previous chemotherapy was of major importance. A similar finding has been reported by Brugger et al.[6] Patients with autografts containing more than 5 x 10^6/kg CD34+ cells had significantly less cycles of previous chemotherapy. This finding is of clinical relevance because patients eligible for high-dose therapy and PBSC autografting should be identified as early as possible during the course of their disease to ensure blood stem cell harvesting before hematopoiesis is compromized by multiple cycles of chemotherapy.

Following high-dose conditioning therapy, including two TBI-containing regimens which are considered as myeloablative [7], the G-CSF-exposed blood stem cells were capable of restoring hematopoiesis. In patients autografted with more than 5 x 10^6/kg CD34+ cells, platelet recovery was observed after a median time of only 10 days. The kinetics of hematological reconstitution in our patients exclusively reflect the restorative capacity of the blood stem cell autografts, because no additional bone marrow support or hematopoietic growth factors were given post-transplantation.

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Table 1 Patients Characteristics

Number	**34**	
Age (years)	**36 (18 - 53)**	
Male/Female	**19/15**	
	No. of courses steady-state / post-chemotherapy	
Total	**10**	**33**
Diagnosis		
ALL	**1**	**1**
Solid Tumors	**2**	**7**
Hodgkin's disease	**1**	**13**
NHL	**3**	**9**
Multiple myeloma	**3**	**3**
Disease status prior to PBSC collection		
CR	**4**	**4**
PR	**4**	**26**
Active disease	**2**	**3**
Bone marrow involvement		
at any time	**4**	**12**
during harvesting	**3**	**7**
Previous chemotherapy		
No. of cycles	**6.5 (0 - 21)**	**7 (0 - 21)**

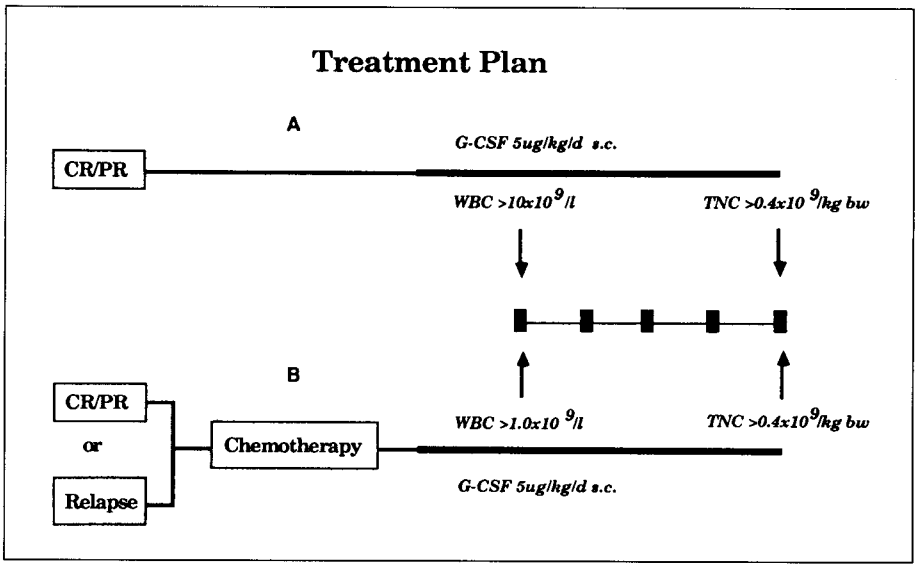

Figure 1: Treatment plan for peripheral blood stem cell collection in the "steady-state" (A) or "post-chemotherapy" (B) patient group.

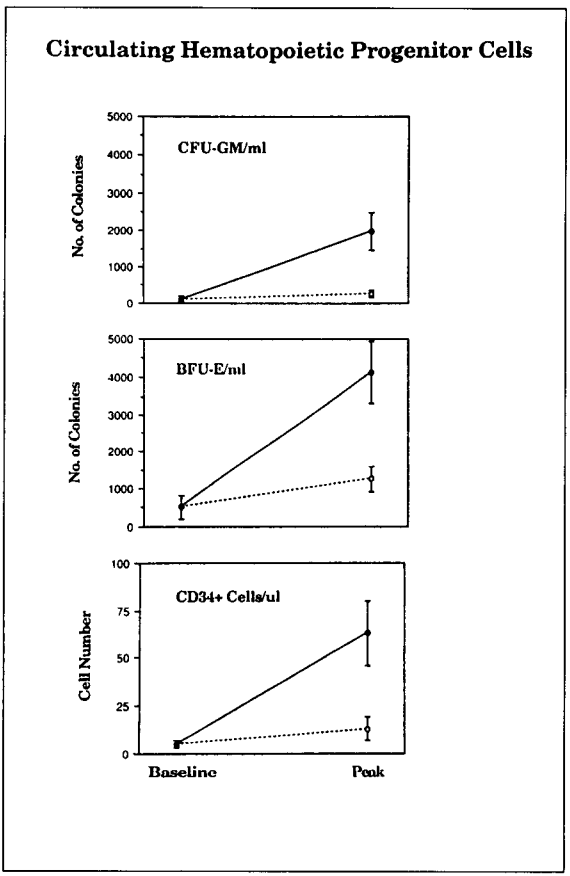

Figure 2: Number of circulating hematopoietic progenitor cells before and during G-CSF administration. The mean peak values for the "steady-state" (- -o- -) and "post-chemotherapy" (—o—) patients are shown.

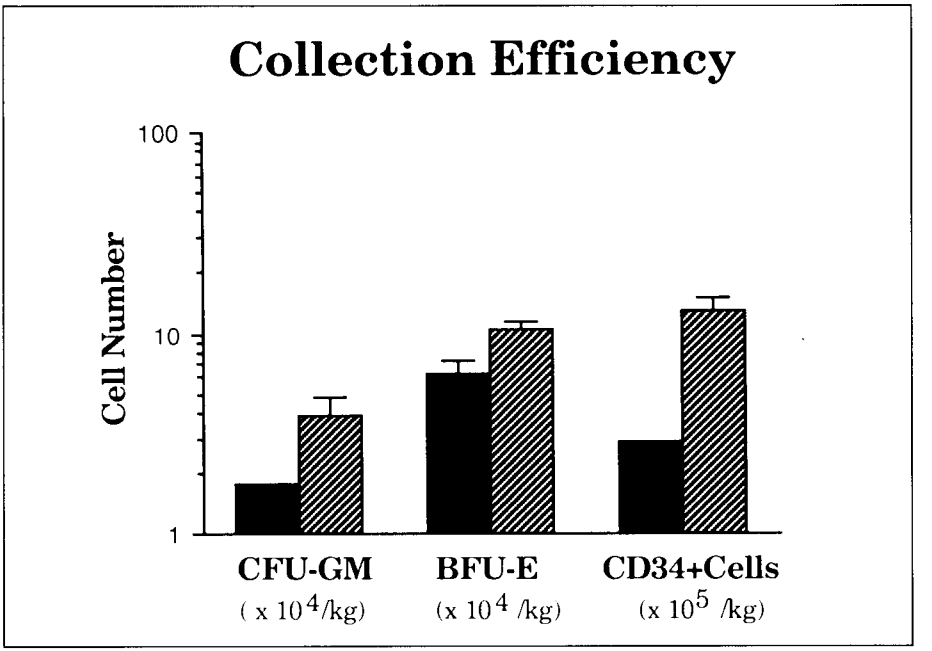

Figure 3: Collection efficiency per leukpheresis in the "steady-state" (■) and "post-chemotherapy" (▨) patient group.

EFFECTS OF TWO SCHEDULES OF ADMINISTRATION OF RH-GM CSF IN THE COLLECTION OF PERIPHERAL BLOOD STEM CELLS (PBSC) OF LYMPHOMA PATIENTS.

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INTRODUCTION

Marrow harvest is feasible in most lymphoma patients and it has been the major source of stem cells for autograft [1]. Nevertheless, marrow aspirate is sometimes impossible, particularly in patients with prior marrow involvement, marrow fibrosis, or in case of prior extended field radiotherapy involving the pelvis. Peripheral blood stem cells (PBSC) are an alternative to bone marrow in such patients [2,3]. Moreover, it has been speculated that the contamination of the transplant by the residual disease in case of prior marrow involvement might be reduced by collecting stem cells in the peripheral blood rather than in the marrow [4]. PBSC were initially harvested during a steady phase of haematopoiesis [5], but 6 to 14 cytapheresis were necessary for each collection. The number of cytapheresis can be reduced if collection is performed within the 6 to 10 days following a short aplasia induced by a conventional course of chemotherapy [6].

Recombinant granulocyte macrophage colony stimulating factor (rh GM-CSF)is a potent haematopoietic growth factor which has been shown to reduce the duration of neutropenia after conventional chemotherapy [7] and after myeloablative-autograft regimens [8,9]. Recent data demonstrate that rh GM-CSF dramatically increases PBSC circulation in peripheral blood [10,11]. Anyhow, we found no data that indicates the better way to deliver rh GM-CSF for an optimal PBSC collection. A major issue is to determine whether a long course of rh GM-CSF before collection is necessary to collect PBSC or if a short course of growth factor can give the same result. Another problem is to establish whether it is essential to collect PBSC during the haematological recovery phase following aplasia - which needs a particular attention to the haematopoietic kinetics -, or if rh GM-CSF is able to give rise to the circulation of a sufficient quantity of PBSC high enough to sustain engraftment.

In a prospective randomized trial, we have investigated two schedules of administration of rh GM-CSF given subcutaneously for PBSC harvest :in one arm (A), rh GM CSF was given from the end of chemotherapy until the completion of leukapheresis performed as soon as WBC reached $10^9/1$ (recovery phase). In the other arm (B), rh GM-CSF was administered after complete haematological recovery defined by stable WBC and platelet counts. We report herein the results of the collection procedures as well as the efficacy in terms of engraftment of both types of PBSC harvested.

MATERIALS AND METHODS PATIENTS.

Patients with histologically confirmed lymphoma assigned to receive autologous transplantation were eligible. In this single-institution trial, patients were randomized before the beginning of the preparative chemotherapy. Ten patients had been initially randomized between the two arms (A and B), 5 in each arm, when the very bad haematological reconstitution after transplant in arm B (see below) prompted us to stop the randomization, and to continue the collection according to the arm A, which gave a rapid haematological reconstitution.

Twenty one patients, 19 men and 2 women were included in the protocol. Ten were randomized, 11 were arbitrarily assigned to arm A. Median age was 36 years (range 16-67). Eight patients had Hodgkin's disease. The remaining 13 patients had non Hodgkin's lymphoma classified as high grade in 5 patients and low grade in 8 patients. A history of prior marrow involvement was present in 18 patients, either at diagnosis (10 patients) or at relapse (8 patients). Seven patients were in first complete remission (CR) at the time of inclusion. Five patients had received extended field radiotherapy. At the time of inclusion, median haemoglobin level was 12.1 g/dl (range 100-134 g/dl), median ANC was 4.4 10^9/1 (range 2.0-10.5 10^9/l), median platelet count was 300 10^9/1 (range 141-741 10^9/1). The characteristics of the patients were not different between the two groups (table 1).

HARVESTING PROCEDURES (FIGURE 1).

All patients had PBSC collected after a conventional chemotherapy regimen named "MINE regimen": mitoxantrone 10 mg/m^2/d day 1-2, etoposide 100 mg/m^2/d d 1-2-3, ifosfamide 1500 mg/m^2/d d 1-2-3 and methotrexate 100 mg/m^2/d d 1. They all received rh GM CSF (Schering Plough®/Sandoz® Laboratories) subcutaneously, at the dosage of 5 mg/kg body weight/day. Patients assigned to arm A (recovery phase arm) received rh GM-CSF from day 5 after the beginning of chemotherapy until the end of the harvesting procedures which began as soon as WBC were 10^9/1. Those who were assigned to arm B (steady phase arm) received rh GM-CSF after at least one week with a normal haemogram following aplasia until completion of stem cells collection (Figure 1). Leukapheresis were initiated three days after the start of rh GM-CSF administration. Peripheral blood stem cells were collected with a Haemonetics® model V50 apheresis device (Haemonetics®, Braintree, MA) using the lymphosurge program of the V50 processor. The duration of each procedure was about 3 to 4 hours. Cytapheresis products were centrifuged to eliminate platelets. The estimated minimal number of nucleated cells collected required for graft was determined according to our past series of patients grafted with PBSC, and was at least 5 x 10^8 nucleated cells per kg body weight. The buffy coats were frozen in teflon capton bags with 10% dimethyl sulfoxyde and stored at -196° C.

TRANSPLANTATION

Seventeen of the twenty one patients were autografted, 13 in arm A, 4 in arm B. Three patients of arm B were transplanted with PBSC collected according to the above procedures. The fourth patient was transplanted with bone marrow

cells because of the bad haematological recovery observed in the first three patients of this arm. Conditioning regimens were either fractionated-TBl/Cy in 6 patients, or BEAM regimen in 11 patients.

STATISTICAL ANALYSIS

The c^2 test and the non parametric Mann-Witney test were used to compare the main clinical and biological parameters between the two groups of patients. The stepwise multiple regression procedure was used to determine factors associated with the number of CFU-GM collected. The product-limit estimates of Kaplan and Meier and the log rank method were used to test the difference of duration of cytopenia between the two groups.

RESULTS

Harvesting procedures (table2). The duration of neutropenia was significantly shorter (p=0.04)in arm A (0 to 10 days, median 6 days) than in arm B (7 to 13 days, median 12 days). The deepness of neutropenia was not influenced by the administration of rh GM-CSF with a median nadir of PMN of 0.39 x 10^9/1 (range 0.01-1.45 x 10^9/l) in arm A versus 0.14 x 10^9/l (range 0.01-0.24 x 10^9/l) in arm B. In arm B, after CBC had reached a plateau, rh GM-CSF was began 3 days before leukapheresis, whereas a 4 to 16-day stimulation by rh GM-CSF (median 10 days) preceded leukapheresis in arm A. Thus, the period of time between the beginning of chemotherapy and the collection was greater in arm B (30 to 36 days, median 35 days) than in arm A (7 to 28 days, median 15 days). Two to five leukapheresis were necessary in arm A to harvest this amount of cells versus 3 to 4 in arm B. No significant difference was found between the two arms when considering the number of nucleated cells and of CFU-GM, whether the number of cells was expressed as an absolute number or as the number of cells per leukapheresis.

The number of nucleated cells and of CFU-GM cells collected in both arms were comparable to that of a personal historical series of 10 patients whose PBSC had been collected during the recovery phase from aplasia without the support of a growth factor. The only parameter which correlated to the number of CFU-GM cells collected was the number of circulating CFU-GM cells before chemotherapy (p=0.001). There was no difference between the two arms of patients for this parameter. Opposite, age, time from diagnosis, disease, remission status, CBC parameters before MINE regimen , arm of randomization, prior marrow involvement were not found relevant covariates.

Transplantation (figures 2,3).

In both arms, the duration of severe neutropenia with less than 0.5 x 10^9/l polymorphonuclear cells was short with a median time of 12 days in arm A (range 7-28) and 12, 17, 20 days for patients within arm B who where transplanted with PBSC. The patient of arm B who received marrow transplant had a 10-day neutropenia. The duration of leukopenia was similar to the duration of neutropenia in both arms. Nevertheless, the 3 patients in arm B who received PBSC all experienced a secondary drop of WBC and PMN counts for a period of time ranging from 40 to 280 days. These three patients received rh GM-CSF after this secondary aplasia without significant improvement of CBC parameters.

None of the patients included in arm A experienced such an evolution, WBC and PMN reaching a plateau in one step. Platelet recovery differed dramatically between the two arms :the median time to achieve 50 x 10^9/l was short in arm A (11 days , range 6 to 85 days), whereas severe thrombocytopenia was much more longer for patients who received PBSC in arm B (60, 254, 365 days). The patient grafted with marrow had a 8-day thrombocytopenia. This patient was kept in her arm of randomization for the analysis of the haematological recovery, though she was eventually grafted with marrow. Despite an improvement of data of recovery in arm B, difference in the kinetics of WBC and of PMN are statistically significant between arm A and B.

When looking at the haematological recovery of 10 historical patients who had been autografted with PBSC collected during the recovery phase from aplasia without growth factor support after transplantation, no history of a secondary drop of the neutrophil count was reported, and the duration of severe neutropenia was found comparable to that of arm A.

DISCUSSION

Based on preliminary data showing that PBSC circulation peaked during the haematological recovery phase from aplasia [12-15] and publications reporting that collection could be dramatically enhanced by administration of growth factors [10,11], the objective of this study was to determine if a 5-day stimulation of the bone marrow by rh GM-CSF without taking advantage of the recovery from aplasia could produce a sufficient collection of PBSC to graft patients. The trial was initially randomized between the two arms. In the reference arm (A) cells were collected during recovery from aplasia with a long stimulation by rh GM-CSF starting after completion of chemotherapy. In arm B, it was speculated that the enhancement of PBSC circulation would be powerful enough to collect an amount of cells at least equivalent to the number of cells obtained with our classical PBSC collection procedure (collection during the recovery from aplasia). The former procedure was in fact more costly and more heavy to manage than the latter.

MINE regimen was our reference chemotherapy regimen used to collect PBSC in our historical study, and it had been shown to produce steep aplasia and recruitment of PBSC in peripheral blood. Ten patients had been previously successively grafted with PBSC using MINE regimen as preparative regimen for collection and the amount of 5 x 10^8 nucleated cells/kg body weight per graft was proved to be sufficient to obtain engraftment before starting this trial. Thus, the initial aim of the study was to compare the efficacy of collection in terms of number of nucleated cells and of CFU-GM collected.

Initial data obtained in the first 10 randomized patients were encouraging since there was no evidence that a long stimulation by the growth factor with collection during recovery from aplasia gave better results than a short stimulation of the bone marrow by rh GM-CSF during a stable haematological phase. Hence, no significant difference was found between the two arms in terms of number of nucleated cells or of CFU-GM cells collected, and the yield per leukapheresis was similar in both arms.

All of the three patients included in arm B who were autografted with PBSC experienced a very poor marrow reconstitution that prompted us to stop inclu-

sion in this arm. The evolution of WBC and PMN counts is remarkable since following a first wave of neutrophils which occurred within the same time than the complete recovery in arm A, a secondary aplasia developed, unresponsive to a reintroduction of rh GM-CSF given subcutaneously. Prolonged thrombocytopenia was observed in these patients, without the first wave observed on the WBC and PMN curves. The history of these three patients was uninspired when looking at parameters such as age, CBC parameters before graft, history of prior marrow involvement, duration of previous chemotherapy, prior radiotherapy which could have generated a marrow micro-environment impairment. Opposite, the recovery from neutropenia and of thrombocytopenia was peculiarly rapid and steady in arm A, whereas the number of cells grafted were comparable in terms of nucleated cells and CFU-GM. The absence of difference between the two groups of patients in terms of clinical parameters, therapeutic procedures and number of CFU GM grafted suggests that PBSC collected in arm B are more mature than in arm A. The number of 7-day CFU GM assay does not reflect the number of pluripotent stem cells and is dependent of the harvesting procedure.

CONCLUSION

Rh GM-CSF can mobilize PBSC for collection by cytapheresis. Nevertheless, mobilization of pluripotent stem cells is dependent on the duration of stimulation by growth factors. One must be cautious when comparing the number of PBSC collected with various harvesting procedures evaluated by the 7-day CFU GM assay.

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Table 1. Patient Characteristics (Patients grafted in brackets)

n	all patients 21 (17)	arm A 16 (13)	arm B 5 (4)	p
age (median)	16-67 (36)	16-67 (36)	26-55 (42)	0.57
sex M/F	19/2 (15/2)	15/1 (12/1)	4/1 (3/1)	0.42
Hodgkin's disease	8 (7)	6 (5)	2 (2)	
low grade NHL	8 (7)	6 (5)	2 (2)	0.97
high grade NHL	5 (3)	4 (3)	1 (0)	
marrow involvement at diagnosis	10 (8)	8 (6)	2 (2)	1.0
Marrow involvement at relapse	8 (6)	5 (3)	3 (3)	0.32
previous extended field radiotherapy	5 (3)	4 (3)	1 (0)	1.0
BEFORE MINE REGIMEN				
	(median)	(median)	(median)	
HGB level g/l	100-134 (121)	100-134 (122)	103-122 (117)	0.12
PMN (giga/l)	2.0-10.5 (4.4)	2.0-10.5 (4.5)	2.6-5.0 (3.8)	0.58
platelets (giga/l)	141-741 (300)	160-741 (335)	141-400 (183)	0.19
CONDITIONING REGIMEN				
TBI/Cy	6	4	2	0.58
BEAM	11	9	2	

Table 2. PBSC harvest.

	arm A	arm B	p
duration of aplasia after "MINE" CT (days)	0-10 median 6	7-13 median 12	0.04*
nadir PMN after "MINE" CT (109/l)	0.01-1.45 (0.39)	0.01-0.24 (0.14)	0.4
# CFU-GM in the peripheral blood before CT (pre-test)	4-848 (211)	0-164 (41)	0.06
exposure to GM-CSF before starting cytapheresis (days)	4-6 median 10	5	
time to 1st cytapheresis after CT (days)	7-28 median 15	30-36 median 35	
# cytapheresis	2-5	3-4	0.32
# nucleated cells collected (108/kg)	5.63±8.91	4.86±9.03	0.64
# nucleated cells/cytapheresis (108/kg)	1.63±2.9	1.33±2.6	0.71
# CFU-GM collected (104/kg)	6.51±14.89	3.21±6.43	0.28
# CFU-GM/cytapheresis (104/kg)	1.79±3.48	0.92±1.21	0.45

Figure 1

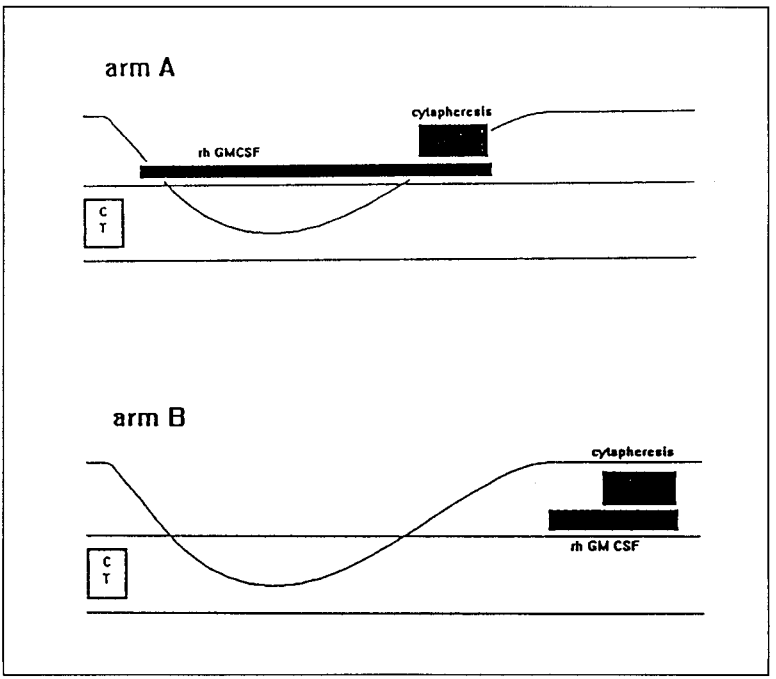
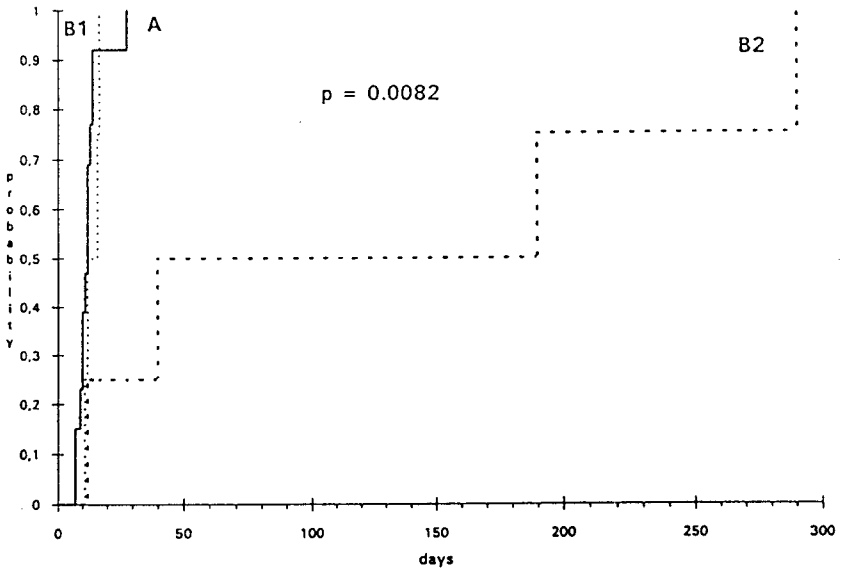
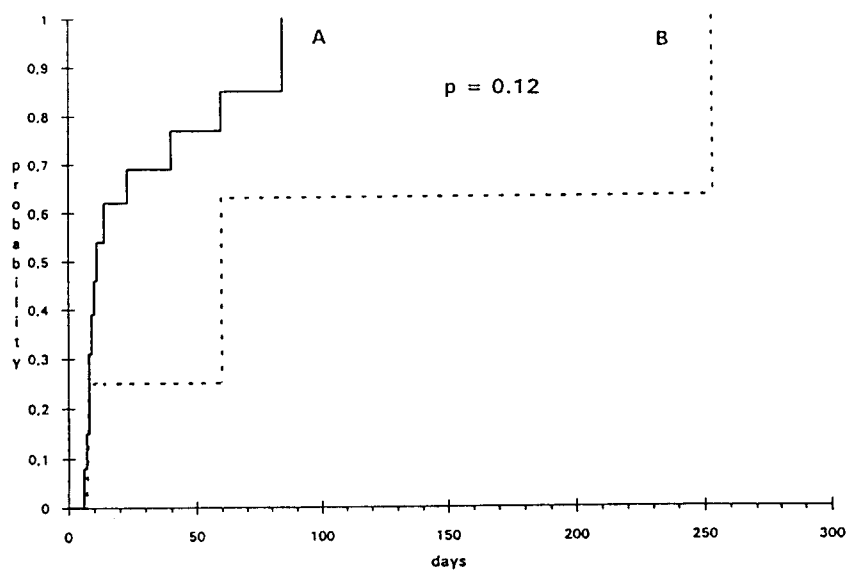


Figure 2



PMN recovery (PMN > 0.5 10^9/l) versus time : d0 is the day of transplantation ; A : arm A ; B1 : first peak of PMN followed by a secondary neutropenia < 0.5 10^9/l ; B2 : stable PMN count > 0.5 10^9/l.

Figure 3



Platelet recovery (Plt > 50 10^9/l) versus time : d0 is the day of transplantation ; A : arm A ; B: arm B.

Summaries

AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA

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Results of numerous single arm studies of intensive therapy and ABMT for AML suggest an apparent advantage over optimal conventional chemotherapy. A recent unpublished update of 8 trials of ABMT for AML in first remission (CR-1) involving a total of 425 patients indicates a disease-free survival (DFS) range of 48-58 percent at a median follow-up ranging from 18 to 96 months.

Also, 5 trials contributing a total of 209 patients with AML CR-2 at a minimum median follow-up of 34 months after ABMT had DFS from 14 to 53 percent. Although these data are gratifying, and are suggestive of the superiority of ABMT, several confounding patient-, disease- and treatment-related variables make comparisons with cohorts treated with chemotherapy alone, problematic. Such variables include age, white blood cell count at diagnosis, cytogenetic abnormalities and FAB type. A major problem in analyzing transplant data relates to the issue of time censoring of patients who receive transplants. Patients who achieve remission and relapse before a scheduled transplant are invariably excluded from the transplant cohort but are frequently included if assigned to a chemotherapy group. This proportion may be as high as 20 percent.

In view of the issues raised above, it was particularly appropriate to focus on current prospective trials of ABMT for AML in the leukemia session of the Symposium.

Four trials were reviewed: the U.K. MRC10 trial, the EORTC-GIMEMA trial, the GOELAM trial and the BGMT study.

A. Burnett reported that the MRC10 trial has accrued over 1200 subjects with an overall CR rate of 82 percent (children included). Of interest, actuarial relapse was below 10 percent within the first 6 months after achieving CR. Over 90 patients have been randomized to the ABMT arm and analysis will be based on intention to treat. The study will be analyzed in late 1994 and should provide a comparison of DFS and relapse rate for patients undergoing allo-transplants, ABMT or chemotherapy. Results of this large and important study will be awaited with interest.

R. Zittoun provided a preliminary analysis of the EORTC-GIMEMA Study (AML 8A) comparing allo-BMT, ABMT and intensive consolidation for AML CR-1. A total of 988 subjects were registered and 581 (67 percent) achieved CR. 119 underwent allo-BMT while 239 were randomized to ABMT (119) or intensive consolidation (120). Actuarial DFS at 3 years favors ABMT, although results are not statistically significant: ABMT, 50 ± 11.2 percent vs 31 ± 1.4 percent for intensive consolidation. Results with allo-BMT were not different with DFS of 51 ± 11.4 percent. Overall survival is similar in all groups.

R. Harousseau reviewed the results of a prospective study organized by the GOELAM Group. 318 patients with newly diagnosed AML were registered and 78 percent achieved CR. 56 underwent allo-BMT, 54 were randomized to intensive consolidation and 52 to the ABMT arm. At a median follow-up of 34 months DFS appears similar between the chemotherapy and ABMT groups (48.7 vs 47.6 percent, respectively).

Finally, J. Reiffers outlined results of a prospective trial on behalf of the French BGMT Group. After one consolidation, patients were randomized to ABMT or to maintenance chemotherapy. 204 patients were entered, 162 achieved CR (80 percent) and 77 were randomized to maintenance (38) or ABMT (39). Actuarial DFS at 3 years was not significantly different: 48.3±8.5 percent for ABMT and 38.9±8.4 percent for the maintenance arm.

A review of these trials indicates that several points deserve emphasis. First, the proportion of subjects registering at induction who were randomized to an ABMT arm is quite small and relatively consistent from trial to trial: from 12 to 19 percent. This confirms the need to enter very large numbers of patients at the outset in order to address questions related to DFS after transplant. Second, although data are preliminary, there are no striking differences between conventional therapy or transplant, whether autologous or allogeneic. Longer follow-up will be required and may disclose the superiority of one type of treatment. In two of the 3 studies, the trend was in favor of ABMT over conventional intensive consolidation therapy. Unfortunately, one difficulty in conducting prospective trials over extended periods (4 to 5 years) is that previously unrecognized prognostic factors may be identified and found not to be controlled for in the study. Moreover, big improvements (>15 percent) in chemotherapy or ABMT in the interim, although unlikely to occur, could make the studies obsolete. One possibility here is that marrow purging will be shown to be of clinical benefit possibly from gene marking studies or analysis of marrow transplant registry data. Of relevance, C. Gorin provided an update on the EBMTG experience and indicated no benefit of marrow purging from an overall evaluation of 1483 transplants. However, a retrospective analysis identified a subgroup with AML CR-1 that underwent marrow purging and had a reduced relapse rate and improved DFS. None of the prospective studies reviewed at the Symposium employed ABMT with purged autografts.

Despite these potential limitations, the investigators who designed and conducted these trials should be complimented since the data generated are likely to lead to conclusions of greater validity than can be derived from single arm studies with small numbers of subjects. It will be very interesting to hear the final reports on these trials.

SUMMARY OF THE NON HODGKIN LYMPHOMA SESSION DURING THE "SIXTH SYMPOSIUM ABMT" IN HOUSTON (DECEMBER 1992)

Professor Thierry PHILIP

First I want to thank Karel DICKE in the name of all the participants for this very nice meeting and I like also to thank Karel for the good organization and friendly period that we had in Houston.

I. As far as Nodular Lymphoma reports and posters are concerned we may go back with the following messages:

- we have no major prognostic factors after marrow transplantation except the very curious report from Boston that "no CR" before marrow transplant is a favorable factor.
- bone marrow transplantation did not eliminate (14;18) translocation but some people with persisting abnormality may be long term survivors.
- survival is equivalent with bone marrow transplantation and peripheral blood stem cell transplantation.
- survival is equivalent with or without total body irradiation.
- survival is equivalent for different histology at least for follicular mixed and follicular small cleaved.
- reports of very late relapses were made during the conference, specially by Pr GORIN with one case of a ten years relapse after marrow transplantation.

CONCLUSION:

Nodular lymphoma is certainly a major field for bone marrow transplantation in the future. It is still debatable whether we should use this modality of therapy at the time of first CR or at the time of first relapse.

II. As far as intermediate and high grade Lymphomas are concerned we may be going back with the following messages:

- Lymphoblastic Lymphomas are now widely grafted in 1st CR. Unfortunately, we have no proof of the utility of this modality of therapy and I will strongly recommend to organize prospective and randomized studies to definitively prove the utility of marrow transplantation in this setting.
- for non-lymphoblastic lymphomas in 1st CR, the major study is coming for the LNH Group and in the bad prognostic group of intermediate grade patients, an intermediate report was done at ASCO 1992, showing no difference between bone marrow transplantation and normal conventional maintenance therapy in this kind of lymphoma. However, we have to stress that this publication was very early and that a difference may be shown in one or two years from now.
- we all agree that bone marrow transplantation in 1st PR is a very good indication. Some evidence coming from Italy is in favor of this modality of therapy in a randomized study and this is probably one of the consensual indications for marrow transplantation at this time.

- as far as relapses are concerned: resistant relapses are still a bad indication for marrow transplantation and sensitive relapses are probably the best candidate for this modality of therapy. Sensitive relapses are patients where 1st CR is absolutely demonstrated and include only CR and PR after two courses of rescue conventional chemotherapy. In this setting the results are now between 40 and 60% long term survival at 5 years and the current PARMA randomized study is trying to demonstrate definitive interest of this modality of therapy.

I want to stress again that it is somewhat unethical not to put patients in the PARMA protocol because we are not able yet to demonstrate the utility of this therapy. Some insurance did not reimburse in the States this modality, which is probably the best one, and we need very few patients now to complete the PARMA study with a definitive conclusion.

- last point: primary refractory patients are still very bad candidates for marrow transplantation.

In this field, allegeneic and autologous bone marrow transplantation are equivalent despite two reports that allegeneic decreases the number of relapses. A lot of published papers (especially from the Nebraska team) show that peripheral blood stem cell is also equivalent to autologous marrow transplantation in this setting.

CONCLUSION:

Non-Hodgkin's lymphoma is still one of the major fields for bone marrow transplantation and we all hope that in the near future several randomized studies will definitively establish this therapy in the field.

SUMMARY: AUTOLOGOUS MARROW TRANSPLANTATION FOR SOLID TUMORS

Roger H. Herzig, M.D.

PHASE I STUDIES

Nearly twenty years ago, the Phase I single agent dose escalation studies began. Alkylating agents were chosen because of their properties of little non-myeloid toxicity, a log-linear dose response relationship could be demonstrated in vitro cell lines and in conventional dose clinical studies, and they are generally non-cross resistant. These sequential, single agent studies defined the toxicity and feasibility of dose-intensive therapy with marrow support. While novel and unusual non-myeloid toxicity was discovered, more importantly, a further dose-response for most agents was demonstrated. The Phase I studies left questions of scheduling open, but it did lead to other trials using multiple agents and/or multiple courses. What still remains is how to increase the therapeutic index of increasing the efficacy while decreasing the toxicity of these dose-intensive regimens.

PHASE II STUDIES

The Phase II studies of dose-intensive therapy demonstrated efficacy in a number of tumors. These studies were generally conducted in patients with advanced, refractory disease. Overall, a high response rate was seen, usually in excess of 60% to 70%, of which 20-40% of the responses were complete. Of note, about 10-15% of the patients achieved significant disease-free survival of more than a year. The promising results in these trials led to the use of dose-intensive therapy earlier in the course of the disease. The solid tumors that fall into this category, in which the dose-intensive therapy is probably effective, include breast, ovary, melanoma, testicular cancer, Ewing's sarcoma, and neuroblastoma. A second group of tumor types in which the dose-intensive therapy is possibly effective include colon, lung, glioblastoma, and metastatic carcinoma adenocarcinoma of unknown primary. The results in these patients showed high response rates but fewer patients with complete responses or with long-term, disease-free survival. It would also probably be beneficial to use a dose-intensive approach in these patients earlier in their treatment. There are some tumor types in which the Phase II trials provided either insufficient data or the tumors were unresponsive to high-dose therapy. Few patients with Wilm's tumor have been treated, however, dramatic responses in some of these patients have been documented. There has been a renewed interest in the non-Ewing's sarcomas, especially in the European community, and again responses are seen even after conventional therapy has failed. In the category of tumors that are unresponsive include patients with renal cell carcinoma. None of the patients in several series with this diagnosis responded to high-dose therapy.

PHASE III STUDIES

There are a very limited number of Phase III prospectively randomized trials using dose-intensive therapy with marrow rescue for solid tumors. Currently, the ones with the highest profile are the high-risk stage II-III breast cancer studies that are being carried out collaboratively in the national cooperative groups. Two such trials are underway. There have been no interim analyses of survival or recurrence performed for these trials. Patient accrual is slow, but continues at a reasonable pace. The preliminary data on toxicity of the treatment (from the CALGB trial) has been acceptable. There is also a Phase III study for advanced breast cancer randomizing complete responders to conventional therapy to early verses late transplant. No results are available. At this point, Phase III trials need to be carefully designed with appropriate questions asked. Not all questions will need a Phase III trial; in some instances, it would be more appropriate to devote more time and attention to increasing the therapeutic index rather than asking inappropriate questions in a Phase III trial.

THERAPEUTIC INDEX

In order to increase the therapeutic index, one must decrease the toxicity and/or increase the efficacy of the treatment. Currently, efforts for decreasing toxicity have been aimed at supportive care issues, mediators of toxicity, and attenuation of dose. The proliferation of hematopoietic growth factors, both before and after the dose-intensive therapy, has made a small but significant impact on the infectious complications following the dose intensive therapy. Erythropoietin can reduce red blood cell transfusion requirements. As more growth factors develop, the beneficial effects on other lineages may be similarly shown. Other investigations have sought to block the mediators of toxicity, for example, by blocking tumor necrosis factor with pentoxifylline. Mixed results have been achieved with this approach, but the concept of blocking the mediators of toxicity is valid. Finally, efforts at optimizing the dosing schedule could decrease the toxicity. Attenuation of dose frequently reduces response, however, combining agents that are hopefully synergistic with sub-additive toxicity will result in lower toxicity and increased efficacy. Other ways at increasing efficacy could also include multiple courses of either single or multiple agents, better drugs, and obviously treating patients earlier in the course of their disease. The dose-intensive approach should not be an alternative to Hospice. Patients that have a better performance status with less tumor bulk are likely to do better with a dose-intensive approach.

OTHER ISSUES

During the meeting, much had been discussed concerning the source of stem cells: bone marrow, peripheral blood, or both. The question should not be which is better, but rather how can we most effectively use this supportive care measure to provide the best effect. In future meetings, we may even want to change our title from autologous transplantation to something else because the stem cell support is really a supportive care issue, not a therapeutic one.

As this treatment modality becomes used earlier, detection of minimal, residual disease will play an increasing role. It will also be an important way for negative selection of a stem cell product, i.e., to select cells that do not have tu-

mor. The obverse of this coin is positive stem cell selection. Marrow and peripheral blood can be fractionated providing purer preparations of stem cells. Preliminary data was presented with the CD-34 positive selection of stem cells as an adjunct in treating women with breast cancer. Recovery of hematopoietic function was not impaired.

These techniques also lead to the possibility of in vitro expansion of hematopoietic cells. Once peripheral blood or marrow have been harvested, small amounts could be expanded in the laboratory for subsequent use. Application of other areas of transplantation, especially in the area of gene therapy, would naturally follow.

INDICATIONS FOR AUTOLOGOUS BONE MARROW TRANSPLANTATION IN HODGKIN'S DISEASE

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During the Sixth International Symposium on ABMT, some of the most pertinent questions on the place of high-dose therapy in patients with Hodgkin's disease have been discussed. To this round-table participated: G.L. Phillips (Vancouver), T. Ahmed (New York), A. Vriesendorp (Houston), A.M. Carella (Genoa). The crucial point was to define which patient population was most likely to benefit from HDT/ABMT procedure. All the authors agreed to systematically subdivide poor prognostic patients into those who never enter CR, those who have a CR of less than 12 months and those who have a first CR longer than 12 months but who ultimately relapse. The characteristics of these primary refractory patients seem related to the amount of tumor burden. For the vast majority of these truly resistant lymphomas, conventional salvage yields poor results, and better results might now be achieved only with dose escalation and marrow or peripheral stem cell rescue. In those patients who do get an initial but short CR, salvage therapy at relapse with conventional dose chemotherapy yields a poor outcome. In this particular situation, high dose chemotherapy with ABMT can offer superior results. If, however, patients have initially been treated with a four drug regimen only, e.g. MOPP or ABVD, the outcome may be different. Following CR with MOPP alone at relapse, salvage with ABVD gives 65% of CR of which 35% remain alive and well at five years.

The above represents the patients entering a first CR which lasts less than a year but a further category of poor prognosis patients is characterized by patients whose disease relapse occurs after an initial CR longer than one year. In this subgroup, treatment with the same chemotherapy protocol as employed in induction can induce a second CR in about 60-70% of cases with a 5 year freedom from second relapse of 50%.

A number of characteristics have been associated with unfavorable remission rates, duration, and overall survival in patients with advanced-stage disease. They include B symptoms, older age than 40 years, mediastinal bulk, multiple extranodal sites of involvement, low hematocrit, high serum lactate dehydrogenase (LDH) and high erythrocyte sedimentation rate (ESR).

Patients presenting with advanced disease and other unfavorable prognostic factors, achieve complete remission at a relatively lower rate, relapse more frequently, respond poorly to salvage therapies and, as a whole, have a rather lower chance of cure. Using the prognostic model of Gobbi et al (22), patients with unfavorable HD (age >40 years, mediastinal bulky disease, B symptoms, more than one extranodal site of disease, MC or LD histology, high ESR, low albumin levels) have a 10-year survival rate of about 20%.

As recently reported in 185 newly diagnosed adults with advanced HD

treated at Memorial Hospital in New York between 1975 and 1984, the major factors negatively affecting the duration of complete remission were high serum LDH, age >45 years, mediastinal mass greater than 0.45 of the thoracic diameter, two or more extranodal sites, inguinal node involvement and low hematocrit. Patients with two or more unfavorable characteristics were much more likely to fail treatment than those with none or only one unfavorable factor. The presence of B symptoms was not associated with a significantly poorer survival; however, there was only a small percentage of patients with B symptoms in this study. In other important studies, B symptoms and age more than 40 yeas were consistently associated with a lower probability of remaining in CR. Similar results have been reported by Proctor, who has used a numerical prognostic index for clinical use in the identification of poor-risk patients. Ninety-two consecutive patients from one center (Newcastle upon Tyne) were used to construct a numerical index based on disease stage (Ann Arbor), age, hemoglobin and absolute lymphocyte count. This index provided a useful criteria to identify those patients with unfavorable prognosis and predestined to die of disease. Recently, high serum levels of CD30 and the soluble interleukin-2 receptor have been suggested to indicate a poor prognosis. In addition to these disease-related prognostic variables, such as dose intensity, may affect the outcome.

On the basis of published data we judge that the definition of high risk for patients with advanced stage, bulky disease, and constitutional symptoms is appropriate. However patients with this unfavorable pattern of presentation represent only a minority of cases, accounting for about 5% of all patients with HD. Eleven out of 201 patients with HD enrolled in different clinical trials by the Italian Lymphoma Study Group (Gruppo Italiano per lo Studio dei Linfomi, GISL) in the last four years fit the above mentioned criteria and can be classified as high risk patients, and are probably suitable for an aggressive treatment followed by ASCT.

IS THERE A ROLE OF ABMT IN FIRST REMISSION?

According to the good results in terms of survival and tolerance achieved in leukemia and non-Hodgkin's lymphomas when appropriate timed aggressive chemoradiotherapy is followed by ABMT in first CR or PR, the same strategy has been applied by Carella in very poor prognosis HD patients. In this trial patients with HD were selected on the basis of the most unfavorable prognostic features currently considered. The Genoa preliminary study has involved patients with many of the previously mentioned factors and even worse such as more than two extranodal sites of disease combined with mediastinal mass greater than 0.45 of the thoracic diameter at the level of the carina, high level of LDH and B symptoms. The excellent results of this study should be viewed as preliminary, even if these results have been recently confirmed from EBMTG analysis and will be presented at the next EBMT meeting in Garmish (Germany). Of course, more patients and longer follow-up are needed to define accurately the curability of very poor prognosis HD with ABMT. The use of ABMT as consolidation treatment does not seem to be justified for the time being for the majority of patients with Hodgkin's disease. This experience does not allow to make firm conclusions regarding the place of HDC in the management of HD. Despite the fact that this study was small and there were no other reported se-

ries of ASCT in first complete remission of Hodgkin's disease patients, the results seen in these 15 high-risk patients are considerably better than those observed in patients with such poor prognostic features (21 patients have been up to now inserted in Genoa: 19/21 alive and well at a median of 4 years!). Such a strategy will concentrate the toxicity of ABMT on an appropriate group of patients and maximize the antineoplastic effects of the conditioning regimen by utilizing such intensification before a refractory disease status develops and before additional and ineffective conventional salvage treatment adds to the risk of eventual toxicity. The results of many teams suggest that patients who never responded to conventional therapy and are completely refractory to chemotherapy are also bad candidates for ABMT and should be spared this aggressive procedure and relative toxicity. The finding of an increased death rate in heavily pretreated patients with Hodgkin's disease emphasizes the need not to postpone the ABMT too long. On the contrary, recent use of ABMT in lymphoblastic lymphoma in first CR demonstrated usefulness for survival and, overall, the death-rate was far lower.

Therefore, we feel that ABMT when employed in first CR should probably be the treatment of choice for patients with disseminated HD who achieve CR after first line therapy; moreover by reducing the BCNU dose and with the use of hemopoietic growth factors (G-CSF or GM-CSF) we could avoid the risks of interstitial pneumonitis and of prolonged neutropenia.

A phase III study comparing ABMT vs no further therapy in patients with high risk HD in first CR could finally provide substantial arguments to the possible role of ABMT in HD. In this way an international trial has been recently organized.

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