Autologous Bone Marrow Transplantation

Proceedings of the Fourth International Symposium

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PROCEEDINGS OF THE FOURTH INTERNATIONAL SYMPOSIUM

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Contents

Session I - Leukemia

A. ACUTE LEUKEMIA - AML

Session III - Solid Tumors

A. BREAST CANCER

xvii

Session VII – Supportive Care

xix

Preface

Continuing progress is being made in the field of autologous bone marrow transplantation (ABMT). The momentum of this success has been realized by the Fourth International Symposium on Autologous Bone Marrow Transplantation, which was held in Houston, Texas, on August 18-20, 1988.

When we consider the results presented at this meeting with those of the last meeting, *definite* progress has been made in leukemia, myeloma, sarcoma, and breast cancer. Isn't it exciting to see a change in the natural history of these diseases? Quality of life is being maintained for our BMT patients--a treasure which is the target of our thoughts and actions.

The participants of this meeting have made both the symposium and the proceedings a great success. The discussions are invaluable and will significantly add to new study designs. This is an important gain factor of the symposium: communication among those whose contributions define the area of ABMT.

As before, I would like to dedicate this symposium to our patients, who contributed significantly to its success. Their endurance and positive attitude have been an inspiration to us all.

Karel A. Dicke

Acknowledgments

SESSION I - LEUKEMIA

A. ACUTE LEUKEMIA - AML

DOES HIGH-DOSE INTENSIFICATION WITH AUTOLOGOUS BONE MARROW RESCUE CONTRIBUTE TO LONG-TERM DISEASE-FREE SURVIVAL IN ACUTE MYELOGENOUS LEUKEMIA IN FIRST REMISSION?

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INTRODUCTION

Acute myelogenous leukemia is a chemo-responsive disease; complete remission rates of 50-80% are consistently obtained with current induction regimens (1-7). However, the median remission duration is only 12-15 months, and the median long-term relapse free survival is only 15-25%. Evidently, the conventional regimens fail to eradicate the leukemic clone in the majority of patients. Early intensification with high-doses of ARA-C has produced 3-year disease-free survival rates of 30-45% (1,5,6) which are probably due to improved cytoreduction as compared to the conventional anthracycline + ARA-C induction regimens.

Searching for an optimal strategy that would achieve maximal cytoreduction, we have combined an intensification with high-dose ARA-C prior to bone marrow harvest followed by late intensification with the high-dose chemotherapy regimen of cyclophosphamide, BCNU and VP-16 (CBV), in conjunction with bone marrow rescue. We are reporting the results obtained in 18 such patients, with a minimum follow-up of 31 months for patients still in remission. The results are compared to those of 9 patients who received an identical regimen except for the omission of CBV-ABMT.

MATERIAL AND METHODS

Patients

The bone marrow transplant group consisted of 18 adult patients (10 females, 8 males) with acute myelogenous leukemia in their first

remission. Eligibility was restricted to patients with age <56 years, performance status <2 in the Zubrod scale, and normal renal, hepatic, cardiac and pulmonary function, who were not eligible for allogeneic bone marrow transplantation. Cytogenetic analysis at the time of diagnosis showed that 6 patients had karyotypes associated with good prognosis (8): 3 inversion 16 and 3 t (15;17); 9 had intermediate prognosis karyotypes: 7 diploid, 1 t (8;21) and 1 45X-Y; and 3 had poor prognosis karyotypes: 2 +8 and 1 with insufficient metaphases.

Chemotherapy

The treatment schema is shown in Figure 1.

Figure 1

TREATMENT OF AML IN CR1

PROGRAM OUTLINE

INDUCTION:	AMSA-OAP
PRE-BMT INTENSIFICATION:	HD ARA-C + AMSA
MAINTENANCE:	AD-OAP
HARVEST	AD-OAP
	AD-OAP
HIGH DOSE THERAPY	CBV + ABMT
MAINTENANCE:	AD-OAP X 3
	AMSA-OAP X 3
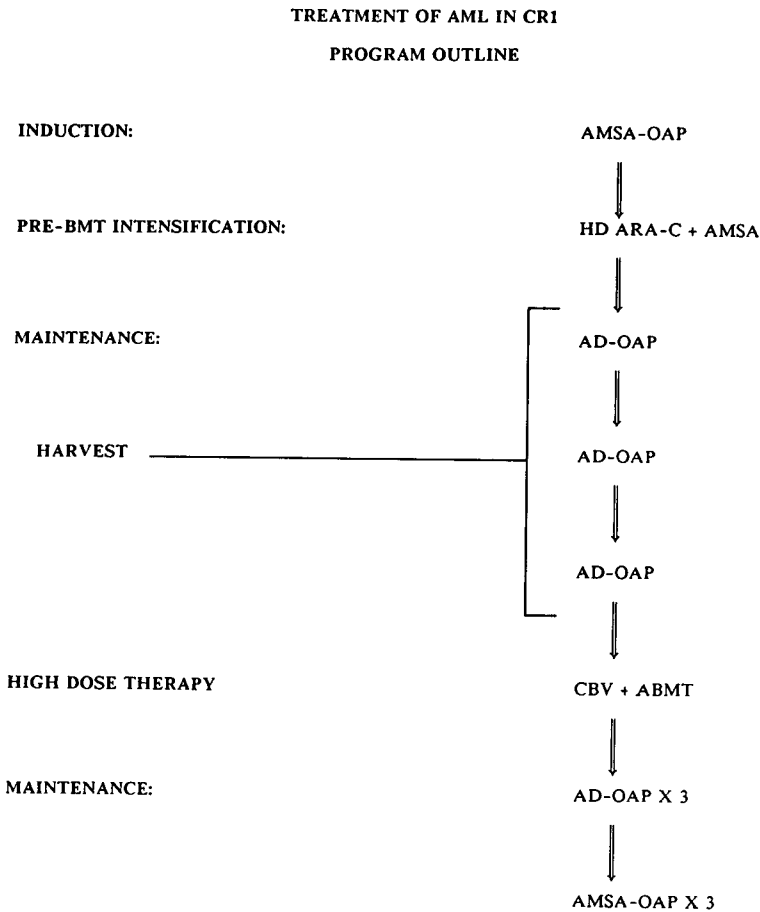
Figure 1. Treatment of AML in CR1 Program Outline

Bone Marrow Transplantation

Bone marrow was collected after patients had recovered from early intensification with high-dose ARA-C (median 106 days, range 68-161). The median time between bone marrow harvest and transplantation was 47 days (14-141). We tried to collect a minimum of $1x10^8$ cells/kg body weight, and $10x10^3$ GM-CFC/kg (9). Five patients needed two separate bone marrow collection procedures to obtain adequate numbers of cells and GM-CFCs. A median of $1.84x10^8$ nucleated cells/kg (0.85-3.14) and $23.0x10^3$ GM-CFC/kg (2.66-43.25) were infused.

Control Group

Nine patients received identical treatment but for the substitution of one cycle of AMSA-OAP in the place of CBV-ABMT. Three of them were randomized to not receive CBV, in the initial part of the study when randomization was used. The other six refused CBV for financial or personal reasons (three patients each). See Table 1 for patient characteristics of this group.

Statistical Methods

Remission duration was calculated from the date of achievement of CR to date of relapse or last follow-up. The method of Kaplan and Meier was used to plot remission duration curves. The duration of remission in distinct patient groups was compared using the generalized Wilcoxon test. The only additional patient that received CBV and ABMT in this protocol has been previously reported (10) but she is excluded from this analysis because she received multiple cycles of maintenance therapy, with an interval between bone marrow collection and ABMT of 15 months. She relapsed at 44 months and is still alive, with persistent disease at 45+ months.

RESULTS
Remission Duration: CBV-ABMT Group

The median remission duration is 30+ months (7-52+)(Figure 2). Ten patients remain alive and disease-free with a median remission duration of 38+ months (31+-52+). Eight patients have relapsed, with a median remission duration of 18 months (7-24), of which seven have died and one is alive in second remission. The latest relapse was seen at 24 months, and all patients still in remission have already surpassed this time mark (minimum remission duration: 31+ months).

Table 1. Patient Characteristics

CBV Group:				
NO. OF PTS:	18 (10F, 8M)			
MEDIAN AGE:	35 (21-51)			
FAB SUBTYPE:	M1:	5	M4:	5
	M2:	3	M5	2
	M3:	2	UNK:	1
CYTOGENETICS: (RISK GROUPS)	GOOD:			4
	INTERMEDIATE:			9
	POOR:			4

CONTROL GROUP				
NO. OF PTS:	9 (F,M)			
MEDIAN AGE:	32 (22-48)			
FAB SUBTYPE:	M1:	1	M4:	2
	M2:	2	M5:	0
	M3:	3	UNK:	1
CYTOGENETICS: (RISK GROUPS)	GOOD:			4
	INTERMEDIATE:			3
	POOR:			2

CBV: cyclophosphamide, carmustine, etoposide; **NO**: number; **PTS**: Patients; **FAB**: French-American-British; **F**: female; **M**: male; **UNK**: unknown

Control Group

The median remission duration is 19 months (9-54+)(Figure 2). Three patients remain alive and disease-free, all with a remission duration of 54 months. Six patients have relapsed, with a median time to relapse of 16 months (9-22). All relapsed patients have died.

The following factors were evaluated to determine their relationship with remission duration in the CBV-ABMT group: age, sex, FAB type, induction regimen, cytogenetics, time to achieve CR, number of cycles needed to achieve CR, interval between CR and bone marrow harvest, interval between bone marrow harvest and transplantation, and interval between CR and transplantation. The
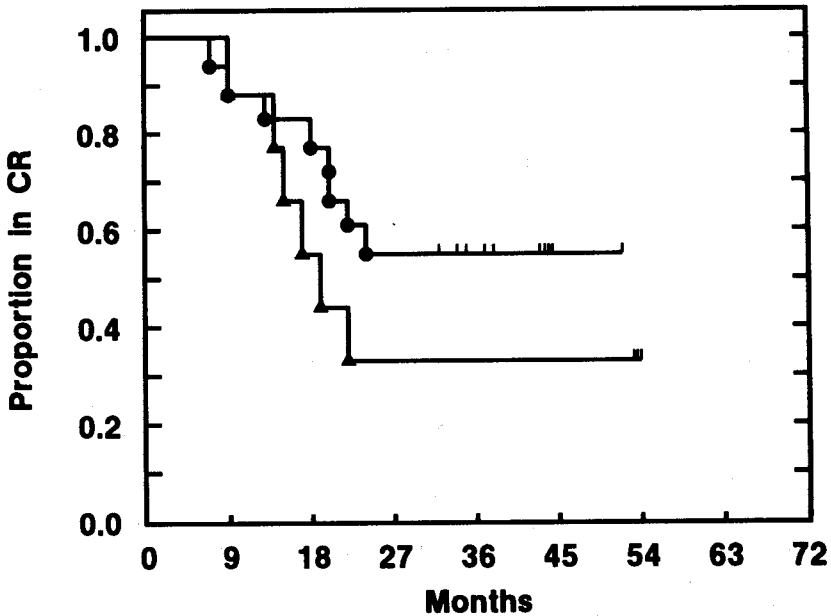
Figure 2. Acute Myelogenous Leukemia in CR1 Remission Duration (• = BMT, N: 18; ▲ = No BMT; N:9). Legend: Duration of first remission for patients with acute myelogenous leukemia. There is a trend for improved remission duration in the ABMT group.

patients with an interval between CR and ABMT of less than 6 months (N: 5) had a significantly shorter CR duration than those with longer intervals (P= 0.04). The logistic regression model PCR1, which calculates the probability of staying in CR for one year in patients treated with conventional chemotherapy (11) was also evaluated as a prognostic indicator in this group. There was a trend for improved remission duration (P= 0.07) for patients with PCR1 >0.60. All the other factors evaluated were found not to be associated with improved remission duration times.

There is a trend for improved CR duration on the CBV-ABMT group (P= 0.17) as compared to the control group. In the control group, two patients had PCR1 values <0.60, and both relapsed. As shown in Table 1, there was no significant difference in the distribution of cytogenetics characteristics between the control and the ABMT groups.

Hemopoietic Recovery in the ABMT Group

One patient had leukemic relapse by day 28 post ABMT, before

full recovery of blood counts, and is not evaluable. Recovery times are described in Table 2. The median red cell transfusion requirement was 5.5 units (2-19) and the median number of platelet transfusions was 7 (2-19).

Toxicity of the ABMT Group

No patients died as a consequence of the late intensification treatment. Table 3 describes the toxicity seen in this regimen.

DISCUSSION

Recent treatment programs have used high-doses of ARA-C as intensification therapy early after achieving CR, with projected disease-free survival rates of 30-45% at 3 years (1,5,6); however, the median follow-up of these series is limited, with relatively few patients beyond two years. If these regimens indeed improve the proportion of long-term disease-free survivors, it may be assumed that high-dose ARA-C intensification increases cytoreduction. According to the Goldie-Coldman hypothesis (12), this early reduction of the total tumor cell load would also decrease the emergence of resistant clones. Based on this reasoning, we incorporated early intensification with high-dose ARA-C to our regimen.

We collected the bone marrow during the maintenance cycles that followed the early remission intensification with high-dose ARA-C. This enabled us to store bone marrow that had experienced maximal cytoreduction, maintain a low tumor load prior to transplantation, and allow the patient to recover from the toxicities of high-dose ARA-C. This was followed by late intensification with CBV, and six cycles of post-transplantation therapy.

Table 2. Hematopoietic Recovery

Landmark	Median (Range) in Days
ANC 0.1 x $10^9/\mu l$	22 (11-34)
ANC 0.5 x $10^9/\mu l$	31 (15-48)
ANC 1 x $10^9/\mu l$	35 (21-61)
PLT 20 x $10^9/\mu l$	22 (14-46)
PLT 50 x $10^9/\mu l$	29 (15-64)
PLT 100 x $10^9/\mu l$	36 (17-100)

Abbreviations: **ANC:** absolute neutrophil count; **μl:** microliter; **PLT:** platelets.

Table 3. Toxicity of CBV-ABMT

		NUMBER OF PATIENTS	
		Total	Grade 3-4
Fever of Unknown Origin		5	0
Sepsis	Gram negative		5
	Gram positive		3
	Candida		1
Pneumonia		4	1
Cellulitis		6	1
Nausea/Vomiting		13	1
Stomatitis		7	1

The original protocol design included randomization to receive or not receive CBV and ABMT, but many patients were hesitant to receive this then new therapy. Consequently, only volunteer patients were included. It must be noted, however, that the proportion of patients with unfavorable cytogenetics and the distribution of PCR1 values of the ABMT group are not significantly different to that of our general leukemia population or to the control group. The differences in the remission duration curve between this two groups suggest a contribution of the CBV-ABMT. Also, when our results are compared to those obtained with early intensification with high-dose ARA-C (1,5,6), their percentage of long-term disease-free survivors followed for more than two years seems to be smaller, which again implies therapeutic activity of CBV-ABMT.

Our results also compare favorably to other groups that utilize autologous bone marrow transplantation (13-18). A recent communication from the European Bone Marrow Transplantation Group (19) shows a significant improvement of disease-free survival on the group of patients treated with a TBI containing regimen whose bone marrow was purged "ex vivo" with mafosfamide, when compared to the group that received unpurged bone marrow (56% vs 32%). This implies that the efficacy of high-dose chemotherapy and autologous bone marrow transplantation may be improved by decreasing the leukemic cell load in the infused marrow. With similar disease-free survival rates between this approach and our regimen, it may be that the administration of high-dose ARA-C prior to bone marrow collection acts as an "in vivo purging".

The very tolerable toxicity of CBV and ABMT is another significant aspect of this trial. There were no treatment related deaths,

whereas other transplantation groups which have reported treatment-related death rates of 19-22% (14-16). The incidence of severe non-hemopoietic toxicity was low. The hemopoietic recovery was fast, showing that prior exposure to high-dose ARA-C does not compromise the regenerative potential of the bone marrow. We optimized the bone marrow collection to obtain adequate numbers of GM-CFCs, which predict rapidity of engraftment (9). It should also be noted that we avoid the use of TBI (which can cause late complications such as cataracts and pulmonary fibrosis), without apparent decrease of therapeutic efficacy.

We can speculate that the following reasons may explain the high percentage of long-term survivors achieved with our regimen:

a) The additive cytoreduction from high-dose ARA-C and CBV over the initial leukemic cell kill from induction chemotherapy.

b) The use of intensification with high-dose ARA-C prior to bone marrow collection, effecting a better "in vivo purging" than previous studies that used less intensive post-remission chemotherapy regimens.

c) The combination of a) and b) should result in a very low load of clonogenic leukemic cells after CBV-ABMT, which can be more effectively controlled by the post-transplantation chemotherapy and the body's immune surveillance mechanisms.

d) No patients died as a result of the high-dose cytoreduction.

CONCLUSIONS

We have treated 18 adult AML patients with a program that combines induction chemotherapy, early intensification with high-dose ARA-C, and late intensification with high-dose CBV and ABMT. This doubly intensified regimen has resulted in a 56% long-term disease-free survival in patients followed for a minimum time of 32 months, with very tolerable toxicity and no treatment related deaths. There is a trend for improved CR duration when these patients are compared to controls treated with identical therapy except for the use of CBV-ABMT. A multivariate logistic regression model may better define the patient population that benefits from this regimen. If these promising findings are confirmed with larger, randomized studies, this treatment strategy may be incorporated to the management of newly diagnosed patients with acute myelogenous leukemia.

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     Transplantation, Chamonix, France, April 10-14, 1988

AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR ACUTE LEUKEMIA: Optimal Timing and Mafosfamide Treatment

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Enrico Madon, and Piero Bernabei

INTRODUCTION

The studies using pharmacological purging for autologous bone marrow transplantation (ABMT) are rapidly developing because the encouraging results obtained in acute leukemia (AL) patients (1,2). The major problem of ABMT is to detect in marrow suspension the clonogenic residual leukemic cells (3,4). The first step for a successful ABMT is strictly dependent on optimal disease remission; to well define the complete remission phase it must be necessary to employ combined techniques as cytological-histological examination, cytogenetic studies, immunological cell-surface typing and pre-transplant "in vitro" cell-cultures.

Major efforts of our study are to use increasing doses of Mafosfamide for "ex vivo marrow purging, compatible with marrow reconstitution, to evaluate the best time for marrow harvest and transplantation and to focalize the correlation between the free survival rate and pre-transplant regimens. The analysis has been done in 187 cases of acute leukemia (104 acute non lymphoid leukemia: ANLL and 83 acute lymphoblastic leukemia: ALL) collected from 13 different Italian teams.

MATERIALS AND METHODS

In 74 patients we used standard doses of Mafosfamide (ASTA-WERKE-Bielefeld-FRG) ranging between $80-100\mu g/2x10^7$ cells for "ex vivo" marrow purging. In 16 patients, starting 1986, the "ex vivo" purging has been performed with individual doses of drug, established on a marrow sample in each patient 10-15 days before the harvest.

The sensitivity of hemopoietic tissue to the drug was evaluated by the feeder layer colony growth (Gordon technique) (5,6); "optimal dose" of Mafosfamide concentration was defined as sparing 50+10% of the undifferentiated type I colonies at 6th-7th culture-day. The type II and type III colonies reflect the more differentiated compartment that do not interfere in the kinetics of the engraftment. The doses of Mafosfamide used vary from $80-140\mu g/2x10^7$ cells (7).

The marrow cells in all patients were collected in a range of $2-3x10^8/kg$ ideal body weight from the posterior iliac crests; the buffy coat, after centrifugation, was removed and resuspended in 20% of autologous plasma plus 80% TC199 medium, with a cell concentration of $2x10^7/ml$. The Mafosfamide was added to the cells and the suspension was incubated at 37°C for 30 ' with periodic gentle agitation. The reaction was stopped by cooling to 4°C. The cells were centrifuged at 2800 RPM for 10 '; the supernatant was removed and the mononuclear cells resuspended at concentration of $4x10^7/ml$ in a solution containing 55% irradiated autologous plasma, 35% TC199 and 10% DMSO. For cryopreservation we used a programmed biological freezer (Nicool 416). See patients' characteristics in Tables 1-2.

The pre-transplant regimens in ANLL were cyclophosphamide (Cy) 50 mg/kg ideal body weight /4 days or 60 mg/kg/2 days plus total body irradiation (TBI) 10 Gy single dose or 12 Gy fractionated dose or Busulphan (Bu) 4 mg/kg/4 days plus Cy, as described above (8). The majority of ALL patients were treated with Cy-TBI as above.

Before reinfusion, marrow cells were thawed by a rapid immersion in a water bath at 37°C. The viability of cells was evaluated by the Trypan Blue dye exclusion test; the cells were infused into the patients 24-36 hours after the stopping of pre-transplant regimen at doses ranging from 0.9 to $2x10^8/kg$. Engraftment was documented by the daily evaluation of hematologic recovery. None of the patients received chemotherapy after ABMT.

All patients were hospitalized in laminar air flow rooms. During post-transplant aplasia, patients were supported with platelets and packed RBC transfusions that were previously irradiated (15-30Gy). A broad spectrum of antibiotics, antimycotic and antiviral drugs, and gamma immunoglobulin were administered until the patients were free of fever and the total leukocyte count rises above $1x10^9/1$ with more than $0.5x10^9/1$ neutrophils. The disease free survival was calculated by the Kaplan & Meier method (9); differences between groups were determined by means of the Log rank test.

Table 1. Distribution of Patients: FAB Classification %

	ALL	ANLL
1	20.5	22.2
2	77.1	20.2
3	2.4	24
4		17.3
5		12.5
6		3.8

Abbreviations: FAB, French-American-British; ALL, Acute Lymphoblastic Leukemia; ANLL, Acute Non-Lymphoblastic Leukemia.

Table 2. Age Distribution (Years)

	ALL (83)	ANLL (104)
Median	20	36
Range	(2-57)	(2-52)
<15	30.1	12.5
Adult 15-45	66.3	72.1
>45	3.6	15.4

Abbreviations: ALL, Acute Lymphoblastic Leukemia; ANLL, Acute Non-Lymphoblastic Leukemia.

RESULTS

The disease free survival (DFS) in ANLL was 45% (median follow-up 10 months, range 1-76 months): 51% in CR1 at 76 months, 36% in CR2 (median follow-up 10 months, range 1-56 months).

The DFS in ALL was 33%: 38% in CR1 (median follow-up 12 mos., range 1-76 mos.) and 27% in CR2 adult patients (median follow-up 10.5 mos., range 1-40 mos.); 30% of the ALL patients are younger than 15 years. When we analyze the impact of marrow purging we observe for ANLL a DFS of 44% vs. 33% in ALL.

When comparing the CR1 purged (P) to CR1 unpurged (UP) group in ANLL we demonstrate a DFS of 53% (median follow-up 15.5 mos., range 2-52 mos.) and 38% (median follow-up 9 mos., range 1-76 mos.) respectively. The same analysis in ANLL CR2 patients shows a DFS of 39% in the purged group (median follow-up 20 mos., range 1-40 mos.) vs. 33% in unpurged patients (median follow-up 10 mos.,
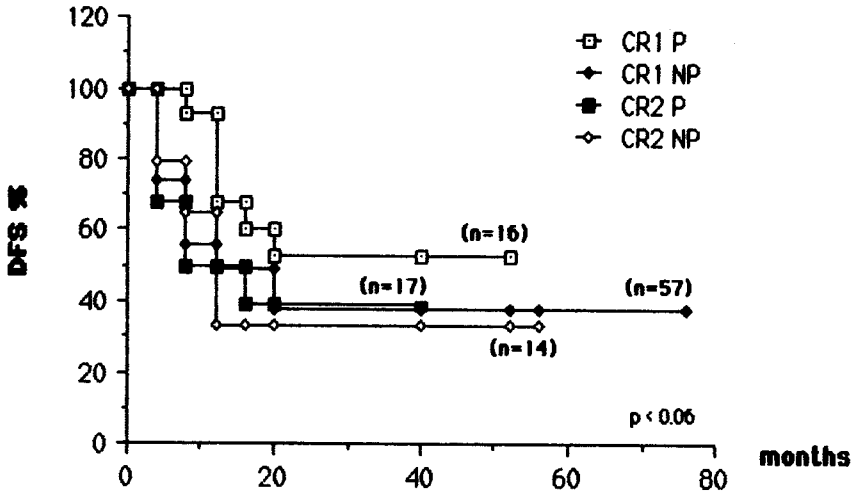
Figure 1. Disease free survival after autologous bone marrow transplantation in acute non-lymphoblastic leukemia in patients grafted in first and second complete remission (CR1,CR2) with mafosfamide treated (P) and untreated (NP) marrow.

range 1-46 mos.) (Figure 1). In ALL the DFS in CR1-P was 44% (median follow-up 10 mos., range 1-76 mos.). The data for DFS in CR1-UP are not available for the analysis (8 pts. with short follow-up). The results in All-CR2 demonstrate a DFS of 33% (28% in adult pts.) in purged group (median follow-up 8 mos., range 1-48 mos.) vs. 32% (27% in adult pts.) in unpurged patients (median follow-up 8 mos, range 1-35 mos.). Sixteen patients grafted in CR1 and CR2 (10 ANLL and 6 ALL) after marrow purged with high individual dose of Mafosfamide, show a DFS of 80% (median follow-up 12 mos., range 3-24 mos.) (Figure 2).

The evaluation of DFS related to pre-transplant regimens shows that the percent of free-surviving ANLL-CR1 patients autografted after Bu-Cy was 53% (median follow-up 11.5 mos., range 1-52 months) vs. 47% (median follow-up 9 mos., range 1-76 mos.) for other regimens comprising Cy-TBI. In CR2 the DFS after Bu-Cy was 61% (median follow-up 9 mos., range 1-40 mos.) vs. 36% in Cy-TBI and/or other regimens (median follow-up 9 mos., range 1-56 mos.).

In ALL patients the Bu-Cy pre-transplant regimen (12 pts.) shows a 56% DFS in CR1 (median follow-up 10 mos., range 1-20 mos.) vs. 39% (median follow-up 10 mos, range 1-76 mos.) obtained with Cy-TBI.

In ALL-CR2 patients the DFS for Cy-TBI was 38% (median follow-up 8 mos., range 1-48 mos.); for Bu-Cy treated patients no data are available for statistical analysis.

The data regarding CR-ABMT interval show that in ANLL patients grafted less than 6 mos. post CR1 the DFS was 40% (median
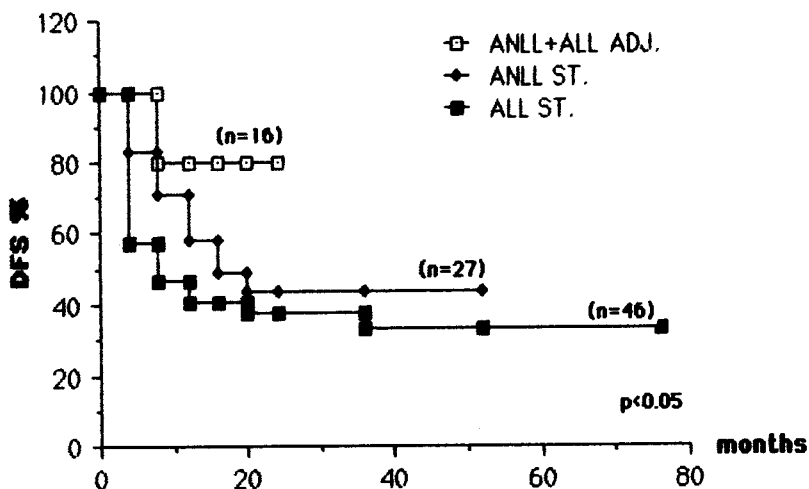
Figure 2. Disease free survival after autologous bone marrow transplantation in acute non-lymphoblastic and acute lymphoblastic leukemia in first and second remission using standard (st.) and adjusted (adj.) dose of mafosfamide.

follow-up 10 mos., range 1-52 mos.); for intervals >6 mos. the DFS was 63% (median follow-up 10 mos., range 1-76 mos.). Similar results are obtained in CR2-ANLL demonstrating a DFS of 24% and 79% for intervals CR-ABMT < 6 and > 6 mos. respectively (Figure 3).

For ALL groups the DFS in patients transplanted at < 6 mos. was 39% in CR1 at 52 mos. (median follow-up 8 mos., range 1-52 mos.) and 26% in CR2 (median follow-up 9 mos., range 1-24 mos.). When considering CR-ABMT intervals of > 6 mos. the DFS was 49% in CR1 (median follow-up 12 mos, range 1-76 mos.) and 55% in CR2 (median follow-up 8 mos., range 1-48 mos.).

In all patients the presenting complications in post transplant clinical course included bacterial, viral, and fungal infections in 20%, 11%, and 8% of patients respectively; interstitial pneumonitis (1.5%), liver VOD only in ANLL patients (2%), and hemorrhagic cystitis (7%). In 5 patients we observed CNS symptoms, and 3 patients developed cutaneous reactions as moderate GVHD. Hemorrhagic episodes occurred in 14 patients. Cardiac toxicity was observed in 8% of patients and ARDS in 2 patients. One patient developed a cataract.

Other complications during the aplasia period, all of which were mild and transient, included mucositis (70%) diarrhea (30%), renal and hepatic dysfunctions, respiratory distress.

Thirty-one patients developed complications and died before discharge from the centers: the causes of death were septicemia (8 patients), cardiac failure (9 patients), acute renal failure (3 patients), intracerebral hemorrhage (7 patients), liver VOD (2 patients), ARDS
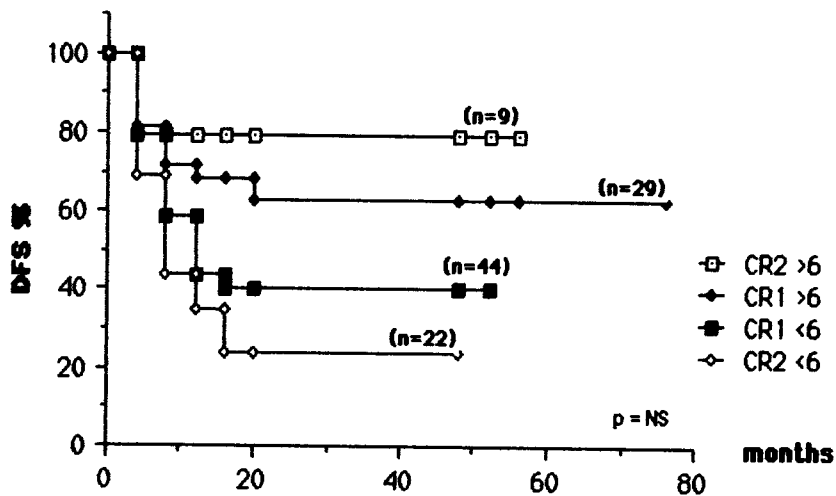
Figure 3. Disease free survival after bone marrow transplantation in acute non-lymphoblastic leukemia in first (CR1) and second (CR2) complete remission in relation to interval between complete remission and graft (>6 mo. and <6 mo.)

(2 patients). Three patients died of septicemia within two years after ABMT, two patients of interstitial pneumonitis, one of viral hepatitis. Recurrent leukemia was the cause of death in 47 patients. The kinetics of engraftment were: for ALL, WBC>$1x10^9$/l 18 days (range 12-70), platelets > $50x10^9$/l 41 days (range 20-199); for ANLL, WBC > $1x10^9$/l 21 days (range 15-55), platelets >50 x 10^9/l 60 days (range 22-302).

DISCUSSION

Autologous Bone Marrow Transplantation (ABMT) is effective in ANLL and in poor risk ALL (45% and 33%) (10). If we analyze the data of ANLL and ALL groups, transplanted in CR1 or in CR2 we observe impressive results, even better when we transplant in CR1.

The "ex vivo" pharmacological purging performed with standard-dose Mafosfamide demonstrates that ANLL is more responsive (44%) than ALL (33%) to the treatment.

The actuarial DFS rate in poor-risk ALL patients is 44% in the purged CR1 group; because of the low number and short follow-up in unpurged CR1 patients, the efficacy of the purging approach was not demonstrable, any significant difference is observed in CR2 groups. In contrast, for patients with ANLL in first remission the DFS in the purged group is 53% vs. 38% (p <0.06) of unpurged patients demonstrating a significant effect of "in vitro" marrow treatment (11). In second remission there is a trend in favor of

purged marrow for ANLL but the difference is not statistically significant.

In the last two years a group of AL patients was treated with programmed purging with increasing doses of Mafosfamide; the DFS is very impressive: 80% at 24 mos. In this group of patients the kinetics of engraftment is similar to that observed in patients with marrow treated with standard doses of the drug.

We have analyzed the timing for bone marrow transplantation. The DFS is better for patients where the interval CR-ABMT was longer than 6 mos.: 63% in CR1-ANLL and 79% in CR2. Similar results are obtained in ALL CR1 and CR2.

The pre-transplant regimen Bu-Cy demonstrates better results in ANLL either in CR1; the same protocol used in CR2 ANLL is the recommended treatment (61% vs. other regimens). Cy-TBI is the regimen of choice in ALL.

In conclusion ABMT using marrow "ex vivo" treated with Mafosfamide following Bu-Cy gives a DFS of 53% demonstrating an active effect of purging for removing residual disease in CR1 ANLL; the analysis of data shows a trend in favor of ALL CR1 treated marrow (44% at 76 mos.).

The marrow purging at this time is a technical approach that has demonstrated positive results in CR1-CR2 ANLL and CR1 ALL but randomized studies are needed to definitely clarify the effect of Mafosfamide treated marrow on DFS in AL (12).

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TIMING OF BONE MARROW TRANSPLANTATION FOR ADULTS WITH ACUTE NONLYMPHOCYTIC LEUKEMIA

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INTRODUCTION

The timing of marrow transplantation in the course of treatment of acute nonlymphocytic leukemia (ANL) is an important and difficult choice faced by patients and physicians. In this article the issues surrounding this decision will be discussed.

Chemotherapy

Any discussion of the timing of marrow transplantation must take into account the outcome of treatment without transplantation. Studies of chemotherapy for younger adults have reported widely divergent results. Some of the variation is likely due to differences in treatment regimens, but bias in the selection of patients, small study size, and limited follow-up almost certainly contribute. The most reliable studies are probably those in which patients are entered at diagnosis (to eliminate or reduce patient selection bias), include large numbers of patients (>100), and in which patients are all followed for at least two years. As shown in Table 1, there have been some studies recently published which fulfill most of these criteria, and show in general that of those patients achieving complete remission (CR), approximately 20% to 30% can be expected to remain in CR for more than two years (1-6). Most treatment failures are due to recurrent leukemia, although with the more aggressive treatment regimens used currently, anywhere from 5% to 15% of remission patients die of toxicity while in remission. Once patients relapse, few, if any, can be cured by further chemotherapy without marrow transplantation.

**Table 1. Results of Selected Large Studies
of Treatment of Adults with ANL in CR**

Form of Therapy		No. of CR pts	% in CCR	Source
Chemotherapy				
Consolidation	Maintenance			
None	Ara-C*	151	25% @ 60 mo	Rai et al, 1981[1]
None	Ara-C*	356	22% @ 36 mo	Yates et al, 1982[2]
+/-DAT	Ara-C, 6TG	146	21% @ 24 mo	Cassileth et al, 1984[3]
+/-DAT	Various	374	24% @ 36 mo	Buchner et al, 1985[4]
DAT	Ara-C, 6TG	757	20% @ 48 mo	Rees et al, 1986[5]
None	Various	329	22% @ 36 mo	Preisler et al, 1987[6]
Allogeneic Marrow Transplantation Preparations				
CY + TBI		231	46% @ 60 mo	Clift et al, 1987[7]
Various		447	45% @ 60 mo	Champlin et al, 1987[8]

*Various other drugs added to ARA-C

Results of Allogeneic Marrow Transplantation

For the purposes of this discussion, allogeneic transplantation can be considered for patients age 50 or less who have an HLA-genotypically or phenotypically identical family member, or a family member mismatched at a single HLA locus (A, B, or D), to serve as a marrow donor. A number of studies describing the results of allogeneic marrow transplantation in such patients have been published (see Table 1). Approximately 45%-50% of patients transplanted in first remission remain alive three years later (7,8). Many reports of marrow transplantation for ANL suffer from the same weaknesses as the chemotherapy studies in that patients were not identified at diagnosis, enrollment was small, and follow-up often was limited. In the few studies where patients were identified at diagnosis (9,10), and in those with large numbers of patients (7,8) and adequate follow-up, the results were consistent with 45%-50% of patients alive more than five years after transplant.

Unlike chemotherapy, marrow transplantation can cure some patients who have relapsed from initial chemotherapy. From Seattle we reported that five-year disease-free survival was 30% for 54 patients transplanted during untreated first relapse, 28% for 49 patients transplanted during second remission, and 21% for 29 patients transplanted while in chemotherapy-resistant relapse (7,11). These results have several implications. First, there is no reason to delay transplant beyond first relapse or second remission, since results deteriorate. Second, since the outcome in first relapse is as good as in second remission, there is no apparent reason to subject first relapse patients to reinduction prior to marrow transplantation, since some may not achieve a complete remission, and those that do are not better candidates than they were prior to reinduction. Of course there may be important logistic problems such as identifying a donor, finding a transplant bed, and arranging financing, which may delay transplantation, therefore requiring further chemotherapy to maintain the patient until transplantation. The third implication of results of salvage transplantation is that a strategy of initial chemotherapy, followed by transplantation at the time of first relapse, may yield an outcome almost as good as transplantation during first remission. This is illustrated in Figure 1. Shown are the outcome of marrow transplantation in 220 patients transplanted in first remission in Seattle, and the outcome of 392 patients <age 50 treated on two sequential Southwest Oncology Group (SWOG) trials. A third line shows what would have happened if every patient who failed the SWOG trial were transplanted in untreated first relapse. Since 30% of the relapsing patients would be salvaged, the cumulative result of chemotherapy and salvage transplantation would approach that of first remission transplants. Of course, in reality, not all the failing patients will be transplanted. Some (5%-15%) will have died of treatment-related toxicities rather than relapse, some will have acquired additional illnesses, such as severe hepatitis, precluding transplantation, and others will relapse so explosively that they never make it to transplant. Because of these concerns, transplantation in first remission is, in our view, the preferred approach.

Since the predominant causes of failure of chemotherapy and transplantation differ, we asked whether it might be possible to identify factors predictive of outcome in order to distinguish patients likely to benefit more from early transplantation from those in whom chemotherapy might be preferred. In terms of continued disease-free survival, early transplantation was preferred for every identified patient category, although this advantage was least apparent for older men who achieved a complete remission with one cycle of chemotherapy. This analysis, however, did not take into account the fact that some relapsing patients could be salvaged with transplantation (12).

'SURVIVAL' AFTER ACHIEVING FIRST REMISSION
Projected effect of treatment strategy

Chemotherapy only (SWOG) _____ Transplant 1st remission ....
Transplant untreated 1st relapse _ _ _ _
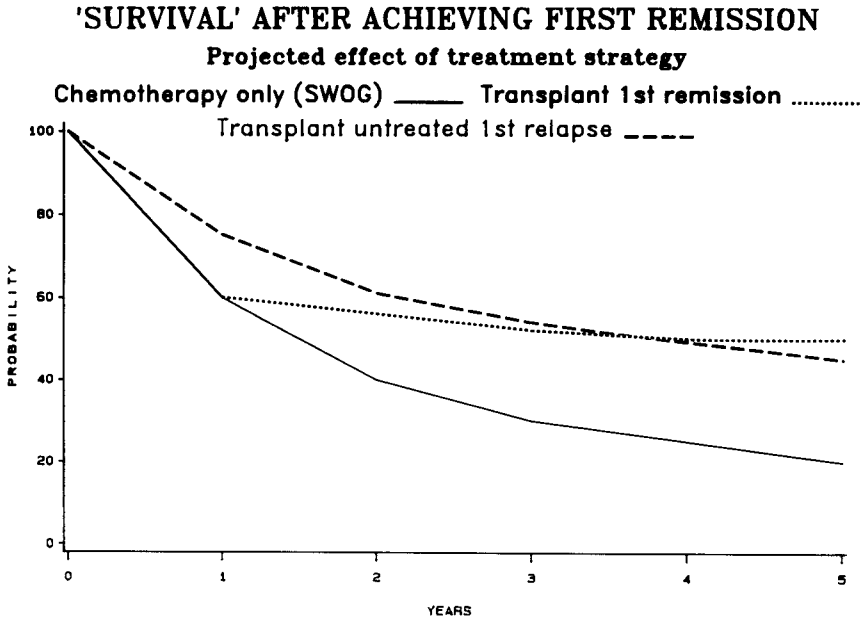
Figure 1. Shown are the Kaplan-Meier probabilities of survival after achieving a first CR for 220 patients transplanted in Seattle while in first CR (- - - - -), 392 adults less than age 50 treated with chemotherapy on two sequential SWOG trials (_____), and the theoretical survival of the SWOG patients if every patient who failed chemotherapy were transplanted from a matched sibling at first untreated relapse (-- -- -- --).

In summary, for patients with ANL who have recently achieved a complete remission and have an appropriate donor, transplantation during first remission is probably preferred. But for some patients, transplantation at the time of first relapse may represent an appropriate time is a reasonable alternative.

Autologous Marrow Transplantation

It is more difficult to reach conclusions about the timing of autologous transplantation. None of the reported studies of autologous transplantation for patients in first remission identified patients at diagnosis, and thus selection bias may have been substantial. These studies generally consisted of small numbers of patients, and follow-up was limited. For these reasons, results have been highly variable, with three-year survivals ranging from 20% to 60% [reviewed in reference 13]. Thus, any advantage of autologous transplantation in first remission over continued chemotherapy has yet to be definitively demonstrated. Further, it is unknown how many patients with ANL who relapse from conventional chemotherapy can be salvaged with

autologous transplantation later in their course. Two reviewed reports of autologous transplantation for ANL in second remission have shown surprisingly good results with 30% of patients alive in remission (14,15). These reports dealt with small groups of selected patients and follow-up was short. As with allogeneic experience, transplantation in untreated first relapse may represent an appropriate time to consider autologous transplantation. However, there are no reports of autologous transplantation at this disease stage. We have transplanted, and followed for at least one year, nine such patients using marrow stored during first remission. All patients were prepared with cyclophosphamide-TBI preparative regimens. Three of the nine patients remain alive at 15, 16, and 69 months post-transplant. Of interest, two of the three survivors, including the patient alive in remission beyond five years, received first remission marrow untreated in vitro. Of the six that have died, two did so due to leukemic relapse and four died with infectious complications.

The published data available to date are too limited to allow conclusions about the appropriate timing of autologous transplantation in the management of patients with ANL. Whether transplantation is better than chemotherapy alone remains in question, and it is certainly unknown if the outcome of transplantation in first remission is superior to that achieved with chemotherapy plus later autologous transplantation at first relapse or second remission. These questions can best be addressed by a prospective study in which all patients are identified at diagnosis and those who achieve a complete remission and have no donors have marrow stored while in remission and are then randomized to autologous transplantation in first remission vs. continued chemotherapy with transplantation offered after relapse. Several studies with this or a similar design are currently underway.

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Discussion 1 - Session 1A (AML)

Dr. Goldstone: Dr. Buckner, does patient referral to Seattle have any effect on the length of time between remission induction and transplantation? Have you broken down the data in 0 - 3 months, 3 - 6 months, etc.?

Dr. Buckner: Tony, 75% of the patients are transplanted within 3 months after remission induction. Interval between CR induction and transplantation has no influence on outcome after allogeneic transplantation. We do not have enough experience with auto transplantation to draw conclusions. We believe that transplants are most meaningful when done early.

Dr. Goldstone: Dean, you mentioned that allo-BMT is the golden standard. How do you compare this with chemotherapy when basically the allogeneic patients are a selected group of patients?

Dr. Buckner: We already did a comparative study in Seattle. Follow up is over 5 years. In the allogeneic arm, we identified the patients at time of diagnosis. Even when we include the patients with suitable donors who were lost before transplantation due to relapse or refused the long term survival in this arm is approximately 50%.

Dr. Gulati: What about autotransplantation? Is there any difference in outcome dependent on the blast cell infiltrate?

Dr. Gorin: As far as I know there are no disease-free long- term survivors after autotransplantation in relapse.

Dr. Dicke: Dr. Rizzoli, is there any difference in outcome after transplant in second CR between patients who relapsed on therapy and those who relapsed after chemotherapy?

Dr. Rizzoli: I do not know. However, after achieving CR2, patients who received chemotherapy prior to transplant did better than those who waited for transplantation without chemotherapy.

Dr. Dicke: Dr. Rizzoli, the other interesting point is that patients treated immediately after achieving CR2 do more than those already in CR for several months. Is that because the bad prognostic patients have been eliminated? Your overall survival curve is surprisingly good, 35%.

Dr. Rizzoli: We need to keep in mind that with conventional chemotherapy cure in CR2 is possible. But this is no more than up to 20%. We think that the results with autotransplantation are much better.

MEROCYANINE-540 MEDIATED PHOTOSENSITIZATION IN COMBINATION WITH MAFOSFAMIDE FOR EX-VIVO BONE MARROW PURGING

Maria Teresa Marchetti-Rossi, Giovanni Sparaventi, Annunziata Manna, and Adolfo Porcellini

INTRODUCTION

To assess the efficacy of ex-vivo cleansing procedures, we have recently developed a highly sensitive in vitro clonogenic assay (1) which involves mixing 5% of established leukemic cells (CCRF-SB or K562) with an excess of human bone marrow cells (HBMM). In our previous studies we were able to detect with our limiting dilution assay (LDA) as much as 6 log reduction of clonogenic cells in simulated remission marrows purged with either ASTA-Z 7557 (AZ) or Merocyanine (MC-540).

MATERIALS AND METHODS

In this study we tested the efficacy of a combination of AZ and MC-540 on co-cultures of 5% of CCRF-SB or K-562 cells with an excess of HBMM cells.

To this end the cell mixtures were adjusted to 20×10^6/ml cells with TC199 medium, placed in plastic tubes and incubated with Mafosfamide as previously described (1).

The Mafosfamide treated suspensions were then diluted to 4×10^6 cells/ml and incubated, in Petri dishes, with $15 \mu g$/ml of MC-540 and exposed to light for 90 minutes as previously described (1).

Limiting Dilution Analysis

At the end of the incubation period with AZ alone or with AZ

followed by MC-540-mediated photosensitization, 10^4 cells from those treated were plated in microculture wells. Decreasing cell numbers of the same line used to contaminate the HBMM cells were added to the 10^4 purged cells contained in the microwells and incubated for 13 days. Twelve hours before harvesting, cells were pulse-labeled with 1 μC of tritiated thymidine. The cells were then harvested with a Skatron apparatus and incorporated thymidine was quantitated by scintillation counting.

Wells that exceeded the mean of the control wells (with no added leukemic cells) by at least 3 SD were scored as positive (responders), while wells with tritiated thymidine incorporation less than this threshold were scored as nonresponders (negative).

LD assay techniques usually involve adding decreasing numbers of cells, as enumerated by LDA, containing an unknown proportion of target cells to microculture wells containing irradiated cells (Figure 1). Hence, when 0 cells are added to the wells (thus containing only irradiated cells), no cell growth, as measured by ^{3}HTdr uptake, will be attained. In other words for 0 cells added, we will necessarily obtain 100% of negative wells. By definition therefore, the ML regression line will necessarily include the origin (Figure 1). The number of target cells is then obtained by the Poisson distribution equation (assuming that we are dealing with a homogenous cell suspension):

$$F(n) = U^n \times {}^{-U/n}$$

is the probability of n responder cells (RC)/well when the mean number of RC at that cell density is U.

The probability of obtaining a culture with no RC is:

$$F(o) = e^{-U}.$$

$$\text{At } U=1 \quad Fo = 0.37$$

Therefore when N cells/well = 37% non-responding cultures, the frequency of RC = 1/N.

In our case, however, the cell layer (Figure 2) consists of the purged cell suspension, thus containing a number of leukemic cells that was originally determined (5%) but unknown after purging. On this cell layer, containing an unknown number of leukemic cells (or none in the case of complete purging), decreasing numbers of leukemic cells are seeded. According to the 0 term of the Poisson distribution equation:

$$(Fo = e^{-U})$$

the proportion of negative wells is a negative logarithmic function of the number of leukemic cells added to each well.
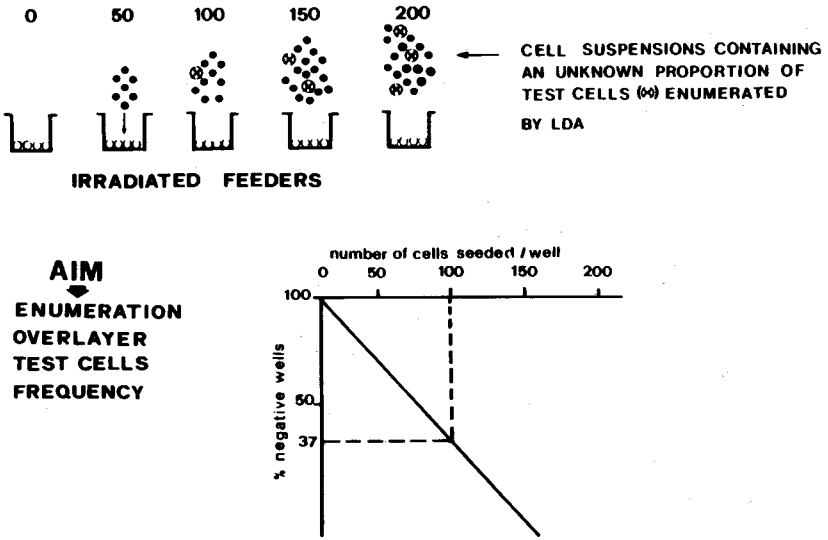
Figure 1.
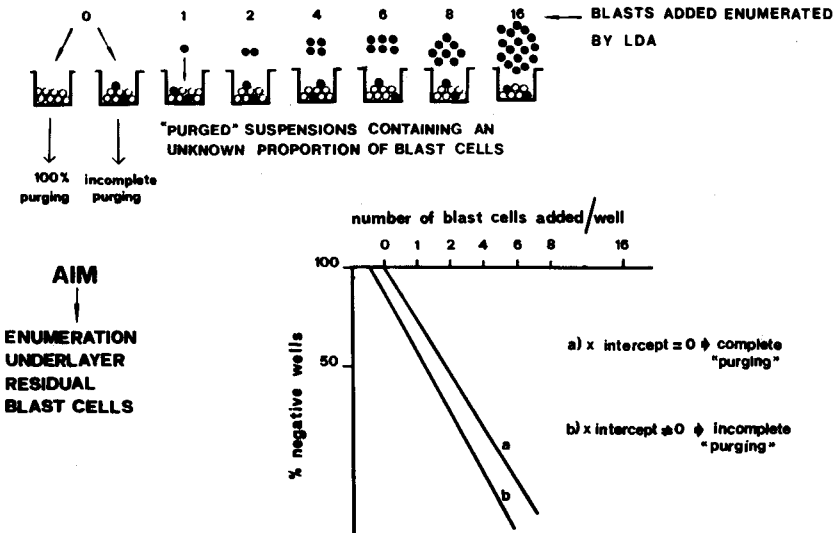
Figure 2.

a. <u>The purging was complete</u>: In this case the cell layer will no longer contain any leukemic cells. Thus for 0 leukemic cells added we will have 100% negative wells, and the ML regression line will necessarily include the origin.

b. <u>The purging was not complete</u>: Then some leukemic cells remained in the purged suspension and were seeded in the microcultures. The wells with 0 blasts added will show some degree of incorporation; in other words for 0 blasts added we will have some positive wells. The ML regression line, in this case, is expected to pass, not through the origin, but through that point on the negative ordinate determined by the number of percent negative wells per 0 blasts added, determining also an X intercept on the negative abscissa. This X intercept of the line will represent the number of blast cells that would have to be removed from the wells in order to produce 100% negative wells.

RESULTS

Treatment with AZ alone produced a total elimination (about 6 logs) of B-cell acute leukemia cells (CCRF-SB), whereas nearly 1.66 logs of K-562 acute myelogenous blasts were still present in the cell mixtures after treatment.

The cloning efficiency (CE), calculated as the slope of the regression line when the ordinate is the natural log of percent negative wells, was 22% (CCRF-SB) and 37.5% (K-562).

When the CCRF-SB contaminated cell suspensions were treated with AZ followed by MC-540 photosensitization, we could not demonstrate any further improvement in terms of log kill of leukemic cells, as 100% purging of this line was already attained with AZ alone. However, the CE increased to 24.4% after purging with both agents, clearly indicating a reduced number of false-negative results, or in other words, an improved cleansing, albeit beyond the detection level of our assay (1,2).

The 95% confidence intervals (CI) for the value of the x-intercept, calculated as described by Cox and Hinkley (3), were -0.27 to 0 after cleansing with AZ alone and -0.5 to 0 after cleansing with both agents.

Treatment of K-562-contaminated cell suspensions with AZ and MC-540 in combination produced a total elimination of myelogenous leukemic cells, and the CE of this cell line increased from 37.3% (AZ alone) to 62%. Again the 95% CI ranged from -0.24 to 0.

In conclusion the drug combination AZ and MC-540 has proved highly efficient in killing established leukemia cells lines as compared to treatment with single agents; previous studies have shown that in

experimental models AZ (4) or MC-540 (2,5) do not impair the ability of marrow cell suspensions to restore hemopoiesis; evidence that also the combination of AZ and MC-540 is not harmful to pluripotential stem cells has already been presented (2); thus this purging protocol may provide a new approach for removing malignant cells from autologous marrow cells suspensions.

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INTRINSIC LEUKEMIC PROGENITOR CELLS' SENSITIVITY TO CRYOPRESERVATION: Incidence for Autologous Bone Marrow Transplantation

Maria Alessandra Allieri, Manuel Lopez, Luc Douay, Eric Martini, Jacqueline Deloux, H. Lan NGuyen, M. Catherine Giarratana, Albert Najman, and Norbert Claude Gorin

INTRODUCTION

Cryopreservation of hematopoietic stem cells is a routine technique for autologous bone marrow transplantation. Adequate viability of cryopreserved stem cells is a prerequisite for successful engraftment (1). In our experience, more than 75% CFU-GM and BFU-E are recovered after cryopreservation (2). Cryopreservation of leukemic blast cells is frequently used for immunological studies. For that purpose, its efficiency is well established. However, while blast cell recovery and viability in Trypan Blue staining are good after thawing, simultaneous preservation of the clonogenic cells has not been yet determined. The present study was undertaken to determine the best technique for cryopreservation of leukemic blast cells and to evaluate whether cryopreservation of leukemic blast cells and to evaluate whether cryopreservation is specifically toxic for the populations of clonogenic cells which give rise to CFU-L.

MATERIALS AND METHODS

Isolation of Mononuclear Cells

Bone marrow from 2 healthy control donors and peripheral blood from 3 patients with AML (n=2) and ALL (n=1) at diagnosis were collected in preservative-free heparin. Mononuclear cells (MNC) were isolated over a Ficoll gradient (d=1.077) and adjusted to a final concentration of 30×10^6 cells/ml.

Freezing, Storage and Thawing Procedures

We selected six techniques of cryopreservation which vary for different parameters such as the freezing solution (DMSO concentration, nature of serum, medium), the type of container (polypropylene ampoule or plastic bag) and type of freezing: directly at -80°C or using a programmed freezing technique (Nicool ST 20 or Minicool LC40) with a cooling rate of -2°C/mn. Frozen vials were stored at -80°C or in the gas phase of liquid nitrogen. Details of freezing techniques are described in Table 1. The cells were thawed just before the assay in a 37°C water bath: vials were diluted 1/10 in the thawing solution, washed twice and resuspended in 10% FCS containing medium. Cell viability was determined by the Typan Blue staining.

Table 1. Main Parameters in Six Freezing Techniques

T*	MNC x10E6/ml	Freezing Solution	Container	Freezing	Thawing Solution
1	30 in AB	DMSO 20% AB 10% TC 199 70%	ampoule	direct -80°C	TC 199 90% AB 10%
2	10 to 50 in FCS MEM 80%	DMSO 10% FCS 10%	ampoule	direct -80°C	MEM 80% FCS 10% DEXTRAN 10% DNase
3	30 in 100% FCS	DMSO 20% FCS 80%	ampoule	direct -80°C	IMDM 80% FCS 20%
4	<40 in 100% FCS	DMSO 10% (1/10)	ampoule	direct MINICOOL (-2°C/mn)	IMDM 80% FCS 20%
5	30 in FCS	DMSO 10% FCS 20% TC 199 70%	ampoule	programmed MINICOOL (-2°C/mn)	TC 199 97.5% FCS 2.5% DNase
6	30 in AB	DMSO 20% AB 10% TC 199 70%	Plastic bag	programmed NICOOL (-2°C/mn)	TC 199 90% AB 10%

*Technique: 1) CNTS St. Antoine-Paris, 2) Ontario Cancer Institut-Toronto, 3) Hopital Henry Mondor-Paris, Terry Fox Laboratory-Vancouver, 5) Dana Farber Cancer Institut-Boston, 6) CNTS St. Antoine-Paris.

Frozen Vials were stored at -80° and, only for technique 6, in the gas phase of liquid nitrogen. AB: pool of AB sera; FCS: fetal calf serum; DMSO: dimethylsulfoxid; MEM: minimum essential medium.

Culture Conditions

CFU-GM and BFU-E Assay

Fresh and thawed cells from the normal bone marrows were cultured according to our previously described procedures (1-2).

CFU-L Assay

Assay for CFU-L was performed according to techniques described by Löwenberg et al. (3-4) with some modifications. The MNC were recovered after Ficoll separation. T-lymphocytes were removed by complement lysis using a pool of monoclonal antibodies (CD2-5-7) and baby rabbit complement. T-depleted MNC were placed in 0.36% methylcellulose in Mac Coy's 5A medium supplemented with 30% FCS, 2.5% PHA and for ALL only, 25 units of Interleukin 2 were added. 0.5 and $2.5x10^5$ cells respectively for AML and ALL were seeded in triplicate in 35mm Petri dishes over a stimulating 0.5% agar feeder layer containing $2 x10^6$ irradiated peripheral blood leukocytes. Cultures were incubated for seven days at 37°C in a humidified 5% CO_2 atmosphere. Colonies containing at least 50 cells were counted. Leukemic origin of the clones was assessed by cytological examination and immunological characterization by flow cytometry of the pooled colonies.

Analysis of Results

In order to quantitate the stem cell recovery, results are expressed in terms of number of progenitors per ml and compared to the initial growth of the fresh cells.

RESULTS

Normal Bone Marrow Cryopreservation

Table 2 shows overall efficient cryopreservation of CFU-GM and BFU-E. There were no significant differences in colony recovery between the six techniques.

CFU-L Cryopreservation

Patient data and CFU-L before freezing are depicted in Table 3. Table 4 shows overall deficient cryopreservation of leukemic cells. In spite of a good cell viability and recovery after thawing, recovery of CFU-L was very poor: median = 2 ± 0.6%, 0%, 25 ± 10% respectively. There was no significant difference between the freezing techniques used. However one may point out poorer results with technique 4.

Table 2. Progenitor Recovery in 2 Normal Bone Marrows

Freezing Techniques	1 % recovery		2 % recovery	
	CFU-GM	BFU-E	CFU-GM	BFU-E
1	45	80	44.5	66
2	58	98	46	76
3	75	71	44	64
4	78	64	23	26
5	71	70	47	65
6	24	30	27	44.5
Medians (range)	65 (24-78)	70.5 (30-98)	44 (23-47)	64 (26-76)

Table 3. CFU-L Before Freezing

Patients	Diagnosis	Source of Cells	Blasts %	Colonies /5x10E4 cells
1	LAM2	PB	52	732 ± 71
2	LAM2	PB	63	194 ± 68
3	LAL-T	PB	90	43 ± 17

Cells pooled form colonies from fresh and frozen CFU-L showed the same cytological aspect and immunological pattern that incultivated cells (data not shown).

DISCUSSION

In order to investigate the sensitivity of normal and leukemic progenitors to cryopreservation, six freezing techniques have been studied. While CFU-GM and BFU-E from normal donors were efficiently cryopreserved whatever the technique used, the recovery of CFU-L was always very poor. Indeed, although the mean platting efficiency for CFU-L after thawing was 7.6×10^{-4} and 1.8×10^{-4} in two patients, the absolute number of actually recovered CFU-L, comparatively to normal progenitors, was dramatically low. Our results show that leukemic progenitors are effectively more sensitive to cryopreservation than normal progenitors. Therefore, one may

**Table 4. Recovery of CFU-L after Freezing
in 3 Patients with Acute Leukemia**

Pts	Parameters	Freezing Techniques						Medians (range)
		1	2	3	4	5	6	
1	% cell recovery	36	100	89	69	70	61	65.5 (36-100)
	% viability in TB	80	85	85	75	86	74	82.5 (74-86)
	% CFU-L recovery	1.3	2.5	2.5	1.1	2	1.97	1.98 (1.1-2.5)
2	% cell recovery	53	57	56	73	76.5	--	57 (53-76.5)
	% viability in TB	50	85	61.5	76	80	--	76 (50-85)
	% CFU-L recovery	0	0	0	0	0	--	0
3	% cell recovery	86	100	100	100	100	100	100 (86-100)
	% viability in TB	--	--	--	--	--	--	--
	% CFU-L recovery	22	33	21	4.5	28	29	25 (4.5-33)

suggest either that standard culture technique for in vivo growth of CFU-L are not satisfactory for frozen leukemic cells, or that cryopreservation of CFU-L requires specific conditions which are yet to be defined. Nevertheless, this particular sensitivity of leukemic progenitors to cryopreservation could suggest a possible purging effect in acute leukemia in complete remission.

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AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR CONSOLIDATION OF ACUTE LEUKEMIA IN REMISSION:
Purging or Not Purging? Influence of Pretransplant Intervals

Norbert Claude Gorin, Jean-Philippe Laporte, Luc Douay, Manuel Lopez, and Albert Najman

INTRODUCTION

High dose chemotherapy and/or total body irradiation (TBI) followed by autologous bone marrow transplantation (ABMT) is increasingly used to consolidate patients with acute myelocytic leukemia (AML) and acute lymphoblastic leukemia (ALL) in remission (CR).

In contrast to numerous in vitro studies and experiments in preclinical animal models which have clearly demonstrated the efficacy of purging in reducing leukemic cell contamination of marrow collected in CR or artificially built marrow-tumor cell mixtures, the validity of marrow purging in man has never been established. In some centers, attempts have been made to purge the marrow prior to autografting, while in others, the reinfused marrow has not been manipulated in vitro.

We will report here in a first part the results of our institution (updated as of September 1, 1987) on 76 adult patients, autografted in remission with marrow purged by mafosfamide at a dose individually adjusted (1). In a second part of this paper, we will present the results of the last European survey on 1021 patients in which we found evidence in favor of marrow purging, as well as the beneficial influence of long pretransplant intervals.

THE EXPERIENCE OF THE PARIS-ST ANTOINE BONE MARROW TRANSPLANT UNIT

Material and Methods

Since January 1982 and as of September 1, 1987, 76 patients with acute leukemia received autologous bone marrow transplantation to consolidate complete remission. In our institution, patients are classified as standard risk in the absence of all the following poor risk criteria:

1. At diagnosis: secondary leukemia, presence of a Philadelphia chromosome, leukocytosis > $25x10^9/1$, mediastinal enlargement, extra hematopoietic localization (other than CNS).

2. Prior to ABMT: CNS involvement at any time.

3. At ABMT: evidence for partial remission rather than complete remission, LDH level > 1000 IU.

The distribution of the patients was the following:

- ALL CR1	standard risk:	5
	high risk:	19
- ALL CR2		7
- AML CR1	standard risk:	31
	high risk	7
- AML CR2	and beyond	7

The male/female distribution was 48/28. The median age of the population was 33 years (6-55). Four patients were children (<15 years). Forty-four patients were older than 30 years, including 21 who were older than 40 years. The interval diagnosis to autograft for patients in CR1 was 6 months (2-15). The interval from remission to autograft was 4 months (0.5-12).

Marrow collected in remission was divided in 2 parts: a back-up marrow and a marrow treated with mafosfamide. The dose of mafosfamide to treat the marrow was estimated in each individual patient 15 days prior to marrow collection, by studying the sensitivity of normal progenitor CFU-GM to increasing dosages of mafosfamide in semi-solid cultures. The dose retained for treatment was the CFU-GM LD-95 or 90, respectively for ALL and AML, defined as the dose sparing 5% and 10% CFU-GM. Details of our protocol have

been published elsewhere (1). Treated marrow was then cryopreserved in TC199 medium containing 10% DMSO and 5% human compatible serum. All patients then received the following pretransplant regimen:

- Cyclophosphamide (CY: 60 mg/kg/day x 2 and,

- Total body irradiation (TBI).

Following TBI, the treated marrow as reinfused. No maintenance therapy was then applied to the patient.

RESULTS

Complications

In the immediate transplant period, bacterial infections were the most common complications (sepsis n = 52), followed by viral infections (n = 18), and fungal infections (n = 8). Four patients developed liver veno-occlusive disease and all died (100%). Following discharge of the patients, the principal complication was viral infections (n = 16) including CMV (4), herpes zoster (8), HIV (1), followed by fungal infections (n = 8). Among other complications, our attention was drawn by the occurrence of numerous auto-immune manifestations post-ABMT:

- Monoclonal IgG spike: 3 (resolutive)

- Auto-immune pancytopenia and peripheral neuropathy (resolutive under steroid therapy): 1

- Peripheral bicytopenia cured by splenectomy: 1

- Hepatitis with graft versus host pathological feature: 1

- Primary biliary cirrhosis: 1.

Two patients developed liver veno-occlusive disease and 1 of them died.

Results

Engraftment was much more delayed in AML than in ALL. Recovery to WBC >10^9/l (day 30 vs 10 p <0.001), platelets >$50x10^9$/l (day 100 vs 47 p <0.01), reticulocyte >.1% (day 20 vs 15 p <0.01). Five patients with AML had prolonged (150, 150, 180, 475, 485 days)

thrombocytopenia and 2 thrombocytopenia were persisting at 18 and 20 months post transplant. Of 31 AML patients transplanted in CR1, 8 (26%) died from toxicity, including 5 during the ABMT procedure, and 3 while in persisting complete remission post ABMT (2 viral infections, 1 lung fibrosis). Three patients (13%) only have relapsed 5, 5 and 7 months post transplant. As of September 1st, 1987, 22 patients (65%) have remained in unmaintained complete remission with a follow-up of 19.5 months (1-52). Of these patients, 4 are beyond 3 years, 7 beyond 2 years, and 16 beyond 1 year. Twenty-four patients were transplanted for ALL in CR1 (19 high risk): 3 patients died from toxicity. Seven patients have relapsed at 3, 5, 8, 13, 14, 19, 29 months, and 13 (55%) have remained in complete remission with a follow-up of 15 months (1-51). Four patients are beyond 2 years.

Figure 1 indicates the disease free survival in 24 patients with ALL autografted in CR1. Figure 2 indicates the disease free survival in 31 patients with AML autografted in CR1. Figure 3 compares the disease free probability post ABMT for patients with AML and ALL autografted in CR1.

DISEASE FREE SURVIVAL POST ABMT
ALL IN CRI   n = 24 (19 poor risk)

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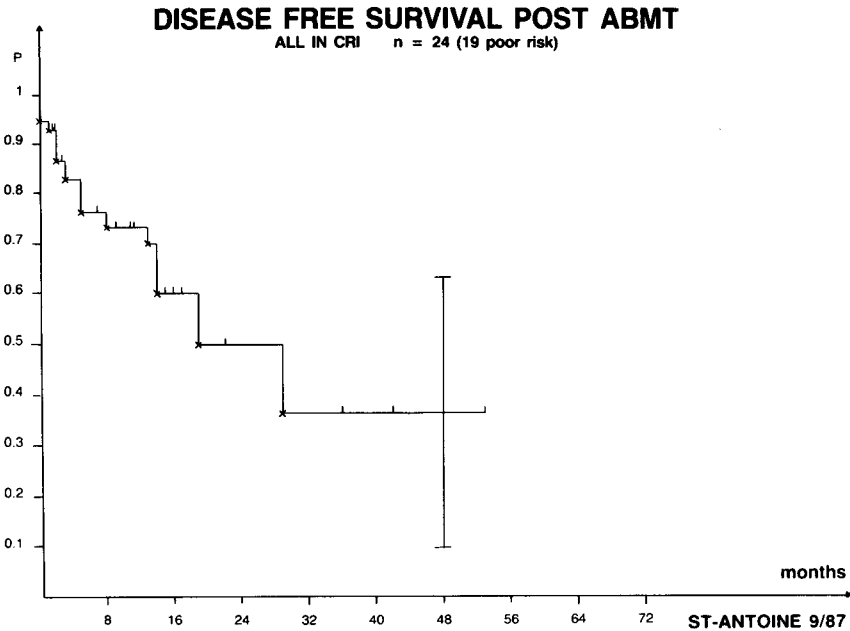
Figure 1. Disease Free Survival Post ABMT
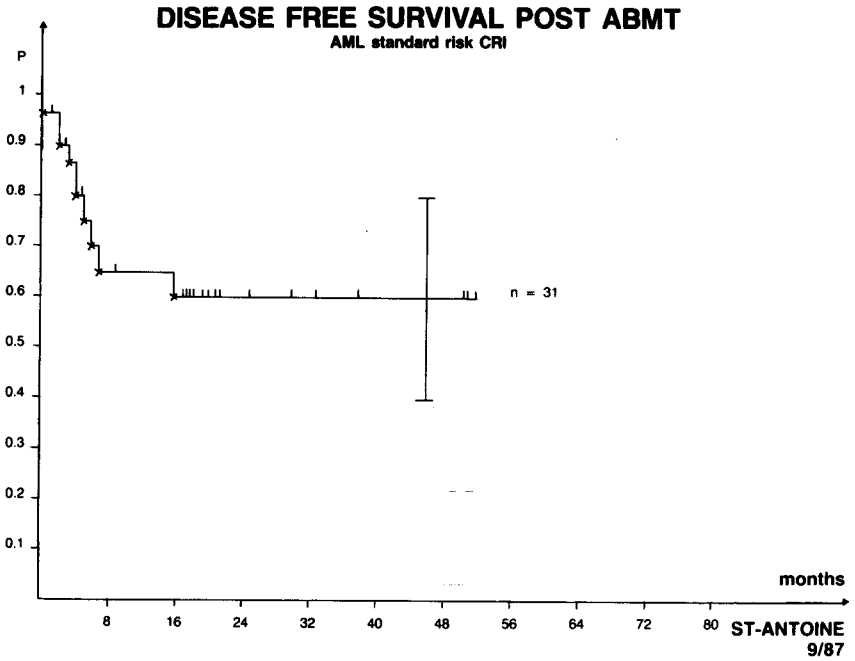
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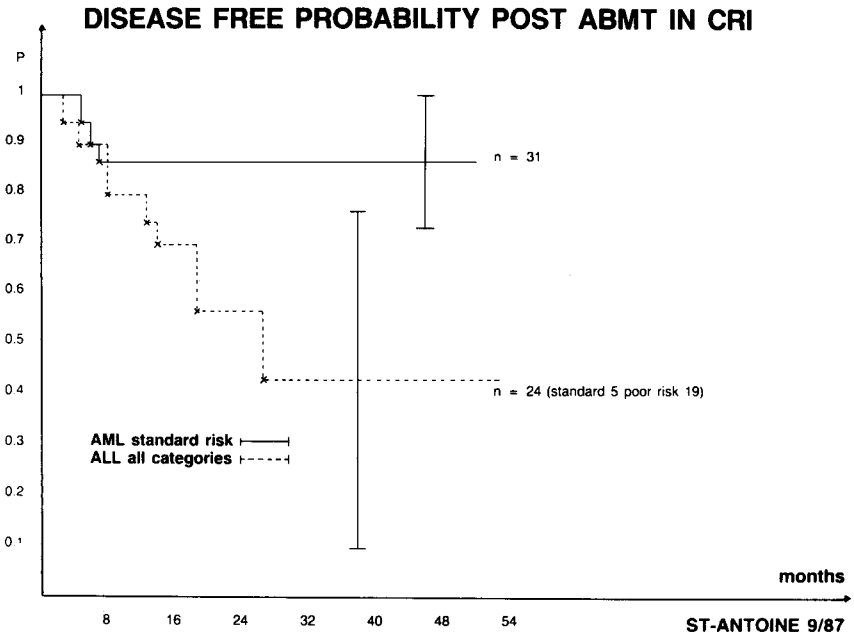
Figure 3. Disease Free Probability Post ABMT in CR1

CONCLUSION

Our interpretation of these results are the following:

1. Results of autografting in AML CR1 look favorable with a disease free survival of 60% from 16 to 50 months. We experienced very few relapses which occurred in the first year post-transplant.

2. Results in poor risk ALL are less impressive, but still favorable when compared to reports on conventional chemotherapy or allogeneic bone marrow transplantation (2).

3. Results in AML are considerably better than in ALL. We wish to pursue this study in view of the particularly very low rate of relapse in our experience.

FIFTH SURVEY OF THE EUROPEAN BONE MARROW TRANSPLANTATION GROUP

This fifth survey was conducted as the previous ones, however, a special effort was made to assess the value of marrow purging and to study the possible impact of the intervals pretransplant on the final outcome.

Material and Methods

In June 1987, all previously reporting teams received a complete print out of their own data for verification and necessary corrections. Modifications were entered in September 1987. New questionnaires (new patients and follow-up forms) were sent to 54 teams of whom 50 had appropriately answered by December 15, 1987. Forms have been reviewed up to January 30, 1988 and further clarification requested for 10%. A considerable effort has been made to ensure the accuracy of the present database prior to the introduction of the new homogenized EBMTG computer program. This analysis has been done on 1021 cases from 50 reporting institutions (see appendix). Distribution according to diagnosis and status was the following: ALL : 436, CR1 standard risk (std): 138, poor risk (pr) : 63, CR2 std: 138, pr : 31. Median age of the population 17 y (0-54), children (<15 y): 43%, adult >45 y: 3%. AML: 540, CR1 std : 353, pr : 40, CR2 std : 108, pr : 6. Median age of the population 32 y (0-67), children: 15%, adults >45y : 15%. Others: 45.

Median interval diagnosis-ABMT : 200 days (55-1290). Among all complications (infections : bacterial 42%, viral 18%, fungal 13%, pneumonitis 9.5%, cardiac failure 3.6%, liver V.O.D. 2.9%), only veno-occlusive disease was reported with a significantly higher

incidence in AML (3.9% vs 1.6% in ALL, p <0.05). Populations studied were stratified in subcategories as follows : ALL, AML and according to the FAB classification, CR1 and CR2, standard and poor risk, adults and children, all grafted patients (median follow up : 700 days) and populations grafted prior to January 1987 (follow up 826 days) and prior to January 1986 (follow up 1026 days). Different pretransplant regimens were studied but TBI was chosen for subsequent studies as the reference. The impact of the interval between CR and transplant was studied by comparing patients who were grafted with intervals CR-ABMT of <3 months, 4 to 6 months, 6 to 9 months and >9 months. In vitro treatments considered were: mafosfamide globally (dose ≥ 50 µg/ml) and mafosfamide dissected in 5 different laboratory techniques including treatment of Ficoll purified mononuclear cells, buffy coat and treatment adjusted to individual patients; monoclonal antibodies (ALL only) and other chemotherapies (highly heterogenous). While studying the value of marrow purging in all possible situations as mentioned above, the following working hypothesis was made: purging efficacy might appear particularly in situations of real minimal residual disease (CR1, post TBI) and/or conversely in the absence of effective previous in vivo purging (interval CR-ABMT <3 months). This hypothesis apparently turned out to be fruitful.

AML

1) The disease free survival (DFS) in the whole heterogenous group of patients autografted in CR1 was 34% at 75 months compared to 32% at 40 months for patients grafted in CR2. According to the FAB classifications, DFS were: M1: 38%, M2: 39%, M3: 45%, M4: 30%, M5: 53% (p = NS) (Figure 4). For AML CR1 std, DFS in relation to pretransplant regimens were: UCH (London): 60%, TBI 45%, at 50 months, Bulsulfan + Cyclophosphamide, BACT or high dose melphalan alone <30% at 20 months (Figure 5).

2) Long intervals pretransplant were associated with better DFS in patients grafted in CR1. For instance, when considering the interval CR1-marrow collection, DFS at 50 months were 38% below 6 months and 60% above 6 months (p = 0.02).

When considering the interval CR1-ABMT, DFS were 26%, 40% and 65% respectively for intervals of <3 mo (63 patients), 4 to 9 months (222 patients) and >9 months (41 patients) (Figure 6). For patients autografted in CR2, utilization of marrow collected in CR1 did not improve the DFS (one reason might be that these patients were grafted very quickly after induction of CR2 with no time for consolidation).
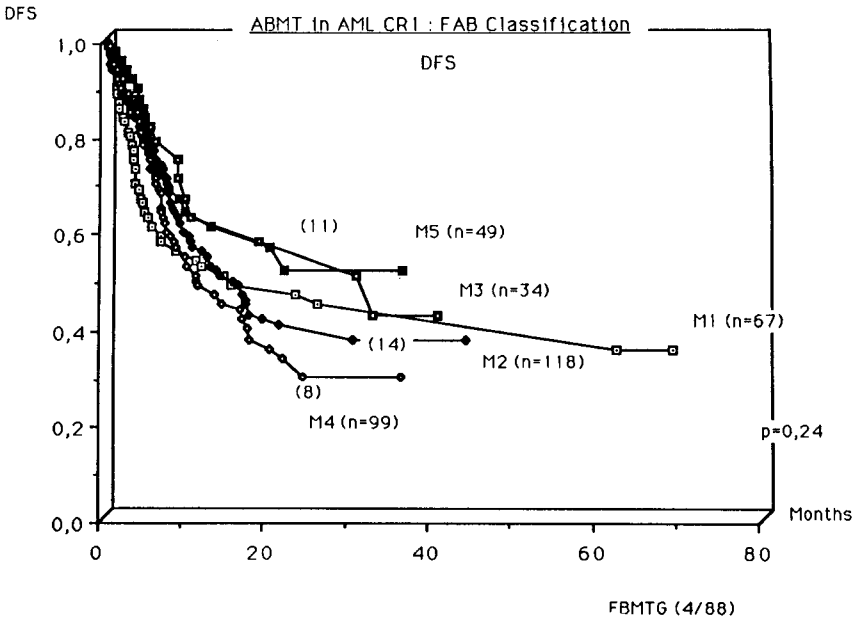
Figure 4. ABMT in AML CR1: FAB Classification
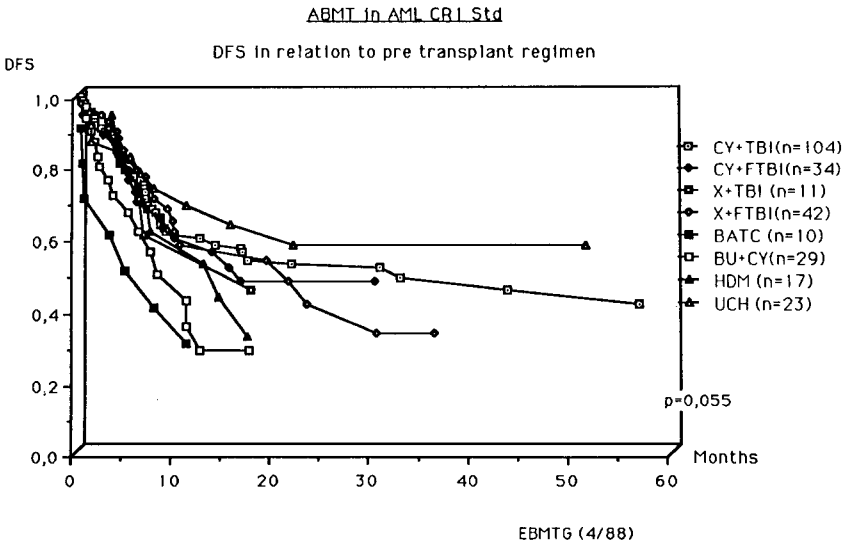
Figure 5. ABMT in AML CR1 Std
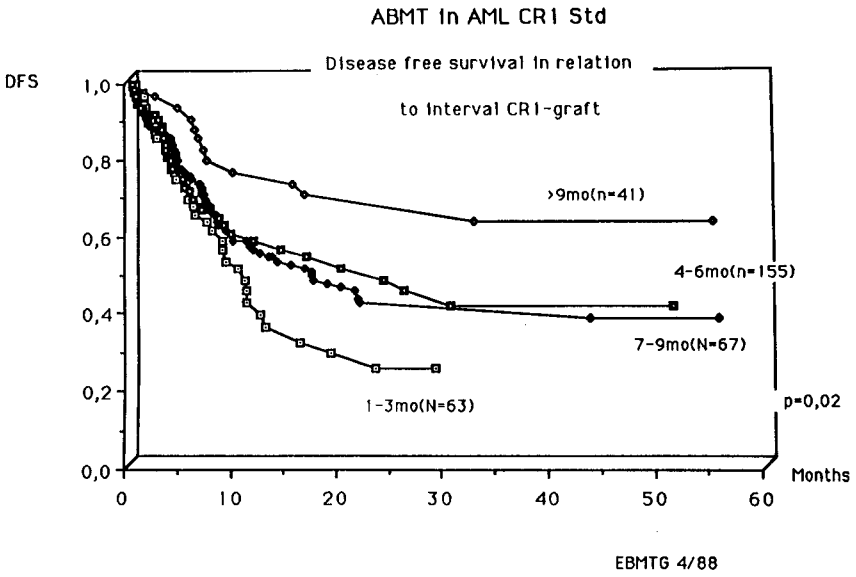
ABMT in AML CR1 Std

Figure 6. ABMT in AML CR1 Std
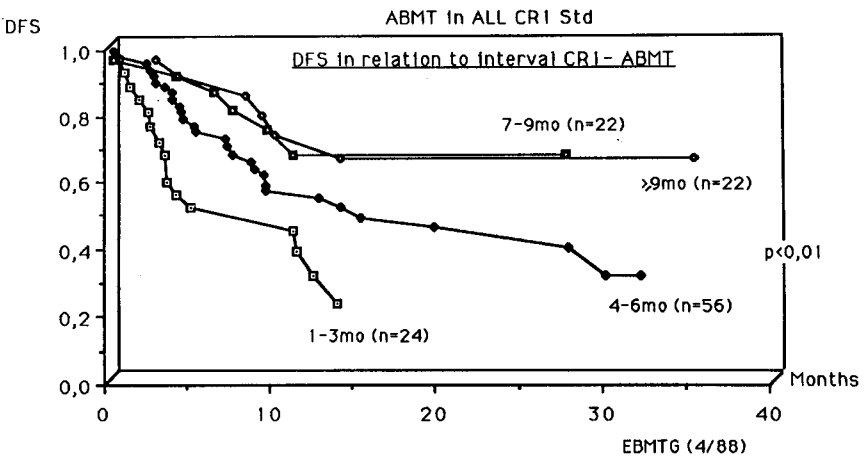
ABMT in ALL CR1 Std

Figure 7. ABMT in ALL CR1 Std

Table 1. Efficacy of Marrow Purging in ABMT for AML

Population	Results DFS (months)		
(Number of Patients)	Mafosfamide	No Purge	P*
CR1 Std (69/237)	52% (43)	30% (75)	0.10
CR1 TBI (57/138)	56% (43)	32% (59)	0.02
CR1 CR-ABMT <3mo (12/47)	40% (23)	20% (16)	0.06
CR1 Std (27/237)	Adjusted 64% (38)	35% (78)	0.009

* MANTEL COX   EBMTG 4/88

3) The impact of marrow purged by mafosfamide was demonstrated or suggested in the following situations (Table 1): CR1 std: DFS if marrow purged with mafosfamide 52% at 43 months vs. 30% in the absence of purge (p = 0.10).

CR1 autografted after TBI: DFS 56% at 43 months vs 32% (p = 0.02).

CR1 autografted less than 3 months post CR1: 40% at 23 months vs 20% (p = 0.06).

CR1 std: marrow purged with mafosfamide individually adjusted vs no purge: 64% at 38 mo vs 35% (p = 0.009).

However, this last comparison remains highly questionable since individual adaptation of the dose of mafosfamide for marrow purging was done in only 2 institutions (Paris, St Antoine and Tours, France).

ALL

1) The DFS for patients autografted in CR1 std (more than 80% received TBI) was 40% at 60 months. For patients grafted in CR2, results were considerably better in children (40% at 45 months: 103 patients) than in adults (20% at 20 months: 52 patients).

2) Long intervals pre–ABMT were associated with better DFS. In ALL CR1 std DFS were respectively 30% and 68% for delays CR1–ABMT ≤ 6 months and/or ≥ 7 months (p <0.01) (Figure 7). In ALL CR2, the DFS was 68% at 45 months in patients whose marrows had been collected ≥6 mo post CR2 as compared to 25%

at 20 months for smaller intervals or ABMT done with marrow collected in CR1 (Figure 8).

3) There was a trend in favor of marrow purging with mafosfamide in the following situations (Table 2): CR1 poor risk: - mafosfamide vs monoclonal antibodies : DFS 63% at 21 months vs 39% (p = 0.06) (Figure 9) - mafosfamide individually adjusted vs MoAB 82% at 20 months vs 40% (p <0.05).

CR1 with interval CR1-ABMT ≤ 3 months : mafosfamide vs MoAB 51% at 22 months vs 44% (p = 0.15).

These data however in opposition to AML remain controversial since the number of patients in the groups studied are small and all other comparisons have not shown any advantage of marrow purging.
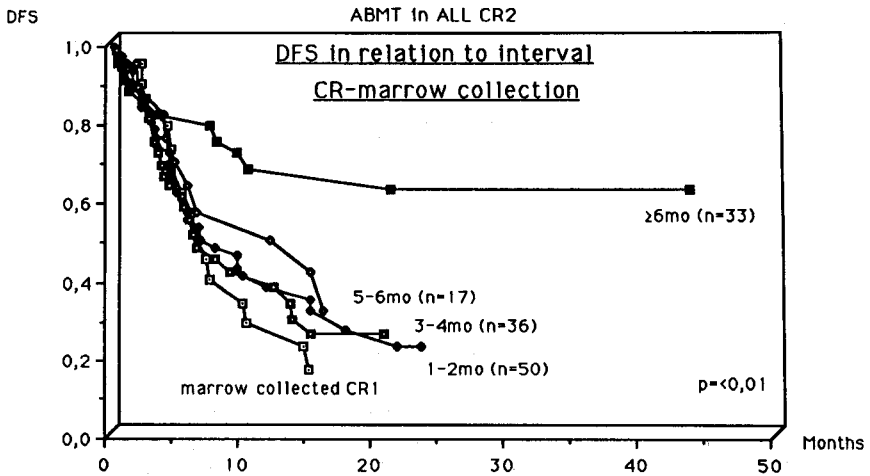
Figure 8. ABMT in ALL CR2   EBMTG (4/88)

Table 2. Efficacy of Marrow Purging in ALL

Population (Number of Patients)	Mafosfamide	Results DFS (months) Monoclonal Antibodies	No Purge	P*
CR1 poor risk (23/18)	63% (21)	39% (18)		0.06
CR1 poor risk (12/18)	adjusted 82% (20)	40% (18)		<0.05
CR1 CR-ABMT ≤3mo(11/9/7)	51% (22)	44% (3)	42% (3)	0.15 disputable

*MANTEL COX   EBMTG 4/88
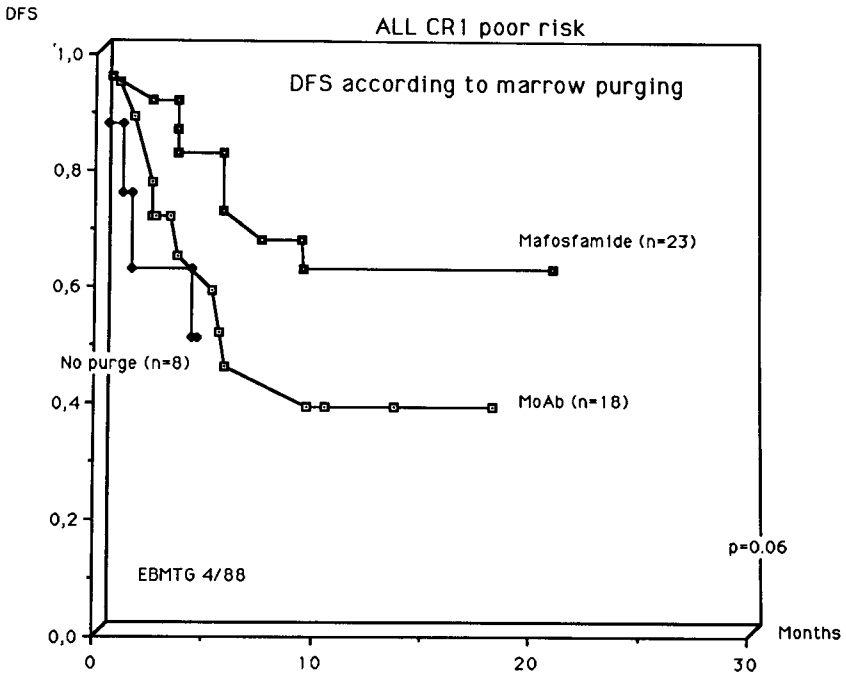
Figure 9. ALL CR1 Poor Risk

CONCLUSIONS

1) Autologous bone marrow transplantation using marrow purged by mafosfamide, following TBI, produces DFS of 58% in AML CR1, 40% in ALL CR1, 32% in AML CR2 and 42% in childhood ALL CR2.

2) Marrow purging by mafosfamide now appears to be effective or likely so, in selected situations: such as CR1, post TBI, presence of poor risk factors, interval CR-ABMT <3 months, with results in AML being more suggestive. We propose that these results in these particular situations reflect the efficacy of marrow purging when the residual disease is really minimal and/or the absence of a previous sufficient in vivo purging renders in vitro purging necessary/effective and evaluable. Efforts should now be conducted towards the generation of randomized protocols studying the value of marrow purging by mafosfamide, in AML CR2 and possibly AML CR1 autografted after TBI and poor risk ALL CR1. Outside such randomized studies, we feel that abstention of

marrow purging should no longer be approved/accepted in all situations.

3) Recommendations of today may include:

TBI as the standard pretransplant regimen (BU + Cy not yet evaluable). Delay CR-ABMT not < 3 months to allow minimum in vivo purging. In vitro purging with mafosfamide.

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SELECTION OF CD34+ MARROW CELLS FOR AUTOLOGOUS MARROW TRANSPLANTATION

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INTRODUCTION

Remission marrow stored for patients undergoing autologous marrow transplantation (AMT) may contain undetected malignant cells which represent a potential cause of relapse. A variety of techniques have been developed to deplete tumor cells from marrow prior to transplantation. Alternatively, methods for selectively isolating the small fraction of hematopoietic progenitor cells responsible for engraftment may provide marrow free of tumor cells for AMT. Successful separation of normal hematopoietic progenitor cells from malignant cells requires: 1) a marker that identifies normal precursor cells but not malignant cells and 2) a method that can isolate the marked cells in the absence of tumor cells.

We have developed a column immunoadsorption method for cell separation that relies on the high affinity between the protein avidin and the vitamin biotin ($K_D = 10^{-15}$M.)(1-4). Marrow cells are first labeled with a mouse monoclonal antibody directed against the antigen of interest; and second, are labeled with biotin-conjugated goat anti-mouse antisera. Cells are then passed over a column of avidin-coated beads which will bind antibody labeled cells and allow unlabeled cells to pass through the column. Bound cells are recovered by mechanical agitation. Avidin-biotin immunoadsorption can therefore be used both for positive selection and for elimination of specific cell populations from marrow. Importantly, the technique can be adapted to separate the large number of cells required for AMT.

Several laboratories have generated monoclonal antibodies (12-8,

My-10, BI-3C5, and ICH3) that recognize the CD34 antigen (5-8). This antigen is present on 1 to 4% of human marrow cells including virtually all unipotent and multipotent colony-forming cells and precursors for these colony-forming cells that can be detected in long-term marrow cultures (LTMC). We have recently demonstrated in baboons that CD34+ marrow cells selected by immunoadsorption with antibody 12-8 are capable of engrafting lethally irradiated recipients (9). Marrow depleted of CD34+ cells failed to reconstitute normal hematopoiesis in the 2 baboons studied. The data suggest that the hematopoietic progenitors responsible for engraftment are CD34+.

Therefore, we investigated the expression of the CD34 antigen by a variety of malignant cell types. We also measured the capacity of immunoadsorption with antibody 12-8 to select CD34+ marrow cells and not tumor cells. Based on these studies, immunoadsorption with antibody 12-8 was used to enrich hematopoietic progenitors from the marrow of patients with metastatic breast cancer. These preclinical studies provide the basis for the future clinical application of CD34+ marrow cell selection to AMT.

RESULTS

CD34 Expression in Different Malignancies

Reactivity of antibody 12-8 with hematopoietic and non-hematopoietic malignancies was evaluated using either immunoperoxidase staining or flow microfluorimetric techniques (Table 1). The CD34 antigen was not detectably expressed by tumor cells from patients with breast carcinoma or neuroblastoma, nor did we detect it on the myeloma or lymphoma cells studied. In contrast, leukemic cells from 30% to 50% of patients with acute nonlymphocytic leukemia (ANL) and acute lymphocytic leukemia (ALL) expressed the CD34 antigen.

Tumor Cell Depletion After Positive
Selection with Antibody 12-8

We determined if selection using 12-8 could effectively separate CD34+ marrow cells from fluorescein labeled CD34-negative lymphoma cells that had been mixed with normal marrow cells. The number of tumor cells present in marrow before immunoadsorption and in the recovered 12-8+ population was determined by fluorescence microscopy. This assay can detect as few as one fluorescent cell in 10^6 unlabeled marrow cells (1). In two experiments the absolute number of labeled tumor cells detected in the enriched cell population was reduced by 3 logs, compared to marrow prior to separation (Table 2).

Table 1. CD34 Expression in Different Malignancies[a]

	No. Positive/No. Tested
Breast Carcinoma	0/10
Neuroblastoma	0/6
Lung Carcinoma	4/9[b]
Follicular Lymphoma	0/11
Large Cell Lymphoma	0/25
Lymphoblastic Lymphoma	0/2
Multiple Myeloma	0/7
Acute Lymphocytic Leukemia	13/33
Acute Nonlymphocytic Leukemia	11/24

[a]Marrow cells from pts with multiple myeloma and acute leukemia were stained with either antibody 12-8 (IgM) or a control monoclonal antibody H12C12 (IgM, anti-Thy1.2) followed by fluorescein-conjugated goat anti-mouse IgM antisera and analyzed by flow microfluorimetry. Samples were considered positive if more than 5% of the cells were stained by antibody 12-8. Cells were considered stained if their level of fluorescence was greater than 98% of cells stained with the control antibody H12C12. Lymphomas and solid tumors were evaluated by immunohistology using cryopreserved tissue specimens. Tissues were stained by a three step avidin-biotin-peroxidase complex technique. Samples were stained wih either antibody 12-8 or control antibody H12C12 followed by biotin-conjugate or goat antimouse IgM antisera and avidin-peroxidase. Negative control tissues included normal human tonsil and spleen. Splenic tissue from a patient with chronic myelogenous leukemia was used as a positive control. [b]The four positives were squamous cell carcinomas. Small cell undifferentiated ᴄarcinoma and adenocarcinoma were non-reactive.

Table 2. Efficiency of Tumor Cell Depletion After Positive Selection with Antibody 12-8[a]

Expt.	Cell Fraction	Total Cell No.x10[6b]	% Marrow[c]	Tumor Cells Total No. x 10[6d]	Log Removal[e]
I	Unseparated	150	5.7	8.6	--
	Adsorbed	1.3	0.6	0.008	3.0
II	Unseparated	168	3.9	6.6	--
	Adsorbed	1.0	0.8	0.008	2.9

[a]Fluorescein labeled tumor cells from either the HSB2 (Experiment I) or Daudi (Experiment II) lymphoma lines were mixed with normal marrow cells and subjected to immunoadsorption with antibody 12-8. The number of tumor cells was determined with an inverted fluorescence microscope in the unseparated cell fraction (Unseparated) and column bound cell fractions (Adsorbed); [b]Total nucleated cell number present before and after immunoadsorption; [c]Percentage of marrow containing tumor cells; [d](Total nucleated cell number) (% marrow) (100); [e]Log:
(Total Tumor Cell No. in Unseparated Fraction)
(Total Tumor Cell No. in Adsorbed Fraction)

Enrichment of Hematopoietic Progenitors

Studies with baboons demonstrated that immunoadsorption could be used to enrich CD34+ cells from large numbers of marrow cells in sufficient quantities for transplantation (9). The immunoadsorption procedure can be further scaled up to separate the larger number of cells required for bone marrow transplantation in humans. Studies were performed using marrow from patients with metastatic breast cancer and the adsorbed cells were stored for later reinfusion (Table 3). In these experiments $11-16 \times 10^9$ marrow cells were separated by column immunoadsorption with antibody 12-8. After immuno-adsorption, $50-260 \times 10^6$ adsorbed cells were recovered that were 65-85% CD34+. These cells were significantly enriched for precursors that generated CFU-GM from 2 to 6 weeks in LTMC. The unadsorbed cells that passed through the columns were markedly depleted of progenitors.

CONCLUSIONS AND FUTURE DIRECTIONS

The CD34 antigen is expressed by 1-4% of human marrow cells including nearly all hematopoietic progenitors detectable by in vitro assays (5-8). We have demonstrated previously that CD34+ marrow cells were capable of reconstituting hematopoiesis in lethally irradiated baboons (9). The two animals from these studies who received CD34+ marrow cells isolated by immunoadsorption followed by flow microfluorimetric sorting are clinically well with normal hematologic parameters greater than one and two years post-transplant.

Using antibody 12-8, we could not identify the CD34 antigen on most solid tumors. Watt et al reported similar findings using the anti-CD34 antibody BI-3C5 (8). In the present study, CD34+ cells isolated by immunoadsorption with antibody 12-8 from the marrow of patients with metastatic breast cancer are enriched for precursors of CFU-GM in LTMC. This selection method produced a 3 log reduction of tumor cells in the 12-8+ cell population. In a preliminary clinical trial, autologous CD34+ cells have been infused into patients with recurrent metastatic breast cancer who received high dose chemoradiotherapy. Successful engraftment has been observed, but further follow-up of additional patients is needed to evaluate this procedure (unpublished observations).

The CD34 antigen was found on tumor cells from many patients with ALL and ANL as has been observed by other investigators (5,6,8,10). The usefulness of anti-CD34 antibodies may therefore be limited by their potential reactivity with normal and malignant stem cells in these diseases. Additionally, it is possible that, for other hematopoietic malignancies such as lymphoma and myeloma, the

**Table 3. Marrow from Breast Cancer Patients:
Hematopoietic Progenitors Detected After
Immunoadsorption with Antibody 12-8**

	Unseparated Cell	Unseparated Cell	Cumulative CFU-GM LTMC weeks 2-6[a]		
PT	No.x10[6]	No.x10[6]	Unseparated[b]	12-8+ enriched[c]	12-8+ depleted[d]
1	15,000	260	240	21,369	13
2	11,000	110	439	44,640	12
3	13,000	50	81	2,948	22
4	16,000	170	97	13,071	ND[e]
5	13,000	168	659	32,758	35

aCumulative CFU-GM per 4×10^6 cells used to initiate LTMC
bUnseparated marrow cells
cColumn bound cells
dCells passing through column
eNot done

malignant stem cell may be a primitive lymphohematopoietic CD34+ progenitor (11,12). Thus, it is possible that the CD34 antigen is on the rare malignant progenitors in myeloma and lymphoma even though the antigen is not detected on the majority of tumor cells. If this is the case, it will need to be determined whether subsets of CD34+ cells capable of in vivo engraftment can be distinguished from those containing malignant progenitors. Bernstein et al. have utilized clonal markers (glucose-6-phosphate dehydrogenase) in patients with ANL to demonstrate that normal precursors of colony-forming cells detectable in LTMC can be separated from malignant progenitors based on cell surface antigen expression (13). Similar studies for ALL, lymphoma and multiple myeloma should be possible using appropriate clonal markers to distinguish cells of normal and malignant origin as well as assays to detect lymphoid progenitors.

Despite significant depletion, tumor cells remain in the adsorbed cell fractions after immunoadsorption with antibody 12-8. It is unknown if these remaining tumor cells will contribute to relapse. Nevertheless, attempts are being made to develop improved cell selection devices that increase the selectivity of CD34+ cells and reduce nonspecific tumor cell binding. The capacity of CD34+ cells to reconstitute long-term hematopoiesis and the usefulness of transplanting CD34+ cells in humans will remain to be determined in future clinical trials.

ACKNOWLEDGMENTS

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BONE MARROW PURGING WITH MONOCLONAL ANTI-MYELOID ANTIBODIES IN THE TREATMENT OF AML

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INTRODUCTION

The data from the International Bone Marrow Transplant Registry (IBMTR) has shown that 20 to 35% of patients with AML in 2nd to 4th complete remission are alive without evidence of leukemia greater than 3 years after allogeneic transplantation (1). Most patients are under the age of 45 because the mortality rates for allogeneic transplants in patients with AML increases as the age of the patient increases. One of the prime causes of the increased mortality relates to the higher incidence of graft versus host disease (GVHD) that occurs in the older patients (2). In addition, only one in three patients will have an HLA-matched donor available for an allogeneic transplant. As a result, an alternate approach to transplantation has been initiated, involving the use of the patient's own bone marrow harvested in remission prior to treatment of the patient with high dose therapy.

Autologous bone marrow transplantation begins with the removal of the patient's remission bone marrow under sterile conditions through multiple aspirations from marrow-bearing sites. The quality of the patient's remission at the time of the harvest is important, as studies with the L1210 leukemia model in the mouse have shown that there can be as many as 10^9 residual leukemia cells at the time of a clinical remission (3). We have been investigating the efficacy of the elimination of these residual occult leukemia cells through purging of the bone marrow with monoclonal antibody and complement treatment ex vivo.

CLINICAL RESULTS

At the Dartmouth Hitchcock Medical Center, we have been investigating the use of monoclonal antibody purging of bone marrow

(4). Two antibodies, PM-81 and AML-2-23, are directed against antigens associated with myeloid cells (5,6). PM-81 is an IgM antibody that binds to the lacto-N-fucopentaose III (LNF III) moiety on myeloid cells, an oligosaccharide expressed on glycolipids and glycoproteins. It is designated as CD 15 in the International Workshop cluster designation for monoclonal antibodies. AML-2-23 is an IgG2b MoAb that reacts with a 55kD glycoprotein designated as CD 14, primarily present on AML M4 and M5 blast cells. Greater than 90% of myeloid leukemia blast cells express at least one of these antigens (7,8). Both antibodies are cytotoxic in the presence of complement. Moreover, they are cytotoxic to leukemia progenitor cells in at least two-thirds of patients tested (9). They are minimally toxic to normal myeloid precursors by the CFU-GM assay, but have no suppressive effect on BFU-E colony formation. Therefore, they appear to be ideal candidates to eliminate residual leukemia cells from remission marrow prior to ABMT.

To date we have transplanted 27 patients in 1st, 2nd and 3rd remission (Table 1). Each has received cyclophosphamide 60 mg/kg for 2 days, and 1200 cGy of TBI (200 cGy b.i.d. for 3 days) prior to ABMT. The mean follow-up time is 19 months. One of the 3 patients in 1st remission at the time of transplant has relapsed at 9 months but is currently alive undergoing re-induction therapy (Table 2). Eight of the 18 patients in 2nd remission have relapsed at times ranging from 3-21 months. Seven of these relapsed patients have died. One patient received a second autologous transplant and is alive in complete remission 24 months after the 2nd ABMT. Three patients died of bleeding problems secondary to poor platelet engraftment at 3, 3, and 8 months. Five patients are alive in complete remission (28%) from 2-48 months after transplant.

One of 6 patients in 3rd remission has relapsed at 5 months. Two remain alive (33%) although only 1 is in complete remission. We have seen no correlation between the rate of hematologic recovery and the number of nucleated bone marrow cells or CFU-GMS infused.

Table 1. Patient Characteristics

Remission	Number of Patients	Male	Female	Age (range)
1st	3	2	1	34-43
2nd	18	11	7	11-53
3rd	6	3	3	28-53

Table 2. Outcome of ABMT

Remission	Number of Patients	Early Death	Relapse	Survival(%)	Months
1st	3	0	1	3 (100%)	12-22
2nd	18	3	8	5 (28%)	2-48
3rd	6	2	1	2 (33%)	8-42

Engraftment data is shown in Table 3. Platelet recovery appears to be quite slow with this method of marrow treatment, however this has been a general problem found in all types of autologous transplants, especially in patients with AML.

We have done a second ABMT in 4 patients. Two patients had the previous ABMT with marrow purged with 4-hydroperoxy-cyclophosphamide (4HC) and two had received MoAb-treated marrow in our program. All were reinduced into remission and then underwent a second ABMT using marrow harvested just prior to the second ABMT. One patient in 2nd CR died early due to fungal sepsis. One of 3 patients in 3rd CR died early while undergoing treatment. One has relapsed at 4 months after transplant and one remains in continuous CR at 24 months. Engraftment in these patients appears to be quite slow.

DISCUSSION

Autologous bone marrow transplantation is increasingly being investigated as a treatment for patients with acute myelogenous leukemia. The morbidity and mortality of the procedure appear to be substantially less than that seen in the allogeneic setting. The results of transplantation in identical twins (syngeneic transplantation) serves as a model of the best that may be achieved in the autologous setting, since normal marrow is given to the patient. In such patients with AML in relapse 30% have been reported to achieve long-term survival (10). The major treatment failure here results from relapse of the leukemia, suggesting that an inadequate ablative regimen is at least part of the problem.

Our major complications relate to problems with engraftment. Recovery of platelet production to normal levels is often delayed and in some patients, does not occur. This is not a problem unique to the use of purged marrow. It is not known if this is a problem related to stem cells or related to the changes in the hematopoietic microenvironment.

Table 3. Engraftment Data
Average days (range) post ABMT to achieve

Remission	PMNs> 500/ul	Hab/10 g/dl	pH>50 k/ul
1st CR	30 (21-46)	42 (35-87)	38 (33-96)
2nd/3rd	32 (12-75)	35 (33-64)	47 (40-150)

In our patients in 2nd or 3rd remission, ABMT appears to offer a chance for long-term survival not seen with present second-line standard chemotherapy regimens and should be considered a viable option for patients under the age of 55. Our in vitro purging of bone marrow with monoclonal antibodies PM-81 and AML-2-23 is currently being studied in patients in 2nd CR by the Cancer and Leukemia Group B (CALGB). We will also be evaluating the feasibility of using this therapy in patients at the time of 1st relapse, as studies in the allogeneic setting have suggested the results are similar to those achieved in 2nd remission (11). Our data on patients in 1st remission is too small to draw any conclusions at the present time. A randomized study between standard therapy and ABMT will be important to further evaluate the place of autologous bone marrow transplantation in the early treatment of AML. Additionally, ongoing studies evaluating the role of purging techniques in ABMT are necessary.

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CHEMOPURGE WITH 4-HYDROPEROXYCYCLOPHOSPHAMIDE AND VINCRISTINE IN SECOND OR SUBSEQUENT REMISSION OF ACUTE LEUKEMIA: Is Inversion Rate an Appropriate Parameter?

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INTRODUCTION

Purging bone marrow in vitro ("ex vivo") has been developed in an attempt to remove residual tumor cells which, when reinfused following high-dose chemotherapy or radiation, could lead to earlier relapse of the patient's disease. For acute leukemia, a variety of methods have been tried (1); in particular, incubation with cyclophosphamide derivatives (2,3) has been popular. At The University of Texas M. D. Anderson Cancer Center (UT-MDACC), a combination of 4-hydroperoxycyclophosphamide (4-HC) and vincristine (VCR) has been used, based on model cell kill studies using established leukemic cell lines (4). However, there are difficulties in interpreting survival data from such studies. First, large numbers of patients are needed to detect a significant difference between purged and unpurged bone marrow or even chemotherapy alone, especially in first remission, where a significant fraction may remain in remission for years (5). Second, there is a need for long follow-up to determine if there is a true increase in disease-free survival. Finally, the question of selection bias always enters: the need for patients to have a cellular marrow selects for those with longer remissions and possibly slower-growing disease.

The use of "inversion rates" in second remission (CR2) was introduced to circumvent these problems (6). This is based on the trend for each subsequent remission in a patient to be shorter than the one previous. This method of data interpretation was used in analyzing nine patients treated with standard cyclophosphamide/

BCNU/etoposide (CBV) (7) in second or subsequent remission followed by transplantation with marrow purged with 4-HC and VCR. This was a study designed to ascertain engraftment following chemopurge. A discussion of the advantages and pitfalls of using "inversion rates" and CR2 purging follows.

METHODS

Patients' marrows were harvested in remission under general anesthesia from the iliac crests and/or sternum. After centrifugation, the buffy coat was removed and layered onto Percoll. The 40/60% interface was saved and resuspended in Hank's Balanced Salt Solution supplemented with 2-10% fetal calf or autologous serum at a final cell concentration of 5×10^6/ml. 4-HC was added to a final volume of 5 ug/ml and VCR to 1 or 5 ug/ml, and the solution was incubated 60 minutes in a shaking water bath at 37°C. After incubation, the sample was washed and frozen; a reference sample was taken to measure CFU-GM. Patients not yielding 5000 CFU-GM/kg had a second procedure done (8).

Patients were treated with cyclophosphamide 6 g/m^2, BCNU 300 mg/m^2, and etoposide 750 mg/m^2. Marrow was reinfused intravenously 48-72 hours after the last dose of etoposide. When possible, patients were treated in the Protected Environment (sterile isolated laminar air flow rooms).

RESULTS

Patient characteristics are summarized in Table 1. The patient with "mixed" disease had Tdt and cALLA positive cells which were trace peroxidase positive, and a granulocytic sarcoma developed contemporaneously with the leukemia. All patients engrafted satisfactorily and did not require reinfusion of an unpurged "back-up" bone marrow. Their median rate of engraftment is compared with those of patients treated with CBV and unpurged ABMT in first and subsequent remissions in Table 2; early deaths and relapses (before two months) are excluded.

Table 3 updates the disease-free survival of each patient; note a single "inversion" in Patient 1, and that Patient 9 is too early to determine. These are compared with patients in second or subsequent remissions who received CBV and unpurged ABMT in Table 4. The "chemo" column consists of data from those patients who received chemotherapy alone in second remission but received CBV/ABMT in third remission.

Table 1. Patient Characteristics

	Age	Sex	Diagnosis	CR #
1	59	M	AML	2
2	67	M	AML	3
3	49	F	APL	2
4	35	M	AML	3
5	19	M	ALL	2
6	34	M	ALL	3
7	17	M	ALL	2
8	62	F	ALL	3
9	22	F	Mixed	2

Table 2. Median Days (Range) to Recovery

	CR1	CR2	PURGED
500 Granulocytes	29 (14-55)	23 (14-55)	**30 (20-48)**
1000 Granulocytes	35 (17-78)	32 (16-81)	**38 (20-64)**
50,000 Platelets	29 (12-70)	29 (13-75)	**33 (21-56)**
100,000 Platelets	35 (17-100)	35 (15-98)	**49 (24-194)**

DISCUSSION

As a phase I study of engraftment, purging with 4HC/VCR has been successful. Adding other drugs or different purging agents may be possible. However, at this time, the inversion rate is no different than that seen with unpurged marrow or chemotherapy alone. This could be a limitation of the conditioning regimen or of the actual purge.

Table 3. Remission Duration, Months

Patient	CR1	CR2	Post ABMT
1	16	--	17
2	5	2	2
3	17	--	5
4	13	5	4
5	27	--	4
6	13	5	2
7	2	--	2
8	14	7	4
9	33	--	20+

Table 4. Population Characteristics

	"Chemo"	ABMT	Purged
Total	22	9	14
Early Deaths	--	2	0
Too Early	--	0	1
CR1 (Months)	21	18	14
	(3–28)	(3–29)	(2–23)
Post-RX (Months)	6	5	4
	(1–93)	(3–44+)	(2–20+)
Total Inversions	3	3	1
Rate	21%	14%	13%
Total CR	0	3	1

"Inversion rates" were introduced in an attempt to detect biologically significant responses in second or subsequent remission leukemia. It has the advantages that each patient is his or her own control as far as disease and patient characteristics, so that selection bias may be ruled out. Furthermore, since a patient only has to

remain in remission as long as his or her previous remission to be considered positive, follow-up can be shorter. However, as seen in the Results Section, there are pitfalls with using "inversion rates". It does not guarantee freedom from disease. Its upper confidence limit (roughly 25%) is close to the lower confidence limit of about 35% for syngeneic transplants, who are the ultimate in tumor-free cell infusions; this makes for a very narrow range in which to reveal the maximum potential of purging. Finally, large numbers of patients are needed still to show improvement over other methods. "Inversion" tells only part of the story.

Furthermore, attempting to purge patients in CR2 may be selecting for more favorable patients in and of itself. For one thing, patients must recover adequate marrow cellularity before leukemia relapses in order to undergo this procedure, eliminating patients with rapid tumor growth rates. In addition, the set of patients benefiting from marrow purge may be quite narrow in CR2: those with high tumor burdens at the time of purge may fail anyway due to inadequacy of the conditioning regimen to eliminate their remaining systemic tumor entirely, while those with very minimal tumor burdens may do well even without the purging. In CR1, although a measurement like "inversion rate" cannot apply, the patients are more likely to remain in remission long enough to undergo a purging procedure and are more likely to have tumor still responsive to current conditioning regimens. The value of purging may have to be found yet in accruing many more patients in CR2 AML (where inversion rates seem to be the highest), in using better conditioning regimens when available, and in aiming these procedures for patients in first CR deemed at risk of relapse.

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AUTOLOGOUS TRANSPLANTATION WITH CHEMOPURGED BONE MARROW IN PATIENTS WITH ACUTE NONLYMPHOCYTIC LEUKEMIA

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INTRODUCTION

Autologous bone marrow transplantation (BMT) may be an alternative to allogeneic BMT for the treatment of acute nonlymphocytic leukemia (ANLL). We have previously described the results of autografting using marrow treated *ex vivo* with 4-hydroperoxycyclophosphamide (4HC), an active alkylating agent in aqueous solution, in patients with ANLL in second or third remission (CR2 or CR3) (1). This report includes an update of our studies of autografting with chemopurged marrow in patients with ANLL in CR2 and CR3 and presents preliminary results of autologous BMT in patients with ANLL in first remission (CR1).

METHODS OF STUDY

Patients

Eighty-eight patients with ANLL (17, CR1; 59, CR2; 12, CR3) were included in this study. Patients underwent autologous BMT in CR1 at a median of 4+ months (range 2+-15+) after attaining CR1. In patients transplanted with 4HC-treated marrow in CR2 or CR3, the median duration of CR1 was 15 months (range, 2-96) and the median duration of CR2 or CR3 at the time of autografting was 2.5+ months (range, 1.5+-6.0+). All protocols for autologous BMT were approved

by the Joint Committee on Clinical Investigation of The Johns Hopkins Medical Institutions, and informed consent was obtained from all patients.

Marrow Collection, Processing, and Infusion

For each patient, an attempt was made to aspirate at least 4×10^8 nucleated marrow cells/kg from the iliac crests. Approximately 70% of the collected marrow was treated *ex vivo* with 4HC at a concentration of 60 ug/mL (2 patients), 80 ug/mL (2 patients), or 100 ug/mL (84 patients). The remainder of the collected marrow was incubated with a lower dose of 4HC or without drug as a reserve marrow. The marrow buffy-coat was incubated for 30 min at 37°C with 4HC, as previously described (1). The final incubation hematocrit was adjusted to 5-10%. After incubation, the marrow cells were centrifuged, resuspended, and frozen in 50 mL polyolefin bags in a controlled-rate freezer. The 4HC-treated marrow was kept in a liquid nitrogen freezer until infusion, when it was rapidly thawed in a 37°C water bath, and injected intravenously at a rate of 10-15 mL/min.

Preparative Regimens

Eighty-six patients received high-dose busulfan and cyclophosphamide (CY), as used for pre-transplant conditioning before allogeneic BMT for ANLL at this institution (2). Two patients with a history of CNS leukemic involvement received intravenous CY (50 mg/kg/day for four days) followed by total body irradiation (300 rad/day for four days, with lungs shielded after 900 rad).

RESULTS

Post-Transplantation Clinical Course

Eleven patients (two in CR1, eight in CR2, and one in CR3) died with documented or presumptive bacterial or fungal sepsis during aplasia or early hematologic recovery, one to seven weeks after BMT. One patient with ANLL in CR2 had nonfatal sepsis with *Klebsiella pneumoniae* at the time of marrow infusion, had persistent marrow hypoplasia, and died with Gram-negative sepsis 5 months after BMT. Two patients (one in CR2, one in CR3) died with interstitial pneumonitis 3 and 8 months, respectively, after BMT: one due to cytomegalovirus and one of presumably idiopathic etiology. Three patients (one in CR2, 2 in CR3) died with hepatic veno-occlusive disease five to seven weeks after autografting.

Hematologic Reconstitution

Two patients with ANLL in CR2 failed to demonstrate hematologic reconstitution after autografting with chemopurged marrow. One of these patients had no reserve marrow available for infusion and died with Gram-negative bacterial sepsis 5 months after BMT; the other had satisfactory hematologic reconstitution after infusion of untreated reserved marrow 38 days after the 4HC-treated autograft and is in unmaintained CR2 21 months after infusion of reserve marrow. Satisfactory hematologic reconstitution occurred in the remaining patients; neutrophil count exceeded $0.5 \times 10^9/L$ and platelet count exceeded $50 \times 10^9/L$ at medians of 29 days (range, 12-99) and 45 days (range, 18-259), respectively after BMT. A mean of 5.03 ± 1.41 (range, 0.0-71.3) x 10^3 GM-CFC/kg was infused. The logarithm of granulocyte-macrophage colony-forming cell (GM-CFC) content of the 4HC-treated marrow graft could be correlated with the time to hematologic recovery (3).

Leukemic Relapses and Disease-Free Survival

Thirty-six patients (5, CR1; 27, CR2; 4, CR3) had hematologic relapses at a median of 6.2 months (range, 1.8-20.2) after BMT. The actuarial relapse rates were 46% in CR1, 64% in CR2, and 55% in CR3, but these differences are not statistically significant (Figure 1).
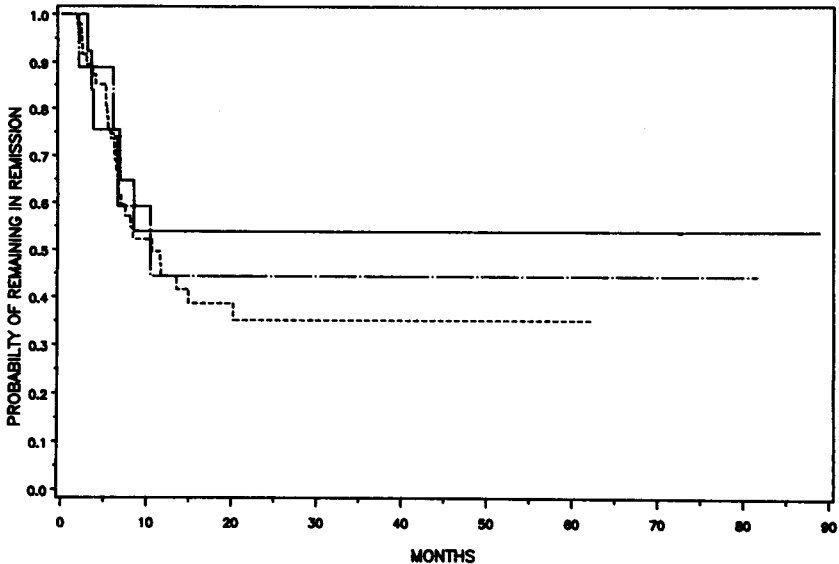
Figure 1. Analysis of relapse rates in 88 patients undergoing autologous BMT with 4HC-treated marrow for ANLL in CR1 (n=17; solid line), CR2 (n=59; dotted line), or CR3 (n=12; dot-and-dash line).

Thirty-five patients (10, CR1; 20, CR2; 5, CR3) are in unmaintained remission at a median of 19.8 months (range, 0.4-88.8) after autologous BMT with 4HC-treated marrow. Disease-free survival was 47% in patients transplanted in CR1 and 28% in CR2 or CR3 (Figure 2). The durations of post-BMT CR exceed the durations of CR1 ("inversions") in 13 of the 25 patients in unmaintained CR2 or CR3 after autografting; in contrast, inversions were observed in only two of 31 patients who relapsed after autologous BMT for second- or third-remission ANLL.

DISCUSSION

Intensive antileukemic therapy followed by autologous BMT with 4HC-treated marrow may be associated with long-term disease-free survival in patients with ANLL in CR2 or CR3, in whom conventional therapy is not curative. The actuarial relapse rate is similar to that seen with syngeneic BMT, and the disease-free survival is comparable to that observed after allogeneic BMT for ANLL in CR2 or CR3. The preliminary results of autologous BMT with 4HC-treated marrow in patients with ANLL in CR1 are also encouraging; the disease-free survival is superior to that seen after autografting
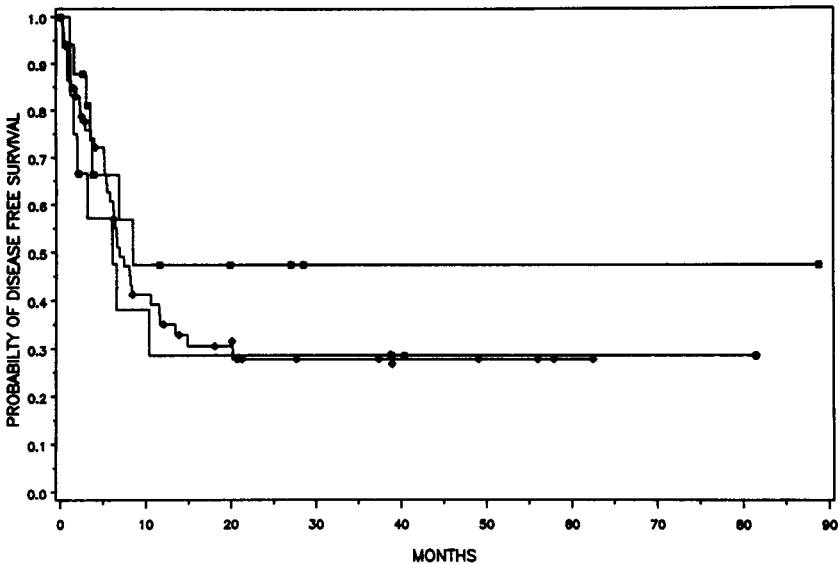
Figure 2. Analysis of disease-free survival in 88 patients undergoing autologous BMT with 4HC-treated marrow for ANLL in CR1 (n=17; squares), CR2 (n=59; diamonds), or CR3 (n=12; circles). Individual living patients are indicated by the symbols.

in CR2 or CR3 and resembles that seen after allogeneic or syngeneic BMT for ANLL in CR1. Nevertheless, the role of autologous BMT and the need for purging of the autograft in first-remission ANLL are controversial. Randomized prospective studies, in which disease-free survival and leukemic relapse rates will be compared in patients with newly-diagnosed ANLL who receive the same intensive remission-induction chemotherapeutic regimens and then either additional (or no further) chemotherapy or autografting with chemopurged marrow, will be needed to resolve this issue.

Exposure to 4HC substantially inhibits the growth of committed and multilineage human hematopoietic progenitor cells *in vitro*, although more primitive blast cells may be less sensitive to the drug. In our study, the number of GM-CFC cells infused in 4HC-treated marrows was greatly reduced, but hematologic reconstitution was nevertheless correlated with the GM-CFC content of the infused marrow cell suspension. Although the recovery of neutrophil and platelet counts in recipients of 4HC-treated marrow was significantly delayed when compared with hematologic reconstitution in recipients of untreated allogeneic or syngeneic marrow, similar delays have been described by other groups in patients with ANLL who undergo autologous BMT with untreated or drug-incubated marrow.

Patients who relapse after autologous BMT for ANLL may have had a failure of the intensive pre-BMT conditioning regimen to destroy residual leukemia *in vivo* or may have had incomplete elimination of leukemic cells from the marrow cell suspension by the *ex vivo* 4HC treatment. Current techniques do not allow one to determine with certainty which of these aspects of the antileukemic regimen was inadequate. However, early recurrences of leukemia (within 6 months after autografting) suggest a failure to eliminate tumor from the marrow inoculum, while relapses occurring later may more likely be due to residual disease *in vivo* (as is the case for leukemic recurrence after syngeneic BMT). Furthermore, preliminary data from our institution suggest that patients with ANLL in whom the recovery of marrow GM-CFC after 4HC treatment exceeds 1% of pre-treatment values are at higher risk for leukemic relapse, presumably on the basis of inadequate cytotoxicity of the drug to both neoplastic and normal hematopoietic progenitor cells (4). The administration of more intensive cytoreductive regimens before autologous marrow rescue may therefore be required to eliminate more leukemic cells *in vivo* and to offset the loss of allogeneic graft-versus-leukemia effect. Strategies for optimizing the *ex vivo* marrow treatment with maximally-tolerable concentrations of single agents such as 4HC, development of multiple-drug purging regimens, and combined immunopharmacologic methods may further eradicate residual leukemic cells from autologous marrow suspensions in patients with ANLL.

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Discussion 2 - Session 1A (AML)

Dr. Gorin: Since both Alan and Karel quoted the results of the European Bone Marrow Registry, let me present the conclusions. In the subpopulation of patients transplanted in CR treated with TBI (540 patients), purging of the marrow has a favorable effect on outcome provided that transplantation occurs early after onset of CR (within 3 months). The longer the time interval between onset of CR and transplant, the better the outcome. I am delighted that Alan came to similar conclusions after analysis of the same European data. Karel, how important do you think maintenance chemotherapy is after transplant?

Dr. Dicke: I do not know. I think you can only find out by randomization. We reasoned that there might be a chance that not all leukemic cells are eliminated either from the graft or after CBV.

Dr. Burnett: Now if you consider the pretransplant chemotherapy you give, Karel, you said that high dose Ara: C most probably is very important. There is another drug which is AMSA. And the reason why I am referring to AMSA, is that the Rome team, using the BAVC regimen for patients with AML transplanted in 2nd CR, have excellent results. Can you comment on that?

Dr. Dicke: I agree, AMSA may also play an important role in cytoreduction. It may well be that the combination AMSA and Ara: C might be the most effective regimen as preintensification. In future studies we would like to increase the dose of Ara: C to 15 g instead of 12 g which we use at the moment.

Dr. Rizzoli: According to the presentation of Karel Dicke, where pre-BMT intensification may be responsible for increasing disease-free survival, did you, Alan, evaluate the role of post remission intensification in your group of patients?

Dr. Burnett: Unfortunately, that data is not available in the European data base. Those patients, who have (had) 4 post remission pulses, are the ones who did not relapse. The MRC trial took that question "in vivo" purging on board.

Dr. Hagenbeek: I think the concept of chemotherapy after autologous transplantation is an interesting one -- to get rid of the few residual leukemic cells that might cause the relapse. But I think we all are aware that the young graft is extremely vulnerable to aggressive chemotherapy. So my question to Karel Dicke: how well is chemotherapy tolerated?

Dr. Dicke: In some patients we had to cut the doses of adriamycin in half. Four to 6 doses of chemotherapy were administered.

BAVC REGIMEN AND AUTOLOGOUS BONE MARROW TRANSPLANTATION IN PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA IN REMISSION: An Update

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Valeria Giarnieri, Roberto La Tagliata, Alfredo Covelli,
Alessandra Micozzi, and Franco Mandelli

INTRODUCTION

Patients with Acute Myelogenous Leukemia (AML) in first remission have a 15-25% probability of cure (1). Better results are reported in children, where 30-40% are probably cured (2). After relapse, prognosis both in children and adults is very poor, with less than 5% of long term survivors (LTS).

New regimens of intensive polychemotherapy have been used as post-remission treatment to improve the percentage of cured patients (3). The results, although preliminary, demonstrate the feasibility of these new strategies with a significant increase in the proportion of LTS.

These intensive cytoreductive post-remission therapies have been utilized either alone or followed by allogeneic or autologous stem cell reinfusion. Indeed rescue with autologous or allogeneic stem cells allows the use of supralethal chemo/radiotherapy regimens (4).

Over the last years Autologous Bone Marrow Transplantation (ABMT) has been performed more and more often in AML patients both in I or II CR (5).

In I CR transplanted patients, a 40-50% probability of LTS has been reported. However, results from different centers are not easily comparable because of heterogeneity of population and methods (induction treatment, conditioning regimens, consolidation of remission, timing of transplant, type of disease, purging procedures).

ABMT instead is an elective treatment in AML in II CR since the

20-30% chance of LTS in transplanted patients is clearly superior compared to the results achieved with chemotherapy alone (6).

We report 71 patients affected by AML in I or II CR treated with BAVC high dose chemotherapy regimen followed by cryopreserved autologous bone marrow reinfusion. The toxicity and the therapeutic efficacy of this approach will be analyzed.

PATIENTS AND METHODS

Seventy-one patients (47 males and 24 females) with a median age of 24 (range 1-54) entered this study. The classification of AML was performed to FAB criteria.

Fifty patients were transplanted after achieving I CR and twenty-one after achieving II CR (Table 1).

All patients treated in I CR received an induction treatment consisting of a combination of Daunorubicin (DNR) +ARA-C (3+7 schedule) followed in all but 3 patients by a consolidation therapy. ABMT was performed after a median of 4 months (range 1-14) from CR. Most patients in II CR received an induction treatment including high dose ARA-C followed by various consolidation. ABMT was performed after a median of 2 months (range 1-14) from II CR.

Neither I CR nor II CR patients received further chemotherapy after ABMT.

Table 1. Patients Details

	I CR	II CR
No. Patients	50	21
Median Age	29	23
Sex		
Male	35	12
Female	15	9
Median Time from CR to ABMT		
Months	4	2
Range	1 - 14	1 - 24

Bone Marrow Processing

The techniques of marrow collection, cryopreservation and reinfusion have been previously described (7). Patients transfused in I CR underwent marrow collection immediately prior the starting of pretransplant regimen. Of patients transplanted in II CR 16 underwent marrow collection immediately prior the starting of pretransplant chemotherapy and 5 were harvested during I CR. A median of 1.6×10^8 nucleated marrow cells per kilogram of body weight (range 0.2-3.0) were collected from posterior iliac crests while patients were under general anesthesia. In seven patients marrow was ex vivo treated with S-47 MoAb + complement (8).

Preparative Regimen

All patients received prior bone marrow reinfusion, a four drug schedule (BAVC) consisting of BCNU (800 mg/m^2 on day -6), AMSA (150 mg/m^2 on days -5,-4,-3), VP-16 (150 mg/m^2 on days -5,-4,-3) and ARA-C (300 mg/m^2 c.i. on days -5,-4,-3), followed after 2 days by bone marrow infusion.

Supportive Care

Patients were nursed in a reverse isolation single room, with a central venous catheter placed for the administration of fluids, chemotherapy and blood products. Broad spectrum antibiotics were given for fever during aplasia adding amphotericin B when a documented fungal infection or a persistent fever was demonstrated. All patients at risk for the recurrence of herpes virus infection received prophylactic intravenous acyclovir.

All blood products were irradiated with 2,000 rad before infusion to prevent possible graft-versus-host reactions.

Toxicity

Conditioning regimen was well tolerated and only various degrees of nausea and vomiting were observed during administration of chemotherapy. Nineteen patients developed severe oral mucositis which generally resolved at the time of bone marrow engraftment. No episodes of severe hemorrhage were observed. Fifty-six patients had fever during aplasia: in 31/71 patients (43%), these fevers were associated with positive cultures for bacteria (24 patients) or fungus (7 patients). Two patients died in aplasia 1 month after bone marrow reinfusion (both from fungal sepsis) and one patient died from pulmonary thromboembolism after engraftment on day +21.

In 7 out of 22 patients under 15, clinical symptoms of pulmonary distress, including rapidly progressing dyspnea, dry cough and

tachypnea were observed. Pulmonary function tests revealed arterial hypoxemia, marked reduction of residual volume and total lung capacity and decreased CO_2 diffusion. Low-dose steroid treatment was given, resulting in disappearance of all clinical signs and X-ray normalization in all patients.

Hematologic Reconstitution

All evaluable patients had complete engraftment and hematological reconstitution. The median time required to attain an absolute neutrophil count in excess of $0.5 \times 10^9/l$ was 18 days (range 10-46) for patients in I CR and 18 days (range 11-35) for patients in II CR. A platelet count exceeding $50 \times 10^9/l$ was observed after a median of 36 days (range 14-301) in cases treated in I CR and 26 days (range 15-95) in II CR. No correlation was observed between the number of nucleated bone marrow cells or granulocyte-macrophage colony-forming cells reinfused and the rate of hematologic recovery.

RESULTS

As of June 30, 1988, 68 out of the 71 treated patients were evaluable for the duration of CR. Hematologic relapses occurred in 25 of 48 patients treated with ABMT during I CR and in 5 of 20 patients treated during II CR. Median time to relapse was 6 months with a range of 1-25 and 2-6 for patients transplanted in I CR and II CR respectively. Twenty-three patients (48%) are alive in unmaintained I continous complete remission (CCR) with a median follow-up of 20 months (range 2-44). Fifteen patients (75%) are alive in II unmaintained CCR with a median follow-up of 20 months (range 2-44). A conversion was observed in 11 patients transplanted in II CR. Projected probability of disease free survival (DFS) at 52 months of the 50 patients treated in I CR is 40% while DFS at 45 months of 21 cases transplanted in II CR is 68% (Table 2).

DISCUSSION

In our experience, 71 patients affected by AML in remission were treated with a high-dose chemotherapy regimen followed by ABMT. BAVC regimen showed an acceptable toxicity with less than 5% transplant procedure related deaths.

Sixteen patients (11 in I CR and 5 in II CR) are alive in CR for over 24 months after ABMT.

As concerns the anti-leukemic efficacy, results are reported in patients treated at different times of the disease; according to general

Table 2. BAVC Regimen Results

	I CR	II CR
No. Patients	50	21
Early Deaths	2	1
No. Relapses	25	5
Median Time to Relapse:	6 months	
Range (Months):	(1 – 25)	(2 – 6)
No. CCR	23	5
No. CCR > 24 months	11	5
Conversions	--	11

experience a 40% of CCR at 4 years is shown in patients treated in I CR. Although the median follow-up is still relatively short, a resonable number of patients were analyzed resulting in a proportion of surviving patients almost comparable to that achievable with allogeneic bone marrow transplantation and probably higher than that reported with conventional chemotherapy. Impressive results were obtained in our patients treated in II CR; 15 patients are in CCR after a median follow up of 20 months with a probability of DFS of 68%. Eleven patients achieved a II CR duration longer than the first one.

No conclusion can be drawn about the value of ABMT in patients transplanted in first CR because of the heterogeneity of populations and only randomized trials now in progress will answer this question. Concerning patients transplanted in II CR the value of ABMT is more easily assessed since chemotherapy does not lead in such cases to long-term survivals.

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RESULTS OF TWO DIFFERENT CONDITIONING REGIMENS FOLLOWED BY ABMT IN ADVANCED ACUTE LYMPHOBLASTIC LEUKEMIA

Paolo De Fabritiis, Alessandro Pulsoni, Antonella Sandrelli, Silvia Tosti, Lorenzo Coppola, Rita Pinto, Matilde Rolla, Anna Cipriani, and Giovanna Meloni

INTRODUCTION

Intensive regimens of chemotherapy in patients with acute lymphoblastic leukemia (ALL) give a probability of cure of 60 to 75% in children, while in adults the results are worse with less 30% of long term survivors (LTS) (1-2). The relevant number of leukemic relapses make the role of post-remission chemotherapy crucial in terms of maintaining long term disease free survival. More critical is the treatment of these patients after relapse. Different approaches with allogeneic bone marrow transplantation (BMT) employed variously during the course of the disease have provided, in patients who have an HLA compatible donor, an opportunity for cure of leukemia although graft-versus-host related complications and viral infections account for most of mortality after transplantation (3). Indeed BMT is an elective treatment especially in patients either refractory to a first line induction therapy or relapsing within 18 months from first remission. Autologous bone marrow transplantation (ABMT) has been widely used in recent years as an alternative approach in patients with advanced ALL and a 40% LTS probability has been reported by several groups (4). We describe 30 patients affected by advanced ALL treated with BMVC or BU+CY conditioning regimens followed by purged autologous bone marrow reinfusion. The toxicity and the therapeutic efficacy of the two different regimens will be analyzed.

PATIENTS AND METHODS

Patients

Thirty patients entered this study (Table 1). Median age was thirteen years (range 4-40) and 21 were males. Twenty patients were treated with BMVC conditioning regimen (5) consisting of BCNU (800 mg/m^2 on day -6), Mitoxantrone (Mito) (12-18 mg/m^2 on days -5,-4,-3), Vp-16 (150-300 mg/m^2 on days -5,-4,-3) and ARA-C (300 mg/m^2 continuous infusion on days -5,-4,-3). ASTA-Z pretreated marrow was reinfused on day 0. Ten patients received the BU+CY conditioning regimen (6) consisting of Bulsufan (BU) (4mg/kg/day over 4 days) and Cyclophosphamide (CY) (50 mg/kg/days over 4 days) followed after 48 hours by stem cell reinfusion.

Nine patients treated with BMVC and nine patients with BU+CY conditioning regimens were autografted in CR II; six patients treated with BMVC and one patient with BU+CY conditioning regimen were autografted in CR III.

One child (BMVC regimen) was treated in CR I obtained after a second line induction chemotherapy; four children (BMVC regimen) were transplanted in I hematological remission after multiple meningeal or testicular relapses.

All patients were treated in a protected environment and received bowel decontamination with nonabsorbable antibiotics. A central venous catheter was placed in all patients for the administration of fluids, antibiotics and chemotherapy.

Table 1. Patients Details

	BMVC	BU+CY
No. Patients	20	10
Median Age	13	13
Range	4-40	7-27
Sex		
Male	14	7
Female	6	3
Median Time from CR to ABMT		
Months	2.5	2.5
Range	1-6	1-7
Status of Disease		
CR I	1	--
CR II	9	9
CR III	6	1
CR III after isolated CNS or Testis involvement	4	--

After hematological reconstitution, patients received a standard maintenance chemotherapy consisting of weekly i.m. Methotrexate, daily oral 6-Mercaptopurine, monthly pulses with Vincristine and Prednisone and monthly intrathecal Methotrexate.

Collection of Marrow, in Vitro Purging and Cryopreservation

Bone marrow was harvested from the posterior iliac crests under general anesthesia. A volume corresponding to $2-4 \times 10^7$ nucleated cells/kg was cryopreserved serving as backup marrow. From the remaining volume a buffy coat was obtained and adjusted to a final cell concentration of 2×10^7 cells/ml with RPMI 1640. Cells were incubated with ASTA-Z 7654 (ASTA WERKE, Bielefeld, FRG) at a concentration of 100-120 μ/ml for 30' at 37°C with gentle shaking. After incubation the suspension was cooled, washed twice, resuspended in autologous plasma with TC 199 and cryopreserved with 10% DMSO in a programmed biological freezer.

The toxicity of ASTA-Z in vitro purging was evaluated in each patient by establishing before and after the treatment long term bone marrow cultures and by the evaluation of colonies in semisolid medium.

RESULTS

Toxicity

Both protocols were well tolerated; apart from nausea and vomiting, severe mucositis appeared in four patients treated with BU+CY and after BMVC regimen especially when Mitoxantrone at 18 mg/m^2 and VP-16 at 300 mg/m^2 were employed. Twenty-seven patients had fever during aplasia with documented microbiological infection in 17 cases.

Six patients treated with BU+CY conditioning regimen developed hemorrhagic cystitis within two months from transplantation.

Clinical Results

BMVC Regimen

The median day for recovery of PMN to $0.5 \times 10^9/1$ was 22 (range 13-32) and of platelets to $50 \times 10^9/1$ was 32.5 (range 15-120). Two patients died during aplasis from sepsis, one patient died in CR 5 months after ABMT from progressive viral hepatitis. Thirteen patients relapsed after a median of 4 months (range 1-31). Four

patients are in continuous complete remission (CCR) with a median follow-up of 13.5 months (range 9-22).

BU+CY Regimen

All patients showed hematological reconstitution and no toxic deaths were observed during aplasia. The median day for recovery of PMN to $0.5 \times 10^9/1$ was 17 (range 13.27) and of platelets to $50 \times 10^9/1$ was 68.5 (range 22-210).

Four patients relapsed after a median of 3 months (range 2-7) from ABMT and six patients are in CCR with a median follow-up of 4 months (range 2-26) (Table 2).

DISCUSSION

Although the number of patients and the shortness of follow up make this study only preliminary, some considerations can be drawn from our experience. Supralethal therapy and autologous bone marrow transplant may represent a promising alternative approach in advanced ALL especially if a residual leukemic cell purged marrow is reinfused. From our and other studies (7-8) it is clearly demonstrated that in vitro treatment with immunological agents could eliminate the majority of neoplastic cells in an "in vitro model" which resembles the in vivo situation; unfortunately no technique is available at the moment to demonstrate the absence of leukemic cells in vivo after chemotherapy and the source of blast cells at the time of relapse.

Table 2. Results

		BMVC	BU+CY
No. Patients		20	10
Early Deaths		2	--
Deaths in CR		1	--
No. Relapses		13	4
Median Time to Relapse		4	3
	Range	1-31	2-7
C C R		4	6
Median Follow-up		13.5	4
	Range	9-22	2-26

Two different conditioning regimens have been employed in our experience. Two transplant related deaths were observed in the BMVC group. Actuarial disease free survival in this protocol shows no surviving patients at 31 months because of a late relapse; indeed 13 patients relapsed and only 4 are alive in CR. Different results have been obtained in BU+CY regimen group: no toxic deaths were observed and 6 out of 10 patients are alive in CCR from 2 to 26 months after transplant. Obviously a longer follow-up and a larger number of patients are needed to verify the value of this interesting schedule also in ALL.

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THE STATUS OF AUTOGRAFT FOR ACUTE
MYELOID LEUKEMIA

Alan K. Burnett and Anne Morrison

High-dose chemotherapy or chemoradiotherapy supported by autologous marrow transplantation in first and second remission is becoming increasingly widely used, but all clinical results so far reported are anecdotal. These studies (1-10), which in some cases now extend to follow-up of more than 6 years, while encouraging, do not firmly establish a role for autograft in AML in remission. Larger multicentre prospective clinical studies are required to answer a number of relevant questions.

The major criticism of the autograft data is that the encouraging results may have been achieved on patients who already have an improved prognosis by virtue of being in remission for 5-6 months on average, prior to the autograft, thereby avoiding the period of highest relapse risk. It has been suggested by at least one group (11) that equivalent results can be achieved with chemotherapy alone when a comparison was made of patients who have been in remission for similar periods. While such a comparison serves to make the point that the longer the autograft procedure is delayed into remission, the more difficult it will be to demonstrate a reduced relapse probability, a comparison between one group (i.e. chemotherapy) who have *all* reached, say 6 months in remission, with a combined autograft group whose average pre-autograft remission interval is 6 months, is of limited validity because the latter contains a substantial number at shorter intervals, and by the same arguments, presumably at higher relapse risk, which might be more influential on outcome than the proportion with longer intervals who are at the reduced relapse risk. The pattern of relapse following autograft, where relatively few events have been noted beyond 12-15 months, may be different from the familiar continuous relapse risk seen following chemotherapy, suggesting that autograft is having an impact.

Since no conclusions can be reached on the data available so far, perhaps the most constructive use which be made of it is to suggest which are the major questions which prospective trials should address. Partly with this end in view, an analysis was done of 263 first remission and 61 second remission patients recorded in the EBMT Database all being autografted before November 1986(12), the analysis being carried out with a minimum follow-up of 16 months (range 16-90 mo: median 33 mo) for first remission and 15.5 months (range 15.5-82 mo: median 34 mo) for second remission patients.

AUTOLOGOUS TRANSPLANT IN FIRST REMISSION

Of the first remission patients who had a median age of 31 years (range 1-62), 146 had a TBI based ablative protocol of whom 96 had an "unpurged" autograft while in 50 the marrow was treated chemically with the cyclophosphamide derivative (mafosphamide). No differences were apparent between fractionated and non-fractionated irradiation or whether they were combined with cyclophosphamide or another agent such as Melphalan. These groups of patients had an event-free survival of 54% with the possibility of remaining leukemia being 61%.

Of the 117 patients who received a chemotherapy protocol, of whom 24 received a purged marrow and 15 had a "double" autograft, the event free survival was 50% and the prospect of remaining leukemia free 52%. However, the relapse rates within the chemotherapy group were significantly greater in recipients of the TACC (64%) (7) or high-dose Melphalan (67%) (9) than of the UCH (36%) (4) or BAVC (45%) (6) regimens (p=0.02). Purging was confined to the TACC patients, but any possible benefit was obscured by the inability of that protocol to prevent relapse.

When an irradiation protocol was used, 47% of the 96 patients who received unpurged marrow relapsed compared with 34% of the 50 patients whose marrow was purged (p=0.02). Although the event free survival (45% vs 63%) was not significantly different, these data suggest a trend in favor of purging, at least in these irradiated patients.

PRE-AUTOGRAFT DELAY

In an earlier analysis of unpurged autografts (13), a correlation between reduced relapse rate and delay in remission before the autograft was demonstrated. This could be either because patients have a reduced risk of relapse anyway, which can be demonstrated in chemotherapy schedules (14) or that the patients had more cytoreduction prior to the autograft which reduced the relapse risk

post-ABMT. There was a considerable range of delay in these 263 patients (median 143 days; range 16-694). When they were stratified into 5 periods of pre-autograft delay (Figure 1), no significant difference was noted until the delay was 12 months. Of these 17 selected patients, 83% remain in remission. It might be expected that patients who took a long time to enter remission might have a poor prognosis for autograft, but in this analysis there was no difference in outcome in those patients who entered remission promptly (i.e. within one month and those who took 2-3 months). Although there was a tendency for FAB subtype M3 to do better (as with chemotherapy) this was not a significant benefit (Figure 2) but the M4 subtypes did significantly worse than other subtypes. There is no data on the influence of cytogenetics or a preleukemic phase.
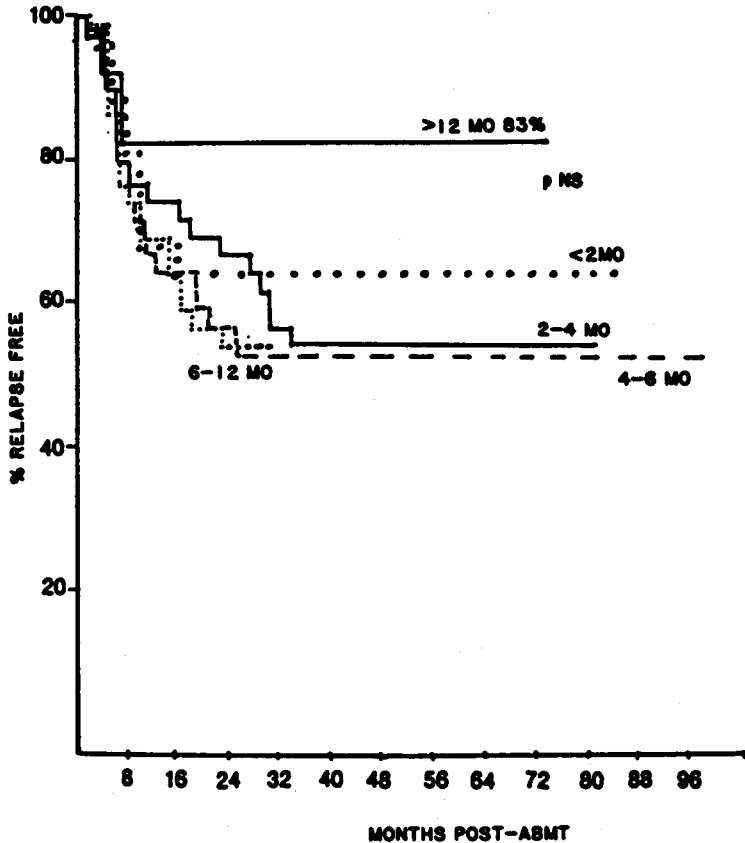
Figure 1.
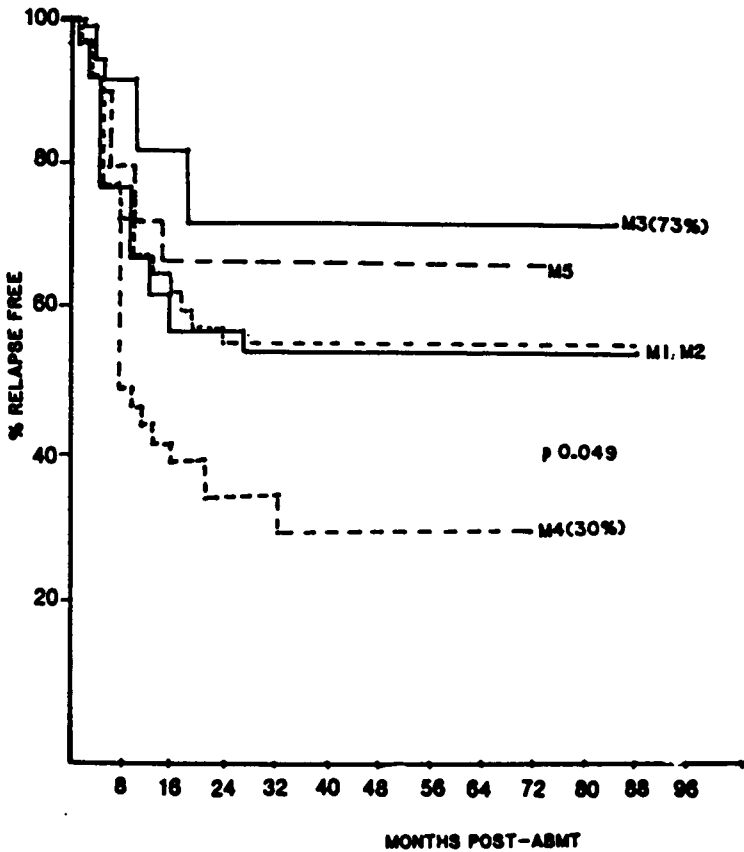
Figure 2.

AUTOLOGOUS TRANSPLANT IN SECOND REMISSION

Of the 61 patients in second remission at the time of autograft, 33 were given a TBI based protocol of which 15 received purged marrow. There was no overall difference in outcome between radiotherapy or chemotherapy ablation or whether or not the marrow had been purged. The overall survival of these patients is 43% (median follow-up 34 mo.), with a relapse free probability of 46%. It is noteworthy that, in this database, there is no significant difference in relapse rate or survival in patients autografted in first or second remission. Relatively few patients received the Bulsulphan/Cyclophosphamide protocol employed by the Baltimore group, but it is of interest to note the surprisingly good outcome for patients treated with the BAVC

Regimen by the Rome Group. Seven of 9 patients remain in CR at the time of this analysis. A subsequent report (15) shows 14 of 18 patients remain in CR at a median of 11.5 months after an autograft. While this second remission data is encouraging, it may well be that these patients are highly selected because they had long first remission. It is not clear, for example, how many patients in this database have "inverted" which, together with prolonged follow-up, will be the most valid assessment.

IDENTIFICATION OF PATIENTS AT POOR RISK FOR AUTOGRAFT

The M4 subgroup appear to have a higher risk of relapse (70%) following autograft, as has been reported following allograft (16). This analysis does not strongly support the view that performing the autograft early, i.e., within two months of achieving CR, results in a higher relapse rate compared with later in the first year, by which time the risk of relapse has diminished. Apart from the comments made about individual schedules, insufficient is known about those patients to indicate whether or not cytogenetic or other prognostic factors can select subgroups for whom autograft is most appropriate.

We have recently used the Long Term Bone Marrow Culture system (17) to evaluate stored aliquots of the autografts given to patients who have now had 6-72 months follow-up principally in an attempt to identify the subgroup of patients who, despite fulfilling morphological and in some cases cytogenetic criteria of remission, regenerated with leukemia. These data indicate that the generation of CFU-GM's from this system by 13 patients who have not relapsed was not different from normal individuals till 6 or 7 weeks in culture when it significantly deteriorated. However, in 10 patients who subsequently relapsed - including those patients who regenerated adequately after the autograft - had significantly inferior CFU-GM generation than normals, with little or no growth beyond 4 weeks in culture. If the patients with growth beyond 4 weeks are compared with those less than 4 weeks, the relative relapse free survivals are 85% (n=13, median follow-up 32 months) compared with 29% (p=0.01). Such assessment pre-autograft may be valuable if corroborated in more patients.

FURTHER ISSUES FOR AUTOGRAFT IN AML

The considerable experience of a number of groups has established that the procedural related mortality associated with autologous BMT is low (around 5%) and is well tolerated in patients, aged up to their mid-fifties. Most groups who have used effective ablation report a

relapse free probability of 45-55%, which, as the series are now more durable, appears to persist. However, the superiority over chemotherapy cannot be categorically claimed until a large trial is conducted. There are a variety of questions which could be addressed. The most important is whether the addition of autologous BMT to an adequate chemotherapy schedule in first remission confers survival advantage. A subsidiary question could be whether the autograft should be undertaken early in remission, which would given the clearest answer but create considerable logistic problems if necessarily conducted on a multicentre basis, or later around 3-6 months.

Since relapse remains the reason for failure there is considerable scope for improved protocols. These could be indicated in single arm studies and tested in second remission. The introduction of purging as a variable raises considerable technical and logistic problems and will be difficult to answer in first remission since the relapse rate after autograft is very similar to that reported for syngeneic transplants for AML (18). It will be necessary to subject the data presented here to multivariate analysis before concluding that the statistical benefit shown for purging in this analysis for the first time is real. It is probable that such studies should be conducted in second remission.

The encouraging second remission data may suggest that the overall comparison between autograft and chemotherapy should be between autograft in first remission versus chemotherapy plus autograft for those who relapse and achieve a second remission. Some of the questions form the basis of some national protocols which hopefully will not miss the opportunity to incorporate prognostic factors into these studies.

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HIGH DOSE ETOPOSIDE, MELPHALAN, TOTAL BODY IRRADIATION AND AUTOLOGOUS BONE MARROW TRANSPLANTATION IN PATIENTS WITH HEMATOLOGIC MALIGNANCIES

Armand Keating, Morel Rubinger, Simon Sutcliffe, Christopher Rajan, and J. Gerald Scott

Results with syngeneic marrow transplantation for hematologic malignancies (1) indicate that, despite hematopoietic rescue with normal marrow, regimens employed to eradicate the malignancy remain suboptimal.

A new regimen comprising agents active in the hematologic malignancies, especially in resistant disease, may therefore be of value.

Etoposide (VP-16), a semisynthetic congener of epipodophyllotoxin with cytotoxicity for lymphoma, leukemia, testicular cancer and small cell lung cancer has been evaluated in phase I (2) and Phase II (3) studies. Dose escalation studies have determined that the main limiting factor, apart from myelotoxicity, was mucositis (2). The maximum tolerated dose was 2,400 mg/m^2. High dose etopside (60 mg/kg) and total body irradiation (TBI) was used in patients undergoing allogeneic bone marrow transplantation by Blume et al(4), who found minimal toxicity and good clinical response with an actuarial disease-free survival of 43% in advanced acute leukemia.

High dose melphalan (140 mg/m^2) with AMT rescue has also been used in acute leukemia in remission and relapse by Maraninchi et al (6) who showed an overall complete remission rate of 79%. Toxicity was minimal and confined to gastrointestinal side effects.

This report details our experience with the first twenty patients receiving high dose etoposide, melphalan, total body irradiation (TBI) and autologous marrow transplantation in hematologic malignancies.

METHODS

Patients

Twenty patients were studied. There were 12 females and 8 males. The median age was 33.5 years. Eleven patients had acute leukemia, 4 with acute myeloid leukemia (AML) in first remission, and six with AML in second and subsequent remission. One patient was in stable PH chromosome-negative phase after chemotherapy for PH-positive chronic myeloid leukemia in lymphoid blast crisis. Six patients had relapsed non-Hodgkin's lymphoma (NHL) (3 immunoblastic, 3 intermediate grade) and one patient had relapsed Hodgkin's disease. All lymphoma patients had disease partially or fully responsive to salvage chemotherapy consisting of at least 2 cycles of the DHAP protocol (6). Further tumor debulking was achieved either with an additional cycle of DHAP or with involved field irradiation or both. One patient with advanced resistant testicular cancer was also included.

The median time from diagnosis to autologous marrow transplantation was 13 months (7-68 mos) for acute leukemia, and 10 months (7-36 mos) for lymphoma. Two patients with lymphoma were transplanted in complete remission and the remaining 5 in partial remission.

Marrow Harvesting

Remission status of the bone marrow as assessed in all patients 7 days or less prior to bone marrow harvest by morphologic, cell surface immunophenotyping, cell culture and cytogenetic studies.

Bone marrow harvesting was performed in the operating room under general anesthesia by multiple needle aspirations from both posterior iliac crests. The harvested marrow was filtered through two gauges of stainless steel mesh, enriched for nucleated cells by processing on a Ficol-metrizoate gradient using an IBM blood cell separator and frozen in a step-wise fashion at $1°$ per minute in 10% dimethylsulfoxide and autologous plasma. All cryopreserved autografts were stored in the liquid phase of liquid nitrogen at $-196°C$. The harvested marrow was not manipulated *ex vivo* in order to remove occult malignant cells.

Etoposide, Melphalan, TBI (EMT) Regimen

Etoposide was given in a dose of 60 mg/kg at day -3 (day 0 is the day of marrow infusion) in the first 13 patients and at day -4 in the remainder (7 patients) by continuous infusion over 5 hours in 5 litres of normal saline. The infusion bottles were changed hourly to ensure stability of the drug. Patients were hydrated with normal saline at 250 ml/hr for the following 96 hours.

At days -2 or -3, melphalan at 140 mg/m^2 in 100 ml normal saline was given intravenously for 30 minutes.

Total body irradiation consisted of 500 cGy in 13 patients and 300 cGy in 7 patients delivered as a single midplane dose at 50 cGy/min. The latter group of patients with AML in second or subsequent remission are to undergo a second autologous marrow transplant with the same conditioning regimen (VP-16 60 mg/kg, melphalan 140 mg/m^2, TBI 300 cGy).

Cryopreserved marrow was thawed rapidly in a 40°C water bath at the bedside and infused through a Hickman-type central venous catheter. Patients were nursed in isolation rooms on a low bacteria diet. Prophylactic trimethoprim-sulamethoxizole (TDS bid) and acyclovir (5 mg/kg i.v. bid) were administered from day -1. All patients received irradiated blood products and CMV seronegative patients received CMV seronegative blood products.

Toxicity was graded according to criteria established by the World Health Organization (7) and performance status was graded according to the E.C.O.G. score (8).

RESULTS

A total of 21 marrow transplants were performed on 20 patients. One patient underwent a double autologous transplant. All patients received a remission marrow autograft. At the time of analysis with a median follow-up of 188 days, 17 patients were alive, 15 in continuous complete remission with an E.C.O.G. score of 0 to 1. In two patients, the post-marrow transplant remission is longer than the first remission. Four patients have relapsed, including the case with testicular cancer, 2 with acute myeloid leukemia and one with large cell lymphoma. Three patients have died, two in relapse at day 83 and 245 and one of interstitial pneumonitis at day 68 with no evidence of malignant disease. There was no mortality during hospital admission for transplantation (day 0 to 60).

Engraftment Data

The patients received autografts containing a mean of 3.1 ± 0.85x10^8 nucleated cells per kilogram patient weight with a range of 2.19 to 6.22x10^8 nucleated cells/kg counted at harvest.

All assessable patients (n=18) successfully engrafted, with the mean

and median values for peripheral neutrophil and platelet counts recorded below in days:

	Neutrophils		Platelets	
	0.5×10^9/L	1×10^9/L	20×10^9/L	50×10^9/L
Mean	29.8±6	37.4±8	37.8±14	53.3±32
Median	29	37.5	34	39.5
	(19-43)	(23-57)	(17-80)	(20-136)

All patients are platelet transfusion independent.

Complications

Most patients experienced mild nausea, vomiting, diarrhea and oral mucositis (WHO grade ≤2). No patient required intravenous morphine for pain control. Three patients had prolonged vomiting but none required parenteral nutrition. Transient cholestatis jaundice (grade 4) was encountered in one patient. Fever was documented in 18 of the 21 transplants leading to positive blood cultures in 7 cases. Sepsis with hemodynamic compromise occurred in 3 patients, but all recovered with antibiotic therapy. Three patients remained afebrile and did not require intravenous antibiotics.

DISCUSSION

Numerous intensive treatment regimens in association with autologous marrow transplantation have been employed in patients with hematologic malignancies (9). Studies have assessed the roles of high dose etoposide (3), etoposide and TBI (4) and high dose melphalan (5,10). Our treatment combination of high dose etoposide, melphalan, TBI and autologous marrow transplantation has been remarkably well tolerated by the first twenty patients with hematologic malignancies. Moreover, apart from the low morbidity, there was no mortality during the early post-transplant period. Only one patient died of a probably treatment-related complication. Hematologic recovery was similar to (10) or more rapid than reported by other groups (11). All patients have engrafted and do not require transfusions of blood components.

We conclude that further studies with the EMT regimen are warranted in hematologic malignancies in order to assess remission duration and long-term survival. Since extramedullary toxicity has

been mild, dose escalation of melphalan and/or TBI may confer additional benefit.

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IN VITRO OR IN VIVO TREATMENT OF LEUKEMIA TO PREVENT A RELAPSE AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION?

Anton Hagenbeek, Frank W. Schultz,
and Anton C. M. Martens

Relapse of leukemia remains the major problem after autologous bone marrow transplantation (ABMT) in first complete remission of acute myelocytic leukemia (AML). At three time points during the course of leukemia treatment crucial measures can be taken with the aim to eradicate the leukemic cell population, i.e. , at the time of 1) remission-induction chemotherapy; 2) manipulating the autologous marrow graft to eliminate contaminating leukemic cells, and 3) conditioning prior to ABMT. In a previous contribution to this Symposium the efficacy of a variety of conditioning regimens was presented employing the BN rat acute myelocytic leukemia (BNML) as a model for human AML (1). In summary, either the combination high-dose cytosine arabinoside-cyclophosphamide-total body irradiation or high-dose busulphan followed by cyclophosphamide were found to be most effective, both inducing a greater than 10 log leukemic cell kill. The present paper will address to the question what the chance is that leukemic cells in the graft will contribute to a leukemia relapse after ABMT. Both preclinical and clinical data are used to construct a mathematical model from which this chance can be derived.

MATERIALS AND METHODS

Rats

The experiments were performed with the inbred Brown Norway rat strain produced in the Rijswijk colony. Male rats between 13 and 16 weeks of age and a mean body weight of 200 g were used.

The Rat Leukemia Model (BNML)

The origin, classification, transplantation procedure, and growth characteristics of the BNML model have been described in detail elsewhere (2, 3) . Leukemia was induced in a female Brown Norway rat by 9,10- dimethyl-1,2-benzanthracene. The disease shows a reproducible growth pattern upon intravenous cellular transfer within the Brown Norway rat strain, and it is cytologically and cytochemically similar to human acute promyelocytic leukemia. Further analogies with the human disease are: 1) slow growth rate (10^7 BNML cell kill after 18-23 days; growth fraction, 0.60-0.40); 2) severe suppression of normal hematopoiesis because of an absolute numerical decrease in the number of normal hematopoietic stem cells; 3) diffuse intravascular coagulation; 4) prolonged blood transit time of leukemic cells (34-36 hours); 5) response to chemotherapy as in human AML; 6) presence of clonogenic leukemic cells (including in vivo and in vitro colony formation); 7) low antigenicity; and 8) no evidence for a virus as an etiologic agent.

The BNML is generally accepted as a relevant model for human AML and a number of clinical applications have emerged during the past years as summarized in Table 1.

RESULTS AND DISCUSSION

In the BNML model it is known that 25 leukemic cells are needed to induce leukemia in 50% of normal recipient rats after i.v. injection (ED_{50}; Figure 1). If a total inoculum of e.g. 1000 leukemic cells is regarded as 40 ED_{50} units, an average of 20 BNML cells would grow out in vivo. Indeed after i.v. injection of 10^7 BNML cells, 2 x 10^5 leukemic cells were recovered at the start of leukemia growth process in the major target organs for leukemia growth in the rat, i.e. , bone marrow, spleen and liver. If an inoculum contains xED_{50} units , with each unit having a chance of 0.5 to grow out and cause overt leukemia, the chance that leukemia will not or will develop is 0.5^x or $1-0.5^x$ respectively. If these mathematics are applied to experimental data derived from experiments where the incidence of leukemia was related to the i.v. injection of graded low numbers of leukemic cells, there is a perfect agreement.

Table 1. Preclinical BNML Studies* Applied in Human AML

Diagnosis/detection

- monoclonal antibodies (MoAbs)
- fluorescence-activated cell sorting (FACS)[4]
- flow karyotyping[5,6]
- heterogeneous distribution of "minimal residual disease"[7,8]
- computer simulation of leukemia growth[9]

Treatment

- high-dose cytosine arabinoside (time sequential treatment)[10,11]
- pharmacokinetics of cytostatis agents[12]
- conditioning regimens prior to BMT
    - (fractionated) TBI[13]
    - supralethal chemotherapy[14]
    - combinations[15]
- in vitro chemotherapy/MoAb treatment of autologous marrow grafts[16-18]

*Radiobiological Institute TNO, Rijswijk, The Netherlands and 18 Leukemia Research Centers in Europe, United States and Canada.

number of BNML cells injected i.v.
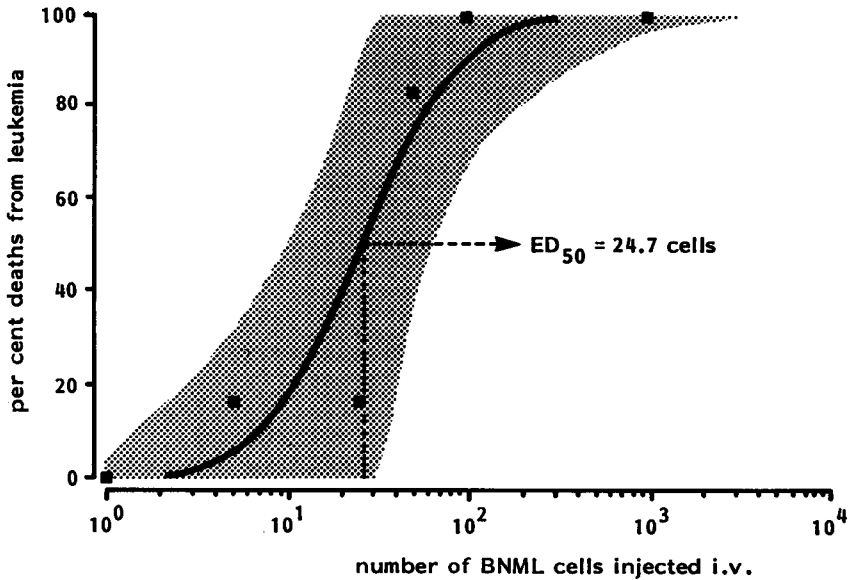
Figure 1. Determination of the ED_{50} value for the Brown Norway rat acute myelocytic leukemia (BNML). Probit analysis with 95% confidence limits.
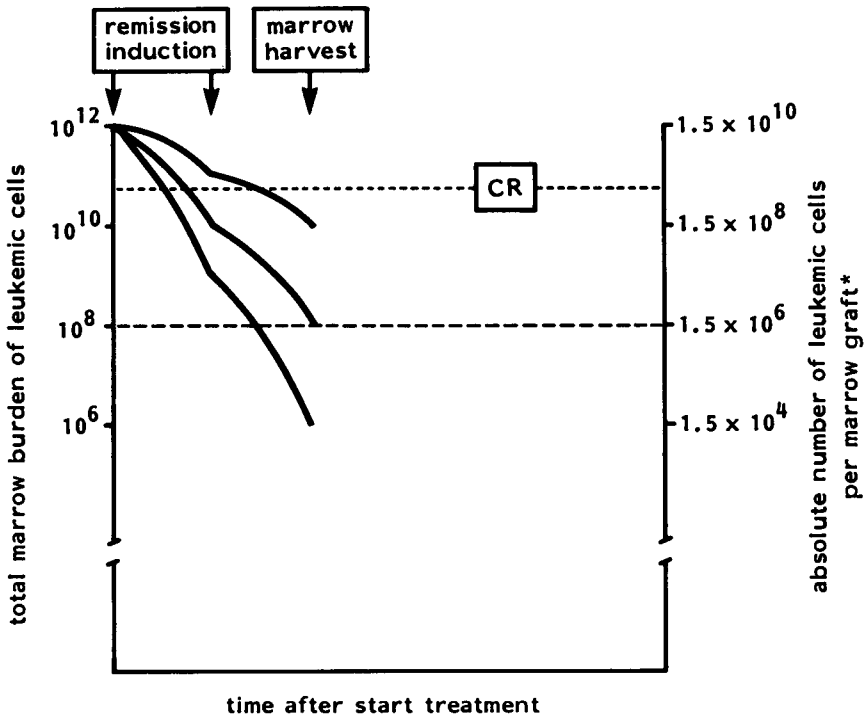
* graft: 2×10^8 cells/kg (BW: 75 kg)

CR : "complete remission"

Figure 2. Relationship between the log leukemic cell kill induced by remission-induction chemotherapy and the AML cell content of the autologous marrow graft.

With the ED_{50} model computer simulations can be performed yielding the chance that leukemia will develop from injected leukemic cells as a fraction of 1) the number of cells injected, and 2) the ED_{50} value. The ED_{50} for human AML is not known.

It seems reasonable to assume that remission-induction treatment on the average induces a 4 log leukemic cell kill (Figure 2). Thus, an autologous marrow graft will contain 1 leukemic cell per 10^4 normal marrow cells, or 10^6 in a graft containing a total of 10^{10} cells (2×10^8 cells/kg; 70 kg patient). In the BNML it was found that 1% of in vivo clonogenic leukemic cells survive cryopreservation (19). Furthermore, from in vitro studies with human AML it is concluded that only 0.1–1% of all leukemic cells can be considered to be clonogenic. Taken these two factors together, only 10–100 clonogenic leukemic cells out

of 10^6 are reinfused with the marrow graft. This is step-by-step illustrated in Figure 3. Assuming now an ED_{50} value for human AML to be 1000 or 10,000 clonogenic cells, which seems realistic based on previous BNML studies (20,21) , it can be calculated employing the computer simulation model that the chance that reinfused leukemic cells indeed cause leukemia is 1-10% or 0.1-1%, respectively (Table 2) .

1. overt AML $\sim 10^{12}$ leukemic cells

2. surviving fraction after remission-induction : 10^{-4} (10^8 leukemic cells)

graft size : 10^{10} cells (2 x 10^8/kg; 70 kg)

total leukemic cells in the graft : 10^6

0.1 - 1% clonogenic : 10^3- 10^4

1% surviving cryopreservation : 10 - 100
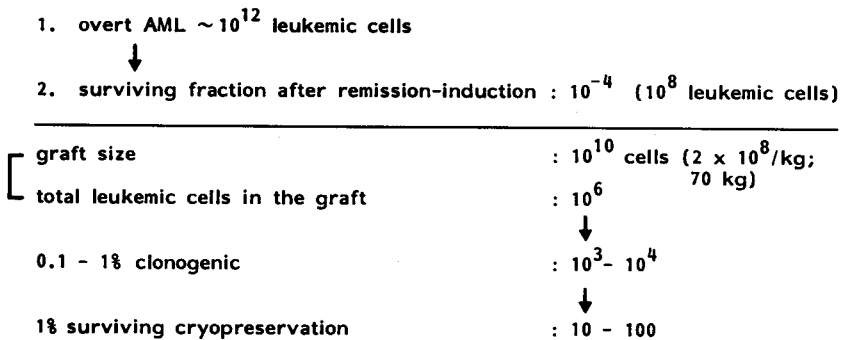
Figure 3. The number of in vivo clongenic AML cells infused with the autologous marrow graft: The hypothesis.

Table 2. Theoretical Chance (%) of Leukemia Development as a Function of Assumed ED_{50} Value and the Number of Leukemic Cells in the Human Autologous Bone Marrow Graft

ED_{50} value	number of leukemic cells in graft								
	10	15	100	150	500	1000	1500	5000	10000
10	50.0	64.6	99.9	100.0	100.0	100.0	100.0	100.0	100.0
100	6.7	9.9	50.0	64.6	96.9	99.9	100.0	100.0	100.0
1000	0.7	1.0	6.7	9.9	29.3	50.0	64.6	96.9	99.9
5000	0.1	0.2	1.4	2.1	6.7	12.9	18.8	50.0	75.0
10000	<0.1	0.1	0.7	1.0	3.4	6.7	9.9	29.3	50.0

The theoretical probability of leukemia development, i.e., the chance that at least one unit of ED_{50} cells yields a cell to grow out, is given by $(1-0.5^X) \times 100\%$, where X denotes the number of ED_{50} units injected.

Thus, to prevent a leukemia relapse after ABMT major emphasis should be given to more effective pretreatment of the patient. An interesting alternative may be post-ABMT treatment with low-dose alkylating agents or biological response modifiers. Employing these, it has been shown to be feasible to eradicate the few remaining logs of leukemic cells surviving after ABMT without jeopardizing the marrow graft (15, 22).

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HIGH-DOSE BUSULFAN AND CYCLOPHOSPHAMIDE:
A Successful Preparative Regimen for
Bone Marrow Transplantation

Robert B. Geller, Andrew M. Yeager,
Rein Saral, and George W. Santos

Preparative regimens for bone marrow transplantation (BMT) in patients with acute leukemia must provide both marked anti-tumor effects and "space-making" properties. Besides providing physical "space" in the marrow, the BMT cytoreductive regimen may enhance engraftment and proliferation of hematopoietic stem cells by stimulation of humoral and microenvironmental factors. For allogeneic BMT, the preparation regimen must also provide significant immunosuppression in the recipient to facilitate engraftment(1,2). In addition to these requirements of antitumor, "space-making," and immunosuppression, the ideal regimen, should be tolerable to the patient – easy to administer, have predictable pharmacokinetics, and acceptable toxicities to non-hematopoietic organs.

Ionizing radiation in the form of total body irradiation (TBI) has impressive anti-tumor effects on lymphohematopoietic tissues and is capable of both immuno-suppression and "space-making" requirements for BMT. However it can produce substantial damage to other organs, the severity of which is related to dose and dose rate of TBI. As a single agent, TBI was not successful in sustaining engraftments or curing refractory disease(3). Cyclophosphamide (CY) is a potent immunosuppressant agent in both man and animal models. Preclinical studies in rodents confirmed that CY used as a single agent produced profound marrow hypoplasia which was rapidly reversed by infusion of syngeneic or allogeneic marrow, and that the frequency and persistence of chimerism was directly proportional to dose of CY administered. Over several weeks, the host cells were eliminated, eventually leaving only donor hematopoietic grafts, suggesting that

CY alone is not an optimal space-maker(4). Busulfan (BU), an alkylating agent with specificities against myeloid tissue is an excellent space-maker with marked antitumor properties against hematopoietic tissue, but has only negligible immunosuppression effects(5).

Because of the inadequacy of single agents as effective conditioning regimens for allogeneic BMT, combinations of agents have been attempted (Table 1). A regimen of CY followed by TBI has been adopted by Seattle for transplantation of the acute leukemias - engraftment has been very successful but relapse rates have been high especially in patients transplanted in second or subsequent remissions(6). The occurrence of interstitial pneumonia was high, correlating with single dose intensity. Memorial Sloan-Kettering Cancer Center has employed hyperfractionated TBI with lower relapse rates in children with acute nonlymphocytic leukemia (ANLL)

Table 1. Preparative Regimens in BMT for Patients with ANLL

	Relapse % (at 3 years)	
Preparative Regimen	CR1	CR2,3,ER
CY/TBI (Seattle)	25	30-60
HFTBI/CY (MSKCC)	0	15-70
HDAra-C/FTBI (Cleveland)	20	20
HFTBI/VP (City of Hope)	(?)	30
BU/CY (Baltimore)	15	30
BU/CY (Columbus)	0	40

CR = Complete Remission; ER = Early Relapse; CY = Cyclophosphamide; TBI = Total Body Irradiation

CY/TBI = CY (60 mg/kg IV qd x 2 d) followed by TBI (920 - 1575 cGy)

HFTBI/CY = TBI (120 cGy x 11 doses tid x 4 d) followed by CY (60 mg/kg IV qd x 2 d)

HDAra-C/FTBI = Ara-C (3 gm/m^2 IV q 12 hr x 12 doses) followed by TBI (1000 cGy or 1200 cGy in 200 cGy BID x 6 doses)

HFTBI/VP = TBI (120 cGy x 11 fractions over 4 d) followed by Etoposide (60 mg/kg x 1 dose)

BY/CY (Baltimore) = BU (16 mg/kg in 16 divided doses over 4 d) followed by CY (50 mg/kg IV qd x 4 d)

BU/CY (Columbus) = BU (16 mg/kg in 16 divided doses over 4 d) followed by CY (60 mg/kg IV qd x 2 d)

transplanted in first and second remissions; however patients transplanted in subsequent remissions had a significant (70%) relapse rate(7). Our regimen with BU followed by CY has a very low relapse rate in patients with ANLL transplanted in first remission and more acceptable relapse rates in subsequent remissions and early relapse (8,9) Toxicity has been minimal except for moderate mucositis which was reversible, and veno-occlusive disease (VOD) which is also observed with our CY/TBI regimen(10). A new regimen of BU with lower doses of CY has recently been reported by Tutschka et al (11). No relapses have been seen in 11 patients with ANLL transplanted in first or second remissions. Of 14 patients transplanted with refractory leukemia and in relapse, 5 have relapsed (medium follow-up 1 yr).

More patients will need to be followed for longer periods of time to adequately compare this newer BU/CY regimen to the one currently employed by our group. Studies with high doses of cytosine arabinoside followed by fractionated TBI have also produced reasonably low relapse rates, however toxicities of this regimen included interstitial pneumonitis, skin rashes, diarrhea, and central-nervous-system dysfunction(12). A regimen of hyperfractionated TBI followed by high-dose etoposide has been reported in advanced leukemias with a relapse rate of 30% and acceptable toxicity(13).

Allogeneic bone marrow transplants in first remission have been successful with relatively low relapse rates, however allogeneic transplants in advanced ANLL are complicated by a substantial risk of leukemia relapse. Therefore it is not surprising that autotransplants for these advanced diseases are associated with even higher relapse rates. Results of autologous BMT conditioning regimens in patients with ANLL need to be analyzed in the context of leukemia-free survival after syngeneic BMT where the recipient is assured of receiving leukemia-free marrow and results are not effected by the effects of graft-versus-host disease or graft-versus-leukemia. The relapse rates following syngeneic BMT are in the 50% range using TBI containing regimens (14). Our experience with syngeneic BMT preceded by BU/CY is too small to generate comparable data but based on this limited experience and our results in autologous BMT, a comparable relapse rate is seen. Our experience shows that there is a significant difference in the relapse rate between first remission patients with ANLL treated with BU/CY and allogeneic transplants compared to those receiving autologous transplants (p=.009) (Figure 1).

Our results for transplants in second and third remissions and early relapse show a similar significant difference in the probability of remaining in remission between allogeneic transplants and autologous transplants (p=.007)(Figure 2).

Because of an increased number of relapses in patients with ANLL transplanted in second and third remissions and early relapses, we are developing a new preparative regimen incorporating busulfan,

BU CY PREPARATIVE REGIMEN IN FIRST CR
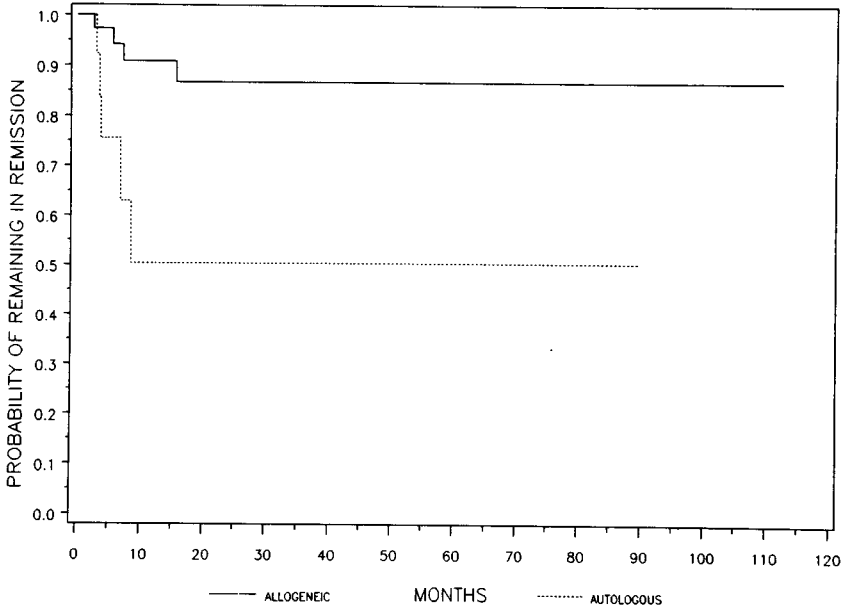
Figure 1. Comparison of the probability of remission for patients with ANLL treated in first complete remission who received allogeneic transplants (54 patients), and autologous transplants (16 patients) following a preparative regimen of BU/GY (p=.009).

cyclophosphamide, and escalating doses of etoposide. This regimen includes busulfan 4mg/kg/day in four divided doses for four days (total 16 mg/kg) followed by one day of etoposide (escalating doses starting at 10 mg/kg) and cyclophosphamide 60 mg/kg/day for two days. At present our dose of etoposide is 40 mg/kg and no significant toxicities other than mild to moderate mucositis have been seen. Once this phase I regimen has been completed it will be evaluated in phase II trials.

In a prospective study of the pharmacokinetics of busulfan in 28 patients undergoing marrow transplantation, a correlation existed between busulfan dose levels (area under the concentration versus time curve) and the occurrence of VOD(15). Because of a concern regarding the occurrence of VOD with high doses of busulfan, a randomized trial has begun to assess the feasibility of therapeutic monitoring and dose modification in patients receiving busulfan in their preparative regimens.

BU CY PREP REGIMEN IN CR2, CR3, AND ER
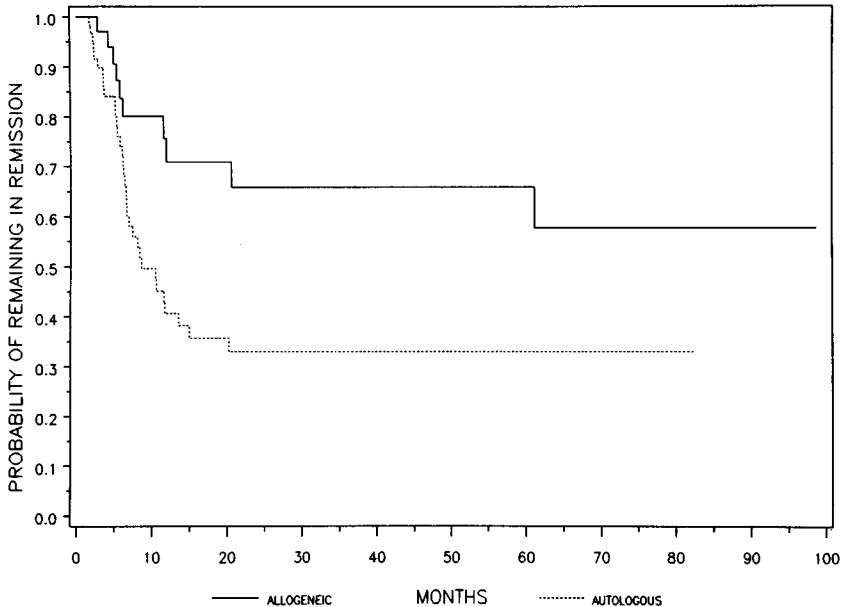
Figure 2. Comparison of the probability of remission in patient with ANLL treated in second and third remissions and early relapse who received allogeneic transplants (53 patients) and autologous transplants (78 patients) following a preparative regimen of BU/CY (p=.007).

Finally, another potential advantage of the BU/CY preparative regimen is in the treatment of patients who have developed secondary ANLL following intensive chemoradiotherapy. We have reported on 5 patients who have undergone BMT, four allogeneic and one autologous, for ANLL in first remission following treatment for Hodgkin's disease(16).

All patients received intensive combination chemotherapy and extensive radiotherapy for their Hodgkin's disease. Of the five patients, two including the one who received an autologous transplant, are disease-free 833 and 830 days, respectively. One patient relapsed after being disease-free 536 days, and the other two died of complications of graft versus-host disease. None of the five patients had increased toxicity associated with the preparative regimen.

In summary, a preparative regimen of high dose BU/CY for BMT is effective in the treatment of patients with ANLL. Patients who may not be a candidate for preparative regimens incorporating TBI, may still benefit from aggressive therapy with BU/CY following either

autologous or allogeneic BMT. In the future we hope to improve our results in second and third remissions and early relapse with an intensive preparative regimen incorporating etoposide with BU/CY. To decrease the toxicity, we are studying the potential role of dose modification with the monitoring of appropriate drug levels of busulfan.

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BLOOMSBURY TRANSPLANT GROUP EXPERIENCE WITH DOUBLE AUTOLOGOUS BONE MARROW TRANSPLANTATION IN FIRST REMISSION ACUTE MYELOID LEUKEMIA

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John D. M. Richards, and David C. Linch

INTRODUCTION

The results of the Bloomsbury Transplant group's use of Double autografting in acute leukemia updated to 1987 showed that the protocol gave encouraging results in first remission AML (1). We now present the updated results of 42 adult AML patients in first remission autografted in this centre of whom 19 have completed both parts 1 and 2. In addition we have analyzed the toxicities of the protocol and the reasons for failure to proceed to the 2nd graft.

PATIENTS AND TREATMENT PROTOCOLS

All patients less than 55 years with AML M1-M6 in continuing first remission treated in this institution or referred for transplantation entered the study unless there was any evidence of significant organ dysfunction. Patients with a preceding bone marrow abnormality were acceptable when the protocol was devised but since the review in 1986 have no longer been treated on this protocol. One patient (UPN 285) with a preceding chloroma, though in remission and treated with ABMT, has not been included in the analysis. Induction and consolidation therapy was not standardized due to referral after induction therapy but in most cases was based on the Medical Research Council AML 8 or 9 protocols. Virtually all the patients were induced with Daunorubicin, Cytarabine and 6 Thioguanine on a DAT 1+5 or 3+10 protocol and given one or two courses of

consolidation with the same agents. Remission was established by morphological and karyotypic analysis of the bone marrow immediately prior to each harvest. Bone marrow was harvested and cryopreserved as previously described (2). No ex vivo manipulation was undertaken.

The U.C.H.1. conditioning regime was used for both grafts in all patients, bone marrow was reharvested after part 1 as soon as satisfactory regeneration was achieved. (Neutrophil count $>1.5 \times 10^9/l$ and Platelet count $>100 \times 10^9/l$.) ABMT 2 was then performed shortly afterwards using the same regime.

U.C.H.1: Bone Marrow Returned on Day 0

B.C.N.U.(5;1,3,bis(2 chloroethyl)-1-nitrosourea) 300 mg/m^2 i.v. day -5.

CYCLOPHOSPHAMIDE 1.5 g/m^2/day i.v. on day -5,-4,-3.

DOXORUBICIN 50 mg/m^2 i.v. on day -5 only.

6 THIOGUANINE 100 mg/m^2 b.d. p.o. on day -5 to -1 inclusive.

CYTARABINE 100 mg/m^2 b.d. i.v. on day -5 to -1 inclusive.

The autologous bone marrow was thawed and reinfused via an indwelling Hickman catheter 24 hours after the completion of the chemotherapy. The mean number of nucleated cells frozen was 1.82 x 10^8 per Kg of the recipients weight.

All patients received care in reverse barrier nursing in single rooms. Laminar flow or filtered air rooms were not used for these patients. Irradiated blood products were not used.

The median age of the whole group was 40.1 years, and for those completing a double graft was 33.7 years. There were 20 males and 22 females. The median interval between remission and ABMT was 119 days (range 57-337) for the whole group, 139 days (range 69-337) for those receiving only a single graft and 112 days (range 57-230) for the group who later received part 2. The median number of days between remission and ABMT 2 was 182 days (range 116-294). Haematological reconstitution is delayed after part 2 compared to part 1 and the details of this are presented in Table 1.

RESULTS

Forty two patients entered the study. Nineteen patients completed both ABMT 1 and 2 and 16 remain in continuing complete remission (C.C.R.) with a median follow-up of 647 days. Fifteen patients

Table 1. Haematological Reconstitution after UCH 1

	Leucocytes 1×10^9/L	Neutrophils 0.5×10^9/L	Platelets 50×10^9/L
ABMT 1	18 Days	21 Days	31 Days
ABMT 2	21 Days	26 Days	38 Days

completed a single graft and 9 remain in C.C.R. with a median follow-up of 580 days. Three of those who relapsed regenerated from the procedure with leukemia. Five patients with an antecedent bone marrow disorder who had a single graft all relapsed within one year and as stated above this treatment is no longer considered for this group of patients. Twenty-five patients out of 42 in the whole group remain in continuing complete remission with a median follow-up of 753 days. The event free survival curves for the combined group and the three patient groups are presented in Figures 1 and 2. Twenty patients did not progress to a second graft and the reasons for this are presented in Table 2.

TOXICITY DATA

Procedure Related Mortality

There were 3 early deaths (7%) all of which occurred during the first procedure, the details are as follows:

UPN 220:   Cerebral hemorrhage during the thrombocytopenic phase.

UPN 264:   Hepato-renal syndrome.

UPN 328:   Pulmonary hemorrhage during recovery phase from *Aspergillus* lung infection.

Morbidity

Bacterial sepsis during the neutropenic phase either documented or treated empirically with broad spectrum antibiotics occurs in the majority of cases. The organisms isolated were most commonly line associated coagulase negative Staphylococci and enteric gram negative bacilli. No deaths have occurred due to bacterial sepsis. Interstitial

Event free survival AML
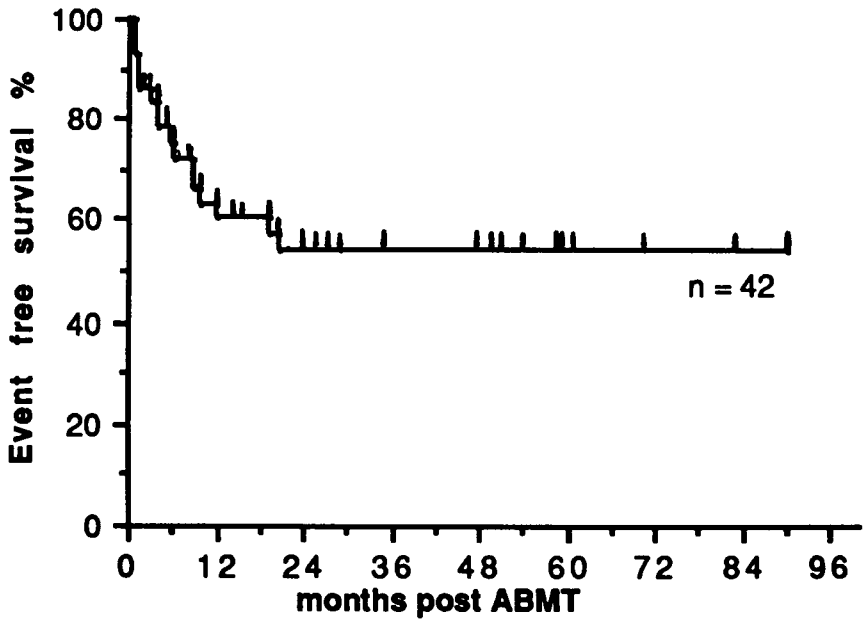
Figure 1. Event-Free Survival after ABMT in First Remission AML with UCH 1 Chemotherapy.

Table 2. Reasons for Cancellation ABMT 2

Reason	Numbers/Outcome	
Slow Recovery	9	(5 remain in remission, 4 relapsed)
Cardiac	2	
Hepatitis	1	
Refused	3	(1 remains in remission, 2 relapsed)
Relapse Before Part 2	4	
Pseudomonas Infection in Part 1	1	

Event Free Survival : double vs single
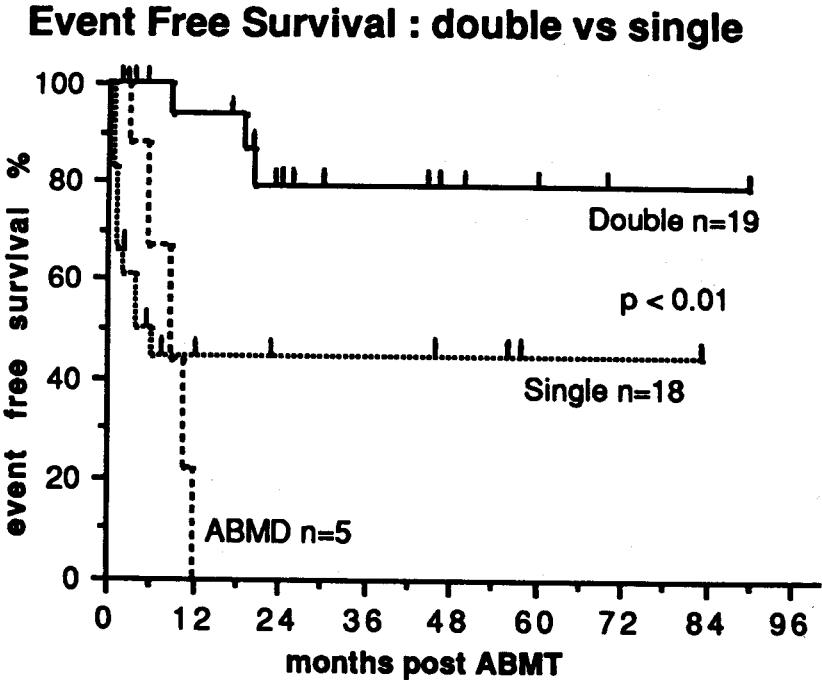
Figure 2. Event-Free Survival after Single or Double ABMT with UCH 1 Chemotherapy in First Remission AML and Single ABMT in Patients with an Antecedent Bone Marrow Disorder.

pneumonitis has occurred in 4 cases but has resolved with appropriate treatment in all cases. One case was of presumed *Pneumocystis carinii* pneumonia (UPN 218) and responded to high dose septa, one case of drug induced (?BCNU) pneumonitis (UPN 245) responded to high dose steroid therapy and two cases of CMV pneumonitis who both responded to treatment with a combination of Sandoglobulin and D.H.P.G. (Gancyclovir) (UPN 307 & 323). This incidence of around 10% pneumonitis after ABMT for AML has not been described previously from our unit.

Hematological recovery has been complete in all long-term survivors.

DISCUSSION

The rationale for Double ABMT when the marrow is reharvested between the two procedures is to achieve an in vivo purging effect.

There is a statistically significant difference (p < 0.01) in the disease-free survival of those patients who received single and double grafts. The contribution of the second graft is unclear as there are a number of features operating to select for longer survival in the double ABMT group. Firstly, only those patients who engrafted quickly after part 1 proceeded to part 2 and rapid normal regeneration may be a marker of future freedom from relapse. Secondly, the long interval from remission to completion of therapy for the double group means that these patients have been longer in remission. There were insufficient patients who refused the second graft, who would otherwise be eligible for it, to be used as a control group. The only definite way of answering this question would be for a randomization step after the second harvest between a second graft and stopping therapy. The factor that must also be considered in this context is that the careful selection of patients for the second graft is likely to be partly responsible for the zero procedure related mortality. Therefore it is our current policy to proceed to the second graft only if there is acceptable recovery from part 1: (Neutrophils > 1.5 x 10^9 and platelets >100 x 10^9 by day 40) and no other factor which would prejudice survival during the neutropenic phase of part 2. As shown in Table 2 delayed recovery is the most common reason for cancellation of part 2 and patient refusal does not play a significant role. All patients who have delayed recovery after part 1 now have their marrow saved if adequate regeneration eventually occurs for use in any subsequent relapse. The incidence of pneumonitis (10%) is low as might be expected for a regime which does not contain total body irradiation. All cases have recovered following pneumonitis which is in marked contrast to the normal outcome after bone marrow transplantation.

CONCLUSION

This study suggests that double autografting may result in an increase in long-term disease-free survival in adult patients with AML in first remission. Approximately 50% of people complete ABMT 1 and 2 but there was still good survival in those who receive a single graft. Careful selection has allowed those who may benefit from part 2 to proceed, while excluding those who would be at increased risk of treatment related mortality.

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DOUBLE UNPURGED AUTOLOGOUS TRANSPLANTATION IN ACUTE LEUKEMIA WITH A MINIMUM FOLLOW UP OF 2 YEARS

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Following the results obtained by allogeneic bone marrow transplantation in acute leukemia, autologous bone marrow transplantation (ABMT) has become a therapeutic alternative to maintenance chemotherapy for patients who had no marrow donors. Because the good record of tolerance and antitumoral effects of Melphalan, we used it as a conditioning regimen for several malignant conditions (1) (2) (3). We then set up a double ABMT protocol with the aim to increase the antileukemic effect and to realize an in vivo purging. We report here a retrospective analysis of 55 patients treated in relapse (RL) or in complete remission (CR) and the results of a cooperative randomized study in acute myeloid leukemia (AML) in first CR. In this study, the results between conventional dose chemotherapy and BMT were comparable.

RETROSPECTIVE ANALYSIS IN ACUTE LEUKAEMIA IN CR OR RL

Patients and Methods

Among the 55 patients who entered the study 30 had acute leukemia in relapse (RL); 9 had acute lymphoblastic leukemia (ALL),

and 21 had acute myeloid leukemia (AML). Twenty-five relapses occurred "on" therapy; 5 "off" therapy. Twenty-five patients received ABMT in complete remission (CR): 4 had ALL, 21 had AML (Table 1).

Conditioning Regimen

o ALL: Before their first ABMT, the patients received 140mg/m^2 of Melphalan and afterwards 10 mg/m^2 of Methotrexate (MTX) was administered intravenously (IV) on days +1 +3 +6 +11; then weekly from day +32 to day +102 post ABMT, MTX was given alternately in IV and intrathecal administration according to the Seattle schedule of allogeneic transplantation. Before the 2nd ABMT, the patients were conditioned with the CBV protocol as previously described (Cyclophosphamide 1.5 g/m^2/day x 4, BCNU 300 mg/m^2 x 1, VP16 125 mg/m^2 x 4).

o AML: Before ABMT, the patients received 140 mg/m^2 of Melphalan given as an IV bolus during hyper hydratation. After hematopoietic recovery, the second marrow was harvested after which the patients received one to three cycles of chemotherapy with VP16 100 mg/m^2 x 2, Cytarabine (ARA) 100 mg/m^2 x 5 before the 2nd ABMT.

Marrow Procedure

During CR a minimum of 2 x 10^8 medullary cells/kg was aspirated. After the first ABMT, when the granulocytes reached more than 1000/mm^3 and platelets more than 100,000/mm^3 a 2nd marrow collection was done and cryopreserved before the 2nd ABMT.

Supportive Care

Patients were managed in single room with usual supportive care including blood product irradiation.

Results

Leukemia in Relapse

Twenty-two of the 30 patients (73%) achieved a CR after high dose Melphalan (HDM). Two/twenty-two patients died in CR, 2 months after the first ABMT respectively of interstitial pneumonitis

Table 1. Clinical Data: Antileukemic Response, Relapse and Survival
after Double Autologous Bone Marrow Transplantation

Status at ABMT	n	Age	M/F	CR	2nd ABMT	Relapse	Toxic Death	OUTCOME Alive	CCR
ALL in RL	9	12 (14-51)	4/5	8 (88%)	4	$6(3)^a(3)^b$	$2(1)^a(1)^b$	--	--
AML in RL	21	42 (14-67)	10/11	14 (66%)	7	$9(5)^a(4)^b$	$3(1)^a(2)^b$	2	$2(75^a\text{-}85^b)$
AL in RL	30			22 (73%)	11 (30%)	15	5	2	2
ALL in CR	4	13	1/3	--	4	$1(0)^a(1)^b$	0	3	$3(44^b\text{-}57^b\text{-}60^b)$
AML in CR	21	32	14/7	--	13	$15(6)^a(9)^b$	0	9	$5(25^b\text{-}28^a\text{-}29^a\text{-}34^b\text{-}65^b)$
AL in CR	25			--	17 (68%)	16	0	12	8

a: after the 1st ABMT
b: after the 2nd ABMT
ABMT: Autologous Bone Marrow Transplant. ALL: Acute Lymphoblastic Leukemia. AML: Acute Myeloblastic Leukemia. CR: Complete
Remission. RL: Relapse. AL: Acute Leukemia.
CCR: Complete Continuous Remission. n: Number of patients. M: Male. F: Female.

(ALL) and fulminant hepatitis (AML). Eight patients relapsed early after the 1st BMT ≤4 months (3 ALL, 5 AML), did not receive the 2nd BMT and died. One patient (AML) refused the second BMT and is still in CR 75 months after BMT. Eleven patients (7 AML, 4 ALL) underwent the 2nd procedure. Three patients (1 ALL, 2 AML) died from sepsis at 1, 1, and 3 months respectively from interstitial pneumonitis in two patients and from disseminated aspergillosis in the third one. Seven patients (3 ALL, 4 AML) relapsed at a median of 10 months (7-60) after the 2nd BMT and died. Only one patient is alive in continuous complete remission (CCR) after 85 months (Table 1) .

Leukemia in Complete Remission

No toxic death occurred; 6 patients (AML) relapsed early (<4 months) and did not receive the 2nd ABMT. In two patients (AML) the platelet count did not exceed $100 \times 10^3/mm^3$ and did not undergo the second procedure and are alive in CCR > 28-29 months. Seventeen patients (68 %) (4 ALL-13 AML) underwent the 2nd procedure. Ten patients (1 ALL-9 AML) relapsed at a median of 14 months. One patient died in 1st CR 17 months after 2nd BMT of HIV induced by unscreened blood products. Six patients (3 ALL-3 AML) are in unmaintained CCR with a median follow up of 50 months after BMT (range 25-65) (Table 1).

Engraftment

We previously reported (4) that the duration of aplasia was generally longer after the second ABMT than the first in 24 analyzed patients among the 28 who received two autografts (Table 2).

Table 2. Comparison of Hematologic Recovery Between First and Second Autologous Bone Marrow Transplantations of 24 Patients who Received Two Autografts

Diagnosis	Days to Recovery of	First ABMT Median Value Range	Second ABMT Median Value Range
ALL n=6	Nucleated Cells/Kgx10^8	1.8 (1.2-2.4)	1.85 (1.8-2.8)
	Granulocytes>500/mm^3	17 (12-23)	17 (14-28)
	Platelets>50,000/mm^3	23 (15->50)	35 (12-42)
AML n=18	Nucleated Cells/Kgx10^8	2.5 (0.6-5)	1.9 (1-6.1)
	Granulocytes>500/mm^3	12 (5-48)	15 (9-102)
	Platelets>50,000/mm^3	21 (10-96)	35 (14->240)

ALL: Acute Lymphoblastic Leukemia
AML: Acute Myeloblastic Leukemia

Discussion

These results confirmed that HDM alone (>140 mg/m^2) is able to induce a high remission rate in leukemic patients in relapse; the 73% CR rate is similar to that obtained after combination of drugs with or without total body irradiation (TBI) (5) (6) (7) (8) (9). However the duration of response was short; 8 patients relapsed within 4 months and could not have 2nd BMT. Although all the ALL patients who received ABMT during relapse, subsequently relapsed and died, 2 patients with AML in relapse remained disease free more than 6 years after BMT. These results are close to those obtained with allogeneic BMT under the same conditions (10) (Figure 1). When the patient underwent transplantation during CR, the projected probability of disease free survival is 30% at 5 years (Figure 1). At the present time our results are comparable to those of other investigators who are using more aggressive conditioning regimens (11). These results prompted us to conduct a prospective study among similar patients to compare the benefit of double ABMT versus post remission chemotherapy in AML 1st CR.
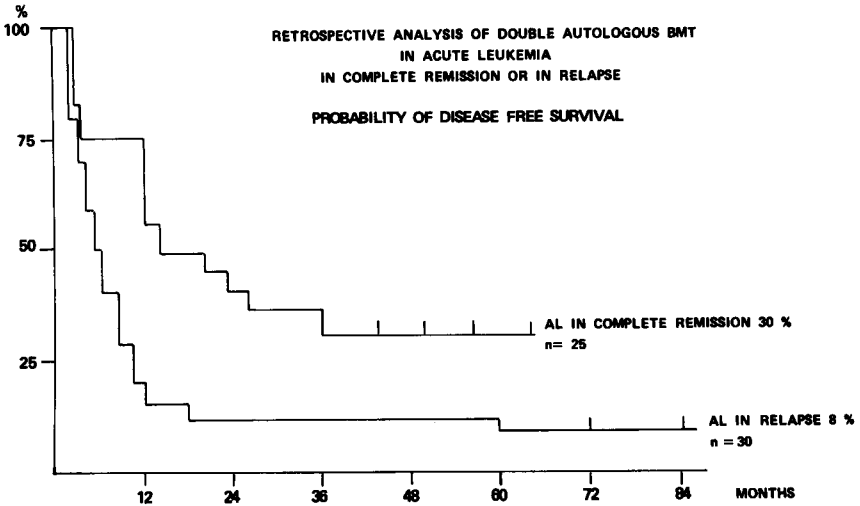
Figure 1. Disease Free Survival in Acute Leukemia (AL) in Complete Remission or in Relapse After Double Autologous Bone Marrow Transplantation

PROSPECTIVE COOPERATIVE STUDY GROUP

Patients and Methods

From January 1981 to December 1986, 124 unselected adult patients with acute myeloid leukemia (<50 y) entered the study from three French centers. From January 1981 to December 1983, 40 patients received induction therapy with cytarabine (ARA) 100 mg/m^2 x 7 with continuous infusion (cp), Daunorubicine (DNR) 60 mg/m^2 x 2, Thioguanine (6TG) 400 mg/m^2 x 2. Consolidation at day 30 consisted of ARA 100 mg/m^2 x 3 subcutaneously (sc), 6TG 50 mg/m^2 x 3, for 3 courses. From January 1984 to December 1986, 84 patients received induction therapy with ARA 100 mg/m^2 x 10 (cp), DNR 60 mg/m^2 x 3. Consolidation at D30 consisted in ARA 100 mg/m^2 x 7 (sc), DNR 60 mg/m^2 x 3. Eighty patients (64%) achieved CR after induction therapy, 10 patients were excluded from analysis, 22 patients had HLA identical siblings and underwent allogeneic BMT. Forty-eight patients were randomized after consolidation (day 60) to receive either double ABMT as previously described for AML or chemotherapy. Sequential post remission chemotherapy consisted for patients from January 1981 to December 1983 of 4 courses till D240 of DNR 40 mg/m^2, ARA 100mg/m^2 x 3, 6TG 100 mg/m^2 x 3 and for patients from January 1984 to December 1986 of 4 various courses till D160: 1) VP16 50 mg/m^2 x 5, Amsacrine 40 mg/m^2 x 5, 2) ARA 100 mg/m^2 x 5, DNR 40 mg/m^2 x 2, 3) ARA 6 gr/m^2 x 2, 4) Purinethol 500 mg/m^2 x 5, Vincristine 2 mg/m^2, MTX 7.5 mg/m^2 x 5, Prednisone 100 mg/m^2 x 5 (POMP).

RESULTS

o Twenty-two patients received allogeneic BMT (median age 26 y, range 6-42, 10 M/12 F) within 3 months after diagnosis. Five patients died in CR. Four of them died at 1, 2, 3 months from acute graft versus host disease, one died 16 months after BMT from untreated chicken pox. Three patients relapsed at 4, 5, 16 months after BMT (the two first patients had received a T cell depleted marrow). Fourteen patients are in CCR within a median follow up of 28 months after diagnosis (range 22-73).

o Twenty-eight patients received sequential chemotherapy: (median age 38 y, range 14-50; 14 F/14 M). Two patients died in CR from sepsis 4 months after diagnosis. Twenty-two patients relapsed in a median of 8 months (range 4-15), after diagnosis. Four patients are in first unmaintained remission with a median follow up of 36 months after diagnosis (range 21-51 months).

o Twenty patients entered the double transplant procedure (median age 33, range 16-47), 10 M/10 F). No toxic death occurred.

Three patients relapsed before 3 months and died. Six patients had delayed recovery which prevented a second bone marrow harvest, 4 of them relapsed before 10 months, 2 of them didn't have full hematologic recovery until 12 months after the 1st ABMT, did not undergo the 2nd BMT and are alive 26-62 months after diagnosis in CCR. Eleven patients received a 2nd BMT within 5 months after the first one (range 4-9). Six patients relapsed at a median of 5 months after the 2nd ABMT (range 3-14) and at a median 12 months after diagnosis. One patient died in CR 23 months after diagnosis from HIV induced by unscreened blood products. Four patients are in CCR with a median follow up of 30 months (28-68 months).

DISCUSSION

At the present time, in our prospective study of unselected patients in 1st CR, the data (Figure 2) show with a median follow up of 32
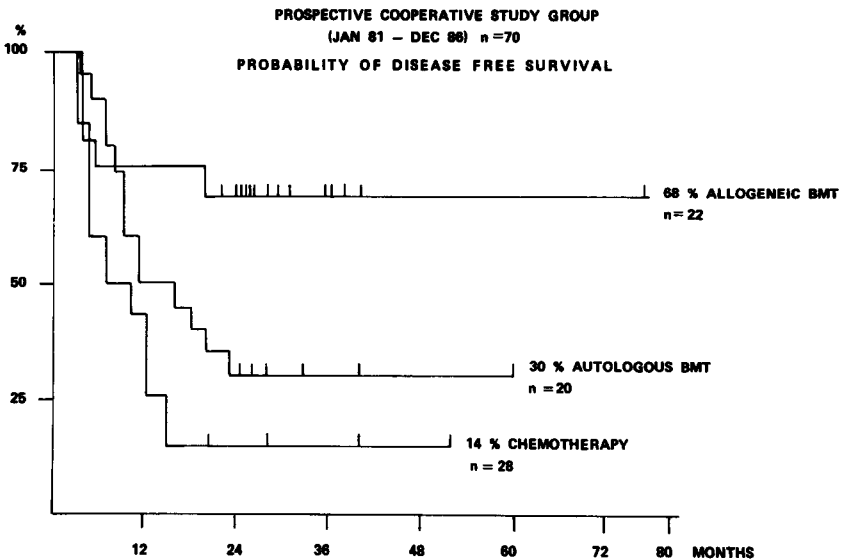
Figure 2. Disease Free Survival in Acute Myeloid Leukemia in First Remission. Result of a Prospective Cooperative Study of 70 Unselected Patients. (BMT = Bone Marrow Transplant)

months (22-73) that there is no significant difference between chemotherapy and double ABMT in terms of DFS (14%-30%) relapse (84%-65%) or survival (21%-39%). There is no difference between allogeneic and autologous BMT in terms of DFS (68%-30%) and survival (68%-39%) but there is a higher probability of relapse for autologous BMT (65%-16%; $0.5 < p < 0.01$). There is a also an advantage of allogeneic BMT compared to chemotherapy in terms of DFS (68%-14%; $p < 0.01$) relapse (16%-84%; $p < 0.01$) and survival (68%-21%; $0.5 < p < 0.01$). These results, in the field of ABMT, could be compared with those reported in other series (12)(13)(14)(15). However, they are not satisfying and should lead to the discussion of some critical points in the aim to improve this strategy in AML. The first ABMT was performed earlier (2 months) in the overall treatment protocol than those reported in other series, the reason for this schedule was the rational of a 2nd ABMT in the goal of realizing an in vivo purging. But only 11/20 patients (55%) underwent the 2nd ABMT among the other 9; 3 relapsed early (<4cm), 6 had delayed hematologic recovery which prevented a new harvesting, and 4 of them relapsed.

Nevertheless, we still believe in the benefit of a <u>double</u> intensification and go on to randomize at D100 after an intensive consolidation between sequential chemotherapy and a unique ABMT with a conditioning regimen of high dose Busulfan and Melphalan (16). Post transplant treatment remains debatable. Chemotherapy has been tried with no clear results (17). Interleukin 2 could be a fascinating strategy to enhance graft versus leukemia in ABMT.

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Discussion 3 - Session 1A (AML)

Dr. Gorin: Bob, let me make a couple of remarks. Five, 6 years ago, at the time you designed the study, I suggested that one consolidation may not be enough for in vivo purging and I suggested to include purging of the marrow. Since you transplant early you are in the worst situation when we take the ABMT data into account.

You showed a delayed hematopoietic recovery after auto BMT compared with allo BMT. It is interesting that the BFU: E population is smaller in autografts than in allografts which might be compatible with the data of Meisner, suggestive for the fact that autotransplants contain considerably less number of CFU megas.

Dr. Lowenberg: We have a consolidation course after remission induction. Adding more consolidation courses may mean a loss of time and of quality of the harvest. The dose of consolidation is low taking into account what we know now. According to Hagenbeek's calculations in the rat model, the leukemic cell population in the graft is not significantly large and may not play an important biological role. We put more emphasis on the value of the conditioning regimen as a cause of relapse.

Dr. Buckner: You had 15 relapses before transplant. That is worrisome. Did you have a logistic problem? In a similar study in Seattle, only 1 patient of the 40 patients relapsed during the time interval between remission induction and transplant. This was a study to determine the efficacy of allogeneic transplantation in first remission taking every patient with an HLA identical sibling donor.

Dr. Lowenberg: I think the relapses are not a logistic problem but rather a consequence of the natural history of the disease. When 50% of the patients relapse within one year, there will be a significant number of relapses during the first 4 months after remission induction.

Dr. Dicke: Are those relapses included in the study?

Dr. Lowenberg: Patients who relapsed before transplant were not included either in the autologous or in the allogeneic arm.

Dr. Buckner: Have you included in the study a treatment arm in which patients were treated with conventional dose chemotherapy alone without transplantation?

Dr. Lowenberg: No, we did not. I agree that this has to be done and this has been included in our new study, the succession of this one. The purpose of this study is to compare transplantation with no transplantation.

Dr. Thomson, San Antonio: Could someone comment, please? What is now perceived to be the standard timing for harvesting marrow in first remission?

Dr. Lowenberg: I think there is no good way yet to define the optimal timing of harvest, as along as we cannot qualify the graft by more refined means. We do not know what the leukemic cell contamination is and there is no good way to assess regenerative capacity of the graft. These two criteria would really determine the optimal timing. What is the best balance between the two? At the moment we have to rely on clinical data and the clinical data do not give a clear answer in my mind. It is our policy as soon as a CR is reached to give at least one additional course of therapy and then harvest as soon as possible. It is a practical approach only.

SESSION I - LEUKEMIA

B. ACUTE LEUKEMIA - ALL

ABMT VERSUS CHEMOTHERAPY IN HIGH RISK ACUTE LYMPHOBLASTIC LEUKAEMIA

H. Grant Prentice, Alan K. Burnett, Bengt Simonsson, Malcolm K. Brenner, David J. Gottlieb, Th. Totterman, Martin Gilmore, Helen Heslop, Joyce E. Reittie, Alessandra Bianchi, Jean–Philippe Grob, and Ian M. Hann

We have undertaken autologous BMT in 21 patients classified as high risk in first complete remission (CR). All were transplanted following remission induction and intensification therapy. In each case the marrow was purged using monoclonal antibodies selected on the basis of the presenting leukaemic phenotype. The actuarial leukaemia free survival is 68% at 5 years. In parallel studies we have demonstrated the presence of activated natural killer (NK) and lymphokine activated killer cells (LAK) after ABMT (as with allogeneic BMT) in contrast to their absence after chemotherapy. In addition we find the spontaneous secretion of cytokines including gamma interferon (IFN) and tumour necrosis factor (INF) after ABMT.

We suggest that the NK/LAK cells and the cytokines they secrete may play a role in the eradication of minimal residual disease (MRD) after ABMT and that the benefits of ABMT may be, at least in part, attributable to these phenomena.

Furthermore, we have shown that these effectors can all be safely enhanced after ABMT by the in vivo administration of recombinant interleukin 2 (IL2). These latter studies have to date been carried out only in acute myeloblastic leukaemia whilst the effects of IL2 on lymphoid malignancies await full evaluation.

PATIENTS, MATERIALS AND METHODS
Patients

Twenty-one consecutive patients meeting our criteria for poor prognosis seen between January 1984 and September 30th, 1987 were treated in a cooperative study at centres in London, Glasgow or Uppsala. Ages ranged from 3 - 55 years (1 < 15 yrs) median age was 26. This analysis was updated to June 1st, 1988. The diagnosis of ALL was based on morphological, cytochemical and immunological examination.

The high risk criteria in CR1 were: age ≥ 16, an observed remission induction time ≥ 28 days (24 days in Glasgow), WBC >30 x 10 ^{9}/1 (20 x 10^9/1 in children in Glasgow), B-ALL, FAB L3-morphology, central nervous system (CNS) leukaemia at presentation, presence of the Ph^1-chromosome or co-expression of myeloid markers.

Pre-Transplant Treatment

Children were treated according to BFM protocol[1], adults in Glasgow and London were mainly treated according to MRC/UKALL protocols[2] and adults in Uppsala according to Swedish national protocol for ALL. Remission induction treatment at diagnosis and at relapse included vincristine and prednisolone with an anthracycline and L-asparaginase. Whenever possible, an attempt was made to study the quality of remission using immunological criteria. In T-ALL, remission was defined as the absence (<0.01%) of surface CD7 (RFT2) and nuclear terminal nucleotidyl transferase (TdT) double positive cells among mononuclear cells in blood or marrow[3]. In Uppsala, for immunological remission of c-ALL a maximum of 0.03% of surface CD10 (anti-CALLA; see below), TdT double positive cells were allowed in peripheral blood[4]. Patients in haematological but not immunological remission are included as 1st CR cases.

Harvesting and Handling of Marrow

Bone marrow was harvested according to standard techniques up to a maximum of 3 months prior to ABMT. A total of 2-5 x 10^8 nucleated cells/kg (400-1990ml in citrate-heparin solution) was collected on 1-3, median 1.3 occasions. The separation of buffy coat and mononuclear cells (MNC) on Lymphooprep® (Nycomed, Oslo) gradients in the COBE (formerly IBM) 2991 cell washer has previously been reported[5].

Residual tumour cells were purged using cytolytic monoclonal antibodies (MAb) plus rabbit serum as source of complement and more recently baby (30 day) rabbit serum (TDRC, Serological Reagents Ltd., East Grinstead, Sussex, U.K). The following MAbs

were used for purging in c-ALL: RFAL3 (anti-CD19, IgM isotype)[6] alone or combined with SB4 (anti-CD19, IgM)[7] kindly supplied by Drs. P. Poncelet and C. Bouloux, Sanofi Recherche, Montpellier. The MAb combination increases purging efficacy[8]. For purging of marrow in T-ALL we used the anti-CD7 MAb RFT2 (of IgG2a class)[6]. The binding of MAbs and efficacy of lysis was pre-tested in microplates on blast cells obtained at presentation in some cases[9].

Transplant Procedure

Patients were treated in single isolation rooms with or without efficiency particulate (HEPA) filtration. During the neutropenic phase they received "sterile" food, oral gut decontamination and prophylaxis against Herpes simplex virus reactivation (if seropositive) with acyclovir.

The preparatory regimen for ABMT in Uppsala was prednisone 100mg/m^2 days -6 and -5, teniposide 200mg/m^2 day -6, vincristine 1.5mg/m^2 day- 6, ara-C 500mg/m^2 days -6 to -2, cyclophosphamide 40mg/kg days -4 and- 3, daunorubicin 30mg/m^2 day -6 and TBI 750 rad (mid-line dose 15cGy/ml) day -1. In Glasgow the chemotherapy part of the preparatory regimen was ara-C 2g/m^2 b.d. x 3 and cyclophosphamide 60mg/kg. All patients received preparatory TBI, i.e. 6 x 200cGy (over 3 days) (mid-line dose rate 26cGy/min) with lung shielding from 1100cGy. The Royal Free Hospital ablative therapy was cyclophosphamide 60mg/kg days -4 and -3 followed by TBI 750cGy (mid-line dose rate 15cGy/min) on day 0.

Studies on NK/LAK Cells and Cytokine Production

Blood derived mononuclear cells (MNCs) and/or large granular lymphocytes (LGLs) were prepared from samples taken before and 2 to 12 weeks after ABMT using Ficoll or discontinuous Percoll gradients. Similar samples were taken from patients after undergoing chemotherapy for remission induction or consolidation therapy for acute leukaemia and from normal volunteer controls. Target cell chromium release was studied at various effector to target ratios against K562, HSB-2 T cell line and a lymphoblastoid (EBV transformed) cell line (as well as against AML cells). Gamma interferon and TNF were assayed as previously described[10]. These assays were also performed in patients on the parallel IL2 study (see below). In sex mismatched transplants, circulating LGLs were probed for Y chromosome via in situ hybridisation to determine host or donor origin. Assays of NK/LAK cells and of cytokine production were also studied with the in vitro addition of IL2.

In a phase 1-2 study of the effects of recombinant interleukin 2 (rIL2) in minimal residual haematologic malignancy, we have administered the cytokine to patients in partial (n = 3) or complete (n

= 6) remission of acute myeloid leukaemia. Six patients were treated after combination chemotherapy, 3 following autologous bone marrow transplantation (ABMT). Fifteen courses of IL2 were given.

RESULTS

Engraftment in ABMT Study

The mean number of mononuclear cells (MNC) and CFU-GM infused were 0.59 x 10^8/kg and 5.6 x 10^4/kg respectively. Following purged ABMT the mean time to recover ≥ 1 x 10^9/1 WBC was 26 days and ≥ 50 x 10^9/1 platelets was 90 days. No failure to engraft was seen in this group of patients.

Post-Transplant Causes of Failure and Disease-Free Survival

The patient who received high dose (3gm/m^2 x 6) acyclovir in conditioning died from pulmonary toxicity attributed to this drug (this protocol was then discontinued). One died from acute Hepatitis B related (pre-existing) liver necrosis at day +120. Six patients have relapsed to date (2.5 - 10 months) after ABMT. Thirteen patients remain in CCR at 8-59 months (median 24 months). The actuarial projected leukaemia-free survival is 68% at almost 5 years.

NK/LAK Cells and Cytokines after ABMT

Following marrow transplantation, MHC unrestricted cytotoxic lymphocytes rapidly recover and have an activated pattern of target cell killing. In addition they secrete cytokines including gamma interferon and TNF. We find that lymphocytes with the capacity to kill MHC non-identical virus infected and leukaemic targets are generated 4-6 weeks after autologous BMT and allogeneic TCD-BMT but do not appear after treatment with chemotherapy alone. PBLs taken from patients studied following autologous BMT had a mean cytotoxicity against an EBV transformed lymphoblastoid cell line of 13.8%, in contrast to a mean cytotoxicity of 3.7% for patients treated with combination chemotherapy (p = 0.001). Alloantigen stimulation through blood product exposure was identical in the two groups.

Studies with IL2

Toxicity was severe in two initial patients treated with escalating dose infusions for <10 days, but when infusions at constant dose (0.5 - 2.0 x 10^6U/m^2/day) were restricted to 3 - 5 days, adverse effects were largely limited to fever and nausea. IL2 had no significant effect on haematological recovery but produced lymphopenia associated with a reduction in the percentage of circulating CD4+ and

CD8+ lymphocytes, but not in the percentage of CD16+ (NK) cells. The proportion of cells expressing the low affinity IL2 receptor (CD25, Tac) increased from 4% to 13% (p = 0.03) during IL2 infusion. Natural killer (NK) activity (measured by Cr^{51} release) increased from a mean of 18.5% pre-treatment to 40.5% (p = 0.001, ET ratio 50:1) during infusion. LAK activity against an NK resistant EBV-transformed lymphoblastoid cell line increased from 6.1% pre-treatment to 14.8% during infusion (p = 0.001). Addition of patient PBM to cryopreserved myeloid leukaemia blast cells prior to IL2 infusion produced <10% inhibition of blast cluster and colony growth. During infusion, leukaemia clusters were inhibited by 47% and colonies by 70% (p = 0.005)[11].

DISCUSSION

Consensus would suggest a long term leukaemia free survival of about 30% for patients at high risk as defined in this study.[12] At almost 5 years the projected CCR rate in our study is 68% suggesting substantial benefit to these patients. This approach is also available to patients lacking a suitable donor for allogeneic BMT and probably safely to a greater age than could be contemplated for allogeneic grafting. The main cause of failure after ABMT has been leukaemia relapse (6 of 21). This result includes one patient who was found to have immunological (but not haematological) evidence of disease prior to BMT.

Transplant related mortality is generally low in ABMT but was seen in two patients in this series; one treatment related, the other due to hepatic necrosis attributable to pre-existing Hepatitis B infection.

A surprising observation in these studies has been the finding that endogenously generated lymphokine activated killer cells circulate in the blood of patients following ABMT[13]. In this respect the disregulated immune recovery shows striking similarities to that seen after T cell depleted allogeneic BMT[14,15]. These cells can be shown to have activity in vitro against both NK and LAK targets. In addition, there is spontaneous secretion of cytokines especially IFN and TNF[10] which can be shown to have synergistic activity against AML (but not ALL)[16]. The in vivo effects of these cells and cytokines cannot, as yet, be determined but it is not unreasaonable to suggest that they may contribute to the eradication of MRD. The activated killer (AK) immune system disregulation following repopulation of the immune system is mediated by both the CD16+ CD3- NK subset and also by CD16- CD3+ T cells[13]. Endogenously generated AK cells may contribute to the lower risk of relapse after both autologous and allogeneic BMT compared to intensive chemotherapy alone.

Finally, we have shown that IL2 can safely be infused to patients after ABMT (for AML) and that this cytokine is capable of enhancing

the activities of NK/LAK cells and increasing levels of endogenously secreted INF and TNF[11].

To evaluate ABMT in the treatment of ALL will require a major international collaborative effort and to determine the role of chemoradiotherapy and the possible effectors we describe here is a truly daunting task.

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ALLOGENEIC VERSUS AUTOLOGOUS MARROW TRANSPLANTATION FOR PATIENTS WITH ACUTE LYMPHOBLASTIC LEUKEMIA IN FIRST OR SECOND MARROW REMISSION

C. Dean Buckner, Jean E. Sanders, Roger Hill, Paul Martin,
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Finn B. Petersen, Rainer Storb, William I. Bensinger,
Ronald Berenson, Keith M. Sullivan, and E. Donnall Thomas

INTRODUCTION

Marrow transplantation is the best treatment for most patients with acute lymphoblastic leukemia (ALL) once a relapse has occurred. Allogeneic marrow transplantation results in long-term disease-free survival in 20-60% of patients with ALL in second marrow remission (1,2,3). Autologous marrow transplantation has been reported to result in survivals at 1 to 2 years of 20-50% (4,5,6). Allogeneic and autologous marrow transplants have also been performed for patients with ALL in first remission with poor risk factors with 35 to 50% of patients becoming long-term disease-free survivors (4,5,7,8). The preliminary results of an ongoing comparison of allogeneic and autologous marrow transplantation for patients with ALL in first or second marrow remission are reported here. Details of the results of allogeneic marrow transplants in patients with ALL in remission from this institution have been published (1,2,7) and details of autografting for similar patients are presented in this publication by Sanders et al. (9).

MATERIALS AND METHODS

All patients with ALL in first or second marrow remission who received unmodified allogeneic marrow transplants from HLA

identical siblings or who received autologous marrow transplants between January 1983 and February 1988 are included in this report. Extramedullary relapses were not considered in the classification of pretransplant remission status. Data were analyzed as of 8/1/88. One patient receiving an autograft while in first marrow remission was prepared with busulfan and cyclophosphamide (Cy). All other patients were treated with regimens containing Cy and total body irradiation (TBI). Marrow for autografting was incubated with monoclonal antibodies and complement when immunophenotypes were known and appropriate (10). Ten of 14 patients receiving autografts in first remission had marrow purged *in vitro* (4 anti-CALLA, 2 anti-T, 2 anti-B1, 1 anti-CALLA + anti-T and 1 anti-CALLA + anti-B1). One patient transplanted in second remission had marrow stored while in first remission while all other patients transplanted in second remission had marrow harvested in second remission. Thirteen of 23 patients in second marrow remission had autologous marrow incubated *in vitro* (10 anti-CALLA antibody, 1 anti-CALLA plus anti-T, 1 anti-B1 and 1 4HC). All autologous marrows were cryopreserved. Marrow was infused within 24 hours of the last day of TBI and the day of infusion was designated day 0. All protocols and consent forms were approved by the Institutional Review Board of the Fred Hutchinson Cancer Research Center, and all patients gave informed consent.

RESULTS

Table 1 presents patient characteristics and results for patients transplanted in first marrow remission. Eight of the 14 autografted and 13 of 27 allografted patients are alive and in remission 165 to 2006 days after transplantation. The 2-year probabilities of relapse for autografted and allografted patients are 36% and 41% respectively.

Table 2 presents the patient characteristics and results for patients transplanted in second marrow remission. Four of 23 autografted and 15 of 43 allografted patients are alive and free of disease 137-1786 days after transplant. Twenty-five of 43 patients who received allograft in second remission did not develop significant acute or chronic GVHD and the probabilities of survival, relapse and event-free survival at 2 years for these are 49%, 60% and 33% respectively.

The probability of relapse for autografted patients (75%) as compared to allografted patients without graft-versus-host disease (GVHD) (60%) was of borderline statistical significance, p = .09 (Wilcoxon) and .057 (Mantel Cox). Eighteen of 43 patients developed significant acute and/or chronic GVHD and the probabilities of survival, relapse and event-free survival at 2 years are 48%, 41% and 36% respectively.

Table 1. Patient Characteristics:
ALL First Marrow Remission

	Autologous	Allogeneic
No. of Patients	14	27
Age (Range)	19 (7-40)	19 (1-50)
Diagnosis to Transplant (Months)	16 (3-122)	10 (4-100)
Marrow Purged	10 (6 Alive DF)[1]	--
Marrow Not Purged	4 (2 Alive DF)	--
Extra Medullary Relapse	8 (4 Alive DF)	10 (6 Alive DF)
No Extramedullary Relapse	6 (4 Alive DF)	17 (7 Alive DF)
Transplant Deaths[2]	2 (14%)	6 (22%)
Relapses	4 (29%)	8 (30%)
Alive	9 (64%)	16 (59%)
Alive Without Relapse	8 (57%)	13 (48%)
Survival[3]	55%	57%
Relapse[3]	36%	41%
Event-free Survival[3]	54%	46%

[1] = DF = Disease-Free
[2] = Deaths due to Non-Leukemic causes
[3] = 2-year probability estimates

Table 2. ALL Second Marrow Remission

	Autologous	Allogeneic
No. of Patients	23	43
Age (Range)	15 (4-37)	15 (4-40)
Duration of 1st Remission	25 (3-64)	24 (3-88)
Extramedullary Relapse	8 (2 Alive DF)[1]	17 (7 Alive DF)
No Extramedullary Relapse	15 (2 Alive DF)	26 (7 Alive DF)
Marrow Purged	13 (1 Alive DF)	--
Marrow Not Purged	10 (3 Alive DF)	--
Relapse Off Therapy[2]	7 (2 Alive DF)	15 (7 Alive DF)
Relapse On Therapy[3]	16 (2 Alive DF)	28 (8 Alive DF)
Transplant Deaths[4]	3 (13%)	9 (23%)
Relapses	16 (70%)	18 (42%)
Alive 8 (35%)	8 (35%)	21 (49%)
Alive Without Relapse	4 (17%)	15 (35%)
Survival[5]	27%	46%
Relapse[5]	75%	55%
Event free Survival[5]	22%	34%

[1] = DF = Disease-Free
[2] = Relapsed more than 3 months after therapy was discontinued
[3] = Relapsed within 3 months of discontinuing therapy
[4] = Deaths due to Non-Leukemic causes
[5] = Probability estimates at 2 years

Three of the 4 patients who have not relapsed received unpurged marrow and 1 received an anti-CALLA treated marrow. An additional recipient of unpurged marrow as in remission 3.5 years before relapse.

DISCUSSION

Kersey et al. compared auto- to allograft in 91 patients with ALL in first through 4th remission (4). The number of relapses including extramedullary relapses was used to classify patients by remission number. In the Minnesota study 20% of autografted and 27% of allografted patients became long-term disease free survivors (4). Post transplant relapses were the most frequent cause of failure in both groups. Patients who did not develop GVHD and patients receiving autografts had a 79% probability of relapse while patients who developed GVHD had a 37% probability of relapse. Those results are similar to the results reported here.

In the present study the results of transplantation in first remission are identical for recipients of auto and allograft. Six patients in the current study were transplanted with autologous marrow prior to any relapse and 4 are surviving 165 to 2006 days after transplant. Seven of 26 patients receiving allograft in first marrow remission without a prior relapse are alive and free of disease. It is unknown whether transplantation in first remission is superior to intensive chemotherapy followed by transplantation after first relapse. In the absence of a multicenter cooperative trial this issue will remain unresolved.

The probability of event-free survival at one year for patients transplanted in second remission was 43% for allograft recipients and 22% for autograft recipients and at 2 years the probabilities were 34% and 22% respectively. More patients and a longer follow-up will be needed for final comparison.

The major cause of failure in transplants for ALL is relapse due to residual disease. Autografted patients transplanted in second marrow remission had a higher relapse rate than patients receiving allograft who did not develop acute or chronic GVHD (75% vs 60%). This increase in relapses could be due to marrow contamination with leukemic cells or to an allogeneic effect present in allograft recipients even in the absence of GVHD. Long-term disease-free survival was achieved without purging which will make clinical evaluation of the antileukemic effectiveness of purging difficult. The fact that the majority of patients relapse even when given normal marrow also makes the evaluation of marrow purging difficult. An evaluation of marrow purging cannot practically be undertaken, because of the large numbers required, until the probability of relapse using normal marrow is reduced to below 25% (11).

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THE ROLE OF AUTOLOGOUS BONE MARROW TRANSPLANTATION IN THE TREATMENT OF CHILDHOOD LYMPHOBLASTIC LEUKEMIA:
Experience of the Italian Pediatric Study Group

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INTRODUCTION

Bone marrow transplantation has been increasingly used as a potential curative therapy in children with acute lymphoblastic leukemia (ALL) of poor prognosis or with ALL in second or subsequent remission. Recent reports showed the possibility of disease free survival for more than 60% of patients with ALL in second remission after allogeneic bone marrow transplantation (1). For patients without a matched donor, alternative methods of transplantation are currently being explored. One approach involves the use of mismatched family donors or matched unrelated donors. Autologous bone marrow transplantation (ABMT) is also being investigated for patients who lack a matched donor (2). This approach has been widely used by the European Bone Marrow Transplantation Group in 103 children with ALL. A disease free survival of about 42% for patients in second remission was obtained (3). Since leukemic cells almost certainly contaminate remission bone marrow, methods to eradicate leukemic cells in vitro have been developed. The strategies for in vitro removal of contaminating leukemic cells include the use of pharmacologic agents or monoclonal antibodies.

PATIENTS AND METHODS

The current report concerns 23 patients with ALL who received ABMT between January 1986 and May 1988 in 4 centers of the cooperative study group. The patients age ranged from 4 to 16 years (median 8). Nine were female, 14 male. Four patients with poor risk ALL were in the first remission, 11 patients were in second remission, 8 patients were in third or subsequent remission. Site of relapse prior to transplantation was bone marrow in 9 patients, nervous system disease in 4, and isolated testicular relapse in 1. Either consecutively or simultaneously, 4 children had recurrent bone marrow and central nervous system disease, and 1 had bone marrow involvement and testicular disease. Table 1 shows other clinical characteristics of the study. Cellularity and remission defined as less than 5% blasts were determined in all patients prior to the harvest. Bone marrow was harvested under general anesthesia to obtain 1 to $5x10^8$ nucleated cells per kilogram body weight. Five bone marrows were purged using incubation with Asta-z 100 mcg/ml for 30 minutes (4) and 16 using incubation of Vincristine 1 mcg/ml and methylprednisolone 3 mg/ml for 30 minutes (5).

Another two bone marrows were purged with monoclonal antibodies. Eighteen patients received pretransplant conditioning consisting of cyclophosphamide (1800 mg/mq for 2 days) and total body irradiation (1200 cGy given twice daily at a dose of 200 cGy). Fifteen out of these 18 patients were given Vincristine by continuous infusion (4 mg/mq in 5 days)(6) also, and another two patients had Aracytin (2 g/mq twice daily for 3 days). Three patients had pretransplant conditioning Busulfan (4 mg/Kg daily for 4 days) and Cyclophosphamide (50 mg/Kg daily for 4 days). High dose Aracytin (3 g/mq every 12 hours for 8 times consecutively) and total body irradiation (1440 cGy given in 3 daily fraction of 120 cGy) were given to 2 patients with central nervous system isolated disease.

RESULTS

Twenty-two patients engrafted as defined by recovery of the white blood counts to > $1x10^9/1$ and platelet counts to >$50x10^9/1$. One patient failed to achieve bone marrow recovery and died for sepsis 51 days after transplantation. Ten of the 23 patients relapsed from 1 to 9 months post transplant (median: 5 months). One patient who developed a pulmonary embolism died in complete remission 3 months after ABMT. The cumulative disease free survival (7) is 53% with a plateau at the ninth month (Figure 1). Eleven patients are still alive without leukemic relapse 1 to 28 months after ABMT (median 15 months).

Table 1. Characteristics of 23 Patients

	1CR	2CR	>2CR	TOT
N. of patients	4	11	8	23
Sex (M/F)	2/2	7/4	5/3	14/9
Age at transplantation (median yr)	6.5	6.0	10.5	8.0
Duration of first remission (median with range mo)	–	22 (5–36)	36.5 (16–84)	24.0 (5–84)
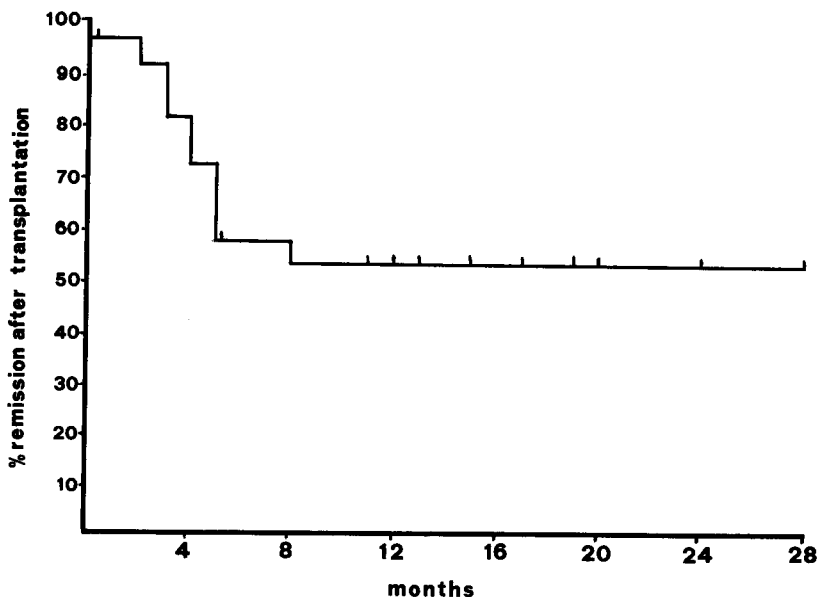
Figure 1. Life table plot of disease free survival for 23 children with ALL after ABMT.

DISCUSSION

Treatment of ALL in children with a poor prognosis or after relapse, by ABMT, is now considered to be better than traditional chemotherapy (8). This report shows that the relapse after ABMT occurred within nine months. The fact that there were only two leukemia unrelated deaths meant that the toxicity of the conditioning regimens was acceptable. The chemical purging did not interfere with the hematological recovery except in one case. The satisfactory results

obtained in terms of disease free survival and the low toxicity must lead to studies to verify the efficacy of different methods of conditioning and purging in further studies.

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AUTOLOGOUS MARROW TRANSPLANT EXPERIENCE FOR ACUTE LYMPHOBLASTIC LEUKEMIA

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INTRODUCTION

Most patients with acute lymphoblastic leukemia (ALL) who relapse while receiving first line treatment regimens are unlikely to be cured. High dose chemoradiotherapy and allogeneic marrow transplantation has been used to successfully treat these patients.[1-5] Since only 25–30% of patients have suitable donors many centers are evaluating the use of autologous marrow transplants (AMT).[6-10] This report details the Seattle experience with AMT for patients with ALL.

METHODS

From January 1983 to February 1988, 46 consecutive patients with ALL who had relapsed at least once at any site and 6 who had not relapsed but were at high risk for relapse on conventional therapy were treated with high dose chemotherapy or chemoradiotherapy and an infusion of cryopreserved autologous remission marrow. Table 1 shows pre-transplant characteristics. Extramedullary relapses were not considered in classification of pretransplant remission status.

All patients had remission status determined by bilateral iliac crest aspirates within 2 weeks of storage. The 28 patients for whom the lineage of leukemic cells had been determined by immunophenotyping had autologous marrow treated *in vitro* with either anti B1 (3), anti CALLA (18), a panel of anti T (4), anti CALLA plus anti T (2) or anti CALLA plus anti B1 (1) monoclonal antibodies plus complement.[11]

**Table 1. Autologous Marrow Transplantation for Acute
Lymphoblastic Leukemia: Patient Characteristics**

	First Marrow Remission	Second Marrow Remission	Third Marrow Remission	Marrow Relapse
Number Patients	14	23	8	7
Years of Age:				
median	18.5	12	8	17
(range)	(7-40)	(4-37)	(2-16)	(6-29)
WBC at Diagnosis ($x10^3$) median (range)	8.8 (0.3-68)	25 (2.6-244)	22 (0.8-612)	125 (6.1-870)
Extramedullary Relapses:	8	8	2	5
Sites: CNS	7	8	2	3
Testes	4	2	0	0
Other	1	0	0	2
Type of ALL:				
T-cell	3	1	1	1
B-cell	3	1	0	0
CALLA	4	11	3	1
Null	4	10	4	5
Diagnosis to BMT or 1st BM Relapse (months) median (range)	17 (4-120)	25 (3-64)	29 (3-50)	19 (7-115)

Of 23 second remission patients, one had marrow stored in first
remission and 22 in second remission. All 8 patients transplanted in
third remission had marrow stored in third remission and 5 of 7
transplanted in relapse had marrow stored in first remission.
Techniques of cryopreservation have been described.[12] Transplant
preparation was cyclophosphamide (CY) and total body irradiation
(TBI) given as 10.0 Gy in a single exposure (3) or 12.0-16.0 Gy
fractionated over 4-7 days from dual [60]Cobalt sources at 5-8
cGy/min. (33) or 14.4 Gy given in 1.2 Gy/doses three times/day for
4 days from a GMV linear accelerator at 12 cGy/min. with lung
shielding and electron beam boosts to blocked areas plus 4.0 Gy
testicular boost (16). Three patients received busulfan, 16 mg/kg and
CY, 120 mg/kg. Cryopreserved marrow was thawed rapidly and
infused immediately following the last dose of TBI or 36 hours after
the last dose of CY. Protocols and consent forms were approved by
the Institutional Review Board of the Fred Hutchinson Cancer
Research Center and all patients gave informed consent. Data were
analyzed as of August 1, 1988.

RESULTS

Results are in Table 2. Of the 52 patients, 12 (23%) survive disease-free and all 12 were transplanted in first or second marrow remission. The small number of patients does not permit an accurate analysis of factors predictive of survival beyond remission status. Eight of 14 first remission patients survive disease-free 174-2,050 days. These 8 include 4 with prior extramedullary disease, 2 given non-purged marrow, and 2 given TBI three times/day. Four of 23 second remission patients survive disease-free 529-1,210 days. These include 2 with prior extramedullary disease, 3 given non-purged marrow and 3 given TBI three times/day. None of the 15 transplanted in third marrow remission or in relapse survives.

Table 2. Results of Autologous Marrow Transplantation for Acute Lymphoblastic Leukemia

	First Marrow Remission	Second Marrow Remission	Third Marrow Remission	Marrow Relapse
Number Patients	14	23	8	7
Preparative Regimen*:				
CY+10.0 Gy	1	1	0	1
CY+2.0 Gy/dx6	1	2	2	2
CY+2.25 Gy/dx7	6	8	4	3
CY+2.0 Gy BID x 4	0	0	2	0
1.2 Gy TID x 4 + CY	5	11	0	0
BU + CY	1	1	0	1
Purged Marrow:	10	13	4	2
Relapse	2	11	3	2
Days	132,298	63-279	112-120	114,449
Disease-Free Survival	6	1	0	0
Days	174-2015	882		
Non-Purged Marrow	4	10	4	5
Relapse	2	5	4	4
Days	127-197	44-1311	82-408	48-165
Disease-Free Survival	2	3	0	0
Days	174-997	529-1210		

CY = Cyclophosphamide, 60 mg/kg/day for 2 days, 10 Gy = 10.0 Gy TBI in a single exposure, 2.0 Gy/d x 6 = 2.0 Gy TBI/day for 6 days, 225 Gy/d x 7 = 2.25 Gy TBI/day for 7 consecutive days, 2.0 Gy BID x 4 = 2.0 Gy TBI twice a day for 4 consecutive days, 1.2 Gy TID x 4 = 1.2 Gy TBI from 6 MV linear accelerator at 12 cGy/minute three times a day for 4 consecutive days, BU = busulfan, 4 mg/kg/day for 4 days.

*((TBI) administered at 5-8 cGy/minute from ^{60}Co sources)

The most common cause of death was recurrent leukemia. Relapse occurred from 48-480 days (median 108) for 13 of 15 patients in third remission or relapse and from 44-1,311 days (median 154) for 20 of 37 in first or second remission. The actuarial probability of relapse for all 52 patients was 70% at 1 year and for the 37 first and second remission patients was 59% at one year. Previous extramedullary disease, transplant preparative regimen or use of purged or non-purged marrow did not influence time to relapse. The second most common cause of death was hemorrhage in 3 patients in second remission and 1 in third remission who were refractory to platelet transfusions. These patients failed to achieve >20,000/mm^3 platelets at time of death on days 21, 62 and 96. One transplanted in relapse died of liver failure due to venocclusive disease and 2 transplanted in first remission died from cytomegalovirus pneumonia.

The probability of disease-free survival for all 52 patients is 25% at one year. The probabilities of disease-free survival for the 14 transplanted in first or the 23 in second remission are 54% and 22% respectively.

DISCUSSION

These results confirm previous reports that some patients with ALL who have failed conventional chemotherapy or who are at high risk to fail can achieve prolonged disease-free survival following the use of high dose chemoradiotherapy and AMT.[6-9] All patients were considered "high risk" due to age, high white blood count or previous medullary or extramedullary relapse. The chance for cure of these patients with further conventional chemotherapy was poor. The role of marrow transplantation for patients beyond first marrow remission has been established. In the present study 4 of 6 patients transplanted before any relapse survive. The role of transplantation versus continued intensive chemotherapy for patients in first complete remission remains to be resolved. A prospective study comparing high dose chemoradiotherapy and marrow transplantation to the best intensive chemotherapy followed by transplantation in those who relapse is needed to resolve this issue.

The major reason for treatment failure was disease recurrence with an actuarial relapse rate of 70%. This relapse rate emphasizes the need to develop better preparative regimens and possibly improved methods of marrow purging. The most promising preparative regimen for allogeneic transplant recipients has been evaluated by Brochstein, et al. where TBI is given in three daily fractions for 4 days followed by cyclophosphamide.[5] Whether this regimen will result in fewer relapses after AMT for ALL will require more patients and a longer follow-up. The other factor which may contribute to the high relapse rate is the infusion of marrow contaminated by leukemic cells. The

number of patients in this study is too small and the patients too heterogenous to permit comparisons of purged and non-purged marrow. For meaningful evaluation of marrow purging techniques, the relapse rate following infusion of unpurged marrow needs to be less than 25% or an extremely large number of patients would need to be entered into a randomized study. The observation that the majority of patients whose marrow was stored and transplanted while in first marrow remission have not relapsed implies that these marrows may have been the least contaminated by residual leukemic cells. The high relapse rate for patients in second and third remission may be related to relative leukemic cell contamination of marrow and/or an ineffective preparative regimen. The high relapse rate for patients whose marrows were stored in first remission but were transplanted in relapse suggests that the preparative regimen was ineffective in eradicating leukemia in the patient.

In the present study, patients who were transplanted in first or second marrow remission had a better outcome than those in third marrow remission or in relapse. This is in contrast to results from the University of Minnesota where the number of remissions did not predict outcome.[6] The two studies, however, are not directly comparable due to the different methods used for classification of remission number. In the present study previous extramedullary disease did not influence relapse or disease-free survival.

For the patient with ALL who has had a medullary or extramedullary relapse or who is felt for other reasons to be at high risk for relapse, AMT offers a possibility of prolonged disease-free survival.

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AUTOLOGOUS BONE MARROW TRANSPLANTATION USING MONOCLONAL ANTIBODY PURGED MARROW IN ACUTE LYMPHOBLASTIC LEUKEMIA

Bengt Simonsson, Gudmar Lönnerholm, Bengt Smedmyr, Thomas Tötterman, and Gunnar Öberg

INTRODUCTION

In ALL patients, with poor prognosis on chemotherapy, BMT has been employed with encouraging results (1,2), and for those who lack a matched sibling ABMT is currently explored as an alternative treatment (3,4). In this paper we report a single center study with 27 high risk ALL patients given myeloablative therapy and autografted with bone marrow harvested in remission and purged with MAbs selected on the basis of the leukemic phenotype.

PATIENTS AND METHODS

Twenty-seven consecutive patients with ALL (1 relapse, 9 high risk CR1, 14 CR2, and 3 CR3), were autografted between May 1985 and April 1988. The study was updated August 1, 1988. The male/female ratio was 15/12 and the median age 13 (3-55) years. High risk criteria in CR 1 were: remission induction time > 28 days, peripheral blast count > 30 x 10^9/l, FAB L3-morphology, CNS-leukemia and presence of Ph'-chromosome. Children were treated according to DFM protocol (5) and adults according to Swedish National Protocol for ALL. Eight of 17 CR2-3 patients had relapsed extramedullary (3 CNS, 4 testis, 1 lymphnodes). Remission was defined both morphologically and immunologically (6). Bone marrow, 2-5 x 10^8 nucleated cells/kg, was harvested in remission (except in the relapse case). Residual tumor cells were purged twice, after pre-testing in microplates, using baby rabbit serum complement and the MAbs RFT2 for T-ALL and RFAL3 alone or combined with SB4 for c-ALL. RFAL3 and RFT2 were provided by Prof. G. Janossy, Royal Free Hospital, London, and SB4 by Drs. P. Poncelet and C. Bouloux,

Sanofi Recherche, Montpelier. The purged marrow was stored at -196° C (7).

When in remission patients received at least two consolidation treatments with Vincristine 1,5 mg/m^2 x 1, Daunorubicine 30 mg/m^2 x 1, Prednisone 60 mg/m^2 x 5, Etoposide 100 mg/m^2 x 5 and Ara-C 100 mg/m^2 b.d. x 5. The myeloablative regimen was Prednisone 100 mg/m^2 days -6 and -5, Teniposide 200 mg/m^2 day -6, Vincristine 1,5 mg/m^2 day -6, Ara-C 500 mg/m^2 days -6 to -2, Daunorubicine 30 mg/m^2 day -6, Cyclophosphamide 40 mg/kg days -4 and -3, and TBI 750 cGy (midline dose rate 15 cGy/min) day -1. The numbers of reinfused MNC and CFU-GM were 0.27 (0.13 - 0.89) x 10^8/kg and 4.2 (1.1-10) x 10^4/kg respectively.

RESULTS

Treatment of leukemic cells with MAbs and complement resulted, except in 1 case, in > 4 log target cell killing. After purging in full scale no TdT-positive cells were detected.

All patients engrafted. Median time to neutrophil count > 0,5 x 10^9/l was 26 days and to platelets > 50 x 10^9/l 37 days. Fifteen percent of the patients had not reached this platelet count level after 90 days. No patient died of transplant related complications. All patients had fever during aplasia, 3 together with bacterial septicaemia and 4 with pneumonia. One patient had VOD which completely reversed, and 2 patients interstitial nephritis, which in one case was fully reversible. One patient, in remission, died in pneumonia 8 months after ABMT.

The patient transplanted in relapse engrafted, but died in a new relapse after 2 months. Figure 1 shows a 53% leukemia free survival from CR for 9 patients autografted in CR 1. Median time from CR to ABMT was 7 (3-12) months. Four patients have relapsed 6,5-17,5 months and 1 patient, in CR, died in pneumonia 20 months after achieving CR. One relapsed patient went into a new CR after BMT.

Figure 2 shows two different curves of the 17 patients autografted in CR 2-3; the proportion of relapses in previous CR and the probability of remaining in the present CR (in which ABMT was performed). The time from diagnosis to relapse range 5 - 70 months and the median duration of present CR is 34 months. The effect of ABMT can also be illustrated by comparing previous CR time with time in new CR including time post ABMT. If the latter is longer than the former the patient is said to have "inverted," suggesting a beneficial effect of ABMT. Five patients have "inverted" with another 8 patients yet to achieve the "inversion point." Table 1 shows that both the great majority of relapses (5/7), and "inversions" (5/5) have occurred in patients with "fast disease" (i.e. relapsed on chemotherapy).
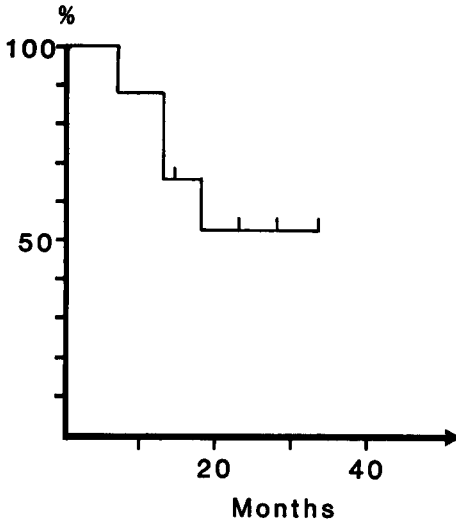
Leukaemia free survival from CR1
in 9 autografted HR–ALL–patients

Figure 1.

Table 1. Outcome of ABMT in ALL CR 2-3

Course prior to ABMT	N	Status post ABMT
Relapse <u>on</u> therapy	9	CCR 4 Relapse 5
Relapse <u>off</u> therapy	8	CCR 6 Relapse 2

Inversion rate = 5/17.

Figure 2.

DISCUSSION

   The hematological reconstitution data and low procedure mortality
(0%) in our study suggest that purging the RFAL3, RFT2 and SB4 is
harmless to normal bone marrow stem cells confirming previous
results with MAbs (3,8). The final outcome after ABMT in our
patients is not yet known due to relatively short follow-up time.
Historical controls in Sweden for high risk CR1 patients and in
Germany for patients beyond CR1 have a < 5% cure prospect (9,10).
A prospective Swedish study (11) reported a disease-free survival of
4 and 31% for children CR2, relapsed on or off therapy respectively,
which is also inferior to our results in similar patients. Five of our 17
CR 2-3 patients have so far "inverted," suggesting that ABMT may
offer an advantage to these patients.

NATIONAL SWEDISH ALL-STUDY
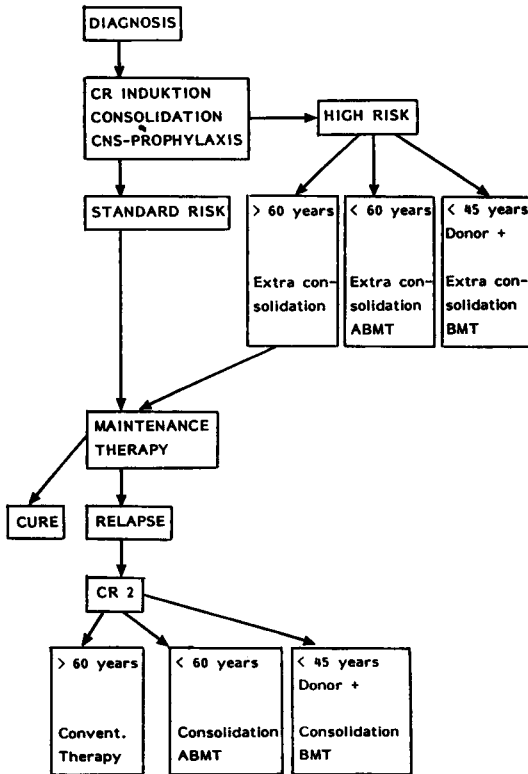
Figure 3.

This and other studies indicate that it is important to identify the therapeutic impact of ABMT in high risk ALL. This will only be possible in the context of large studies such as those currently underway with several European and American collaborative groups. The Swedish National ALL study started in June 1986. The aim of this study is to collect all adult ALL-patients in Sweden and treat them according to the same protocol (Figure 3). On August 1, 1988, 65 patients have entered the study and a preliminary report will soon be published.

CONCLUSIONS

- No adverse effect of in vitro purging on bone marrow reconstitution.

- Low procedure mortality (0%).

- Few other severe complications.

- "Inversions" in CR2-3, showing an effect on ABMT.

- Need for comparative studies.

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AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR CHILDHOOD AND ADULT ACUTE LYMPHOBLASTIC LEUKEMIA IN FIRST OR SECOND COMPLETE REMISSION: 1988 Update

J. Y. Cahn, E. Plouvier, M. Flesch, and P. Herve

We report here our updated results of a pilot study initiated in 1980 (1) to assess the value of ABMT for ALL in CR in patients whose outlook with further chemotherapy was poor. Only patient lacking a sibling with identical human leucocytic antigens was included in the study if remission was obtained.

Patients

From 1980 to 1987, thirty-six patients (25 males, 11 females) underwent ABMT for ALL in CR. Among 13 adults (mean age: 24 years, range: 15-40), nine were treated in CR1 and four in CR2. Among 23 children (mean age: 8 years, range: 3-14), two were treated in CR1 and 21 in CR2. Out of the 21 children grafted in CR2, nine had relapsed while on therapy and 12 while off therapy.

According to the FAB classification their subtypes were as follows: 21 L1, 14 L2 and one L3. Immunological phenotype was available in 29 patients: CALLA positive phenotype in 19, T-phenotype in nine and B-phenotype in one. The type of relapse before BMT for children grafted in CR2 were marrow in 16 cases, CNS in one, testis in four.

Marrow Procedures

Details on harvesting and in-vitro treatments have been described previously (1). Among children treated in CR2, the marrow had been collected in CR1 for eight and in CR2 for 13 patients. The marrow cells were purged in 19 patients by chemical methods: mafosfamide for 18 (40-50 ug/1x10^7 mononucleated cells/ml) and deoxycoformycine plus deoxyadenosine for one. In 15 patients the

marrow was purged by monoclonal antibodies followed by rabbit complement mediated cytolysis according to their blasts' phenotype (when available): CD10 ± CD19 (10 patients), CD2 + CD5 + CD7 (5 patients); in both cocktails, two rounds of complement were used.

Preparative Regimens

Thirty patients received fractionated TBI (12-13.2 gy, 6-8 fractions) followed by cyclophosphamide (2 x 60 mg/kg). Three children received polychemotherapy alone and, after 1985, three patients with high-risk factors received the TAM protocol (2) consisting of fractionated TBI followed by high-dose cytarabine and melphalan. The median interval between CR and ABMT was 4.7 ± 1.7 months for adults grafted in CR1 and 5.8 ± 4.2 months for children grafted in CR2.

RESULTS

Two toxic deaths occurred within three months post BMT (two children): one from cerebral hemorrhage and one from septicemia. Engraftment occurred in the 35 evaluable patients but platelet count higher than $100 \times 10^9/1$ was not reached in three cases. The mean recovery time of neutrophils over $0.5 \times 10^9/1$ was 24.8 days after chemical purging (range: 13-35) and 29.4 days after immunological purging (range: 20-58). The mean recovery time of platelets over 50 $\times 10^9/1$ was 31 days after chemical purging (range: 18-68) and 50.2 days after immunological purging (range: 19-190). Among nine adults treated in CR1, leukemic relapses were observed in four (44%) with a median time until relapse of 11.7 months (range: 3-31 mo) after ABMT. Five remain disease free in unmaintained remission from 12+ to 53+ months (mean: 28.2+ mo) post ABMT. All adults treated in CR2 relapsed. Among the 19 evaluable children treated in CR2, eight (42%) relapsed with a mean time until relapse of 8.3 months post-transplant (range: 5-19 mo). Eleven remain in unmaintained disease free with a mean follow-up of 45+ mo (range: 27+ - 72+ mo). The actuarial disease free survival curve of this group of patients is shown on Figure 1.

DISCUSSION

–   No firm conclusion can be drawn from our experience in first remission ALL, a French multicentre randomized study has started in October 1986 (LALA 87) to compare early ABMT with maintenance chemotherapy in adult ALL but follow-up is still to short.

ABMT for childhood ALL in CR2 (19 pts)
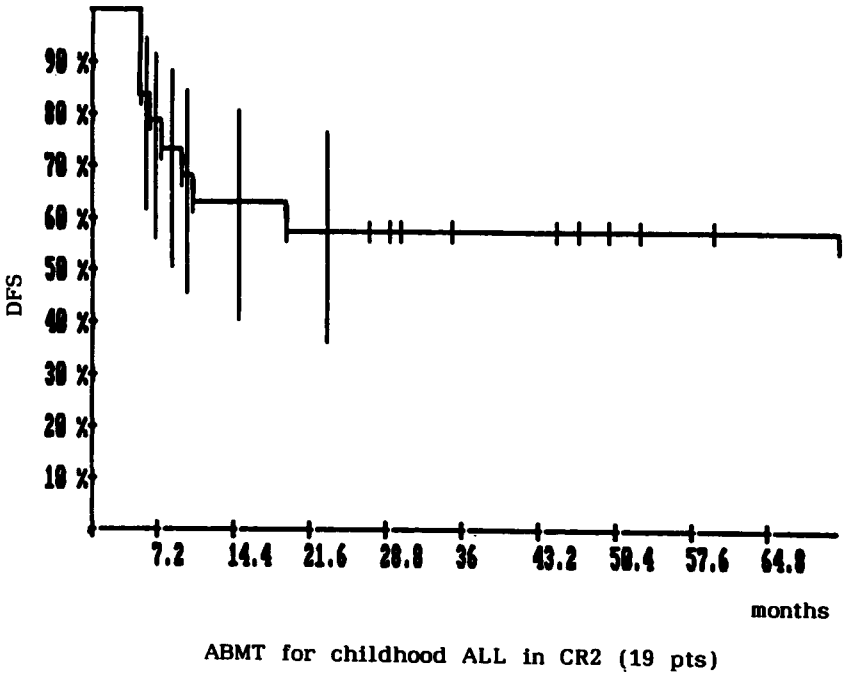
Figure 1.

- In childhood ALL in CR2 the results obtained in our group are better than those obtained with chemotherapy (3): we observed a 40% relapse rate when the first relapse occurred late "on" or "off" therapy. However, these results must be confirmed by controlled trials with homogeneous therapy after first relapse to avoid the problem of selection and the heterogeneity of the patients. On the other hand, results obtained with standard preparative regimen are very poor in patients with early relapse (<1 year). For this high-risk group, new conditioning regimens are under study (2) as well as new purging methods using combinations of monoclonal antibodies and chemotherapy. Moreover mismatched BMT or phenotypically identical BMT using unrelated donors should be proposed as an alternative to ABMT.

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B43/CD19-PAP IS AN EFFECTIVE IMMUNOTOXIN AND SHOWS CONSIDERABLE CLINICAL POTENTIAL FOR BOTH EX VIVO AND IN VIVO IMMUNOTHERAPY OF B-LINEAGE ALL

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INTRODUCTION

Currently, the major challenge in bone marrow transplantation (BMT) for ALL is the development of more effective pretransplant conditioning strategies (1). In addition, effective ex vivo elimination of residual leukemic blasts form remission marrow grafts may be important for a successful outcome in autologous BMT.

Immunotoxins (IT) provide an opportunity for highly efficient "search and destroy tactics" against malignant cells bearing the relevant target surface antigens (2-6). Over the past 4 years, the therapeutic potential of IT containing pokeweed antiviral protein (PAP) in the treatment of human leukemias has been rigorously researched in our laboratory (7-12). We are planning to conduct our initial clinical trial of PAP IT in B-lineage ALL patients using B43-PAP directed against the B-lineage specific CD19 antigen. Herein, we will discuss our rationale in using B43-PAP, describe the preparation of clinical batches of B43-PAP and present experimental data on the antileukemic efficacy of B43-PAP alone and in combination with 4-hydroperoxycyclophosphamide (4-HC) versus an alternative ex vivo marrow purging protocol which employs BA-1/CD24, BA-2/CD9, and BA-3/DC10 in combination with exogenous complement (C') and 4-HC.

RESULTS AND DISCUSSION

Expression of CD19 Antigen in B-Lineage Leukemias Versus Normal Tissues and the Potential of CD19-PAP IT

CD19 (Bp95) is the most reliable B-lineage specific surface marker (13). At the University of Minnesota, we used B43/CD19 MoAb to

Table 1. Expression of CD19 in Leukemias

Diagnosis	Cases reactive with B43/CD19 MoAb	
B-lineage ALL	99.5%	(383/385)
B-lineage CLL	100.0%	(28/28)
B-lineage PLL	100.0%	(6/6)
B-lineage HCL	100.0%	(12/12)
T-lineage ALL	1.0%	(1/97)
T-lineage CLL	0.0%	(0/2)
AML	0.0%	(0/40)
CML	0.0%	(0/10)

The expression of CD19 on leukemic cells was analyzed by indirect immunofluorescence and flow cytometry, as described previously (13).

test for expression of CD19 in 581 leukemia cases (Table 1). Notably, 383 of 385 (99.5%) B-lineage ALL cases were CD19+. By comparison, 346 cases (89.9%) were BA-3/CD10+ and 355 cases (92.2%) were BA-1/CD24+. B43/CD19 reacted with 28 of 28 B-lineage CLLs, 12 of 12 HCLs, 6 of 6 PLLs, and 1 of 3 CML cases in lymphoid blast crisis. In contrast, none of the 40 AML or 10 CML (3 myeloid blast crisis, 6 chronic phase, 1 accelerated phase) cases, and only one of 97 T-lineage ALL cases was B43/CD19+. Using Fluorescence activated cell sorting and colony assays, we have shown that CD19 antigen is expressed on B-lineage leukemic progenitor cells from B-lineage ALL patients (13) as well as their early progeny in PHA-LCM or BCGF stimulated cultures (14,15). Notably, there are a relatively large number of high affinity CD19 binding sites on B-lineage ALL cells (30,000-100,000 molecules/cell; Kd = 1 nM) and CD19 can undergo antibody-induced internalization (13). The opportunity is thus provided for using B43/CD19 MoAB to deliver toxins to B-lineage ALL cells. Our studies to date indicate that CD19-PAP IT are extremely potent inhibitors of B-lineage leukemic cells (7-10,16,17). CD19 is not found on normal bone marrow progenitor cells CFU-GM, BFU-E, CFU-MK, CFU-GEMM (13). Furthermore, B43 (CD19) MoAb shows no cross-reactivity with T-lymphocytes, NK cells, granulocytes, monocytes/macrophages, erythrocytes, or platelets (13). Importantly, B43 does not cross-react with any of the normal non-lymphohematopoietic tissues including the liver, kidney, lung, brain, and blood vessels (13). The potential problem of damaging normal life-sustaining tissues is a major concern of IT therapy (2,3). The absence of the CD19 antigen on normal non-hematopoietic tissues or hematopoietic progenitor cells and its expression on leukemic progenitor cells render CD19 IT especially attractive for

immunotherapy. Furthermore, B-lineage leukemic cells do not shed CD19(13). This is important since: 1) immune complexes may be formed as a consequence to antigen shedding and cause renal toxicity, and 2) shed antigen molecules may bind the IT and impair its homing. These findings indicate CD19 MoAbs should receive initial consideration when designing effective immunotherapy for the treatment of B-lineage ALL. A potentially significant problem in using IT in vivo is related to the presence of carbohydrate residues in the toxin moieties (2). Kupffer cells in the liver express receptors for carbohydrates which may result in rapid clearance and short activity of IT as well as a significant liver toxicity. In this regard, the use of PAP IT are of special interest because PAP lacks carbohydrate residues. Another potential problem in the in vivo use of IT is the development of antibodies by host B cells against the mouse MoAB and/or the toxin moieties (2). B43/CD19-PAP IT is very attractive in this regard as well because they are pan-B reagents with an exceptional toxicity to most B-lineage cells. Importantly, B43/CD19-PAP was more effective against CD19+B-lineage ALL cells/cell lines than PAP IT directed against CD10, CD20, or CD22 antigens (16).

Preparation of B43-PAP

B43 MoAB has been produced in the ACUSYST-Jr automated hollow-fiber cell culture system (Endotronics, Coon Rapids, MN). In brief, the flowpath containing one hollow fiber bioreactor is inoculated with 2x108 viable IST-1586 hybridoma cells (parent myeloma cell line: X63-Ag8.653) secreting B43/CD19 MoAB (IgG1, kappa) in a volume of 100 ml of RPMI supplemented with 10% FBS. During each production run, the feed rate is steadily increased from 1L/day to 20L/day and the medium recirculation rate is increased from the initial rate of 50 ml/min to 500 ml/min in response to increased consumption of glucose, declining pH, and increased production of lactic acid. Cell metabolism is continually monitored on line via dissolved oxygen and pH problems as well as daily sampling for glucose and lactic acid concentrations, and the supernatant is harvested at a rate of 100 ml/day. B43 MoAb is purified from the harvested culture supernatants by using the Affi-Gel Protein A MAPS II MoAb purification system kit (Bio-Rad Laboratories) in combination with size exclusion chromatography on a HPLC (TSK 3000) column and Bio-Gel concentrator resin, as described (13). We usually obtain 0.3-0.5 mg of purified B43 per ml of the harvested supernatant. Hence, the amount of purified B43 MoAb produced each day ranges from 30 mg - 50 mg. PAP is purified from PHYtolacca americana leaves harvested during spring, as previously described (18). One kg of leaves is blended with 1 L of water and the mixture filtered through cheesecloth. This crude extract is fractionated gy a 40-100% ammonium sulfate precipitation,

dialyzed, and filtered through a 1x12.5 cm bed of washed DEAE cellulose contained in a Buchner funnel. The subsequent fraction is applied to a 2.5x20 cm column containing S-Sepharaose equilibrated with 20 mM potassium phosphate, pH 6, the column is washed with 200 ml of the same buffer, and bound protein eluted with a linear salt gradient from 0 to 500 mM KCl in the equilibration buffer. PAP elutes as the largest protein peak at approximately 0.12 M KCl and is dialyzed against 20 volumes of deionized water with one change and lyophilized. Yields of pure PAP varies from 60-170 mg/kg of leaves. We use the cross-linking agent N-succinimidyl 3-(2-pyridyldithio) propionate (SPDP) to introduce a cleavable disulfide bond into the MoAb via the primary amino groups. 2-iminothiolane is used to introduce reactive sulfhydryl groups into the toxin moieties following reaction with primary amino groups under mild, aqueous conditions (16,17). A thiol disulfide exchange reaction between the 2-pyridyl disulfide protected groups introduced into the MoAb and free thiol groups on the toxin forms the basis for our conjugation procedure to produce covalently linked MoAb-toxin conjugates (Figure 1).

Preparation of Immunotoxins
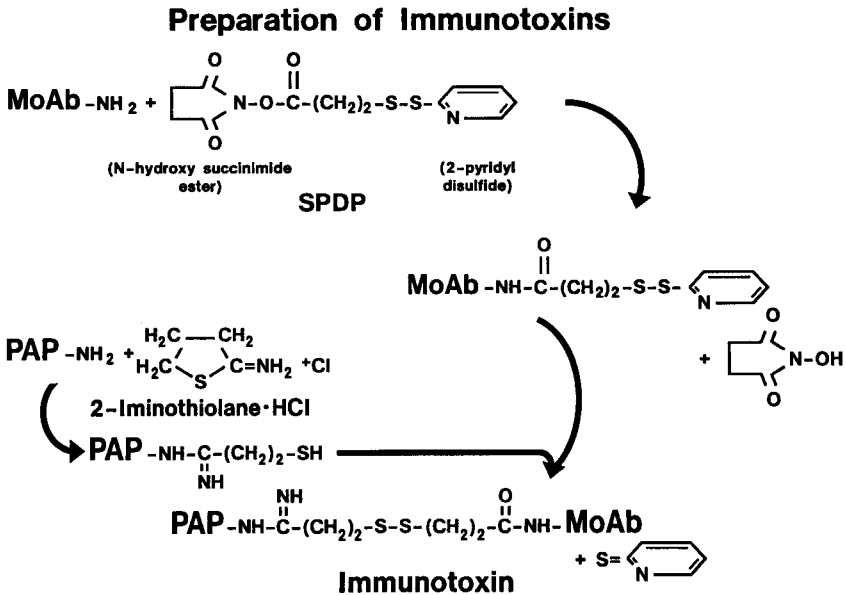
Figure 1. Preparation of PAP Immunotoxins. We use the cross-linking agent N-succinimidly 3-(2-pridyldithio) priopionate (SPDP) to introduce a cleavable disulfide bone into the MoAb via the primary amino groups. 2-iminothiolane is used to introduce reactive sulfhydryl groups into the toxin moieties following reaction with primary amino groups under mild, aqueous conditions, as previously described (16,17). A thiol disulfide exchange reaction between the 2-pyridyl disulfide protected groups introduced into the MoAB and free thiol groups on the toxin forms the basis for our conjugation procedure to produce covalently linked MoAB-toxin conjugates.

Specifically, B43 MoAb (5 mg/ml) is first reacted with a 3-fold molar excess of SPDP (a freshly made solution of 64 mM concentration in DMSO, diluted 1:10 in 40 mM sodium phosphate buffer containing 150 mM sodium chloride, pH 7.5) for 30 minutes at room temperature. PAP is reacted with a 3-fold molar excess for 2-iminothiolane for 30 minutes, desalted, and concentrated. Modified B43 MoAb containing dithiopyridyl groups is mixed with modified PAP containing reactive sulfhydryl groups (molar ration of PAP/B43 = 5/1) and incubated overnight at 4°C.

Following this conjugation reaction, B43-PAP is separated from unconjugated PAP and free B43 MoAb by size exclusion chromatography and affinity chromatography, respectively (16,17). The purity and composition of B43-PAP IT is assessed by SDS-PAGE. B43-PAP batches contain one molecule of B43 IgG and 1-2 molecules of PAP. No free toxin is detected on PAGE under non-reducing conditions. Free antibody contamination is estimated to be <10% by gel electrophoresis (Figure 2).

Anti-Leukemic Efficacy of B43-PAP IT

B43-PAP is very effective against clonogenic blasts from B-lineage ALL cell lines as well as fresh leukemic progenitor cells from B-lineage ALL patients and kills 3-4 logs of leukemic blasts with minimal toxicity to normal bone marrow progenitor cells (8-10,16). A recent evaluation of the anti-leukemic efficacy of various ex vivo marrow purging protocols at the level of leukemic progenitor cells has revealed that some of the current protocols may be suboptimal (11). The current Minnesota ex vivo marrow purging protocol for B-lineage ALL employs the MoAB BA-1 (anti-CD24), BA-2 (anti-CD9), BA-3 (anti-CD10/CALLA) in combination with exogenous complement (C') and the in vitro cyclophosphamide congener 4-hydroperoxycyclophosphamide (4-HC) (11,19). Notably, an number of B-lineage ALL cases are negative for BA-1, BA-2, and BA-3 but positive for B43. Furthermore, there is evidence for the existence of $CE19^+CD10^-CD24^-$ leukemic B-lineage ALL progenitor cells (14,15,20).

Therefore, the use of a CD19 IT such as B43-PAP may offer advantages over the use of BA-1,2,3 + C' + 4-HC. In the present study, we compared the anti-leukemic efficacy of B43-PAP and B43-PAP + 4-HC to the anti-leukemic efficacy of BA -1,2,3 + C' + 4-HC against fresh leukemic progenitor cells from B-lineage ALL patients. As shown in Table 2, in 3 of 5 cases it was as effective as BA-1,2,3 + C' + 4-HC. These promising results taken together with our previous studies on the anti-leukemic efficacy of B43-PAP IT recommend the clinical evaluation of B43-PAP for ex vivo marrow purging in autologous BMT for B-lineage ALL.
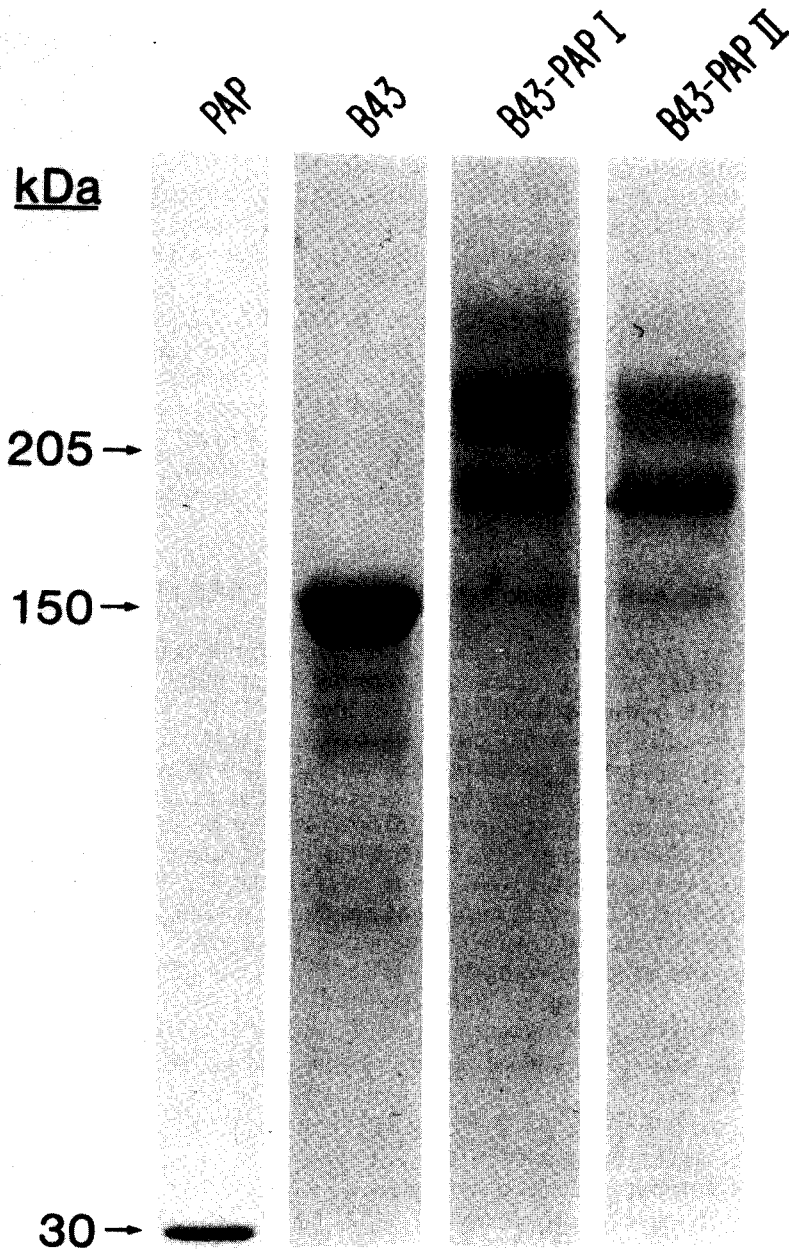
Figure 2. Biochemical Analysis of B43/CD19-PAP Immunotoxins. SDS-PAGE of two B43-PAP IT batches on a 5% running gel under non-reducing conditions according to Laemmli. The gels were stained with Coomassie Blue and the positions of the molecular weight markers were indicated on the left. Controls included unconjugated PAP as well as unconjugated B43 MoAB.

Table 2. Anti-Leukemic Effects of BA-1,2,3 + C' + 4-HC Versus B43-PAP + 4-HC Against Clonogenic B-Lineage ALL Cells

| | % KILL | |
Patient	PA-1,2,3 + C' + 4-HC	B43-PAP + 4-HC
OT	36	>99
DG	87	98
WB	>96	>96
HD	88	98
JC	>98	>98

The efficacy of purging protocols against clonogenic B-lineage ALL Cells was determined in colony assays. Results are presented as % kill of clonogenic ALL blasts. 4-HC was used at 10 μg/ml, B43-PAP at 1 μg/ml, and BA-1,2,3 MoAb at 10 μg/ml. The incubation time was 30 min for 4-HC and 8 hrs for B43-PAP. The treatment protocols have been detailed in previous reports (10,16,19).

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DETECTION OF MINIMAL RESIDUAL DISEASE IN ACUTE MYELOGENOUS LEUKEMIA BY RNA-IN SITU HYBRIDIZATION

Mary Jean Evinger-Hodges, Jorge A. Spinolo, Irene Cox, Verneeda Spencer, Pedro Nieto, and Karel A. Dicke

INTRODUCTION

Two major constraints in the detection of minimal numbers (< 1%) of tumor cells are: 1) the lack of technology available with sufficient sensitivity and 2) the identification of specific tumor markers. For the past decade considerable effort has been made to use molecular technology to help us resolve both of these problems. Since these tumor cells are part of a heterogenous population of cells, the use of Southern and/or Northern blotting techniques have not enabled us to detect < 1% contaminating tumor cells unless pre-selection is used increasing our sensitivity to 0.1 % at best.

Addressing the first question of sensitivity of tumor cell detection, there are two methods presently available which claim to have the ability to detect abnormalities at the level of 1/50,000-100,000 cells. The first of these techniques, polymerase chain reaction, is based on the amplification of a chromosomal abnormality. The limitations of this technique are 1) the need for identification of a consistent chromosomal abnormality in the tumor cells, 2) the necessity of cloning each identified abnormality in order to obtain the specific probe required for this analysis and 3) the inability to individualize the cells in which this abnormality is present for further characterization.

A second possiblity is the identification of an abnormality which is detectable at the single cell level. Recently we reported the development of an RNA-in situ technology which permits the detection of specific mRNAs within individual cells. This technology enables us to identify cells with the abnormal expression of any marker gene at a level of 1/50,000 cells. Adaptions of this technology are presently underway to permit analysis of these cells by flow

cytometry which would reduce the time and labor required for these tests while increasing the number of cells which can practically be analyzed and would provide an objective, quantitative assessment of the sample studied.

In addressing the second question of tumor cell markers, there are numerous reports of aberrant proto-oncogene expression in tumor cells and more specifically in acute myelogenous leukemia (AML) (1-9). Several groups, in addition to ourselves, have identified at least one gene, MYC, which is present at unusually high levels in the peripheral blood and bone marrow cells of untreated and/or relapsed AML patients (10-15). Through the application of our modified RNA-in situ hybridization technology we have identified the presence of abnormal cells as defined by gene expression in 7/10 AML patients studied recently after remission induction, but often at a much lower frequency than that found in untreated or relapse AML (15). Several, but not every patient, in which we found this abnormal group of cells have relapsed; in contrast, none of the patients whose bone marrow cells were found to be normal in their expression of MYC and/or SIS have relapsed since this study was completed. These results led us to question whether the presence of such an abnormal population of cells could be an early predictor of relapse in AML.

The median disease-free survival in AML is 12-15 months and relapses occur after two years in only 15% of cases. If the abnormal cell population is composed of clonogenic leukemic cells, we would not expect to find them in our AML long term survivors after bone marrow transplantation. The results of this study will help us determine what the significance might be of such a population of cells in the bone marrow of leukemia patients.

MATERIALS AND METHODS
Cells

Fresh human normal bone marrow cells were obtained from hematologically normal donors. Leukemia marrow samples were obtained from patients either at diagnosis or while in relapse, from remission patients whose marrow was being stored for future transplantation, or from follow-up examinations of the marrow of long term survivors after high dose chemotherapy and bone marrow transplantation. In all cases bone marrow buffy coat preparations were used for cytospin preparations.

RNA-In Situ Hybridization

A very sensitive and rapid RNA-in situ hybridization procedure was performed as described earlier (16). Briefly the buffy coat cells

are suspended in medium containing 2% serum and then deposited on slides as a cytospin preparation. The cells are fixed with 75% ethanol/20% acetic acid for 15 minutes at room temperature. Hybridizations were performed in 50% formamide at 52°C, followed by extensive washes in 1X SSC, 0.5X SSC and finally 0.1X SSC containing RNase. The slides were cover slipped in 50% glycerol/50% phosphate-buffered saline before being viewed under a fluorescence microscope.

Probes

Single-stranded RNA probes were generated for both MYC and SIS from the Amprobe system of Amersham (Arlington Heights, IL). These probes were sized between 200 and 400 base-pairs (16) and then labeled with Photobiotin^{TM} (BRL, MD). Detection of the biotin-labeled hybrids was performed by the addition of FITC-labelled streptavidin (BRL, MD). Unbound streptavidin was removed by large volume washes in 0.1X SSC containing 0.1% Triton X-100.

Quantification

The level of fluorescence/cell was used as an initial estimate of expression levels in these studies. Studies using cell lines have demonstrated that this method has the sensitivity to distinguish between a gene expressed at 5-10 copies per cell and one present at 20 copies per cell (16). In these experiments over-expression was defined as a greater than 3-fold increase in the level of fluorescence present in a cell as compared to that found in the brightest cell present in any normal bone marrow currently assayed under the same hybridization conditions.

RESULTS

We have described in detail in an earlier manuscript the sensitivity of our RNA-in situ technique (16). This technology has been applied to the detection of minimal tumor cell contamination in bone marrow samples. The expression levels of 3 genes, MYC, SIS, and RAF, were studied at the single cell level in both untreated and relapsed AML patients (Table 1). In these studies MYC appeared to be abnormally expressed in a variable percentage of bone marrow cells in nearly 100% of AML untreated and relapsed patients examined. Similar results were obtained for SIS, although at a slightly lower frequency. In contrast, cells containing abnormally high levels of mRNA for RAF were present only sporadically in this patient group (Table 1). The expression of these genes at such high levels was not detectable in any normal bone marrow cell examined. Recently, we have tested

**Table 1. Frequency of Abnormal Gene Expression
in Untreated/Relapsed AML**

Patient	MYC	SIS	RAF
Untreated	10/10	8/10	0/10
Relapsed	15/15	12/15	2/12
Normal			
Untreated	0/15	0/15	0/15
Solid Tumor*	0/8	0/8	
On Chemo			
Solid Tumor*	0/8	0/8	
ABMT			

*No Bone Marrow Involvement

8 solid tumor patients without bone marrow involvement in their disease, with samples taken before treatment, after high dose chemotherapy and at several time points after autologous bone marrow transplantation and we were unable to identify cells expressing MYC and SIS at such high levels.

As one line of evidence that the cells we are examining actually belong to the leukemic cell compartment, comparisons were made with the percentage of blast cells determined morphologically in these AML patients. As shown in Table 2, the percentage of cells overexpressing either MYC or SIS at least equals, and often exceeds, the number of blast cells present in the marrow.

AML Remission

In an earlier study (15) of 10 AML patients who were morphologically determined to be in remission, we detected in 7/10 of these patients a subpopulation of cells which contained a high expression level of MYC and/or SIS as compared to normal bone marrow through the application of RNA-in situ hybridization. Based on these results, the clinical status of these 10 patients has continued to be followed over the past year. Of the 7 patients in which an abnormal population of cells was identified, 5 have now relapsed.

Table 2. Comparison of % Blast with % Gene Overexpression in AML Bone Marrow

Patient	% Blast	% MYC	% SIS
1	7.2	70	70
2	24	25	25
3	40	90	90
4	49	30	50
5	51	50	50
6	60	75	75
7	63	85	90
8	65	65	90
9	76	70	75
10	78	75	80
11	81	85	0
12	96	>90	>90

All of these patients were examined within 2-8 months after remission induction. Those patients who relapsed did so within 5-6 months after testing positive for abnormal gene expression (Table 3). At this time the 2 patients of this group in which the presence of an abnormal subpopulation of cells was detectable continue to be in remission 3 months and 1 year, respectively, after testing. None of the 3 patients whose bone marrow cells appeared normal by this assay for gene overexpression have relapsed over this same time period.

AML Long Term Survivors

The significance of this abnormal cell population in the eventual clinical stability of the AML patient is still undetermined. To help us answer this question, we have also examined bone marrow samples of 10 AML patients who are long term survivors after bone marrow transplantation. The median CR duration at the time of examination for this group was 38 months with the individual remissions ranging from 14-78 months. The presence of an abnormal cell population expressing MYC at high levels as found in AML short remission patients does not occur in this patient group. However, in 3/10 patients a high level of SIS expression alone was detectable in a variable percentage of cells. At this time, none of the 3 patients identified with this abnormality at the RNA level have been classified as having a recurrence of leukemic cells in the bone marrow by conventional morphological criteria.

**Table 3. Overexpression of MYC and SIS
in AML Remission Marrow**

Patient	% MYC	% SIS	Present Diagnosis
1	75	80	Relapse
2	8	2	Relapse
3	5	2	Relapse
4	0.2	0.2	Relapse
5	0.1	1	Relapse
6	2.5	2.5	CR
7	0.5	0	CR
8	0	0	CR
9	0	0	CR
10	0	0	CR

DISCUSSION

We had previously described the high frequency of bone marrow cells with MYC and/or SIS overexpression in untreated/relapsed AML patients as compared to normal bone marrow cells which express these genes at very low levels (15).

The percentage of these abnormal cells as defined by aberrant gene experession parallels or exceeds the percentage of blast cells found in the marrow of these patients. The continued presence of such abnormal cells at very low frequency in greater than 70% of remission marrows examined from patients within a year after remission induction led us to question the significance of these cells in the eventual clinical stability of the patient. To help us determine this answer, we obtained bone marrow samples from 10 AML long term survivors (median CR duration 38 months; range 14-78 months) and examined these samples by RNA-in situ hybridization to see if any MYC or SIS overexpressing cells could be detected.

We find that the occurrence of such a population of abnormal cells is a rare event in this patient group. Whereas the presence of such abnormal cells was detectable in 70% of the AML patients examined shortly after remission induction, we found such an abnormality to be present in only 3/10 long term survivors. Interestingly, although we find MYC to be overexpressed in every short term remission patient in which we find SIS overexpression, this was not the case in any of the long term remission patients examined. Of the 3/10 patients in which SIS was expressed at high levels, none had MYC present at levels higher than normal; nor do we find any evidence of relapse in this patient group.

Table 4. Abnormal Expression of MYC and SIS in AML Long-Term Survivors After Autologous Bone Marrow Transplantation

Patient #	% MYC	% SIS	Present Diagnosis
1	–	–	CR: 3 yrs
2	–	–	CR: 3 yrs
3	–	0.01	CR: 7 yrs
4	–	–	CR: 3 yrs
5	–	80	
6	–	–	CR: 1^+ yrs
7	–	–	CR: 4 yrs
8	–	–	CR: 5 yrs
9	10	5	
10	–	80	relapse

Since SIS encodes for the B-chain of the growth factor PDGF, it is conceivable that it is the unregulated response of these myeloid cells from AML patients to PDGF stimulation which is responsible for the high levels of MYC mRNA detected within these cells. In the long term survivor population, it is possible that this lack of high MYC expression is indicative of a regulated response of these cells to growth factor stimulation. In this case we would not expect these patients to relapse unless there is an increase in MYC expression similar to that found in AML relapse patients. These last patients will continue to be closely followed for any signs of relapse and possible correlation of relapse with increased MYC expression.

Initial evaluation of these long term survivors gives us an indication that detection of abnormal gene expression may well be a parameter which can be used to monitor remission duration in AML patients after BMT. Based on the data presented in Table 4, it appears that patients with long lasting remissions do not have a population of hematopoietic cells which express MYC at high levels. We are currently doing prospective studies in recently diagnosed patients to better understand the incidence of gene overexpression in the longitudinal course of the disease, their response to treatment modalities (including bone marrow transplantation) and their value as predictors of long term remission.

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Discussion 1 - Session 1B (ALL)

Dr. Kersey: Let me begin and ask if there are any questions for Grant?

Dr. Kersey: Grant, you presented some very interesting data in terms of NK cells and this, of course, raises some very important questions in terms of comparing autologous vs. syngeneic vs. allogeneic transplantation and clearly makes it evident that if these results are real, which I believe they are, one cannot make simple comparisons between these various groups.

Dr. Prentice: Can I say something before you ask your question, Karel? It is obvious that special attention should be given to the cell populations causing graft vs. leukemia, a phenomenon which occurred after allogeneic transplantation. That is ... Dicke and Spitzer, 1986, stimulated us!

Dr. Dicke: Thank you for this remark. I completely agree with that. Can I return a question? You are using, for purging, a monoclonal antibody cocktail?

Dr. Prentice: Not always. The antibodies were selected on the basis of the phenotype and the target cell killing. Sometimes a cocktail was used where it gave the best target cell killing.

Dr. Dicke: So, how did you do that? What did you use?

Dr. Prentice: George Janossy's assessed the phenotype, the target cell killing and in some cases the antigen density. And it was on the basis, and these patients were entered only on that basis, that the phenotype was identified and the right antibodies were selected. That is how we selected patients in this study.

Dr. Dicke: Was there no effect on hematopoietic engraftment?

Dr. Prentice: One patient of the 54 failed to engraft. That patient was the one who had brain necrosis. There was no explanation for graft failure. Actually the in vitro BM culture in that patient was probably one of the best.

Dr. Lowenberg: Grant, in the allogeneic patients, were the NK cells, which were the killers, donor or recipient?

Dr. Prentice: Always donor derived. We have proven that. We know that some populations in our allogeneic series are certainly host derived. What we are seeing, like everybody else, recipients treated with depleted cells, mixed chimeric states. These early cells, however, were always of donor origin.

Dr. Lowenberg: Grant, you intend to suggest that the major part of the antileukemic efficacy in the auto BMT and the allo BMT programs differ because of different immunological mechanisms being involved. I was surprised to note that the NK activation and the cytokine production occurred in the autologous recipients as well as in the allogeneic recipients.

Dr. Prentice: We were a bit surprised, but those are the facts. I think it is very interesting because it may be the explanation of why people are not seeing a difference between different conditioning regimens and how patients are being cured beyond first remission as well.   But how then do you explain the general observation that relapse is higher, for example, in patients with AML following autografting then following allografting. Certain of the effects differ in the patients having allografting vs. autografting. For instance, the NK activity rather than lymphokine activating killer is higher after allografting than autografting. And it may be that there is a different role of T-cells in those two settings as well. So there are differences but the surprising thing for us was the activity of both NK and lymphokine activated killer cells in autologous transplants. I would suggest to you that they may be playing a major role. I certainly do not find any convincing evidence that the conditioning regimen is what cures the patient. I am not saying it is not important, I think it is critical to achieve a good state of minimal residual disease to allow the real effectors to cure the residual leukemia.

Dr. Kersey: I would like to make a comment and see how Ross and Grant react to this. In viewing the data that I know that exists from around the world, as far as transplantation either autologous or allogeneic transplantation for ALL, the only group that I am convinced that we know that transplantation is better than

chemotherapy is in patients who have relapsed and have relapsed in less than 18 months after going on initial chemotherapy. This group of patients as Ross showed us from the St. Jude data does very poorly with chemotherapy. Clearly a number of those patients have been salvaged, both the autologous and allogeneic transplantation. In no other group am I convinced that we have yet shown that either autologous or allogeneic transplantation is preferable to chemotherapy. You want to respond to that?

Dr. Pinkerton: I think I agree entirely. Part of the problem is a consistent second line chemotherapy regimen. In the last 5 or 6 years, most centers including our own have been through the gambit of high dose Ara: C plus or minus whatever for trying to salvage patients for grafting. So getting data such as St. Jude's, where you have got a large cohort of patients given a single second line chemotherapy which you can use to get an idea of survival without transplant is very difficult. The question about the timing of relapse, it is of interest that a number of the allograft series including my own study, and I seem to show that there is no cut off here, early relapses are doing about the same as the late relapses which is very different from the chemotherapy alone regimens. But I agree with John Kersey really that the data is so limited to make any further conclusions.

Dr. Reading: I had a question for Grant. Do you interpret the immunological stimulation you see in autologous transplants as coming from ... by just removing cells and putting them back in or from radiated allogeneic blood products as support for these patients?

Dr. Prentice: We have addressed the problem of allogeneic blood products in a paper that will be coming out later this year. We have also looked at viral reactivations, neither of those hold up. There is no correlation with blood products, no correlation with the viral reactivations which in any case are MC restricted. I think that is a real phenomenon associated with destroying the host immune system and if you like recapitulating the restructuring of the immune system, it is all down to disregulation. We have not done any syngeneic grafts so we do not have any data on those. I would not be surprised if there is a difference between autografting and syngeneic grafting. That is a guess though.

Dr. Dicke: Ross, I like the randomized study you are proposing very much. I am a little bit surprised though that you doubt about adding purging in this. When you want to transplant in second remission and also harvest in second remission, then most likely at the time of harvest, your leukemic cell contamination of the bone marrow might be relatively high. So why take the risk of leukemic cell contamination when there is a real good possibility of removing it.

Dr. Pinkerton: I think the decision about whether we are going to attempt to introduce purging component has not yet been decided. Again the difficulty, this is going to be a national multicenter regimen, multicenter study and trying to standardize techniques of purging in several centers is problematic.

Discussion 2 - Session 1B (ALL)

Dr. Dicke: Dean, do you or John want to comment on what seems to be a contradiction of the data? John indicated that in ALL in second remission, allograft recipients without GVHD and autograft recipients have indistinguishable relapse curves, suggesting that the source of relapse in those patients is the patient, not the BM. In second remission you show a significant difference suggesting that relapse from the marrow plays a role in ALL in second remission. Those are opposite conclusions I believe.

Dr. Buckner: I am not sure with the small number of patients in either group.

Dr. Pinkerton: One explanation for the lack of any difference between the allograft and the autograft in first remission patients would be that the majority were cured before they got to the high dose procedure. What sort of outcome would you be expecting from that particular induction and perhaps consolidation regimen without any kind of high dose procedure for those sort of patients?

Dr. Buckner: There is no way of knowing in those first remission patients whether they are cured or not cured. We do not have a comparable control group. These patients were transplanted basically because the referring physician felt that further chemotherapy would not cure the patient.

Dr. Pinkerton: The 40% plus cure rates in adult ALL is achieved; is it not by the Hoelzer regimen?

Dr. Buckner: But at least 40% of these patients had already had an extramedullary relapse and I am not sure you cure 40% of those patients. That is our more common reason for doing a transplant in first marrow remission. That is where our data is different from other people's data.

Dr. Gale: I just want to say one thing on that point though, Dean, something I think which is not generally appreciated; which is, although the overall outcome of ALL in first remission in adults is about 30 or 35% if one takes patients under the age of 25 or 30, which is the typical transplant patient, and takes patients with white counts under 20,000, any study will show you 50% cures with chemotherapy. So it is quite possible the cured are already cured and those that do not die, because they have an auto or allo transplant, do the same.

Dr. Buckner: I was not making the point that we were curing more patients in first remission than chemotherapy, I was just presenting the data for a very highly selected group of patients showing you the outcome. I am not claiming it is better than chemotherapy.

Dr. Pinkerton: Grant, it has been said today that you cannot be graft versus leukemia effect without GVHD. And this of course is relevant to ABMT. Shake your head or not, but that is what has been said twice. So I will just show our T-cell depleted data suggesting that you can get an antileukemic effect without GVHD. We did 28 AML patients in first remission with T-cell depleted marrow. The relapse risk is 5%, the leukemia free survival is 78%. Since I am not here next week, Bob can you defend T-cell depletion? That is now upside down, that is deliberate. Just because I showed the Marsden data upside down and the wrong way around, my slides are in that way, too. I show this data for two reasons, to show that you can get an antileukemic and an apparent antileukemic affect without GVHD, and because I am leaving tomorrow and try again.

Dr. Gale: Yes, I would like to carry that banner. There is a very large analysis that Mary Horowitz presented at the Tamaron meeting and will present at ISEH. About 2,000 patients transplanted for acute leukemia. And it is very clear that there are 3 separate antileukemic affects associated with BMT: contribution of T-cells, an allogeneic affect which is independent of GVHD, and an antileukemic affect based on GVHD.

Discussion 3 - Session 1B (ALL)

Dr. Buckner: I just want to ask one question. Is your conclusion from this that you should store and transplant patients after 7 months?

Dr. Gorin: No, I think my conclusion would be between them. I am convinced that if you do the transplant very early, most probably you avoid in vivo purging and I would expect that this would be harmful for the patient and therefore we say at least 1 and in our institution, 2 or 3 consolidation courses are necessary before you do the harvest and the transplant.

Dr. Buckner: But all of your statistics showing is that between 1 month and 6 months, patients are becoming ineligible for autologous transplant by relapsing.

Dr. Gorin: The medium population in fact has been transplanted between 4 and 7 months. And this is the population which is responsible in fact for the results around the 38 to 40%.

Dr. Gale: I have a slide for this. But I think what Dean is trying to say is what is shown here. Here is a typical survival curve which has a 25% outcome at the end. And if you just throw blocks of time, as time censoring, you see the first censoring was done at 7 months because that was one of the figures that Claude had in a previous publication. You see the 25% goes to 45%. It is the same exact group of patients; we have just gotten rid of all the patients who relapsed in the first 7 months. So the same trial would show 45%. And if you look at the next block which we threw in at 14 months, again based on data that Claude published, the same group of patients would look like they had 60% cures. Nothing has happened. There has been no in vivo purging, no in vitro purging, no nothing. We just are throwing in blocks of time censoring of patient data. What is the

evidence that BMT is giving the chemotherapy patients in remission a change in outcome? I have yet to see a statistic which could not be explained by manipulation of data? It does not make any difference.

Dr. Spitzer: And what about second remission?

Dr. Gale: Well I do not want to interrupt Claude's lecture; your institution has recently published the study and I have a slide of it that I will show on Saturday saying that for 1973 and 1986, approximately 20% of patients who achieved a second CR, became 3- to 5-year long-term, disease-free survivors (20%). Keating is the first author. The slope of the curve is very steep. Something like 30% of the patients relapsed in the first three months. If you looked at the data shown by Andrew Yeager today, in AML in second remission, the median interval from achieving a second remission to a transplant was 5 months. So about 40 or 45% of the patients from the MDA data would have been censored. Now if you take the 20% survival result in AML in second remission and correct for censoring, it is going to be 40%. That 40% AML in second remission with chemotherapy alone, is obviously not different than any result with auto transplantation. Which is not to say that auto transplantation is not as good as chemotherapy, but simply there are no data to suggest that it is doing anything in that disease that could be explained by time censoring. It could be that the MDA data are wrong. It seems to me that 20% result of chemotherapy in AML in second remission is a very good result. But, as you know, many of those data are not actuarial they are actual.

Dr. Goldstone: Bob, if for instance in adult ALL, unlike in perhaps AML, there was evidence that the chemotherapy from remission in the first few months to the time of auto transplant actually helped keep the patient in remission, then there would be an argument that waiting could get a better result 6 or 7 months out because you had less tumor around, was actually a good idea.

Dr. Gale: Well, Dr. Gulati's institution, Sloan-Kettering, is the only one that has presented an analysis of a short course of intensive chemotherapy 6 months, L16M, against a longer course of 3 years, L16. And those results are precisely the same. So if what you are saying is true, and it could be, it would extend only for the first 6 months. But I think the burden of proof is the other way around. For someone to show that their results cannot be explained by censoring – if it cannot be explained, then you can have a lot of rationale. I would ask Claude the question since we do not have any evidence that continually treating patients with AML in remission changes their outcome. Can you imagine why, other than censoring, it would make a difference of how long patients were treated before they got an

autotransplant? We know that a patient with AML is treated for 2 months or 3 years; median remission duration and survival are unchanged. So why would waiting a year have any affect other than allowing patients, who are going to relapse, to relapse? What would be the biological basis of it?

Dr. Gorin: Well, I do not know. You say that there is no evidence that the survival of disease-free survival is better in AML considering conventional chemotherapy whether they received consolidation courses or not.

Dr. Gale: Patients go into remission and get 1 or more consolidation courses.

Dr. Gorin: Right. It may turn out that the disease-free survival is the same but at the moment you collect the marrow there may be some differences. And I think, if you collect the marrow immediately after -- let's say -- 1 single induction course, without any consolidation, you may very well collect -- I would say -- 10^8 or 10^9 leukemic cells. In fact, you might collect partial remission marrow.

Dr. Gale: I do not think any of us are talking about a patient who just goes into remission, but we are talking about a patient who gets 1 or 2 courses, which is essentially 1 or 2 months. I do not think there is anyone who has any data that would suggest that the treatment after the first two months of remission in anyway effect the ultimate outcome. Now if there was an effect on minimal residual disease, you would expect the median remission duration to be different.

Dr. Prentice: I can think, immediately, of two possible explanations. One would be an immunological one. It may be that the immune constitution of the marrow taken later might be superior in some way or other. Another one, if you do not like the immunologic explanation, is hemopoietic competition, because the quality of the marrow that you collect later is probably superior to the quality of the marrow you collect early. Maybe, it is a matter of who gets to the niches first.

Dr. Spitzer: Can I just ask Claude this same thing I keep coming back to. We heard from Seattle there are cures greater than 23% on relapse with identical twins. What is the explanation? There is only about 1 long-term disease-free survivorship using autologous bone marrow at relapse. And yet the BM is collected at the time you said is ideal in the first remission study.

Dr. Prentice: Right. But the patient is treated in relapse.

Dr. Spitzer: Like the identical twin cures.

Dr. Prentice: Right. But I mean this is, anyway, a very good mean to obtain a CR. You can do that. You can take the marrow collected and everybody will agree that we have 75 to 80% CR.

Dr. Spitzer: And with certain chemotherapy protocols, 50-60%.

Dr. Prentice: But all the patients that have been treated until now, of course, did not receive maintenance chemotherapy. Maybe some (have) in your institution. This is not the answer, I know. What you are asking is a comparison with the syngeneic. I do not know. But my view would be to add additional maintenance chemotherapy or do a second transplant.

Dr. Spitzer: But does that support Bob's supposition that nothing has been shown in autologous? I am just being controversial, but I could support it.

Dr. Gale: Dean said the relapse rate and I agree with him. (Regarding) twins transplanted with advanced leukemia, the actual relapse rate is 70%. And Claude said that all of the patients relapse. Now given the number of patients in those 2 groups, I guess there cannot be more than 15 twins done that have this actuarial 70% relapse rate. The 95% confidence intervals reach 100% relapse. So Claude's failure to find any disease-free survivors and Dean's statement about 70% relapses are quite compatible statements. Obviously, the other explanation is that the autotransplants are bringing in more leukemia in advanced disease.

Dr. Weiner: I was just intrigued by a preliminary analysis that I heard Claude give several months ago. The influence of in vivo vs. in vitro purging was addressed on the duration of remission in the transplanted patients. You demonstrated that in vitro purging was effective for those patients transplanted shortly after attaining CR.

Dr. Gorin: Thank you Roy. The data have been presented in this conference. If you say that we are only censoring patients and those who are transplanted late are doing well, just because they were cured before ABMT, then how do you explain exactly what Roy said -- that you find an advantage of purging which is very easily demonstrable for those who are transplanted early and, you do not find it anywhere later.

Dr. Gale: The proper comparison has to be for people who are in remission for comparable periods of time. Purging vs. not purging.

SESSION II - LYMPHOMA

AUTOLOGOUS BONE MARROW TRANSPLANTS PURGED WITH MONOCLONAL ANTIBODIES AND COMPLEMENT IN B-CELL LYMPHOMAS: A Review of 47 Cases

Marie Favrot, Iréne Philip, Frank Chauvin, Valérie Combaret, Gilles Clapisson, Fathia Mezziane, Pierre Biron, George Janossy, and Thierry Philip

In experimental models, the purging with anti-B cell monoclonal antibodies (MoAb) and complement has been shown to eliminate at least 3 to 4 log of Burkitt cells (1,2). The same procedure has been used for patients with B cell lymphoma entered in an autologous bone marrow transplantation (ABMT) protocol (3-5). We shall review 47 cases of ABMT whose autografts have been purged with monoclonal antibodies and complement (MoAb + C'), and analyze the toxicity of the procedure on progenitor cells, as well as the hematological and immunological recovery after grafting. In 29 patients pathological BM has been observed at least once during the course of their disease and 10 had detectable BM micrometastases on the day of harvest, thus the usefulness of the purging procedure will also be discussed.

MATERIALS AND METHODS

Patients

The clinical parameters of the 47 patients are described in Table 1. High dose chemotherapy was given according to the BEAM protocol (BCNU, etoposide, aracytine, melphalan) or the BEAC protocol (BCNU, etoposide, aracytine, cyclophosphamide), and one of the 2 patients with pre-B leukemia received misulban-endoxan as previously described (5).

Table 1. Clinical Characteristics of the 47 Patients

Disease	Number of Patients	Status at ABMT					No. of patients with positive BM during the course of the disease	No. of patients with positive BM at harvesting and ABMT
		PR	PD	1CR	SR	RR		
BURKITT	37	1	1	16	14	5	21	6
PRE-B LEUKEMIA	2			2			2	
INTERMEDIATE GRADE LYMPHOMA	3	1		1	1		2	1
FOLLICULAR LYMPHOMA	5	2			3		4	3

Table 2. Total BM Mononuclear Cells and CFU.GM Harvested and Reinjected in MoAbs and Complement Purged BM in B Cell Lymphoma

	MN cells/kg x 10^8	CFU.GM/kg x 10^4	CFU.GM/2 x 10^5 MN Cells
Harvested BM			
Before Purging	2.79	32	250
(median range)	(0.72-4.86)	(0-140)	(0-860)
Reinjected BM			
After Purging	0.72	8	200
(median range)	(0.25-3.39)	(0-27)	(0-760)
Recovery	25%	25%	
After Thawing	0.54	3.4	140
(median range)	(0.13-1.2)	(0-20)	(0-600)

Methods

BM harvest and purging procedures have been also described previously (1-3). Briefly, after Ficoll separation mononuclear (MN) BM cells were resuspended in Hanks solution at $2x10^7$ cells/ml with calcium and magnesium and incubated for 20 minutes at 24°C with MoAbs (see below) at appropriate dilution and washed. Samples were then treated twice with baby rabbit C' (Pasteur, Lyon, France) for 60 minutes at room temperature, with one wash between the two treatments; the C' was used at 1/3 final dilution. DNase (2.5 U/ml) was added during the two courses of C' incubation. Finally, cells were resuspended in the freezing solution.

Monoclonal Antibodies (1,2)

Y29/55, a pan-B non clustered MoAb, was kindly provided by Dr. Forster; AL2, a CD10 MoAb (anti-CALLA), was provided by A.M. Lebacq, and RFB7, a CD20-like pan-B MoAB, was supplied by one of us (G. Janossy). Finally SB4, a CD19 MoAb, was given by J.C. Laurent (Sanofi, Montpellier, France). The BM was treated with Y29/55 alone in 7 cases, with 729/55 + AL2 in 6 cases, with Y29/55 + RFB7 + AL2 in 16 cases and with RFB7 + SB4 in 18 cases. The toxicity of this procedure was evaluated by the clonogeneic efficiency of the CFU.GM progenitors in agar.

Immunological Follow-Up

The T cell ratio and percentage of NK cells were evaluated by immunofluorescence (IF) analysis on a microscope. The proliferative index of T cells after 5 days of PHA-stimulation at 0.5 µg/ml was measured by thymidine incorporation. IL2 secretion by PBL after 72 hours of stimulation with PHA was measured by the capacity of the supernatant to induce the proliferation of the IL2-dependent cell line, $CTL2$, compared to a standard medium. NK function was measured in a ^{512}Cr release assay on K_{562} cell line (40:1 ratio).

Detection of BM Micrometastases

Patients had 2-4 trephine biopsies and 2-4 aspirates at diagnosis, relapse and on the day of harvest. After Ficoll separation of the BM cells for the purging procedure, $2x10^6$ cells were analyzed with a single IF method using 2 pan-B MoAbs RFB7 and SB4. In Burkitt lymphoma, cells were cultured in a liquid assay, as previously described (ref. 6,7 and see also M.C. Favrot, this volume). Malignant cells are detectable from day 8 of culture by cytology and the establishment of a cell line allows to confirm the positivity of the BM samples, as previously shown in details (6,7).

RESULTS

As shown in Table 2, the purging procedure was not toxic for progenitor cells when assayed for their clonogenic efficiency (CFU.GM/2x10^5 cells). Nevertheless, a significant loss of MN cells was seen and both the recovery of total MN cells/kg and CFU.GM/kg was about 25% of the harvest. In terms of toxicity, no difference was observed between the four combinations of MoAbs used for the purging procedure. After re-infusion into patients, their hematological recovery was quick: the median time to reach 0.5x10^9 granulocytes/1 was 24 days (range 10-42 days) and the median time to reach 50x10^9 platelets/1 was 27 days (range 8-60 days) Only one patient had delay to engraftment and be presented with an excess of CD8+, leu7+ cells in the blood and the BM. This patient was then treated for 6 days with intravenous injections of CD8 MoAb (provided by A. Bernard) at 0,2 mg/kg/day (8). On CD8 treatment in vivo, the CD8+, leu7+ circulating cells disappeared from the circulation, and both granulocytes and platelets recovered within 2 weeks after the start of injections.

The immunological recovery is summarized in Table 3. Before the graft the T cells functions, evaluated by the number of T5 and T8 cells, the proliferative response to PHA and IL2 secretion was subnormal, so was the NK function. The b cell function, assessed by the level of immunoglobulin secretion, was subnormal (serum IgG levels in the normal range IgM and IgA levels at the lower limits of the normal range). After grafting the patients had T cell deficiency for up to one year and there were variations between patients. The recovery of normal T cell functions did not correlate with a lower incidence of relapse. The NK function was maximal between days 60 and 90 but decreased in patients after 3 months. B cell functions, as assessed by Ig levels, were normal from day 60 post graft.

As shown in Table 1, 29 patients had pathological BM at least once in the course of their disease, but 10 only had detectable BM micrometastases on the day of harvest (Table 4). One patient had intermediate grade B cell lymphoma and 3 nodular B lymphoma: micrometastases were detected on the biopsies but both cytological and immunological analyses of the aspirates were normal. The membrane marker analysis of BM cells in the autograft was not sufficiently sensitive for the objective characterization of these malignant cells, and neither the contamination of the autograft nor the elimination of malignant cells by the purging procedure could be confirmed by this method. On the other hand, in the 6 patients with Burkitt lymphoma both aspirates and biopsies were negative while the liquid culture of harvested BM cells in five cases revealed the malignant contamination. In one case the cytogenetic analysis only was positive (see M.C. Favrot, this volume). The five autografts which contained detectable malignant cells before purging have

Table 3. T, NK and B Cell Functions with A MoAbs and Complement Purged Autograft in B Cell Lymphoma

	Pre-Graft	Post-Graft Day 30	Day 60 - 90	Day 90 - 180	>12 Months
Ratio CD4/CD8	1.6 (0.43 - 7)	0.5 (0.17 - 1.23)	0.75 (0.3 - 1.5)	1 (0.32 - 2.7)	0.6
Proliferative Index of PHA Stimulation	21000 (407-78207)	2400 (162-16261)	1807 (1008-28011)	6618 (527-56057)	7200
IL2 Secretion	1.9 (0.01 - 6.07)	0.11 (0 - 0.55)	0.7 (0 - 3.94)	0.5 (0 - 1.51)	0.75
NK Function	9.6 (3.7 - 34)	4.5 (0 - 25.9)	15 (2.9 - 24.5)	6.6 (6.3 - 29)	2.3
IgM (g/l) (0.6 - 1.5)	0.54 (0.1 - 0.78)	0.5	1.4	0.8	
IgG (g/l) (1.7 - 15)	8 (4.3 - 15.9)	6.2	7.6	11	
IgA (g/l) (1.2 - 2.5)	1.01 (0.23 - 2)	1	0.91	1.5	

Table 4. Clinical Characteristics of the 10 Patients with Positive BM at Time of Harvesting

Patients*	Diagnosis	Status at ABMT	Positive Tests on the BM at Time of Harvesting	Evolution Post-Graft
B.A.	BL	SR	Cytogenetic analysis	Toxic death
(46)	BL	RR	Liquid culture	PD (died day 27) CNS + BM
(27)	BL	RR	Liquid culture	PD (died day 40) head and neck
(60)	BL	RR	Liquid culture	PD (died day 25) CSF+ BM+ blood
(51)	BL	RR	Liquid culture	Relapse (day 28) BM + abdomen
(52)**	BL	RR	Liquid culture	PD (died day 13) before graft reinjection abdomen + BM
B.C.	Intermediate L.	PR	1/4 biopsies	NED (24 months)
D.D.	Nodular L.	PR	1/4 biopsies	NED (12 months)
D.C.	Nodular L.	PR	1/4 biopsies	NED (36 months)
V.P.	Nodular L.	SR	4/4 biopsies	Relapse (13 months) BM + nodes

* - Patients' numbers correspond to those in the next publication during this conference (see M. FAVROT)

** - Patient 52 received BEAM therapy but progressed before graft reinjection.

become negative after the purging procedure when tested with the same sensitive detection system. Before the purging the number of malignant cells was between 10^{-3} and 10^{-6} in the different samples and less than 10^{-6} in the different samples and less than 10^{-6} BL cells were left in the autograft after the purging.

Among the 3 patients with nodular B cell lymphoma who had a positive biopsy at time of BM harvest, 2 are live disease-free (12 and 36 months post graft) and one relapsed 13 months after the graft in the lymph nodes and the BM. The patient with intermediate grade B cell lymphoma is also alive disease-free at 24 months. In these 4 patients, the tumor cells were still sensitive to chemotherapy at time of ABMT (3 partial remissions after induction therapy and 1 sensitive relapse).

As a contrast, among the 6 BL patients one was considered to be in sensitive relapse but died of toxicity just following ABMT; and the other five patients were in progressive disease at time of BM harvest. These patients have relapsed within 6 weeks post graft.

DISCUSSION

The purging procedure with anti-B MoAbs and complement in B cell lymphoma is itself harmless to the BM but is usefulness is still debatable. In this series of 47 patients we have clearly demonstrated that the purging was efficient for 5 patients with Burkitt lymphoma, and in spite of this (see also M.C. Favrot, this volume), the patients were refractory to conventional therapy and relapsed post-grafting due to the failure of high dose chemotherapy to control their disease. For this reason, patients with BM involvement should not be considered for such therapeutic protocols in Burkitt lymphoma. In the other B cell lymphomas the methods of detection of malignant cells are not accurate enough to permit the quantification of malignant B cells in the autograft. the purging was justified in the 4 patients with BM micrometastases on the day of BM harvest but it was no possible to correlate the favorable outcome after ABMT to the efficiency of the purging procedure. Consequently, in B cell lymphoma the purging procedures should be reserved at least at this point to patients who had a pathological BM at least once during the course of the disease (27 out of 48 in this series of high risk patients).

As already observed after ABMT (see Combaret, this volume), patients had a prolonged and severe T cell defect after ABMT. Interestingly, B cell functions were normal within 60 days post grafting despite the purge of BM with pan-B monoclonal antibodies. NK functions were soon normalized after grafting and showed high values for 90 days then decreased after 3 months.

ACKNOWLEDGMENTS

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HIGH-DOSE THERAPY AND AUTOLOGOUS BONE MARROW TRANSPLANTATION IN PARTIAL REMISSION AFTER FRONT LINE INDUCTION THERAPY IN LYMPHOMA: The Best Indication for ABMT in 1988?

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Patients who fail to achieve even a minor response to front line chemotherapy regimens are, without doubt, the best example of refractory disease (1, 2, 3, 4, 5, 6, 7). Those who partially respond to front line regimens, but who develop progressive disease while still undergoing treatment, can also be considered as having refractory lymphoma (7). However, those patients who only achieve partial response to front line treatment but who continuously responded to each course of therapy, represent a well-defined group and a very important one to recognize early because they still have chemosensitive and not refractory lymphoma. Patients who still respond to chemotherapy but who only achieve partial response after front line treatment are rarely cured with further conventional therapy (7, 8, 9, 10). However, if they are quickly identified at the time of plateau of their response to therapy, a change in chemotherapy and the use of the dose effect relationship can theoretically result in durable response (11).

Seventeen such patients with NHL are reported in this study. They all received high dose therapy when in partial remission during induction therapy. The 81% without progression at 51 months post graft strongly support the efficacy of early intensive therapy in these patients.

PATIENTS AND METHODS
Patients

The 17 patients are shown in Table 1. There were 11 children and 6 adults between 3 and 57 years old (median 8 years old). These 17 patients can be divided into three groups:

1. Ten patients, as shown in Table 1, had persistent disease in the abdomen after front line therapy. They all had surgically proven active disease before high dose therapy and ABMT (patients 1 to 8, 16 and 17 in Table 1).

2. Two patients (patients 9 and 15 in Table 1) had persistent active disease in the bone marrow at time of harvesting and ABMT.

3. Five patients (patients 10 - 14 in Table 1) had persistent facial palsy for a stage IV lymphoma after front line therapy, and ABMT was indicated on clinical symptoms except for case 14 were malignant cells were still present (95% reduction from the diagnosis) in the cerebro-spinal fluid (CSF).

METHODS
Preparative Regimen and Supportive Care

Several preparative regimens were used in this multicenter study (Centre Leon Berard Lyon and associates, 8 patients; Institut Gustave Roussy Paris, 7 patients; Besancon Hospital, 1 patient; and Nancy Hospital, 1 patient). However, the regimens were very similar (i.e. 11 BACT as previously described (12, 13), 4 BEAM as previously described (14) and 2 with BEAC and BUSULFAN/CYCLO-PHOSPHAMIDE as previously reported (15, 16, 17)). The technique of bone marrow aspiration, cryopreservation and reinfusion has previously been described (4, 12).

Patients received care in single rooms according to each institution's protocols for supportive care. Prophylactic antibiotics were not given routinely but broad spectrum antibiotic coverage was instituted promptly when fever developed during neutropenia.

Amphotericin B was used in patients with persistent fever after three to seven days of conventional antibacterial therapy. Red cell and platelet products were irradiated and central venous lines were used in all patients.

Table 1. Details of the 17 Patients' Status, Response and Outcome after High Dose Therapy

Pt	Age	Sex	Diagnosis and Stage (Murphy Staging)	Previous Therapy	Length of Induction Therapy	Status at Harvest	Status at ABMT	Conditioning Regimen	Status Post ABMT	Complications	Date of Graft and Outcome as of November 1, 1987
IGR 1	7	F	Burkitt Stage III	CPM-ADR MTX-ASP VCR-PRED	3 months	PR	PR positive Abdominal biopsy	BACT	CR	Sepsis Interstital pneumonitis	16/01/84 Alive disease free
IGR** 2	8	M	Burkitt Stage III	CPM-ADR MTX-ASP VCR-PRED	3 months	PR	PR positive Abdominal biopsy	BACT	CR	CMV pneumonitis	07/07/83 Alive disease free
IGR 3	4	M	Burkitt Stage IV BM	CPM-ADR MTX-ASP VCR-PRED	4 months	PR	PR postive Abdominal biopsy	BACT	CR	Septic shock Strepto.	10/10/83 Died in CR 19/10/83
IGR 4	8	M	Burkitt Stage III	CPM-ADR MTX-ASP VCR-PRED	8 months	PR	PR positive Abdominal biopsy	BACT	PD	Pulmonary Emboly	21/09/83 Relapse day 60 Died 12/83
IGR 5	15	M	Burkitt Stage III	CPM-ADR MTX-ASP	4 months	PR	PR postive Abdominal biopsy	Busulfan Cycloph.	CR	- -	17/07/85 Alive disease free
CLB 6	36	M	Diffuse Intermediate Grade Mixte Stage III	CPM-VCR MTX-ADR BLEO	6 months	PR	PR positive Abdominal biopsy	BEAM	CR	Herpes	10/10/84 Alive disease free
HOPD** 7	7	M	Burkitt Stage III	CPM-ADR MTX-ASP VCR-PRED ARAC-VP16	4 months	PR	PR positive Abdominal biopsy	BEAM	CR	Herpes	23/05/85 Alive disease free

Table 1. (Continued)

Pt	Age	Sex	Diagnosis and Stage (Murphy Staging)	Previous Therapy	Length of Induction Therapy	Status at Harvest	Status at ABMT	Conditioning Regimen	Status Post ABMT	Complications	Date of Graft and Outcome as of November 1, 1987
BES 8	16	M	Lymphoblastic Lymphoma Stage III	CPM-ADR BLEO-VCR PRED-MTX	7 months	PR	PR postive Abdominal biopsy	BACT	CR	Hepatic and Uremic syndrom	27/07/83 Died in CR 5/84
CLB 16	42	F	Diffuse Intermediate Grade Large Cell Stage III	CPM-ADR BLEO-VCR PRED-MTX VP16-ARAC BCMI-TG	7 months	PR	PR postive Abdominal biopsy	BEAM	CR	--	16/02/87 Alive disease free
CLB 17	32	M	Intermediate Grade Large Cell T Stage III	MTX-BLEO ADR-VCR MG-IFOS VP 16	10 months	PR	PR positive Abdominal biopsy 2 Mime NE	BEAC	CR	--	04/11/86 Alive disease free
CLB** 9	4	F	Burkitt Stage IV BM	VCR-CPM ADR-MTX ASP-PRED-ARAC	3 months	PR	PR positive Marrow aspiration	BACT	CR	Interstitial pneumonitis	22/12/80* Alive disease free
CLB 15	52	F	Intermediate Grade Mixte Stage III	BLEO-MTX CPM-ADR MG-IFOS-VP16	7 months	PR	PR positive Marrow biopsy	BEAM	CR	Bleeding life threatening	10/01/86 Alive disease free

Table 1. (Continued)

Pt	Age	Sex	Diagnosis and Stage (Murphy Staging)	Previous Therapy	Length of Induction Therapy	Status at Harvest	Status at ABMT	Conditioning Regimen	Status Post ABMT	Complications	Date of Graft and Outcome as of November 1, 1987
CLB** 10	6	M	Burkitt Stage IV CNS	VCR-CPM ADR-MTX ASP-PRED-ARAC	5 months	PR	PR residual facial palsy	BACT	CR	CSF bleeding	10/01/83 Alive disease free
NANC** 11	3	M	Burkitt Stage IV CNS	VCR-CPM ADR-MTX ASP-PRED-ARAC	4 months	PR	PR residual facial palsy	BACT	CR	- -	16/12/83 Alive disease free
IGR** 12	5	M	Burkitt Stage IV CNS & BM	VCR-CPM ADR-MTX ARAC-BCNU TG-PRED-ASP	3 months	PR	PR residual facial palsy	BACT	PD	Blastic Meningitis	17/10/82 Died in PD 1/83
IGR** 13	6	M	Lympho- blastic T Stage IV CNS & BM	VCR-CPM ADR-PRED ARAC-MTX	8 months	PR	PR residual facial palsy	BACT	CR	Cutaneous Stephylo.	21/02/83 Alive disease free
CLB* 14	57	M	Interme- diate Grade Diffuse Mixte Stage IV CNS	VCR-CPM ADR-PRED ARAC	2 months	PR	PR residual facial palsy + CSF	BACT	CR	- -	17/09/82 Alive disease free

IGR = Institut Gustave Roussy; CLB = Centre Leon Berard; HOPD = Hopital Debrousse; NANC = Hopital Nancy; BES = Hopital Besançon;
M = Male; F = Female; BM = Bone Marrow; CNS = Central Nervous System; CPM = Cyclophosphamide;
ADR = Adriamycine; MTX = Methotrexate; ASP = Asparaginase; BCR = Vincristine; PRED = PRednisone; ARAC = Aracytine; VP16 = Etoposide;
CDDP = Cisdichloroplatinum; BLEO -= Bleomycine; BCNU = Bichloronitrosourea; TG = Thioguanine; MG = Methylgag; IFOS = Ifosfamide; PR
= Partial Response; CR = Complete Response; NE = Non Evaluable; PD = Progressive Disease; CSF = Cerebrospinal Fluid; WBC = White Blood
Count; POLY. = Polynuclears; ABMT = Autologous Bone Marrow Transplantation; Nov. = November.
* = Theroic date of graft, ** = Previously reported in references 3 and 16.

Statistics

Actuarial disease free survival curves were not possible because patients had persistent disease at time of ABMT. Actuarial progression free survival curves were plotted according to the method of Kaplan and Meier (18) until evidence of progressive disease was documented. Analysis was performed as of July 1st, 1988 and deaths from toxic effects were included.

RESULTS

Two patients (IGR4 and IGR12) died in progressive disease. Patient IGR12 was clearly a partial responder to 3 months of induction therapy but despite BACT showed a blastic meningitis at day 13 post ABMT. Patient IGR4 was in clinical CR when discharged from ABMT and died with a local abdominal relapse at day 60 post ABMT.

The other 15 patients were in clinical CR after ABMT. Two (BES1 and IGR3) died in CR from therapy related complications (Table 1) whereas 13 are still disease free from 8 months to 6 years, 3 months post ABMT. Median observation time for the 17 patients is 4 years, 3 months and median observation time for the survivors is 27 months. The failure rate for response in the whole group is 11%. In the group of 5 patients with neurological symptoms, 4 out of 5 achieved clinical remission of their symptoms from day 10 (3) to day 30 (1) post-ABMT whereas one progression was observed.

Recovery after high dose therapy was rather quick with a median of 14 days to recover 1000 white cells, 18 days to recover 500 granulocytes and 29 days to recover 50,000 platelets. Patient CLB9 showed also a quick recovery but without ABMT as previously described (19).

Morbidity was high in this small group of patients with only a median of 4 months of therapy before ABMT and with no total body irradiation in the conditioning regimen. Six of 17 had life threatening complications (3 pneumonitis with ventilation, 1 emboli, 2 bleeding including 1 CSF and 1 with immediate surgery needed) and 3 had minor complications (Table 1: 2 herpes, 1 staphylococcus infection). No patients are alive with sequelae and the quality of life for survivors is excellent.

As shown in Figure 1, the overall survival plateau is 71% at a median of 27 months post ABMT. If toxic deaths in CR are considered as censored-data, as in Figure 2, the progression free survival is 81% at 51 months.

Figure 1. ABMT in PR (overall survival)

DISCUSSION

Survival for patients responding to initial chemotherapy but not in remission after induction is very poor in all published reports with no more than 5% alive disease free at 2 years (5, 8, 9, 10). It must be remembered that several situations can mimic the presence of lymphoma, and bulky abdominal or mediastinal masses at presentation can particularly cause problems. After therapy these masses can shrink without complete disappearance (7). Re-biopsy has shown that frequently these residual masses consist of just fibrotic tissue without any viable tumor explaining why patients reported with no CR after induction become long-term disease free survivors. Only 25% of these residual masses represent active disease (7, 20), but in this group the survival is extremely poor (5, 7, 20).

In this report, 10 patients had surgically proven active disease in the abdomen after induction. One relapsed, two died of therapy related toxicity and 7 are alive disease free. Despite the small number, this appears better than any published report on such a group of patients. The 2 patients with marrow disease also showed active minimal residual disease after induction and are alive disease free after high dose therapy. The 5 patients with neurological symptoms were not proven to have active disease except CLB14 where lymphoma cells were shown in CSF before ABMT.

figure 2
NHL ABMT in PR
Progression free Survival
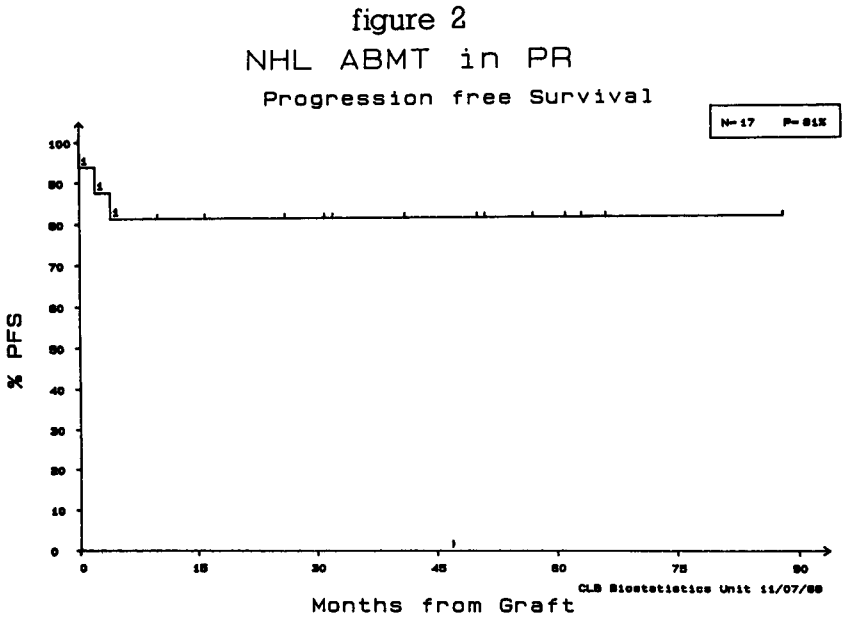
Figure 2. ABMT in PR (survival without evidence of progression)

However, one patient progressed at day 13 post ABMT and was, with no doubt, a very good partial responder to 3 months of induction therapy. The 3 others had facial palsy and the clinical symptoms disappeared 10 days (for 2 patients) and 30 days (1 patient) post BMT. We believe this to be a good evidence of active disease and also of response to high dose therapy.

Although a toxic death rate of 11% may be acceptable in the setting of high dose therapy and ABMT, the very high morbidity rate of this group of patients is of great concern. However, it is not wise to reduce the dose of these conditioning regimens. Biron found with BEAM a reduction of dose was associated with a reduction of response rate (14). The conclusion of this report is clearly in favor of high dose consolidation for partial responders after front line induction therapy. Because of the high morbidity with ABMT, it would be of value to study prospectively the respective role of one course of massive therapy with ABMT versus 2 or 3 courses of a less aggressive, well-defined salvage regimen. This should be done in a prospective and randomized study.

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OPTIMAL TIMING FOR ABMT IN INTERMEDIATE- AND HIGH-GRADE NON-HODGKIN'S LYMPHOMAS

Enzo Pavone, Frank Chauvin, Pierre Biron, and Thierry Philip

Even though malignant lymphomas can be considered among the most sensitive disorders to chemotherapy and radiation therapy, there still exists a significant fraction of adult patients for whom intensive therapy and bone marrow transplantation can be discussed. Advances in the area of bone marrow transplantation have been associated with numerous reports of very promising pilot studies (References in 1). However, no clear indication based on a randomized study is found in the world literature. The purpose of this review is to define the optimal timing for autologous bone marrow transplantation in intermediate and high grade non Hodgkin's lymphomas (NHL).

Fifty diffuse NHL are observed every year for a population of 1 million adults (2). Twenty of them are observed in patients over 60 years old at diagnosis and 10 are localized at time of first symptoms. Thus only 20 diffuse NHL/year/1 million adults can be considered for bone marrow transplantation (BMT). Even with restricted indications, as many as 7/year/1 million can be considered for BMT in first complete remission, 2/year/1 million for BMT in first partial remission, 4/year/1 million for BMT in sensitive relapses, 2/year/1 million for BMT in nonexplosive resistant relapses and possibly also one additional 2/year/1 million adults will mean between 300 and 500 indications in France and between 1,200 and 2,000 indications in the United States (1,2). As a comparison, only 700 BMT for lymphomas were recorded in the world between 1981 and 1985 and only 500 autologous bone marrow transplantations were performed in France in 1987 (highest number of ABMTs in one single country in Europe) (2). THe necessity to clearly define indications for BMT in this disease is then clear and could be considered as an emergency for public health in developed countries.

OPTIMAL TIMING FOR ABMT IN CR1 FOR INTERMEDIATE
AND HIGH GRADE LYMPHOMAS

Optimal timing for ABMT in Burkitt lymphomas was previously extensively reviewed (3,4). Optimal timing for lymphoblastic lymphomas will be reviewed in detail by Milpied at this meeting. However, intermediate grade lymphomas according to the international classification are the most common NHL in adults (5). The majority of reported regimen are able to produce 60% long term survival in this group (6). It is important to consider therefore that 10% of the patients will never reach first CR and will progress on induction therapy, that 8% will only be in partial response after induction and that approximately 8% will die early of toxicity (6). If these patients and patients over 60 years old at diagnosis are excluded, survival curves of 70-75% are common and indications for BMT very unsecured.

The selection of bad prognosis groups is thus mandatory if BMT is considered in first CR. It is now widely accepted to define as candidates for prospective studies patients less than 55 years old at diagnosis, with at least 2 extra nodal localizations or a tumor of at least 10 cm at diagnosis, with a bad Karnofsky (< 70%) or with bone marrow or CNS disease at initial presentation (6). This group is reported with an expected survival with conventional regimen of 55% at 3 years (B. Coiffier, personal communication). Only prospective and randomized studies are acceptable in this field. They should avoid protocols with high toxic death rates and they should include at least 150 patients in each arm. The European NHL groups are currently on the way to study in a randomized multi-institutional European trial this group of patients (C. Gisselbretch, Chairman).

NO INDICATION FOR ABMT IN PRIMARY REFRACTORY
PATIENTS EXCEPT PROSPECTIVE EXPERIMENTAL STUDIES

The term refractory lymphoma has frequently been utilized in an ambiguous context and thus needs to be better defined. In describing the results of salvage studies, the frequently used statement "patients who have failed front line regimens" is not appropriate. The setting in which these patients "failed" is much more important than the fact they failed. Those patients who fail to achieve a major response to front line chemotherapy regimens are without doubt the best example of refractory disease.

As shown in Figure 1, 34 such primary refractory patients were reported in 1987 from major BMT centers in the world (1). These 34 patients who never entered complete remission in the course of their disease underwent transplantation while their disease was progressing during conventional or salvage therapy. Two patients died early,

34 Primary Refractory NHL

(Philip et al. New Eng J. Med. 1987 316 1493-1498)

N=34   P=0%

Response Rate 75% CR rate 9/32   28%
PR rate 16/32   47%
Median duration of response 180 days

% Survival

CLS Biostatistics Unit 13/07/88
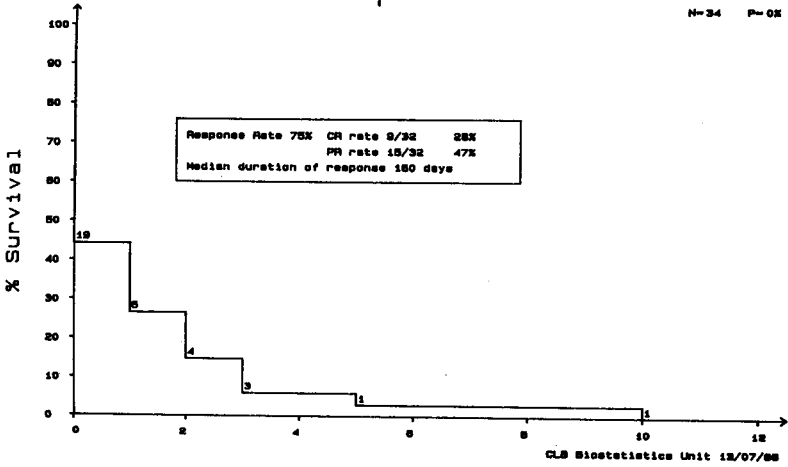
Figure 1. 34 Primary Refractory NHL

NHL ABMT in PR

Event Free Survival

N=17   P=71%

% Event Free Survival

Months from Graft

CLS Biostatistics Unit 11/07/88
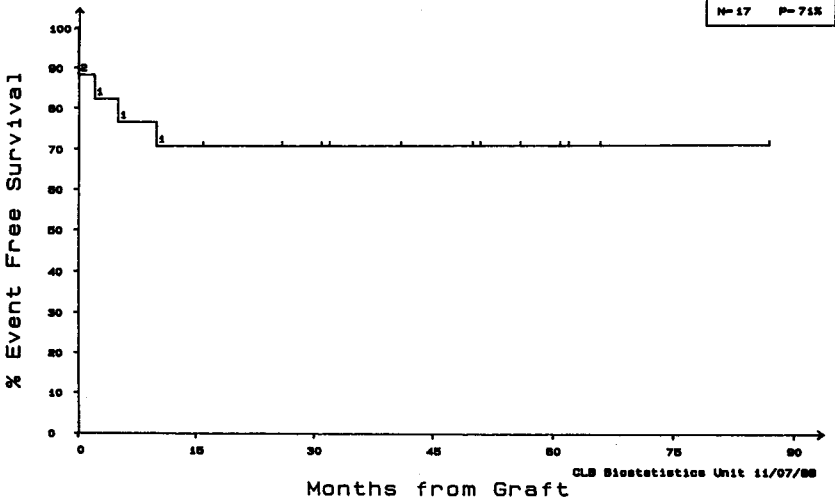
Figure 2. NHL ABMT in PR Event Free Survival

before their response could be assessed, and they were excluded from the evaluation. Twenty-four of the remaining 32 responded to high-dose therapy (9 had a complete response, 15 a partial response, and 8 no response; response rate, 75%), confirming the dose-response effect even in patients with highly resistant disease. However, the median duration of response was only 160 days, and no patient was alive and free of disease at three years (Figure 1). In 1988, these patients will not be cured with BMT and, despite their very high response rate, they are not good indication for BMT.

PARTIAL RESPONDERS TO FIRST LINE INDUCTION THERAPY AND CHEMO-SENSITIVE HIGH-RISK PATIENTS. THIS IS PROBABLY THE BEST INDICATION FOR BMT IN NON HODGKIN'S LYMPHOMAS

The best reported results in the world literature for patients in PR after induction are Coiffier's with 27% survival (B. Coiffier, personal communication from NHL 85). The MBACOD group had reported 14% of such patients alive at 2 years and the ProMace-Mopp group 15% survival at 30 months (see Hartmann, this meeting, for reference). Before undertaking treatment for partial responding lymphoma, it is mandatory to establish as certainly as possible the existence of such a problem (7). An effort to obtain tissue to establish the presence of tumor should be a requisite.

As shown in Figure 2 and as reported in this meeting by Hartmann, pilot studies with ABMT were able to report 71% disease free survival at 90 months for a group of 17 patients, all with proven active lymphomas at time of BMT. These preliminary results should be confirmed but BMT can be strongly recommended in 1988 for these patients if a biopsy shows active lymphomas after 4 courses of a conventional induction regimen.

OPTIMAL TIMING FOR ABMT IN RELAPSES OF NHL

This group of patients was extensively reviewed previously by our group (1,5,6). In summary, two distinct situations are observed.

- Patients who previously reached CR1 on conventional rescue protocol are called RESISTANT RELAPSES. Twenty-two of such patients were reported in 1987, as shown in Figure 3. Response rate was good (i.e. 59%) with quite high CR rate (45%). However, survival for this group is poor (15%) and should be improved, Considering an individual progressing at relapse under conventional therapy, BMT is probably the only chance of cure and can be highly recommended. An

22 RESISTANT RELAPSES

(Philip et al. New Eng J. Med. 1987 316 1493-1498)

N=22   P=14%

Response Rate 58%   CR rate 10/22   45.5%
                    PR Rate 3/22   13.6%
Median duration of response 160 days

% Survival

CLB Biostatistics Unit 12/07/88
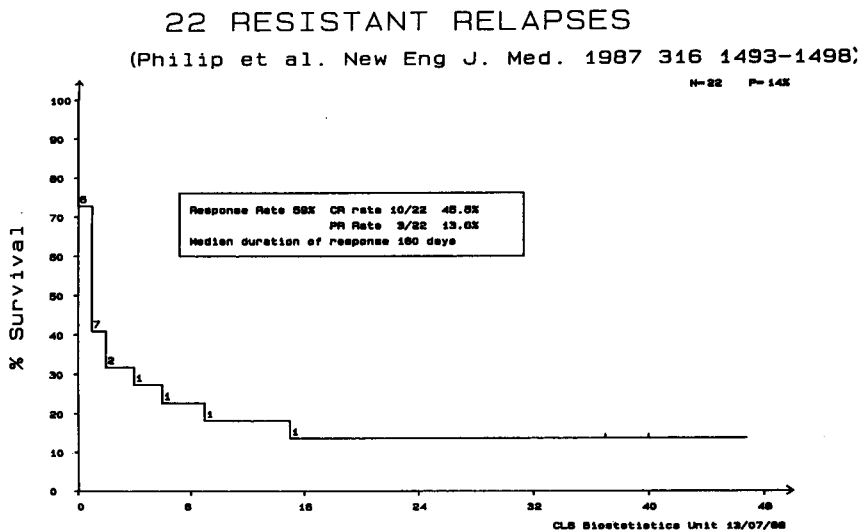
Figure 3. 22 Resistant Relapses

improvement of these results can be expected with the use of biological response modifiers immediately after transplantation.

 - Patients who previously reached CR1 on conventional therapy and then relapsed and who are still responding to conventional rescue protocols are called SENSITIVE RELAPSES (1). As shown in Figure 4, 43% are in CR2 at time of BMT and 86% of the others are transformed to CR2 with the conditioning regimen.   Forty percent of these patients are long term survivors after BMT with a price of 20% toxic deaths (1). Reports of 20% survival at 2 years in this group without BMT (5) (Coiffier, personal communication) and a high prevalence of this group in ABMT reported pilot studies has led to a large questioning about selection criteria (6). A randomized study (i.e. Parma protocol) is currently raising the question of the role of BMT in this group (see the Parma protocol in this conference report).

   In conclusion, sensitive relapses are probably very good group for BMT but randomized studies are need to show whether BMT is the best available strategy for this group of patients.

44 SENSITIVE RELAPSES

(Philip et al. New Eng J. Med. 1987 316 1493-1498)
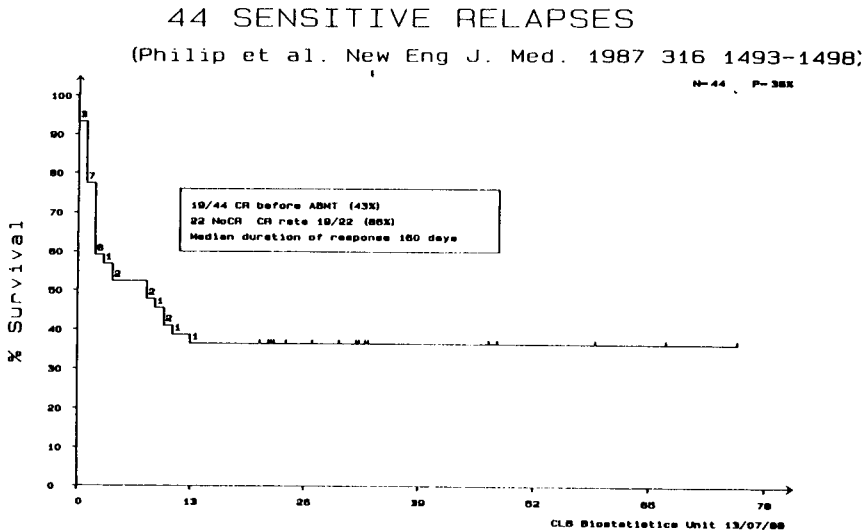
Figure 4. 44 Sensitive Relapses

CONCLUSIONS AND PROSPECTIVE

Indications for BMT in non Hodgkin's lymphomas as reviewed in this paper can lead to 2,000 or 3,000 BMT every year in the United States. Randomized studies are needed to convince the medical community and also ... insurance companies. In August 1988:

- Randomized studies are necessary and welcome. They should all be considered as high priorities.

- PR with positive biopsy and sensitive relapses are the only group of patients in which BMT may be the best therapy available for diffuse lymphomas, despite the lack of proof with prospective studies.

- Primary refractory patients and resistant relapses are not good indications and should be a group eligible for phase II studies.

- Non-randomized studies in CR1 are probably unethical and certainly unwise.

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AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) IN NON-HODGKIN'S LYMPHOMA (NHL) AND HODGKIN'S DISEASE (HD): The Role of Tumor Cell Resistance versus Tumor Bulk in the Timing of Therapy

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Patients with intermediate or high grade non-Hodgkin's lymphoma (NHL) who fail primary combination chemotherapy -- either by failing to achieve a complete remission (CR) or relapsing after an initial CR -- are rarely cured by conventional-dose salvage therapy(1). A similar generalization can be made for patients with Hodgkin's disease (HD) who relapse within one year of primary therapy or fail after the first conventional-dose salvage regimen(2). On the other hand, supra-intensive therapy along with autologous bone marrow transplantation (ABMT) has resulted in the long-term survival and probable cure of 20-60% of patients(3-5). A number of questions need to be answered before this approach to the treatment of lymphoma can be optimized. This report of patients with NHL and HD treated with ABMT at our institution will focus on the implications of tumor sensitivity and tumor bulk for the timing of ABMT in lymphoma.

CLINICAL STUDY

Between May of 1984 and June of 1987, 12 patients with intermediate or high grade NHL and 7 patients with HD were treated with ABMT. Selection criteria were: failure of primary therapy, age <60 years, adequate marrow cellularity with no microscopic tumor, and no intercurrent infection. The median age of this group of patients was 28 years (range 9-56) with 14 male and 5 female patients. Most patients had relatively advanced disease at the time of diagnosis;

11/19 with stage IV, 13/19 with B symptoms, and 5/19 with marrow involvement. All patients had failed prior chemotherapy, with 13/19 patients receiving more than one regimen. Prior regional radiotherapy was given to 7/19. Most importantly, 18/19 patients began their transplant regimen with measurable or evaluable disease.

The preparative regimen consisted of total body irradiation(TBI) followed by cyclophosphamide (CY) -- the order followed the scheme devised by Memorial Sloan-Kettering Cancer Center(6). Regional radiotherapy (1200-2000 cGy) to areas of bulky disease was administered in 9 patients prior to their preparative regimen. The TBI was given twice daily for 4 days(185 cGy fractions) followed by high dose CY (60 mg/kg x 2 in the first 7 cases and 50 mg/kg x 4 in the next 12 cases). Cryopreserved autologous bone marrow was infused 48 hours after the last dose of CY. Post-transplant care was as previously described (7).

The analysis of results focuses on 2 features of the patients' status at the time of ABMT -- the clinical sensitivity of disease and the tumor bulk. Three levels of clinical sensitivity were defined: primary refractory(R1) patients failed to achieve a CR on initial therapy, secondary refractory(R2) patients achieved a CR but relapsed and progressed on conventional-dose salvage treatment, and sensitive(S) relapsing patients achieved an initial CR and either responded to salvage therapy or were not tested.

Four of the group were classified as R1, 5 as R2, and 10 as S. Bulky tumor was defined as a tumor mass >3 cm(TB+). Thirteen patients were TB+, 5 were TB- but with measurable disease and 1 patient was in second CR after reinduction therapy.

Two outcome variables were analyzed; initial response and continuous complete response (CCR) or relapse-free survival(RFS). There appears to be no difference in outcome between HD and NHL and the diagnoses are combined in the results presented.

RESULTS

The major treatment-related complication was infection with 3 patients dying within 2 months of ABMT. Two of these patients had tumor at post-mortem and are non-responders. One of the early deaths was a complete responder, with no microscopic tumor at post-mortem. One patient, who had a CR, developed fatal pneumocystis pneumonia and ARDS at 6 months.

Initial response was as follows. Five of the 19 patients were non-responders; 2 died of early infection, 2 died of progressive tumor, and 1 patient is alive with slowly progressive disease after a partial response. Among the 15 complete responders; 2 died of infection, 1 relapsed and died of progressive tumor, 1 patient had a local relapse outside the margin of his regional radiotherapy and is alive in

subsequent continuous remission after surgical resection and additional regional radiotherapy. Ten patients remain in CCR. These patients are shown in Figure 1, which plots the actuarial RFS of the entire group -- with a plateau at approximately 55%.

The impact of the clinical sensitivity of tumor and tumor bulk at the time of bone marrow transplantation to the response variables is shown in Tables 1 and 2. The first table shows the relationship of tumor bulk (TB) on initial response and long-term outcome. Table 2 shows the relationship between tumor sensitivity (TS) on initial response and long-term outcome. Figure 2 shows the impact of these two variables on the actuarial RFS.

DISCUSSION AND CONCLUSION

This series confirms other studies, showing a significant salvage rate for patients with malignant lymphoma treated with ABMT (1-2). It should be pointed out that this result is achieved at the cost of significant treatment-related mortality. It is unlikely, therefore, to be applicable to initial remission patients unless prognostic factors can reliably identify complete responders who have a high probability of relapse -- in the range of 70-90%.
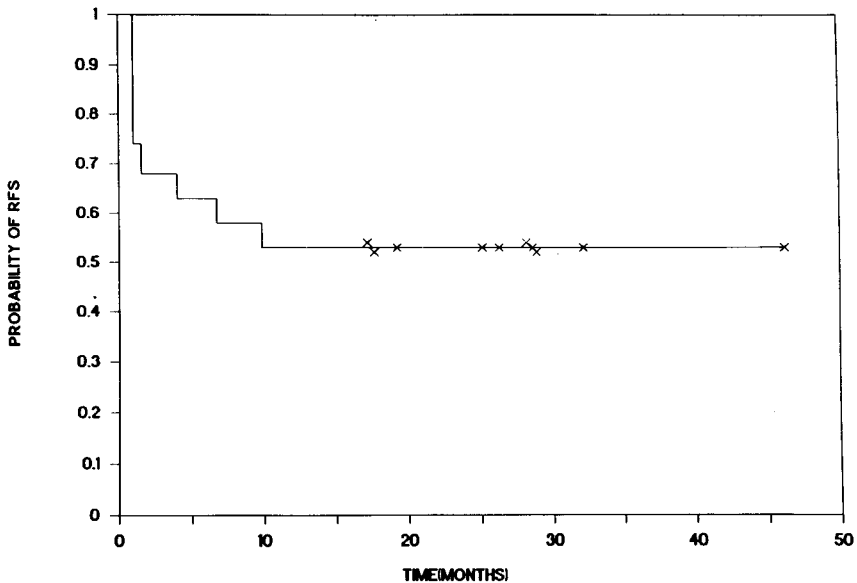
Figure 1. Actuarial relapse-free survival (RFS) of entire group. Symbols on curve indicate censored patients.

**Table 1. Initial Response and Long-Term Outcome (RFS) of Patients
with and without Bulky Tumor at the Time of ABMT**

	Initial Response		Long-term Outcome	
Tumor Bulk	CR	NR	CCR	Other
TB-	5	1	5	1
TB+	9	4	5	8

Abbreviations: TB- =   bulky tumor absent
                TB+ =   bulky tumor (>3 cm) present
                CR   =   complete response
                NR   =   no response (includes partial responders)
                CCR =   alive and in continuous complete remission

**Table 2. Initial Response and Long-Term Outcome (RFS) of Patients
with Sensitive Versus Primary Refractory Vers Secondary
Refractory Tumor at the Time of Relapse Prior to ABMT**

	Initial Response		Long-term Outcome	
Sensitivity	CR	NR	CCR	Other
S	9	1	8	2
R1	4	0	2	2
R2	1	4	0	5

Abbreviations: S   =   sensitive tumor at relapse
                R1   =   primary refractory relapse
                R2   =   secondary refractory relapse
                CR   =   complete response
                NR   =   no response (includes partial responders)
                CCR   =   alive and in continuous remission
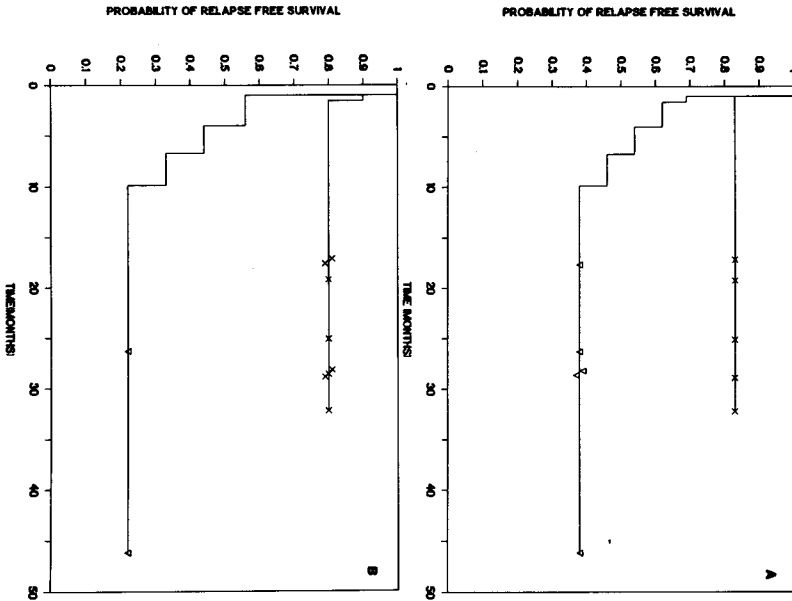
Figure 2. Actuarial RFS of patients grouped according to prognostic variables. A) RFS for patients with (∇)versus without (x) bulky tumor, B) RFS for patients with sensitive (x) versus refractory (∇)tumor at time of relapse.

The principle focus of this discussion concerns the need for conventional dose reinduction therapy to establish minimal residual disease prior to marrow-ablative ABMT regimens. Tumor mass and tumor sensitivity to cytoreductive therapy are clearly related to the success of ABMT(8-9). The assessment of the independent role of these two variables is difficult, since they are closely correlated. For example, all of our refractory patients had bulky tumor. In the absence of additional evidence, we believe that the optimum time to employ effective marrow-lethal regimens is at the earliest possible opportunity -- before disease-refractory cells have been selected by treatment that is less than maximally intensive. We contend that the lessons taught by BMT in treating AML have frequently been misinterpreted. Since the best results for allogeneic BMT in AML have been obtained in first CR, it has been assumed that this result relates to treatment at a time of minimal residual disease. On the other hand, data from Seattle suggest that allogeneic BMT in initial relapse is at least as good as in second CR (10), suggesting that tumor mass may be less critical than tumor sensitivity.

Although our small series cannot prove this contention, we believe two features of the results are consistent with this conclusion. First,

our overall RFS of 55% is equivalent or better than most reported series -- in a group where 18/19 patients began ABMT with measurable or evaluable disease. Second, the difference between RFS for patients with sensitive versus refractory relapses is larger than the difference in RFS of patients who are TB- versus TB+. Although tumor mass may play an independent role in the outcome of ABMT, the potential confounding of this variable with tumor sensitivity requires controlled, prospective clinical trials to determine the independent role of these two variables.

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AUTOLOGOUS BMT FOR PATIENTS WITH POOR-PROGNOSIS LYMPHOMA AND HODGKIN'S DISEASE

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INTRODUCTION

Clinical trials were initiated at Memorial Sloan-Kettering Cancer Center in 1981 to evaluate the role of intensive chemotherapy followed by autologous bone marrow transplant (AuBMT) for patients with poor-prognosis Lymphoma, Hodgkin's disease and Acute Non-Lymphoblastic Leukemia (ANL). The following two subgroups of patients with non-Hodgkin's lymphoma were identified as having poor-prognostic features in our study: [1] newly diagnosed patients with large-cell lymphoma presenting with bulky mediastinal and/or abdominal tumor mass >8 x 8 cm, or patients with a LDH level >500 U/mL, and [2] patients who had failed aggressive initial combination chemotherapy trials. Our initial AuBMT trials involved the use of hyperfractionated total body irradiation (HF-TBI) and cyclophosphamide (CTX). In our previous trial, the results of AuBMT performed in first remission were compared with a historical control group of patients with similar prognostic features who were treated with conventional chemotherapy (1). The results of AuBMT performed in remission were also compared prospectively with AuBMT performed after failure or relapse on combination chemotherapy. The data showed an improvement in survival for patients who received AuBMT in remission. The patients who got transplanted after relapse showed a significant subsequent relapse rate (1). Therefore, attempts were made to decrease the relapse rate by adding VP-16 to the previous conditioning regimen of total body irradiation and cyclophosphamide (2).

Most previous trials involving AuBMT have involved patients who

have failed conventional chemotherapy, and have resulted in 20% to 30% of patients achieving long-term survival (3-8). Most of the protocols have used hematoablative dosages of cyclophosphamide before total body irradiation (3-8). At this institution, the use of hyperfractionated total body irradiation (HF-TBI) followed by cyclophosphamide is felt to reduce the incidence of interstitial pneumonitis in patients with leukemia undergoing allogeneic BMT, and the doses used were hemato- and immunoablative (2,9). In this study, we use the same combination of HF-TBI and cyclophosphamide for patients with lymphoma, but the patients were rescued with an autograft instead of an allograft. The data suggest that the new regimen of HF-TBI, VP-16, CTX is superior to our previous HF-TBI, CTX protocol (10). Preliminary results also show significant improvement in the survival of patients with refractory or relapsed Hodgkin's disease when AuBMT is performed after conditioning with [A] Total Nodal Irradiation (TNI), VP-16, cyclophosphamide regimen or [B] BCNU, VP-16, cyclophosphamide regimen (11,12). Results of our clinical trials for ANL are also briefly detailed (13).

PATIENTS AND METHODS

Fourteen were previously untreated patients (Arm 1) with poor-prognosis large-cell lymphoma who received L-17M induction chemotherapy with AuBMT as initial treatment. Seventeen had AuBMT after relapse (Arm 2) from various chemotherapy protocols and received HF-TBI and CTX 23 patients received HF-TBI, VP-16, CTX (Arm 3). The data for Arm 1 and Arm 2 has been published in detail (1). Details for patients on Arm 3 are provided in this paper. The data analysis is as of July 31, 1988. The patients were evaluated at Memorial Hospital and, after providing informed written consent, were treated with Institutional Review Board (IRB) approved protocols. Staging work-up included confirmation of pathological diagnosis and stage. The highest pretreatment serum LDH level (normal up to 225 IU/mL) recorded before any treatment is listed in Table 1 for Arm 3. Bone marrow (BM) aspirate and biopsy specimens were evaluated for cellularity, morphological presence of lymphoma, colony-forming unit-granulocyte macrophage (CFU-GM) and burst-forming unit-erythroid (BFU-E) growth, and surface marker analysis for immunoglobulins as described (1).

Bone marrow harvest was performed under general anesthesia and heparinized BM was enriched for mononuclear cells by unit gravity sedimentation in the presence of 0.8% hydroxyethyl starch (HES). BM harvest with no suggestion of involvement by lymphoma at presentation was cryopreserved in 6% HES, 5% dimethylsulfoxide (DMSO), and 4% human albumin at 40 to 50 million cells/mL (14). Patient with probable BM involvement at presentation had BM

Table 1. Characteristics of Lymphoma Patients Undergoing TBI, VP-16, CTX with AuBMT

Patient	Age, Sex	Histo-logy	Clinical Stage at Presentation	Initial LDH	Initial Marrow Involved	No. of Prior Rx Programs Protocol (Response)	CR achieved pre-AuBMT	LDH pre-AuBMT	Pre-AuBMT Marrow Involved	Sur-vival (mos.)
1 JC	35,M	N+DML	III	192	ND	2 COP-BLAM(PR); CVP(PR)	No	154	ND	43.2+
2 RM	39,M	DHL	IV	280	Yes	3 L17M(MR); m-BACOD(PR); RT	No	241	ND	34.5
3 KB	33,M	NPDL	IV	NA	Yes	3 CVP(PR); COP-BLAM(MR); RT	No	161	ND	30+
4 BP	42,M	DHL	III	1632	Yes	3 BACOD(CR); m-BACOD(PR); RT	No	171	ND	30+
5 JP	26,M	DHL	IV	321	ND	2 m-BACOD(CR); L-20(CR)	Yes	150	ND	27.3+
6 KS	32,F	DHL	IV	NA	Yes	2 L-17,m-BACOD(CR); L-17(CR)	Yes	158	ND	26.9+
7 DB	39,M	N+DML	III	>600	ND	2 CHOP(PR); COMLA(PR)	No	155	ND	26+
8 WM	35,F	NPDL	IV	WNL	Yes	2 CHOP(PR); Pro-MACE-MOPP(CR); CHOP(Consolidation)	Yes	140	ND	24.8+
9 JL	42,M	DHL	III	NA	ND	2 m-BACOD(PR); L-20(PR)	No	191	ND	18.2+
10 WP	29,M	NPDL	IV	173	Yes	2 CVP(PR); CHOP(CR)	Yes	217	ND	17.2+
11 RSc	40,M	DML	II	186	ND	1 MACOP-B(PR)	No	160	ND	15.1+
12 HEc	44,M	NPDL	IV	NA	Yes	2 MACOP-B(PR); CHOP(CR)	Yes	199	ND	6.9+
13 TM	25,F	NPDL	III	125	ND	2 Pred(PR); RT	No	306	ND	6.2+
14 JF	42,M	DHL	II(Bulky)	NA	ND	3 m-BACOD(PR->PD); L-20(PR); CDDP/VP-16(PR)	No	163	ND	1.8+
15 CH	20,M	DHL	IV	NA	ND	2 MACOP-B(PR); CHOP(PR)	No	181	ND	1.3+
16 MS	44,M	NPDL	I	NA	ND	6 RT; CytPred(MR);CHOP(MR); COP-BLAM(PR); RT; BleoVcr	No	211	ND	3.4
17 LB	38,F	DHL	II(Bulky)	NA	ND	2 MOPP&CHOP(PR); m-BACOD(PR)	No	349	ND	2.7
18 RD	30,M	DHL(T)	II(Bulky)	NA	ND	2 COP-BLAM(CR); COP-(B)LAM(PR)	No	162	ND	.5
19 SM	42,F	DPDL	IV	NA	ND	4 CHOP(PR); Ara-C/MTX(PR)	No	174	ND	.5
20 RSm	33,M	DHL	IV	NA	ND	3 m-BACOD(PD); L-20(PR); RT	No	179	ND	.4
21 JR	40,M	DHL	IV	405	ND	4 MACOP-B(PR); V-VAMP(PR); PlatAraCDex(MR); RT	No		ND	.3
22 AZ	45,M	DHL	II(Bulky)	NA	ND	2 CHOP(CR); COPVP16Bleo(PR)	No	161	ND	.3
23 HEa	20,F	DHL	IV	513	ND	3 RT; m-BACOD(?CR); CHOP(PR)	No	269	ND	.2

aliquoted into two parts. Sixty percent of the BM was purged with 4-hydroperoxycyclophosphamide (4-HC) (100um) and 4.25 or 8.5 of VP-16 (15-17) for 60 minutes at 37°C and cryopreserved; this aliquot was used first for hematopoietic reconstitution of the patient. The other 40% was cryopreserved to be used as back-up for the purged BM. Cell dose and viability of the BM is summarized in the Table 3 for Lymphoma patients receiving TBI, VP-16, and CTX. After marrow harvest, patients received one or two cycles of combination chemotherapy. Often these patients were reinduced before AuBMT. The therapy used for reinduction and its response are also detailed in Table 1. Patients with residual tumor mass after induction chemo-therapy received radiation boost to the area of bulky disease (usually 300 rad/d for four days) before initiation of cytoreduction. Patients were then admitted to single rooms for transplant. HF-TBI (total dose 1320 rad) was administered in 11 fractions over four days. Followed by VP-16 250 mg/m^2 daily for three days. After vigorous hydration, patients received cyclophosphamide IV for two days at 60 mg/kg/d. Cardiac and urinary functions were carefully monitored, and after 48 hours cryopreserved BM was thawed in a water bath at the bedside and rapidly reinfused without any further treatment. Hydrations was maintained until hemoglobinuria subsided (the few RBCs present in the cryopreserved BM lyse during the thawing procedure).

Patients with relapsed or refractory Hodgkin's Disease (usually after multiple drug combinations ± RT) had the BM harvested and received 1-2 months of conventional salvage treatment with an attempt to reduce tumor burden. Sites of bulky disease were considered for boost RT (1200 rads over 4 days) prior to beginning conditioning regimen as described in Table 2 (11,12). Patients with AML received the same therapy as patients with HD described above, all patients with AML received 4-HC, VP-16 purged BM (13, 15). Moderate to severe mucositis was managed with frequent mouth care and antifungal medications. Patients were placed on total parenteral nutrition until they were able to eat. All blood products were irradiated to 3,000 rad to prevent transfused lymphocyte mediated graft vs. host disease. Patients usually required a 5 to 6-week hospital stay and were then followed in the outpatient clinic with physical and laboratory examination until full recovery. Post-AuBMT prophylaxis for Pneumocytis carinii pneumonia consisted of double strength trimethorprin sulfamethoxazole orally twice per day begun on day 50 for a total of 90 days, with frequent monitoring of the complete blood count (CBC).

RESULTS AND DISCUSSION

The characteristics of all patients who received AuBMT are described in Figures 1 - 3. Fourteen patients with poor-prognostic

Table 2. Conditioning Regimens for Patients with Relapsed or Refractory Hodgkin's Disease

Days	If No Previous RT Prior to BMH	If Previous RT
-10	TNI 167 rads TID	--
-9	TNI 167 rads TID	--
-8	TNI 167 rads TID	BCNU 250 mg/m^2 IV
-7	TNI 167 rads TID	VP-16 250 mg/m^2 IV
-6	TNI 167 rads TID	VP-16 250 mg/m^2 IV
-5	VP-16 250 mg/m^2 IV	VP-16 250 mg/m^2 IV
-4	VP-16 250 mg/m^2	VP-16 250 mg/m^2 IV
-3	CTX 60mg/Kg IV	CTX 50mg/Kg IV
-2	CTX 60mg/Kg IV	CTX 50mg/Kg IV
-1	REST	REST
0	BM INFUSION	BM INFUSION

Table 3.

Protocol	# of Pts	Median No. Viable Cells /kgx10^8	Median Days for Count Recovery		
			WBC>1000	ANC>500	PLAT>50x10^3
NHL TBI+CTX no purge	17	3.42(1.62)*	12(10-19)	13(8-20)	23(13-40)
NHL TBI+CTX 4-HC purge	14	2.3(0.98)	17(11-42)	17(12-42)	30(22-121)
NHL TBI+VP-16, CTX no purge	6	2.75(1.5)	16.5(15-23)	16(13-24)	30.5(27-40)
NHL TBI+VP-16, CTX 4-HC & VP-16 purge	10	2.0(1.2)	14(11-31)	14(13-39)	40.5(28-67)
HD TNI+VP-16, CTX	12	3.1(1.4)	13.5(10-28)	14(10-30)	29(17-50)
AML TBI+VP-16, CTX 4-HC & VP-16 purge	10	2.0(1.4)	39(27-53)	37(16-43)	84(42-269)
HD BCNU, VP-16,CTX	12	2.8(1.5)	19.5(10-27)	19.5(10-27)	27.5(17-44)

*Minimum cell dose per kg x 10^8 in brackets
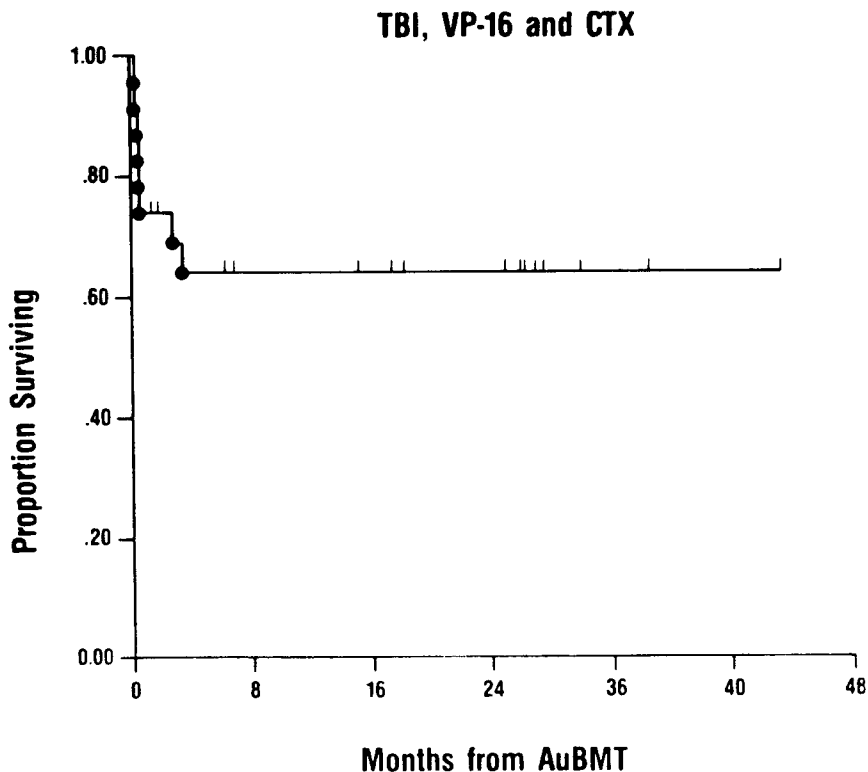
Figure 1. Lymphoma Patients Receiving TBI, VP-16, CTX, and AuBMT

features received AuBMT while they were in CR (6) or PR (8) after L-17M induction (Arm 1). Seventeen patients received AuBMT only at relapse/failure with HF-TBI, CTX conditioning (Arm 2). The survival of the two groups is detailed in previous paper (1). Eleven of the 14 patients in Arm 1 are disease-free with a median follow-up of 49+ months. The results on Arm 2 showed a very high relapse rate (8/15 patients) therefore, VP-16 was added to HF-TBI, CTX in our current regimen. So far, 23 patients have received transplant with this new regimen, and the results are presented in Table 1 and Figure 1. The new protocol of HF-TBI, VP-16 and CTX is slightly more toxic (predominantly mucositis) and has a very low relapse rate with a median follow-up of 24.8 months. The hematopoietic engraftment of patients on Arm 3 was comparable to patients on Arm 1 and Arm 2 and is summarized in Table 3.

Patients with Hodgkin's disease who have relapsed after multiple combination chemotherapy (no previous RT) received AuBMT with total nodal irradiation (TNI), total dose 2004 rads over 12 fractions in four days. VP-16 and cyclophosphamide conditioning regimen in a manner similar to the patients with non-Hodgkin's lymphoma
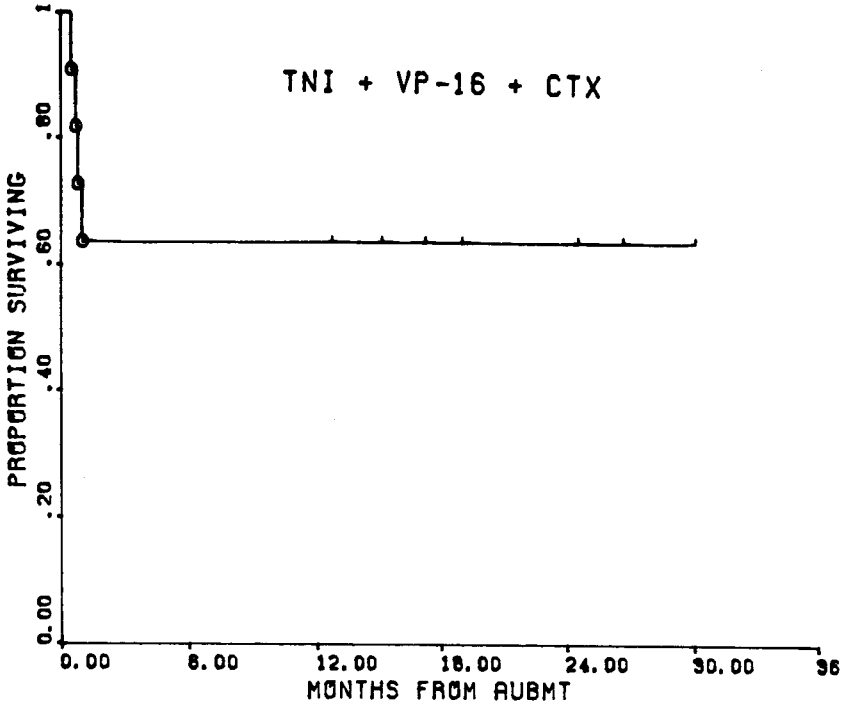
Figure 2. HD Patients Receiving TNI, VP-16, and CTX
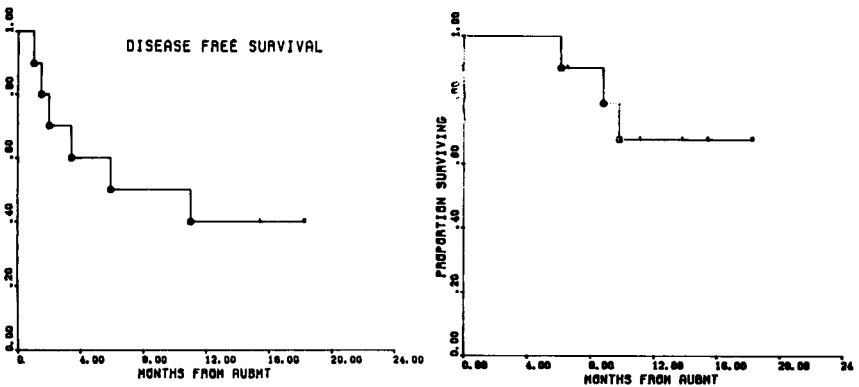
Figure 3. HD Patients Receiving BCNU, VP-16, and CTX

described above. The results are presented in Figure 2. Patients with Hodgkin's disease who have relapsed after radiotherapy and combination chemotherapy received conditioning regimen of BCNU, VP-16 and cyclophosphamide as described in Table 2 and the disease-free and overall survival is described in Figure 3. The number of patients with Hodgkin's disease entered onto the trial as described in Table 2 is too small to make a strong statement regarding the long-term benefit of one protocol over the other. So far, the patients entered onto the BCNU protocol tend to be heavily pre-treated and do have a slightly higher relapse rate.

Patients with Acute Myeloblastic Leukemia received HF-TBI, VP-16 and CTX as per the protocol for patients with non-Hodgkin's lymphoma (13). Overall 3/9 patients in second CR are alive at 41+, 24+ and 11+ months, one of two patients who needed high dose Ara-c plus antracycline to achieve 1st CR is alive for 33+ months and only one out of four patients in first CR is doing well with median follow-up of 6+ months. Several factors are responsible for the hematopoietic recovery of the patients. Various factors being investigated for hematopoietic reconstitution include the:

1) Effect of primary disease on subsequent engraftment.

2) Residual toxicity of therapy prior to BM harvest.

3) Quality and quantity of BM harvested, cell number, viability, CFU-GM and BFU-E dose, etc.

4) BM purging.

5) Effect of BM cryopreservation, it may even be different for malignant vs. normal hematopoietic cells.

6) Microenvironment during the time of early transplant effect of various infections, various drugs (especially antibiotics) and vitamins levels, etc.

As shown in Table 3, our engraftment data suggests that patients with AML take a longer time to engraft than patients with non-Hodgkin's lymphoma who received comparable conditioning and BM purging condition. The implication of the above finding is now being investigated in further detail.

In summary, our data suggests that VP-16 is a useful addition to the AuBMT conditioning regimens and has improved the disease-free survival of patients with relapsed lymphoma and Hodgkin's Disease. Further variants of the conditioning protocols detailed above will help optimize the use of AuBMT for various disease.

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Discussion 1 – Session II (Lymphoma)

Dr. Gulati: The hypothesis we have been going by is that if the purging conditions are not too toxic, and as long as we do not find any major toxicity in what we are doing, and consider we have reasonable lab data for this, we continue to purge the marrow if the marrow is involved. For example, in this group of 30 I showed you, there were 8 patients with nodular lymphoma and about half of them had a marrow which was grossly involved. By giving them chemotherapy the marrow had become negative. Yes, it is possible that the marrow -- if we had frozen and had not purged it -- would have been all right. But 7 out of those 8 nodular lymphomas are alive at this time and I think purging had some role.

Dr. Geoff Herzig: Are you still giving the involved field irradiation in large cell lymphoma before the TBI and are you getting much problems in the chest?

Dr. Gulati: Yes, we do use boost irradiation. About 60% of these patients do get it. Overall the success was around 70% but a few deaths did occur and people with residual disease got irradiation.

Dr. Kaizer: Just a quick clarification. On the VP-16, cytoxan and TBI protocol, outside of the regional radiation, did the patients receive any conventional dose chemotherapy to cytoreduce them before the bone marrow transplant?

Dr. Gulati: Yes. We have always believed that the transplant does not help with bulky disease. We always try to shrink the disease down. And, (considering) all the data I showed you, about 80% of the patients were responsive lymphomas to the therapy we chose. If one therapy did not work, we moved on an other. So, the major emphasis is in cytoreduction prior to transplant.

Dr. Gorin: Did you try your freezing technique on blast cells? And, I am curious to know what are the results.

Dr. Gulati: I clearly feel that the blasts behave a little bit different. We did not use fresh blasts, we used cell lines like HL60. There is a 2-3 log cytoreduction just by freezing and thawing in DMSO. But it is hard to technically and scientifically say that is a meaningful experiment. Clearly, if we could grow the blasts a little better it will be good to do that experiment with pure blasts. We do freeze all our cell lines and various fresh leukemia cells in this medium and it grows very well afterwards.

Dr. Jagannath: Subie, how many patients come referred to you for transplant, but actually go through alternative programs, do not respond and never make it to the transplant?

Dr. Gulati: I would say about 30%.

Dr. Keating: Two questions, 1) have you looked at long term culture generation from your cryopreservation technique and 2) what has been your experience with MACOP-B relapses using your protocol?

Dr. Gulati: I'll answer your second question first. The MACOP-B has been essentially no major problem to us. If they have failed MACOP-B we usually put them on the L17 type protocol, try to cytoreduce them and go ahead with transplant. In terms of the other question, we really (haven't) done too many long-term cultures after freeze thawing.

Dr. Goldstone: I am not so sure that the question of sensitive and resistant relapse which is accepted now for NHL is the same for Hodgkins disease. We do not give our Hodgkins patients a further therapy to see if they are sensitive or resistant. And there is no data for instance from the BMTG which suggests a difference between so called sensitive and resistant refractory patients with Hodgkins disease. Our finding is that you can convert more Hodgkins patients with so-called resistant disease into CR from high-dose therapy than you can with NHL.

Dr. Philip: As I recall, the data you presented at the European meeting did indicate a prognostic role for tumor bulk. And that may actually be a reflection of the resistance of the tumor to cytoreductive therapy.

Dr. Goldstone: Would anyone like to ask any of the speakers in this session about timing of graft for ABMT?

Dr. Dicke: Herb, you concluded that to try to achieve minimal residual disease with chemotherapy may lead to resistant clones. However, when you look at the data available already I think it is just the opposite. When you look again at the oat cell studies done by us, where we try to come in up front with high-dose combination chemotherapy in limited disease and compare these data to induction chemotherapy followed by the high-dose treatment, I think there are real significant differences between those two groups. There are differences in kinetics between the bulky disease and small disease. When we leave out the TBI and when you give drugs the accessibility of these drugs, bulky tumor is completely different than when you do that in minimal residual disease. So, I think there are arguments in favor of reducing the tumor.

Dr. Kaiser: Karel, I do not need to deny that tumor mass or tumor bulk may not be an important variable in determining the results of therapy. I think this needs to be subjected, however, to randomized clinical trials in order to answer the relative importance of tumor mass versus clinical resistance. Now in terms of your oat cell studies, for example, I would contend that you may just simply have selected the sensitive disease by the prior therapy and you were lucky not to have selected for enough resistance cells.

Dr. Phillips: Tony -- a comment about timing. Most of the results recorded have been with older primary chemotherapy regimens in our hands; (i.e.) CHOP, which produced remission in at least large cell lymphoma patients in 50 or 60% of the patients and cured 30%. I think the situation though has and is changing. The Vancouver results with MACOP-B, which now include nearly 200 large cell patients, suggest a complete remission rate of 85% and perhaps a cure rate of 65%. So I think when one says you cannot salvage patients with CHOP that do not have complete remission, you should not plan on salvaging these other patients. And the same thing might hold to Dr. Philip's comment about the partial responders. Someone who has a partial response to CHOP that might be curable might be someone who would be cured by MACOP bleo. And the final point is that if one chooses patients in first remission who have a 50% chance of relapse -- I have asked Joe Connors to calculate that -- he said that he thinks it is about 10% of his patients. It is acutally more than 10% of his patients, but his worst prognostic finding was age over 65 which is something we usually would not accept for transplantation anyway.

Dr. Goldstone: Thierry, many of the ideas that you and some of the rest of us developed 4 or 5 years ago about NHL and sensitive and resistant patients after therapy were always given the CHOP regimen, we don't know whether this is true with the newer regimens.

Dr. Philip: That is a very difficult question. First lets go to Burkitt's lymphoma disease which I know quite well. In Burkitt's lymphoma, five years ago the conventional chemotherapy regimen was about 40% survival rate and the salvage rate with ABMT at relapse was about 50%. Now, with conventional chemotherapy, the long-term survival rate is 85% and I just reviewed the 26 relapses out of 200 patients which occurred in the French LMB protocol ... a protocol with only Burkitt's lymphoma, only children, and with about 80% long term survivors. And, in this group of 26 relapses, which I think is the best current protocol in Burkitt's lymphoma, half of the patients were not grafted. Of those the majority of them died so quickly that they could not be grafted. In this group of non-grafted patients, some patients died 5 or 6 months after relapse. These, of course, should have been grafted. The other half were grafted. In the group of non-grafted patients, the survival is 0%. The other half was grafted and the survival is 30%. Then I think the salvage rate is less than it was before but I think it is very, very clear that in Burkitt's lymphoma, at the time of relapse, only aggressive therapy with BMT can cure this patient. For the intermediate grade lymphoma, I can tell you that with the current prospective trial of the PARMA study, the results are still the same and, if the patient came from -- lets say -- the DOT protocol, which is quite the best protocol currently available in Europe, the response rate at time of relapse is 50%. And I do not think it will change a lot.

Dr. Gulati: I just wanted to continue to answer Gordon Phillips question. Yes, we are using MACOP-B as a front line for lymphomas. Actually we have a randomized trial and all of our good risk patients go on MACOP-B. We have about 6 patients who have relapsed after MACOP-B who are going through transplant. Two of them are finished, the others are going to go through soon. So, with time, we will be able to tell whether MACOP-B was better now. But, so far, it does not look very good.

Dr. Velasquez: Let me just contribute to this discussion in terms of the relapse of lymphoma experience at MDAH. At this point, we have more than 220 patients treated with either DAP or EDAP which is just an addition of VP-16 to the original DAP regimen. At this point, over 60% of our patients are referred after failing MACOP-B and the possibility of responses are practically the same regardless of whether they have been treated with CHOP or MACOP-B before. One of the major concepts which we hear this morning -- I certainly agree completely with it -- is tumor bulk goes straight ... directly to the response to salvage regimen and probably to BMT. Secondly, the second factor for remission duration is the LDH. This counts in front line therapy as well as relapse therapy.

Dr. Frei: Yes, very briefly, my comment has to do not with the timing of the ABMT but rather with the radiotherapy. If there is a generalization in cancer chemotherapy, it is that previously irradiated tumors respond very poorly to chemotherapy. And, accepting that, it is the wrong time to give it. It is also a difficult time to give it because things are dynamic and field sizes and delivery rates and so forth are complex. And I would suggest that it would be given afterwards as I think somebody else suggested. If there is time for a question, it would be: is there evidence that irradiation of sites of bulk disease is changing relapse patterns? The relapse pattern is almost always at pretreatment sites of bulk disease.

Dr. Goldstone: The very heterogenous sort of inexact registry data that we have in ABMT for NHL appears to show, maybe, that if you get a CR from the transplant, it does not make any difference when you give radiotherapy after that. But if you get a PR and then convert some of these patients to CR with the radiotherapy that may make a difference. I do not know any other transplant data about that.

Dr. Philip: The question is very good. The only thing we know is that 75% of the relapse after BMT are at local size of initial bulky disease. I think very clearly from the world literature TBI containing regimen is comparable with the non-TBI containing regimen. And the second point is that we know -- and I agree with Tony -- that it does not work after BMT. Last point ... if you look at very old data, radiation is (the) best drug for NHL. No drugs have ever been able to give response rates comparable with radiation. That is why I think it is worthwhile to try radiation before ABMT to try to control the local disease. But that is not proven.

Dr. Goldstone: Thank you very much. We must close this session.

BONE MARROW TRANSPLANTATION FOR ADULT POOR PROGNOSIS LYMPHOBLASTIC LYMPHOMA

Noel Milpied, Norbert Ifrah, Mathieu Kuentz, Dominique Maraninchi, Philippe Colombat and Jean-Luc Harousseau

Lymphoblastic lymphoma (LBL) is now recognized as a separate entity, included in the high grade category of the Working Formation for non-Hodgkin's lymphoma (1). The main clinical features are the young age of patients, a high incidence of mediastinal involvement, and bone marrow and meningeal spread during the course of the disease (2). The morphologic and immunologic features are those of immature T cells (3).

Since the report by Levine et al (4), it is now well established that high complete remission rate (between 80 and 95%) can be achieved in adult patients using multiagent chemotherapeutic programs analogous to those used in acute lymphoblastic leukemia (5,6). The next step is to prolong disease free survival to such a point that cure of the disease may be expected. It is clear that maintenance protocols incorporating the concepts common to ALL programs are of benefit. In this respect, the report of a 40% 5-year disease free survival with a modified LSA_2 $-L_2$ protocol (4) represents an impressive improvement compared to previous results achieved with less intensive treatments (7). However in Levine's report, the median follow-up of the patients was only 14 months and, of 5 patients with Ann Arbor stage IV disease only one remained in first CR at 12 months. More recently, the report of results of consecutive regimens used at Standford (5) and at the Memorial Sloan - Kettering Cancer Center (6) demonstrate that long term survival (56% at 3 years and 54% at 5 years respectively) can be achieved with intensive chemotherapeutic protocols. Both studies however isolated a subgroup of patients with bad risk fracture leading to a high failure rate due to disease

progression. In Coleman's study (5) the bad risk group (i.e. patients with Ann Arbor stage IV disease with bone marrow or CNS involvement or initial serum LDH concentration > 300 UI/L) had an actuarial 5-year FFR at 19% only, compared to 94% for good risk patients. In Slater's study (6), bad risk features were age > 30, high WBC counts, failure or delay to achieve CR. The 5-year survival for patients with bad risk features ranged between 19% and 40%.

Thus, for patients with poor prognostic features as defined above the use of very intensive treatments followed by marrow transplantation early during the first CR was though justified and performed in 26 consecutive patients in 5 different French Centers between October 1982 and October 1987.

PATIENTS CHARACTERISTICS (TABLE 1)

The diagnosis of LBL was based on light microscopy examination of pathologic specimen. An immunophenotypic analysis for T and B

Table 1. Patients Characteristics

	Autologous	Allogeneic	Total
Number	13	13	26
Age in years	16-39	15-43	16-43
Median	23	22	23
Sex			
Male	11	9	20
Female	2	4	6
Disease extension			
at diagnosis			
Mediastinal mass	13	12	25
Pleural effusion	10	7	17
Pericardial effusion	2	1	3
Kidney	--	1	1
Skin	1	--	1
Bone marrow	5	6	11
CNS	3	1	4
Staging (MURPHY)			
III (Serous +)	6 (4)	7 (4)	13 (8)
IV	7	6	13
Immunophenotyping			
T (Mc Abs)	10	8	18
NP	3	5	8
Treatment before Transplant			
"ALL TYPE"	9	11	20
"NHL TYPE"	4	2	6
CNS Prophylaxis			
IT chemotherapy	11	8	19
IT chemotherapy and			
CNS irradiation	2	5	7

cell antigens was performed in 18 patients. Blasts were less than 30% in the bone marrow in stage IV. According to Murphy criteria (8), 13 patients were stage IV and 13 stage III at time of diagnosis (Table 1). Of the latter, 8 had serous thoracic involvement and would have been staged as IV according to Ann Arbor Criteria.

TREATMENT BEFORE TRANSPLANTATION (TABLE 1)

Five different multiagent protocols including anthracyclines were used to obtain a CR. Twenty received an ALL type chemotherapy according to the French protocols LALA (9), (10 pts), FRALL (10), (8 pts) or LMB (11), (2 pts). For 6 patients CR was achieved using NHL-like protocols: CHOP 1 pt and AMACOP 5 pts. The time to reach a CR ranged between 15 and 90 days (median (30). The patients with initial CNS involvement received a CNS irradiation at a dose ranging from 15 to 18 Gy. No irradiation to bulk disease was performed at any time before transplantation.

BONE MARROW TRANSPLANTATION

Thirteen patients with an HLA identical sibling received an allogeneic BMT. As part of a multicentric protocol of GVHD prophylaxis, 3 donors' marrows were T-cell depleted with anti T-cell monoclonal antibodies and complement. The time of marrow harvesting and type of marrow purging for patients with no identical donor who underwent an autologous BMT is mentioned in Table 2. Of the 9 patients whose marrow was purged, 7 had initial involvement of either marrow or CNS. The conditioning regimen consisted of high dose chemotherapy with either cyclophosphamide (120 mg/kg) or high dose Melphalan (140 mg/m^2) followed by either single exposure 10 Gy or fractionated 12 Gy TBI. For allografted patients, the GVHD prophylaxis consisted of standard Methotrexate in 1, Cyclosporin in 2, Cyclosporin and short Methotrexate in 7 and an association of T-cell depletion and Cyclosporin in 3.

RESULTS

For the entire group of patients (Figure 1), the median observation time is 22 m (1 to 69). Of these, 18 patients remain alive in first CCR from 9 to 69 m after BMT with a median follow-up time of 25 m. The actuarial 4-year DFS is 70% (±9% SE). The actuarial 4-year DFS is 70% (±3% SE) with the last relapse at 10 m. Nine out of 13 autografted patients and 8 out of 13 allografted patients remain in 1st CCR 9 to 69 m (median 26 m) and 19 to 39 m (median 25 m) respectively. Eight out of 13 patients with Murphy stage IV and 10 out of 13 with Murphy stage III remain in CCR from 10 to 39 m (median 33 m) and from 6 to 69 m (median 22 m) respectively. The

main reason for failure was relapse. This occurred in 6 patients
(Table 3) between 1 and 10 months after BMT. The mediastinum was
the initial site of relapse in each case and 4 of 6 relapsing patients had
serous thoracic involvement at diagnosis. Two patients died after
allogeneic BMT, one with severe acute GVHD and one of unexplained
sudden death. No toxic death occurred after autologous BMT. For
surviving patients, the Karnofsky score is 90 to 100%.

Table 2. Bone Marrow Transplantation

	Autologous	Allogeneic	Total
INTERVALS (DAYS)			
From start of treatment to CR	19-76	15-90	15-90
(median)	(30)	(30)	(30)
From CR to Harvest	15-120	--	--
	(65)		
From CR to BMT	60-120	50-330	50-330
	(120)	(80)	(100)
MARROW TREATMENT			
ASTA Z	6	--	6
T Cell Mc Ab	3	3	6
None	4	10	14
CONDITIONING REGIMEN			
Cy + 10 Gy TBI	7	2	9
Cy to Fract^d TBI	2	8	10
HDM + Fract^d TBI	4	3	7
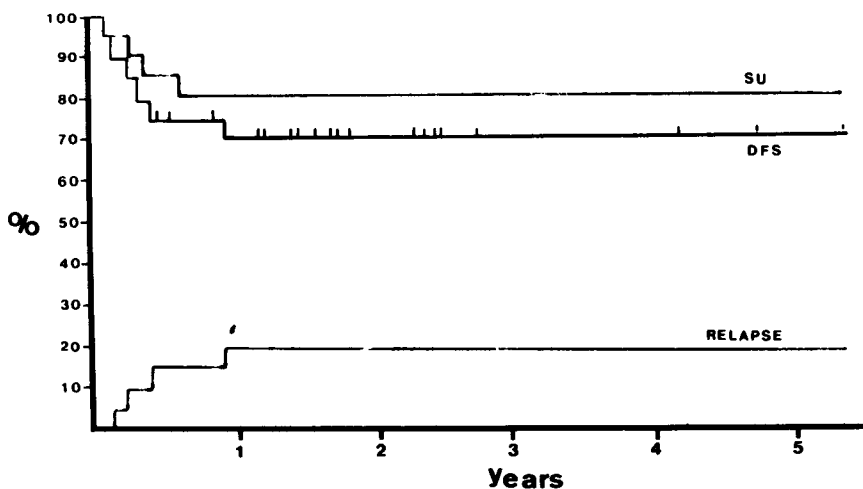
Figure 1.

Table 3. Relapses

Age (Y)	Staging	T.X. /	Time (D) to CR	BMT	Marrow Purging	Cond. Regimen	Site of Relapse
18	IV (Serous)	AMACOP	30	90	ASTA-Z	Cy + 10	Mediastin
16	IV (Serous)	FRALL	28	140	ASTA-Z	Cy + F^d	Mediastin
30	IV (CNS)	AMACOP	30	120	Mc -AB	Cy + 10	Mediastin
33	III	LALA	76	200	ASTA-Z	HDM + F^d	Mediastin
28	III (Serous)	FRALL	28	110	- (ALLO)	Cy + 10	Mediastin
21	III (Serous)	LMB	36	80	- (ALLO)	Cy to F^d	Mediastin

CONCLUSION

It is clear that retrospectively there is a great heterogeneity regarding treatment, marrow purging, conditioning regimen in this study. However the point is that intensive chemoradiotherapy followed by bone marrow rescue in first CR of adult poor prognosis LBL produces results comparing favorably with those achieved with modern multiagent chemotherapy for the same patients. Several questions remain to be answered: the source of marrow to be used, the need for purging if autologous, the way to improve control of mediastinal disease. These questions can be answered in a prospective multicentric protocol.

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HIGH DOSE THERAPY FOR MULTIPLE MYELOMA

Bart Barlogie, Karel A. Dicke,
and Raymond Alexanian

About 25 years have passed since the introduction of melphalan and prednisone (MP), but the prognosis of patients with multiple myeloma has not been advanced despite extensive clinical trial research with combinations of alkylating agents, anthracyclines, glucocorticoids and nitrosoureas (for review, see 1). Even the VAD regimen that was highly effective in MP-refractory myeloma has not prolonged remission or survival times compared to more standard drug combinations (Figure 1) (2,3). The lack of durable disease control from VAD despite rapid tumor mass reduction and improvement in signs and symptoms may be the result of preferential cell kill of mature tumor cells, while MP provides more effective inactivation of myeloma progenitor cells (Figure 2).

Based on pilot experience with high dose melphalan in newly diagnosed and refractory myeloma by McElwain and co-workers (4), our group initiated similar trials initially with high dose melphalan alone and subsequently with added total body irradiation (TBI) supported by autologous bone marrow transplantation (ABMT) (5,6,7). Once the feasibility and efficacy of this approach were demonstrated in refractory disease, it was also applied to patients with VAD-sensitive myeloma in first and second remission (Table 1). To date, high dose therapy has been administered to 81 patients, a third of whom were older than 60 years with typical immunoglobulin isotope distributions (Table 2).

In **VAD-REFRACTORY MYELOMA**, the incidence of tumor cytoreduction by >75% increased significantly with the addition of TBI without a concomitant rise in early mortality (first 2 months after therapy); similarly, relapse-free and overall survival were extended by added TBI (Table 3). A long duration of primary drug resistance (>1 year) as well as hypodiploidy or low plasma cell RNA content had
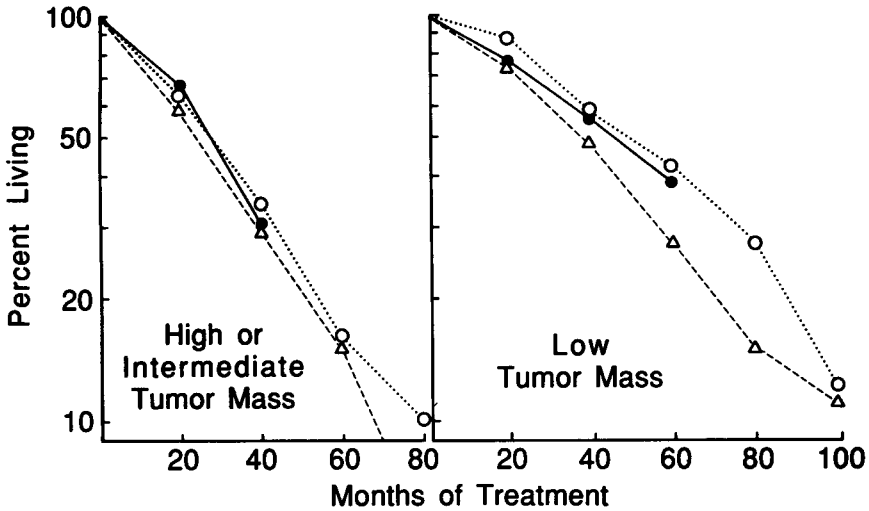
Figure 1. Unchanged survival over a 15-year period of clinical investigation at M.D. Anderson Hospital using standard melphalan-prednisone (triangles), combinations with bolus adriamycin (open circles), and VAD (closed circles).

been associated with resistance to dexamethasone pulsing and VAD (1), whereas high dose alkylating agent therapy especially when combined with TBI appeared equally effective whether or not the aforementioned features were present (Table 4). High serum LDH levels (>300 U/L), present either prior to or induced by high dose therapy, identified a high risk group with short relapse-free and overall survival; in contrast, patients with low LDH levels derived marked clinical benefit especially from added TBI (Table 5 and Figure 3) (8).

Durations of disease control and survival were not affected by marrow plasmacytosis or the timing of marrow harvest in relationship to tumor mass kinetics, supporting our contention that reinfusion of mainly terminally differentiated tumor cells would not affect patient outcome adversely.

The marked clinical efficacy of melphalan/TBI in VAD-refractory myeloma prompted similar studies in **VAD-SENSITIVE DISEASE**. Surprisingly, additional cytoreduction from high dose consolidation treatment was observed infrequently and occurred more slowly (Table 6 and Figure 4).

Myeloma Cell Phenotype, Kinetics, and Drug Sensitivity
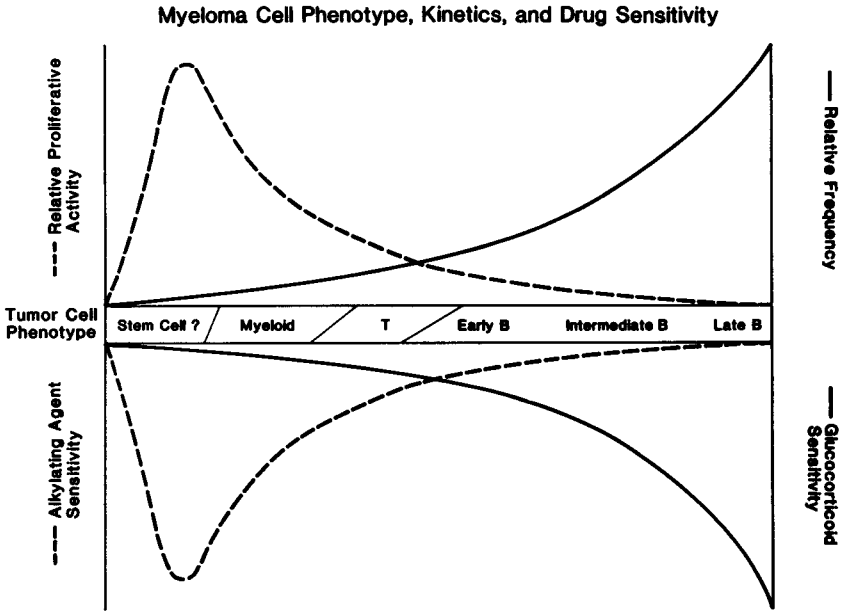
Figure 2. Model of myeloma ontogeny. On the basis of available phenotype and molecular data, a DNA-diploid myeloma stem cell early in hemopoiesis is postulated with sequential abortive commitment to megakaryopoiesis, erthryopoiesis, granulopoiesis and finally B cell lineage. Terminally differentiated B cells (plasma cells) represent the dominant tumor phenotype usually with DNA-aneuploidy and frequently asynchronous expression of earlier stages of differentiation. Proliferation is highest at an early commitment stage with greater sensitivity to melphalan, whereas plasma cells (producing a variety of cytokines) are kinetically inactive and highly sensitive to glucocorticoids.

The failure to achieve frequent complete remissions with marrow-ablative therapy applied in remission is not understood; potential mechanisms include a highly drug-resistant tumor subpopulation, unfavorable tumor cell proliferative characteristics during plateau phase of disease, or the reestablishment of a benign monoclonal gammopathy condition which may be a more frequent precursor of overt myeloma than previously expected (1). Follow-up is too short to determine the long term benefit from marrow-ablative therapy administered in remission.

Toxicity was mainly related to profound and sustained neutropenia with sepsis and/or pneumonia, whereas extramedullary toxicity (stomatitis, diarrhea) was of moderate degree and short duration with melphalan-containing programs but severe with thiotepa that was administered to 13 patients when melphalan was not available (see Table 1; Figure 5).

Table 1. Development of High Dose Therapy
for Myeloma at MD Anderson Hospital

Disease Status	Drug	REGIMEN Dose (mg/m2)	TBI 850cGy	BMT	N
VAD-Resistant	HDM	≤100	-	-	43
	HDM	140	-	+	7
	HDM	140	+	+	15
	HDT	750-900	+	+	1
VAD-Sensitive 2nd Remission	HDM	140	+	+	1
	HDT	750-900	+	+	9
1st Remission	HDM	140	+	+	2
	HDT	750-900	+	+	3
TOTAL					81

HDM, high dose melphalan
HDT, high dose thiotepa

Table 2. Patient Characteristics

Parameter	RESISTANT No TBI	TBI	SENSITIVE All TBI
N	50	16	15
Age > 60 years	16	3	5
Ig G	29	7	9
A	10	4	4
Other	11	5	2

Table 3. High Dose Therapy for VAD-Resistant Myeloma
- Clinical Outcome -

TBI	N	% Response	%Early Death	Relapse-Free Survival (mos)	Survival (mos)
NO	50	46	18	3	5
YES	16	81	19	15	13
P		.03	.003	.09	

Table 4. Variables Affecting Response to Salvage Therapy

Treatment	Resistant to	% Responding (No. Treated)	
		Unresponsive for ≤ 12 mos + Favorable DNA/RNA*	Other
DEX	MP	32(28)	17(18)
VAD	MP	56(32)	14(14)
HDM without TBI	MP & VAD	56(27)	35(23)
TBI + HDM or HDT	MP & VAD	79(14)	100(2)
Total		52(101)	26(57)

*not hypodiploid, RNA Index ≥4

Table 5. Variables Affecting Remission and Survival Times after Salvage Therapy

TBI	N	Median Relapse-Free Survival (mos)			Median Survival (mos)		
		LDH, Low-Low*	Other	P	LDH, Low-Low	Other	P
No	50	7	3	.04	10	4	.009
Yes	16	18	6	.03	35+	8	.06
P		.06	.006		.18	.19	

*LDH,Low-Low: LDH <300 U/L pretreatment and within 14 days after treatment
Other: LDH ≥300 U/L pretreatment and/or wihin 14 days after treatment

Table 6. High Dose Therapy for Myeloma - Sensitive vs. Resistant Disease -

Disease Status	Regimen	N	%Responding	Median T 1/2 (days)
Resistant	No TBI	50	46	6
	+ TBI	16	81	9
Sensitive	+ TBI	15	33	12

HIGH DOSE THERAPY FOR MULTIPLE MYELOMA
Relapse-Free Survival

No TBI

+ TBI

N	Relapsed	
10	10	● LDH, Low-Low
7	7	▲ LDH, High
		ꞁ Non-Failure
		p = .06

N	Relapsed	
7	6	● LDH, Low-Low
6	5	▲ LDH, High
		ꞁ Non-Failure
		p = .03

Proportion

Months After Treatment

A

HIGH DOSE THERAPY FOR MULTIPLE MYELOMA
Survival

No TBI

+ TBI

N	Dead	
18	11	● LDH, Low-Low
18	17	▲ LDH, High
		ꞁ Non-Failure
		p = .01

N	Dead	
8	2	● LDH, Low-Low
8	6	▲ LDH, High
		ꞁ Non-Failure
		p = .06

Proportion

Months After Treatment

B

Figure 3. Relapse free (**A**) and overall survival (**B**) of VAD-refractory myeloma after treatment with high dose melphalan with or without TBI. Longest disease-free and overall survival were achieved in patients whose serum LDH levels did not exceed 300 U/L within 2 weeks of therapy, whereas patients with higher levels rarely derived clinical benefit. Prognostic importance of LDH applied whether or not TBI was added.

TUMOR MASS CHANGES AFTER HIGH DOSE THERAPY
WITH TBI & BMT FOR MULTIPLE MYELOMA
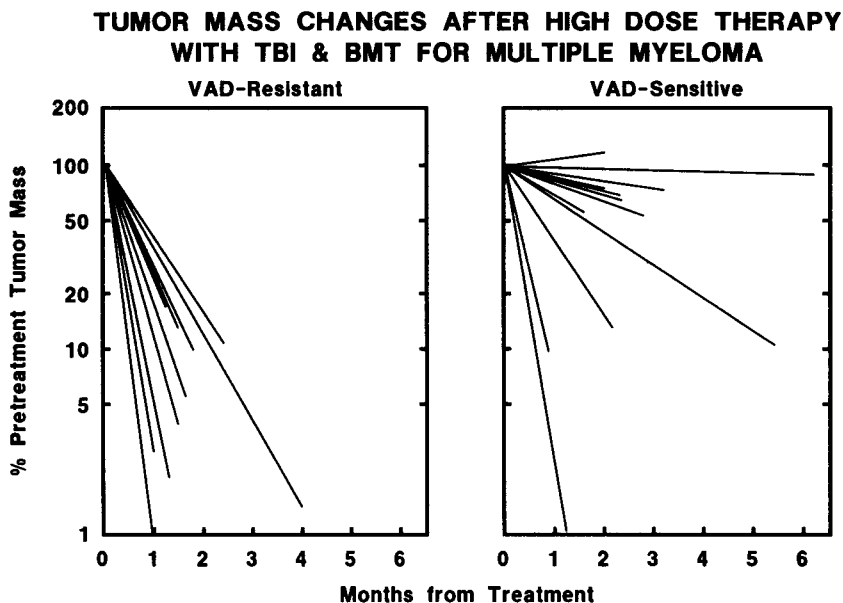
Figure 4. More frequent, speedier and more marked tumor cytoreduction from TBI-containing high dose therapy in VAD-resistant vs VAD-sensitive myeloma. Note that a higher percentage of patients with VAD-sensitive disease received thiotepa instead of melphalan (see Table 1).

In summary, a chemoradiotherapy program with autologous hemopoietic stem cell support has been developed that produced marked tumor cytoreduction in patients with active multiple myeloma. When reserved for patients with good performance, this treatment can be administered with relative safety even to elderly patients up to age 70; thus, the overall mortality was under 10% and all patients treated in remission survived. Its application in drug-responsive myeloma will hopefully effect long term disease control especially in patients treated in first remission.

The persistence of monoclonal gammopathy in the majority of patients is not understood at present but does not appear to originate from tumor cells reinfused with marrow autografts.

HEMATOLOGIC RECOVERY AFTER HIGH DOSE THERAPY FOR MULTIPLE MYELOMA
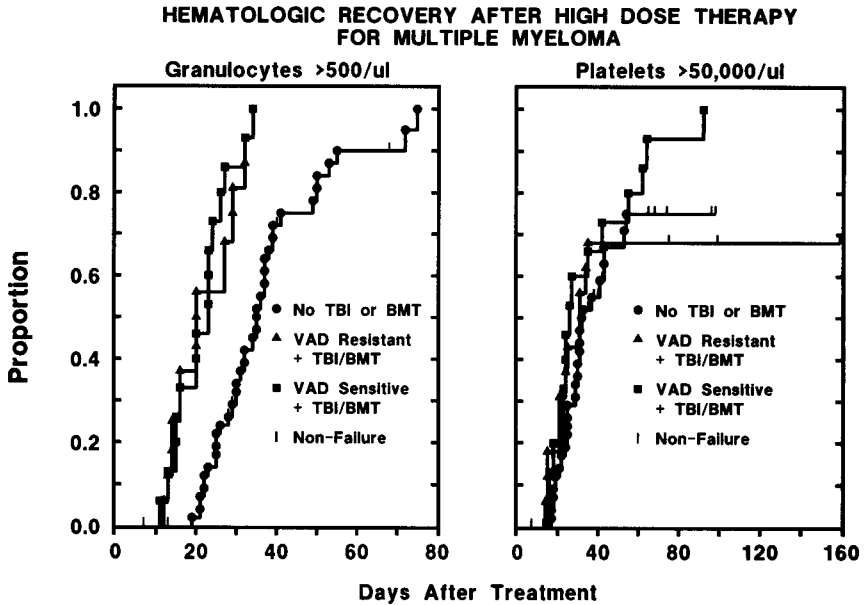
Figure 5. Hematologic recovery after high dose therapy for various treatment programs including high dose melphalan alone (closed circles) and BMT-supported TBI-containing high dose therapy for VAD-resistant (closed triangles) and VAD-sensitive myeloma (closed squares). Note the significantly faster granulocyte recovery with BMT support despite higher doses of melphalan and added TBI when compared to melphalan alone (closed circles)

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OPTIMAL TIMING OF AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR PATIENTS WITH HODGKIN'S LYMPHOMA

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In the last twenty years, the prognosis of patients with advanced Hodgkin's Lymphoma has modified dramatically due to the development of new combination chemotherapy protocols (1,2).

Although such protocols revolutionized the treatment of advanced Hodgkin's Lymphoma, at least one third of the patients do not achieve complete remission or relapse precociously within 12 months. For such patients, the prognosis is very poor, especially for patients resistant to MOPP/ABVD. The newer conventional salvage therapies do not seem to modify the prognosis of these patients. Because of the poor results with conventional salvage therapies, clinical trials were begun to evaluate the role of high-dose therapy followed by autologous bone marrow transplantation (ABMT) for patients refractory d'emblee to the first line therapy or relapsed to first, second and third-line protocols.

Here we describe our results with 72 patients who received ABMT in Italy.

MATERIALS AND METHODS

Patients with advanced Hodgkin's lymphoma received high-dose therapy/ABMT from July 1981 to July 1987 and no data collected

after July 1987 is included in this analysis. The patients were evaluated at Division of Hematology (Genoa).

Eligibility criteria included normal bone marrow function in the absence of marrow involvement, Karnofsky performance status ≥40% and resistance to MOPP and then later to radiation and ABVD or CEP (Lomustine, Etoposide, Prednimustine) (3) or to the alternating regimen of MOPP/ABVD ± CEP and radiation, as indicated by either progressive disease during therapy or relapse disease after an initial complete remission.

The clinical characteristics of the patients are shown in Table 1. There were 50 males and 22 females with a median age of 26 years (range, 12-51). Nodular sclerosis histology was the most prevalent subtype (49 patients). Fifty patients had extranodal disease, and the majority of patients had B symptoms (47 patients). Eleven patients received ABMT in first relapse, 23 had progressive disease while receiving either alternating MOPP/ABVD plus radiation protocol or MOPP followed by ABVD and CEP protocols, and 36 patients had recurrent disease ranging from three months to 45 months after complete remission duration less than one year.
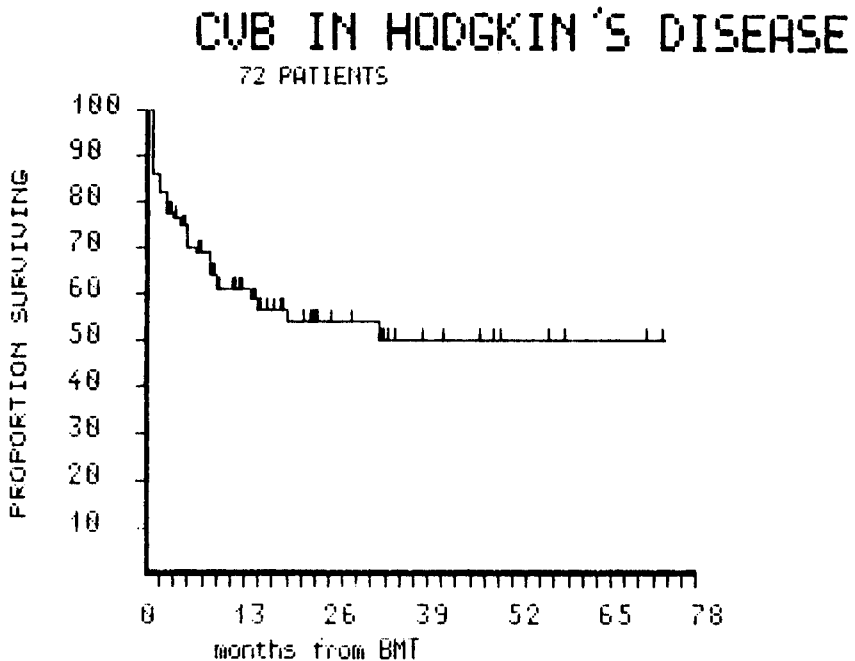
Figure 1. Actuarial disease-free survival for patients with Hodgkin's disease after Autologous Bone Marrow Transplantation.

Table 1. Patients' Characteristics

Total		N. of Patients
		72
Stage of disease at 1st presentation		
	Nodes ≥ 2	48
	Extranodal 1	18
	Disease > 1	6
B symptoms		47
Histology		
	NS	49
	Others	23
Karnofsky performance status		
	70% - 100%	20
	40% - 60%	52
Sites of recurrent Hodgkin's disease		
	Nodes ≥ 2	20
	Extranodal 1	39
	Disease > 1	11
Prior therapy		
	MOPP/ABVD*+RT±CEP	31
	Others**	41
Status at the time of HDC/ABMT		
	First Relapse	11
	Progressive Disease	23
	Resistant Relapse	13
	Sensitive Relapse	23
	First Complete Remission	2

*alternating: MOPP regimen was alternated with ABVD regimen every other month.

**MOPP+RT/ABVD+RT; CCVPP+RT+ABVD±CEP; MA/MA+RT+CEP(CAD).

LP: Lymphocytic predominance; NS: Nodular sclerosis; MC: Mixed cellularity; LD: Lymphocytic depletion; MED: Mediastinal bulky; CCVPP: Lomustine, Vinblastine, Procarbazine, Prednisone; RT: Radiotherapy; CEP: Lomustine, Etoposide, Prednimustine. For Resistant-Relapse and Sensitive-Relapse see Evaluation and Statistical Analysis.

Evaluation and Statistical Analysis

The patients who never achieved a complete remission after first line treatment and even after second and/or third-line therapies, were considered *progressive disease* patients at transplantation. *Relapsing patients* were first treated according to a conventional salvage protocol

and were classified according to their responsiveness to this protocol as having had either "resistant-relapse" when no response or disease progression were observed immediately before high-dose therapy and autologous transplantation or "sensitive-relapse" when at least a partial response was observed immediately before transplantation (4).

Complete remission was defined as the disappearance of clinical and radiological evidence of Hodgkin's Lymphoma. Partial remission was defined as a reduction of 50 percent or more in measurable disease for at least one month.

No complete remission patient received maintenance treatment while patients achieving partial remission always received subsequent radiotherapy.

Disease-free survival was calculated from the day of marrow transplantation (day 0) and was analyzed as of February 10, 1988. Actuarial disease-free survival curve was plotted according to the method of Kaplan and Meyer (5).

Supportive Care and Treatment Protocol

In all patients, a central venous catheter was inserted 12 hours before high dose therapy. The patients were maintained in single rooms, received prophylactic allopurinol, antiemetic, and oral non-adsorbable antibiotics and antimycotic and were given intravenous hydratation until day 5 after transplantation. Urine output was maintained at 150 ml/hour. The high dose therapies consisted of a modified CVB protocol (6) (Table 2).

Table 2. Drug Schedules and Doses for the Chemotherapy Regimens used in Italy

Drug (Regimens)	Total Dose (MG/MQ)		Route	Time
CVB-1				
Cy	6000	in	I.V.	30 min.
VP-16	600 }	two	I.V.	120 min.
BCNU	600	days	I.V.	Push (H/D) 30 min.
CVB-2				
Cy	6000	in	I.V.	30 min.
VP-16	1000 }	four	I.V.	120 min.
BCNU	800	days	I.V.	Push (H/D) 30 min.

**Table 3. Results with CVB Regimens in Patients
with Advanced Hodgkin's Disease**

	Evaluable Patients	CR	PR	CR+PR	Median CR Duration (mo.)	CCR
FR	9	6 (67%)	2	8 (88%)	16	4
PR	22	10 (46%)	6	16 (72%)	19	7
RR	12	3 (25%)	8	11 (92%)	34	2
SR	22	12 (55%)	7	19 (86%)	8	4
FCR	2				3	2
	67	31 (46.2%)		54(80.5%)		19 (61.2%)

RESULTS

Tumor Response and Survival

The outcome of high-dose in relation to the patients' characteristics before transplantation are summarized in Table 3. Thirty-one (46.2%) patients achieved complete remission with a median duration of 26 months and twenty-three (34.3%) patients achieved a partial remission with a median duration of nine months, for an overall response rate of 80 percent. Nineteen out of 31 complete responder patients subsequently relapsed three to 28 months (median, 7 months). Eleven patients failed to respond and died in progressive disease one to 10 months (median, 6 months) after transplantation. The analysis of the progression sites showed that 82 percent of relapses occurred primarily at the initial site of disease, whereas, only 18 percent occurred in other sites and in no patient did relapse occur in the marrow.

Toxicity

High dose therapy produced significant neutropenia and thrombocytopenia in these heavily pretreated patients. All patients had a white blood cell count less than $0.5 \times 10^9/L$ for a median of 14 days (range 8-34 days) and a platelet count less than $20 \times 10^9/L$ for a median 20 days (range 14-57). Fever during neutropenia, which warranted intravenous antibiotic and antimycotic therapy, was present in all patients.

There were ten treatment-related deaths; two patients died of Pseudomonas aeruginosa septicemia, and another patient died of

Candida pneumonitis; one patient died in complete remission of cerebral hemorrhage two months after transplantation. The autopsy, effected in this case, confirmed the complete remission state. Four patients died of cardiac toxicity and other three patients of interstitial pneumonitis. All these cases received ABMT as fourth line therapy.

Eight patients had severe mucositis and other three cases had herpes virus simplex generalized infection, well controlled with intravenous Acyclovir.

Nausea, vomiting, liver enzymes and/or alkaline phosphatase elevations were observed in all patients.

DISCUSSION

Hodgkin's lymphoma is a responsive neoplasia both to radiotherapy and chemotherapy. Today, fifty to seventy percent of patients with advanced disease can be considered cured with MOPP or MOPP/ABVD protocols. However, all patients cannot be cured with the presently available primary therapies. On the other hand, the optimal management of Hodgkin's lymphoma resistant or relapsing after primary intensive therapy, remains to be established. At present, several salvage protocols have been tested for these patients, but the results are controversial and must be interpreted with caution (7).

High-dose therapy and autologous bone marrow transplantation is a promising new approach for these patients. According to the results of pilot studies from the U.S.A. and the European Bone Marrow Transplantation Group (EBMTG), approximately two-thirds of end-stage patients can expect to achieve a complete remission after high-dose therapy.

The aim of our study, which was started more than seven years ago, was to improve by high dose therapy salvage results in advanced heavily pretreated patients. In this cooperative pilot study we showed that it is possible to achieve a high response rate (80%) with an acceptable proportion of early deaths in 72 resistant Hodgkin's lymphoma patients with very bad prognosis. Superimposable results have been obtained by others in similar patients (8,9).

All these results, as well as those concerning smaller groups of patients, are similar to ours and prove the validity of such procedure in inducing complete remission; however, the high rate of relapse suggests that it may be useful to treat these patients earlier, in first relapse or in second complete remission after standard therapy before ABMT, or in first complete remission in very high risk patients.

Clearly, the question of whether high-dose therapy and autologous bone marrow transplantation will eventually supercede new conventional dose salvage chemotherapy, would be answerable only by a controlled clinical trial recently in progress.

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TIMING OF AUTOLOGOUS BONE MARROW TRANSPLANTATION IN HODGKIN'S DISEASE:
The Experience of the Bloomsbury Transplant Group

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INTRODUCTION

Autologous bone marrow transplantation (ABMT) following intensive therapy is being used increasingly in relapsed Hodgkin's disease. The value of such therapy can only be ascertained in randomized controlled trials, but if a meaningful result is to be obtained from these trials then care must be taken in their design. ABMT has an unavoidable procedure related death rate and therefore can only be justifiably used in situations where a significant improvement over conventional therapy is achieved or where conventional therapy is clearly unsatisfactory. We described here the two data bases used in the design of a current prospective randomized trial of intensive therapy and autologous rescue: the experience of high dose therapy and ABMT at University College, London, and the experience of the British National Lymphoma Investigation (BNLI) trials in advanced Hodgkin's disease.

Phase I/II Studies of Autografting in Hodgkin's Disease in Bloomsbury

The Bloomsbury transplant group has now treated 72 patients with advanced, relapsed Hodgkin's disease by intensive therapy and ABMT. There were 42 males and 30 females with a median age of 30 (range 13-59) years. All patients had active disease at the time of ABMT. Five patients (7%) had primary resistant disease showing relapse

through first line alternating chemotherapy. All other patients had received at least two regimens of chemotherapy and 35 (48%) had received radiotherapy in addition. Thirty-six patients had never achieved complete remission (CR). The median time from diagnosis to ABMT was 20 (range 7-95) months. Three early patients received a total body irradiation (TBI) containing regimen, six others received the UCH 1 protocol(1) but all subsequent patients have received the BEAM protocol. Of 63 patients treated by BEAM 44 have a follow up of greater than one year and have been reported in detail (2). Twenty-three patients had previous extra nodal disease and 21 had extra nodal disease at the time of ABMT.

Twelve patients had disease infiltrating lung and eleven had massive mediastinal disease with a tumour mass greater than 10 cm in diameter. Three patients had previous bone marrow involvement but there was no evidence of bone marrow involvement at the time of harvest as assessed by histological examination of bilateral iliac crest trephine biopsies. All patients who had failed to respond to alternating front line therapy, or failed any two treatment modalities and who were deemed fit to undergo high dose therapy by members of the transplant team received this treatment. Bone marrow was harvested and cryopreserved as previously described (3) and a Hickman central venous catheter inserted under the same anaesthetic.

RESULTS

The response to high dose therapy was assessed clinically and by computerized axial tomography at three months post ABMT. Of the 44 patients with a minimum follow up of one year,15 (34%) achieved CR within three months. A further 23 patients (52%) achieved PR. Two of these patients had slow resolution of their disease and were in CR by six months post ABMT. Seven patients received post graft radiotherapy to sites of residual disease and five subsequently entered CR. Overall therefore by six months post ABMT 22 patients (50%) were in CR and 4 further patients have remained free of tumour progression. Two patients have relapsed from CR at seven and nine months from ABMT. The remaining 20 patients remain in unmaintained remission and all have a Karnovsky score of 100%. The overall survival of these 44 patients is shown in Figure 1. The major toxicity of the procedure was significant bone marrow suppression with a median of 24 days (range 14-68) to reach a neutrophil count greater than $0.5 \times 10^9/1$ and 32 days (range 13-54) to reach a platelet count greater than $50 \times 10^9/1$. Six patients (9.5%) treated by BEAM have died during the aplastic phase of the procedure. We have investigated the use of in vivo recombinant granulocyte macrophage colony stimulating factor (rh GM-CSF) in patients receiving BEAM for resistant Hodgkin's disease (4) in an attempt to reduce this period
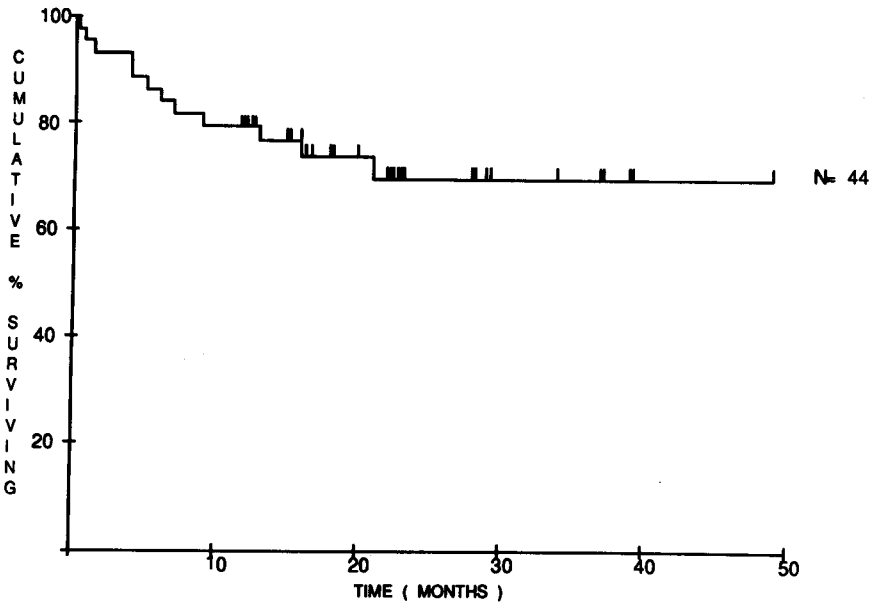
Figure 1. Overall Survival of ABMT in Hodgkin's Disease Using Beam Chemotherapy

of neutropenia. From June 1987 to March 1988 all such patients were offered rh GM-CSF. Twelve patients received rhGM-CSF at doses of 100-400 mg/m^2/day. In those evaluable patients there was more rapid regeneration of total leucocyte and neutrophil count compared to concurrent and historical controls. Platelet recovery was not affected. Two patients (16%) who received rhGM-CSF died within 21 days of marrow re-infusion, one of progressive Hodgkin's disease and one of toxic colitis.

The European bone marrow transplant group (EBMTG) registry now contains details on 270 patients with Hodgkin's disease treated by high dose therapy and ABMT (5). There were 253 adults and 17 children; 16% had ABMT performed in first or subsequent CR and 34% never achieved CR; 46% of patients achieved CR post ABMT and a further 39% achieved partial response to therapy. The procedure related mortality was 17%.

Selection of Patients and Timing of ABMT in Hodgkin's Disease

The preliminary results of very intensive chemotherapy are very encouraging but must be carefully compared to the results obtained with less aggressive therapy. We have used the British National lymphoma in investigation (BNLI) experience of over 600 patients

treated by MOPP like regimens (6) as a data base for designing future studies. Using factors shown to have prognostic significance it is not possible to define a group of patients with sufficiently poor prognosis to merit high dose therapy at presentation or in first CR. Future studies should therefore take place with patients who have failed first line therapy. Those who fail to achieve CR on first line therapy have a poor survival although this group may be decreasing because of the introduction of alternating and hybrid regimens. The prognosis for those patients who relapse after obtaining a CR is considerably better than those who fail to achieve CR but it is not possible to define a group with a sufficiently poor prognosis at first relapse to be suitable for ABMT. The role of reinduction therapy before ABMT must be considered for it might either demonstrate drug sensitivity or reduce tumour volume. The lack however of any satisfactory salvage protocol mean neither aim can be reliably achieved in all patients who may benefit from ABMT.

CONCLUSIONS

These results and observations lead us to believe that ABMT may be of value in the treatment of relapsed or resistant Hodgkin's disease and therefore this must now be tested in the context of a randomized controlled trial. A BNLI study has been initiated to address this question and is studying the uses of dose escalation in Hodgkin's disease in three groups of patients – those who fail alternating first line therapy, those who fail two treatment modalities and those who fail to obtain CR on a MOPP type regime if there is high grade histology or high presenting ESR. These patients will be randomized between BEAM chemotherapy and ABMT or intensive salvage therapy without ABMT using the same drugs (mini-beam). Randomization and treatment will be immediately on relapse or failure of induction and there will be no reinduction treatment.

Many of the patients referred to us for high dose therapy had been previously given more treatment than this. Statistical analysis of our data failed to show any difference in outcome for those patients who had received more than two modalities of therapy against those who had failed alternating regimens or only two modalities. It remains likely that these patient groups are not comparable. Those patients who fail to respond to front line therapy and come quickly to high dose therapy may have an intrinsically different natural history than those patients with more indolent, chronically relapsing disease. Any difference between these groups may only become apparent in future randomized studies. One advantage of a chemotherapy based high dose regimen is the feasibility of using radiotherapy to sites of residual disease. Such an approach has achieved durable complete remissions in a further 22% of patients who achieved a partial response to

BEAM. Unfortunately, only 27% of partial responders were eligible for such treatment as the remaining patients had already received maximal doses of radiotherapy to areas of residual disease. However eight of our patients who had a residual mediastinal mass at three months after ABMT did not receive radiotherapy for this reason and such patients may be disease-free despite the persistence of a mass on the CT scan(7).

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TIMING OF HIGH DOSE CBV AND AUTOLOGOUS BONE MARROW TRANSPLANTATION IN THE MANAGEMENT OF RELAPSED OR REFRACTORY HODGKIN'S DISEASE

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INTRODUCTION

High dose chemotherapy with or without total body irradiation (TBI) and bone marrow transplantation (BMT) is being used with increasing success in the management of relapsed Hodgkin's disease (1-10). We had reported encouraging results with high dose cyclophosphamide, carmustine, etoposide (CBV) and BMT for the management of Hodgkin's disease relapsing after MOPP and ABVD chemotherapy (11). We present here an update on 62 patients who had undergone CBV and autologous BMT and look at their prognostic features and optimum timing for transplantation.

PATIENTS AND METHODS

The first two patients were treated in a pilot study. All other patients were entered consecutively on protocol at two referral institutions. Informed consent for participation in the study was obtained from each patient.

Marrow cellularity of 15% or more with no tumor infiltration on bilateral iliac cross-biopsies was a prerequisite for autologous marrow storage. One patient had marrow relapse after bone marrow harvest;

another patient, with marrow disease, received allogeneic BMT from human leukocyte antigen (HLA) matched and mixed lymphocyte culture (MLC) non-reactive sibling.

Patients entered in the study had good organ functions. The Zubrod performance status was 0 in 36 patients (58%), and ≥ 1 in 36 patients. Two-thirds of the patients with performance status ≥ 1 had constitutional (B) symptoms.

PRIOR THERAPY

All but three patients had received both a MOPP-like regimen and a doxorubicin-containing program. Twenty-four patients had not achieved a complete response to the initial combination chemotherapy. The patients had failed a median of two (range 1-6) chemotherapy regimens.

Forty-one patients had previous radiation therapy; the mediastinum was the most frequent site (50% of patients) of prior radiation therapy. Of these, 31 patients had progression of disease within the irradiated field at the time of transplantation.

DISEASE STAGING

Twenty patients had only nodal disease, 40 patients also had extranodal involvement, and two patients were intensified immediately upon achieving a third complete remission with conventional dose salvage chemotherapy. Among the patients with extranodal disease, 27 had a single extranodal site, 12 had 2, and one had three extranodal sites.

Nodal involvement was assessed individually for each site and defined as extensive involvement as follows: for peripheral lymph node bearing area, nodal mass >5 cm; for mediastinum, presence of any size mediastinal mass by chest radiograph or chest CT; for abdomen, nodal mass >5 cm or simultaneous pelvic and para-aortic disease.

The estimation of tumor burden was made as previously described for advanced diffuse large cell lymphoma (12). Low tumor burden was defined as no more than one extranodal site of disease and no more than one area of extensive nodal involvement, or as two extranodal sites but no area of extensive nodal disease. High tumor burden was defined as two or more areas of extensive nodal disease, or as three or more extranodal sites, or as one area of extensive nodal disease plus two extranodal sites.

THERAPY

The high dose chemotherapy schema is given in Table 1. The total dose of etoposide was 450 mg/m^2 in the first, pilot patient; then, the

dose was escalated to 600 mg/m^2 in 21 patients, 750 mg/m^2 in 19 patients and, finally, 900 mg/m^2 in 21 patients.

After a 2-day rest period, autologous BMT was done on day 7. Further details of transplantation and patient management have been reported elsewhere (11).

STATISTICS

The method of Kaplan and Meier was used to obtain survival and progression-free survival curves. Survival was calculated from the day of BMT (day 0) to the day of last follow-up or death.

Progression-free survival was calculated from day 0 to the date of documented progression of the disease or death. Four patients died within a month after transplantation before complete hematopoietic recovery, from treatment related toxicity, and were considered to be failures.

All but three patients had follow-up of more than two years (20-81 months, median 31 months).

PROGNOSTIC GROUPS

Ten clinical factors, determined at the time of entry into the high dose chemotherapy program, were analyzed for their possible role as prognostic indicators. Dichotomous categories for these factors were as follows.

For both age and time from diagnosis to BMT, patients were divided into two groups at each median. For performance status, patients were categorized as good (Zubrod 0) versus symptomatic (Zubrod ≥ 1). Patients were divided into two groups by the number of previous chemotherapies they had failed (≤ 2 versus >2). For response to first chemotherapy, the patients were grouped according to complete response or no complete response. For extent of disease, patients were classified as low tumor burden (43 patients) or high tumor burden (19 patients). For site of relapse, patients were grouped as nodal disease only (22 patients) versus extranodal disease, with or without nodal disease (38 patients).

For relapse after previously administered radiation therapy, the patients were grouped as relapse within the radiation field (31 patients) versus relapse outside the field of radiation therapy or no prior radiation therapy (31 patients). Patients were also categorized as relapsing while on therapy (45 patients) or off therapy (17 patients).

Table 1. Chemotherapy Schema for High-Dose CBV and BMT

Drugs	Day -6	Day -5	Day -4	Day -3	Day -2	Day -1	Day 0
Cyclophosphamide 1.5g/m^2/d	X	X	X	X			
Carmustine 300 mg/m^2	X						
Etoposide 100-150 mg/m^2/q12h	X	X	X				
BMT							X

RESULTS

Following high-dose CBV 29 (47%) patients were in CR, 19 patients achieved a PR and 10 patients had progressive disease. There were 4 (7%) treatment related deaths. Of the 29 patients in CR, 9 patients had relapsed and one died in remission 5 months after transplantation. Six of the 19 patients in PR achieved a CR after additional local radiation therapy. Two of these 4 patients had relapsed subsequently.

The progression-free survival and overall survival for 62 patients with relapsed Hodgkin's disease treated with CBV and BMT is shown in Figure 1. The median survival from the day of transplantation is 26 months. Twenty-three patients are alive and free of disease with a median follow-up time of 31 months.

RISK GROUPS

Each of ten clinical factors (age, sex, time from diagnosis to BMT, performance status, number of chemotherapy regimens failed, response to initial combination chemotherapy, nodal or extranodal site of relapse, relapse on or off therapy, relapse within previously irradiated areas, and tumor burden) was examined individually for its relationship to overall survival time. Four of these factors were found to be statistically significant (Table 2): performance status, number of
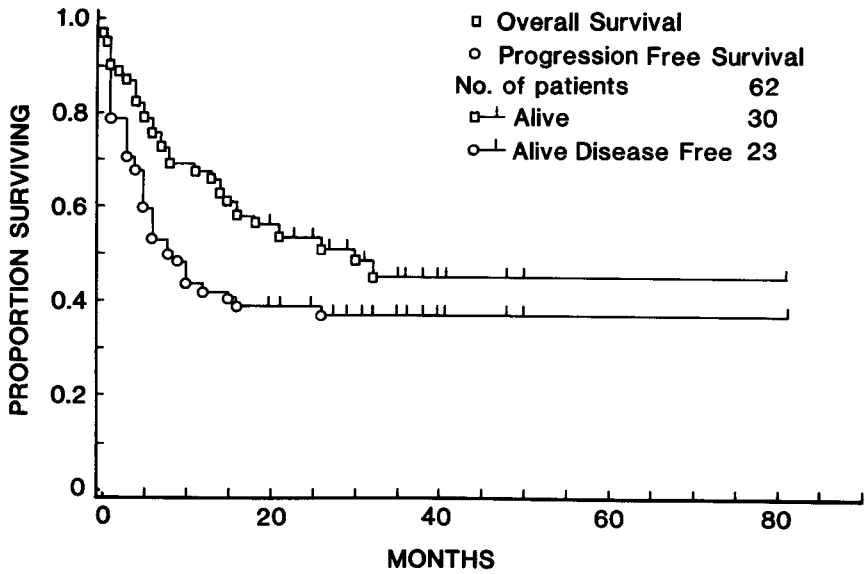
Figure 1. Survival and progression-free survival for all patients with relapsed Hodgkin's disease treated with CBV + BMT.

Table 2. Clinical Factors and Their Effect on Survival Time

Risk Factors	Group	No. of Pts	CR (%)	Median Survival (mo.)	p value
1) Performance status	0	36	54	NR*	<0.001
	≥1	26	31	7	
2) No. of prior chemotherapy regimens failed	2	34	65	NR	0.008
	>2	28	25	13	
3) Tumor burden	Low	43	58	NR	0.005
	High	19	21	7	
4) Response to prior radiation therapy	None or outside field relapse	31	52	NR	0.02
	In field relapse	31	42	14 mo.	

* NR = Not reached
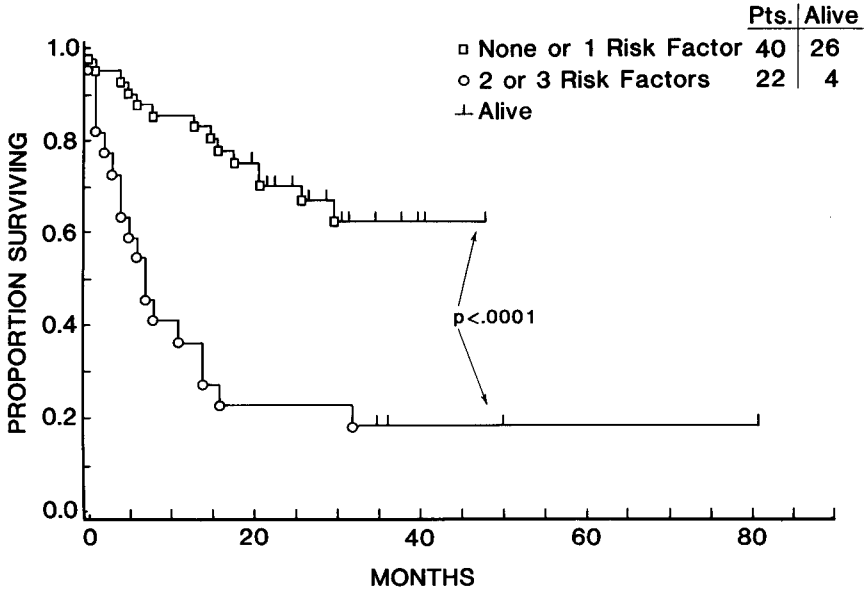
Figure 2. Survival curves for patients with relapsed Hodgkin's disease treated with CBV + BMT. The risk groups, as defined in the text, show a marked difference between patients with no or 1 risk factor and those with two or three risk factors.

prior chemotherapy regimens failed, tumor burden, and in field relapse after prior radiation therapy.

A multivariate analysis (Cox's proportional hazards model) was performed to determine a set of independent factors related to differences in survival times. Of the four factors which were significant as a single variable, only three were retained in the multivariate model, i.e.: performance status, tumor burden at the time of BMT and number of previous chemotherapies. Figure 2 shows the survival in two groups of patients having none or one versus two or all three of these risk factors. The difference in survival between patients with none or at most one risk factor and those with two or three of these risk factors is highly significant (p <0.0001).

Achievement of CR is an important factor for long-term survival; Table 3 shows the decreasing response rate as the risk (determined by the three factors mentioned above) increases. Among the patients with none or one risk factor, 60% achieved a CR, whereas among patients with two or more risk factors, only 23% achieved a CR (Table 3).

Table 3. Risk Groups for Patients with Relapsed Hodgkin's Disease

No. of Risk Factors	No. of Patients	No. in CR (%)	Survival at 2 Years
0	18	14*(78%)	89%
1	22	10*(45%)	55%
2	15	5 (33%)	27%
3	7	0	14%

* Includes 1 patient in third remission

DISCUSSION

High dose CBV combination chemotherapy in combination with BMT induced CR in 45% of patients with persistent or relapsed Hodgkin's disease after receiving a MOPP-like regimen and a doxorubicin based program. These response rates compared favorably with the results obtained with other high dose carmustine programs reported in the literature (5-10). The remission duration after CBV is remarkably different from the remission duration after normal dose salvage therapy in the MOPP-ABVD failures (13-15). Of the patients who received CR, 66% are still in remission ranging from 20 months to 6 years. The median remission duration has not yet been reached with the median follow-up of 31+ months.

None of the patients who had achieved a CR with CBV were given additional radiation therapy. However, six of the 19 patients in partial remission after CBV who had one area of residual nodal disease were rendered disease free with additional involved field radiation therapy. Of these, four are still in complete remission from 20+ to 40+ months. Radiation therapy has been shown to induce durable remission in patients who relapse with Hodgkin's disease at nodal sites, especially after a long disease free interval (16-17). Adjunctive use of radiation therapy is applicable only to a minority of our patient population.

Relapsed lymphoma which is still responsive to conventional chemotherapy is more likely to benefit from high-dose therapy intensification (18). Our present study extends this observation to relapsed Hodgkin's disease but not all patients were challenged with conventional chemotherapy prior to CBV and transplantation. Therefore, it was not included in the multivariate analysis.

The three adverse risk factors, namely, high tumor burden, more than two chemotherapy treatment failures and poor performance status (Zubrod 1 or greater), allowed us to categorize patients into two prognostic subgroups: a good prognostic group and a poor prognostic group. The good prognostic group included 65% of our patients, who had at the most one of the adverse risk factors. The projected three-year survival was 63%. There was only a single treatment related death in this group. The poor prognostic group contained 35% of our patients, who had two or all three adverse risk factors. Their projected three-year survival was 18%. These results would indicate the need for consideration of high dose CBV + BMT early in the course of management of patients with relapsed Hodgkin's disease failing a MOPP- and an ABVD-like regimen, while the tumor burden is still low and before tumor cells become resistant to multiple chemotherapeutic agents.

ACKNOWLEDGMENTS

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HIGH DOSE CHEMOTHERAPY REGIMENS FOR PATIENTS WITH ADVANCED HODGKIN'S DISEASE

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INTRODUCTION

Treatment of advanced Hodgkin's disease represents a triumph of modern combination chemotherapy (1-3). More than 50% of patients with advanced Hodgkin's disease are cured with first line chemotherapy. Patients who fail initial therapy or who relapse, however, represent a different subgroup. Salvage chemotherapy for these patients is suboptimal (4). High dose chemotherapy with autologous bone marrow rescue has been shown to produce a high complete remission rate in patients with advanced relapsed Hodgkin's disease (5-13). However, most studies have been carried out with very advanced disease or on patients with primary refractory disease.

MATERIALS AND METHODS

Forty-six patients with advanced Hodgkin's disease have been treated with intensive chemotherapeutic regimens along with autologous bone marrow transplantation at Westchester County Medical Center/New York Medical College. These patients were eligible to enter this program if they had biopsy proven Hodgkin's disease with evidence of recurrence after initial chemotherapy, no evidence of marrow involvement at the time of marrow harvest, and were felt to be capable of withstanding intensive chemotherapy by the bone marrow transplant team. Informed consent approved by New York Medical College and Institutional Review Board was obtained from all patients. Standard response criteria were used and included:

Complete remission (CR): complete disappearance of all clinical, radiographic, and biochemical evidence of tumor for at least one month.

Partial remission (PR): >50% decrease in diameters of measurable lesions for one month.

Progression: greater than 25% increase in tumor size.

While anesthetized, at least 10 ml of bone marrow per kilogram of the patients body weight was aspirated from posterior and, if necessary, anterior iliac crest. For patients undergoing sequential marrow harvesting, approximately 20 ml/kg was collected. Marrow was mixed with 200 ml of tissue culture medium TC199 (Gibco Laboratories) and 40-120 x 10^3 heparin, filtered twice through wire meshes, then collected in sterile plastic bags of 400-500 ml. It was refrigerated at 4°C for 2-5 days or cryopreserved using hetastarch and DMSO and then stored at -80°C. No ex-vivo manipulations were carried out.

Following marrow storage, chemotherapy was initiated. The chemotherapy regimens used are outlined in Table 1. For the last 18 months, patients deemed to be at high risk for relapse were entered into a program for sequential myeloblative chemotherapy regimen: patients were initially treated with BCNU 400/m^2, VP-16 1800/m^2, cyclophosphamide 5 gm/m^2; upon count recovery patients were then retreated with ThioTEPA 900/m^2, vinblastine .4-.5 mg/kg, ± cytarabine 3-6 gm/m^2 and a second bone marrow transplant was carried out. Aggressive antiemetic regimens were used. One such regimen is outlined in Table 2. Bone marrow was infused intravenously 12 to 48 hours after completion of chemotherapy. All patients were given irradiated blood products. For patients without evidence of previous cytomegalovirus infections, blood products from donors sero- negative for cytomegolavirus were used. Cotrimoxazole was given for prophylaxis against *Pneumocystis carinii*.

Patient Characteristics

Of forty-six patients entering these studies, 37 underwent a single bone marrow transplant and 9 underwent the double bone marrow transplant regimen. Patient characteristics are outlined in Table 3. Briefly, the median age was 28 (range 16 - 48) and the median Karnofsky score was 80. Thirty-six patients had evidence of progressive disease while receiving conventional chemotherapy, four had sensitive relapses and six had relapsed while off therapy. Twelve patients had primary refractory Hodgkin's disease demonstrating resistance to initial chemotherapy. Patients had previously received a median on 3 regimens (range 1-7) and had been treated with a median of 10 different chemotherapeutic regimens (range 4-12).

Table 1. Chemotherapy Regimens

	BEC - 1
BCNU	$450\text{-}600/M^2$
ETOPOSIDE	$1500\text{-}2000/M^2$
CYCLOPHOSPHAMIDE	120 MG/KG

	BEC - 2
BCNU	$400/M^2$
ETOPOSIDE	$1800/M^2$
CYCLOPHOSPHAMIDE	$5\ GM/M^2$

	TAVE
THIOTEPA	$900/M^2$
VELBAN	0.4-0.5 MG/KG
±ARA-C	$3\text{-}6\ GM/M^2$

**Table 2. Antiemetic Regimen for Patients
Receiving Aggressive Chemotherapy**

| METOCLOPRAMIDE | 50 MG IV
THEN 25 MG/HR X 60 HRS |
|---|---|
| DIPHENHYDRAMINE | 25 MG IVP Q4H X 18 DOSES |
| METHYLPREDNISOLONE | 125 MG IVP Q4H X 18 DOSES |
| LORAZEPAM | 1-2 MGQ12H X 6 DOSES |
| SCOPOLAMINE PATCH | Q72H |
| CLONIDINE | 0.1-0.2 MG Q12H X 10 DOSES |

Table 3. Patients Characteristics

NO. ENTERED	46
MALES	25
AGE: MEDIAN	28
(RANGE)	(16 – 48)
KPS: MEDIAN	80
(RANGE)	(30 – 100)
NUMBER OF PRIOR CHEMOTHERAPY REGIMENS	
MEDIAN	3
(RANGE)	(1 – 6)
PRIMARY REFRACTORY	12
REFRACTORY TO LAST REGIMEN	36
SENSITIVE RELAPSE	4
RELAPSING OFF THERAPY	6

Nineteen patients had received at least 2 of the drugs used in the study and six had had all three drugs.

Of the forty-six patients entered into these studies, 23 were treated with the BEC I protocol. Of those, 10 achieved a complete remission (43.5; 95% CI 23-64). In addition, 5 patients had a partial remission (21.7%; 95% CI 5-39%). The median duration of complete remission for these patients was 6 months (range 2 - 13+ months). Of 23 patients treated on the BEC II protocol, 22 are currently evaluable for response. Eleven (50%; 95% CI 29-71%) achieved a complete remission. Another 5 patients (23%; 95% CI 5-40%) achieved a partial remission. The median duration of a response in this program has not been reached, with patients being alive from 3+ to 14+ months. Nine patients treated with the BEC II protocol were subjected to a second bone marrow transplant with the TAVe regimen. Five of those patients (55%; 95 confidence; 23-88%) are in continuous complete remission from 8+ to 14+ months post transplant, and one patient is too early post second BMT. Eleven patients with disease refractory to primary therapy participated in these studies. Four achieved a

complete remission. Responses were short in duration for these patients and lasted for an average of 3 months.

Stomatitis, nausea and vomiting were common toxicities. Nausea and vomiting were fairly well controlled and anticipatory nausea and vomiting was almost non-existent. Hepatotoxicity was more frequently noted in patients receiving high dose BCNU ($600/m^2$) or high dose ThioTEPA (900 mg/m^2). Myalagias and ileus were noted in patients receiving vinblastine infusions.

Significant neurologic toxicity was seen in patients receiving concomitant ThioTEPA and cytarabine. Thus cytarabine was deleted from the TAVE program.

Liquid stored bone marrow provided sufficient support in that time to recovery to WBC > 1.0×10^3/cu mm or a granulocyte count >500/cu mm was 18 days (range 11 - 43) post transplant. Count recovery was slower for patients undergoing a second bone marrow transplant, and may have been influenced by early attempts at re-harvesting marrow prior to each transplant.

Pulmonary dysfunction was more common in patients undergoing higher dose BCNU therapy. Seven of 12 patients (58%; 95% confidence; 30-36%) had interstitial pneumonia following high dose BCNU therapy. This effect was seen in only 4 of 27 patients receiving 400 mg/m^2 or less of BCNU. Similarly the toxic death rate was lower for patients receiving 400 mg/m^2 of BCNU versus 600 mg/m^2; i.e. 0 of 25 versus 5 of 12 (p <0.05). These effects are tabulated in Table 4.

DISCUSSION

It is unclear at this time whether autologous bone marrow rescue is necessary (14). Autologous bone marrow transplantation probably accelerates recovery of hematopoiesis (15). It is possible that the high dose drug regimens used in various protocols do not cause irreversible bone marrow toxicity. It appears unlikely that autologous marrow transplantation is an absolute requirement for hematopoietic recovery. Autologous marrow transplantation however, may shorten the total duration of pancytopenia. This however, has not been proven in controlled clinical trials. Hematopoietic growth factors may be useful in making autologous transplantation safer (16). Preliminary evidence from our labs indicates a lower response, *in vitro*, to colony stimulating activity in terms of CFU-C/BFU-E production by bone marrow from patients with Hodgkin's disease (17). Improved methods of stem cell collection and storage could help accelerate hematopoietic recovery following aggressive chemotherapy (18).

The toxic death rate seen in patients receiving aggressive chemotherapy is in part related to BCNU dosage. As shown in Table 4, higher doses of BCNU are associated with a higher toxic death rate

Table 4. Toxic Death Rate (TD)
Interstitial Pneumonia (IP)

	# Entered	# IP (CI%)	TD
BCNU 600			
	12	7 (58%; 20-86)	5 (41%; 14-70)
BCNU 450			
	9	5 (55.6%; 23-88)	1 (11%; 0-32)
BCNU < 400			
	25	4 (14.8%; 1-28)	0 (0%;0-0)

CI: 95% confidence interval

and an increased risk of interstitial pneumonitis. Thus, while lower doses of BCNU may be preferable, this purpose may be self defeating in that the full therapeutic dose of BCNU cannot be utilized. Other agents should be evaluated for high dose therapy beside BCNU.

Sequential myeloablative regimens are certainly toxic but may be useful for highly selected subgroups of patients with advanced Hodgkin's disease (19). The three toxic deaths noted in our program included one secondary to sepsis from pseudomembranous colitis and two due to non-engraftment following use of marrow obtained at a second harvest after the first and prior to the second bone marrow transplant. Patients who have survived the double transplant regimen continue to enjoy disease free survivorship on no maintenance therapy.

New programs will have to focus on identifying patients at a higher or a lower risk of relapse following autologous marrow transplantation for Hodgkins' disease and will thus have to be tailor made for various respective subgroups. Patients with poor pulmonary reserve should receive lower dose BCNU contain regimens or regimens containing no BCNU at all. Patients with primary refractory disease rarely, at least in our experience, have durable complete remissions. For them entirely new approaches have to be devised.

Marrow transplantation is now an accepted mode of therapy for advanced Hodgkin's disease and should probably be employed earlier in its course.

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Discussion 2 - Session II (Lymphoma)

Dr. Goldstone: Lymphoblastic lymphoma, despite being a fairly rare subtype, has been one that has caused, maybe, an inordinate amount of discussion. It has been the lymphoma in which patients are most likely to be transplanted in first CR. And the data that has been previously presented from the AMBTG would suggest that patients transplanted in first CR have done really quite well. That is part of the stimulus for what Dr. Armitage will share with us in a moment. As we start out this discussion, I think it is worthwhile to remember that lymphoblastic lymphoma from the point of view of the clinician does not represent just one disease. Any kind of lymphoblastic lymphoma in childhood seems to do quite well, as do most children with immature lymphoid malignancies in childhood. Lymphoblastic lymphoma in adults can probably be subdivided into two groups. Patients who have either a low LDH and do not have CNS involvement do especially well and, with any sort of chemotherapy regimen including CHOP-like regimens, well over half of those patients are cured now in a number of studies. Patients who have any of the bad prognostic factors do incredibly poorly, with 10 or 20% survival in almost all series. Despite the fact that they go into a CR promptly, they relapse and die and have provided us with a stimulus with the possibility of utilizing high-dose therapy and autotransplantation earlier in the course of the disease. So, for the first part of the general discussion, I would like Dr. Armitage to develop a little about the rationale of where the place might be for high-dose therapy in this disease and early in its treatment. Then we will go ahead with some of the other comments.

Dr. Armitage: Here, as you said, we do have a group of patients that can be clearly defined who are high risk in first CR, high risk for failure and are good candidates for a new approach. And what I would say is that this is a particularly good group to look at the question of intensification without BMT and addressing the issue also

of local disease aside from transplant, because many of the failures are in the CNS and the mediastinum. Also, local sites will not necessarily be addressed effectively by just the standard marrow transplant regimen. So that it may (very) well be that in this situation intensification of therapy and addressing those local sites, without a marrow transplant, would be an equally effective approach and the risk of marrow involvement in the autologous transplant. Because many of these patients at high risk have marrow involvement to begin with, I think (that) is another point in favor of that. And we can give very intensive therapy. Some of the same chemotherapy along transplant regimens that have been used could be given in the non-transplanted group. And that would allow you -- if you chose -- to address the question of TBI which is the only modality which we actually need to do with the autograft. And you could look at that as part of the question.

Dr. Dicke: I come back to the third point of your discussion, that is, TBI or no TBI. The fact that you did not find or see differences in the European studies between TBI and chemotherapy might be that in the TBI containing regimens the mortality of these programs was high.

Dr. Philip: No, I think the fact that you cannot see any differences at the moment is because the numbers are too small. But all the evidence we have on procedure-related mortalities, there is no difference in lymphomas with TBI regimens than with chemo-regimens.

Dr. Philip: The only real question is to know if we are allowed, in fact, to go so quickly to the question of CR1 patients. Because, really, I think that the two slides that Tony shows are very important slides and that confirms some of my slides in the French group and I think it is quite obvious that cyclophosphamide and TBI did not give good results. But particularly if you look at relapse of lymphoblastic lymphoma. The relapsed lymphoblastic lymphoma are doing very poorly with about 25% long-term survival which is not what we are able to achieve with non TBI regimen in B lymphoma. Then my question is to know if the first step should not be to go back to relapse again and to find the real correct protocol to be used in CR1.

Dr. Kersey: I would just like to comment on the question of whether or not it is appropriate to transplant children and adolescents with lymphoblastic lymphoma in first CR. The childrens cancer study group in the U.S. has now data on more than 300 children adolescents with lymphoblastic lymphoma. The overall long term disease-free survival in that group of patients is 70%. The worst group of all is the group that is greater than 15 but that group has a disease-free survival

of 50%. And there are no really bad factors in the total group, in otherwords, bone marrow involvement is not a bad prognostic factor, CNS involvement is not a bad prognostic factor, the only other factor of interest is actually race, in terms of predicting outcome. I think those data, if they are similar to others data, would suggest that probably there is not a group of patients, children, adolescents, who should be transplanted in first CR.

Dr. Kaizer: The French data on LL that were presented gives me an opportunity to come back and answer Tom Frei's question from before and that is the role of regional radiation in bulky tumor. You saw in the French data that most of their relapses were in the mediastinum. I can comment on the Rush series and the initial series from Hopkins of 25 patients that were published in BMT. Those patients who received regional radiation, and who relapsed, had regional relapses they which were outside of the treatment field.

Dr. Pinkerton: In the U.K. National CCSG study stage III and stage IV are treated just like T-cell ALLs and the outcome is excellent for stage IIIs and IV patients with bone marrow involvement. And we feel there is no indication for considering randomized studies or anything in these sort of patients.

Dr. Goldstone: Allright, so an area of some controversy with obviously more difficulty in identifying candidates for such a study in children than in adults where at least with the results that have been seen around the world for adults, thus far we could identify bad risk patients. Now we want to change pace and go from undifferentiated lymphoid malignancies to terminally differentiated T-cell to B-cell malignancies and Dr. Barlogie will present to us results about something that is fairly new and still unusual sounding and that is autologous transplantation in multiple myeloma.

Discussion 3 - Session II (Lymphoma)

Dr. Kaiser: In your British data base, did you look at the impact of relapse within the first year after therapy?

Dr. Goldstone: Yes, that has been presented before and there is no difference between those two groups in overall long-term survival on the BNLI data base. We do not believe that those patients relapsing early necessarily do worse.

Dr. Carella: In the patients who received regional radiation post-transplant, were those all pathologically proven residual disease?

Dr. Goldstone: No. Largely on clinical examination and scanning.

Dr. Jagannath: Tony, in your GM-CSF patients, did you look at number of febrile days? Days of fever has been a side effect of GM-CSF in other studies.

Dr. Goldstone: Yes, the number of febrile days are fewer. The number of antibiotics were not fewer simply because the patients were on antibiotic protocol which demanded that once given antibiotics they stayed on them for a set number of days. So you could not assess the difference in need of antibiotics in those particular patients.

Dr. Hagenbeek: Tony, can I reiterate what you had said one more time? That -- you said patients who achieved CR to MOP and subsequently relapsed are not the candidates right away for BMT, but not primary refractory if they have progressive disease on MOP which is a different issue?

Dr. Goldstone: Yes.

Dr. Herzig: Tony, just to comment about long-term follow-up. We had treated 26 patients with Hodgkin's disease with cyclo-

TBI, of which 10 are alive. The minimum follow-up is 3 years, and the maximum follow-up is 7 years. We have seen 3 relapses all in nodular sclerosing patients at the sites of original disease at approximately 3 years after transplant. So I think that underlines your concern about long-term follow-up. Conversely, some of those patients are beyond 5 years, free of disease. So they may well be cured. I think you have also answered Herb's question, at least in Hodgkin's disease, about partial responders. The two reasons for continuing to look at those patients is that some of them may be spot welded -- if you will -- into remission, such as you have done. The second reason is we have seen patients that have had persistant radiographic abnormalities -- especially nodular sclerosis disease in the medistinum -- even beyond 2 years that have normal performance statuses, normal gallium scans, etc., etc. We have coded those people as complete responses if they have made it a year.

Dr. Jagannath: Tony, you also made a point that most studies have short follow-up and the role of bone marrow transplant is still not yet proven in Hodgkin's disease. I would like to contradict that. I already presented you the MIME data. Even in the patients who achieved CR, there is a consistent relapse pattern. And then we saw the data published on CEP by Bonnadonna's group, where they had a 33% CR. If you take only the complete remitters, median duration of CR was only 17 months. If you plot disease-free survival for our patients who achieved CR, median is never achieved. And the third point is we also have this data on mini CBV which is a kind of another study -- where you are trying to do in a third salvage situation a conventional chemotherapy. You just fail. I think that the BMT role is pretty much established that as a very definite role in a third line situation.

Dr. Goldstone: I think -- actually you and I are not disagreeing. The point, however, I am really making is that we have no proof that the transplant yet is curing the patients, because that needs longer follow-up. But I am of the same view that it appears to be doing them some good because they are staying disease-free longer. Another point that I am trying to make is even if they do relapse, I believe some of them are still treatable. So, it is the proof of cure that we have not yet got -- because of follow-up. I agree with you. It looks as though we are beginning to get proof of the benefit from this high dose procedure at that time.

Dr. Bitran, Chicago: I have got one question for Dr. Carella and maybe I missed this. Was the mini CBV versus CBV times 2 a randomized study?

Dr. Carella: No.

IS THERE AN OPTIMUM CONDITIONING REGIMEN FOR PATIENTS WITH LYMPHOMA UNDERGOING AUTOLOGOUS BONE MARROW TRANSPLANTATIONS?

James O. Armitage and Philip J. Bierman

INTRODUCTION

Autologous bone marrow transplantation is an increasingly utilized treatment modality for patients with lymphoma who cannot be cured with standard chemotherapy regimens. Long term disease-free survival can be achieved, even in patients who have become refractory to chemotherapy administered at "traditional" dosage levels(1). In allogeneic bone marrow transplantation, the antitumor activity might be related in part to an immunologic effect of the new bone marrow. However, in autologous transplantation the antitumor effect of the treatment is presumed to be entirely related to the high dose treatment regimen and the bone marrow is given simply to rescue the patient from the severe myelosuppression. It seems highly unlikely that all high dose therapy regimens would be equally efficacious. Unfortunately, there are no randomized, perspective trials comparing the available high dose regimens.

The presently used preparative regimens for autologous bone marrow transplantation for patients with lymphoma are actually fairly similar. This is because there are only a few agents whose toxicity is sufficiently restricted to myelosuppression to allow significant dose escalation. Almost all preparative regimens include one or more alkylating agents. Other agents that are frequently utilized include etoposide and cytarabine. The latter drugs can be delivered in very high doses without marrow infusion when used as single agents. Finally, radiation is a common component of preparative regimens. This might be included as total body radiotherapy or localized radiation delivered in higher doses before or after the autologous

marrow infusion. Although, we lack completed, prospective studies comparing these approaches, there are a number of questions on which some data is available. These include the type, timing, and toxicity of radiotherapy, the intensity of the chemotherapy regimen utilized, and the effect of previous exposure to drugs in the preparative regimen.

Is There a Dose Response Effect at this Dosage Level?

Most, but not all, preparative regimens for autologous bone marrow transplantation are severely myelosuppressive and lead to prolonged marrow aplasia. Although many patients would eventually recover hematopoiesis after their administration if they survived prolonged cytopenias, hematopoiesis is presumably restored more rapidly by infusion of the autologous bone marrow. The fact that hematopoietic cells can survive even the most aggressive treatment regimens is reflected in the frequent persistence of host hematopoietic tissue after high dose total body radiotherapy and allogeneic bone marrow transplantation (2).

Available preparative regimens for autologous bone marrow transplantation which utilize the same agents are not all equally intense. For example, Table 1 illustrates three variations on the "CBV" regimen that was developed at MD Anderson Hospital (3). The other two regimens are the variation utilized by the group in Genoa and other Italian investigators (4), and the higher dose version utilized in Vancouver (5). The dose cyclophosphamide varies by approximately 20%, but the dose of etoposide varies by more than 300%. Since higher doses might lead to increased toxicity, the presence or absence of a dose response effect in the tumor at this dosage level is an important issue. Table 1 presents the results with these three dosage levels in treating patients with Hodgkin's disease. As can be seen, the more intense regimen utilized in Vancouver seems to have a higher complete response rate without much increase in early, fatal toxicity. However, the patients in these three studies are not necessarily comparable. The patients treated in Vancouver apparently included a higher proportion of patients who had failed only one chemotherapy regimen and were in good clinical condition. A prospective trial would be necessary to determine which treatment approach is superior.

Does Previous Exposure to Drugs in the Preparative Regimen Effect the Outcome?

In general, the primary chemotherapy regimens for patients with lymphomas utilize the most active available agents. If these agents could be dose escalated safely, they could also be included in preparative regimens for bone marrow transplantation. However, the

importance of previous exposure to a drug at a lower dose in the outcome of autologous bone marrow transplantation has not been clearly answered.

I reviewed the results for autologous bone marrow transplantation in the first 40 patients with lymphoma that we treated. These patients all have a greater than three-year follow-up. The results are presented in Table 2 in relation to whether or not the patients had been previously exposed to one of the drugs used in the bone marrow transplant preparative regimen and whether or not they were classified as good risk or poor risk. To be considered a good risk, a patient had to have a tumor that was responsive to the last chemotherapy regimen administered at traditional doses, a Karnofsky performance score of at least 80, and no bulky tumor (i.e. any tumor mass > 7 cm).

Table 1. Effective of Dosage Level in CBV Regimens for Relapsed Hodgkin's Disease

Investigators	Dose (gm/m^2)* C	B	V	Number of Patients	CR %	ED*%
MD Anderson/UNMC	6.0	0.3	.75	62	47%	7%
Italy	6.0	0.6	.60	45	56%	4%
Vancouver	7.2	0.6	2.40	55	80%	11%

*C - cyclophosphamide; B - carmustine; V- etoposide; ED - early death

Table 2. Effect of Previous Exposure to Drugs in the Preparative Regimen

Group		Total Pts	Currently Alive (%)	Continuously Free From Relapse (%)
Previous Exposure:				
	Yes	23	7 (30%)	5 (22%)
	No	17	7 (41%)	7 (41%)
Good Risk Patient:				
	Yes	20	13 (65%)	12 (60%)
	No	20	1 (5%)	0 (0%)

As can be seen there is a slight, but not significant advantage for patients who have not been previously exposed to one of the drugs in the preparative regimen. However, by far the most striking variable in predicting outcome was whether or not the patient fit into the good risk group. The tremendous importance of the patient characteristics on outcome of autologous transplantation is an important factor in the need for a prospective, randomized trail to identify the best treatment approach.

What is the Place of Radiotherapy in Preparative Regimens for Autologous Bone Marrow Transplantation?

Total body irradiation (TBI) is often, but not always included in preparative regimens for autologous bone marrow transplantation. In Hodgkin's disease radiotherapy has been utilized less frequently because a high proportion of patients with relapsed Hodgkin's disease will previously have had thoracic radiation therapy. However, most patients with non-Hodgkin's lymphomas have not been previously irradiated, and TBI is more frequently utilized. The exact contribution that TBI makes to preparative regimens for autologous bone marrow transplantation, however, remains unclear. In one large study involving 100 patients undergoing autologous bone marrow transplantation in centers in Europe and North America, TBI was administered to 39 patients and 61 received only high dose chemotherapy (1). Patients were not randomly assigned to the treatment groups. The rate of long term disease free survival was almost identical in the two groups, but TBI was associated with a higher early death rate (i.e. 28% vs. 16%). At the University of Nebraska Medical Center we have noted a higher rate of pulmonary toxicity in patients who receive TBI in their preparative regimen for autologous bone marrow transplantation. This information is presented in Table 3. Thirty-one percent of 54 patients with a variety of malignancies who received TBI in their preparative regimen developed a syndrome of diffuse pulmonary infiltrates, hypoxia, and alveolar hemorrhage documented by bronchio-alveolar lavage. In contrast only 14% of 87 patients who did not receive radiotherapy in their preparative regimen developed this syndrome. Although the diseases being treated were not randomly distributed, this difference is significant (i.e. $p = .01$). These data suggest that TBI is accompanied by more toxicity than some other agents that might be included in bone marrow transplant preparative regimens. Of course, this increase toxicity might be overcome by a greater antitumor efficacy.

Localized TBI is also often included in the treatment of patients with refractory lymphoma undergoing autologous bone marrow transplantation. This might be administered before the transplant as part of the immediate pre-transplantation treatment as in the BEAC

Table 3. Occurrence of Pulmonary Toxicity After Autologous Bone Marrow Transplantation at the Univ. of Nebraska Medical Center

TBI	Number of Patients	Occurrence of the Syndrome Diffuse Alveolar Hemorrhage (%)
Yes	54	17 (31%)
No	87	12 (14%)

regimen (6) or given later after recovery from myelosuppression to the sites of previously bulky disease. We and other investigators have utilized both treatment approaches and both can lead to long term disease free survival. There is no data available comparing the merits of these two approaches.

CONCLUSIONS

At present there is not one preparative regimen for autologous bone marrow transplantation for patients with lymphomas with clearly superior efficacy. In choosing a regimen for the treatment of any particular patient or group of patients, both toxicity and antitumor efficacy are important and need to be considered. The relative importance of toxicity increases as better risk patients are treated. The best regimen will likely vary for different histologic subtypes of lymphoma and with the timing of the transplant during the course of the disease. In any particular clinical setting identifying the best regimen will probably require prospective, comparative clinical trials.

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AUTOLOGOUS BONE MARROW TRANSPLANT CONDITIONING REGIMENS FOR NON-HODGKIN'S LYMPHOMA

Gordon Phillips, Joseph W. Fay, Roger H. Herzig, Hillard Lazarus, Fred C. LeMaistre, Steven N. Wolff, and Geoffrey Herzig

INTRODUCTION

Previous studies (1) have demonstrated that intensive therapy plus autologous bone marrow transplants (AuBMTs) can cure some otherwise incurable patients with progressive intermediate-grade (IG) or high-grade (HG) non-Hodgkin's lymphoma (NHL). The challenge now facing investigators is to improve upon these results with more of the many patients who might benefit from AuBMT therapy.

Extrapolating from the pioneering studies of Thomas et al. (2), it is likely that AuBMT regimens would substantially decrease disease recurrences when given to NHL patients in first remission. However, this strategy might actually decrease the excellent survival noted with newer chemotherapies (3) in certain NHL patients by increasing toxic deaths. Therefore, while AuBMT in first remission may be indicated for identifiable poor-prognostic groups in some NHLs, the high durable response rates demonstrated with newer chemotherapies suggest AuBMT will be appropriate for only a small number of first-remission NHL patients. Since AuBMT regimens will thus more frequently be applied to NHL patients not in first remission, current treatment regimens must be improved.

Evaluation of regimens consisting of intensive therapy plus AuBMT is hampered by difficulty in determining the reason for relapse following AuBMT; that is, whether the conditioning regimen was inadequate or whether the transplanted marrow was contaminated with occult malignant cells. However, recurrence due to contaminated AuBMT is considered a lesser problem than regimen inadequacy, since similar relapse rates have been noted with unpurged autologous or

normal-donor BMT in one large series of patients with NHL and Hodgkin's disease (4). For this reason, the potential of relapse due to contaminated AuBMT will not be considered in this discussion.

SPECIFIC TREATMENT REGIMENS

Between 1977 and 1988, we treated 128 NHL patients with the AuBMT regimens described herein. Virtually all had IG or HG NHL progressive after primary chemotherapy, and all were considered incurable with conventional therapy. Patients were excluded for age >65 years, abnormal marrow histology at harvest, or medical problems that would preclude intensive therapy; otherwise, patients were unselected. Most were refractory to conventional chemotherapy (i.e., they were induction failures or had disease which progressed despite therapy) and fewer than 5% were treated while responding to secondary chemotherapy.

Our treatment regimens and "optimal" dose schedules are summarized in Table 1. Some of the results noted in Table 2 were with patients receiving slightly different doses of these agents. All marrows were cryopreserved. While the majority were not purged, selected patients have recently received chemo-separated AuBMTs.

Cyclophosphamide Plus Total Body Irradiation (CY+TBI) (Table 1A)

Initially, we used cyclophosphamide (CY) 120 mg/kg followed by unfractionated total body irradiation (TBI) 1000 cGy. Later, we used fractionated (f) TBI in 1200, 1400 or 1600 cGy doses, with the 1400 cGy regimen considered optimal. A total of 35 NHL patients were so treated; 50% achieved complete remission (CR). Six patients (17%) remain in CR at a median 4.0 years, including 4 in continuous CR. Two > 5-year survivors died of non-lymphomatous causes. The toxic death rate was 23%.

Cyclophosphamide Plus Total Body Irradiation Plus Involved-Field Radiotherapy (IF-RT)

In the above series, ˜30% of failures were focal. In 1981 we consequently first gave IF-RT (1500-2400 cGy) immediately before CY-fTBI (1200 cGy) to selected patients. Both the CR rate (69%) and the disease-free survival (DFS) rate (31%) was increased (albeit not significantly), and in-field relapses were uncommon. However, the design of the study precluded a definite analysis of IF-RT. In this (and the previous) study, fatal interstitial pneumonitis (IP) was seen chiefly in patients who received additional mediastinal radiotherapy (RT); in the combined NHL and Hodgkin's disease (HD) series, 7 of

Table 1. Conditioning Regimens for AuBMTs[1] in Non-Hodgkin's Lymphoma

A. Cyclophosphamide Plus Total Body Irradiation (CY+TBI)

		Day					
Agent	Daily Dose	-5	-4	-3	-2	-1	0
CY	60 mg/kg	*	*				BMT
TBI	400 cGy			*/*	*/*	*/*	*/-

B. Etoposide, Cyclophosphamide Plus Total Body Irradiation (VP+CY+TBI)

		Day							
Agent	Daily Dose	-7	-6	-5	-4	-3	-2	-1	0
VP16-213	1800 mg/kg	*							B
CY	60 mg/kg			*	*	*			M
TBI	400 cGy					*/*	*/*	*/-	T

C. Cyclophosphamide, Carmustine (BCNU) Plus Etoposide (CBV)

		Day						
Agent	Daily Dose	-6	-5	-4	-3	-2	-1	0
CY	1.8 g/m^2	*	*	*	*		Rest	B
BCNU	0.6 g/m^2					*		M
VP16-213	0.4 g/m^2	*	*	*				T

D. Etoposide, ThioTEPA Plus Cyclophosphamide (VP+TT+CY)

		Day									
Agent	Daily Dose	-9	-8	-7	-6	-5	-4	-3	-2	-1	0
VP16-213	1000 mg/m^2	*								Rest	B
TT	300 mg/m^2		*	*	*						M
CY	50 mg/m^2					*	*	*	*		T

[1] Autologous bone marrow transplantation

Table 2. Conditioning Regimens for NHL[1]: Results

Regimen	No. Pts	%Refr[2]	%CR[3]	%Rel[4]	%Trd[5]	N, A&W[6]: Median and (Range in Years)
CY+TBI±IF-RT	67	69	57	75	19	16:4.0(2.8-6.5)
VP+CY+TBI	30	NA	60	--	20	13:0.9(0.2-1.7)
CBV	5	80	--	--	20	--
VP+TT+CY	26	NA	45	44	38	5:1.2(0.7-2.2)
Totals	128	NA	54	NA	24	34

[1]Non-Hodgkin's lymphoma.

[2]%Refractory: Induction failures and disease progressions despite chemotherapy (primary and secondary).

[3]%Complete response.

[4]%Relapse.

[5]%Treatment-related deaths.

[6]Number, alive and well.

25 such patients developed fatal IP, compared with only 2 of 68 patients who did not receive the additional mediastinal RT (p <0.01).

Etoposide, Cyclophosphamide Plus Total Body Irradiation (VP+CY+TBI) (Table 1B)

An obvious next step was to add a third agent. Etoposide was attractive because it was not used in then-current primary regimens, had a limiting toxicity (mucosal) which did not severely overlap with that of CY+TBI, and had been given in high doses, alone and with either cyclophosphamide or fTBI. A concurrent attempt was made to optimize the CY dose; this was found to be 60 mg/kg daily x 3, and we have recently determined the fTBI dose of 1000 cGy as optimal. While it is difficult to compare this series with CY+TBI, adding etoposide did not clearly change either initial response or toxic events. However, early results regarding disease-free survival appear promising; longer follow-up will be required to determine eventual recurrence rates.

Cyclophosphamide, Carmustine (BCNU) Plus Etoposide (CBV) (Table 1C)

While previous studies had suggested that TBI is important in conditioning for some NHL (5), we tried this regimen because TBI is

excessively toxic, ineffective or inconvenient for some patients. Moreover, combination chemotherapy regimens (without TBI) were proving effective in HD. Unfortunately, working with a small number of NHL patients, we did not find our modification of the original (6) CBV regimen (i.e., cyclophosphamide 7.2 g/m^2, BCNU 0.6 g/m^2 and etoposide VP16-213 2.4 g/m^2) to be effective.

Etoposide, ThioTEPA Plus Cyclophosphamide (VP+TT+CY) (Table 1D)

Our group has also investigated high-dose ThioTEPA (TT) (7), an agent attractive for use in NHL protocols for various reasons, including its excellent penetration of the central nervous system (CNS). Although these results were slightly inferior to those with other regimens, the true effectiveness of this regimen may have been obscured because only patients ineligible for VP+CY+fTBI were treated.

CONCLUSIONS

It is difficult to determine the relative benefits of these regimens due to the sequential nature of the studies, differing patient selection criteria, and possibly an increasing use of marrow purging techniques. At present, there is no clear advantage to any of these regimens, although long follow-up is required. In any case, further work is clearly needed. Our current practice is to use VP+CY+fTBI and IF-RT, avoiding the mediastinum. VP+TT+CY is used for patients who have either previously received or require additional mediastinal radiotherapy.

FUTURE DIRECTIONS

If current treatment regimens are suboptimal, how can they be improved? First, while better patient selection (i.e., AuBMT at first sign of disease progression) is important, most BMT centers do not have control over the timing of referral, and results with current treatment regimens cannot be improved by this method unless the medical community acts on this recommendation to improve patient selection. In any case, it is unlikely that this situation will change markedly in the near future, and NHL patients with advanced disease will continue to require AuBMT. Cytoreduction must therefore be improved if durable response rates are to be raised.

One approach is the use of conventional therapy to produce cytoreduction before conditioning; AuBMT regimens are given only to those patients who respond. While this approach will likely increase

the percentage of long-term disease-free survivors, however, there are drawbacks: 1) these conventional regimens are not very effective, thereby limiting patient numbers; 2) such therapy may produce excessive toxicity and thereby preclude AuBMT; 3) since success with AuBMT is limited mainly by resistant tumor cells, which conventional therapy does not destroy, delays caused by administering conventional therapy may actually increase relapses--despite producing a lower tumor burden at AuBMT.

It is therefore likely that improved conditioning regimens must be developed. If conventional chemotherapy drugs are utilized, regardless of dose, they should be chosen for their absence in primary regimens -- a choice that grows more difficult as the number of drugs in primary regimens increases. New agents must be evaluated in systematic Phase I and II trials, and nontraditional agents (e.g., biologic response modifiers, immunoradio-pharmaceuticals, etc.) should be investigated.

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ESCALATING DOSE CYCLOPHOSPHAMIDE, BCNU, AND VP-16 WITH AUTOLOGOUS BONE MARROW TRANSPLANTATION IN RELAPSED HODGKIN'S AND NON-HODGKIN'S LYMPHOMA: A Phase I Trial

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Autologous bone marrow transplantation (ABMT) obviates myelosuppression as the dose-limiting toxicity of selected chemotherapeutic agents and permits exploitation of dose-response effects of these drugs by the use of combination chemotherapy in doses limited only by major organ toxicity. Spitzer *et al* have demonstrated the efficacy of cyclophosphamide, BCNU and VP-16 in conjunction with ABMT in patients with refractory lymphoma employing doses below the maximally tolerated dose of each drug when used as a single agent (1,2). In the current study, doses of cyclophosphamide, VP-16 and BCNU were escalated in a stepwise fashion to determine the maximally tolerated doses of these agents in patients with relapsed Hodgkin's disease and non-Hodgkin's lymphoma.

PATIENTS AND METHODS

Between March, 1986 and July, 1988, 52 patients were treated with cyclophosphamide, BCNU and VP-16 at seven dose levels (see Table 1). Doses were escalated after at least three patients had successfully completed therapy at a given dose level; if grade III or IV toxicity occurred (by WHO criteria) at least five patients were accrued at that level prior to dose escalation. Infectious complications and

Table 1. Dose Levels

	I (n=7)	II (n=4)	III (n=5)	IV (n=9)	V (n=8)	VI (n=7)	VII (n=12)
DRUG (TOTAL DOSE)							
Cyclophosphamide (g/m^2)	4.5	6.0	6.0	7.2	7.2	7.2	7.2
BCNU (mg/m^2)	450	450	450	450	450	450	600
VP-16 (mg/m^2)	1200	1200	1600	1600	1800	2000	2000

reversible oral and esophageal mucositis were not considered contraindications for further dose escalation. Cyclophosphamide and VP-16 were administered in eight divided doses -7 to -3. BCNU was administered as a bolus on day -7 in dose level I; subsequently the dose was divided into four equal daily doses day -7 to day -3. Unpurged autologous marrow was reinfused day 0. All patients had continuous bladder irrigation days -7 to -2. Patients with non-Hodgkin's lymphoma in first or subsequent relapse, or with Hodgkin's disease who had failed two chemotherapy regimens or MOPP/ABVD, were eligible for therapy. Two patients with poor risk non-Hodgkin's lymphoma in first remission are also included. Entry criteria included negative bilateral bone marrow biopsies at time of ABMT, normal cardiac and renal function and pulmonary function tests greater than 70% of normal predicted values. Individual exceptions were made for patients otherwise healthy with pulmonary function of 50-60% predicted. No requirements were made for a minimal disease state. Patients were treated with salvage chemotherapy or focal radiotherapy prior to ABMT on an individualized basis.

RESULTS

Clinical characteristics of the patient population are summarized in Table 2. Adverse prognostic factors for either Hodgkin's disease or non-Hodgkin's lymphoma have been identified in other series (3,4). Toxicity (WHO criteria) by dose level was summarized in Table 3. There have been three treatment-related deaths: one hepatic venoocclusive disease, autopsy documented, at level I; one interstitial pneumonitis at level IV; and one cardiac arrest during marrow reinfusion at level VII. Mild hepatotoxicty occurred at all dose levels but severe elevations in liver function tests were not clearly dose-related and resolved except for the fatal case. Interstitial pneumonitis

Table 2. Patient Characteristics

DIAGNOSIS	n
Non–Hodgkin's Lymphoma	25
Hodgkin's Disease	27
	52

Median Age (Range) 34 (18-66)

Prognostic Factors

Prior Chemo Regimens >2	19
Disease Bulk ≥2 CM	38
No Prior CR	14
Unresponsive to Salvage Chemotherapy	14
Prior Chest Radiotherapy	23

Table 3. Acute Toxicity by Dose Level
(WHO Criteria)

Dose Level	I (n=7)	II (n=4)	III (n=5)	IV (n=9)	V (n=8)	VI (n=7)	VII (n=12)	TOTAL (n=52)
Toxic Death	1	0	0	1	0	0	1	3
Hepatotoxicity (grade 3-4)	1	0	0	1	1	2	0	5
Interstitial Pneumonitis (grade 1-4)	0	0	0	1	0	1	1	3
Mucositis (grade 3-4)	0	1	1	4	7	6	11	30
Infections	0	0	0	4	3	2	5	14
Septicemia	0	0	0	2	2	1	3	8
Focal Pneumonia	0	0	0	0	1	1	1	3
Other	0	0	0	2	0	0	1	3
Hematuria (grade 2)	0	1	0	0	1	1	1	4

occurred in three patients. Severe mucositis became prominent at dose
level IV and occurred in nearly all patients at the highest dose levels.
Infectious complications were also more prominent at dose level IV
and above. Most patients who achieved remission returned to a normal
pretransplant level of function. Chronic toxicity included asympto-
matic decreases in cardiac ejection fraction and pulmonary diffusing
capacity in 5/18 and 11/20 evaluable patients respectively; non-A,
non-B hepatitis in 2 patients; 1 patient with acute non-lymphocytic
leukemia; and 4 patients with localized varicella zoster.

Eighteen patients with non-Hodgkin's (NHL) lymphoma and
twenty-two patients with Hodgkin's disease (HD) had measurable
disease at transplant and are evaluable for response. Of the patients
with NHL, 5 had complete responses (CR), 8 had partial responses
(PR), 2 had stable disease (SD), and 3 had progressive disease (PD)
within 30 days of transplantation. Of patients with HD, 10 had CR,
8 PR, 3 SD and 1 PD. The heterogeneity of patient populations makes
response comparison between dose levels difficult. There was no
clear-cut increase, however, in either CR or CR+PR rates with
increasing dose. Several patients, particularly with HD, had
radiographic residue of uncertain significance; the response rates,
therefore, may underestimate the number of patients who have
inactive disease. Results described in terms of time to treatment
failure (including all responses and toxic deaths) are described in
Figure 1.
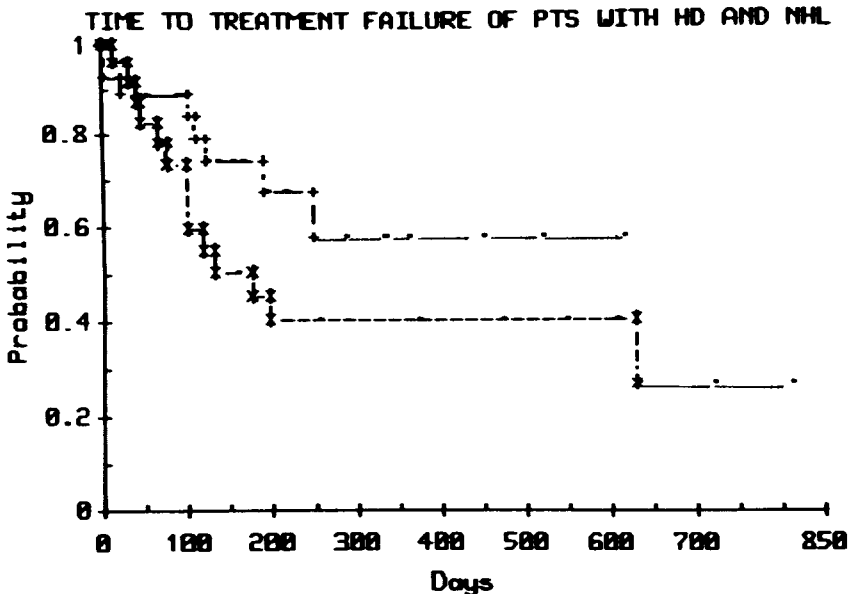
Figure 1. Time to treatment failure of 52 patients with HD (- + -) and NHL (- * -)
treated with escalating doses of cyclophosphamide, BCNU and VP-16.

Currently, 14/25 patients with NHL and 15/27 patients with Hodgkin's disease are free from disease progression with median follow-up of 195 and 168 days respectively.

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CONCLUSIONS

Cyclophosphamide (7.2 gms/m^2), BCNU (600 mgs/m^2) and VP-16 (2 grams/m^2) can be administered with acceptable toxicity to a heavily pretreated patient population on this dose schedule. Severe reversible mucositis occurs in the majority of patients at dose levels above 1.6 grams/m^2 of VP-16. Responses were seen at all dose levels; a dose-response relationship could be determined only by a randomized trial.

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HIGH DOSE CHEMOTHERAPY WITH AUTOLOGOUS BONE MARROW TRANSPLANTATION IN LOW GRADE NON-HODGKIN'S LYMPHOMAS

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Eleven patients with follicular low grade non-Hodgkin's lymphomas (L-NHL) were treated with high dose chemotherapy ± Total Body Irradiation (TBI) and Autologous Bone Marrow Transplantation (ABMT). Five of these 11 patients were follicular mixed (FM) and six patients were follicular small cleaved cell (FSC) lymphomas. Three patients were in first partial remission after conventional chemotherapy and eight in sensitive relapse. Conditioning regimens were BEAM for 7 patients and cyclophosphamide + TBI for 4 patients.

In the eight patients who had bone marrow purging either with ASTA Z (4 cases), or with monoclonal antibodies (4 patients), no toxic death was noted. Three patients relapsed 3 and 11 months after ABMT and eight patients remain in complete remission 6+ to 33+ months after treatment. These preliminary results suggest that intensive cytoreductive therapy followed by ABMT may improve disease free survival in patients with poor prognosis L-NHL.

INTRODUCTION

In the working formulation, low-grade non-Hodgkin's lymphomas (L-NHL) include three histologic entities: diffuse small cell lymphoma, follicular small cleaved cell (FSC) lymphoma and follicular mixed small and large cell (FM) lymphoma. The optimal treatment of stage III-IV of L-NHL has not yet been determined; few data have been reported about efficacity of high dose conventional chemotherapy in L-NHL.

It is known from experimental studies that many tumors, including lymphomas, show a step dose - response effect to cytotoxic drugs. Several previous reports have documented the activity of high dose chemotherapy with or without total body irradiation (TBI) and autologous bone marrow transplantation (ABMT) in patients with advanced lymphomas of poor prognosis or who have failed primary therapy. In non-resistant relapses, a long disease free survival rate of 30-60% can be expected (7-10).

Only few cases of high dose chemotherapy with ABMT in low grade non-Hodgkin's lymphoma has been yet reported (6-9-10). We report here preliminary results about 11 cases of follicular L-NHL who failed conventional therapy.

MATERIAL AND METHODS
Patients

Eleven patients with low grade NHL according to the working formulation were studied between July 1985 and December 1987. Details of the patients are summarized in Table 1: six patients were FSC lymphomas and five FM lymphomas. Staging of disease according to the Ann Arbor classification was determined at diagnosis. Restaging was performed at relapse and just before the ABMT procedure. Five patients had bone marrow involvement at diagnosis, one with leukemic phase. All patients received conventional therapy as initial treatment with different protocols: 1) Chlorambucil (CLB), or Melphalan (MELPH); 2) COP (Vincristine 1 mg/m^2 on day 1, Cyclophosphamide 500 mg/m^2 on day 1, Prednisolone 50 mg/m^2/day, 5 days) monthly; 3) CHOP (The same drugs with Adriamycin 50 mg/m^2 on day 1); 4) CHEP-Bléo (Adriamycin 75 mg/m^2 on day 1, cyclophosphamide 1,2 g/m^2 on day 1, Vindésine 3 mg/m^2 on days 1 and 5, Bleomycin 10 mg/m^2 on days 1 and 5, Prednisolone 50 mg/m^2 on days 1 to 10; 5) M-BACOD (Adriamycin 45 mg/m^2 on day 1, Cyclophosphamide 600 mg/m^2 on day 1, Vincristine 1 mg/m^2 on day 1, Bleomycin 4 mg/m^2 on day 1, Dexamethasone 6 mg/m^2/day for 5 days, Methotrexate 3 g/m^2 on day 14; 6) PROMACE (Etoposide 120 mg/m^2 on days 1 and 8, Cyclophosphamide 650 mg/m^2 on days 1 and 8, Adriamycine 25 mg/m^2 on days 1 and 8, Methotrexate 1.5 g/m^2 on day 15, Prednisolone 60 mg/m^2/day on days 1 to 15) - MOPP (Nitrogen mustard 6 mg/m^2 on days 1 and 8, Vincristine 1 mg/m^2 on days 1 and 8, Procarbazine 100 mg/m^2/day and Prednisolone 40 mg/m^2/day for 14 days.

Three patients were in good first PR, after M-BACOD (two patients) or CHEP-Bleo (one patient), one case with residual disease in nodes, the two others with a minimal residual nodular involvement of bone marrow. Four patients were in second CR and two others in third CR after relapses treated with Etoposide 100 mg/m^2/day and

Table 1. Characteristics of Patients

Case	Sex/Age	Hist.	Stage/Disease Sites at Presentation	Initial Therapy	Results	Relapse	Disease Sites of Relapse or Initial No Response	Therapy of Relapse	Response
1	M/29	FSC	III	M-BACOD	PR	no	Nodes + BM	--	--
2	F/36	FSC	IV (BM)	CLB	PR	yes	Nodes + BM	No. 1 = CHOP (2°cr = 8 months) No. 2 = MIME	PR$_3$
3	F/29	FSC	IV (BM)	Melph	CR	yes	Nodes	CLB + PROMACE MOPP	CR$_2$
4	F/41	FM	IV (BM)	M-BACOD	PR	no	BM	--	--
5	M/38	FSC	IV (Leuk)	Splenectomy	PR	no	BM	--	--
6	M/51	FM	III	COP	CR	yes	Nodes	No. 1 = CHEP Bleo (2° CR = 7 months) No. 2 = VP16 + CDDP	CR$_3$
7	F/38	FM	IV (BM)	COP	CR	yes	Nodes	No. 1 = CHEP Bleo (2° CR = 21 months) No. 2 = VP16 + CDDP	CR$_3$
8	M/42	FM	III	CHOP	CR	yes	Nodes + BM	VP16 + CDDP	CR$_2$
9	F/36	FSC	II	CHOP + RxT	CR	yes	Nodes + Skin	VP16 + CDDP	CR$_2$
10	F/27	FM	III	PROMACE + MOPP PROMACE - Cy TABOM	CR	yes	Nodes + BM	MIME; VP16 + CDDP + MITOX DHAP	PR$_2$
11	M/46	FSC	I	COP + RxT	CR	yes	Nodes	VP16 + CDDP	CR$_2$

FSC: Follicular Small Cleaved Lymphoma
FM: Follicular Mixed Lymphoma
RxT: Radiotherapy

CDDP 20 mg/m^2/day on days 1 to 5 for five patients. One patient was in second PR after salvage therapy. One patient was in third partial remission for a second relapse of nodular NHI treated by MIME (Methyl Gag 500 mg/m^2 on days 1 and 14, Ifosfamide 1 g/m^2 for 5 days, VP$_{16}$ 100 mg/m^2 for three days, Methotrexate 30 mg/m^2 on day 3) with residual involvement in nodes and bone marrow.

Complete remission was defined as the disappearance of all signs and symptoms related to the disease for a minimum of one month. Partial response was defined as a reduction of more than 50 percent in all measurable disease for a minimum of one month.

BONE MARROW PROCEDURES

Bone Marrow Harvest, Cryopreservation and Reinfusion

The technique was used as described by Appelbaum et al. (1) with slight modifications. The bone marrow was aspirated 1 to 24 months prior to transplantation and contained a mean of 2.3 x 10^6 nucleated cells per kilogram (range 0.4 to 4.9 x 10^8/kg) and 8.6 x 10^4 GM-CFC per kilogram (range 0.2 to 48 x 10^4/kg).

Ex Vivo Treatment

Two techniques were used for the ex vivo purging of bone marrow: chemical purging with ASTA Z for 4 patients and immunological purging with two pan B monoclonal antibodies in four cases.

Immunological Purging (3)

Briefly, samples were treated with cocktails of two pan B monoclonal antibodies either RFB$_7$ + SB$_4$ from Sanofi (IgM isotype, 1/50 final dilution). Samples were incubated 20 minutes with monoclonal antibodies and then twice with baby rabbit complement (from Institut Pasteur), with one wash between the two complement treatments. Complement was used at 1/3 final dilution and DNase I (pancreatic DNase, sterile, non-pyrogenic, from Sigma CC, Saint Louis, USA) 5 units/ml was added to each complement treatment.

Chemical Purging (40)

To obtain an optimal in vitro bone marrow cleansing we performed 2 weeks before harvesting an individual pre-test to determine the dose of ASTA Z corresponding to 95% destruction of CFU$_{GM}$ (LD 95). Purging was performed after adjustment to 20 x10^6 nuclear cells per

ml in TC 199 medium (Flobio) with an haematrocrit of about 5%. ASTA Z doses corresponded to the LD 95. Treatment lasted for 30 min at 37°C and bone marrow cells were washed twice before freezing.

CYTOREDUCTIVE REGIMEN

Under hyperhydration (3,000 ml/m^2/day) seven patients received combination chemotherapy consisting of BCNU 300 mg/m^2, IV on day 1; ARA C 100 mg/m^2 every 12 hours on days 2 to 5; VP$_{16}$ 100 mg/m^2 every 12 hours on days 2 to 5 and Melphalan 140 mg/m^2 IV on day 6. Two of these patients received also radiotherapy in involved fields.

Four patients received fractionated TBI on days -7, -6, -5, and cyclophosphamide 60 mg/kg on days -3, -2. Total doses of fractionated irradiation were 12 Gy with 18 MEV X-rays at a dose rate of 13 rads per minute. The lungs ere shielded to a dose of 8 Gy. Patients were hydrated adequately and were given mensa (to prevent hemorrhagic cystitis), Furosemide, Metopimazine and Methylprednisolone (to prevent vomiting).

RESULTS

Antitumor Effect (Table 2)

The details of each patient are summarized in Table 2. Four weeks after ABMT all patients were evaluable. No toxic death was noted.

Three patients received transplants in PR with residual disease in bone marrow. All achieved CR and are well and alive 9, 29 and 33 months after ABMT.

Eight patients were grafted after relapse; four in second CR, two in third CR and two in partial remission achieving CR after ABMT. Of these patients, only three developed a recurrent disease. One presented generalized lymphadenopathies three months after ABMT and died despite further therapy ten months after ABMT. The second one presented nodal and bone marrow relapse eleven months after ABMT and is alive 35 months after ABMT. The last one relapsed sixteen months after ABMT in lomboaortic nodes. The remaining five patients are still in unmaintained CR 6 to 21 months after ABMT.

Hematologic Toxicity

Details of hematologic toxicity are given in Table 3. All patients developed profound leukopenia and thrombocytopenia. Granulocyte counts of <0.5 x 10^9/l lasted 8 to 40 days from the day of ABMT.

Table 2. Evolution of Patients

Case	Delay Between Diagnosis and ABMT	Duration 1st CR	Duration 2nd CR	Status at ABMT	Conditioning Regimen	In Vitro Purging	Response	Current Status
1	6			PR_1	BEAM+RxT	Mo (Y2955+RFB7)	CR	CR + (29+)
2	26	12	8	PR_3	BEAM	Mo (SB4+RFB7)	CR	Relapse (16) alive in CR_4
3	39	26	/	CR_2	BEAM	Mo (Y2955 = RFB7)	CR	Relapse (11) alive with disease (35+)
4	6	/	/	PR_1	BEAM	Mo (SB4 + RFB7)	CR	CR + (9+)
5	12	/	/	PR_1	TBI + CPM	Mafosfamide	CR	CR + (33+)
6	34	19	8	CR^3	BEAM	- -	CR	Relapse (3) dead of disease (10)
7	32	5	21	CR_3	BEAM + RxT	- -	CR	CR + (21+)
8	25	15	/	CR_2	TBI + CPM	Mafosfamide	CR	CR + (13+)
9	39	26	/	CR_2	TBI + CPM	Mafosfamide	CR	CR + (8+)
10	52	19	/	PR_2	TBI + CPM	Mafosfamide	CR	CR + (8+)
11	28	20	/	CR_2	BEAM	- -	CR	CR + (6+)

Recovery to 50 x 10^9/l platelets occurred between 15 days and more than six months after transplantation.

Non-Hematologic Toxicity

Characteristics of the non-hematologic toxicity are summarized in Table 3. Nausea, vomiting, diarrhea and mucositis were mostly of a mild degree. Abnormal liver function tests were observed in two patients, consisting of slight and transient elevation of liver enzymes. Fever (> 38°C) developed in nine patients and was of unknown origin in seven patients. Only two patients developed documented bacteremia with staphylococcus epidermidis.

No hepatic veno-occlusive disease or pneumonitis was observed.

DISCUSSION

Adequate treatment of disseminated low grade non-Hodgkin's lymphoma remains unsolved (2, 5, 8). Despite a high initial remission rate, there is a continuous relapse rate and no plateau in survival is obtained. Thus, many approaches of treatment have been used with similar results: deferral of treatment until clinical indications are manifest, single agent chemotherapies, total lymphoid irradiation, Interferon. The first results with polychemotherapy were very disappointing and few data have been reported about treatment of L-NHL with chemotherapy regimens more intensive than CHOP.

Recently, Young et al (11) reported results of a randomized trial which compared "watch and wait" and aggressive therapy with PROMACE-MOPP (Prednisone, Methotrexate, Doxorubicin, Cyclophosphamide, Etoposide + Mechlorethamine, Vincristine,

Table 3. Hematologic and No Hematologic Toxicities

Case	WBC <1x10⁹/ℓ	PN <0.5x10⁹/ℓ	Platelets <50x10⁹/ℓ	Mucositis	Nausea Vomiting	Diarrhea	SGOT/SGPT
1	17	20	18	3	1	2	1
2	40	40	>180	1	2	0	0
3	17	14	24	1	2	0	0
4	12	13	30	1	3	0	0
5	14	8	21	2	1	0	1
6	20	13	16	2	1	0	1
7	7	10	19	1	2	2	0
8	38	18	106	2	2	1	0
9	18	18	15	2	2	1	0
10	28	28	>133	2	2	1	0
11	15	17	200	0	2	0	0

Procarbazine, Prednisone) plus total nodal irradiation in advanced indolent lymphomas. Although survivals are not significantly different between the two arms, disease-free survival rates are significantly different between the two arms, disease free survival rates are significantly different (51% versus 12%) at 4 years; it seems that aggressive initial therapy offers the prospect of patients being continuously disease-free.

Many authors have reported effectiveness of high dose chemotherapy and ABMT in advanced or relapsed non-Hodgkin lymphoma. The prognostic value of resistant and non-resistant relapse has been defined and these studies show that a disease free survival rate between 30 percent and 60 percent can be expected in non-resistant relapses (7-10) of intermediate or high grade NHL. Few data are known about the value of ABMT in L-NHL. Lu et al(6) reported one case of nodular poorly differentiated lymphocytic NHL who died on day 13 post ABMT in complete remission. Recently, Takvorian et al (10) published ABMT in 49 NHL with 6 nodular L-NHL. Seventeen out of 20 patients with intermediate or low grade NHL remained in complete remission but no specific data were given about these 6 ABMT patients. Schouten et al (9) reported good results in 7 out of 9 patients with nodular lymphomas grafted with TBI + Cyclophosphamide conditioning regimen with marrow or peripheral blood stem cells. In our study, we report results about ABMT in 11 cases of L-NHL of bad prognosis with conventional chemotherapy. Actually, only three patients relapsed -- three, eleven and sixteen months after graft.

The optimum conditioning regimen in ABMT of L-NHL is not yet defined. Total body irradiation and Cyclophosphamide seem to be efficacious in the report of Takvorian (10). In our study, the four patients treated with TBI and Cyclophosphamide are well and alive. However, four of seven patients treated with chemotherapy alone are actually incomplete remission and high dose chemotherapy seems to be able to eradicate disease in nodular lymphomas.

Moreover, this study suggests that:

1. Nodular lymphomas can be cured with high dose chemotherapy and ABMT;

2. High dose chemotherapy and ABMT seem to be as effective in bad prognosis L-NHL as in high grade NHL. But further data are required.

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AUTOLOGOUS BONE MARROW TRANSPLANTATION IN LOW GRADE NON-HODGKIN'S LYMPHOMA

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INTRODUCTION

The majority of patients with low grade NHL generally present with disseminated disease, usually due to bone marrow involvement, and although indolent, they are unlikely to be cured with standard therapy (1-4). The overall median survival is approximately 8 years in the best of series. The development of histologic transformation to an intermediate or high grade NHL is reported to occur in 15-70% of cases and is associated with a poor prognosis (5-8). Although most patients often achieve CR with conventional therapy, the median duration of CR is between 12 and 24 months, and most patients ultimately relapse with DFS at 5 years with only 25% survival. High dose chemotherapy with or without total body irradiation, and ABMT can salvage patients with relapsed high and intermediate grade NHL. For patients with low grade NHL, a relatively small number of patients have been treated using this approach. The early reports, with a total of only 5 patients, examined the use of marrow transplantation in low grade lymphoma which included both syngeneic and allogeneic BMT (9-11). In these limited studies, early toxic deaths were a major problem. There are several problems associated with ABMT in low grade NHL including the long natural history of indolent NHL, the high frequency of bone marrow involvement, the decreased marrow reserve and potential prolonged engraftment from previous therapy, and resistance to therapy following multiple relapses.

ABMT in Relapsed Low Grade (and Follicular) NHL

The use of ABMT in low grade lymphoma has been limited as compared to other histologies. Gorin et al. reported on two patients treated with bulky disease. One patient died early from toxicity; the other patient remains in CCR at 45+ months following ABMT (12).

Three larger series have treated relapsed patients with high dose therapy and ABMT. The University of Nebraska has transplanted 9 patients (median age of 39, range from 31-59) with relapsed follicular NHL (13). The histologic subtypes of these patients included 2 with small cleaved cell, 2 with mixed small cleaved and large cell and 5 with large cell type. The median time from original diagnosis to ABMT was 25 months (range from 5-47 months). These patients were generally still responsive to therapy. All achieved CR, with 6 of the 9 patients in continuous CR from 246^+ to 1385^+ days after ABMT. Two patients had local relapses at 370 (with diffuse large cell lymphoma) and 380 days post ABMT, and both received local XRT. One additional patient died in CR at 66 days of sepsis.

At the Dana-Farber Cancer Institute, patients with relapsed low grade NHL have been treated with cyclophosphamide and TBI followed by ABMT with anti-B cell monoclonal antibody purged marrow (14,15). Twenty patients with a median age of 41 have been treated as of 4/1/88. Twelve had follicular small cleaved type, 6 with follicular mixed small cleaved and large cell type, and two with diffuse intermediate lymphoma. The median time from diagnosis to ABMT was 31 months (range from 10-104). All patients were responsive to standard therapy. At the time of intensification 8 patients were in clinical CR and 12 in PR, with 10 of the 12 having persistent marrow involvement. Four patients have relapsed at 4, 9, 12, and 20 months following ABMT, and there was one toxic death at day 20. One of the relapsed patients was reinduced into remission with local radiotherapy. Although the follow-up is generally short, fifteen patients remain in continuous CR from 2^+ to 36^+ months post ABMT, with only 2 patients beyond 12 months. Of the patients who are in continuous CR, 10 patients have exceeded their prior maximum DFS. However, no conclusions can be made from this study because the median DFS with standard therapy is 12 to 24 months.

The last series of patients originates from St. Bartholomew's Hospital in London, where 22 patients with relapsed follicular lymphoma have undergone high dose chemoradiotherapy with anti-B1 purged ABMT (personal communication A. Rohatiner, T.A. Lister). These patients have been in second or subsequent remission. Eighteen of the patients remain in CR from 1+ to 36+ months. Only three patients have relapsed and there was an early toxic death, and one suicide in CR.

ABMT Following Histologic Transformation

Histologic transformation of low grade NHL is associated with a poor prognosis. The University of Nebraska has treated 9 patients (median age of 42) with histologic conversion with high dose therapy and ABMT (13). The patients were heterogeneous for response to salvage therapy with 4 of 9 not responsive. Eight of the patients had transformed from follicular to diffuse patterns. Early toxic deaths in 5 patients did not permit evaluation for a response to therapy. Of the remaining 4 patients, there were 3 CRs and 1 PR. However only 1 patient remains in continuous CR at 99^+ days, one relapsed at 90 days and the other died of sepsis at 102 days. All of the patients who achieved CR with high dose therapy were previously responsive.

At Dana-Farber, 15 patients (median age 39) with a history of low grade lymphoma who have undergone histologic transformation have been treated with high dose chemoradiotherapy and with anti-B cell monoclonal antibody purged ABMT (14,15). Eight patients had converted from either follicular small cleaved cell type or follicular mixed to diffuse large cell, 3 to a diffused mix, 2 to a diffuse small cleaved, and 2 to follicular large cell type. All patients were still responsive to conventional therapy, with 7 of the 15 patients in clinical CR and 8 in PR (6 of the 8 with residual marrow involvement). Three of the patients have relapsed at 2, 4, and 8 months post ABMT, while 12 patients remain in continuous CR from 5^+ to 64^+ post ABMT with 6 patients out over 12 months. Of patients in continuous CR, 8 have exceeded their previous maximum DFS. These two studies suggest that patients with a histologic conversion who are still responsive to salvage therapy may still be further salvaged by high dose therapy and ABMT.

Future Directions

There are several challenges of ABMT in low grade NHL; these include patient selection, the issue of purging and the ablative regimen. The timing of ABMT is being examined in several studies. Low grade NHL may be likened to CML where timing of BMT appears to be critical. The follow-up for the current studies is short and it is unknown whether multiply relapsed patients or responsive-patients with histologic conversion can have prolonged DFS following ABMT. The St. Bartholomew's study is attempting to address whether ABMT in second remission results in prolonged DFS. A study at Dana-Farber, with previously untreated patients with stage IV nodular lymphoma is examining ABMT in first remission. These studies are not going to demonstrate that low grade NHL is curable with ABMT, however they should be able to demonstrate whether the duration of remission which is between 12 and 24 months with standard therapy is prolonged by more intensive treatment.

ACKNOWLEDGMENTS

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EX-VIVO BM PURGING IS EFFICIENT IN BURKITT LYMPHOMA BUT HIGH DOSE CHEMOTHERAPY IS INEFFECTIVE IN PATIENTS WITH ACTIVE DISEASE IN THE BONE MARROW

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Major advances have been made in the treatment of Burkitt lymphoma (BL) and the survival rate has been raised by 75% with conventional therapy (1,2). High dose chemotherapy and ABMT is thus reserved for cases of very bad prognosis, including stage IV with CNS involvement at diagnosis, partial response to induction therapy, primary refractory disease and relapse (3,4). In such patients we have previously shown that the BM could contain residual malignant cells (5-7) and that the use of a purging procedure could be a safe and ethical means to avoid re-injection of malignant cells with the autograft (8,9). However, it remained fundamental to demonstrate that this procedure is useful for the patient and whether the post-graft relapses are due to failure of the high dose chemotherapy regimen or to a failure of the ex-vivo purging process. BL cells are highly proliferative in culture and the use of a liquid cell culture assay enables us to detect down to 10^{-6} residual BL cells in the BM. We report here the observation of 13 patients in progressive disease (i.e. either primary refractory disease or resistant disease) at time of BM harvesting and high dose chemotherapy; 10 received an autograft and 3 received an HLA-matched allograft. All had cytologically and histologically normal BM, although in 6 of them the liquid cell culture assay allowed the detection of malignant cells in the autograft before the purging procedure. This assay was always negative after the purging. The analysis of these 13 patients leads us to conclude that high dose chemotherapy is ineffective in patients grafted in progressive disease, and that ex-vivo purging is efficient but with very limited indications in BL.

PATIENTS AND METHODS

Patients are described in Table 1. Clinical status and criteria of evolution are defined by clinical examination (Murphy classification), radiological parameters and biological ones (LDH), lumbar puncture and BM examination, as previously described (1).

BM Harvesting and Complement Lysis Procedure

The autologous BM was harvested 2 to 3 weeks before the beginning of high dose chemotherapy in patients with resistant relapse or primary refractory disease (Table 1). After Ficoll separation mononuclear cells were treated with monoclonal antibodies (MoAbs) and two cycles of rabbit complement as previously described (8,9). The cocktail of monoclonal antibodies was progressively modified when more effective antibodies became available. AL3 recognizes CALLA (CD10 cluster), RFB7 is a CD20 like MoAb, SB4 a CD19 MoAb and Y29/55 a non-clustered pan-B MoAb (8,9). Al3 was kindly provided by A.M. Lebacq, RFB7 by G. Janossy, Y29/55 by K. Forster and SB4 by Sanofi.

Detection of BL Cells in the BM

All patients had at least 1 aspirate at diagnosis, relapse or at time of progression and 2 trephine biopsies and 2 aspirates on the day of BM harvest for cytological and histological examinations. The liquid cell culture assay was performed at diagnosis and at relapse when patients were in our institute; it was systematically performed on the BM harvested for the autograft, before and after the purging. Briefly, $4x10^6$ BM cells were poured into 5 ml supplemented RPMI medium in a 25 cm^2 plastic culture flask with MRC5 feeder layer and incubated at 37°C in a 5% CO_2 atmosphere. After 10 days of culture, the cytological examination of a centrifuged smear enabled to detect growing BL cells in the sample. If BL cells are not detectable, samples are kept 2 more weeks before one can conclude that the test is negative. If malignant cells are detectable, samples are kept until cells lines can be obtained which allow one to assess objectively the presence of malignant cells (5). By serial dilutions, the limit of detection in this assay has been shown to be of 1 BL cell in 10^6 to 10^7 normal ones (6,7).

RESULTS

All 13 patients were in progressive disease both at time of BM harvest and high dose chemotherapy. Three were primary refractory disease and 10 were resistant relapse despite effective induction including adriamycine and salvage therapy. Ten patients received

Table 1. Clinical Status and Therapeutic Regimens for the 13 Patients

Patient No. (staging at diagnosis)	Previous Chemotherapy	Status at BM Harvesting and Prior Massive Therapy (localization of disease)	High Dose Chemotherapy	Status After Massive Therapy
50 (st.III)	VCR-CPM-ADR PRED-MTX	Primary refractory (abdomen)	BACT	PD: abdomen (died day 46)
44 (st. III)	VCR-CPM-ADR-MTX VP16-CDDP-CYT	Primary refractory (abdomen)	BEAM	PD: abdomen (died day 11 before ABMT)
38 (st. III)	VCR-CPM-ADR PRED-CYT-HD MTX IT MTX-VP16	Primary refractory (abdomen)	Misulban CPM	PD: abdomen (died day 38 before ABMT)
14 (st. III)	VCR-CPM-ADR PRED-CYT-ASP HD MTX-TG	Resistant relapse (abdomen)	BACT (IGR)	PD: abdomen (died day 39)
15 (st. III)	VCR-CPM-ADR PRED-HD MTX	Resistant relapse (CNS)	BACT (IGR)	PD: CNS + BM (died day 54)
36 (st. III)	VCR-CPM-ADR MTX-MG-ARAC IFOS-X RAY	Resistant relapse (abdomen and CSF)	BCNU Misulban CPM	PD: abdomen +CNS +BM (died day 20)
40 (st. IV CNS)	VCR-CPM-ADR HD MTX-PRED-IT MTX VCR-VP16-CYT	Resistant relapse (abdomen)	Misulban CPM Melphalan	NE toxic death (day 13)

Table 1. Clinical Status and Therapeutic Regimens for the 13 Patients (Continued)

Patient No. (staging at diagnosis)	Previous Chemotherapy	Status at BM Harvesting and Prior Massive Therapy (Localization of disease)	High Dose Chemotherapy	Status After Massive Therapy
46 (st. IV CNS)	VCR-CPM-ADR HD MTX-PRED-IT MTX VP16-CYT	Resistant relapse (CNS)	BEAC	PD: CNS + BM (died day 27)
27 (st. III)	VCR-CPM-ADR CDDP-VP16-ASP	Resistant relapse (head and neck, abdomen)	BEAM	PD: head and neck (died day 40)
60 (st. III)	VCR-CPM-ADR MTX-PRED MG-ARAC-IFOS-VP16	Resistant relapse (CSF + BM)	Super MIME VCR-CPM + TBI	PD: CSF +BM +blood (died day 25)
*51 (st. III) first graft	VCR-CPM-ADR-PRED ARAC-HD MTX-VP16	Resistant relapse (abdomen)	BEAM	Relapse: abdomen +BM (day 28)
51 (st. III) second graft	id + BEAM and autograft	Resistant relapse after 1st AMBT (abdomen + BM)	Super MIME VCR-CPM + TBI	PD: abdomen +BM +CSF (died day 45)
53 (st. IV CNS + BM)	VCR-CPM-ADR VP16-ARAC-HD MTX MG-IFOS	Resistant relapse (nodes and CSF)	BEAM TBI	PD: nodes +CSF +BM (died day 42)
52 (st. IV BM)	VCR-CPM-ADR VP16-ARAC-MG IFOS-VP16	Resistant relapse (abdomen)	BEAM	PD: abdomen + BM (died day 13 before graft)

*Patient 52 had a first course of chemotherapy followed by a purged autologous BMT; he relapsed and received a second course of high dose chemotherapy followed by an allogeneic BMT (interval between the two grafts: 50 days)

Table 2. Graft, Purging Procedure and Assessment of the BM for the 13 Patients

Patient Number	Autograft (BM purging) or Allograft	BM Culture on the Autograft Before Purging	BM Culture on the Autograft After Purging	BM SINCE DIAGNOSIS Culture	BM SINCE DIAGNOSIS Cytohistology	BM AFTER MASSIVE THERAPY Culture	BM AFTER MASSIVE THERAPY Cytohistology
50	auto (unpurged)	-	-	n.d.	-	n.d	-
44	auto (unpurged)	-	-	-	-	-	-
38	auto (RFB7 + AL3 +Y29/55)	-	-	-	-	-	-
14	auto (unpurged)	-	-	n.d.	-	n.d.	+
15	auto (Y29/55)	-	-	-	-	+	+
36	allo	-	-	-	-	+	+
40	allo	-	-	+ (at diagnosis) (4 months before)	-	-	-
46	auto (RFB7 + SB4)	+	-	-	-	+	+
27	auto (Y29/55+AL2)	+	-	-	-	-	-
60	auto (RFB7 + SB4)	+	-	n.d.	+ (at relapse) (2 months before)	n.d.	+
51 (1st draft)	auto (RFB7 + SB4)	+	-	(-)	-	+	+
51 (2nd draft)	allo			+ (relapse after 1st graft)	+	n.d.	+
53	allo	***+	-	-	-	n.d.	+
52	auto (RFB7 + AL2 + Y29/55) (not re-injected)	****+	-	+ (diagnosis)	-	+	-

*Pt 51 relapsed in the BM after the 1st course of high dose chemotherapy followed by ABMT; a 2nd course of chemotherapy was then given, followed by allogeneic transplantation 2 wks later. **In pt 53, the BM was harvested and purged; the liquid cell culture assay was positive before the purging procedure and negative afterwards but the patient had a compatible donor & he was given an allogeneic BMT 2 weeks after. ***In pt 52, the bone marrow was harvested & successfully purged; he received the 1st part of the high dose chemotherapy program (BEAM) but died of progression before the scheduled cyclophosphamide + TBI treatment & the BM was not re-injected.

high dose chemotherapy followed by autologous BMT and 3 had high dose chemotherapy and HLA-matched allogeneic BMT (Table 2). All 13 relapsed or were in progressive disease 45 days post graft. Patient 51 relapsed after ABMT; he received a subsequent second course of high dose chemotherapy followed by allogeneic BMT and he died in progression (Table 1). Six patients (Nos. 50, 44, 38, 14, 15 and 36) had a normal BM as assayed by cytohistological analysis and liquid cell culture assay from diagnosis to the time of high dose chemotherapy (Table 2); however, two progressed in the BM after high dose chemotherapy, including one who received an allograft. Thus, relapse or progression after the graft, even with BM localization, were not due the reinjection of malignant cells. Of note, patients 50, 44 and 14 were grafted with unpurged BM. In one patient, the BM was cytohistologically normal at diagnosis and relapse but the liquid cell culture assay was positive at diagnosis; he received high dose chemotherapy followed by an allograft and died of toxicity with a normal BM. In six patients (Nos. 46, 27, 60, 51, 53 and 52) malignant cells were detectable in the autograft before the purging by the liquid cell culture assay whereas cytohistology was negative. The liquid cells culture assay was negative in all 6 after the purging; four patients received the purged autologous BM; one received an allograft and the last one (no. 52) died of progression before the autograft reinjection. After high dose chemotherapy, all had progressive disease in the BM as well as in the other sites of the disease, including both patients who did not receive the autograft. Of note,and as mentioned above, patient 51 was given a second course of high dose chemotherapy followed by allogeneic BMT but still relapsed in the BM.

Cytohistological examination of the BM was negative in the 6 cases of positive culture, the initial contamination then ranged from 10^{-2} to 10^{-7}. Malignant cells were accurately detected in the culture one to two weeks after the beginning of the test by a cytological examination. Finally, if one considers the other BL patients in the series of 61 entered in the ABMT program, 18 were in first complete remission, 3 in partial remission, 27 in sensitive relapse; none of them had malignant cells in the BM detectable by the liquid cell culture assay.

DISCUSSION

The use of a liquid cell culture assay has allowed us to demonstrate the presence of rare BL cells in the BM of 6 patients and their elimination by the purging procedure. Relapse or progression were observed after autografts as well as allografts and were therefore not due to the re-injection of malignant cells but to the failure of high dose chemotherapy. Of note, the presence of malignant cells in the

BM after the post-graft relapse was a common feature and cannot thus be taken as an argument in favor of the failure of the purging, as it has often been pointed out. The resistance of malignant cells to conventional therapy, as proved by progression of the disease under appropriate induction or salvage therapy, must lead to the exclusion of the patients from autologous or allogeneic BMT programs. Any new therapeutic strategy must be based on different mechanisms of antitumoral activity and one thinks in particular of immunotherapy.

As previously published, when the BM was cytologically normal and the cell culture assay was negative at diagnosis (with 1 exception in 293 samples analyzed), it was positive in 40% cases at relapse. In 61 patients entered in the ABMT program after induction therapy or salvage therapy, the liquid cell culture assay was found positive only in the 6 cases described above and can thus be taken as an objective criteria of resistance to therapy. Such criteria can be very important for BL in which the evolution of the disease is extremely rapid and any therapeutic decision taken in an emergency; the positivity of the liquid cell culture assay in the BM can precede any obvious local progression by a few days.

In our series of 61 BL patients, purging was proved to be necessary and efficient in 6 patients who, however, could no longer enter an ABMT program. In all others the BM liquid cell culture assay was negative. For patients whose BM was pathological at any period of their clinical evolution (i.e. diagnosis or relapse) it is ethical to continue to use a purging procedure. In opposition, in patients whose BM was always normal, as assessed both by cytological examination and by our liquid cell culture assay, the purging of the autograft is unnecessary. With such restricted indications, only 17 patients in our series of 61 would have received a purged BM.

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DETECTION OF MINIMAL RESIDUAL DISEASE BY POLYMERASE CHAIN REACTION

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Emil J Freireich, and Sanford A. Stass

Successful induction therapy has resulted in high remission rate in patients with hematologic malignancies. Disease recurrence, however, remains a major problem. Relapse from clinically undetectable residual disease is the most likely mechanism. Detection of minimal residual disease is very difficult. A new technique, polymerase chain reaction (PCR) has made it possible to detect extremely small numbers of neoplastic cells with chromosomal translocations. Two typical examples of this application are the t(14;18) characteristic for follicular lymphoma (FL) and the t(9;22) characteristic for chronic myelogenous leukemia (CML).

The t(14;18) occurs in about 90% of FL(1). It involves reciprocal translocation of a putative oncogene, bcl-2, residing at chromosome 18q21 and the immunoglobulin heavy chain (IgH) gene residing at chromosome 14q32(2). By means of Southern blot analysis, it has been shown that about 60% of FL have breakpoints on chromosome 18q21 clustering within a 2.8 kilobases (kb) Eco RI-Hind III restriction fragment which is named the major breakpoints clustering region (mbr) (3-6). By means of DNA sequencing, the breakpoint are tightly clustered within 150 bases pairs (bp) of each other, which is named molecular breakpoint hot spot (7-9). The breakpoint on chromosome 14q32 is also very consistent from case-to-case. It always occurs a the 5' end of one of the J segments of IgH gene (7-9).

Taking advantage of these unique characteristics, we made two oligonucleotide primers. Primer 18q21(+) was derived from (+)-strand 18q21 sequence 5' to the molecular breakpoint hot spot. Primer JH(-) was derived from the common sequence located at the 3' end of each J segment (10). These primers were expected to flank the crossover site of the t(14;18) in about 60% FL. Except for the cases that breakpoints occur immediately 5' to J6, we expected the amplified DNA fragments would not be uniformly in one size since our JH(-) primer could also anneal to the other J segments 3' to the

crossover site. In order to confirm that the PCR amplified DNA fragments indeed contained the t(14;18) hybrid sequences, we used an "internal" oligonucleotide probe, 18q21(+)II, which was derived from the (+)-strand 18q21 DNA 27 bases 3' to Primer 18q21(+)(10).

Since one of our primers was derived from chromosome 18 and the other one was derived from chromosome 14, they would anneal to two different chromosomes in case of normal karyotype. In such situation, only one new copy of 18q21(+) DNA and one new copy of JH(-) DNA could be synthesized. When there was t(14;18), these two primers would anneal to the crossover site of the translocation. Exponential amplification of the t(14;18) hybrid sequence could, therefore, be generated.

PCR assay is highly sensitive. We performed a mixing experiment using a DNA sample from a lymph node which was known to have the t(14;18) breakpoint occurring within the mbr region and a DNA sample from a normal lymph node. The t(14;18) hybrid DNA sequence was easily detectable even at the dilution of 1:100,000 (Figure 1). Our assay is not only very sensitive but also very specific. We have analyzed two samples with the t(14;18) 3' to the mbr region and more than 10 samples without the t(14;18). None of them were amplifiable.

We then utilized this highly sensitive assay to address a very fundamental question: are there small numbers of circulating cells carrying the t(14;18) in patients with follicular lymphoma? We first selected 10 blood samples from 8 patients who had follicular lymphoma and the t(14;18) breakpoint within the mbr region. These samples were obtained in various clinical status: 3 before treatment, 2 at partial remission and 5 at complete remission. These samples appeared to be normal morphologically. They were analyzed both by Southern blot analysis for bcl-2 gene rearrangement and the PCR assay. Only two samples were positive by Southern blot analysis. In contrast, 3 out of 3 samples obtained before treatment and 2 out of 2 samples obtained at partial remission were positive by PCR, indicating that there were small numbers of cells carrying the t(14;18) in circulation when there were disease detectable clinically. Even when patients achieved clinical complete remission, we could detect circulating cells carrying the t(14;18) in 4 out of 5 instances by PCR.

The major limitation of the PCR technique is that it can only amplify a short DNA fragment. This approach, therefore, is not applicable to chromosomal translocation with variable molecular breakpoints. It is also not immediately applicable to the amplification of RNA. Ph' chromosome, the t(9;22), has been observed in more than 90% of CML(11). Even though the breakpoint on chromosome 22 cluster within a small DNA fragment designated as bcr (12,13), the breakpoint on chromosome 9 occur at variable positions up to more than 100 kb 5' to the second exon of the c-abl oncogene (14,15). Despite the variability of the molecular breakpoint at the DNA level,
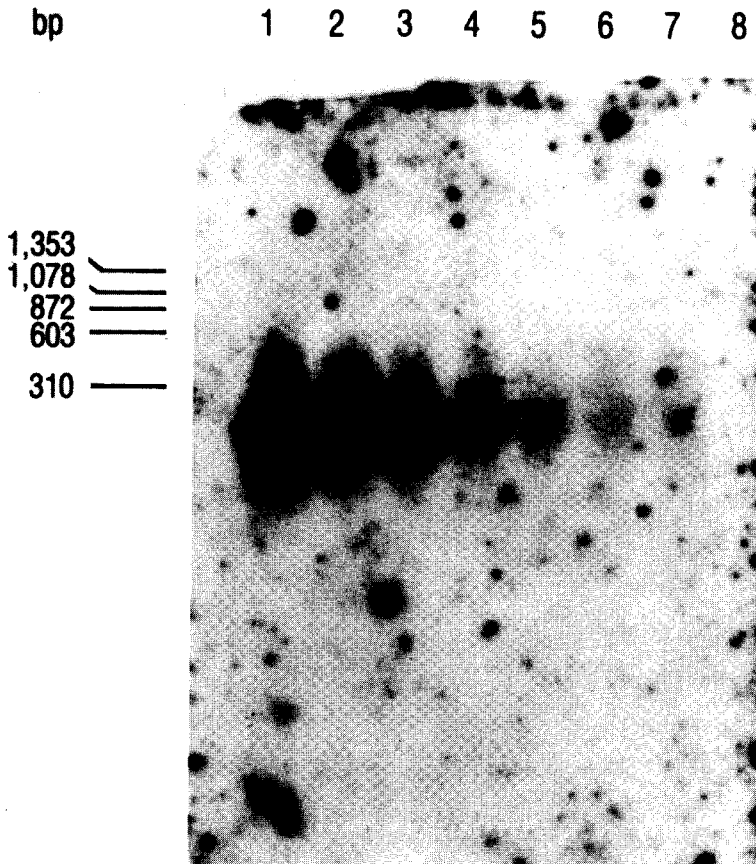
Figure 1. The sensitivity of the PCR assay to detect cells with the t(14;18). A DNA sample known to have the t(14;18) occurring within the mbr region was first diluted with a normal DNA sample in various ratios and then PCR was performed using primers 18q21(=) and JH(-) and Taq DNA polymerase (Perkin-Elmer Cetus) for 45 cycles as described (18). The PCR amplified Samples (5 microliters) were loaded to a 2% alkaline agarose gel, size fractionated by electrophoresis (60V for 3 hours) and then transfered onto a nylon membrane. A 5'-end radiolabeled probe 18q21(+)II was used to hybridize with the membrane followed by washing with 2XSSPE/0.1%SDS at room temperature for one hour and at 65°C for 15 minutes. Autoradiography was carried out with a single intensifying screen for 16 hours. Lane 1: 1 microgram of DNA sample which was known to have t(14;18); Lane 2 1:10; Lane 3 1:100; Lane 4 1:1000; Lane 5 1:10,000; Lane 6 1:100,000; Lane 7 1:1,000,000; Lane 8: normal control. Size markers are labeled at the left side.

the fused bcr/abl gene is transcribed into two types of chimeric mRNA: one with abl exon 2 linked to bcr exon 2 (designated as L-6 junction) and the other one with abl exon 2 linked to bcr exon 3 (designated as K-28 junction)(16).

Figure 2. The reverse transcriptase-mediated sequence amplification assay to detect the chimeric bcr/abl transcripts in Ph'-positive CML. Lane 1: normal control; Lane 2: CML in blast crisis; Lanes 3-6: CML in chronic phases; Lanes 7-9: CML in cytogenetic remission. Size markers are labeled on the right side.

Taking advantage of these unique characteristics, we have modified the PCR technique to amplify sequences equivalent to the chimeric bcr/abl transcripts in Ph'-positive CML. We made an oligonucleotide primer, abl(-), which was antisense to the second exon of the c-abl gene. By means of reverse transcription, the first strand cDNA was constructed. PCR was then carried out after adding Primer bcr exon 2(+) which was derived form the (+)-strand of the second exon of the bcr region. The expected cDNA fragments amplified were 80 bp in case of L-6 junction and 155 bp in case of K-28 junction. Synthetic oligonucleotide antisense to L-6 junction and K-28 junction mRNA were used to detect the chimeric bcr/abl sequences (17). This assay is very specific. We have analyzed 9 RNA samples obtained from Ph'-positive CML patients and correlated the

results with the chromosomal breakpoint at the DNA level. The L-6 junction sequence (80 mers) was detected in 3 out of 3 samples with the DNA breakpoint immediately 3 ' to the second exon of the bcr region. The K-28 junction sequence (155 mers) was detected in 6 out of 6 samples with DNA breakpoint occurring within the third intron of the bcr region. Furthermore, the RNA samples from normal lymph nodes were not amplifiable. This assay is also highly sensitive. The chimeric bcr/abl transcripts can easily be detected at a dilution of 1:10,000. We also analyzed 5 blood samples obtained from 5 Ph'-positive CML patients who were in cytogenetic remission from interferon therapy. Even though no bcr region rearrangement could be detected by Southern blot analysis, minimal residual bcr/abl transcripts were detected in all of these 5 samples by PCR (Figure 2).

We conclude from our studies that PCR is a powerful technique which can be used to detect small numbers of neoplastic cells with the t(14;18) or the t(9:22). It has the potential to identify a subpopulation of patients who are at high risk of relapse.

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Discussion 4 - Session II (Lymphoma)

Dr. Appelbaum: A question to Dr. Wheeler. How did you give the BCNU, in one dose or in several doses?

Dr. Wheeler: At the first dose level, the BCNU was given as a single dose on day 1 and, at all subsequent dose levels, BCNU was given in equally divided doses -- daily times 4 doses.

Dr. Jagannath: This is a very interesting question because, on one hand, we are trying to escalate the dose on high-dose therapy to show a dose response curve and question of toxicity.

Dr. Wheeler: The way we have usually given BCNU has been as a single infusion over 6-hour period usually on day 2 following VP-16. We give VP-16 followed by BCNU infusion followed by high-dose cyclophosphamide.

Dr. Peters: I have a comment. We have had an interest over the years in trying to develop or looking at different therapeutic regimens. And, as I listen today, there is one interesting feature that seems to prevade all of what is going on both in our own institution and in other places -- and that is that we are attempting to ask pharmacologic questions, and we do not have the pharmacologic data. Basically there is a substantial variation in the pharmacology and the pharmacokinetics among each of these drugs in the patient populations and in the disease settings in which we are testing them. And we are trying to compare, among very heterogenous regimens in a variety of different patients in different disease settings, patients who have had different pre-treatment programs in which we have no pharmacokinetic data. It may be time for us to consider doing more of this and, furthermore, getting to the point of really attempting to do molecular dosimetry or attempting to monitor how much injury we actually create to the tumor as an attempt to determine really what we are doing.

Dr. Carella: I would like to make a short comment to Dr. Goldstone's presentation. At the Tumor Institute in Milano, we consider people relapsing for Hodgkin's disease after one year a very good risk class. So, in developing a new program for high-dose treatment for Hodgkin's in relapse, we should perhaps consider this point. And, the second one is, when we developed at the tumor institute high-dose regimens and we treated patients with ABMT, we decided deliberately not to use GM-CSF in Hodgkin's disease because of uncertainties about the nature of the disease cell. It is probably a macrophage according to most of the opinions. So, I am asking Dr. Goldstone how he solved this problem?

Dr. Goldstone: We were not clever enough to think about it, Mr. Chairman. And we cannot see any clinically adverse affects in the patients who have had GM-CSF given the short follow-ups since then.

Dr. Dicke: The data published by your institute, with the conventional dose data in relapsed Hodgkin's disease -- do you agree first of all -- I think they are the best conventional dose data in the world literature. Do you agree that those data are basically inferior to the high-dose data presented in this conference?

Dr. Carella: Well, this is a difficult question to ask where we don't know really. But we selected, for our program, people relapsing from Hodgkin's disease within 12 months of MOPP and ABVD treatment either donating it or one following the other one. In the other class we are treating, obviously, people not responding completely to first line. Our treatment appears to be superior to standard CEP treatments but the follow-up is very short. The medium follow-up is a little bit higher than one year and we use a completely different program than everybody else is using right now, using high-dose sequential chemotherapy treatment using single agent high doses. So, what we can say right now is that there seems to be a clear cut advantage as compared (to) standard salvage treatment in Hodgkin's disease in this risk population. But we are not sure yet.

Dr. Dicke: I have one more question, Mr. Chairman. That is... I am highly confused about the variation in toxicity with high-dose BCNU containing programs between the Vancouver results and the results reported from New York.

Dr. Ahmed: Of the first 23 patients who were transplanted, 6 of 23 experienced toxic death. The difference between Dr. Phillip's group, perhaps, and our group may be that we have patients with an average of 3 previous regimens and 10 previous drugs prior coming to transplantation. Most of these patients are taken into the transplant

protocol provided they have a DLC of greater than 40%. So that there are people with marginal pulmonary function and the ones we have lost to toxic death have been people with poor performance status and marginal pulmonary reserve.

Dr. Phillips: Tried to explain in an apparent paradox we have very unselected patients but they are probably better patients than anyone else sees. And that is due to the fact that we catch all the patients in British Columbia, which is about 3 million. They all pass under basically Joe Connor's care. And when the patients fail, MOPP, ABVD, hybrid regimen, we see the patients at that time. Now we see all those patients, so in a sense they are not selected. The 90% response rate less than a quarter relapsed at a median of over a year and their toxic death rates are 4%. But those are patients just because they are treated by Dr. Connors that they do not see the nitrosoureas in salvage regimens, spot radiation therapy to buy a little time. Clearly a good group of patients.

Dr. Dicke: Basically, that confirms also Dr. Wheeler's data with a relatively low mortality with the 600 mg of BCNU, isn't it?

Dr. Frei: I think it is going to be very difficult with certain drugs to do dose response studies, particularly in the context that we are doing them here. Dr. Henner has observed (Dana Farber) that for BCNU there can be as much as a 9-fold variation in area under the curve. That is C x T per given dose of BCNU. Now, with that kind of experience, there is so much of a range within dose and across patients that a variation in 2 or 3-fold dose delivered is not likely to show up in the context of dose response with respect to either anti-tumor effect or toxicity. And I would like to underscore, particularly for certain drugs -- ideally for all drugs like Bill Peters said -- I do not think we are going to understand dose response until we can express dose as mg per square meter but also as area under the plasma curve. I think once we do that then the effective dose and bioavailability will be thrown into relief.

Dr. Jagannath: That means you would be explaining the results of Catherine Wheeler, who stated that she could not see any change in the response rate between the dose escalation from level one to level seven, based on the pharmacokinetics?

Dr. Frei: Well, that difference in dose, Cathy, was that you ended up at about 50% higher than you were initially -- if I read it correctly. If that is true and there is a 9-fold variation in area under the curve/given dose, that latter is going to mask anything you might see. I would like to ask Cathy -- because you expressed response rate, CR + PR -- since it is CR that makes a difference, was there a dose effect on CR rate?

Dr. Wheeler: No, there was not. But, the number of CRs, I do not want to make too much about the responses at the different dose levels, because we are talking about patients with Hodgkin's disease and with NHL they are all sort of thrown into together. There are different proportions of each at different dose levels. So, I think all you can say -- very broadly -- is that there is nothing that strikes you enormously. But it is not something that is subject to very close scrutiny, I do not think.

Dr. Peters: To just comment again from Tom's, I would like to confirm this type of data. We have found about 6 to 7-fold variation in BCNU at fixed dose level among now about 70 patients that we have looked at perspectively. And we find 6-fold variations in cyclophosphamide area under the curve. So when one starts taking two drugs, each of which can have a 10-fold difference in the area under the curve for the primary drug, and have metabolites which in fact may also have substantial variation among them, it is going to be very difficult in limited number of patients, 10 max or 12 max, at a given dose level, to make any sense without doing the pharmacokinetics along the way.

Dr. Herzig: Bill, have you found differences in toxicity or response related?

Dr. Peters: We had decided, until one had complete profiles on 75 to 100 patients, that it was meaningless to try to ask clinical correlations, because the statistics would be terrible.

Dr. Herzig: There is a 7 or 10-fold difference in those areas under the curve CXT. If that was important, you ought to be able to see such a large difference in 75 patients.

Dr. Peters: I think the answer is that I do not want to interpret the data until we have it in hand.

Dr. Spitzer: I know that Gordon Phillips mentioned I think more than 1/2 year ago, that the answer to this question can only be addressed by comparative trial and Hodgkin's disease is really the perfect model to us. Whether -- now maybe -- we might call our program mini CBV against super CBV and ask it in a comparative fashion and let us resolve whether above a certain dose there is or is not improvement of therapeutic efficacy.

Dr. Jagannath: Dr. Spitzer, you presented, yesterday, the data on the breast cancer where you said you were able to deliver more chemotherapy within a period of 1 month by giving double transplantation. We talked about single transplant, and the doses

being escalated. How would that compare with the double transplant approach?

Dr. Spitzer: Well, it is complicated. Further, these patients are difficult to give double transplants to because you are taking marrow at a more extended or prior chemotherapy history. So whether you can collect adequate marrow for recovery from two courses of chemotherapy is a bigger question. At least you can give different drugs rather than escalating with the same drugs. Double transplant you get the lymphokine recovery at a time you are administering the second course of therapy. We just have to do, maybe, those studies comparatively -- double transplants versus single -- to prove the efficacy of that approach over one transplant alone.

Dr. Shea: One further comment about the pharmacokinetics... just to finish it up...there are some drugs that we use in the high dose setting in which there is some pretty good pharmacokinetic data. Karen Antman mentioned last night some work that had been done with high dose thiotepa that did show a higher incidence of toxicity and response with escalating dose, as measured by area under the curve, in some of the data she presented last night. And there is a poster that we are presenting today that looks at high dose CBDCA, you know that is a single agent, but CBCDA is potentially an interesting compound because there is very little variation around the curve in terms of looking at the median dose at a given dose level. And I think if you look at drugs that have predictable bioavailability, as you will, I think that is a real advantage in the transplant regimen because it does not only cause problems in terms of toxicity because one person effectively is not seeing 10 times the dose of drug as someone else, but it also allows you better handle on what your likely response should be because, in fact, the drug is reproducibly metabolized.

Dr. Phillips: Actually I do not think CBV is ready to be put in a randomized trial and we are not using that regimen anymore. We just decided that, although it was working well in Vancouver, we were seeing the unique population of patients and we have made some modifications which include a slightly lower dose of BCNU, a different way of giving the etoposide and the addition of cisplatinum. That is a phase I-II study that is ongoing.

Discussion 5 - Session II (Lymphoma)

Dr. Appelbaum: Dr. Friedman, patient selection in indolent lymphoma seems like it is not the key issue. What is the pretransplant regimen you are planning at the Dana Farber?

Dr. Friedman: The patients are getting six cycles of standard CHOP followed by up to 3 sites of involved field radiotherapy followed by cytoxan, TBI.

Dr. Appelbaum: First of all, age cut-off, and second, is there a marrow status you insist on before you harvest and purge?

Dr. Friedman: We do anybody who relapses who is under age 65 and, in terms of the upfront study, the age cut-off is 50. Marrow status -- we have transplanted people with histologic evidence of up to 20% marrow involvement of the intratrubecular space by bone marrow biopsy. And, we are keeping that at the limit.

Dr. Appelbaum: In the upfront study?

Dr. Friedman: Even in the upfront study.

Dr. Gorin: Is there a particular reason why you did not select promace MOPP -- I mean -- as being, maybe, one of the best regimens for these patients?

Dr. Friedman: That is a good question, I am not familiar about the track record outside of the NCI study of the response rate in low grade lymphoma to promace MOPP but I am not so sure that any combination regimen has really been shown to have a higher response rate than even CHOP.

Discussion 6 - Session II (Lymphoma)

Dr. Peterson: I can supplement by telling that we have done autologous transplantation in 16 Burkitt's lymphoma patients in Seattle in resistant relapse. Most of them have received a Pan-B purged marrow. All relapsed shortly after transplant. We have no survivors.

Dr. Dicke: Marie, I would like to ask you, what do you think the level of detection might be in your system? Is that 1 in 100, 1 in 1,000, 1 in 10,000 cells?

Dr. Favrot: No. The level of detection is 1 in 10^6. This has been done both by mixture of normal cells with cell lines and mixture of autologous normal bone marrow cells and the tumor cells from the patient.

Dr. Dicke: If you are able to detect Burkitt cells in patients who are for a longer time in remission, that may shed a whole new light on the remission status of a patient.

Dr. Favrot: But I do not think it is possible but, just because of natural story of Burkitt's lymphoma when patients are in remission for a long time. We all know that they are cured and the liquid cell culture will stay always negative. It means that the time where we find this culture positive is only in patients in relapse or in progressive disease.

Dr. Jagannath: Marie, you showed that you were able to detect the Burkitt's lymphoma cells from the bone marrow. Normally that would be considered -- that they are clonogenic cells because they are able to grow and reproduce. And, at the same time, you showed that these bone marrows, even if they were not purged, did not result in relapse in the bone marrow of the patient. So here, at least in this disease, where the tumor cell is fairly highly clonogenic, you are able

to grow them in the lab very effectively and purging has proven not to be not effective. You want to comment?

Dr. Favrot: No, I do not think that is the conclusion. For the 3 patients where the bone marrow was not purged, those 3 patients never had bone marrow involvement all along the course of the disease; they were all stage III Burkitt's lymphoma. So it means that they did not have any malignant cell in the bone marrow. When there are malignant cells in the bone marrow--we have 6 cases like that-- we were able to show that the purging could eliminate the malignant cells. But in 60% of the Burkitt's lymphoma you do not need to purge the marrow because the patient does not have malignant cell in the marrow.

Dr. Bostrum: I am having a little trouble visualizing how you quantitate the number of Burkitt's cells in this technique. Could you explain that a little better?

Dr. Favrot: It is an extrapolation from the dilution curve that we are doing with the normal marrow and the patients cells. We are not able to quantify the cells without this curve.

Dr. Reynolds: Marie, in culturing marrows, we have occasionally had lymphoblastoid cells grow out with great efficiency that are obviously not malignant since they are from patients without malignancy -- could that be happening in occasional patients of yours and how do you distinguish the two?

Dr. Favrot: It is there in 30% of the cases. But we always kept the samples 3 weeks to get the cell line and conclude that the cells are malignant by immmunological and cytological methods. The advantage is that we are able to make the diagnosis since day 8

SESSION III - SOLID TUMORS

A. BREAST CANCER

AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) AS INTENSIFICATION THERAPY IN BREAST CANCER:
An Italian Cooperative Experience

Giovanni Rosti, Enzo Galligioni, Marzia Argnani,
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Amelia Tienghi, and Maurizio Marangolo

INTRODUCTION

In the last two decades many therapeutic strategies led to improvements in terms of overall responses in advanced breast cancer, but the final outcome still remains very poor. First line chemotherapy regimens show a percentage of responses ranging from 40 and 70%, with few and short lasting complete remissions (CR) (1). Breast cancer is a responsive tumor to chemotherapy, hormonal manipulations and radiation; in recent retrospective studies a steep dose–response relationship regarding remission rate and probably response duration had been suggested (2,3,4). Many papers had been published about the combination of high-dose chemotherapy and ABMT as a support for severe marrow hypoplasia. This year Dr. K. Antman and Dr. R.P. Gale coordinated a world-wide analysis collecting the experiences in this particular field (5). The best results had been reported when ABMT had been programmed after induction therapy (in responsive patients or sensitive relapses) with a CR rate of 71% and an overall response of 91%; 46% of the patients who achieved CR were still disease free for a median of 12-18 months. Thirteen percent of the patients however died of toxicity.

DESIGN OF THE STUDY

In late 1986, we started a cooperative protocol of intensification therapy in previously induced patients with advanced breast

carcinoma. Very early results, including some patients treated after many relapses, had already been presented elsewhere (6); the present report is an update. The aim of the study, which is ongoing, is to evaluate the efficacy of a high-dose regimen and ABMT in terms of maintaining responses achieved with induction therapy, transforming induction results into major responses and evaluating survival duration.

PATIENTS AND METHODS

Patients with advanced breast cancer, not previously submitted to chemotherapy (except for adjuvant CMF), age below 65 years, Performance Status 0-1 (ECOG scale), without bone marrow and/or CNS involvement, negative receptor status or failing hormonal treatment(s), were included in an induction protocol (5-Fluorouracil 600 mg/sqm, Epidoxorubicin 75 mg/sqm, Cyclophosphamide 600 mg/sqm on day 1 every 3 weeks for 4-6 courses: FEC regimen). No dose escalation was planned. After induction treatment a complete restaging was performed with all the investigations used in the staging phase. All patients achieving CR, PR or with stable disease (SD), entered high-dose program. This consisted of Mytomicin 20 mg/sqm push on day -9, Cyclophosphamide 1,400 mg/sqm in Dextrose 5% 250 cc, from day -9 to day -5 and Vinblastine 1.7 mg/sqm C.I. from day -7 to day -3; ABMT on day 0. Mesna was used with Cyclophosphamide to prevent urothelial damage. Double autograft was planned, and the minimum number of nucleated cells/Kg b.w. was 0.5×10^8. Up to now 17 patients are available for FEC response, 16 female and 1 male. Six CR, 5 PR, 2 SD and 4 progressions were achieved. At the time of restaging before harvesting, marrow involvement was evaluated with 2-4 trephine biopsies and anti-EMA antibody detection as described by others (7). All patients undergoing ABMT received gut decontamination with Paromomicin, antifungal prophylaxis with ketoconazole and total parenteral nutrition if required. When PMN decreased below 500/mmc, patients were assigned to a reverse isolation room. Platelet transfusion was planned when bleeding occurred or PLT were below 20,000. Up to July 1988, 9 patients (4 CR, 3 PR and 2 SD) are evaluable for high-dose intensification program (mean age 42 years - range 32-55). The majority of these patients failed previous endocrine therapy.

Toxicity

Nine patients were submitted to 15 courses of high-dose therapy. No toxic death was observed. All patients but one had thrombocytopenia (PLT below 20,000) and PMN decreased below

$500\mu l$ in all courses. Mean time of thrombocytopenia was 12 days while neutropenia lasted 14 days. Fever was present in 10 cases (>38°C) and appropriate combination antimicrobial therapy was given. Severe stomatitis occurred in 4/15 courses. One episode of bronzing as previously described by others occurred (8) (Table 1).

RESULTS AND DISCUSSION

Table 2 shows the results up to July 1988. All patients but three received 2 high-dose courses. The causes for receiving only one graft were case G.V.: too early for second course; case D.M.: meningeal diffusion just before second course; case T.M.: psychological refuse. Two patients improved their previous responses (PR to CR and SD to CR). Three cases relapsed: one already mentioned, for meningeal disease, and the remaining two at 10 and 12 months respectively (bone and liver metastases). The other six patients are alive with no progression at 1+ to 9+ months. Obviously the follow-up is too short to draw definitive conclusions. Our aim is to evaluate 20 patients with ABMT intensification program. We think that rating Stage IV receptor negative for failing endocrine therapy patients, due to brief survival spans of this population, improvements in survival and disease-free period interval may be rapidly seen. The use of ABMT in breast cancer is still controversial and many problems are still unsolved. For example: is purging necessary and if so, which is the best effective approach? Are our methods to detect bone marrow

Table 1. Toxicity

	Days	Range
WBC < 1000/μl	15	8-27
POLYS < 500/μl	14	8-27
PLT < 50 K/μl	12	0-29

Course	#1	Range	#2	Range
Number PLT units	12	0-48	16	0-31

Days with Fever $\geq$ 38°C	5 Days (0-13)
Stomatitis (WHO $\geq$ 3)	4/15 Courses
1 Episode of Skin Toxicity	(Bronzing)

Table 2. Results of Intensification Program

Sites of Disease Pre FEC Therapy	Response to FEC	# HDCT	Response to HDCT	Response Duration (mos)	Site of Relapse
G.V. Bone	PR	1♦	PR	1+	
G.E. HEP	SD	2	SD	8+	
D.M. Nodes	CR	1	CR	1	Meningeal
V.P. Nodes	CR	2	CR	12	Liver
D.S. Nodes,Bone	PR	2	CR	9+	
K.S. Bone, HEP	PR	2	PR	6+	
O.A. HEP	SD	2	CR	10	Bone
P.T. Nodes	CR	2	CR	3+	
T.M. Breast, Skin	CR	1*	CR	3+	

♦Ongoing Second Graft.   *Second ABMT Refused.

micrometastases really adequate? Do we have to include in our trials patients with widespread bone disease but not bone marrow contamination?   Which is or which are the "best" treatment combination(s)? The regimen we employed has shown to be well tolerated in terms of extramedullary toxicity and life threatening events; probably it is less intensive and toxic than other chemotherapy schemes employed at high-doses in various neoplastic disorders (9). We also think that the kind of selected population submitted to ABMT is an important and critical factor affecting the compliance of the patients.   In this study only relatively young women with good Performance Status, not progressing under first line therapy were included. In case of satisfactory results achieved at the end of this and other ongoing trials, we think that ABMT may be tested also in Stage II breast cancer, selected patients as an adjuvant program. A possible population of patients could be: receptor negative, more than 10 auxiliary nodes positive, elevated labeling index and aneuploid DNA content or a combination of these or other unfavorable prognostic features.

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INFLAMMATORY AND METASTATIC BREAST CANCER:
Cyclophosphamide and Total Body Irradiation (TBI) with Autologous Bone Marrow Transplantation (ABMT)

Christian Gisselbrecht, Eric Lepage, Jean-Marc Extra,
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Michel Marty, and Michel Boiron

The prognosis of inflammatory breast cancer (IBC) has improved lately through the use of neo-adjuvant chemotherapy (1). However, less than 40% of the patients can expect to survive without relapse at 5 years. The most important factor for prolonged survival is complete response after initial chemotherapy.

Metastatic breast cancer in premenopausal women has a poor prognosis with a median survival of 1 to 3 years. In order to evaluate in a pilot study the impact of intensive therapy as first line treatment; cyclophos- phamide, total body irradiation and autologous bone marrow transplantation was proposed as consolidation in premenopausal women with inflammatory or initially metastatic breast cancer. High dose single alkylating agent with autologous bone marrow support has been attempted in metastatic breast cancer as salvage chemotherapy, with a response rate of 30% to 40% (2). However, response duration is generally short (2 to 5 months). In an attempt to prolong remission systemic irradiation, a putatively non-cross resistant agent was added to high dose cyclophosphamide in our study (3).

MATERIALS AND METHODS
Patients Characteristics

Patients under 50 years of age with inflammatory or initially metastatic breast cancer were evaluated by history, physical

examination, histopathologic review of breast biopsy, renal and hepatic chemistries, hemogram, ECG, chest X-ray, ultrasonic or CT scan of the abdomen, and posterior iliac crest bone marrow biopsy; hepatic, renal pulmonary or cardiac impairment rendered a patient ineligible for this study. Inflammatory signs were graded according to the Institut-Gustave-Roussy classification (4) and only patients with grade 2 or 3 were included in the study.

All patients are pre-menopausal. All patients had no prior hormonal or combination chemotherapy.

Treatment Regimen

Induction chemotherapy was begun on the day following breast biopsy. Eleven patients had received four cycles at two-week intervals of 4'Epi-Adriamycin 75 mg/m^2 associated with cyclophosphamide 1 200 mg/m^2, 3 patients had received four cycles, at one month interval of standard combination of adrimycin, 5-Fluorouracil, cyclophosphamide and vincristine.

Evaluation of response was performed in all sites previously involved. In the case of partial or complete response with negative bone marrow biopsy, patients underwent local treatment by mastectomy, 2 cycles of methotrexate 3 g/m^2 and folinic acid rescue were administered as soon as possible after surgery. Twenty-five Gy was then delivered to the parietal chest.

Conditioning Regimen

After two weeks rest, high dose cyclophosphamide was infused over one hour at 2.2 g/m^2 on two consecutive days. TBI was administered either in one fraction (8 patients) of 10 Gy with lung protection of 8 Gy or fractionated TBI 2 Gy twice a day up to 12 Gy with lung protection at 8 Gy (6 patients).

Autologous bone marrow was reinfused. Patients were placed in protected environment with gut sterilization.

No adjuvant treatment was carried out after ABMT.

RESULTS

Fourteen patients were included in the pilot study mean age 36 y (range 24-50 y). Nine had IBC (T4 b N1 b M1) and 2 were classified T2 N1 M1. Metastatic sites were bone, 4 patients; bone marrow, 2 patients; contralateral nodes, 1 patient. According to histologic Scarf et Bloom rate, 6 patients were grade 2, 4 grade 3, and 4 undefined. Six patients were positive for estrogen receptors, 3 were negative and 5 were undetermined. After initial chemotherapy, among IBC patients only two had complete clinical response. All other cases

experienced major partial response. Histological complete response after mastectomy was found in only one case. Three patients had negative lymph nodes. All patients could be considered in complete remission after surgery. Among metastatic patients after induction chemotherapy, 4 were clinically partial responders and 1 was a complete responder. After surgery, 3 patients could be considered close to complete remission.

Overall, 12 patients received ABMT in an adjuvant situation. Toxicity of the regimen was as expected with a median duration of neutropenic 1 giga/1 of 21 days and thrombopenia 50 giga/1 of 35 days. No patient died during the period of bone marrow hypoplasia. However, one patient died two months later of cytomegalovirus interstitial pneumonitis. Median survival from the time of ABMT for all patients is 39 months with a median survival for metastatic patients at 18 months (p=0.4). Median freedom from progression estimated from the day of ABMT is 11 months with a significant difference between IBC (37 months) and metastatic patients (9 months) (p=0.04) (Figure 1). Five patients are presently alive beyond 2 years, 4 IBC and one metastatic form who underwent surgical resection of one single bone metastasis.
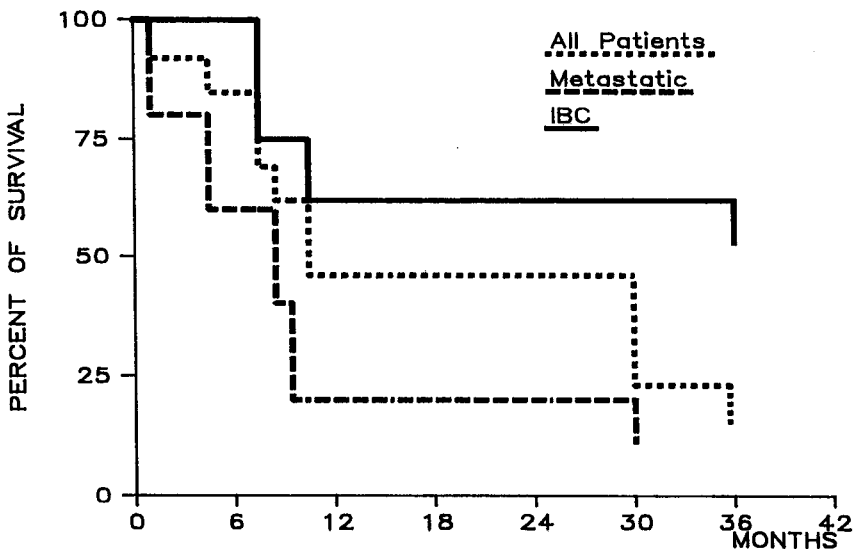
Figure 1. Freedom from progression from the time of ABMT

	J-8	J-7	J-6	J-5	J-4	J-3	J-2	J-1	J-0
Mitoxantrone 12 mg/m^2	X	X	X	X	X				
Cyclophos-phamide 60 mg/kg			X	X					
Melphalan 140 mg/m^2							X		
Bone marrow infusion									X

A:	Metastatic Breast Cancer:	< 50 Y
		Relapse < 2 Y
		Liver/Lung Involvement
B:	Initially Metastatic Breast Cancer:	< 50 Y
		Measurable Site
		Responding Patients
C:	Inflammatory Breast Cancer:	< 50 Y
		C.R. Patients Excluded

Figure 2. Breast ABMT FAG Study

Although the number is still small, it seems that in metastatic breast cancer cyclophosphamide, TBI and ABMT is unable to control long term remission duration, even when used in patients in good response without heavy pretreatment. As far as IBC concerned, the follow-up is still small, it seems that in metastatic breast cancer cyclophosphamide, TBI and ABMT is unable to control long term remission duration, even when used in patients in good response without heavy pretreatment. As far as IBC is concerned, the follow-up is still too short to determine a benefit in survival.

New regimens have to be defined for future pilot studies with ABMT. A French collaborative program in metastatic and IBC will study a regimen consisting of mitoxantrone 12 mg/m^2 x 5 from d-8 to d-4; cyclophosphamide 60 mg/kg x 2 d-6,d-5 and Alkeran 140 mg/m^2 d-2 followed by ABMT (Figure 2).

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HIGH DOSE CHEMOTHERAPY WITH AUTOLOGOUS HEMATOPOIETIC STEM CELL RESCUE IN STAGE IV BREAST CANCER:
The University of Chicago Experience

Jacob D. Bitran, Lynne S. Kaminer, and Stephanie F. Williams

INTRODUCTION

Women with stage IV breast cancer can be significantly palliated with combination chemotherapy. Chemotherapeutic regimens such as cyclophosphamide, methotrexate, 5-fluorouracil (CMF) or cyclophosphamide, doxorubicin and 5-fluorouracil (CAF) can induce objective responses in 40-80% of patients; with complete response rate of 10-20% (1). The median survival of patients with stage IV breast cancer from the time chemotherapy is initiated is only 18 months (2).

There is a sound scientific rationale to explore high dose chemotherapy with autologous hematopoietic stem cell (AHSC) rescue in patients (pts) with advanced (stage IIIB and IV) breast cancer as:

1. Breast cancer is a chemotherapeutic sensitive tumor.

2. A source of AHSC potentially free of tumor cells may be obtained from either the bone marrow cavity or peripheral blood.

3. Finally, it would be possible to utilize this therapeutic approach early in the course of the disease at a time of minimal tumor.

In 1984, we initiated a series of phase I/II trials of high dose alkylating agent chemotherapy with AHSC rescue in patients with refractory stage IV breast cancer in an attempt to identify active combinations. We chose to examine combinations of alkylating agents as:

1. There is a steep dose response curve for many alkylating agents in both animal tumor models and human cancers.

2. There is potential synergy between classes of alkylating agents when used in high dose (3).

3. Cancers that are "resistant" to conventional doses of alkylating agents may respond to increased doses of these agents.

4. Alkylators are an ideal class of antineoplastic chemotherapeutics to be explored in high dose programs as their major dose-limiting toxicity is myelosuppression.

5. Resistance to one alkylating agent does not necessarily confer resistance to others (4).

We wish to summarize our experience with high dose alkylating agents and AHSC rescue in patients with stage IV breast cancer and outline treatment strategies for future studies.

METHODS

From June 1, 1985 to July 1, 1988, we have enrolled 48 women with stage IV breast cancer in phase I or phase II trials employing high dose alkylating agents (AA) either at the University of Chicago Medical Center or Michael Reese Medical Center. Written informed consent, approved by the respective Institutional Review Boards, was obtained in all patients. The median age of these 48 patients was 42 years (range 27-64 years); the median performance status 0 (range 0-1). Twenty-seven patients were previously untreated with chemotherapeutics for stage IV disease and enrolled in a phase II trial of high dose AA chemotherapy used as "intensification therapy" following conventional chemotherapy and are reported separately (5). The remaining 21 patients were enrolled in four successive phase I trials of high dose combination AA chemotherapy. Nineteen of these 21 patients were in "refractory relapse" having failed a median of three prior chemotherapeutic regimens; two patients having relapsed after two prior chemotherapy regimens were in "responding relapse" (partial responses).

A bone marrow harvest was performed in 18 of 21 patients to obtain $2x10^8$ mononuclear cells/kg as described by Gorin (6). Two patients with histologically proven bone marrow involvement, had peripheral blood-derived AHSC collected using a continuous flow cell separator (Fenwall CS-3000) to obtain $6x10^8$ mononuclear cells/kg. One patient had a syngeneic marrow transplant.

Further eligibility criteria included: serum creatinine < 2.5 mg/dl

or creatinine clearance > 40cc/min; serum bilirubin < 6.0mg/dl, cardiac ejection fraction > 40%, marrow cellularity > 30%, and absence of any serious underlying medical condition. All patients required negative serologies for Hepatitis B surface antigen and human immunodeficiency virus.

Treatment Programs

All patients received high dose AA chemotherapy in one of the four successive studies shown below:

Four patients received intravenous (IV) cyclophosphamide (C) 2.5gm/m^2 on days -6,-4,-2 and thiotepa (T) in escalating dose up to 275mg/m^2.

Three patients received IV C 2.5gm/m^2 and T 225mg/m^2 on day -6,-4,-2 and p.o. melphalan 1.5mg/kg on days -7,-6 and -5.

Twelve patients received IV C 2.5mg/m^2 T225mg/m^2 and escalating doses of BCNU up to 200 mg/m^2 on days -6,-4,-2.

Two patients received IV 2.5gm/m^2 and T 225mg/m^2 and mitoxantrone 10mg/m^2 on days -6,-4,-2.

Cryopreserved AHSC in 10% dimethylsulfoxide were rapidly thawed and diluted with acid-citrate dextrose (ACDA) and reinfused to patients on day 0.

Responses

Response to therapy was categorized by the usual criteria as complete (CR), partial (PR) or anything less than partial as no response (NR).

RESULTS

Of the 21 patients treated with the four aforementioned protocols, 18 patients are evaluable for response. Three patients died from septic shock at days +28, +36, +44. Fourteen of the eighteen had objective responses including 4 complete responses and ten partial responses (77% response rate). The median survival for all patients was 6.0 months. The median duration of response was 89 days and five patients are still alive in unmaintained responses at +38, +49, +98, +215, and +232 days.

Hematologic Toxicity

The median time to granulocytes > 500/ul was 20 days (range 14-43 days) and the median time to platelets > 50,000/ul was 49 days (range 28-157 days) after AHSC reinfusion.

Non-Hematologic Toxicity

All patients required antibiotics and amphotericin-B. Infectious complications included staph. epidermitis sepsis (4 patients) and aspergillus pneumonia (1 patient). Hemorrhagic cystitis requiring formalin instillation occurred in 2 patients. Skin toxicity in the form of erythematous rash, bronzing, and pressure hypersensitivity was observed in all patients and thought to be secondary to thiotepa. Nausea, vomiting, diarrhea and mucositis were universal side effects; however, the severest mucositis occurred with the melphalan-containing program.

DISCUSSION

Based on our results and those of others, it is clear that high dose chemotherapy with AHSC support is effective in yielding responses in patients with refractory stage IV breast cancer (7,8). However, the meager duration of response and the costs of such therapy do not justify continued exploration of this approach in refractory patients. Preliminary results from our group, Jones et al and Spitzer et al, using high dose chemotherapy and AHSC support as "intensification therapy" following induction chemotherapy in previously untreated patients (9,10,11) are clearly superior and may yield long-term control of stage IV breast cancer. However, it is clear from studies in patients with Hodgkin's disease or non-Hodgkin's lymphoma that the patients who are ultimately cured are those who undergo high dose chemotherapy or chemo-radiotherapy in CR or minimal tumor burden. The same treatment strategy must be applied in *breast cancer*.

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HIGH DOSE CONSOLIDATION THERAPY WITH AUTOLOGOUS STEM CELL RESCUE IN STAGE IV BREAST CANCER: A Preliminary Report

Stephanie F. Williams, Rosemarie Mick, and Jacob D. Bitran

INTRODUCTION

To date there is no curative therapeutic approach for patients with advanced stage IV breast cancer. The complete response rates achieved with combination chemotherapy in current use are modest and virtually all patients die of progressive disease. Recently several groups have attempted to increase the complete response rate (which is the initial step in rendering even a small subset of stage IV breast cancer patients disease-free), by using high dose alkylating agent chemotherapy followed by autologous bone marrow rescue (1,2). Based on the results in "refractory breast cancer (1,2), we devised the current study in previously untreated stage IV breast cancer patients.

The purpose of this study is to attempt to increase the complete response rate, survival and hopefully eventual "cure" of women with metastatic breast cancer. We have chosen the approach of using an initial cytoreductive outpatient combination chemotherapy regimen followed by high dose intensification with alkylating agents and autologous stem cell rescue in an unselected group of women with Stage IV breast cancer that have not received prior chemotherapy.

METHODS

Patient Characteristics

Twenty-nine consecutive patients with stage IV breast cancer were enrolled onto this study from July 1, 1986 to April 1, 1988, at which

time the study was closed. No patient received prior chemotherapy for metastatic disease. Written informed consent approved by the institutional review board was obtained in all cases. Thirteen patients had received prior adjuvant chemotherapy. Patient characteristics are shown in Table 1.

Study Design and Treatment Regimen

Patients were treated with induction therapy (LOMAC) of cyclophosphamide (C) 1000 mg/m^2 IV, adriamycin (A) 50 mg/m^2 IV, vincristine (0) 1.4 mg/m^2 (maximum 2.0 mg) IV, and methotrexate (M) 200 mg/m^2 IV with Citrovorum factor (L) rescue 25 mg PO every six hours for six doses beginning 24 hours after methotrexate (LOMAC). At the conclusion of induction therapy, all patients were evaluated for response to LOMAC. Patients achieving a complete (CR), partial (PR) or stable (SD) response were eligible for intensification chemotherapy (ICT). Patients with progressive disease on LOMAC were not eligible for ICT. Within two to four weeks after completion of LOMAC, patients with histologically normal bone marrow biopsies underwent bone marrow aspiration and cryopreservation as previously described (3). Seven patients with previous biopsy proven bone marrow metastases underwent peripheral stem cell procurement obtained using a continuous flow cell separator (Fenwal CS-3000). After obtaining autologous cryopreserved stem cells, patients underwent ICT with cyclophosphamide 2.5 gm/m^2 IV and thiotepa 225 mg/m^2 IV on days -6, -4, -2. On day 0, the autologous, cryopreserved stem cells were rapidly thawed, diluted with acid-citrate-dextrose (ACD-A) and reinfused through a Hickman catheter.

RESULTS

Anti-Tumor Response and Survival

Of the twenty-nine patients entered, two patients were found to be ineligible for LOMAC response and are excluded from further analysis because of two different primary tumors (UPN 1012) and renal failure (UPN 1022). Of the twenty-seven eligible patients, four patients obtained a CR, 15 patients a PR, 71% response rate, 95% confidence intervals of 54-88%) 5 patients had SD and 1 patient had no response. The latter patient was not eligible for intensification therapy. One patient (UPN 1006) died of sepsis during neutropenia during her first cycle of LOMAC. In addition, two patients after completing the prescribed course of LOMAC refused further ICT. Thus, twenty-two patients underwent high dose intensification therapy. Three patients are too early to evaluate, thus, 19 are

Table 1. Patient Characteristics and Treatment Outcome

UPN/Age	Prior Adjuvant Chemotherapy	Sites of Metastases	Responses LOMAC	ICT
1000 56	CMF	Liver, Lung	PR	PR
1001 39	L-PAM+5FU	Orbit, Bone	PR	CR
1002 24	CAF+HEXADROL	Lymph Nodes	CR	CR
1003 57	None	Bone, Skin	CR	Refused
1004 39	None	Bone, Lung	PR	CR
1005 44	None (TAMOXIFEN)	Liver	PR	CR
1006 43	CAF	Liver, Bone	NR	Expired
1007 34	CAF	Chest Wall	CR	CR
1008 51	None	Bone, Bone Marrow	SD	PR
1009 29	CMF	Chest Wall, Liver	PR	PR
1010 45	CMF	Chest Wall, Bone	PR	CR
1011 39	CMF	Lung	PR	CR
1012 64	None		NOT ELIGIBLE	
1013 56	None	Lymph Nodes	PR	Refused
1014 30	None	Breast, Liver	PR	CR
1015 45	None	Bone, Bone Marrow	SD	PR
1016 62	None	Chest Wall	CR	CR
1017 35	CAF	Liver	PR	NR
1018 45	CMF-AC (NSABP)	Lung, Liver	NR	NE
1019 46	None	Liver, Lung	PR	CR
1020 42	None	Orbit, Liver, Lung, Bone Marrow	PR	CR
1021 39	CMF	Chest Wall, Lymph Nodes, Bone	NR	Refused
1022 31	CAF	Para-aortic Lymph Nodes	NOT ELIGIBLE	
1023 53	AC	Liver	PR	CR
1024 34	FAC	Bone, Bone Marrow	Sd	NR
1025 38	None	Lung, Skin	PR	PR
1026 53	None	Bone, Bone Marrow, Endocervical, Lung	TOO EARLY	
1027 48	CMF (TAMOXIFEN)	Bone, Lung	TOO EARLY	
1028 51	CAF	Liver, Lung	TOO EARLY	

evaluable for response. At the completion of this therapy there were 11 CRs and 7 PRs (complete response rate 58%, 95% confidence intervals of 36-80%). We were able to convert 13 PRs to 8 CRs and 2 SDs to 2 PRs. One patient progressed on ICT. Two of the CRs died of toxicity from the intensification regimen. One patient (UPN 1010) died of a presumed pulmonary fungal infection on day 73. The second patient (UPN 1011) died of staphylococcus aureus pneumonia and sepsis despite appropriate antibiotics and granulocyte recovery on day 24. At autopsy she had no evidence of metastatic breast cancer.

The median follow-up of the 27 patients is 15 months. The median survival has not yet been reached but the one-year survival estimate is 65% (Figure 1). The median time to treatment failure (TTF) for all patients is 11.8 months (Figure 2).
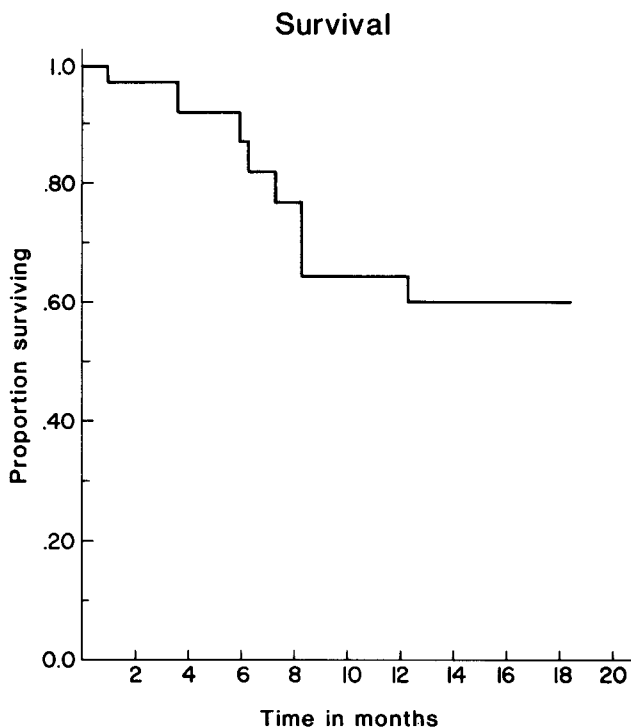
Survival

Figure 1. Overall survival of all patients.

Toxicity

All patients who underwent high dose ICT with autologous bone marrow or peripheral blood derived stem cell rescue had hematologic recovery. Granulocyte counts reached 500/ul in a median of 22 days (range, 11 to 54 days). Platelets exceeded 50,000/ul in a median of 53 days (range, 10 to 140 days).

Non-hematologic toxicities were of variable severity. Three patients died as a result of infections; one during LOMAC and two during ICT. Another patient (UPN 1020) developed pneumocystis carinii pneumonia during LOMAC therapy. She was also on steroids to control swelling of her orbital metastasis. She recovered after high dose Bactrim® therapy and had no infectious complications during ICT.

Other toxicities included nausea/vomiting, diarrhea, alopecia and cholestatic jaundice (in patients on hyperalimentation). One patient (UPN 1015) developed an acute, interstitial pneumonitis (culture-negative) on LOMAC. She recovered uneventfully and underwent ICT without major complications. No patient developed hemorrhagic cystitis or cardiomyopathy.

Time to Treatment Failure
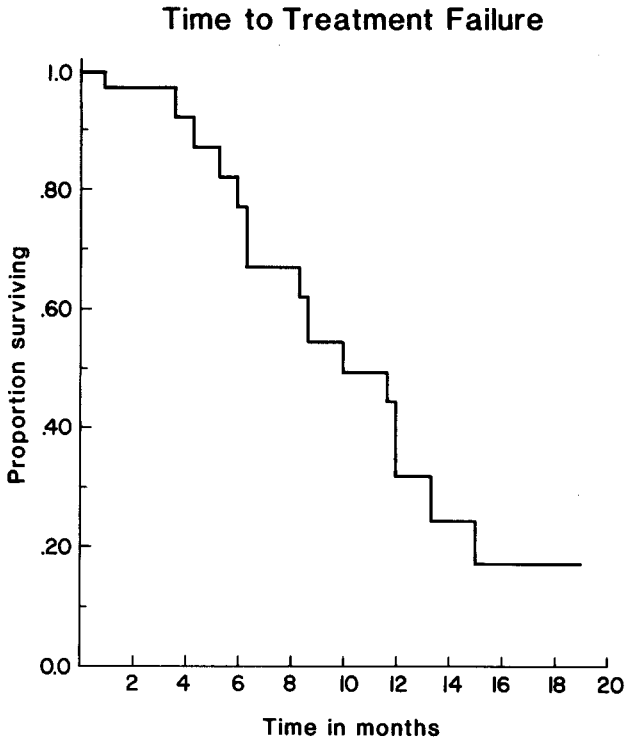
Figure 2. Time to treatment failure (TTF) for all patients.

DISCUSSION

The results of this phase II study demonstrate that despite a dose intensive induction regimen, only four patients (15%) achieved a complete response. However with the use of ICT, the complete response rate was increased to 58%. Thus, ICT was able to convert patients with a partial response to chemotherapy to a complete response. This complete response rate is higher than reported for more standard first-line regimens such as CMF or FAC (4,5). The attainment of a complete response was possible in all subgroups of patients with breast cancer (5 patients with pulmonary metastases and 4 patients with liver metastases attained a CR following ICT). All four patients with chest wall or nodular involvement attained a complete response.

There are several disadvantages with this still experimental approach. First and foremost is the toxicity. Three of our twenty-seven patients (11%) died as a consequence of therapy. All three died from infectious causes; two while neutropenic and one patient after

recovery from neutropenia. One patient developed non-fatal pneumonocystis carinii pneumonia during LOMAC induction. This patient was also being taped from steroids at that time which may be the most important contributory factor. Other infectious complications were mild and not life-threatening during intensification therapy. Secondly, the intensification phase with high dose alkylating agents and autologous stem cell rescue is expensive and requires prolonged hospitalization. Nonetheless, for patients who are in continuous CR and require no further therapy, the benefit of this approach is obvious.

CONCLUSIONS

An approach of initial cytoreductive chemotherapy followed by high dose bialkylator therapy with autologous stem cell rescue lead to high complete responses in women with metastatic breast cancer. Further clinical trials are warranted to improve upon response rates, durations of response and to decrease life-threatening toxicities.

ACKNOWLEDGMENTS

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STAMP STUDIES OF HIGH DOSE
CHEMOTHERAPY IN BREAST CANCER

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INTRODUCTION

The Solid Tumor Autologous Marrow Program (STAMP) has completed a series of laboratory and clinical studies focused on developing an active preparative regimen for breast cancer. Alkylating agents, the core of most high dose combination chemotherapy regimens, generally exhibit steep dose-response curves, (1) varying non-hematologic toxicities (2), non cross-resistance (3), synergy (4), and broad activity (5). In the MCF-7 human breast carcinoma cell line, simultaneous exposure to thiotepa and 4-hydroperoxycyclophosphamide (4-HC) resulted in striking synergy (6) and produced a linear-log dose-response relationship over 4 logs of tumor cytotoxicity in the EMT6 murine mammary carcinoma tumor excision assay in BALB/c mice.

Clinically, an initial evaluation of an empiric combination of cyclophosphamide, cisplatin & carmustine (STAMP I) demonstrated considerable activity in metastatic breast cancer, but also substantial toxicity (7). The encouraging preclinical data above, and the lack of single agent activity of BCNU in breast cancer, prompted a phase I study of high dose cyclophosphamide, thiotepa and melphalan, the most active alkylating agents in breast cancer at conventional doses (8) (STAMP III). Cyclophosphamide and thiotepa proved active with acceptable toxicity, but the addition of melphalan resulted in prolonged life threatening mucositis. The third phase I study (STAMP V) explored the addition of carboplatin to cyclophosphamide/thiotepa.

At standard doses, cisplatin is active in untreated breast cancer. At Indiana, 9/20 (45%) such patients responded (9). A randomized EORTC study of CMF vs. cisplatin/etoposide revealed no significant difference in response rate (10). Platinum compounds in the laboratory are commonly non cross-resistant and synergistic with other agents. CBDCA, unlike cisplatin, can be escalated 5 fold over standard doses with ABMT.

Cyclophosphamide, thiotepa and carboplatin were administered in STAMP III & V by continuous infusion to facilitate pharmacologic studies and to attempt to improve the therapeutic index. High dose cyclophosphamide is significantly less cardiotoxic and immunosuppressive in non-human primates when delivered in divided dose schedules compared to single doses (11) . In preclinical in vitro and in vivo studies, continuous exposure to a given dose of cyclophosphamide (4-HC in culture) or thiotepa is equal or superior to single dose administration. (Teicher BA et al, unpublished observations, 12,13.)

METHODS

Patients aged <55 with metastatic or unresectable cancer were evaluated by history, physical examination, pathologic review, hepatic and renal chemistries, ECG, chest x-ray, pulmonary function tests, computerized tomography of the head and bilateral iliac crest biopsies. Patients with bone marrow or CNS metastases, significant hepatic, renal or cardiopulmonary impairment, performance status >1 or prior pelvic radiation were excluded.

Cyclophosphamide, thiotepa & carboplatin were given by continuous I.V. infusion day-7 to -3 (Table 1). Drug doses were escalated in cohorts of 3 to 5 patients per dose level. After reaching the dose limiting toxicity of cyclophosphamide and thiotepa, the dose of thiotepa was decreased 25% to 680 mg/m2 and 40 mg/m2 of melphalan was divided over 4 days and given i.v. bolus. When mucositis proved unacceptable, melphalan was deleted and carboplatin was added at escalating doses.

RESULTS

Between 1983 and 1988, 43 breast cancer patients were treated on 3 protocols. The median age was 38.

Toxicity
(Table 2)

The median time to recovery of granulocytes (> 500/u1) was 22 days and to platelet independence, 19 days.

**Table 1. Schemata for Stamp I, III, & V
Day from Marrow Reinfusion**

	-8	-7	-6	-5	-4	-3	-2	-1	0
BM Harvest	X								
Reinfusion									X

STAMP I:

	-8	-7	-6	-5	-4	-3	-2	-1	0
Cyclophosphamide	-	-	-						
Cisplatin		-----------------------------							
BCNU					-*				
Melphalan**				↑					

STAMP III:

	-8	-7	-6	-5	-4	-3	-2	-1	0
Cyclophosphamide		-----------------------------							
Thiotepa		-----------------------------							
Melphalan	↑		↑		↑		↑		

STAMP V:

	-8	-7	-6	-5	-4	-3	-2	-1	0
Cyclophosphamide		-----------------------------							
Thiotepa		-----------------------------							
Carboplatin		-----------------------------							

*Last 12 patients given same total dose divided over four days, given BID.
**Dose levels 5-6 only.

Response

Three patients treated on Stamp I were unevaluable. (Two died early, one had no measurable disease.) Data by protocol and prognostic category are shown in Table 3.

Pharmacokinetics

Considerable variation existed in the clearance of thiotepa among patients (coefficient of variation = 44%). The frequency of response or severe toxicity in individual patients correlated with thiotepa dose

Table 2. Deaths/Severe, Life Threatening & Lethal Toxicity for all Patients Entered on the Phase I Trial (including other primarys)

Dose Level: dose in mg/m^2

	STAMP I				STAMP III			STAMP V		
	1-3	4(MTD)	5-6	7	1-3	4(MTD)	5-6	1-2	39MTD)	4
Cyclophosphamide	3875	5625	5625	7500	6000	6000	6000	6000	6000	6000
Cisplatin	150	165	180	180						
Carboplatin								6-800	800	1000
BCNU	450	600	600	750						
Thiotepa					500	720	7-900	720	500	500
Melphalan			40-80				0-40			
Number	9	40	7	5	14	3	5*	9	13	5
Toxic Deaths	0	7	3	4	1	0	1	2	0	0
Herpes zoster	0	4	2	0	0	0	0	0	1	0
CMV	0	0	0	1/1	0	0	0	0	0	0
Bleeding	1	2/2	0	2/2	1	0	2	0	0	0
Creatinine > 2.5	0	2/6	2/3	2	2	0	0	2/3	0	1
Noninf. Pneumonitis	0	4	1/1	0	1	0	0	0	0	0
VOD	0	2/4	1	2/2	0	0	1/1	0	0	0
Bili,SGOT>5x nl		1	1		0	0	1	2/3	0	1
Mucositis	0	1	6	0	2	0	4	6	6	3
Cardiac	0	2	0	0	0	0	1/1	1/1	1/1	0
Cystitis	0	0	0	0	0	0	0	0	0	1
Capillary-leak	0	2	0	0	3	0	1	3	3	0

*Each death had multiple causes.

Stamp III: One patient withdraw day 2 (needed tracheostomy) for H&N tumor.

Table 3. Reponse of Breast Cancer to STAMP Regimens

	I			III			V		
	#	CR	Resp	#	CR	Resp	#	CR	Resp
Number of patients:	16	6	13	8	0	6	16	1	14
No prior chemotherapy	2	1	2	0	0	0	0	0	0
Prior adjuvant therapy	2	2	2	**1	0	1	0	0	0
Response to prior therapy	6	*2	4	1	0	1	2	0	2
No response to prior therapy	6	*1	5	6	0	4	14	1	12
Inflammatory breast cancer*	5	3	5	0	0	0	0	0	0
Percent of patients with response to STAMP therapy:									
No response to prior therapy		17	83		0	67		7	86
All patients		38	81		0	75		6	88

**Progressed on adjuvant chemotherapy

(p=.08 & p=.06) , but was significantly associated with higher AUCs for thiotepa (p=.04 & p<.01) (15).

DISCUSSION

An important area for research in high dose therapy of breast cancer is the development of regimens capable of rendering a patient disease free. We have developed an integrated preclinical and clinical approach to evaluate intensive combinations of alkylating agents. While the pharmacokinetics of thiotepa at conventional and high doses are similar (14), substantial inter-patient variation in the systematic clearance of thiotepa results in markedly different drug exposures (AUC) for patients treated at the same dose levels. The AUC correlated more closely with response and toxicity than drug dose (15). If the determinants of such variability can be defined, more rational and safer dosing schedules can be designed. At maximum tolerated doses, interpatient variability in drug excretion may contribute to morbidity in a significant percentage of patients. Continuous infusion dosing regimens (if at least comparable in activity) would facilitate the use of serum levels to optimize the dose in individual patients (similar to adjustments made for antibiotic therapy).

The three regimens all produced a high response rate in refractory patients although the toxicity of the thiotepa regimens was considerably less than that of STAMP I. Response duration in these phase I study was generally short. In a recent review of 40 patients with failed breast cancer treated with high dose combination regimens, 73% responded. The median duration of the responses observed in the review were 1-4 months. These same preparative regimens, when given to patients responding to an standard dose induction chemotherapy regimen, resulted in an 81% response rate with 64% complete responses (16). We are currently evaluating STAMP V as a preparative regimen in women with metastatic breast cancer responding to standard dose treatment.

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Discussion 1 - Session IIIA (Breast)

Dr. Hortobagyi: I would like to ask Dr. Peters how many patients with Stage II breast cancer have been treated with this strategy?

Dr. Peters: There are now 14 patients on trial; 10 have all completed therapy, the 11th person is engrafting at the present time. The median follow-up is now 11 months for the group that have been treated. It is short.

Dr. Philip: Just a small question for Dr. Peters. I think it is very important that Gary Spitzer made the statement that 13 of these 19 patients which were not in CR before the ABMT went into CR after. And I still do not understand in the Duke data what happened? How many patients were in PR after induction therapy and how many of these PR are converted into CR by the STAMP regimen?

Dr. Peters: Let me answer the question in the following way. Firstly, the transplant regimen by itself produces a 54% CR rate. That is a piece of data I have not heard from any other group. No one has done Phase II data in upfront breast cancer data. Secondly, our conventional dose induction regimen is better than any induction regimen. We produce a 45% CR rate as the induction portion. In that setting the CR rate goes up to 70% after the STAMP regimen. Patients with radiological abnormalities after BMT went to surgery and, of the 9 patients, only 3 had evidence of presence of tumor. So, the others became CR. So, in fact, the numbers are substantial for converting PRs to CR in that setting, but we are not going to change the data except for the setting of calling them pathological complete responses. I think that is the key difference in the study designs. I do not know if that answers your questions.

Dr. Hortobagyi: Your data look very impressive, Bill. I am a little bit skeptical about the statement that your induction regimen is

clearly better than any other induction regimen. I think with the relatively small number of patients you have and with patient selection, you can probably get the same results with other induction regimens. So I hope you are right, but I do not think you have not demonstrated that yet.

Dr. Peters: We are undertaking a randomized trial to try to establish that.

Dr. Hortobagyi: Congratulations, I think that is the way to do it.

Dr. Dicke: I understand the importance of the question of conversion of PR into CR, it is basically a biological phenomenon. However, it may not change the natural history of the disease. When you look at the oat cell studies of our group, of all the PRs which were converted into CRs by the high dose program, none of them were durable. Although it is impressive in terms of response rate, it may not change in those patients the biology of the disease. However, the intensification may make the CR of the patients obtained after induction durable. Do you agree with that, Bill?

Dr. Peters: I think your premise is correct -- that, in fact, the conversion rate is not a very meaningful parameter. What you will need to follow in the long run is the outcome. I do think that it is critical to know what each portion of the regimen does, in terms of toxicity and therapeutic effect. I did not present this data because of time but we have noted a substantial difference in the post transplant toxicity of cyclophosphamide, platinum, BCNU with regard to the difference of the induction regimen of AFM vs. CAF. AFM is a much more intensive regimen in terms of myelosuppression in mucositis than the CAF. AFM by itself, followed by cyclophosphamide, Platinum, BCNU in our hands, has a 2 to 3% incidence of pulmonary toxicity. However, when we use CAF, followed by cyclophosphamide, platinum, BCNU, out of those 10 patients we either have 5 or 6 who have biopsy proven pulmonary drug toxicity. So a difference of 60% vs. 2 to 3%. A difference of 1 drug in the induction regimen.

Dr. Schaeffer: Can I ask either J. Bitran or Jeff Herzig, or maybe one of the others who have used a lot of high dose thiotepa, if they have treated anybody with CNS disease with breast cancer and are there any responses in CNS? Does the drug get there quite well? Is it potentially attractive?

Dr. Bitran: We have treated a couple of patients with breast cancer with CNS involvement. It was in the context of the phase I

study; patients with CNS lesions that had been previously irradiated. With the high dose thiotepa we have been able to show, on two occasions, that you can clear CNS disease. But I am not sure that this is a function of the thiotepa alone. I think others have reported it in breast cancer with conventional chemotherapy. So it just may be using an effective regimen.

Dr. Herzig: We have had responses in melanoma in the CNS but I do not recall specifically in the few patients with breast cancer, whether any of them had CNS metastases, so it can produce a response in the CNS. No reason to think that breast cancer would not respond to it too.

Dr. Peters: We have explored cyclophosphamide, platinum, thiotepa. In two patients with CNS metastases, one of them actually with prior radiation therapy, cyclophosphamide, platinum and thiotepa produced a profound CNS toxicity. Interestingly, 1 patient did not relapse at the original lesion site but relapsed as carcinoma meningitis. And the other person progressed in the CNS after treatment.

Dr. Gale: Obviously, many of these patients relapse following these high dose chemotherapy programs, I would like to ask the speakers, how is life after transplantation? How well can you evaluate these patients afterwards?

Dr. Bitran: There is life after transplantation. Patients can be paliated very well with conventional drugs, given in conventional dose. We have seen secondary responses and, I believe, in the patients who have relapsed only 2 or 3 have died. So, one can continue to treat these patients.

Dr. Herzig: I am certainly convinced that there is a dose response relationship that has been demonstrated for breast cancer without alkylating agents and that there is very suggestive data toward the idea that we might be able to produce curative therapy in patients with metastatic breast cancer by dose escalation. I think that is the answer, but we are going to need to provide stronger data and support before this is going to gain any popularity. And whenever we talk about this with our other medical oncology colleagues, they say how many patients are cured. They say why should we put our patients through this toxicity and hospitalization for prolongation of disease-free survival. I think all of us agree with that. It is not a question of an increase in the median duration of remission by 3 to 6 months, or changing the complete response rate. Those things aren't worthwhile. It is the question whether we can cure any of these patients. Also, it looks like in several of the regimens -- I mean it is clear that you

cannot intensify the individual regimen any longer and it seems that there are two ways to go -- one is the approach that Gary (M.D. Anderson people have taken), which is to do 2 transplants. I would rather see it done with 2 transplant regimens, rather than half of a transplant regimen 2 times...also, preferably with different drugs in the two regimens. And the other thing is that there is still a lot of room for pretransplant intensification with drugs that are active in breast cancer that are not really appropriate for a transplant regimen, like the anthracyclines. I would actually put platinum in that same category. I do not think platinum is a really appropriate drug for a transplant regimen when you can give it at its maximum dose without a transplant, and the others you could come up with as well. I think there is a lot of room for movement in that direction.

Dr. Bitran: I was wondering if anyone has any data on the patients who are responders vs. non-responders, in terms of -- let us say -- MDR gene expression or resistance markers, etc.? Number 1 and number 2 is in the double transplant setting at Anderson, what about using drugs which are non-cross-reactive for the second transplant?

Dr. Spitzer: Obviously, that is why we devised this strategy. First of all, we do not have any MDR data although we are about to do this on the bone marrows of these patients -- not necessarily on the initial tissue. Second, we have quite a bit of experience with our high dose mitoxontronea, thiotepa, and VP-16 (although we are going to drop the VP-16) on something like 20+ patients at this moment and do have a high response rate.   One of our plans is to introduce that particular program eventually as the second go-around. And that is why we think this idea of tandem transplants is a very flexible approach. We would think mitoxontronea and thiotepa may be non-cross-resistant drugs. Karel Dicke is a little bit more excited than I, and he thinks we should do 2 CVPs and then mitoxontronea and thiotepa.

DOSE INTENSIFICATION USING HIGH-DOSE COMBINATION ALKYLATING AGENTS AND AUTOLOGOUS BONE MARROW SUPPORT FOR THE TREATMENT OF BREAST CANCER

William P. Peters, Roy B. Jones, Elizabeth J. Shpall, and Jeffrey Shogan

SUMMARY

The use of high-dose cyclophosphamide, cisplatin and carmustine (CPA/cDDP/BCNU) and autologous bone marrow support (ABMS) will produce frequent and rapid responses in metastatic breast cancer. In advanced resistant breast cancer, this approach will produce complete remission in about 25% of patients but the duration of unmaintained remission is short. However, the use of high-dose combination CPA/cDDP/BCNU and ABMS in early metastatic breast cancer will produce complete responses in more than half the treated patients, wfth a limited number of these remissions being durable. Intensive non-transplant induction therapy, followed by autograft consolidation, further increases the frequency of complete remission and the durability of the response. These findings and early pilot data in the adjuvant treatment of primary breast cancer indicate that CPA/cDDP/BCNU and ABMS in the appropriate setting is effective therapy and warrants systematic clinical trials to further define treatment effects and to reduce toxicity.

INTRODUCTION

The optimal treatment of breast cancer remains the subject of controversy and conceptual dispute. During the past decade, evidence

has been accumulating for a steep dose response effect in the treatment of breast cancer. Careful analysis of clinical trials of chemotherapy in metastatic and primary breast cancer, coupled with substantial clinical and laboratory investigations in other neoplastic diseases, suggests that the dose response effect for most non-hormonal neoplastic agents is steep for both therapeutic and toxic effects. With careful selection of treatment programs, it appears that one may improve the therapeutic results in breast cancer by dose intensification.

Various investigators have utilized the term "dose intensification" or "high-dose therapy" in differing manners. As used by most investigators, exploring the concept in animal or *in vitro* models, the term refers to dose escalation over a defined time period. In most clinical settings, the occurrence of myelosuppression, or other toxic side effects of cancer chemotherapy has resulted in the application of intermittent therapy, i.e., chemotherapy applied on a regular basis, e.g., monthly. In order to compare different treatment schedules, Hryniuk and Bush[1,2] have utilized the concept of relative dose in testing, that is, the dose of antineoplastic chemotherapy administered over a given cycle of chemotherapy, normalized to a "standard" therapeutic regimen. In the case of breast cancer, this has generally been to the original Cooper regimen.[3] This term, however, is not generally applied to experiments employing a single dose of intensive chemotherapy which is not to be introduced repetitively. In most studies utilizing high-dose combination chemotherapy and autologous bone marrow support, the treatment is used only once. However, a calculation of dose intensity in this fashion for our combination of cyclophosphamide, cisplatin and carmustine yields a relative dose intensity of 12.85 compared to the original Cooper regimen. There are other investigators who utilize reduced doses of chemotherapy applied more than once, although the necessity of autografts in these clinical trials is questioned and the possibility of increasing relapse through a reduced dose intensity has been raised.

It is likely that it will be valuable to calculate the relative dose intensity, by using pharmacologic "area under the curve" (AUC) and maximum plasma concentration (C_{max}), as a monitor of dose intensity between therapeutic programs and among patients. The importance of this approach is emphasized by the observation of substantial pharmacodynamic variability among patients and various drugs.[4,5,6,7] Unfortunately, at this time, this approach is limited to only a few centers and will likely be first utilized for evaluating high-dose pulse experiences. However, because of the relative insensitivity of most solid tumors to chemotherapy, the threshold dose for killing malignant cells, i.e., the "minimum tumoricidal dose," is often close to the C_{max} achieved with standard dose therapy. For this reason, it may be that the importance of pharmacodynamic variation will be greatest in patients treated with conventional doses in which marrow supportive

efforts are not utilized. Ultimately, the most precise way to define dose administration is through the use of molecular dosimetry in which the direct damage occurring to normal and malignant tissue nucleic acid for a given dose of chemotherapy is compared among treated patients. Although this type of approach appears to be possible with currently available techniques, it has not been extensively investigated and is technically very complex.

The optimal mode of dose intensification has not yet been defined. At least two modes of dose intensification should be recognized: (1) "intermittent" total dose intensification can be achieved by applying a lower dose of chemotherapy continuously over an extended period of time; e.g., Cooper-type chemotherapy; (2) "pulse" dose intensification refers to the administration of the maximum tolerated dose over a very short period of time, allowing time for organ recovery in the interim; for example, high-dose combination chemotherapy at maximum tolerated dose with autologous bone marrow support. Other approaches to dose intensification can be considered variants of these two basic approaches.

Most *in vivo* experimental models suggest that the optimal method of dose intensification, in terms of maximizing tumor kill, is to apply the dose as a single administration. Careful experimentation by Skipper, Schabel and Griswold in murine models has demonstrated this effect for several tumor models and antineoplastic agents.[8,9] The effect is most prominent with the alkylating agents.

Our efforts over the last six years has been to systematically and quantitatively evaluate high-dose combination alkylating agents and autologous bone marrow support to determine the therapeutic and toxic effects of this approach. We will present here observations on 98 patients with breast cancer treated with high-dose cyclophosphamide, cis platinum, and carmustine (BCNU) and autologous bone marrow support (ABMS). These results indicate that high-dose combination cyclophosphamide, cisplatin and carmustine with autologous bone marrow support utilized in the appropriate patient population will produce frequent and rapid complete responses which appear superior to conventional therapeutic programs. While follow-up is yet short, there is accumulating evidence that the treatment program is effective.

METHODS

Patient Population

From January, 1985 through August, 1988, 136 patients with breast cancer were entered on autologous bone marrow transplant protocols at the Duke University Medical Center. This analysis will deal with 98 patients with breast cancer treated between January, 1985

and June, 1988 who received high-dose combination alkylating agents and ABMS. The remaining patients were entered more recently, or received high-dose single agent therapy with ABMS. All patients, except 12, received high-dose cyclophosphamide, cisplatin and carmustine in a standardized therapeutic regimen, as described previously, as their conditioning regimen. Twelve patients who had received prior chemotherapy for metastatic disease received a modified regimen in which either melphalan or thiotepa was substituted for carmustine. These therapeutic regimens were described elsewhere.[10,11,12,13,14,15,16,17,18,19,20,21]

Twenty-two patients were treated with cyclophosphamide, cisplatin and carmustine regimen as the initial chemotherapy for metastatic breast cancer. The treatment program, patients characteristics, and therapeutic results are published elsewhere. Fifty patients with estrogen receptor negative, hormonally insensitive measurable metastatic disease were treated with the "Duke AFM" regimen of intensive adriamycin, 5-fluorouracil and methotrexate followed by high-dose combination cyclophosphamide, cisplatin, carmustine and ABMS.

Fourteen patients with Stage II breast cancer involving 10 or more axillary lymph nodes received induction chemotherapy with augmented conventional doses of cyclophosphamide, adriamycin, and fluorouracil, followed by the same autograft preparative regimen of cyclophosphamide, cisplatin and carmustine.

Results

The outcome of these therapeutic trials is presented in Table 1. The treatment results for the treatment program of high-dose combination alkylating agents applied to different stages in extensive disease settings is presented. When patients with Stage IV breast cancer who have received prior conventional chemotherapy for metastatic disease are treated with a high-dose alkylating agent regimen and ABMS, a 27% complete response rate was obtained. The overall objective response rate was 79%. However, as seen in Figure 1, the time to treatment failure is brief and, perhaps predictably, long-term responses have not been achieved in this patient population. These results are similar to those we reported in breast cancer patients during a Phase I trial of CPA/cDDP/BCNU and ABMS.[22]

However, the finding of frequent and rapid responses in this group of patients with advanced resistant disease prompted a therapeutic trial in patients who had not received prior chemotherapy for metastatic breast cancer. In this group, the overall response rate was 77% (92% for evaluable patients), and the complete response rate was 54%. Again, the time to treatment failure for this single treatment with no maintenance therapy was short, although 15% of the patients remained in continuous, unmaintained remission exceeding 18 months.

**Table 1. Treatment of Breast Cancer with High Dose
Combination Alkylating Agents and
Autologous Bone Marrow Support**

Disease Status	Induction Chemotherapy	ABMT Preparative Chemotherapy	Complete Response	Comments
		Treatment Program		
Refractory		CPA/cDDP/PAM CPA/cDDP/TT	27%	Short duration of Response
1st Relapse		CPA/cDDP/BCNU	54%	Relapse at bulk disease sites, some durable
1st Relapse	AFM	--	45%	Induction; durability not studied
1st Relapse	AFM->	CPA/cDDP/BCNU	70%	Median duration of follow-up 11 mos.
1st Remission	CAF->	CPA/cDDP/BCNU	--	14 patients enrolled

Relapses, when they occurred, were generally at sites of pretreatment bulk disease in excess of 3 cm, or in areas of previous radiation therapy. This finding prompted an exploration of the same transplant regimen after the use of induction chemotherapy to reduce tumor bulk.

This derivative program, called the "Duke AFM program," uses a short induction course of intensive adriamycin, fluorouracil and methotrexate until maximum response, followed by immediate treatment with cyclophosphamide, cisplatin and carmustine and ABMS with surgical restaging and radiation therapy to sites of pretreatment bulk disease. The program results in a complete response rate of 70% of treated patients. Patients who do not achieve a complete response will generally develop progressive disease rapidly. However, patients achieving a pathologic complete remission frequently have durable, unmaintained remissions. Of over 45 patients completing therapy, only four patients achieving complete remissions have relapsed within a median follow-up approaching one year.
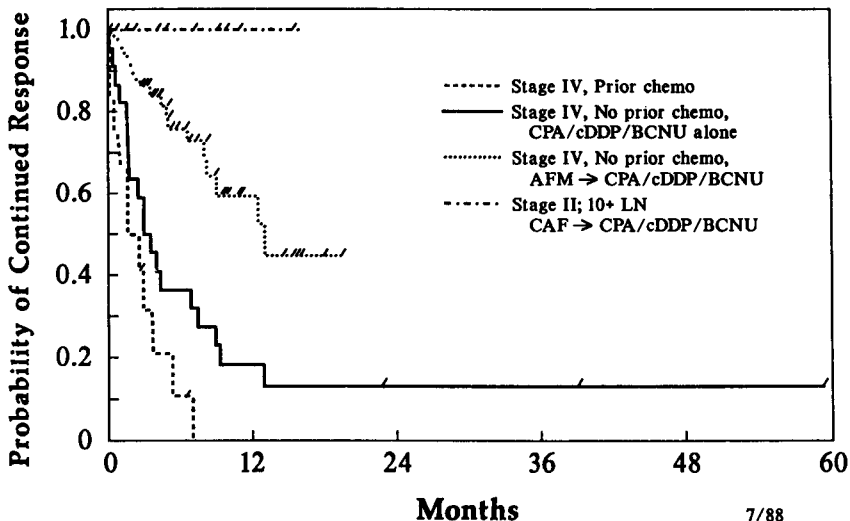
Figure 1. Time to treatment failure for treated patients in months from treatment initiation. Patient treatment characteristics for each line are presented in the inset and refer to the programs described in the text. Tic marks represent patients in continuing, unmaintained remission.

The second major conceptual advance is that the treatment of early and minimal disease states may be associated with reduced toxicity and approved therapeutic efficacy. In our experience, the treatment of patients with Stage II multi-node positive breast cancer is associated with rapid relapse, with a median time to treatment failure of one year, and a median survival of 3.4 years. Because of the substantial risk of early recurrence in this disease, we have reasoned that this is an appropriate setting for the use of high-dose intensification following standard chemotherapy. To be able to have contemporary data available for comparison, we chose the most intensive arm of the current Cancer and Acute Leukemia Group B, Stage II, adjuvant protocol as the induction therapy prior to transplant preparation. This CAF program, for four cycles, has been coupled with high-dose cyclophosphamide, cisplatin and carmustine and ABMS in a back-to-back fashion. Results to date of presentation are encouraging. Of 14 patients entered on trial, 10 completing chemotherapy, there have been no treatment failures and no therapy-related deaths. Follow-up is a median 10 months, and further observation of these patients will be required.

DISCUSSION

The data that is presented in this manuscript provides evidence for three important features of high-dose chemotherapy in the treatment of breast cancer.

First, this is effective therapy. The evidence for this is that there is a high frequency of remission and, more importantly, of complete remissions in the treatment of breast cancer when one is using high-dose combination alkylating agents and autologous bone marrow support. Complete response frequencies of approximately 70% in early metastatic breast cancer are reported in this manuscript and have been observed by other investigators using similar approaches.[23,24,25] Although the follow-up on many of these studies is short, it is clear from our work that some of these remissions may be durable, and that further work is warranted. Other treatment programs rarely will produce complete responses in excess of 30%.

Second, the data suggests that there is an important relationship between the timing of intensive therapy with high-dose alkylating agents and the outcome of the treatment. When this therapeutic strategy is employed in advanced disease, frequent responses can be obtained, but they are rarely durable. As one uses the intensification earlier in the disease natural history, or at a time when tumor bulk is minimized, the therapeutic results are superior. This set of observations is consistent with animal treatment data and with experience in other disease settings.[26,27,28]

Third, these treatment programs are toxic and test the limits of the physiologic tolerance of patients for therapy. Because of their intensity, toxicity, and expense, their application should be restricted at this point in time to centers with demonstrated capacity for undertaking such dose intensification. It is likely, however, that advances in treatment strategy, e.g., by the use of recombinant growth factors, may allow a reduction in the toxicity associated with these therapeutic approaches. We have provided evidence that recombinant GM-CSF can accelerate hematopoietic recovery following high-dose chemotherapy with an attendant reduction in the morbidity and mortality associated with high-dose treatment.[29,30] If such results can be confirmed and extended, it is likely that this therapeutic approach may be more widely utilized and have an even more successful outcome.

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HIGH-DOSE INTENSIFICATION FOR STAGE IV
HORMONALLY-REFRACTORY BREAST CANCER

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INTRODUCTION

Estrogen receptor-negative or hormonally-refractory metastatic adenocarcinoma of the breast is almost uniformly fatal. Patients have a median survival of just over one year from the onset of metastatic disease and very few patients survive over three years with the best conventional therapy.

Past analysis of adjuvant chemotherapy dose intensity suggested dose-response in breast cancer. Similarly, high-dose single-agent or combination chemotherapies in multiply-relapsed breast cancer show response rates much higher than expectations with conventional-dose chemotherapy. Because of the dismal natural history of this metastatic breast cancer subgroup, and the known dose-responsiveness of breast cancer to chemotherapy, we initiated, approximately two and one-half years ago, a pilot study involving high-dose combination chemotherapy intensification with cyclophosphamide, VP-16, and cisplatin (CVP) for ER- or hormonally-refractory metastatic breast cancer. Patients were initially treated with adriamycin combination therapy, marrow was collected, and subsequently almost all patients received two courses of high-dose therapy. Doses were chosen to be modestly decreased for each cycle compared to the doses used for single transplant regimens; over the period of these studies the initial doses were escalated. The initial level (level 1) was an approximately 20-30% reduction of the maximum tolerated dose of this combination as a single course. Subsequently, we have cautiously escalated to level

3, which may be approximately 90% of what would be the maximum tolerated dose (MTD) of this combination as a single bolus. Nevertheless, the cumulative doses over a four-week period encompassing the two courses of therapy represent approximately 1.2 to 1.8 times the dose administered if all the chemotherapy were given in the one setting. This approach was designed to avoid significant extramedullary toxicity. If the slight extra increment in dosage necessary to achieve myeloablation were added, extramedullary toxicities could appear with alarming increase in proportional rate (i.e., veno-occlusive disease, diffuse interstitial pneumonitis, vasculitis) and could be irreversible. We feel that the strategy of using less than ceiling doses of drugs, but with two courses of chemotherapy administration, increases the therapeutic ratio.

RESULTS

To date, 46 patients have been entered at levels 1-3; 7 patients at level 3 are too early to evaluate. In the 39 evaluable patients, the median age was 40 (29-62); approximately one-third of these patients had received prior adjuvant therapy (either hormonal or chemotherapeutic); 60% were ER- and, when ER+, were only minimally positive. Most patients were also progesterone-receptor negative or unknown and 80% were pre- or peri-menopausal. Most importantly, the disease-free interval was a median of just over one year and two-thirds of these had two or more metastatic sites which frequently included lung or mediastinal nodes. Response to induction was typical of most programs, with 9 of 37 (24%) patients having measurable disease achieving CR and a total of 89% CR plus PR. However, at the time of intensification, three patients with partial remission were progressing and four patients were stable. Following intensification the CR rate increased significantly, 23 of 35 evaluable patients having achieved CR (66%). Thirteen of 19 patients in PR prior to intensification converted to CR (68%); two of the total 21 induction PR patients were early deaths and not evaluable for response. Only one of the patients with stable disease or progressive disease at the time of transplant achieved CR. Of the 46 patients to receive therapy, three patients have died (two patients were >50 years old and two had fulminant alpha-hemolytic streptococcus sepsis to initial therapy with empiric antibiotics).

Nineteen patients have relapsed; approximately 50% of these relapsed at the original site of disease only and 80% at the same site and/or new sites. Less than 20% of the patients relapsed only at a previously unaffected site alone. The median progression-free survival (Fig. 1) approximates a year, with eight patients at 70 weeks or greater progression-free while off-therapy one year or more. Median survival should be at least 2 years (Fig. 2). We have examined
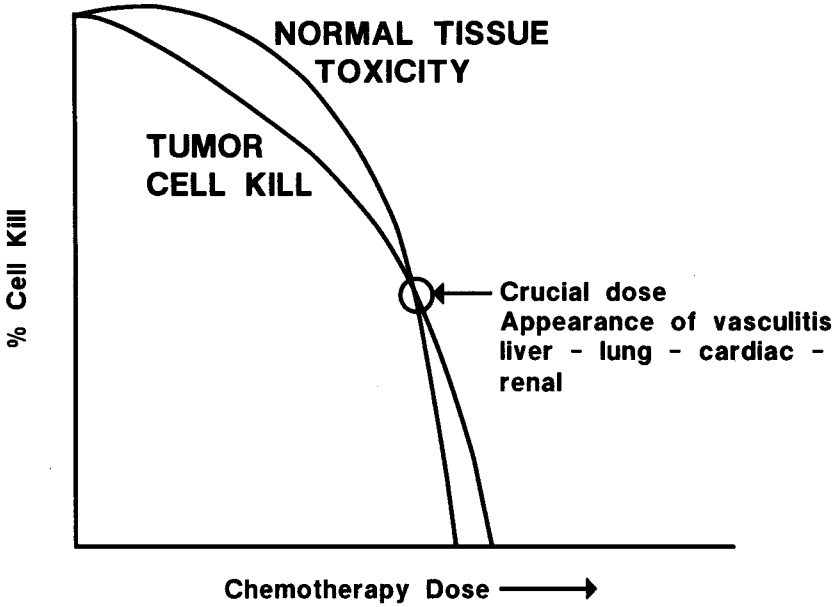
Figure 1. Curve depicting the basis of loss of therapeutic effect with very high-dose therapy.
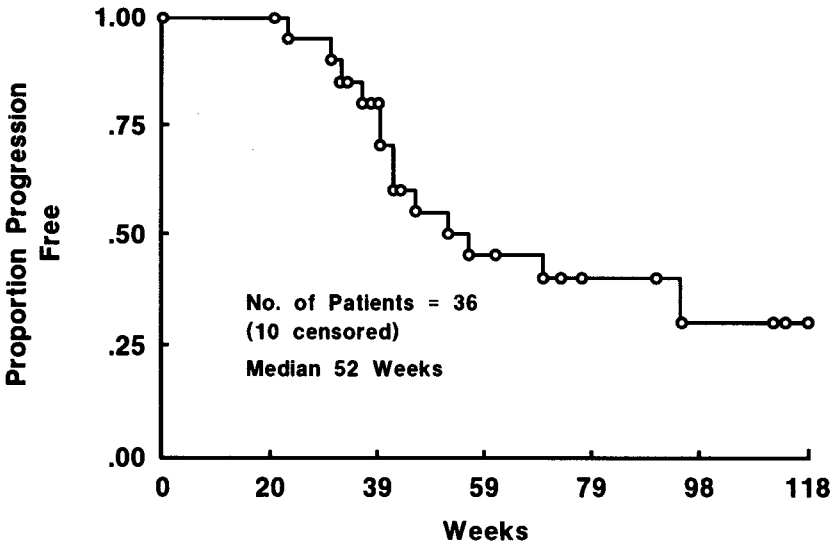
Figure 2. Progression-free survival from induction therapy.

the features of the eight patients who have had significant progression-free survival. Three of these eight patients have exceeded their initial disease-free interval, and all of these patients have had visceral disease and/or lung metastases.

The most prominent toxicity was sepsis, most frequently gram-positive (gram-negative or polymicrobial being quite rare). Grade 4 extramedullary toxicity was infrequent, and there was a low percent of grade 3 toxicity. The most common extramedullary toxicity was nausea and vomiting with a modest degree of mucositis and diarrhea. We noticed no serious pulmonary, cardiac, hepatic, renal, or vascular toxicity.

CONCLUSION

The response rates and CR rates (Table 1) documented with this program have been impressive. It is difficult to compare the efficacy of this program with other programs, but if we were to look at only the patients responding to induction at the time of intensification, the CR rate realized in this subgroup approaches 80%. Obviously, response to induction therapy, intensification, and long-term survival will be most favorable if patients 1) have long disease-free intervals between mastectomy and recurrence of metastatic disease, 2) have received no adjuvant chemotherapy, and 3) have a single site of disease in soft tissue or lymph nodes. Patient selection is always a significant concern, but the implication that this may be a favorable program can be inferred by 1) the impressive conversion to CR of patients who are only partial responders to induction therapy (Figure 3), and 2) the characteristics of some of the patients who are currently the longest progression-free survivors, who had visceral and lung disease and who have shorter previous disease-free intervals. We are also encouraged by the very acceptable toxicity level and low mortality rate in these patients. Further follow-up will be needed to determine whether our longest progression-free survivors have been cured, as well as accrual of further patients, to confirm the low toxicity and mortality so far seen with this program.

Table 1. Intensification Response (35 Patients)

CR	23/35	(66%)
PR	10/35	(29%)
	total	95%

two patients inevaluable (NED at induction)
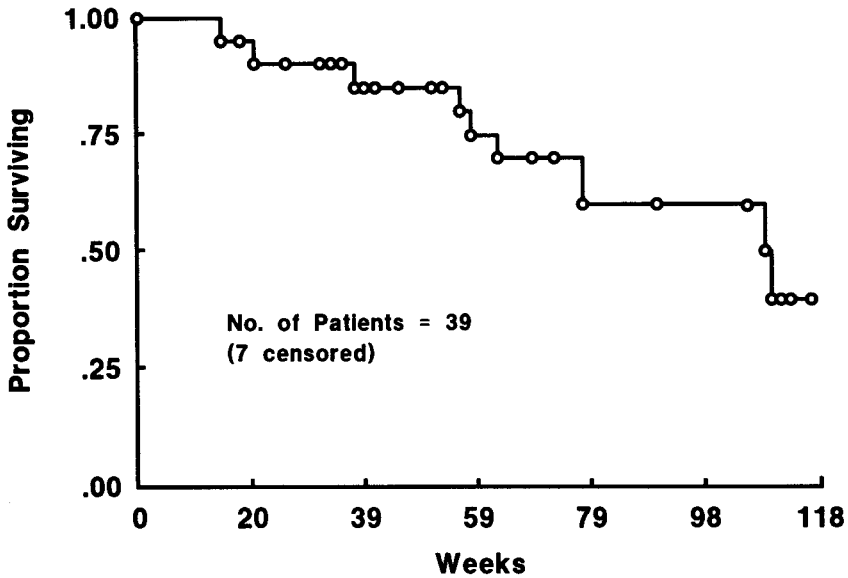
Figure 3. Survival from induction therapy.

ACKNOWLEDGMENTS

AUTOLOGOUS BONE MARROW TRANSPLANTATION AND HEMATOPOIETIC RECOVERY IN SOLID TUMORS

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ABSTRACT

We have treated 59 patients with advanced solid tumors using tandem cyclophosphamide/VP-16/cisplatin (CVP) intensification. Half were randomized to autologous marrow support and half to no marrow support. We have observed delayed neutrophil and platelet recovery in a large proportion of patients in the no marrow infusion arm. Hematopoietic recovery is favorably influenced by marrow infusion. There is no difference in morbidity and mortality between the groups. In 25% of the patients in the no transplant arm, salvage infusion was necessary. Data concerning reinfusion of tumor contaminated marrow is too preliminary.

INTRODUCTION

Intensive chemotherapy followed by autologous marrow support has been shown to induce complete remission (CR) in patients with relapsing Hodgkin's disease, non-Hodgkin's lymphoma, neuroblastoma, and other solid tumors (1-3). Single courses of high-dose combination chemotherapy with bone marrow support has shown an increase in CR rate and prolonged disease-free survival with a potential for cure in some of these patients (4-6). We have applied the above approach in the treatment of adult, non-hematologic malignancies. We have adopted two general principles in treating solid tumors, 1) intensify early in their disease course, preferably after they have shown response to two or three cycles of induction chemotherapy (7), and 2) the use of tandem high-dose chemotherapies separated by one-month intervals (Figure 1). Using this tandem
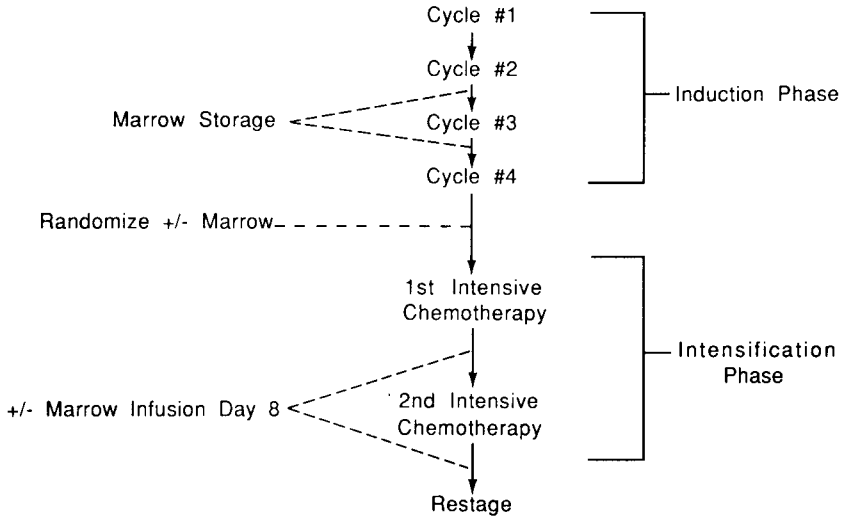
Figure 1. Schema of tandem high-dose chemotherapy for metastatic or advanced cancer.

approach, the total dose of chemotherapy that can be administered over two courses is significantly greater than that which can be given over one course (Table 1). Half of our patients have been randomized to marrow infusion and half to no marrow infusion. We cautiously escalated the dose of our core drugs (cyclophosphamide, VP-16, and cisplatin), observing hematopoietic recovery between the randomized groups.

The questions we addressed were as follows:

1) Is autologous marrow reinfusion necessary at these doses?

2) If there is significant earlier hematopoietic recovery with autologous marrow reinfusion, could the potential benefits associated with this (for example, less viral-transmitted disease, less allo-immunization, less febrile episodes), be outweighed by the risk of reinfusing contaminated tumor cells?

3) Will tumor-contaminated marrow affect hematopoietic recovery?

4) Will patients who receive tumor-contaminated marrow relapse sooner and at different sites than their non-marrow-involved counterparts?

5) Is there a survival advantage for patients randomized to marrow vs no marrow?

Table 1. High-Dose Tandem Combination Chemotherapy

Dose Level		Total Dose After 1 Course	Total Dose After 2 Courses
LEVEL 1	Cytoxan	$4.5g/m^2$	$9g/m^2$
	Etoposide	$750mg/m^2$	$1500mg/m^2$
	Cis-platinum	$120mg/m^2$	$240mg/m^2$
LEVEL 2	Cytoxan	$4.5g/m^2$	$9g/m^2$
	Etoposide	$900mg/m^2$	$1800mg/m^2$
	Cis-platinum	$150mg/m^2$	$300mg/m^2$
LEVEL 3	Cytoxan	$5.25g/m^2$	$10.5g/m^2$
	Etoposide	$1200mg/m^2$	$2400mg/m^2$
	Cis-platinum	$180mg/m^2$	$360mg/m^2$
LEVEL 4	Cytoxan	$6g/m^2$	$12g/m^2$
	Etoposide	$1500mg/m^2$	$3000mg/m^2$
	Cis-platinum	$180mg/m^2$	$360mg/m^2$

Patient Eligibility

Patients with advanced or metastatic solid tumors which were chemotherapy-sensitive and had received less than 10 total courses prior chemotherapy without marrow involvement.

Patient Characteristics

There are 29 patients in the marrow infusion arm, median age 45 (range 30-63), performance status 0 (range 0-1), 72% with breast cancer and 8% with other solid tumors. There are 30 patients in the no marrow infusion arm, median age 41.5 (range 28-66), performance status 0 (range 0-1), 70% with breast cancer and 9% with other solid tumors.

METHODS

We have treated 22 patients at level 1, 26 patients at level 2, and 11 patients at level 3.

Multiple bone marrow aspirations from the iliac crests were performed when the patient was first considered a candidate, preferably after the second course of conventional chemotherapy. To address the problem of possible occult bone marrow contamination with malignant cells, we gave 29 of our patients bone marrow reinfusion on day eight following each intensive chemotherapy, and

in 30 of our patients we did not reinfuse marrow unless they had delayed hematopoietic recovery.

RESULTS

Analysis of recovery of neutrophils to $500/mm^3$ from the start of chemotherapy at level 1 (course 2) and level 2 (course 2) reveals that 38-50% of patients in the no marrow infusion arm have significantly slower recovery, with no detectable difference between the two levels. Level 3 data are still to preliminary for evaluation. Delayed neutrophil recovery, defined by failure to achieve $500/mm^3$ absolute neutrophils by day 28, was more frequent in the group randomized to no marrow infusion. Recovery of platelets to $50,000/mm^3$ from the start of chemotherapy at level 1 (course 2) and level 2 (course 2) (Figures 2 and 3), and were also significantly and markedly slower in the patients randomized to no marrow infusion. Delayed platelet recovery, defined by failure to achieve $50,000/mm^3$ by day 35 post-chemotherapy, was much more frequent in those patients in the no marrow infusion arm. Salvage marrow infusion was necessary in seven patients randomized to the no transplant arm, primarily for delayed hematopoietic recovery. Data on recovery of platelets at level 3 are also too early for evaluation.
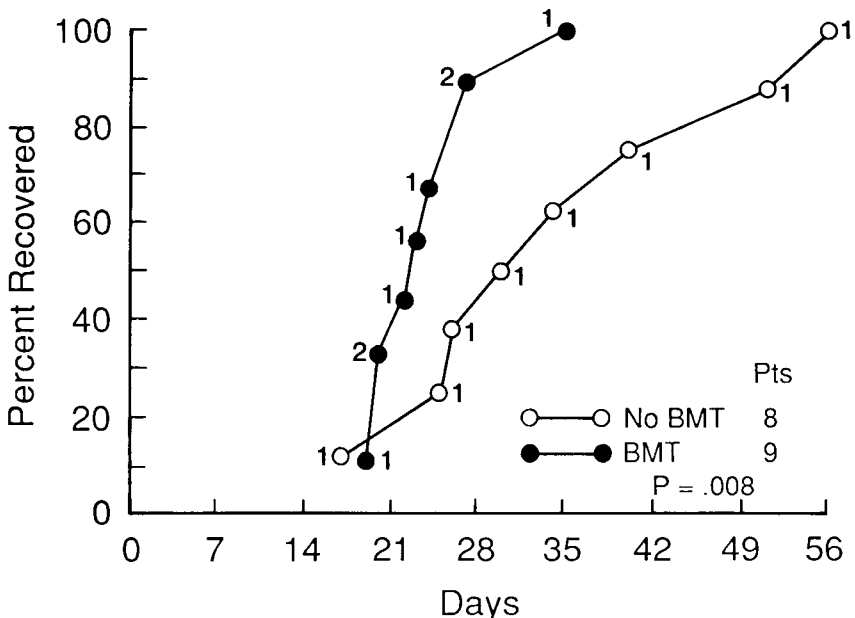
Figure 2. Recovery of platelets to $50,000/mm^3$ from start of level 1 chemotherapy (course 2).
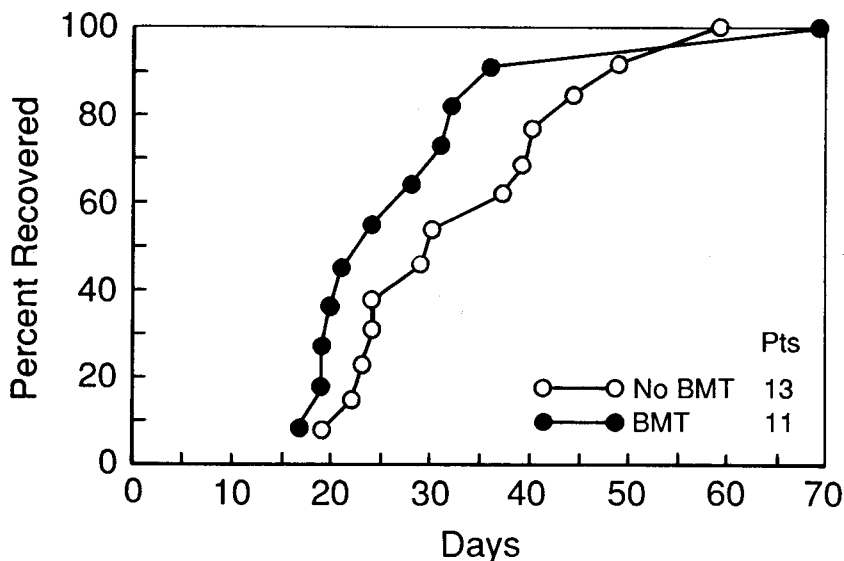
Figure 3. Recovery of platelets to $50,000/mm^3$ from start of level 2 chemotherapy (course 2).

We have observed seven patients with occult malignant cells contaminating their stored marrow by use of monoclonal antibodies reactive to surface antigens and immunoalkaline phosphatase (four in the marrow infusion arm and three in the no marrow infusion arm). In preliminary analysis, there does not seem to be delayed hematopoietic recovery in these seven patients (see chapter by Owen, et al.).

Complications with this therapy have included a 60-80% incidence of fever, 10-20% incidence of pneumonia, and 10% incidence of major bleeding. There have been two deaths in the marrow infusion arm (one from sepsis, the other from ARDS) and two deaths in the no marrow infusion arm (one secondary to progressive cancer, the other secondary to sepsis).

CONCLUSIONS

1) Hematopoietic recovery is favorably influenced by marrow infusion.
2) There is no difference in morbidity and mortality between the two randomized groups.
3) In 25% of the patients in the no marrow infusion arm, salvage marrow infusion was necessary due to delayed hematopoietic recovery.

4) Data concerning reinfusion of tumor-contaminating marrow is too preliminary to reach a conclusion.

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SESSION III - SOLID TUMORS

B. MINIMAL RESIDUAL DISEASE

BONE MARROW METASTASES IN SMALL CELL LUNG CANCER: Detection with Magnetic Resonance Imaging and Monoclonal Antibodies

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Bone marrow (BM) involvement might have prognostic and therapeutic influence in small cell lung cancer (SCLC) (1). Lack of tumor cell detection when minimal involvement is present in the BM might be the cause of some tumor relapses observed after intensive chemotherapy followed by autologous BM transplantation in SCLC (2,3,4). Routine detection of BM metastases in SCLC usually consists of unilateral posterior iliac crest aspiration and biopsy and permits detection of BM involvement in 15 to 30% of patients (5,6). Since magnetic resonance imaging (MRI) and monoclonal antibody (MoAB) immunostaining were recently shown to be very sensitive methods to detect BM metastases, we investigated the value of both MRI and immunological methods to look for the presence of malignant cells in the BM of 28 consecutive patients with SCLC.

PATIENTS AND METHODS

Patients

From April 15, 1987 to April 15, 1988, twenty-eight previously untreated patients with histologically proven SCLC were referred to our institution. Initial staging included fiber optic bronchoscopy, CT scan of the thorax, abdominal ultrasonography and/or CT scan, brain radionucleide and/or CT and radionucleide bone scan (RBS) + BM staging (see below). After completing all of these procedures, 23 out

of 28 (82%) patients were considered to have metastatic disease at the time of diagnosis.

BM Examination

Cytological and Histological Examination of the BM

In all patients, unilateral posterior iliac crest aspiration and biopsy were performed before any treatment.

MoAB Immunostaining (7)

In 26 out of 28 patients, 2 to 3 mls of marrow were harvested in heparin and separated on Ficoll. Two MoABs of the Immunoglobulin (IG) G isotype (UJ 13 A kindly provided by J. Kemshead, 11.14 kindly provided by J.C. Laurent, Sanofi) recognize antigens expressed by cells of neuroectodermal origin, and CD 45 of the IgM isotype (kindly provided by G. Janossy) recognizes the panleucocyte antigen (8).

Double Immunofluorescence Immunostaining on Cell Suspension

Two samples of 10^6 cells in 100 μl phosphate buffer saline (PBS) with 0.1% NaN3 are incubated with the 2 MoABS (one for each sample) in combination with the CD45. MoAB are recognized by TRITC anti-mouse IgM specific and FITC anti-mouse IgG specific second layer (Southern Biotechnology Associates). Samples are analyzed in a fluorescence Zeiss microscope. Malignant cells are recognized by being positive with one of the 2 anti-neuroectodermal antigens MoABS and negative with the CD45.

Alkaline Phosphatase Immunostaining on Cytocentrifuged Smears

Immunochemical staining is performed using an indirect 3-stage immunoenzymatic procedure with alkaline phosphatase (Dakopatts, Copenhagen, Denmark). After fixation with acetone, slides are counterstained with hematoxylin and evaluated under an optical microscope.

The limit of detection of these 2 immunological methods is of one malignant cell in 10^5 normal mononuclear BM cells if 3×10^6 cells are analyzed in double immunofluorescence and 6 smears in alkaline phosphatase.

Magnetic Resonance Imaging

All patients were studied with a .5T superconductive magnet (Magni Scan 5000 Thomson CGR). Contiguous slices of the pelvic

bony structures were obtained including femoral heads, upper extremities of the femur, pelvic bones (iliac, sacrum), and 2 or 3 lower lumbar vertebral bodies.

A T1-weighted spin echo sequence was performed using a TR of 500 ms and a TE of 26 ms. This sequence is known to show normal BM in adults as homogeneous areas of high signal intensity. MR examination was considered to be positive or negative depending on the presence or absence of focal areas of low signal intensity within the normal signal intensity of marrow fat as already described by Shields (9) in lymphomas and by ourselves in SCLC (10).

RADIONUCLEIDE BONE SCAN (RBS)

RBS was performed in 25 out of the 28 patients, 2 hours after a bolus intravenous injection of 9 M Bq/kg hydroxymethyl-diphosphonate labelled with 99 tecnetium.

RESULTS (TABLE 1)

In 7 patients, cytologic examination of the BM was positive. BM biopsy was positive in only 4 out of these 7 patients and could not be performed in one case (no. 20). In one case (no. 23), BM biopsy was positive with negative cytology. Thus, BM involvement was detected with cyto- and/or histologic examination in 8/28 cases: MRI was considered to be positive in all 8 cases, BM involvement appearing as focal areas of low signal intensity within the normal signal intensity of marrow fat (Figure 1); this finding correlated with strongly positive immunostaining in all of 7 of these cases where it was performed with a percentage of malignant cells ranging from 10^{-4} to 6.10^{-1}.

Of 20 patients in whom conventional cytohistology was negative, 2 had both positive MRI and immunostaining, 4 had isolated positive immunostaining. MRI and immunostaining was negative in 12 out of these 20 patients and in 1 case (no. 10), MRI was negative and immunostaining was not done.

MoABS UJ 13 A and 11-14 were shown to stain more than 95% of SCLC tumor samples with very homogeneous staining of malignant cells within the same tumor. Practical limitation to the use of any MoAB against SCLC on BM samples is their reactivity with very few normal cells of the NK lineage. The use of double immunofluorescence analysis permits to accurately distinguish between those few normal cells (stained with UJ 13 A, 11-14 and the anti-panleucocyte MoAB) and malignant cells negative with anti-panleucocyte MoAB.

Table 1. Results of MoAB Immunostaining and MRI Findings Compared to Routine Procedures (Cytology, Histology, and RBS)

Stage	Patient Number	Cytology	BM Biopsy	MoAB Immunostaining	MRI	RBS
ED	1	+	+	ND	+	+
ED*	22	+	+	$+ (10^{-4})$**	+	-
ED	22	+	+	$+ (6.10^{-1})$	+	+
ED	26	+	+	$+ (2.10^{-1})$	+	+
ED	3	+	-	$+ (10^{-1})$	+	-
ED	24	+	-	$+ (10^{-2})$	+	-
ED	15	+	-	$+ (10^{-3})$	+	ND
ED	23	-	+	$+ (10^{-2})$	+	+
ED	25	-	-	$+ (10^{-5})$	+	+
ED	11	-	-	$+ (10^{-5})$	+	ND
ED	12	-	-	-	+	+
ED	17	-	-	-	+	+
ED	16	-	-	-	+	-
ED	20	-	ND	-	+	+
ED	14	-	-	$+ (10^{-3})$	-	-
ED	4	-	-	-	-	-
ED	5	-	-	-	-	-
ED	6	-	-	-	-	-
ED	7	-	-	-	-	-
ED	8	-	-	-	-	-
LD	18	-	-	-	-	-
LD	21	-	-	-	-	-
LD	28	-	-	-	-	-
LD	9	-	-	-	-	ND
ED	13	-	-	-	-	+***
ED	19	-	-	-	-	+***
ED	27	-	-	-	-	+***
LD	10	-	-	ND	-	-

ND: Not Done, +: positive, -: negative, LD: limited disease, ED: extensive disease
*: BM was the only metastatic site
**: the percentage of malignant cells quantified by the immunostaining and present in the BM sample is indicated in brackets
***:RBS was positive in areas where MRI was not performed

RBS showed evidence of cortical bone involvement in 11 cases, 8 of which also had positive MRI. The remaining patients (nos. 13, 19 and 21) with negative MRI were shown to have bone metastases on the sternum or dorsal column, locations not studied by MRI. Four patients had positive MRI and normal RBS. Bone CT scan was performed in 2 of them and no abnormality was found at the location of MRI abnormal areas.

Figure 1.

In one (no. 2) of the 23 patients with extensive disease, BM was the only metastatic site and BM invasion was detected by cytohistological examination as well as with MoAB immunostaining and MRI. All patients with positive MRI and/or MoAb examination of the BM and negative routine examination were shown to have at least one other metastatic site at diagnosis. Thus, no patient was shifted from the "limited disease" group to the "extensive disease" group when adding these 2 new methods to the initial staging.

DISCUSSION

From this series, it appears that analysis of one BM aspirate was positive in 3 cases where biopsy was negative, whereas there was only one example of false negative cytology compared to histological examination of the BM. These results are concordant with previous publications (11) showing that BM aspiration is more effective than BM biopsy in detecting BM metastases in SCLC. On the contrary, some authors (12) claimed the superiority of histologic examination of the BM but, in most instances, these patients underwent a second and even a third biopsy located a few millimeters lateral to the first. This is in accordance with previous reports on many neoplastic diseases including SCLC (13) which showed that the number of

positive BM aspirates and biopsies increases with bilateral examination of the BM. The rate of detection of BM involvement is clearly related to the total amount of BM tissue analyzed, and to the specificity of the method used for detection: the percentage of BM involvement in SCLC patients with negative BM biopsy is 8-11% with the use of in vitro semi-solid culture techniques (14) and 22% with discontinuous gradient sedimentation methods (15).

Our study shows that all patients with cytohistologic evidence of BM involvement have positive MRI and MoAB immunostaining. Thus, there are no false negatives with MRI and immunological detection compared to routine procedures. In 7 out of the 20 patients with negative cytology and biopsy, MRI and/or MoAB were shown to be positive.

Immunological analysis is a highly sensitive method and allows detection of malignant cells in roughly 50% SCLC patients using MoABS directed against SCLC cells surface antigens like SM1 (16) or MOC (Postmus, personal communication). In this study, the concentration of malignant cells by Ficoll separation, the analysis of a very large number of cells and adjunction of objective criteria of malignancy such as double membrane immunostaining with 2 different markers improves the level of detection to 10^{-5} and to identify as malignant, cells with cytological lymphoid-like features. MoAB immunostaining allows a semi-quantitative evaluation of the percentage of malignant cells, and it is to be noted that in the 3 cases where it was positive while routine procedures were negative, the contamination in the aspirate was low: less than or equal to 10^{-3}.

Of the 6 patients with positive MRI and negative cytology and biopsies, 2 showed positive immunostaining of the BM involvement leukaemias, lymphomas, neuroblastomas and SCLC. Furthermore, patterns of positivity (multiple focal areas of hyposignals) tend to confirm the focal nature of BM involvement and rather easily explains the negativity of methods based on the examination of one unique aspiration or biopsy site, including immunological analysis. MRI has the advantage of being painless, being able to explore a wide part of the body and being particularly able to detect very focal involvement. There is a strong need for the demonstration of BM invasion by malignant cells in the precise sites of hyposignals on MRI, considered as positive in patients with normal BM aspiration and biopsy in one posterior iliac crest.

RBS is known to be a highly sensitive but very unspecific test in SCLC. In our study, all RBS positive patients also had a positive MRI except for the 3 cases where bone metastases were detected outside the MRI field of view. In contrast, in 4 patients with positive MRI, RBS was considered to be negative. MRI focal areas of low intensity are located in the fat tissue, that is, in BM itself. In contrast, RBS abnormalities reflect cortical bone involvement. Therefore, no comparison can be made between these 2 methods in terms of

sensitivity and specificity. However, the lack of bone metastases detected with RBS and CT bone scan in MRI positive areas might reflect different patterns of BM and cortical bone involvement in SCLC.

The real necessity of BM sampling in SCLC was recently questioned by some authors (17). According to our preliminary results, routine cytohistological examination of the BM might fail to detect BM involvement in 35% (7/20) patients with SCLC and BM could be a very frequent site of detectable malignant cells in SCLC using MRI and MoAB immunostaining. In our opinion, this finding in a disease where the presence and number of metastatic sites is known to be the more important prognostic factor, is a very strong argument in favor of completing routine examination of the BM by the use of these 2 complementary and non-aggressive methods. Furthermore, if our future results confirm the superiority of cytological and immunological examination of one BM aspiration compared to a good quality biopsy, we could thing of abandoning the latter which is considered as too much aggressive by some authors (17).

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RECENT PROGRESS IN THE DETECTION OF METASTATIC TUMOR IN BONE MARROW BY CULTURE TECHNIQUES

J. Graham Sharp, James Armitage, David Crouse,
Shantaram Joshi, Anne Kessinger, Sally Mann,
William Vaughan, and Dennis Weisenburger

We have previously described the application of culture techniques which permit the detection of occult tumor cells when applied to bone marrow harvests of candidates for autologous marrow transplantation (1,2). In this report, we update our findings regarding the detection of metastatic tumor in the bone marrow of patients with non-Hodgkin's lymphoma (NHL) and breast cancer. We also describe a new approach which permits the detection of abnormal cells suspected to be Reed-Sternberg cells in the bone marrow of patients with Hodgkin's disease (HD).

METHODS

The screens used to filter marrow harvests were used as the source of cells established in long term marrow culture (LTMC). The cells were cultured as previously described for NHL (1) and breast cancer (2). Bone marrow cells from patients with HD were cultured in a medium described by Douay et al. (3).

Cytospin preparations were made from the cultures at regular intervals, blind coded, intermixed with similar preparations from normal donor cultures and examined by the pathologist. Some samples were examined further with special stains or by molecular biologic techniques as described previously (1,2).

RESULTS AND DISCUSSION
Non-Hodgkin's Lymphoma

The initial criterion for suspecting the presence of lymphoma cells in cultured marrow harvests was the long term growth (greater than four weeks) of lymphoid cells with abnormal morphology in liquid cultures. Representative cultures containing such cells were harvested and subjected to additional analyses, including molecular probing for monoclonality of T or B cell receptor gene rearrangements, flow cytometry and fluorescence staining for Epstein-Barr Virus (EBV) associated antigens.

Approximately 50% of bone marrow samples from patients with NHL exhibited long term growth in culture and the production of cells with abnormal lymphoid morphology. However, molecular studies confirmed a monoclonal gene rearrangement characteristic of the primary tumor in only 40% of these cultures, resulting in a potential overall positivity of about 20% of bone marrow samples (8 of 35 evaluated). The isolation of adequate amounts of DNA from relatively small numbers of cultured cells ($2 - 10 \times 10^5$) is demanding and requires careful attention to procedural detail (4). The nature of the cells in the remainder of the cultures is not entirely clear. Some may have been EBV infected lymphoblasts and others normal cells that expanded polyclonally in the presence of lymphokines in the culture medium. In order to counter these problems, samples are now examined for both monoclonality and EBV by molecular probing techniques.

The overall survival of NHL patients whose bone marrow contained occult tumor cells detected by the culture technique and confirmed by molecular probing was similar to that of the entire group of patients whose marrow continued to produce cells for greater than four weeks in culture. Surprisingly, the survival of these patients is better than that of patients whose cells did not grow long term, but the number of patients studied to date is still too small for adequate statistical analysis. These observations suggest that the presence of occult lymphoma cells in the marrow may be less important with regard to prognosis than the long term growth of the marrow cells in culture.

Breast Cancer

Our preliminary results of the detection of suspected breast cancer cells in bone marrow harvests were reported previously (2). An update of the current series of patients is presented in Table 1. It should be noted that this group consists of poor-prognosis patients based on the clinical history and/or pathologic findings. The proportion of patients with stage I to III disease whose marrow harvest contained

Table 1. Detection of Occult Tumor Cells in Bone Marrow Harvests from Poor Prognosis Breast Cancer Patients

Stage	Number of Patients	Biopsy Age Range (median)	Culture Positive at Harvest	Number Positive/Evaluated (Percent)	
I	2	38-43 (40)	0	1 / 2	(50%)
II	8	32-50 (39)	0	6 / 7	(86%)
III	10	29-59 (41)	0	6 / 9	(67%)
IV	15	31-57 (44)	4	7 / 14	(50%)
TOTAL	35	29-59 (41)	4	20/ 32	(63%)

unsuspected tumor cells is surprisingly high (13 of 18 evaluated). However, other recent studies involving extensive pathological analysis have also detected high frequencies of tumor cells in the bone marrow of Stage II and III patients (5-7). It appears that the bone marrow is a frequent and early site of breast cancer metastasis.

Clinically, the follow-up on these patients is short. With a maximum follow-up of 1000 days, there is no difference in the survival of patients whose cultured marrow grew abnormal cells and those whose marrow did not. However, the relapse rate of the former group is significantly higher than that of the latter group (47% versus 9% respectively; $p \leq .04$, Fisher's exact test). However, most patients relapsed at sites other than the marrow where there was prior evidence of disease. Nonetheless, this association suggests that long term culture techniques may be useful for identifying patients who would benefit from additional therapy.

Hodgkin's Disease

When bone marrow from patients with HD is cultured according to the method for other bone marrow samples, the cultures resemble normal bone marrow in culture. Morphologically-abnormal cells have not been detected in such cultures (Table 2). When a method for maintaining peripheral blood stem cells in culture was developed (8), however, an attempt was made to culture marrow from patients with HD using a similar technique. Abnormal cells which resemble Reed-Sternberg cells have been detected in bone marrow cultures from 3 of 17 patients (Table 2). Although we have not yet been able to establish by immuno-cytochemical or other means that these are Reed-Sternberg cells, they have not been observed in cultures from NHL patients or normal individuals.

It is currently difficult to confirm the malignant nature of individual or small numbers of presumed tumor cells grown in culture, particularly for breast cancer and HD. Cytogenetic studies

Table 2. Detection of Morphologically Abnormal Cells Resembling Reed-Sternberg Cells[a] in Bone Marrow Harvests of Normal Donors and Patients with Hodgkin's Disease

Population Examined[b]	Culture Medium[c,d]	Number	Biopsy Positive at Harvest	Abnormal/ Total (Percent)		
ND	RF10H10 HC/2ME	13	0	0 /	13	(0%)
HD	RF10H10 HC/2ME	12	0	0 /	12	(0%)
HD	IMDM/F12.5 H12.5/MI/FA HC/2ME	17	0	3 /	17	(18%)

a. Although these cells morphologically resemble Reed-Sternberg cells, currently we have no proof of their lineage.
b. ND = normal donor; HD= Hodgkin's disease.
c. RF10H10/HC/2ME = RPMI 1640 with 10% fetal calf serum, 10% horse serum, hydrocortisone and 2-mercaptoethanol.
d. IDMEM/F12.5/H12.5/MI/FA/HC/2ME = as described in the methods.

of cultured marrow containing small numbers of such cells from 12 patients have not yielded significant numbers of consistently abnormal metaphases. Molecular and/or immunocyto-chemical techniques using carefully selected probes need to be developed and applied to such samples in the future.

CONCLUSIONS

Our findings indicate that the application of culture techniques to harvested bone marrow permits the detection of occult tumor cells. It appears that these techniques represent a very sensitive method of detecting small numbers of abnormal cells of several different types. The ultimate clinical utility of these culture techniques is still uncertain because of the short period of clinical follow-up. In patients with NHL, the presence of small numbers of occult tumor cells in the bone marrow does not appear to be of major clinical significance. The most important clinical application of these sensitive culture techniques may be in breast cancer, where the information could be useful in selecting patients who would benefit from additional therapy.

ACKNOWLEDGMENTS

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BREAST TUMOR CELL DETECTION IN MARROW

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Gabriel Hortobagyi, Christopher Reading, Karel Dicke,
and Gary Spitzer

INTRODUCTION

Micrometastatic infiltration of the bone marrow of breast cancer patients has been linked to an early recurrence and a poor prognosis for the disease (1, 2).

Monoclonal cytokeratin antibodies have previously been employed in the detection of micrometastatic disease in bone marrow because of the universal presence of cytokeratins in epithelial tumors (3-5). Also, they have shown no reactivity with marrow cells (6-8). However, due to the heterogenous expression of cytokeratins in epithelial tumors, cytokeratin antibodies could be less than optimal in the detection of minimal marrow involvement (9, 10).

In an attempt to overcome this heterogeneity, we have developed and tested a panel of monoclonal antibodies to supplement the cytokeratin antibodies.

MATERIALS AND METHODS

Breast Carcinoma

Fresh breast cancer specimens were collected from 14 breast cancer patients, immediately embedded in OCT medium, and snap frozen in isopentane. Specimens were cut into 4-μm sections, air dried, fixed in cold acetone, and stored at -20°C.

Bone Marrow

Fresh bone marrow was obtained and anticoagulated with 100 IU/ml of preservative-free heparin. From the buffy coat, the mononuclear cells were separated by centrifugation on Ficoll-Hypaque. Next, the erythrocytes were lysed. Cytospin preparations were made with 10^5 cells per slide, fixed in cold acetone, and stored at 4°C.

Avidin-Biotin-Peroxidase

Sections were then incubated for 15 minutes with 0.3% hydrogen peroxide in methanol. The slides were then incubated with 1% normal horse serum for 20 minutes, the primary antibody for 1 hour, biotinylated secondary antibody for 45 minutes, and the ABC Kit (Vector Laboratories Inc., Burlingame, CA) for 30 minutes. The slides were washed in PBS before each incubation. The reaction was developed in a chromogen substrate and counterstained with Mayer's hematoxylin. The positive control was red cytoplasmic staining on a known positive tissue. The negative control consisted of replacing the primary antibody with normal mouse immunoglobulin.

Alkaline Phosphatase

The slides were incubated with 1% normal horse serum for 20 minutes, primary antibody for 30-60 minutes, biotinylated secondary antibody for 20-40 minutes, the ABC Kit for 15-30 minutes, and the substrate, with levamisol, for 15-30 minutes, and then counterstained with Harris's hematoxylin. The slides were washed with PBS with 1% BSA before each incubation. The positive control was the breast cancer cell line T47D.

PROCEDURES AND EXPERIMENTS

In order to determine the reactivity of anticytokeratins, 14 breast carcinoma sections were tested with AE1 and AE3 by the immunoperoxidase methods. Nine cases (64.3%) showed high positivity, which ranged from 85% to 98% positive cells, and 5 cases (35.7%) showed only weak or moderate positivity, ranging from 10% to 60% positive cells. Therefore, we decided that a panel of monoclonal antibodies was needed.

In order to confirm negativity for marrow cells, mononuclear cells from 6 normal donors and 10 with hematological malignancies were tested with the 9 monoclonal antibodies. The alkaline phosphatase method was used instead of immunoperoxidase to eliminate background staining. All nine were negative.

Next, all nine antibodies were tested on the five specimens that had previously shown low or moderate reactivity with anticytokeratins. The percentage of positive cells ranged from 5% to 98%. However, only the monoclonal antibodies 113F1, 260F9, and 317G5 showed consistently high percentages and were chosen for the cocktail.

In order to test the reactivity of the cocktail versus cytokeratin antibodies alone, five cases were stained with the cocktail and with the anticytokeratins alone. The anticytokeratins stained 95% to 100% of the cells, but the cocktail was able to stain 100% in each case and was judged to be superior.

In order to determine the sensitivity of this technique, T47D tumor cells were mixed with marrow cells in ratios from 1:10 to $1:10^6$. Using our method, 1 tumor cell in 10^5 marrow cells could be detected. In Giemsa stains, only 1 tumor cell in 10^3 marrow cells could be detected. Thus, our method is 2 logs more sensitive than the standard Giemsa method.

At present, marrow samples have been collected from 71 stage IV patients, representing a variety of epithelial tumors. Sixty-nine have been evaluated, and of this number, 44.9% (31/69) were positive. Results for each tumor type are given in Table 1.

DISCUSSION

Our goal has been to detect small numbers of infiltrating cancer cells without staining normal marrow cells. Although anticytokeratins alone could stain a relatively high percentage of carcinoma cells, they could not stain 100% of tumors and close to 100% of the cells within these tumors. Therefore, we feel that our cocktail is superior to cytokeratin antibodies alone and also to conventional histological methods.

Table 1. Epithelial Tumor Bone Marrow Data

Dx	Number of cases	Number Eval.	Number Positive	+%	(+No. Eval.)
Breast	63	61	27	44.3%	27/61
Ovarian	2	2	0	0.0%	0/2
Colon	1	1	1	100.0%	1/1
Lung (all)	3	3	2	66.7%	2/3
Anal	1	1	1	100.0%	1/1
Adeno.	1	1	0	0.0%	0/1
Total	**71**	**69**	**31**	**44.9%**	**31/69**

In our patient studies, we have found about 45% of patients with metastatic breast cancer to have positive marrow. However, as more patients are tested, we feel that the percentage of positive cases may be slightly higher.

At this point the biological significance of these cells is not known. Whether they are clonogenic tumor cells capable of reproducing disease elsewhere has yet to be determined.

ACKNOWLEDGMENTS

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Discussion 2 - Session IIIB

Dr. Moore: A few minutes of general questions for all the speakers in this evenings session so far. If you have a question -- if you would come to the microphone as well.

Dr. Dicke: Malcolm, I want to ask you -- your patient with the congenital neutropenia -- you mentioned that you had a persistence of neutrophils in that particular patient. Did you have to give continuous G-CSF or did you see also neutrophils after stopping the CSF?

Dr. Moore: No. I should mention, Karel, in the studies I mentioned they were pre-clinical using the canine (dog) neutropenic model. Basically, as long as the G-CSF was administered, the neutrophils were elevated. As soon as we stopped, the animals reverted to a cyclical pattern of neutrophils. I think we will all be hearing later on in this session some additional data that might relate to some of the clinical aspects. The in vivo administration of G-CSF had very profound effects on a variety of cell lineages including pluripotential stem cells. I wish I knew why. It is a cascade phenomenon, one suspects. There is an induction of other cytokines but I have no direct evidence as to why that should be.

Dr. Gorin: I would like to know if there are any indications that the administration of GM-CSF following ABMT might be harmful if GM-CSF is started on day 0 immediately with the marrow as compared for instance with day 1 or day 5 or day 7. That would be my first question. Maybe I can get the answer and then I will start with the second one.

Dr. Applebaum: We tried to assess that in a few patients. Our concern was the potential harm of giving GM-CSF too early and inducing further committed cells. Bill has his CFU-GM assays, we

have ours. His picked up some peaks in response to GM-CSF where we did not assess that in time as he did. But in giving it on day 7, compared to day 0, the day 7 did not show any enhanced engraftment of myeloid cells and, on platelets, it did not appear to be different from given from day 0 to day 20. The long-term data has to be followed in terms of stability of engraftment years later. From day 7 compared to day 0, in just 4 patients, we did not really find a difference.

Dr. Peters: There is another comment that is relevant here. In fact while I do not think there is any damage or difficulty in starting it early on, it is conceivable that GM or G or, in fact, most growth factors can induce antigens present on the surface and we can show quite clearly that you will induce mole 1, for instance. And we can then show that the cells that acquire that antigen induction will be trapped specifically in the lungs. That might lead conceivably to a deposition of stem cells within the, if it induced that way, in the pulmonary tissue and lost the marrow. In fact we do not see that happen, and you do not see evidence for any delayed engraftment or any long-term problems in the patients when we start 3 hours after the marrow went in.

Dr. Gorin: May I proceed with the second question? It is completely different. If we believe in hemopoietic stem cell competition, I would be interested in knowing if anyone tried GM-CSF in a patient with ALL -- full relapse ALL?

Dr. Bradley: I would like to just make a comment about this issue of competition. Because it goes so much against everything that experimental hematology is evolved before we started putting these agents into humans. We already heard -- I think Dr. Neumanitis speculated that the reduction in CFU-GM might have been because of differentiation pressure -- (that) there has been no evidence of any instructional role of any of these hematopoietic growth factors. They are permissive for differentiation events. So I really do not see that we can exhaust or preempt populations of cells into one lineage vs. another. If somebody can give me some credible information from their clinical trials to the contrary, I could give them a lot of very credible information from experimental studies in other animal systems that would contradict them. I will throw that open, if anyone feels that that is still an issue.

Dr. Gorin: Dr. Peters, while you are still there, could I ask you one question about the neutrophil migration inhibition? What is the reversibility of that? In other words, do you give continuous GM-CSF? (If so, would) you stop neutrophil migration if you give intermittent GM-CSF? Is there any time scale on that?

Dr. Peters: We were naive enough to believe that, in fact, if you administer it as a pulse you might be able to get around the problem. Bring it in, get it out, bring it in, get it out. It does not work that way. No matter whether they are receiving it as a pulse on a 4- hour basis or continuously, the migration is inhibited. There is a marked difference in the recovery of the ability to migrate following cessation of GM-CSF in which the pulse administration appears to lead to a much more rapid recovery of normal granulocyte migration compared to people receiving continuous infusion.

Dr. Spitzer: It appears in looking at all these studies that granulocyte recoveries are equivalent in the transplant situation with all the molecules, but I would like Bill to discuss why they have a discrepancy and also Tony Goldstein that 2 GM studies say that there is no difference in platelet recovery in the transplant situation.

Dr. Peters: I just think the best thing to do is really to wait for the prospective trials.

Dr. Neumanitis: I had a question for Dr. Monroy. The doses that you used of IL-1 for the normal monkeys were .5 ug/kg, where you found optimal effects. And, then, with the transplant monkeys you used 5 ug/kg. Why did you use a log scale higher?

Dr. Monroy: The optimal effect on the platelets were at 1ug/kg/day. We went up to 10, between 5 for the IL-1 beta and 10ug because those were based upon the original murine data for improving engraftment. That is where we started on those. We just need to go back to the lower doses because future studies are going to show the lower doses of IL-1 are much better.

Dr. Moore: If there are no further questions ... are there? I just like to make one little comment. I have not heard today, anywhere, anybody discuss possible inhibitors. I am sure that they may be relevant to the transplant situation in post-cytotoxic treatment. Anyway, thank you very much. Good night.

SESSION III - SOLID TUMORS

C. GLIOMAS

HIGH DOSE BCNU AND ABMT, SURGERY AND RADIOTHERAPY IN GLIOMAS

P. Biron, F. Mornex, P. Colombat, C. Vial, F. Chauvin, M. Veyssere, I. Philip, and T. Philip

INTRODUCTION

High grade gliomas of the brain (anaplastic astrocytoma and glioblastoma multiform) represent the most frequent primary central nervous system (CNS) tumor. Despite conventional treatment including surgical resection, post operative irradiation and adjuvant chemotherapy, the prognosis has remained very poor (1). Post-operative irradiation has significantly increased the overall survival (2). Attempts to improve these results, using adjuvant mono- or polychemotherapy did not improve either the response rate or the survival (3,4,5). Lack of activity of chemotherapy in such tumors is explained not only by a low chemosensitivity but also by a low penetration of the drugs into the CNS. One way to overcome the problem of the so-called "blood brain barrier" is to use the dose effect relationship and especially high doses of chloroethyl nitrosoureas, such as BCNU (1,3-bis(2-chloroethyl)-1-nitrosourea, carmustine), which has been proven to be efficient at conventional dosage (3).

Since 1980 (6) many reports have been published on escalation of doses in gliomas (7,8,9,10). Until now results are controversial, but Johnson (11) recently published encouraging results.

The aim of the study was to evaluate the feasibility, toxicity and efficiency, judged on survival and quality of life, of a therapeutic approach combining:

1. a cytoreductive surgery

2. an early post-operative chemotherapy

3. an early intensification with high dose BCNU followed by autologous bone marrow transplantation (ABMT).

4. a whole brain irradiation followed by a local boost to the tumor bed.

The whole program is scheduled over a 2-month period with the objective to reduce hospitalization time of these poor prognostic patients and to begin radiation therapy in a conventional post-operative delay.

PATIENTS AND METHODS

Between March 1986 and June 1988, 77 adult patients entered the study. Seventy patients have been treated in Centre Leon Berard and 7 in CHR Tours. Patients were age 16 to 64 with a median of 49 years. Thirty-eight were male and 39 female. Seventy-one percent had a good performance status (21 grade 0 and 34 grade 1 of the WHO scale) and 29% had a bad one (11 grade 2, 7 grade 3 and 4 grade 4). Two patients had not undergone surgery and diagnosis was established on arteriography and CT scan criteria. Among the others, 16 had a grade III astrocytoma and 58 a grade IV astrocytoma or a glioblastoma. One patient had a grade I astrocytoma in relapse. Seven patients were treated at time of relapse and 70 patients were treated in initial phase of the disease as a first line treatment.

According to the feasibility of the surgery based on the size of the tumor and its location in the brain, 2 strategies have been used called arm 1 and arm 2 (Table 1):

Arm 1

o Cyto-reductive surgery as wide as possible on day 0.

o Early post-operative chemotherapy on day 3 after histology confirmation, combining:

o VM26, 150 mg/m^2, diluted in 500 ml of 5% dextrose over an 8-hour infusion.

o BCNU, 100 mg/m^2 in a 5 mn IV short infusion, through a peripheral venous access.

o PROCARBAZINE (day 3 to 10), 100 mg/m^2/day, per os.

The bone marrow harvesting was scheduled between day 21 to 24, as previously reported (12). 1×10^8 mononuclear cells/kg were harvested.

Table 1. Gliomas = Arm 1; Gliomas = Arm 2

A_1 GLIOMAS = ARM 1

D 0	Surgery	
D 3	VM 26:	$150\,mg/m^2$
	BCNU:	$100\,mg/m^2$
D 3 to 10	Procarbazine:	$100\,mg/m^2$
D 21	Marrow Harvesting High Dose BCNU:	$800\,mg/m^2$
D 24	Marrow Infusion	
D 45 to 63	Radiotherapy	45 Gy

B_1 GLIOMAS = ARM 2

D -10	Biopsy	
D 1	Marrow Harvesting High Dose BCNU:	$800\,mg/m^2$
D 4	Marrow Infusion	
D 30	Surgery if Indicated	
D 33	VM 26	$150\,mg/m^2$
	BCNU	$100\,mg/m^2$
	Procarbazine	$100\,mg/m^2$
D 45 to 63	Radiotherapy	45 Gy

A buffy coat was performed to reduce the volume to 100-150 ml, then mixed with 4% albumin in a 600 ml Travenol bag (Travenol Laboratories, Norfolk, England) and stored at approximately 6°C. On the afternoon of marrow harvest, the high dose chemotherapy (BCNU: $800\,mg/m^2$) was administered diluted in 100 ml of 5% dextrose over a 2-hour infusion, using a peripheral venous access. BCNU was processed by Mannitol infusion 250 cc and followed by methyl prednisolone $80\,mg/m^2$ x 3 days. The marrow was centrifuged at 1,000 g for 25 minutes, resuspended in 200 ml saline with 4% albumin, and re-infused at body temperature 72 hours after BCNU

administration. The patient was discharged from the hospital on the following day.

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 sustentorial brain, followed by a localized boast of 21 Gy in 7 fractions to the tumor or tumor bed. Every radiotherapy field was designed using the dosimetry CT scan planning system.

Arm 2

If initial surgery was not indicated, or at relapse, a biopsy alone was performed when possible, then patients received directly high dose BCNU, followed by surgery 4 weeks later. They were planned to receive then the early post-operative chemotherapy (VM26, BCNU, PROCARBAZINE) and finally were irradiated in the same delay as in arm 1.

Patients were treated in single rooms, without isolation procedures. The 5 initial patients were hospitalized during the whole post-ABMT period, but quickly supervision was organized on an out-patient basis with an one weekly blood count, hepatic and renal biology, physical staging and chest X-ray.

Evaluation of response in gliomas remains very difficult since the only way to monitor these patients is clinical staging and CT scan data. In this study, the overall survival which allowed a rapid answer due to the poor prognosis of these patients was chosen as the main evaluation criteria. The measure of the quality of life of these patients was also a major objective of the study.

RESULTS

At the moment, 77 patients have been treated: 54 in arm 1, 16 in arm 2 (including 2 patients without surgery) and 7 patients at time of relapse. For patients in arm 1, surgery consisted of a macroscopically complete resection in 24 patients, partial resection in 12 patients and biopsy in 18 patients. For patients in arm 2, surgery consisted of a macroscopically complete resection in 11 patients, partial resection in 1 patient and biopsy in 1 patient. Three patients could not undergo surgery: 2 because of location of the tumor in a high function site of the brain and one because he died of progression one month after ABMT. Only 4 patients underwent surgery after initial biopsy and BCNU.

Early post-operative chemotherapy was difficult to manage due to the number of surgery departments involved and only 15 patients have received this course. Only 10 courses were correctly done (66%). The mean delay after surgery was 5 days and it was finally decided to stop this chemotherapy in March 1987.

Toxicity

Marrow harvesting was easy any time and a mean of 585 cc of marrow blood containing 4×10^8/kg nucleated cells and 1.6×10^4/kg GMCFUC could be collected. But at the beginning several patients (4) presented with decreased consciousness or enhanced neurologic impairment and cranial hyperpressure syndrome. This was supposed to be related to hydration administered during the general anesthesia and harvesting procedure. This problem disappeared when furosemide was systematically added at the end of the anesthesia. Concerning marrow re-injection, in 10 cases aggregates occurred in the infusion line have needed resuspension of the marrow in human albumin.

During the reinjection of BCNU, 13 patients presented at the beginning pain at the site of injection and painkiller (oxycodene) to BCNU infusion is now always added with complete disappearance of this problem.

Immediate tolerance of BCNU was good. We observed 1 sinusal tachycardia, 3 regressive febrile episodes. Nausea and vomiting was mild: 23 grade 0, 18 grade 1, 30 grade 2 and only 6 grade 3. This good tolerance, 53% patients without vomiting, is probably partially due to the short delay between the harvesting under general anesthesia and the BCNU infusion (less than 6 hours). Hematologic toxicity is very mild and is probably one of the most interesting points of this procedure. Only 8% of the patients had a severe aplasia with neutrophil less than 500/mm^3 and 4% with WBC less than 1,000/mm^3. This neutropenia was of less than 10 days duration but fever was observed in 3 patients. We observed a grade 3 toxicity in 20%. In 72%, no neutropenia was observed. Concerning thrombopenia, platelets were less than 25,000/mm^3 in 9% only. Twenty-five percent have a grade 3 toxicity, 11% a grade 2 toxicity and 55% did not experience thrombopenia at all. WBC and PN nadir was maximum on day 28 and platelets on day 21.

The moderate hematologic toxicity of this procedure is probably due to the delayed myelotoxicity of BCNU, giving time to the preserved marrow to be efficient before the maximum toxicity of the drug.

Concerning the non-hematologic toxicity, lung complications were of great concern due to the fibrosis risk of BCNU (7). We observed 24 lung complications. Three were embolisms and 2 of them were fatal. Twenty-one were pneumonitis (27%) with 10 only partial. Two were life-threatening requiring assisted ventilation. Eight occurred in post-graft period (first month), others occurred later in the evolution and often intricated with neurologic degradation and corticosteroid treatments.

Nevertheless 3 patients (4%) after a first period of improvement presented a fibrosis and 2 died of this complication.

Thirty-five patients presented biological hepatic abnormalities: cholestasis and cytolysis. Those modifications were moderate and only one patient experience jaundice, hepatitis and perhaps veno-occlusive disease.   He presented also a pneumonitis and a severe febrile syndrome. We documented during the evolution a CMV infection and the patient died despite anti-CMV immunoglobulin treatment.

Nine patients presented a phlebitis and 3 a pulmonary embolism (already mentioned).

We observed 5 toxidermia, 2 were severe.   The most serious presented extensive cutaneous edema, erythema and desquamation associated with an impressive eosinophilia (26,000/mm^3).

In these patients, symptoms progressively disappeared when anticomitial treatment (phenytoïn) was discontinued.

Renal function remained normal in all patients but 3 with a maximum creatinine level of 352 mmoles/l.

Twenty-three febrile episodes were recorded.   Most of these occurred after the first month and are not related with the treatment program.   Only one patient had a local osseous infection requiring surgical procedure.

Concerning surgery no major problems were recorded, except thromboembolic complications, but this is probably a bias of selection, since only patients with a good performance status were referred to enter the program.   However, coma and death due to an immediate post-operative extradural hematoma was observed in a patient treated in arm 2.

Irradiation was performed as scheduled in 64 patients.   The tolerance was very 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 3 days after the end of the radiotherapy course in a sudden coma; the necropsy showed a cerebral hemorrhage when platelet counts were normal; the remaining part of the tumor was completely necrotic, and no cerebral edema was found.   This type of complication is well known, and classical in cerebral irradiation.

Finally 17 patients had a critical event in the first month after ABMT (22%) and the total toxic death rate is 9% (7 patients): 2 embolisms, 2 pneumonitis, 2 hemorrhages (1 after surgery, 1 after radiotherapy) and 1 CMV infection. We must add an accidental death in relation with the neurological status.

RESPONSE AND SURVIVAL

Evaluation of response after BCNU is difficult because of surgery. Evaluation of the whole program is also difficult in terms of tumor response.   CT scan is not able to evaluate response,seems to be efficient in showing relapse, before clinical status degradation

(enhanced contrast ratio and mass effect are observed in every single patient 2 to 3 months before clinical alterations) (13).

Analysis of survival with a median follow-up of 16 months shows a 25% overall survival at 24 months (Figure 1). Median survival is 11 months after ABMT and 12 months after surgery. The survival of the 70 patients treated in initial phase of their disease (Figure 2) is the same: median survival: 12 months after surgery. Twenty-seven percent surviving at 24 months. It is important to point out that patients treated in relapse have also a median survival of 12 months.

Median survival of the 16 patients in arm 2 is the same and 19% survive at 24 months. So far there is no difference between the two arms (Figure 3). In an intermediate analysis 9 months ago, we noted a trend for better results in patients treated with arm 2, but now the results are completely comparable.

Background for high dose BCNU before surgery is the same as neoadjuvant chemotherapy, and in the field of brain tumors Butti (14) published good results with any one course of conventional dosage BCNU immediately (6 h.) after surgery. The second aim was to improve quality of surgery in patients who had an initial contra-indication.

Among 16 patients, 13 have had a surgical procedure. It is of interest to note that only 2 had a partial resection and 11 could have a macroscopically complete resection (68%) compared to 44% in arm 1. During surgery it was noted a nodular aspect of the tumor and a more marked limit with the normal brain in 9 cases. Furthermore, tumor seemed to be less hemorrhagic in 7 cases. Concerning histopathological observations, all tumors were widely necrosed and the capillary endothelium was very thick. In all patients there remained viable astrocytoma tumor cells: 12 grade IV or glioblastomas (92%) and 1 grade III. Four patients had a biopsy before chemotherapy and a resection after BCNU. No difference in the histopathological grading was found. All had residual viable tumor cells and the major modifications due to the high dose of BCNU seem to concern first capillary endothelium which become thicker with a sclerohyalin aspect and necrosis which seems to be wider. Those observations need of course to be confirmed in a greater number of patients.

Even with results identical to arm 1, this neoadjuvant chemotherapy (arm 2) remains valuable for patients who had an initial contra-indication. Nevertheless many problems arise in such an approach: initial diagnosis of glioblastoma is known before CT in only 4 patients, even if it is confirmed for all at time of surgery. Initial biopsy must be performed which can furthermore allow one to evaluate histo-pathological response to chemotherapy. Secondly histo-pathological diagnosis is perhaps modified by chemotherapy even if in 4 evaluable patients there is no difference in the staging.

Finally surgery is probably easier to perform after chemotherapy but there is not yet a definitive answer to this question.

Figure 1. Gliomas: HD BCNU - Overall survival after ABMT

Figure 2. Gliomas: HD BCNU - Survival Arm 1 and Arm 2
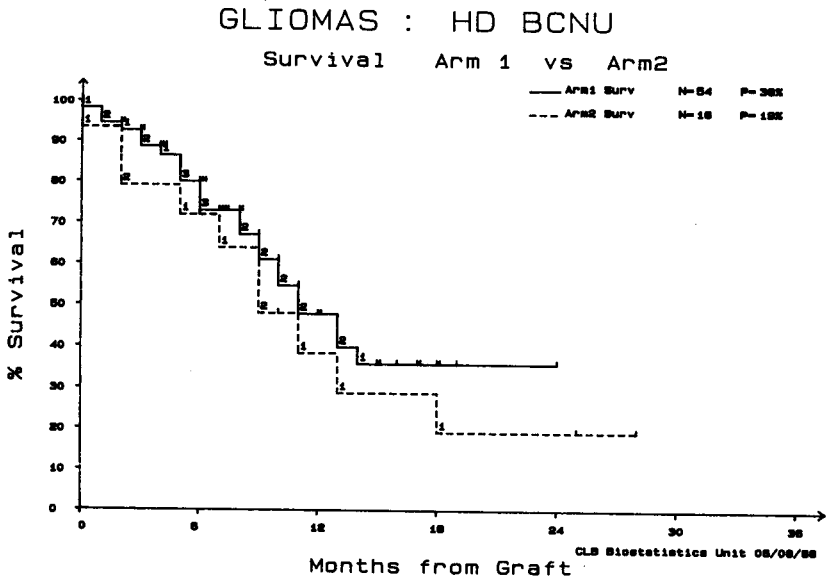
Figure 3. Gliomas: HD BCNU - Survival Arm 1 vs Arm 2
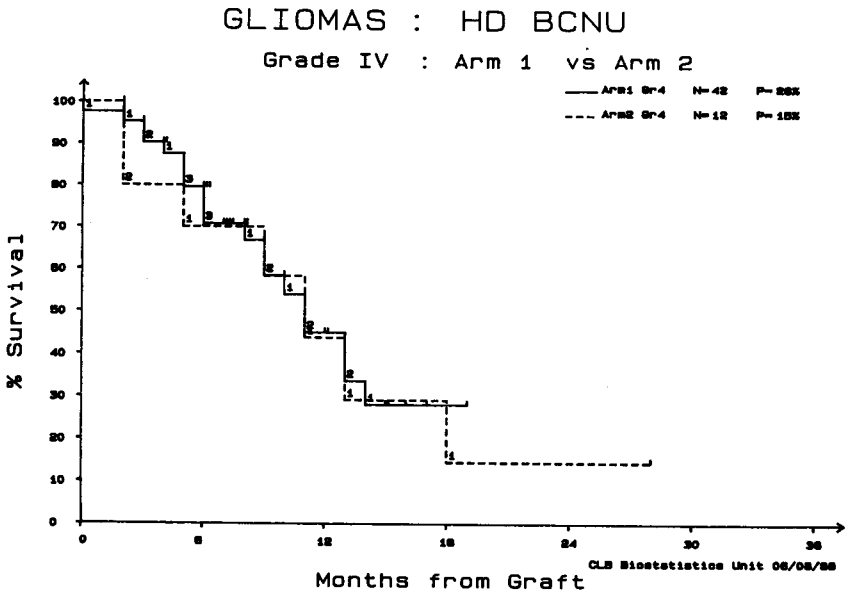
Figure 4. Gliomas grade IV: HD BCNU - Survival Arm 1 vs Arm 2

Histological staging is always of great importance in brain tumor survival. We have analyzed survival of our 54 patients with grade IV or glioblastomas treated in the initial phase of their disease (Figure 4). Median survival is still the same: 11 months after ABMT and 12 months after surgery. Nineteen percent survive at 24 months. There is no difference again between arms 1 and 2.

In gliomas, quality of life is of major concern for the patients, due to the poor prognostic and their neurologic impairment. In an attempt to evaluate the quality of life we have analyzed the performance status (PS) during the first year (Table 2). Most of the patients remained semi-ambulatory until the 9th month. A major degradation of their PS occurs at the end of the first year. Patients fully ambulatory (PS:0 or 1) begin to decrease their activity during the 9th month, and after 12 months only 17% of the patients remain ambulatory. We can assess that for the majority of the patients, the quality of life remains good hardly during one year.

CONCLUSION

In conclusion, this study shows that high dose BCNU can be integrated in the treatment of high grade gliomas. Its toxicity is low and the toxic death rate of the whole program is less than 10%. Feasibility is good. Hospitalization time required by this treatment schedule is short, and after BCNU, patients can be monitored as out patients. Results of the whole procedure remain poor in terms of survival but quality of life is acceptable for such a disease at least

Table 2. Quality of Life – Evaluation of Performance Status

Time of ABMT	Patients Strictly Ambulatory PS= 0 or 1	Patients Semi Ambulatory PS= 0, 1, or 2
3rd month	71%	85%
6th month	57%	73%
9th month	44%	68%
12th month	17%	17%

during the first year, but it is too early to mention potential long term survivors. This technique allow us to consider chemotherapy intensification either by a double graft program or by adding other drugs.

ACKNOWLEDGMENTS

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HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS MARROW RESCUE IN CHILDREN WITH RECURRENT BRAIN TUMORS

Jonathan Finlay, Charles August, Roger Packer,
Robert Zimmerman, Leslie Sutton, James Nachman,
Allan Levin, Patrick Turski, Richard Steeves,
Walter Longo, and Manucher Javid

Brain tumors constitute almost 25% of childhood cancers, second only to the acute leukemias. Although nearly half reflect low-grade astrocytic tumors, the majority are high-grade tumors which have fared poorly with conventional therapeutic approaches of surgery and irradiation. The outlook for children with highgrade astrocytomas (anaplastic astrocytoma and glioblastoma multiforme, (GBM)) is hardly better than for their adult counterparts. Although a Children's Cancer Study Group study has indicated benefit for newly-diagnosed children with high-grade astrocytomas who received adjuvant chemotherapy compared with irradiation alone, it was apparent that children with minimally resected tumors all fared badly (1). Children with recurrent high-grade brain tumors rarely are cured with conventional therapeutic modalities.

We have initiated a pilot study of high-dose chemotherapy with autologous bone marrow "rescue". Because of reported serious neurologic and pulmonary toxicities with high-dose nitrosoureas (2), and since we anticipated most eligible patients will have had significant prior exposure to nitrosoureas, we elected to evaluate thiotepa and etoposide (VP-16). These drugs were chosen based upon pharmacologic data indicating excellent penetration into CSF and/or brain tissue following intravenous administration (3,4,5) as well as upon phase I data reported in gliomas and/or other solid tumors (2,6).

Table 1. Patient Characteristics

Pt No.	Age/Sex	Tumor Type	Location	Prior Surgery	Prior Irradiation	Prior Chemotherapy
1	11/F	Glioblastoma	Temporal	Resection x 3	5600 cGy-Local	"8-in-1" x 11 cycles
2	11/M	Malignant Germ Cell	Pineal Leptomeningeal	Resection x 3	Cranio-Spinal	Velban/Cisplat/BLEO x 6 cycles VCR/Actino/Cytoxan x 3 cycles
3	5/M	Malignant Ependymoma	Cerebellum Spinal Cord	Resection x 3	5100 cGy-Local	"8-in-1" x 11 cycles
4	21/M	Malignant Melanoma	Temporal	Resection x 1	None	None
5	13/M	Glioblastoma	Parietal Cerebellum	Resection x 2	5600 cGy-Local	"8-in-1" x 6 cycles
6	14/F	Glioblastoma	Temporal	Resection x 2	4000 cGy Whole Brain 5600 cGy-Local	"8-in-1" x 3 cycles VCR/CCNU/PROCARB x 4 cycles
7	7/M	Glioblastoma	Cerebellum	Resection x 2	5600 cGy-Local	None

Yrs = Years, M = Male, F = Female, VCR = Vincristine, PROCARB = Procarbazine, Velban = Vinblastine, BLEO = Bleomycin, Cisplat = Cisplat, Actino = Actinomycin D, Cytoxan = Cyclophosphamide, "8-in-1" Regimen: Vincristine, Cisplatin, CCNU, Procarbazine, Hydroxyurea, Cytosine arabinoside, methylprednisolone, cyclophosphamide or DTIC.

PATIENTS AND METHODS

Patients were treated between April 1986 and July 1988; four at the University of Wisconsin-Madison, one at the University Chicago Medical Center, and two at the Children's Hospital of Philadelphia. The patients' characteristics and prior therapies are detailed on Table 1.

Surgery Prior to High-Dose Chemotherapy

The protocol in use encourages aggressive de-bulking surgery, as permitted by preservation of neurologic function, prior to the therapy. This was attempted in all seven patients. However, no patient was rendered grossly free of all measureable disease.

Irradiation (XRT) in Conjunction With ABMT Regimen

Because of our desire to evaluate the toxicity of the combination of XRT with ABMT, the protocol initially allowed XRT delivered shortly after marrow re-infusion, to sites of gross residual tumor either previously unirradiated or else for which re-irradiation was deemed appropriate. Patient 1 received 120 cGy twice daily to a total of 2160 cGy involved field (prior XRT of 5600 cGy same local field 5 years earlier). Patient 3 received 5100 cGy to previously unirradiated spinal cord metastasis.

Bone Marrow Harvesting, Storage and Re-Infusion

Patients 1-4 had their marrow stored without cryopreservation at 4°C for approximately 96 hours prior to re-infusion. The bone marrow doses re-infused were between 2.9 and 4.1 x 108 nucleated cells per kg. body weight. Patients 5-7 had their marrows cryopreserved in DMSO, and subsequently rapidly thawed and reinfused, approximately 48 hours after completion of ablative chemotherapy.

Chemotherapy Regimens

The chemotherapy regimens used in each patient are detailed in Table 2. The rationale for giving VP-16 in Patient 1 over the 2nd 24 hour period was to determine if thiotepa clearance was altered by concomitant administration with VP-16; this was not the case and thus thereafter, VP-16 was given after the first dose of thiotepa. Subsequently, the protocol was modified to give VP-16 daily on each of the three days following thiotepa. Intra-Ommaya thiotepa was abandoned based upon data indicating relatively poor CSF distribution after intra-ventricular compared with intravenous administration (3).

Table 2. Chemotherapy Regimen

Pt	IV Thiotepa 3 Hr. Infusion	IT Thiotepa (Ommaya)	IV Etoposide (VP-16)
1	300mg/M^2/d x 2 d	none	1500mg/M^2 over 15 hrs, d 2-3
2	300mg/M^2/d x 2 d	10mg/d x 1	1500mg/M^2 over 15 hrs, d 1-2
3	300mg/M^2/d x 3 d	10mg/d x 3	1500mg/M^2 over 15 hrs, d 1-2
4	300mg/M^2/d x 3 d	none	1500mg/M^2 over 15 hrs, d 1-2
5	300mg/M^2/d x 3 d	none	500mg/M^2/d x 3 d
6	300mg/M^2/d x 3 d	none	500mg/M^2/d x 3 d
7	300mg/M^2/d x 3 d	none	500mg/M^2/d x 3 d

IV = Intravenous, IT = Intrathecal

Evaluation of Response

Head CAT scans with and without contrast were performed prior to administration of ablative therapy and at 1-2 week intervals thereafter. Partial responses reflected complete elimination of contrast-enhancement in tumor areas, and greater than 50% shrinkage of tumor size, with the patient on stable or tapering steroid dose. Complete responses reflected complete elimination of contrast-enhancing areas and of all tumor mass.

RESULTS

Response

Tumor responses are detailed on Table 3. Patients 1 and 3 had concomitant XRT so their responses to ablative chemotherapy alone cannot be assessed. Patient 2 had a reduction in CSF B-HCG from 4976 IU/L pre-chemotherapy to 55.9 IU/L by day 53 post-marrow re-infusion. Patient 4 showed complete elimination of all contrast-enhancement on CT, as well as clinical shrinkage of palpable nodes and chest x-ray shrinkage of lung metastases. Patient 5 showed complete disappearance of a posterior fossa metastasis and diminution of supratentorial CT enhancement by day 28. Patient 6 showed complete elimination of posterior fossa contrast enhancement and minimal tumor mass. Patient 7 showed greater than 70% reduction in tumor size, and complete elimination of contrast enhancement by day 9 following initiation of chemotherapy; by day 28, it was unclear by CT or MRI whether any residual viable tumor remained.

Table 3. Tumor Responses

Pt	CT Scan	Clinical	Duration of Response
1	PR	Stable	PD - Day 272
2	PR	Improved; CSF ↓ B-HCG	PD - Day 81
3	NE	Improved, MRI Spine	PD - Day 115
4	PR	Nodes ↓ Lung Metastatis ↓	PD - Day 83
5	PR/CR	? ↓ CNS Function	PD - Day 61
6	CR	Stable	NED - >Day 54
7	PR/?CR	Improved	NED - >Day 34

PR = Partial Response, NE = Not Evaluable, CR = Complete Response, Pulmonary Metastasis, CSF = Cerebrospinal fluid, CNS = Central Nervous System, NED = No Evidence of Disease, PD = Disease Progression

Toxicity

Toxicities are shown on Table 4. No life-threatening infections developed, despite ANCs less than 1OO/mm3 in all patients for at least 6 days.

All patients experienced skin pigmentation, erythema and desquamation, as anticipated. XRT did not significantly intensify the cutaneous reactions in patients 1 and 3. Oro-pharyngeal mucositis was moderately severe to severe; one patient underwent elective endotracheal intubation for 4 days. Patient 5 had prolonged mucositis compounded by oral HSV infection.

Patient 1 developed liver enzyme elevations and jaundice without synthetic dysfunction, in the face of prolonged hyperalimentation and dilantin. Liver biopsy revealed a picture compatible with acute hepatic graft-versus-host disease.

Patient 5 suffered some general decline in neurologic function of unclear etiology, characterized by weakness and lethargy. All 7 patients left hospital ambulant, except Patient 5, who was discharged home in poor state, with tumor progression.

DISCUSSION AND CONCLUSIONS

We present our early experience with thiotepa, VP-16 and ABMT in children with brain tumors recurrent, in most cases, following extensive prior therapies. Toxicities have been mainly transient and anticipated.

Table 4. Toxicity of Therapy

Pt	Day to Plats. >50,000/mm^3	Day to ANC >500/mm^3	Mucositis	Cutaneous	Hepatic
1	d + 197	d + 70	Severe: Palmar Erythema	Pigmentation BX-GVHD	Yes
2	d + 32	d + 18	Severe: PICU d.11-16	Pigmentation	None
3	·d + 34	d + 28	Severe	Pigmentation	None
4	d + 32	d + 16	Severe: Mild Diarrhoea	Pigmentation Minimal Desquammation	None
5	d + 35	d + 28	Severe: HSV Infection	Erythema	None
6	>d + 54	d + 37	Moderate	Pigmentation	None
7	d + 27	d + 18	Severe	Pigmentation Erythema Desquammation	None

BX = Biopsy, PICU = Pediatric Intensive Care Unit, HSV = Herpes Simplex Virus, GVHS = Graft Versus Host Disease

The achievement of dramatic responses, especially in the GBM patients, has been encouraging. However, responses have not been sustained, although two patients are still without progression early in follow-up.

We plan to pilot children with recurrent GBM with the addition of moderate-dose BCNU (450-600 mg/M2) following the thiotepa and etoposide. Ultimately, we intend to utilize this approach for children with newly-diagnosed GBM.

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ADJUVANT HIGH-DOSE BCNU AND AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR HIGH-GRADE MALIGNANT GLIOMAS

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M. Timothy Smith, R. A. de los Reyes, Mary B. Daly,
David Lamaster, Richard S. Leff, Richard Mercier,
and Gerald L. Messerschmidt

INTRODUCTION

The current standard therapy for high-grade gliomas consists of surgery followed by whole brain irradiation, which often uses a 'boost' to the tumor bed. The role of adjuvant standard-dose chemotherapy, given in addition to irradiation, was recently summarized based upon results from four Brain Tumor Study Group (BTSG) clinical trials (1). Chemotherapy with BCNU (1,3, bis (2-chloroethyl)-1-nitrosourea) produced a small, but statistically significant, improvement in survival. The number of patients surviving long-term (>18 months) was specifically attributed to the use of BCNU.

With data demonstrating a steep dose-response relationship for BCNU (2), several investigators treated patients with recurrent gliomas with high-dose BCNU followed by autologous bone marrow rescue (3,4,5). Among this group of poor prognosis patients, a few treated in this fashion have remained free of disease for up to 84 months. Based on these findings, our group undertook a trial combining standard post-operative irradiation followed by high-dose BCNU and autologous bone marrow transplantation in patients with newly diagnosed high-grade gliomas. Preliminary results have been previously published (6). This article provides follow-up on the initial twenty-five patients reported as well as results for an additional thirteen patients treated on this protocol.

MATERIALS & METHODS

Patients with newly diagnosed high-grade gliomas seen at Wilford Hall USAF Medical Center between September 1983 and October 1987 were eligible for this therapy. Prior treatment consisted only of the initial surgery. Tumor grade was verified to be grade III or IV as outlined by Kernohan et al (7). Bone marrow harvest, cryopreservation, and autologous bone marrow reinfusion were performed as previously reported (6). BCNU was given at a dose of 350 mg/m^2 intravenously, daily, for 3 consecutive days (total dose 1,050 mg/m^2).

The initial twenty-five patients received 60Gy whole brain irradiation. Subsequent patients received 45Gy whole brain irradiation with cone down to the tumor bed to a total dosage of 60 Gy. This change was made when the results of BTSG trial 8001 were reported showing no difference in survival in patients randomized between these two irradiation protocols (8).

Patients were followed closely after completing irradiation with a physical examination, chemistry profile, CBC, and brain CT performed every three months. The overall treatment schema is shown in Figure 1. Survival results were compared to an historical control group treated at our institution as previously described (6).

BIOPSY PROVEN GRADE III OR IV ASTROCYTOMA

↓

BONE MARROW HARVEST WITHIN 3 WEEKS OF DIAGNOSIS

↓

BCNU 350mg/m^2 q d x3

↓

REST FOR 3 DAYS

↓

MARROW REINFUSED

↓

WHOLE BRAIN IRRADIATION 60 gy

↓

FOLLOW UP 3 MONTHS
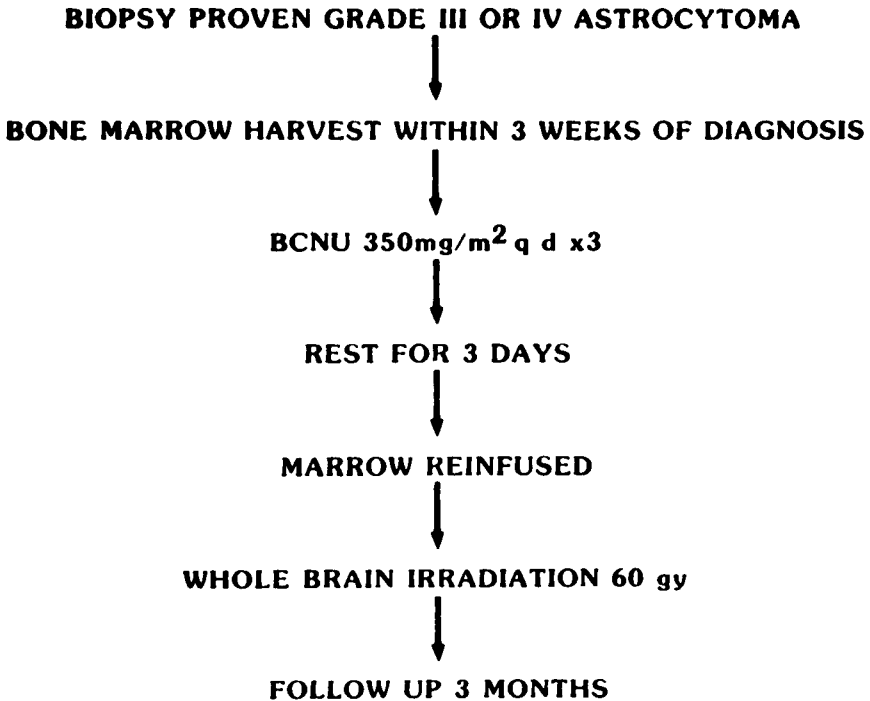
Figure 1. Overall Treatment Schema

RESULTS

Thirty-eight patients, 26 males and 12 females, received adjuvant high-dose BCNU therapy. Patient age ranged from 16-71 years, with a median of 47 years. Histopathologic review revealed 21 grade IV and 17 grade III gliomas. Re-examination of significant prognostic variables following the addition of thirteen patients to the previously reported study group failed to reveal any significant differences between the entire 38 patient study group cohort and our 52 patient historical control group (6).

Treatment related toxicity is summarized in Figure 2. Hematologic toxicity remained acceptable with a median of eight days with absolute neutrophil count of less than 500/M1, and four days with a platelet count of less than 20,000/M1. Four patients had serious infections. Three patients experienced early death due to the cause listed.

TOXICITY

INFECTION

26 PATIENTS HAD FEVER + NEUTROPENIA REQUIRiNG ANTIBiOTICS

12 PATIENTS DID NOT REQUIRE ANTIBiOTICS

9 PATIENTS NEVER DEVELOPED NEUTROPENIA

SERIOUS INFECTIONS

2 - PNEUMOCYSTIS CARINII

1 - FUNGAL PNEUMONIA

1 - BACTERIAL SEPSIS

EARLY DEATHS

1 - BCNU RELATED INTERSTITIAL PNEUMONITIS

2 - VENO-OCCLUSIVE DISEASE OF LIVER

Figure 2. Treatment Related Toxicity

A Kaplan-Meier plot of survival comparing the study group versus the historical control group is seen in Figure 3. The overall median survival for the entire 38-patient cohort is 13 months. Nine patients (24%) remain alive with a median follow-up of 28 months (12-53 months). The median age of survivors is 30 years (18-47 years). Six of the surviving patients had grade III, and three had grade IV histology. Subset analysis revealed that younger patients (<35 years old), grade III histology, and good performance status (≥70% by Karnofsy) were associated with improved survival.

DISCUSSION

BCNU has been the drug most commonly used in the adjuvant setting for the treatment of malignant gliomas. BTSG studies have revealed a small improvement in survival when this agent has been used in non bone marrow ablative doses. Following the observations by Hochberg et al (3) and Phillips et al (4,5) of a few long-term survivors treated with high-dose BCNU at the time of relapse, we studied the application of this concept at initial diagnosis, in addition to standard surgery and irradiation, for patients with grade III and IV gliomas.

SURVIVAL ANALYSIS TABLE
Study Group vs Historical Control
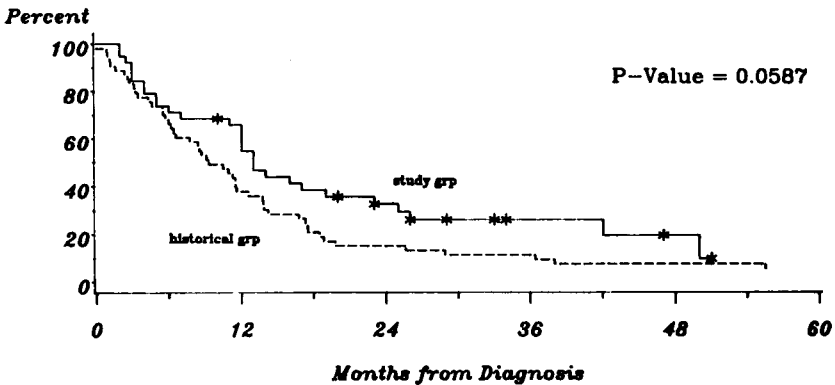
Study group – 38 patients   Historical Control – 52 patients

July 11, 1988   • indicates patient surviving

Figure 3. Actuarial survival for patients in the BCNU treated study group versus the historical control group. Overall median survival for the study group is thirteen months. *Indicates surviving patients.

This combined modality therapy was well tolerated. Hematologic toxicity was minimal. One-third of patients received most of their care as an outpatient as empiric antibiotics were not required. Non-hematologic toxicity was similar to that reported by other investigators with one patient developing interstitial pneumonia and two cases of veno-occlusive disease.

Although our initial results were promising (6), with additional patient experience and more prolonged follow-up, it is now apparent that the addition of high-dose BCNU therapy did not offer any clinically significant survival advantage when compared to our historical control group that was treated with surgery and irradiation alone. With subset analysis, it is apparent that younger patients, grade III histology, and good performance status were associated with longer survival in this non-randomized trial. These clinical characteristics have proven to be prognostically important by the BTSG in larger randomized trials.

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Discussion 3 - Session IIIC (Gliomas)

Dr. Frei: Is the effect on the capillary endothelium something that has been described?

Dr. Biron: Yes, after radiotherapy, but not after BCNU.

Dr. Dicke: Why didn't you include radiotherapy in your protocol?

Dr. Biron: The radiotherapy was done after the BCNU as well as before BCNU, in some patients, in the period immediately after surgery. The dose is equivalent with 60 gray.

Dr. Dicke: So is it, in your opinion, better to do the radiotherapy before or after high dose therapy?

Dr. Biron: That is a very interesting question. Until now I think only Johnson has published a paper where he tried to do BCNU before radiotherapy as all other patients have been treated with BCNU after the radiotherapy. We hoped at the beginning that doing the BCNU before would change the results. This does not seem to be the case in the Johnson study and in our study.

Dr. Frei: You do not say anything about CT scanning. I take it that it was not helpful in evaluating what was going on?

Dr. Biron: The evaluation of the response with CT scan is very difficult and I think the only way to use the CT scan is to know the relapse before the clinical change. But to evaluate the response I think is very difficult due to the CT scan itself and also because the patient has received surgery before.

Dr. Shea: Dr. Biron, how many of that last 20% are disease-free at this point?

Dr. Biron: It is so difficult to be sure that they are disease-free. I cannot be sure anyone could be disease-free.

Dr. Frei: I think a very important aspect about experimental design is that the chemotherapy was given before radiotherapy because given after radiotherapy in this setting puts the chemotherapy in a difficult position with respect to evaluation. So that is an important study.

Dr. Philip: Dr. August, can you give us some detail about the management of the patients. Were they in a sterile environment or normal room?

Dr. August: The patients at the University of Wisconsin were managed in single rooms, with hand washing and no attempts at reduction of gut flora. The patients in Philadelphia -- I cannot speak for the University of Chicago -- are managed as our routine BMT patients in a PE with non-absorbable antibiotics and the whole business. The primary reason is that our ancillary personnel get too confused if we have different protocols for managing different patients. If the patients are HSV sera positive we treat them prophylactically with acyclovir and we give I.V. gammaglobulin to the patients who are CMV sera positive. We protect all patients with CMV negative blood products.

Dr. Frei: Why did you chose to give the thiotepa first followed by etoposide?

Dr. August: At the moment, the drugs are being given together but initially pharmacokinetic studies were being done with thiotepa and they did not want to confuse the picture by the simultaneous administration of the etoposide.

Dr. Herzig: We have used etoposide with thiotepa and reversed the order, gave the VP-16 first at 2400mg/m2 followed by 900 per meter squared of thiotepa for some patients with lymphoma. It is fairly well tolerated and have added cytoxan at 200gr/kg to that regimen as well. The order in which it is given when we reversed the VP-16 and the thiotepa when combined with cytoxan made an enormous difference in stomatitis.

Dr. Pinkerton: I just like to ask you a question about the use of high dose etoposide as a single agent in gliomas. In London, a phase II study has been done and (has) shown very disappointing response rates at $500/m^2$. Do people have experience of this agent as a single agent at 1-1/2 grams?

Dr. August: I personally cannot answer that question. I think the high dose etoposide was partly taken from Dr. Herzig's earlier work. He has had enormous amount of experience compared to Dr. Finley's. The pharmacological data shows good penetration into the CNS.

Dr. Frei: Roger, do you want to reply?

Dr. Herzig: The single agent thiotepa data I have, but I do not have data of the single agent etoposide.

Dr. Pinkerton: Presumably it is adding substantially to the mucositis problem.

Dr. Herzig: It probably isn't because a lot of that is explained I think by the radiation that they had.

Dr. Dicke: When Dr. Messerschmidt was giving a lecture in our institute, he mentioned that when you give radiotherapy first -- prior to the high dose chemotherapy -- that it affected survival negatively compared with radiation after the high dose chemotherapy treatment. Patients did really very badly. And he also saw a tumor lysis syndrome where he showed us scans where you saw basically an increase in volume and then afterwards, within a couple of weeks, a shrinkage. Is that a syndrome which has been noticed by other teams?

Dr. Thomson: Maybe I could clarify on that last question, Dr. Dicke. Basically--in terms of the first question about radiotherapy-- first, it is true that several patients that were treated on the protocol early on received radiotherapy first and then got high dose BCNU afterwards and had a uniformly bad outcome. We were hopeful that would suggest that there might be some alteration in terms of delivery of the drug to the tumor. All of the 35 other patients got the high dose BCNU prior to radiotherapy. As you know from our poster update of the data from yesterday, there was no difference in terms of long-term outcome in our observations. In terms of the tumor lysis actually what I think you are referring to is the single patient that actually was published in the JCO article from May of 1987 where serial CT scans were done every 3 months in these patients and after seeing an initial response at 3 months the tumor apparently grew at 6 months and further shrank at 9, 12 and 18 months. Actually, in our experience -- corroborating the French experience -- the interval over which these tumors get smaller -- and the vast majority did -- there were only 2 that failed to show some radiographic decrease in demonstrable tumor by serial CT scans. It often took 12 months to show a change over time.

Dr. Biron: Among the 16 patients who received chemo-therapy -- first, I only observed 1 real response with decrease of the tumor and nearly disappearance of the tumor at time of surgery. But it is only 1 patient.

SESSION III - SOLID TUMORS

D. TESTICULAR

HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR POOR DIAGNOSIS NON-SEMINOMATOUS GERM CELL TUMORS

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INTRODUCTION

The prognosis of advanced non-seminomatous germ cell tumors (NSGCT) has improved markedly these last years due to various chemotherapy schedules including cisplatin, associated in selected cases with surgery (1,2,3,4). Approximately 70% of patients with advanced disease can be cured, however, there remain a number of patients who do not achieve complete remission (CR) or who relapse. Several multifactorial analysis taking into account clinical and/or biological tumor markers have selected poor prognostic factors at diagnosis which might act as guidelines for a more aggressive therapy (5,6). The objective of the present study was to explore high-dose chemotherapy schedule followed by hematological rescue in selected patients with poor prognosis NSGCT. This approach was justified by the young age of the patients, a chemosensitive tumor with a well-established dose-response relationship (7,8,9,10) and an extremely low rate of bone marrow involvement (11). We adopted a high-dose chemotherapy regimen associating cisplatin, etoposide and cyclophosphamide (PEC protocol). Pharmacokinetic studies of cisplatin and etoposide were undertaken in this trial to determine the potential interference in protein binding between cisplatin and etoposide,the renal clearance of these two drugs, etoposide salivary elimination and to detect active metabolites which could hinder bone marrow recovery.

PATIENTS AND METHODS

From April 1984 through October 1987, 43 patients with advanced NSGCT entered this study and underwent 47 courses of the PEC protocol followed by ABMT. The patient characteristics and previous therapy are shown in Tables 1 and 2. WHO performance status was < 3. All patients had previously received cisplatin-containing chemotherapy. Group I consisted of 12 heavily-pretreated patients with progressive disease despite multiple standard drugs including cisplatin. Group II contained 6 patients classified as having non-resistant relapse, i.e. patients having relapsed after achieving remission with a cisplatin-containing protocol but responding to a second-line regimen. Group III contained 25 patients in first complete or partial remission who received the PEC protocol as consolidation therapy. Inclusion criteria for group III were, at diagnosis: advanced disease and high levels of HCG and/or AFP according to a multivariate analysis (5). The PEC treatment regimen (Table 3) consisted of cisplatin 40 mg/m^2 in 250 ml hypertonic saline at 11 a.m., daily over 60 min on five consecutive days. Etoposide was administered at 350 mgs/m^2 in 500 ml of isotonic saline at 10 a.m., over 60 min on the same five consecutive days.

Cyclophosphamide was administered at 1600 mgs/m^2 in 250 ml of 5% dextrose at 2 p.m. over 60 min on four consecutive days starting on the second day of the protocol. Uroepithelial protection was ensured by hyperdiuresis and Mesna. Surgery for residual disease was considered shortly after ABMT. Blood, urine and saliva were collected from 12 patients undergoing the PEC protocol for pharmacokinetic and metabolic studies.

Table 1. Patient Characteristics

NUMBER	43	(ABMT 47)
MEDIAN AGE	25 years	(13–50)
SEX	39 male	4 female

SITE OF PRIMARY

– Testicular	31
– Ovary	4
– Extragonadal	8

Table 2. Previous Therapy

	cisplatin (1) (median cumulative dose/m^2)	surgery on metastases (positive histology/ number of patients)
Salvage therapy (N 18)	1000 mg (425-1500)	15/18
Consolidation therapy (N 25)	400 mg	

(1) All patients had previously received cisplatin-containing regimen.

Table 3. High Dose Chemotherapy - PEC Protocol

Cisplatin	40 mg/m^2/d	days 1-5
Etoposide	350 mg/m^2/d	days 1-5
Cyclophosphamide + mesna	1600 mg/m^2/d	days 2-5
ABMT		day 8

RESULTS

Antitumoral Response

For better analysis of results, the 43 patients were stratified according to clinical status into the 3 groups described above (Table 4).

Group I (Refractory Disease)

Fifteen courses of PEC followed by ABMT were administered to 12 patients, of whom 2 died of iatrogenic causes. Of the 13 evaluable procedures, there were 1 responses (3 CR, 8 PR) and 2 failures.

Table 4. Response and Progression-Free Survival after PEC Protocol in Poor Prognosis Non-Seminomatous Germ Cell Tumors

	Group I Refractory 12 pt-15 ABMT	Group II Non-Resistant Relapses - 6 pt	Group III Consolidation 25 pt-26 ABMT	Total
NE	2	1	7	10
F	2	0	0	2
CR	3	5	16	24
PR	8	0	3	11
Total Responses CR+PR	11/13	5/5	19/19	35/37
Outcome	2 ED 10 died TP median 7m (2-16)	4 CCR 37,39,40,42 m+ 2 died TP (11,24m)	17 CCR median 18.5 m (4-35) (2 AWD (5-24 m+) 4 died TP (7-10m) 2 ED	

Abbreviations: CR, complete remission; PR, partial remission, F, failure; NE, non-evaluable; ED, early death; TP, tumor progression; AWD, alive with disease; CCR, continous complete remission.

However, in these heavily-pretreated patients with refractory disease, the response was of short duration and all the patients died of tumor progression after a median of 7 months (2-16 m).

Group II (Non-Resistant Relapses)

One of the 6 patients was not evaluable for response because he was in third CR after 3 different multistandard drug regimens associated with surgery for lung metastases with positive histology. The 5 remaining patients achieved CR. A post-ABMT CAT scan showed residual abdominal and liver masses in only 1 patient who underwent a partial hepatectomy and lymphodenectomy. The histology revealed only necrotic and fibrotic tissue. This patient relapsed with abdominal nodes and died of disease 24 months later. Another patient relapsed later with lung metastases and died 11 months post-ABMT. Four patients are alive in unmaintained CR with a follow-up of 37, 39, 40 and 42 months.

Group III (Consolidation of a First PR-CR)

Twenty-six course of PEC were administered to 25 selected poor prognosis patients as consolidation after 2 courses of conventional chemotherapy. Seven patients were not evaluable for response because 5 were already in CR and 2 died of iatrogenic causes. In the 19 other cases, 16 CR and 3 PR were obtained. Post-ABMT surgery was indicated in 16 patients with residual masses: L2 of the 3 patients in PR had viable tumoral cells and 14 had non-active tumoral tissue. Four patients died of tumor progression between 7-10 months, 2 are alive with disease at 5 and 24 months and 17 patients are alive in unmaintained CR with a median follow up of 18.5 months (4-35 m).

Hematological Toxicity

The PEC protocol produced a universal myelosuppression, but with unusually rapid platelet recovery in comparison with the duration of granulocytopenia. The median duration of granulocytopenia (< 0.5 x 10^9/l) was 15 days, (6-37 days), whereas thrombocytopenia (< 20 x 10^9/l) lasted a median 13 days (3-32 days). During the 47 PEC protocol + ABMT procedures we observed 30 episodes of fever of unknown origin and 12 (25.5%) documented infections mainly due to gram positive bacteria. Three patients died of infectious complications, always of fungal origin: 2 cases of septicemia due to candida and 1 case of invasive aspergillosis.

Non-Hematologic Toxicity

Gastro-intestinal toxicity was the most frequent, with moderate or severe nausea and vomiting in 39 cases, diarrhea in 17, mucositis in 37 and oesophagogastritis in 6. Sixteen patients developed peripheral neuropathy. Other non-hematological toxicity included: transient liver dysfunction (5), transient renal dysfunction (2), skin rash (7), inappropriate secretion of anti-diuretic hormone (4) and hemorrhagic cystitis (1). One patient with extra-gonadal NSGCT and a gastric mass died at day 14 post-ABMT of a massive gastrointestinal hemorrhage caused by tumor lysis.

Pharmacokinetic and Metabolic Studies

These results have been published elsewhere (13). Briefly:

1) No in vitro protein-binding interaction between cisplatin and etoposide was observed.

2) No significant accumulation of etoposide occurred during the 5 days of chemotherapy.

3) The renal clearance of etoposide depended on renal function.

4) Cisplatin caused no significant changes in the pharmacokinetic parameters of etoposide.

5) A cytotoxic metabolite of etoposide, the aglycone, was identified in the plasma for up to 48 hours after the last administration of the drug.

6) Etoposide was secreted in the saliva with a saliva to plasma ratio ranging between 0.3 and 25%.

DISCUSSION

Our approach is based on the use of a synergistic combination of high-dose chemotherapy. In effect, experimental data (14,15,16) have shown a high degree of synergy between etoposide, cisplatin and cyclophosphamide. In addition, a dose-response relationship has been reported by Wolff for etoposide (17), Buckner for cyclophosphamide (18), Samson and Ozols for cisplatin (19, 20). This study of high-dose chemotherapy with the PEC protocol showed an impressive response rate. Of 37 evaluable procedures we observed 35 responses (94,6%) including 24 CR (64,8%). The 12 heavily-pretreated patients with refractory disease in group 1 could not be cured, despite a high response reate. The results obtained in group II (6 patients with non-resistant relapse) are encouraging since 4 have a long disease-free survival (37-42 months) and are probably cured. Group III, consisting of 25 patients receiving the PEC protocol as consolidation of a first CR-PR, included 5 patients already in CR after 2 cycles of induction therapy. The results in this consolidation group are encouraging but given the severe treatment-related toxicity this approach should be reserved for selected very poor prognosis NSGCT patients. The hematological toxicity and documented bacterial infections encountered were significant but comparable to other studies using this type of procedure. The major non-hematologic toxicities were gastro-intestinal tract effects and peripheral neuropathy. Four treatment-related deaths occurred during the 47 PEC protocols administered. This mortality rate (8.5%) is quite low compared to other studies with ABMT (21). The presence of a residual cytotoxic etoposide metabolite led us to wait 72 hours after the last injection of etoposide before the bone marrow reinfusion. The secretion of etoposide into the saliva may be a cause for concern since peroxidases in the salivary glands are able to oxidize the drug, giving rise to free radicals possibly involved in mucositis. Animal studies in vitro and in vivo have shown that the concommitant administration of etoposide and N-acetylcysteine does not modify the cytotoxic prooperties of etoposide (Gouyette, unpublished results).

CONCLUSIONS

1) The high-dose PEC and ABMT protocol is an effectve regimen for poor prognosis NSGCT patients. Tolerance is acceptable.

2) Patients with progressive disease have a disappointingly short duration of response; other procedures will be necessary to improve the prognosis.

3) We recommend the PEC regimen for non-resistant relapses.

4) Results of consolidation in patients selected for initial unfavorable prognostic features should be compared with a recognized conventional treatment in a prospective trial.

5) Surgery on residual masses is of great value.

6) Pharmacokinetic andmetabolic studies proved invaluable in determing the timing of ABMT and threw further light on the pathogenesis of mucositis.

ACKNOWLEDGMENTS

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CENTRE LEON BERARD EXPERIENCE OF MASSIVE CHEMOTHERAPY IN NON-SEMINOMATOUS GERM CELL TUMOURS (NSGCT): Analysis of First Regimens in Progressive Disease and VP16-IPM-CDDP (VIC) in Sensitive Patients

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INTRODUCTION

Non-seminomatous testicular cancer (NSGCT) is a privileged model for the study of treatment of solid tumors: chemosensitivity, surgical procedures even in metastatic phase, close monitoring by serum markers (AFP-HCG), allow a very precise control of efficiency of this treatment. Prognosis has been dramatically improved by chemotherapy: 25 years ago probability of death was 75% of the patients in 2 years, probability is now 75% of cure. Currently all patients must be treated for cure.

The dose-effect relationship has been well demonstrated in NSGCT first by Ozols with CDDP (1) and massive chemotherapy and ABMT. Buckner with CPM (2) published 2 CR and 6 PR in 9 patients. Blijham (3) treated 13 patients with VP16 and CPM and at least one other drug and obtained 4 CR. We have published in Houston in 1985 (4) the results of high dose Melphalan (MHD) and MHD + VP16 in 15 patients treated in 5 French institutions. We observed 2 CR and 7 PR. Seven were among the 9 patients receiving MHD + VP16. Pico published results of the European Bone Marrow Transplantation Group (5) collecting 55 patients with 64 ABMT. Several regimens are reported in this study including 46 patients who have been treated with CDDP-VP16-CPM: PEC. Fourteen CR are reported among 35 evaluable patients and 15 patients remained in continuous CR among 21 treated by massive chemotherapy in late intensification in the first part of the disease.

The problem now is to select patients with poor prognosis because most of the patients can be cured by conventional chemotherapy and surgery. Several recent studies (6,7,8) have shown that the major prognostic factor is represented by the extent of the disease. The most precise indicator of this extent in the majority of the cases is the initial level of AFP and HCG. Patients with a bulky tumoral mass have less than 50% of likelihood to be in CR after conventional treatment.

The number of lung metastasis and the size of the node in the abdomen are also a good indicator of the spread of the tumor. Persistence of viable residual tumor cells at time of retroperitoneal lymphadenectomy remain a very poor prognostic factor. Finally the close monitoring of the decrease of serum markers allows one to identify quickly the primary refractory tumors.

We present here the experience of our team since 1982 for NSGCT patients treated by massive chemotherapy and ABMT in a progressive phase of their disease and in a latter period as a late intensification in patients with initial or secondary poor prognostic factors.

PATIENTS AND METHODS

In our center between January 1982 and June 1988, 29 massive chemotherapies have been performed in 26 male patients with NSGCT: 22 testicular cancer and 4 extragonadic (3 mediastinal mass and 1 sacral teratoma).

Median age is 32, ranged from 19 to 50 years and 2 children (1,4).

The initial staging for the 22 testicular tumours is: 3 stage I patients, 3 stage II patients and 16 stage III.

At time of massive chemotherapy the tumoral status was:

- progressive disease in 7 cases: relapses resistant (RR) to conventional rescue treatments;
- partial response (PR) after relapse in 8 cases of tumors sensitive to conventional chemotherapy: sensitive relapse (SR);
- 10 PR after initial treatment and 4 first CR;
- 4 first CR.

We observe 8 relapses on therapy among which 6 were RR and 7 relapses off therapy of which 6 were SR.

All the patients received conventional chemotherapy before intensification. For patients in relapse, the VAB-CDDP regimen was the most frequent since it is the current protocol in our institution. At time of ABMT, 93% had received more than 4 drugs and 70% had received more than 5 courses of chemotherapy. Patients had received a mean dose CDDP of 1400 mg (range 850 to 2,000 mg).

In fact, we must separate our experience into two periods, the first period from January 1982 to November 1985, the second period from

November 1985, the second period from November 1985 until June 1988, because in those two periods, conditioning regimens and patients were not the same.

First Period

We used 3 different chemotherapeutic conditioning regimens:

- VP16 400 to 1,000 mg/m^2 and MHD 140 mg/m^2 in four patients.
- VP 1,000 mg/m^2 and Ifosphamide 12 gm/m^2 in one patient.
- Then VP16 1,000 mg/m^2 and CDDP 200 mg/m^2 in 4 patients after Ozols (1) showed the dose effect relationship for CDDP.

All RR were treated during this period.

Second Period

We added Ifosphamide to Ozols's regimen and 20 patients were treated with a combination of VP 16 1,000 mg/m^2, CDDP 200 mg/m^2 and Ifosphamide 12 gm/m^2. We systematically add Mesna to Ifosphamide to decrease hematuric cystitis risk. The VIC regimen is shown in Table 1. For the 14 patients treated in first PR or CR, VIC was preceded by two courses of CDDP 200 mg/m^2 - VP 16 500 mg/m^2 and 2 to 4 courses of VAB-CDDP.

All but one of the first PR and CR patients, and all but one of the SR patients, were treated in the second period.

All patients were treated in a single room, usually without laminar air flow but always with oral decontamination, sterile food, and protected-approach procedures.

Table 1. V.I.C. Regimen

Days		1	2	3	4	5	6	7	8
Drugs									
VP 16	200 mg/m^2	X	X	X	X	X			
IPM + MESNUM	3 gm/m^2 3,6 gm/m^2		X	X	X	X			
CDDP	40 mg/m^2	X	X	X	X	X			
ABMT									X

RESULTS

ABMT Procedure

Bone marrow transplantation was performed on all patients. The mean number of nucleated cells grafted was 0.85 x 10^8/kg and the mean number of GM-CFU cells grafted is 4.1 x 10^{10}/kg. It must be emphasized that in each of the 29 marrow harvests, at least two marrow aspirations and two bone marrow biopsies were performed on each patient. We never found any viable tumor cells or even necrosis focus in the marrow. One patient had at diagnosis diffuse bone metastasis on bone scan and his marrow was also clear at time of harvesting.

We therefore decided that bone marrow purging was not necessary for NSGCT patients.

Toxicity

Concerning hematological toxicity of the procedure the median duration of aplasia was 14 days with less than 1,000/mm^3 WBC, 16 days with less than 500/mm^3 neutrophils and 11 days with less than 200/mm^3 neutrophils. The median time with platelets less than 50,000/mm^3 was 18 days. The median number of platelet transfusions was 4.2 and of red cell transfusions 3.4.

The median duration of isolation was 19 days and almost every patient experienced fever; 16 febrile episodes of unknown origin. Seven were septicemia with 5 staphylococcus epidermidis, 1 escherichia coli, and one with streptococcus. We observed in 2 patients pneumonitis without need of assisted ventilation. One was not documented and one (due to aspergillus) was regressive after amphotericin B and 6 months of Itraconazole.

Finally median duration of febrile episode (0 > 38°) is 5 days with 15 days of antibiotic treatment. Eleven patients received Amphotericin B.

Non-hematological toxicity is summarized in Table 2. It was mild in all cases even for renal function. Mucositis is the most frequently occurring toxicity. During the use of IPM and Mesna, we never observe macroscopic hematuria, but we did initially observe episodes of lethargy. After we stopped the use of anxyolytic drugs and IPM at the same time, we did not observe further episodes of lethargy.

The most critical event in this series was the occurrence in one patient, who had had a post-traumatic coma years before, of one episode of troncular-encephalopathy with progressive coma 30 days after completion of the chemotherapy. Etiology of this encephalopathy was toxic or viral. This episode was slowly regressive but the patient died months of lung recurrence at 6 months.

Finally tolerance to the conditioning regimen and ABMT procedure

Table 2. Non Hematological Toxicity - WHO Scale

	Grade 1	Grade 2	Grade 3-4
Nausea and vomiting	46%	46%	8%
Mucositis	56%	31%	6%
Renal failure	30%	23%	0%
Liver dysfunction	38%	15%	
Lethargy		15%	
Hematuric cystitis	No macroscopic hematuria		
Encephalopathy	1 patient with anteriority of post traumatic coma presented a very long coma slowly regressive. He finally died of lung evolution.		

was good and we did not observe any toxic deaths. Until now, 9 patients died; all of progressive disease.

RESPONSE AND DISCUSSION

Response cannot be evaluated for the whole group and it must first be according to the pre-ABMT status and secondly according to the criteria of evaluation, which are at least three: 1) para-clinic imaging, 2) biologic markers, 3) surgery. Those three criteria are not always evaluated simultaneously, as residual mass can persist without any elevation of markers and with only necrosis at pathologic status.

On the other hand, all the relapses occurred in our experience between the third and the sixth month.

According to the pre-ABMT status we observed (Table 3):

- seven resistant relapse and progressive disease are evaluable: no CR, three PR and four PD.

- in eight sensitive relapse patients, 7 are evaluable: two CR, four PR, one PD. Seven have been treated by VIC, two died of relapse. Five are still alive: 4m+, 11m+, 14m+, 15m+, 19m+.

- among the 14 cases treated in first PR or CR, one patient received in first PR a VP16-CDDP with a good PR, as only microscopic hepatic metastasis were viable, and he received two more courses of conventional chemotherapy and then a new intensification with VIC.

Table 3. ABMT in NSGCT: Overall Response Rate

STATUS PRE-ABMT PERIOD			CCR	CR	PR	NE	PD
RR in PD	7	1		0	3	0	4
SR	8	1 1 case					1
		2 7 cases		2	4	1	
PR and CR	14	1 1 case			1		
		2 13 cases	4	4	3	2	0

We can analyze 13 patients treated in first PR (nine patients) or in first CR (four patients) who have had intensification by VIC (Table 4). Eight patients were stage 3 with more than five lung metastasis and/or very high level of biologic markers and/or bulky abdominal disease. Two were stage 2 with viable residual mass at time of RPLN. One was stage 1 who relapsed with several lung metastasis as he did not receive chemotherapy after orchidectomy and RPLN and was not followed correctly. Two were mediastinal masses.

The results are as follows. The four patients in CR all remained in CR after ABMT and are still in continuous complete remission. Of the nine patients in PR:

- four achieved CR but one relapsed after four months and died of progressive disease; three are in continuous complete remission.

- three remained in clinical PR, and they all had lung metastasectomy of residual mass. Two were necrotic and one was only mature teratoma at pathological status; all are still alive NED.

- two are not evaluable owing to no macroscopic residual mass beefore ABMT (liver metastasis on biopsy only, residual viable cell in an incomplete abdominal node resection). They both are alive NED.

- We did not observe progressive disease during VIC and ABMT.

The overall response rate of FIC in 16 evaluable patients is: 6 CR (37%), 7 PR (43%), with a total of 80% responding.

These results seem encouraging, with an overall survival of 80% in 20 patients treated by VIC with a median follow-up of 12 months (Figure 1). Survival of the 13 patients with poor prognosis treated in first CR or PR is 88% with a median follow-up of 12 months.

Table 4. ABMT in NSGCT - Response of VIC as Intensification in 13 Patients in First PR or CR

STATUS PRE-ABMT	STATUS POST-ABMT	LONG TERM STATUS
4 CR	4 CCR	Still NED
9 PR	4 CR	1 relapse after 4 months and died in PD 3 are alive in CCR
	3 PR	Metastasectomy: -2 necrosis - 1 mature teratoma. All 3 are alive in CCR
	2 NE	1 microscopic liver metastasis only before BMT. 1 incomplete RPLN with viable cells both alive in CCR.
	No PD	
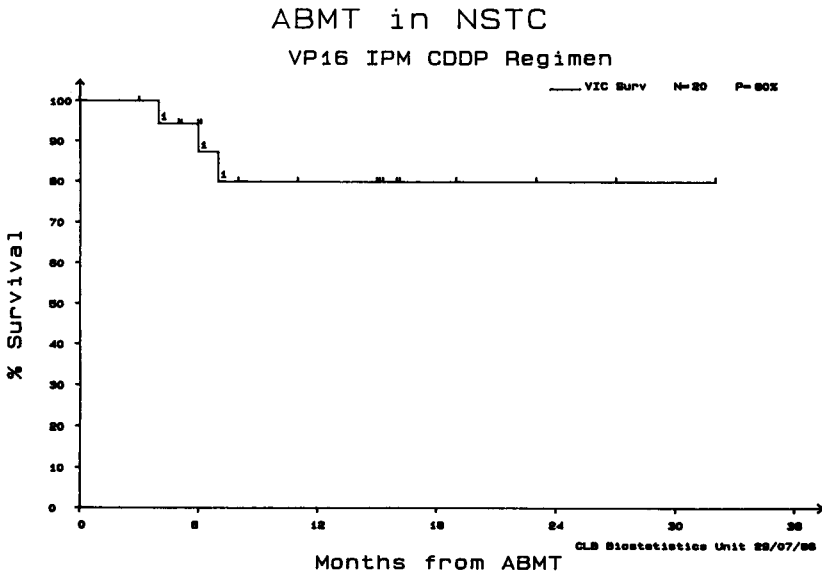
Figure 1. ABMT in NSTC - VP16-IPM-CDDP regimen.

Survival of seven patients treated in sensitive relapse is 67%, but follow-up is only eight months (Figure 2).

These results provoke several points of discussion. NSGCT seems to be the first solid tumor acting as the lymphoma model for two reasons.

Firstly, in sensitive patients, the conditioning regimen resulted in more CR than PR and there are long-term survivors. In addition, even if the conditioning regimens are not the same, we do find a difference in response and survival between resistant relapses, where there were no CR and no long-term survivors, and sensitive relapses where responses are better (two CR and a response rate of 75%) and where we can observe long-term survivors: four patients have a follow-up greater than one year (Figure 3). The second point of discussion is the role of high-dose chemotherapy and ABMT in the treatment strategy of NSGCT since we have observed an 88% survival for patients with poor prognosis stage 3 or 2C. Although this study needs to have a longer follow-up period and more patients to be confirmed, three points can be emphasized:

1. In our previous study, in patients treated by VAB-CDDP alone, we observed a 40% survival of three years in 31 patients with stage III disease.
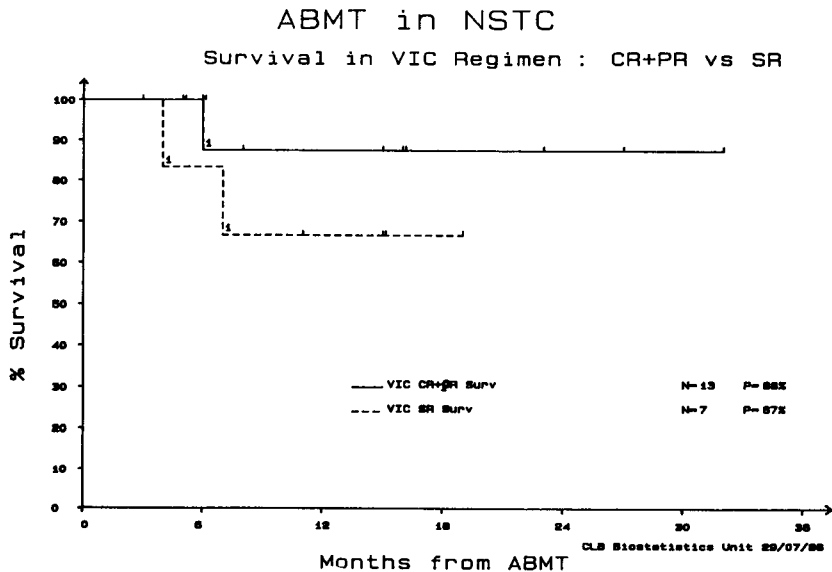
Figure 2. ABMT in NSTC - Survival in VIC Regimen: CR + PR vs SR.

ABMT in NSTC

Survival according to the Status before ABMT
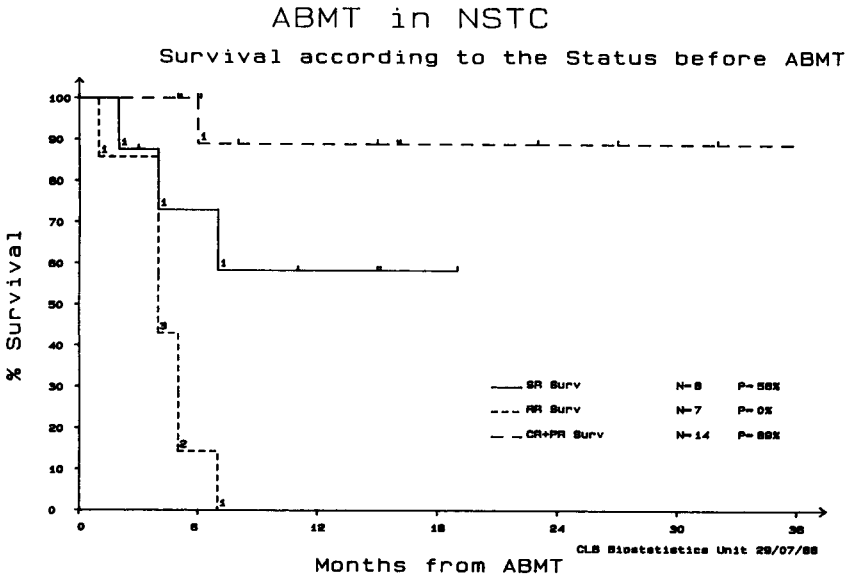
Figure 3. ABMT in NSTC - Survival according to the status before ABMT.

2. A major problem is selection of patients who must be treated by conventional treatment or by intensive chemotherapy. During the same period, 90 NSGCT of the testis have been referred to our institution while we used bone marrow transplant in only 22. Prognostic factors must be evaluated at the beginning and during the treatment of patients, using, a) initial tumor spread, b) initial level marker and close monitoring of their decrease during conventional chemotherapy, and c) wide surgical procedure and pathological status.

3. The VIC regimen gave a good response rate after initial treatment and appears to be well tolerated with no toxic death, which is a primary condition for a first-line treatment used with intent to cure.

4. These results must now be compared with results of recent conventional regimens for extensive stage III disease. One randomized trial in such patients is being conducted by J.P. Droz in Institut Gustave Roussy (Villejuif, France).

In conclusion, we think that results obtained in NSGCT are very encouraging with a conditioning regimen like VIC. The response rate is high compared with low toxicity. It seems to allow long-term

survivors, not only in first-line intensification but also as salvage in still sensitive patients. These results must be compared with conventional treatment in extensive stage III disease. Finally, on a theoretical point of view, NSGCT is the first solid tumor where application of the data from the lymphoma model marks a difference between resistant and sensitive patients.

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PHASE I/II STUDY OF HIGH DOSE CARBOPLATIN/VP-16 WITH MARROW RESCUE IN REFRACTORY GERM CELL CANCER

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Germ cell neoplasms are exquisitely sensitive to chemotherapy agents, in particular cisplatin. The vast majority of patients with metastatic germ cell cancer can be cured with first and/or second line platinum-containing regimens (1). However, the small percentage of patients who either relapse after salvage therapy or are truly cisplatin refractory have an extremely poor prognosis (2). In such patients we performed a phase I/II study with carboplatin (CBDCA) and etoposide (VP-16) with autologous marrow rescue under the following assumptions:

1. High dose carboplatin may be capable of overcoming cisplatin refractoriness;

2. One course of chemotherapy will be insufficient to cure patients with relapsed/refractory germ cell tumor;

3. With high dose CBDCA/VP-16, bone marrow rescue will be necessary.

METHODS

On admission, bone marrow was harvested ($0.48-3.16 \times 10^8$/kg) and cryopreserved in two identical fractions using 10% DMSO, 20% autologous plasma, and a controlled rate freezer (3). Chemotherapy was administered on days -7, -5, and -3; marrow infusion followed on day 0. The VP-16 was kept constant at

400mg/m^2/d (total 1,200 mg/m^2); the CBDCA was escalated during the phase-I part of the study from 300 mg/m^{2}x3 (total 900 mg/m^2, #3) through 1350mg/m^2(#4), and 1,800 mg/m^2(#4) to 2,000 mg/mg^2(#1). For the phase II study a dose of 1,500 mg/m^2 was chosen (#12).

All patients were nursed in HEPA-filtered rooms, and received decontamination of the gastro-intestinal tract with antibacterial and antifungal agents. Total parenteral nutrition was routinely used, and all patients received irradiated blood products.

When the absolute granulocyte count (AGC) was >500/mm^3, the patient was discharged and re-evaluated. Patients who had responded to the regimen (>90% decrease in markers) were re-admitted 7-14 days later for a second, identical, course of chemotherapy with marrow rescue.

RESULTS

From September 1986 through April 1988, 25 patients with refractory nonseminomatous germ cell cancer were enrolled at Indiana University. Their median age was 24 years, with a range from 15 to 46 years. The primary site of germ cell tumor was testis in 19 cases, mediastinum in three, retroperitoneum in two, and ovary in one case. One patient had failed four chemotherapy regimens, 13 patients had failed three regimens, and 10 patients two regimens; one patient had shown progression during the first cisplatin based regimen.

Therapy Results

Twenty-five patients received the first round of chemotherapy. Two died during the pancytopenic phase, and six had refractory disease (<90% reduction in markers) and were not eligible for the second round. Consequently, seventeen patients received the second round. Two patients died during the second episode of pancytopenia. Seven patients entered a complete clinical and biochemical remission (28%), and five patients had a partial remission (20%) for total remission rate of 48%. Five patients continue in complete remission for 3+, 4+, 14+ and 16+ months; in all cases this remission is longer than any previous remission.

Toxicity

All patients developed granulocytopenic fever and needed broad-spectrum antibiotics. Additional toxicities included diarrhea (16% grade III, 52% grade II), increased bilirubin (28% grade III, 28% grade II), mucositis (16% II + III), but no oto-, or neurotoxicity. The four fatalities all occurred during pancytopenia; one patient died d+19 from

massive gastrointestinal bleeding from a tear at the gastro-esophageal junction. The other three patients died from candida septicemia. This candidemia occurred as a primary event (d+5, +15, +20) and not following prolonged use of broad-spectrum antibiotics. In fact, surveillance cultures did not show extensive colonization of the GI tract prior to the fatal infection.

Engraftment

The rate of engraftment was extremely variable, with the interval between marrow infusion and AGC >500/mm^3 ranging from 11 to 40 days (mean 23.5 ± 6.8). There was no difference between engraftment after the first and 2nd rounds for the 15 patients who received two treatments (Table 1). In fact, per individual patient, a clear correlation was found between the two courses of treatment for days AGC >500/mm^3 (r=0.74) (Figure 1).

DISCUSSION

The patient population studied was heavily pre-treated and considered incurable with conventional therapy. The phase I study suggested that CBDCA at 1500 mg/m^2 can be safely combined with VP-16 at 1200 mg/m^2. No attempt was made to escalate the dose of VP-16. These doses were used in the subsequent phase II study. Several interesting observations arose from this phase I/II study.

First, high dose CBDCA/VP-16 had considerable anti-tumor activity in this population of patients, the majority of who were truly cis-platin refractory. Therefore it appears that high dose CBDCA can overcome cisplatin refractoriness(4). Seven patients entered complete remission (28%); several of these patients had extensive disease at the time of treatment. Three patients are now in unmaintained CR for >1 year and have a fair chance of being cured.

Secondly, the toxicity of the therapy regimen was acceptable and manageable. Diarrhea was the most prominent side-effect. Ototoxicity and neurotoxicity were not observed and nephrotoxicity only in cases treated with Amphotericin-B. The high incidence of severe candida infection was worrisome. This complication was not related to colonization of the intestinal tract with yeast or to prolonged therapy with broad-spectrum antibiotics (5). In fact, as shown in Table 2, the incidence was higher than in allogeneic bone marrow transplants receiving the same anti-fungal prophylaxis with nystatin and clotrimazole. Since in CBDCA/VP-16 patients ketoconazole was used, no new cases of severe candida infection have been observed.

Thirdly, engraftment was very similar for the first and second rounds of therapy. As shown in Table 1, granulocyte, platelet and red cell engraftment was almost identical for both treatment courses.

Table 1. Engraftment for the 15 Patients who Successfully Underwent Two Rounds of Chemotherapy/Marrow Rescue

	1st Round	2nd Round
Days to AGC >500/mm^3	23.4 ± 7.2*	23.0 ± 7.8
Days to last platelet transfusion	17.1 ± 5.3	18.5 ± 6.9
# units of platelets	65.0 ± 27.8	65.0 ± 26.4
# units of red cells	9.5 ± 3.2	9.8 ± 3.0

*days (mean ±1 s.d.) after marrow infusion
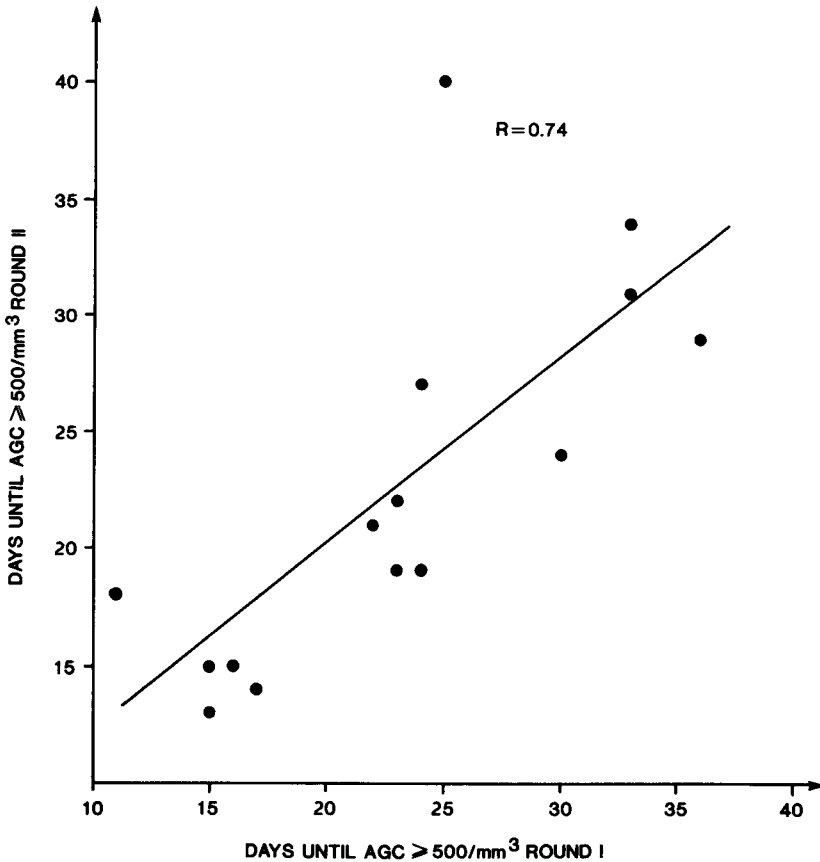
Figure 1. Comparison for days till granulocyte count >500/mm^3 between first and second round of treatment in 15 patients. The correlation is highly significant ($p < 0.01$).

Table 2. Incidence of Severe Candida Infection (septicemia, liver abscess) in Various Populations of Bone Marrow Transplant Patients Treated Under Similar Conditions

nystatin/clotrimazole prophylaxis

allogeneic	3/81	(3.7%)
autologous, non-CBDCA/VP16	0/15	(0 %)
autologous, CBDCA/VP-16	4/23	(17.4%)

ketoconazole prophylaxis

autologous, CBDCA/VP-16	0/20	(0 %)

More importantly, per patient, a close correlation was found for all engraftment parameters between the two courses. This suggests that either a constituent of the marrow-graft, e.g., stem cells, was responsible for engraftment (16), or that repopulation is completely determined by an environment factor. Obviously, we did not prove that autografting was necessary to restore marrow function following high dose CBDCA/VP-16.

Finally, the set-up with two consecutive transplants from single bone marrow collection forms an ideal model to study the kinetics of bone marrow repopulation and of the effect of hemopoietins on engraftment.

Studies are now underway to study the therapeutic effect of high dose CBDCA/VP-16 with marrow rescue earlier in the course of relapsed germ cell tumor. Simultaneously, we are evaluating combinations of high dose CBDCA with other agents in therapy-resistant cases of this tumor.

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Discussion 4 - Session IIID (Testicular)

Dr. Frei: Is the interpretation correct that you did not see more toxicity with the second course?

Dr. Jansen: We did not see more toxicity with the second course, neither hematological toxicity nor GI or (any) other toxicity. The median interval between the 2 marrow infusions was about 5-1/2 to 6 weeks.

Dr. Rosti: We have a study like yours with carboplatinum VP-16 in refractory testicular cancer, just 7 patients. We still transplant patients for the second time even when serum markers were elevated after the first course. In 2 cases, we escalated the doses and we had response in these patients which otherwise we would have called non-responsive. What do you think about that?

Dr. Jansen: I cannot answer your question. I only can say what our routine was. When a patient would come in for a second course, we would draw the markers and that day we would make the decision whether you could get a second course or not -- which meant, at that day, he would still have to fulfill the criteria of more than 90% decrease in the markers as compared to before the start of the first round. To be honest, I cannot remember any patient who left the hospital responding and thought he would come back for the second course who ultimately did not fulfill the criteria. So all our patients were still in the 90% radiation range when they came back. The only exception, perhaps, were patients who already started with low markers with measurable disease and had a marked decrease in their abdomen and a 90% reduction of serum markers. If the patients were coming up with their markers already during their pancytopenia then we call them non-responsive and they would not get a second course. All the patients initially had a dramatic response in their markers but some of them came up very rapidly and we called those non-responders.

Dr. Biron: How many patients did you put off the study because of BM involvement at the beginning?

Dr. Jansen: Basically, one. To be honest, we were not very precise in it. We did not even wait for a BM biopsy. We did an aspirate and a biopsy. If the aspirate was negative, they were eligible and they did not wait for the results of the biopsy. We had 1 patient whose aspirate was clearly positive and we ruled him out.

Dr. Kaizer: With respect to your statement that the carboplatinum overcoming cis-platinum resistance, in terms of myelosuppression, what is an equitoxic dose of cis-platinum as compared to carboplatinum?

Dr. Jansen: Dr. Shea can probably answer that a lot better than I ever could.

Dr. Shea: Basically, it is about 4 to 1.

Dr. Kaizer: So essentially you were giving about twice the myelosuppressive dose of carboplatinum at 1500 as the 200 of cisplatinum that is usually given as a maximal dose.

Dr. Jansen: But I think cisplatinum is not myelosuppressive at all. At least not, to a large extent in our hands, when given as a single drug.

Dr. Spitzer: These durables, CRs, were they CRs at the end of the first course?

Dr. Jansen: One patient was in CR, the second patient had an enormous response and the third patient ... I do not know.

Dr. Spitzer: What is your feeling about the second course? Do you think it is essential?

Dr. Jansen: My feeling is that for the ones who respond very well, it is essential. We have never seen a patient whose markers were coming up even a little bit after the first course before they got the second course who stayed in long remission. So I think ultimately we probably could decide that only the patients who are still going down with their markers or had a response of tumor mass at the time of second course should be the ones who benefit from the treatment, not the ones who have a slight relapse already. Minimal relapse.

Dr. Frei: Our next speaker is Dr. Pierre Biron.

Dr. Jansen: One more question about your patient selection. I am always told, by my Oncology friends, that I do not know anything about testicular cancer; that it is impossible to select in CR who is going to do poorly and who is going to do well. Also the patient in first relapse is mostly still doing pretty well with normal, more conventional, salvage therapy. How many of your patients were treated in first relapse when they were still probably salvageable with other therapy?

Dr. Biron: The patients were treated after 1 salvage regimen.

Dr. Dicke: Well, since the two of you are treating testicular cancer, I want to ask a question which may apply for the future. What I hear from Dr. Logothetis, our testicular cancer expert, is that basically the disease-free survival rate in testicular cancer can be increased when it is possible to extend the induction therapy in the bad prognostic patients -- that means that 3 courses of induction in certain patients is not enough. You have to stop because of severe myelosuppression and, according to Dr. Logothetis, if one would be able to give the fourth or fifth course it may increase survival. So store BM in those patients and continue with the induction therapy but then with BM support. What is the feeling of you and Jan Jansen?

Dr. Biron: We think that we must treat the patient very intensively in the beginning.

Dr. Jansen: We have the feeling that if we want to use BM rescue, we might as well blast the patients very hard once instead of doing that a number of times at a lower dose. We believe that if we want to rescue the poor risk patients who have relapsed once, that it might be better to first put them in remission with more conventional therapy -- for example, 3 courses, then harvest, and then give them one course of high dose chemotherapy. That is a randomized study we are writing now.

Dr. Dicke: Dr. Pico, in how many cases did you see residual tumor at debulking?

Dr. Pico: Right. There were 16 patients that went to surgery after this high dose chemotherapy. Fourteen had negative histology and 2 patients had positive viable cells. And debulking and surgery was important because one of these patients received a second course of high dose chemotherapy after 90% of fibrotic tissue and about 5% viable tumor cells. He is now with a long follow-up.

Dr. Sorzsky: Was there any correlation between the VP-16 in the saliva and the severity of mucositis?

Dr. Pico: That is a good question. We have too few data for definite conditions.

Dr. Frei: There are other drugs such as thiotepa that are secreted into the saliva. The relationship of that to the mucositis that occurs is a very interesting question and challenge.

SESSION III - SOLID TUMORS

E. MELANOMA

TREATMENT OF ADVANCED MELANOMA WITH HIGH-DOSE CHEMOTHERAPY AND AUTOLOGOUS BONE MARROW TRANSPLANTATION

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Metastatic malignant melanoma is difficult to treat because of resistance to chemotherapy. A large number of agents have been studied, with DTIC (dacarbazine) most extensively evaluated. The response rate (complete and partial) in patients receiving decarbazine alone or in combination is about 20% (1,2). We, and others, have explored the use of autologous bone marrow to permit dose intensification of alkylating agents with the anticipation of improving the response in patients with metastatic malignant melanoma. This manuscript will summarize the results of our phase I and II studies with 1,3-bis(2-chloroethyl)-1-nitrosourea (BCNU, carmustine), melphalan (L-Pam), triethylenethiophosphoramide (thioTEPA) and the combination of carmustine and melphalan. A summary of the literature is also provided.

MATERIALS AND METHODS

Patients

Patients eligible for treatment had metastatic melanoma and met the requirements of participation in our Phase I and II trials of intensive chemotherapy and autologous marrow rescue. Informed consent, approved at each institution, was obtained before beginning treatment.

Marrow Processing

Before high-dose therapy was administered, marrow was collected and cryopreserved by standard methods (3). Histology was normal and the marrow was collected a minimum of 4 weeks after previous chemotherapy. It was then cryopreserved in dimethyl sulfoxide and kept at - 196°C in the liquid phase of liquid nitrogen. Three to four days after completing chemotherapy, the marrow was rapidly reinfused intravenously.

Chemotherapy

Dose escalation of the chemotherapy was accomplished by a modified Fibonacci scheme. During the single-agent studies, the total dose was administered over 3 days and given intravenously over 2 hours (BCNU, thioTEPA) or by rapid intravenous bolus administration (melphalan). When the combination of BCNU and melphalan was given, the starting dose was 50% of the maximally tolerated dose determined in the respective phase I single-agent study. Each drug was escalated singly, holding the other drug dose constant. The melphalan was administered for the first 3 days followed by BCNU on the subsequent 3 days.

Evaluation of Response

Responses were defined using standard criteria. Complete response (CR) was the complete disappearance of all measurable disease for more than 1 month; partial response (PR) represented a more than 50% reduction of measurable disease; any response less than partial was considered no response (NR). Duration of response was calculated from the day of marrow infusion. The preliminary results of our phase I and II studies have been previously reported (4-8) and have been updated. For statistical comparisons, the confidence interval method described by Simon, Fisher's exact test, or chi-square method were used (29).

RESULTS

The results of treating advanced melanoma in patients with maximally tolerated doses of BCNU, melphalan, or thioTEPA are presented in Table 1. These doses were determined to be the maximally tolerated doses in the phase I trials. In the Phase I study of thioTEPA, there was a dose-response effect noted at 900 mg/m^2, with a significantly higher response in patients receiving ≥ 900 mg/m^2 compared with patients who received lower doses. The overall response rates for melphalan and thioTEPA were better than for

**Table 1. Three Drugs and Autologous Bone Marrow
Transplantation for Advanced Melanoma: The Effects
of Prior and Extent of Disease**

Drug[a]	No. of Patients	Response		
		CR (%)	PR (%)	Total (%)
Carmustine				
Prior Rx	15	0	2 (13)	2 (13)
No Prior Rx	16	4 (25)	8 (50)	12 (75)
Soft tissue	5	1 (20)	2 (40)	3 (60)
Visceral	26	3 (12)	8 (31)	11 (42)
Total	31	4 (13)	10 (32)	14 (45)
Melphalan				
Prior Rx	16	3 (19)	7 (44)	10 (63)
No Prior Rx	10	3 (30)	5 (50)	8 (80)
Soft tissue	2	1 (50)	0	1 (50)
Visceral	24	5 (21)	12 (50)	17 (71)
Total	26	6 (23)	12 (46)	18 (69)
ThioTEPA				
Prior Rx	31	1 (3)	15 (48)	16 (52)
No Prior Rx	24	3 (12)	10 (42)	13 (54)
Soft tissue	8	3 (38)	3 (38)	6 (75)
Visceral	47	1 (2)	22 (47)	23 (49)
Total	55	4 (7)	25 (45)	29 (53)

[a]Carmustine, 1200 mg/m^2; melphalan, 180-225 mg/m^2; thioTEPA, 180-1575 mg/m^2.
Note: Prior Rx is for patients who received prior therapy before autografting; no prior Rx is for patients who received no therapy before autografting. Soft tissue is skin ± lymph node only; visceral refers to visceral organ metastases.

BCNU. The lower response rate for BCNU can be accounted for by the significantly poorer response in patients who had received previous therapy (13% for BCNU compared to 63% and 52% for melphalan and thioTEPA, respectively, p<.02). In patients who had not received previous therapy before autografting, there were excellent responses with all three agents. The effect of the extent of metastases in which patients with visceral metastases are compared with patients with skin and/or lymph node disease are also presented in Table 1. Not surprisingly, patients with more limited disease tended to respond better. Overall 10/15 (67%) response rate was seen

in patients with only skin and/or lymph node involved; 51/97 (53%) responded with visceral metastases.

The response rates for high-dose BCNU (45%), melphalan (69%), and thioTEPA (53%) are compared with the responses reported with each of these agents used in conventional doses for advanced melanoma (Table 2). In each instance, the response is significantly greater for the high dose with autografting (P <.05 by confidence interval). The median duration of response was similar for all three agents: BCNU, 6 months (range, 2-46+ months); melphalan, 4 months (range, 2-14 months); thioTEPA, 4 months (range, 2-31+ months). However, twice as many patients treated with BCNU had unmaintained responses greater than 1 year compared with patients who received melphalan (15% versus 8%). Patients who received thioTEPA had similar results, with 10% of the patients having a response lasting more than 1 year.

Since there were no overlapping toxicities between the high-dose melphalan and BCNU studies, and since both agents demonstrated significant antitumor responses (with a somewhat better response rate with melphalan and possibly improved duration of response with BCNU), we performed a phase I dose-escalation study of both agents in combination (CARMEL) for patients with metastatic melanoma (7). We began at 50% of the maximal doses form the previous studies: melphalan (90 mg/m^2) and BCNU (600 mg/m^2). Four dose escalations (five treatment levels) were accomplished: melphalan (135 mg/m^2) and BCNU (600 mg/m^2); melphalan (135 mg/m^2) and BCNU (900 mg/m^2); melphalan (180 mg/m^2) and BCNU (900 mg/m^2); melphalan (180 mg/,^{2}) and BCNU (1200 mg/m^2). The pattern of marrow recovery (granulocytes >500/μl and platelets >20,000/μl untransfused) were similar to each agent alone. Increased nonmyeloid toxicity involving the lung (noninfectious diffuse interstitial pneumonitis and adult respiratory distress syndrome) and the gastrointestinal tract (diarrhea) were significantly greater at the highest level (100% of both agents) compared to the previous level (100% melphalan, 180 mg/m^2, 75% BCNU< 900 mg/m^2) or to any lower level. The pulmonary toxicity of the highest level CARMEL was significantly greater than that observed with BCNU alone at 1200 mg/m^2, thus indicating the potential for additive toxicity.

The response rate showed a trend for improved responses with increasing doses, but too few patients were entered at each level to be statistically significant. Overall, there were 58 patients entered, with 6 CR and 28 PR for a response rate of 59%. The extent of disease (skin and/or lymph node involvement versus visceral organ involvement) was not different than that seen with each agent alone: 4 out of 10 patients (40%) with skin and/or lymph node but no CRs, and 30 out of 48 (62%) with visceral organ involvement (including all six CRs). The response rate was not statistically different form melphalan alone, but was marginally better than BCNU alone (p = .08,

**Table 2. Conventional-Dose Versus High-Dose
Therapy for Advanced Melanoma**

Drug	No. of Patients	Response Rate (%)	95% Confidence Interval
Carmustine[a]	110	15	10 - 23
Carmustine/ABMT[b]	31	45	29 - 62
Melphalan[a]	24	17	7 - 36
Melphalan/ABMT[b]	26	69	50 - 83
ThioTEPA[a]	55	16	9 - 28
ThioTEPA/ABMT[b]	55	53	40 - 65

Abbreviations: ABMT, autologous bone marrow transplantation; thioTEPA, triethylenethiophosphoramide.

[a]Conventional dose.
[b]High-dose with autologous bone marow transplantation.

Fisher's exact test). At the maximally tolerated dose (melphalan 180 mg/m^2, BCNU 900 mg/m^2), the response rate (16/27, 59%) was also similar to thioTEPA. The duration of response, however, resembled BCNU with a median duration of 5 months (range, 2-30+ months), with 15% greater than 1 year.

Since there was no pulmonary toxicity with thioTEPA and no overlapping dose-limiting toxicities with melphalan and thioTEPA, we started a new trial combining these two alkylators - MELT. The plan was similar to the CARMEL regimen. Only two patients were entered before the study was temporarily suspended because of problems with the intravenous formulation of melphalan.

A summary of clinical trials using high-dose single agent and high-dose combination of two or more drugs with autologous bone marrow rescue can be found in Tables 3 and 4.

SUMMARY

The results of these studies suggest that autologous bone marrow transplantation will permit moderate increases in doses sufficient to obtain responses where conventional doses fail. Although increased response rates with either single agents or in combination were observed, the duration of the responses were generally of short duration (only about 10% >1 year). Possible solutions to improve the duration of response include multiple courses of high-dose therapy and/or other combinations of chemotherapy or biologic modifier

Table 3. High-Dose Single Agent Trials with Autologous Bone Marrow Transplantation for Advanced Melanoma

Ref. No.	Agent[a]	Dose[b]	No. of Patients	CR	PR	Total	(%)
12	Cyclophosphamide	20 mg/kg	2	0	0	0	--
13	AMSA	600-1000	4	0	0	0	--
14	Nitrogen Mustard	33	4	1	1	2	(50)
15	BCNU	600-750	5	0	1	1	(20)
16	BCNU	1200	6	0	1	1	(17)
4	BCNU	1200	31	4	10	14	(45)
17	L-PAM	100	31	4	10	14	(45)
18	L-PAM	140	20	2	9	11	(55)
19	L-PAM	120-200	3	0	0	0	--
20	L-PAM	140	2	0	2	2	(100)
21	L-PAM	>140-260	27	2	10	12	(44)
22	L-PAM	180	3	0	3	3	(100)
6	L-PAM	180-225	26	6	12	18	(69)
23	ThioTEPA	135-1215	2	0	2	2	(100)
10,11	ThioTEPA	180-1575	55	4	25	29	(53)

[a]AMSA = acridinylaminomethanesulfon-m-anisidide; BCNU = carmustine; L-PAM = melphalan; thioTEPA = thiethylenethiophosphoramide.
[b]mg/m^2 except as noted.

Table 4. High-Dose Combination Chemotherapy with Autologous Bone Marrow Transplantation for Advanced Melanoma

Ref. No.	Agents[a]	No. of Patients	CR	PR	Total	(%)
24	CY, CDDP BCNU ± L-PAM	19	1	10	11	(65)
25	BCNU, L-PAM	17	2	5	7	(41)
7	BCNU, L-PAM	58	6	28	34	(59)
26	CY, ThioTEPA	2	1	0	1	(50)
27	CY, ThioTEPA, L-PAM	1	1	0	1	(100)
28	CY, CDDP, L-PAM	10	1	5	6	(60)
28	CY, CDDP, ThioTEPA	4	0	2	2	(50)

[a]CY = cyclophosphamide; CDDP = cis-platinum; BCNU = carmustine; L-PAM = melphalan; thioTEPA = triethylenethiophosphoramide.

agents. Multiple courses of therapy have been tried in few patients without increased toxicity. No conclusions can be drawn with the small number of patients treated. The results of combinations of high-dose drugs have not been shown to be superior, in terms of response rate or duration of response, to high-dose single agents (7, 24-28).

A more practical approach would be to extend this type of therapy to patients with less extensive disease, but with a poor prognosis for cure. For example, adjuvant therapy for patients with stage II melanoma might be such a group of patients. While conventional therapy has not been shown to be of benefit in the adjuvant setting, it also has not shown significant benefit in patients with advanced disease. One of the prerequisites of adjuvant therapy is a demonstrated effectiveness in the patients with advanced disease. Since high-dose regimens appear to be effective in advanced disease, certain high-risk stage II patients might particularly benefit from adjuvant high-dose therapy and autografting. A prospectively randomized trial involving high-risk stage II (more than 5 positive nodes) patients is now underway at Duke University. A total of 25 patients have been enrolled; it is too early to analyze the results (W. Peters, personal communication).

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SESSION III - SOLID TUMORS

F. OVARIAN CANCER

HIGH DOSES OF MELPHALAN AND AUTOLOGOUS MARROW RESCUE IN ADVANCED COMMON EPITHELIAL OVARIAN CARCINOMAS: A Retrospective Analysis in 35 Patients

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INTRODUCTION

Common epithelial ovarian carcinoma is a frequently fatal malignancy in women usually diagnosed at an advanced stage. FIGO stages III and IV have a poor prognosis with generally a survival rate < 30% at five years (2)(3) even under therapy including debulking surgery and Cis-diamine dichloroplatinium (CDDP) (4)(5)(6)(7)(8)(9). Chemotherapy with alkylating agents has demonstrated activity in this disease; Melphalan given at a standard dosage allowed 47% response rate leading to a moderate improvement of survival (10). Dose response relationships have been demonstrated for alkylating agents including Melphalan (11)(12) whose main dose limitation is its hematological toxicity reversible with autologous bone marrow transplantation (ABMT) (13)(14)(15)(16). The data in these tumors prompted us to start a pilot study of high doses of Melphalan (HDM) followed by ABMT.

PATIENTS AND METHODS

Patients

Thirty-five patients between February 1981 and November 1986 with initial FIGO stage III (30 patients) or IV (5 patients) common epithelial ovarian carcinoma were studied, median age was 46 years (range 20 to 57) . All patients underwent debulking surgery, then

received chemotherapy including at least CDDP - Cyclophosphamide and Doxorubicine (CAP = 23 patients) for a median of 6 courses (range 2 to 12) or Hexamethylamine addition (CHAP = 12 patients).

A second look laparotomy was done in 32 patients at a median of 9 months, range (6 to 20) after diagnosis (three were not restaged because of clinical evidence of progressive disease). Results of initial therapy were evaluated according to residual tumor: 11 complete remissions (CR), 10 residual tumor $\leq$ 2 cm (PR $\leq$ 2 cm), 9 residual tumor >2 cm (PR >2 cm), 5 progressive disease (PD). Between second look and HDM, 3 patients progressed from CR or PR to PD. At time of HDM (median of 3 mo after the 2nd look surgery), 9 patients were treated as a consolidation of CR, 26 patients were treated for a persistent disease; 10 minimal residual disease (PR $\leq$2 cm) , 8 PR >2 cm, 8 progressive diseases.

Melphalan was given by IV bolus through a central venous line during hyperhydratation. Twenty patients received 140mg/m^2; 3 received 180mg/m^2, 10 received 200mg/m^2, 2 received 240mg/m^2. Marrow was collected as described by Thomas et al. (17) and infused 24 hours after HDM. Thirty-one patients received fresh marrow collected immediately before HDM. In 4 patients, marrow cells were cryopreserved with DMSO prior infusion to the patients. In all cases marrow was cytologicaly normal. Patients were managed in single isolated rooms with usual supportive care including blood product irradiation.

RESULT

Response

Out of the 26 patients with residual tumor, 12 patients were evaluable for tumor response to HDM, 3 by surgery and 9 by radiography. Seventy-five percent (9/12) had an objective response to HDM. The 4 complete responders were treated by HDM at 140 mg/m^2, out of the 5 patients who achieved a partial response (diminution of tumor size $\geq$50% for at least 4 weeks), 3 received 140 mg/m^2, 1: 180mg/m^2 and 2: 200mg/m^2. The 3 patients who failed to respond were treated at a dosage of 180 mg/m^2 (1 patient), and 240 mg/m^2 (2 patients).

Hematological Toxicity

All patients experienced aplasia after HDM. Prompt hematological recovery was observed in most patients without statistical difference between "fresh" and cryopreserved marrow (Table 1). Three patients had delayed platelets recovery (counts < 50 x 10^9/l) lasting more than 4 months.

Table 1. Hematological Toxicity

	<<Fresh>> Marrow Rescue	Cryopreserved Marrow Rescue	Total
Number of Patients	31	4	35
Infused Nucleated Cells /kg x 10^8	2 (0.2-4.2)	1.45 (1.4-3.7)	2 (0.2-4.2)
Days with Granulocytes <0.5 x 10^9/l	13 (6 - 45)	18 (9 - 41)	13 (6 - 45)
Days with Platelets <50 x 10^9/l	26 (13 - 300)	72 (16 - 270)	26 (13 - 300)

Extra Hematological Toxicity

The most frequent toxicity was gastrointestinal (mucositis, diarrhea): these complications occurred with a similar frequency in patients receiving HDM at a dosage > or < 180 mg/m^2 and were not influenced by the patient's tumor status. Patients were hospitalized for a median of 36 days (21-70) (Table 2). Three patients died from complications related to the procedure; 2 early deaths at 2 months (aspergillosis, viral hepatitis), one patient died at 10 months from acute myeloid leukemia.

Duration of Response and Survival

Overall, 19/35 patients are alive, 15 with a non evolutive disease, with a median follow up of 23 months (range 8 to 35 months) after HDM.

Eight patients were in progressive disease at the time of HDM : 1 died from toxicity, but with a residual tumor, 5 progressed and subsequently died at a median of 2.5 months (range 1.5 to 28 mo), 2 are alive with a non evolutive disease (NED) (>21, >31 mo); 1 received an additional course of HDM with marrow rescue 11 months after the first course. Out of the 8 patients who had tumor masses greater than 2 cm at the time of HDM, 1 died from infection with a residual tumor, 3 relapsed at 3, 4, 7 months and died at 10, 12, 14 months; 4 are alive NED between 8 and 35 months (median: *24* months); 1 of these patients received additional abdominal radiotherapy 5 months after HDM.

Ten patients had "small" residual masses ($\leq$2 cm) when they received HDM : 1 died from leukemia, 6 relapsed between 3 and 53

Table 2. Extra-Hematological Toxicity

| | MELPHALAN DOSAGE (mg/m2) | | | | | |
	140-180		200-240		Overall	
Number of Patients		23		12		
Absent or Discrete	17	(74%)	7	(58%)		
Mucositis						
Moderate or Severe	6	(26%)	5	(42%)	11	(31%)
Absent or Discrete	18	(78%)	8	(66%)		
Diarrhea						
Moderate or Severe	5	(22%)	4	(34%)	9	(25%)
Number of Patients with Bacteriemia	7	(30%)	4	(33%)	11	(31%)
Median of Hospitalization in Days (Range)	36	(21-65)	35	(30-70)	36	(21-70)

months; 6 patients are still alive -3 NED- with a median follow up of 22 months (range 13 to 54 months).

Nine patients received HDM as a consolidation of a complete remission : 3 relapsed (3, 4, 10 months), 6 are alive NED with a median follow up of 27 months (range 9 to 32) .

Duration of response and survival were not affected by the dose of Melphalan (inferior or superior to 180 mg/m^2). At the present time, the projected progression free survival (PFS) is not statistically different between the patients who received HDM while being in CR or PR $\leq$2 cm in comparison with patients who were in PR > 2 cm or in progressive disease (Figure 1) . Overall, actuarial survival is 47% at 53 months after HDM (Figure 2).

DISCUSSION

Our results confirmed the feasibility of HDM followed by ABMT with a high antitumoral response (75%) in poor risk patients with advanced ovarian carcinomas. This high therapeutic index for such patients confirms the relation between dose and antitumor efficacy in these tumors, which was previously reported with the use of CDDP (18). Few other data are available on the response to high doses of alkylating agents in ovarian cancer, especially after initial therapy by
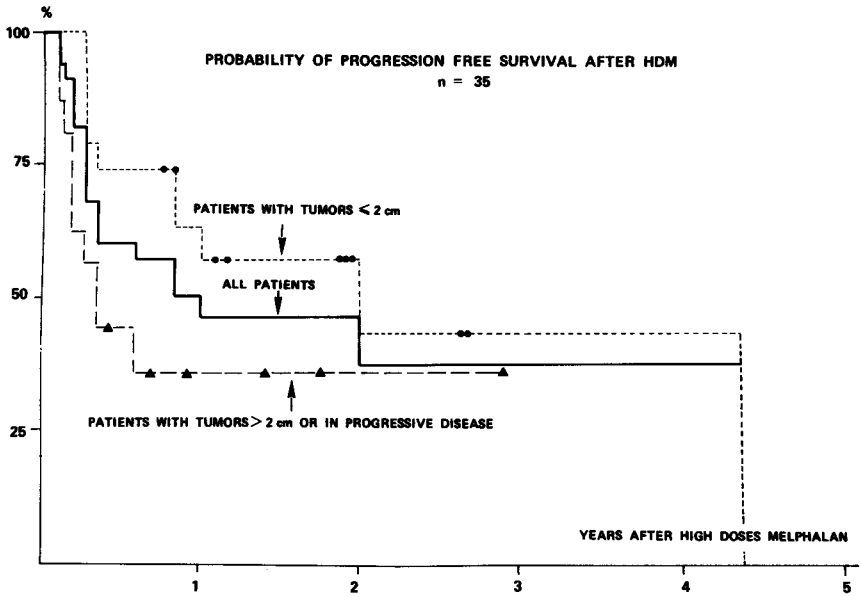
Figure 1. Probability of Progression Free Survival After High Dose Melphalan in Advanced Ovarian Carcinoma
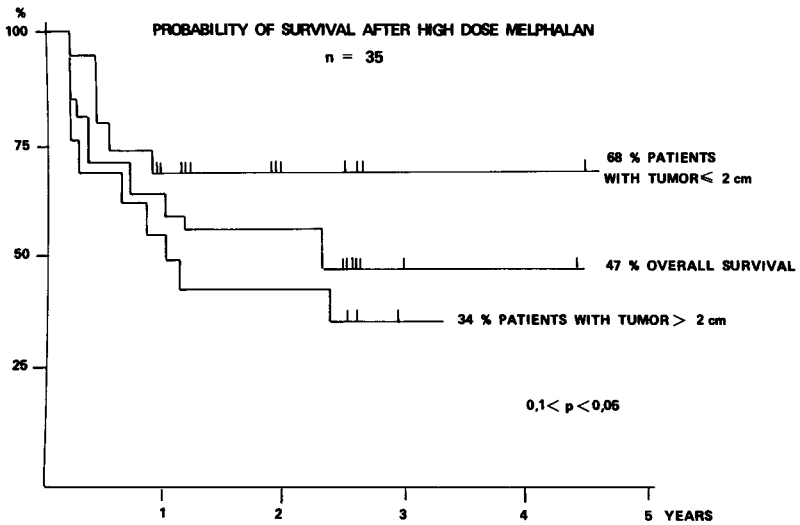
Figure 2. Probability of Survival After High Dose Melphalan in Advanced Ovarian Carcinoma

CDDP (14) (15) (13). Our data on the efficiency of HDM in ovarian carcinoma are comparable to previous reports in other refractory malignancies (13) (14) (15) (16). In this small series of patients it was impossible to distinguish any advantage in the close range between 140 mg/m^2 and 240 mg/m^2. The main adverse effect of HDM is the hematological toxicity reversible with the use of ABMT as well as mucositis and diarrhea but these side effects were never life threatening. However, 8% (3 out of 35) of the patients died from the procedure: 2 died of infection, 1 from secondary leukemia. The occurrence of secondary leukemia after Melphalan has been previously reported (20) (21). Duration of response was short in most of the patients who were treated at a stage of resistant disease: however 2/8 patients treated in progressive disease remain alive NED at 21 and 31 months. These patients were clearly benefited by the therapy HDM and ABMT. Thirteen of the 27 remaining patients are alive NED with a median follow-up 24 months after Melphalan. It is difficult to assess with certainty in this heterogenous group the beneficial effects of HDM and ABMT: however for the subgroup of patients who had tumors > 2 cm before HDM, the present results are definitively encouraging with 4/8 patients NED at a median follow up of 24 months. For other patients with more favorable clinical presentations (CR or PR ≤ 2 cm) the real impact of high dose Melphalan and ABMT on the progression free survival remains to be demonstrated prospectively in a larger cohort of patients in comparison with a more standard but intensive therapy (22).

Possibly the conditioning regimen will be improved by combining high doses of various alkylating agents (i.e. thiotepa) with carboplatin. This will be the focus of our efforts.

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SESSION III - SOLID TUMORS

G. PHASE I STUDIES

A PHASE I STUDY OF HIGH-DOSE CARBOPLATIN

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Emil Frei III, and William D. Henner

INTRODUCTION

The selection of chemotherapeutic agents for use in conjunction with autologous bone marrow transplantation (ABMT) is determined in large part by their toxicity and their ability to demonstrate a steep anti-tumor dose response curve (1,2). Drugs whose major side effects are myelosuppressive offer an advantage over those compounds where nonmyeloid toxicity is dose limiting. Carboplatin (Cis-diammine-cyclobutane-dicarboxylato-platinum 2, CBDCA, NSC 24120) is an active, second generation platinum containing compound whose major toxicity at standard doses has been myelosuppression (2,3). In contrast to the non-myeloid toxicities of cisplatin, CBDCA therefore offers the potential for significant dose escalation when marrow suppression is ameliorated with ABMT. We undertook a phase I study of high-dose CBDCA in order to determine the dose limiting toxicities, maximum tolerated dose, and pharmacokinetics of this agent when administered by continuous infusion with ABMT.

MATERIALS AND METHODS

Patient Selection

Patients with incurable malignancies who had normal bone marrow, liver, and renal function without evidence for CNS or marrow involvement with tumor were eligible for study. All requirements for institutional review were fulfilled.

TREATMENT REGIMEN

Patients received the appropriate dose of carboplatin by continuous infusion over 4 days. Dosages ranged from 375-2400 mg/m^2 with escalations following the successful treatment and evaluation of a minimum of three patients per dose level. Autologous bone marrow harvesting and re-infusion were performed by standard techniques with the institution of ABMT support following the appearance of >14 days of neutropenia and thrombocytopenia (1). Patients received ABMT reinfusion (N=15) 72 hours following completion of chemotherapy.

PHARMACOKINETIC ANALYSIS

Plasma samples were collected in EDTA containing tubes and total and ultrafiltrable platinum measured by flameless atomic absorption spectroscopy (4,5). Plasma area under the concentration curve (AUC) was calculated by the linear trapezoidal method and systemic clearance (CL_{ss}) calculated according to the relationship $CL_{ss} = DOSE/AUC$.

RESULTS

Thirty-three patients were enrolled in this study, 31 of whom are evaluable for toxicity and response (Table 1). There was one early toxic death and one patient was withdrawn due to tumor related hemorrhage after she received 20% of her projected dose.

Table 1. Patient Characteristics and Responses

No. of Patients:			N = 33
Age (Median):			41 yrs
Sex:			Male = 10
			Female = 23
Pts. with Autologous Bone Marrow Transplantation			N = 15
Prior Chemothrapy (Prior Cisplatin/Total)			20/31

Histology	Evaluable Patients[a]	Response	
		CR	CR+PR
Ovarian Carcinoma	11	1	6
Germ Cell Carcinoma	3		2
Breast Carcinoma	2		1
Small Cell Lung Carcinoma	1		1
Other[b]	14		0
Total	31	1	10

aOne pt. with ovarian carcinoma was inevaluable because of premature termination of treatment due to tumor-related hemorrhage after 20 hrs of chemotherapy. A second patient with plasmacytoma was inevaluable for response because of early death from candida sepsis. bTwo pts each with non-small cell lung carcinoma, cervical carcinoma, melanoma, mesothelioma, and soft tissue sarcoma: 1 pt. each with renal cell, rectal, osteogenic, and uterine carcinoma.
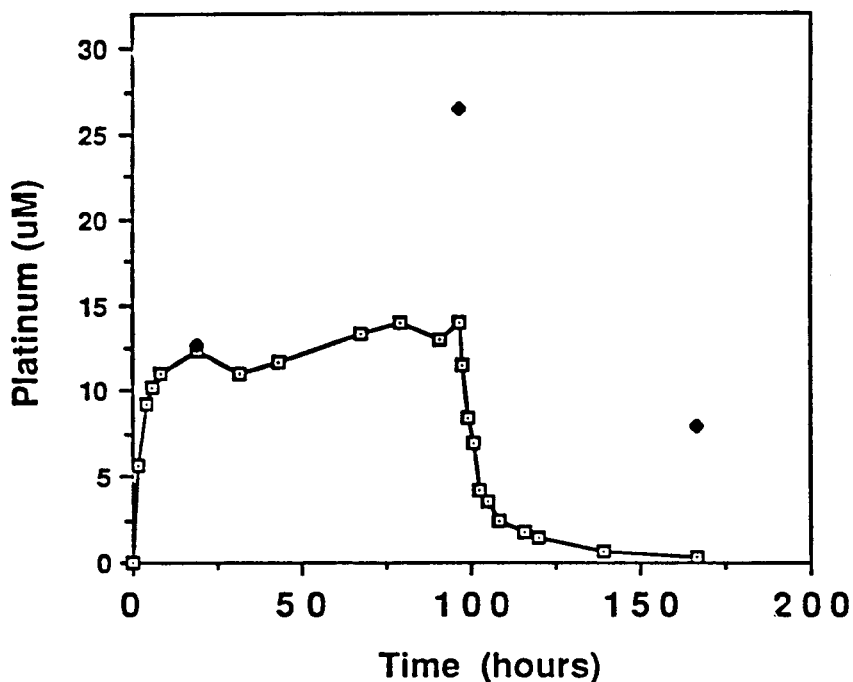
Figure 1. Time course of total ( ) and ultrafiltrable ( ) platinum for a patient treated with 2000 mg/m^2 CBDCA. Drug was infused over 96 hours and followed 72 hours later by bone marrow reinfusion.

PHARMACOKINETICS

Ultrafiltrable platinum concentrations as depicted by the AUC ranged from 221 uM hr to 1545 uM hr. There was a close correlation between AUC and dosage (r=.85, p<. 01) without evidence for saturation of CL$_{ss}$ at high dose. A typical plasma time course is shown in Figure 1, with an abrupt decline in steady state drug levels following completion of chemotherapy (t 1/2 elimination = 113 min). At the time of marrow reinfusion, intact "drug" never represented more than 5% of the peak CBDCA level.

TOXICITY

Hematologic toxicity was significant in all patients treated with 600 mg/m^2 and above (Table 2). Two patients who received 1600 mg/m^2 of CBDCA and all patients treated at higher doses received

ABMT. In this group of patients, the median duration of neutrophils < 500 was 22 days and platelets < 20,000 was 20 days. One patient died of neutropenia-induced Candida sepsis.

Dose-limiting non-hematologic toxicity (Table 3), consisted of hearing loss and hepatic toxicity characterized by biliary stasis and, at the two highest dosage levels, a moderate increase in serum transaminases. Hearing loss at 2000 mg/m^2 and above seemed to be more frequent in patients who had received prior cisplatin chemotherapy (4/6). Additional severe, but infrequent, toxicities are listed in Table 3.

RESPONSES

Thirty-one of the thirty-two patients who completed treatment were evaluable for response. There were 8 responses in patients who had previously responded to cisplatin containing regimens, one response in a patient who was refractory to standard dose cisplatin and one response in a patient with breast cancer which had not been previously treated with cisplatin. There was one complete response and while the median time to tumor progression was short (3 months), 2 patients underwent post treatment resection of residual tumor and experienced disease-free intervals of 9 and 12 months.

Table 2. Hematologic Toxicity

Dose MG M^2	Nadir Average (Range)/MM^3 x 10^3		Duration: Days (Range)	
	Neutrophils	Platelets	Neutrophils	Platelets
(N)				
<600(2)	3.1(2.6-3.6)	238(189-290)	0	0
600(4)	0.76(0-1.7)	19(1-54)	8(0-14)	8(0-13)
900(2)	0.22(0.5-.38)	<20	10(9-11)	6(8-24)
1200(5)	0	<20	13(11-18)	16(4-28)
1600 (6)[a]	0	<20	14(5-28)	16(4-28)
2000(10)[a]	0	<20	24(15-42)	21(10-44)
2400(3)[a]	0	<20	18(15-20)	18(15-22)
	All Transplant Patients: N = 15		22(5-42)	20(4-44)

[a]Two patients treated with 1600m^2 and all patients treated with higher doses of carboplatin received autologous bone marrow support.

Table 3. Non-Hematologic Toxicity

Toxicity		# Cases/Dose	
mg/m^2		$\leq$ 1600 mg/m^2	$\geq$ 2000
		N=19	N=12
[a]Hepatitis/Biliary Stasis	2	p=.002	7
Hearing Loss (> 50%)	1	p=.02	5
Renal (> 30% Decrease)	7	p=.15	9
Acute Renal Failure			1
Pneumonitis			1
Hemorrhagic Colitis			1
Optic Neuritis			1

[a] $\geq$ 3-fold increase in serum transaminase or bilirubin level.

SUMMARY AND CONCLUSIONS

Carboplatin is a clinically active second generation platinum containing compound producing myelosuppression as its major toxicity at standard doses. In the current study, the use of ABMT permitted a 5-fold dose escalation before non-myeloid organ toxicity was observed. The dose limiting toxicities were hepatic dysfunction, hearing loss, nephrotoxicity and isolated cases of acute pneumonitis, hemorrhagic colitis, optic neuritis, and acute renal failure. The high response rate, reproducible pharmacokinetics and acceptable toxicity reported in this study warrant the development of additional high-dose carboplatin containing regimens.

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**Discussion 5 - Session III,E,F,G
(Melanoma, Ovarian Cancer, Phase I Studies)**

Dr. Frei: Roger, when you combine drugs, you did have to sacrifice dose in varying degree. Were there any combinations in which full doses of the individual agents, on the basis of non overlapping toxicity, could be used?

Dr. Herzig: I do not know. We came fairly close with the melphalan and BCNU. It was 75% of the BCNU.

Dr. Philip: The most interesting finding is that when you combine drugs you do increase the response rate but did not increase the CR rate. Probably it is because when you combine the drug, you reduce the dose per drug and as a consequence you reduce tumor cell activity. I think your concepts -- to try to look at 2 BMTs -- (are) the only answer. Do you think it is better to go to double graft with several drugs or will you, as I will conclude from your report, go to single drugs?

Dr. Herzig: With some of these patients, with the extended disease that they have, it is unlikely that you are going to effect a cure. In fact one of the tasks should be that with the results in metastatic disease, such as this, it is sufficient to warrant an adjuvant study for high risk melanoma -- which in fact Bill Peters currently has ongoing. In terms of dose reduction and whether or not you get as good a response, when you look at the patients that got the maximum tolerated dose of a combination of melphalan and BCNU there response rate was not statistically different than the melphalan alone. So we did not sacrifice response rate and it was a limited success in that we at least got the same kind of duration of response with a tail on the curve. But you are right that if dose reduction is necessary, you may end up sacrificing response with that. Now in the thiotepa single agent study we did have a dose response relationship

we actually demonstrated a difference between patients treated with less than 900 compared to over 900. In general, the tact I think would be to combine probably 2 agents. So first, you can probably approximate a 100% of the dose.

Dr. Gianni: We are doing exactly what Thierry Philip suggested. We started 2 years ago with a new concept of protocols in which in order to not sacrifice the dose, we use single agent -- the maximum tolerated dose in schemes in which you sequentially add the second drug as soon as possible after the first. The prototype of such a schema is used in Hodgkin's disease, with which we have treated 24 patients. We use 7 g/m^2 cyclophosphamide then we add vincristine and methotrexate 8 g/m^2 plus citoforum rescue plus platinum and then we finish the scheme with TBI and melphalan.

Dr. Herzig: If you have to accept significant dose reduction in combination and there is a very steep dose response in the tumor, as there maybe in the melanomas, you would not want to make the combination and sacrifice the dose. On the other hand, you give up any possibility of a synergistic interaction if you do not use combinations. The problem in melanoma is that other than the melphalan, BCNU, regimens we have not examined the maximum tolerated dose of the combinations. Combinations have been tried but they have not been escalated to their maximum. Right now the best you could do is to do single agents and multiple transplants with single agents.

Dr. Frei: The issue of dose and combinations is an extremely important one. In addition to using single agents in sequence there is the possibility that certain agents may be combinable without compromising dose. I think that is important to explore as well.

SESSION IV - PEDIATRIC TUMORS

A. NEUROBLASTOMA

NB 87--A NEW PROTOCOL USING AN ALTERNATING NON-CROSS-RESISTANT INDUCTION REGIMEN AND TWO DIFFERENT MODALITIES OF MASSIVE CONSOLIDATION CHEMOTHERAPY IN TREATMENT OF METASTATIC NEUROBLASTOMA IN CHILDREN

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INTRODUCTION

Stage IV neuroblastoma in children over 1 year of age remains one of the major therapeutic challenges in pediatric oncology. Despite advances in conventional regimens (1-3) and the recent introduction of high-dose chemotherapy or chemoradiotherapy with bone marrow rescue, the long term outcome remains poor (4-8). One of the obstacles for progress in the treatment of this disease is the difficulty in comparing results of protocols from different centers and from different countries. This is largely because of a lack of uniform criteria for diagnosis, for staging and for determining response to therapy (9) which will hopefully be overcome by an international consensus (10).

Despite our strong commitment to use consolidation regimens with bone marrow transplantation (BMT) in the treatment of advanced neuroblastoma, it is of note that with the previous protocol used, 17% (7) and 46% (8) of the patients with metastatic disease at diagnosis could not be grafted. This is mainly due to the failure of the induction regimens and surgery to obtain sufficient tumor debulking prior to the use of high dose chemotherapy (HDC) or HDC + total body irradiation (TBI).

The neuroblastoma working group of the Société Francaise d'Oncologie Pédiatrique (SFOP) is mainly composed of two

subgróups: the LMCE group (Lyon, Marseille, Curie Paris, East of France) which developed a consolidation regimen using HDC + fractionated TBI and rescue with autologous bone marrow transplantation (ABMT) purged with immunomagnetic procedure described by J. Kemshead and modified by M. Favrot (7), and the IGR group (Institut Gustave Roussy) which developed consolidation regimen based on HDC with alkylating agents and rescue with ABMT purged with Asta-Z (8). Both groups are also involved in the design of multimodal chemotherapy induction regimens and phase II studies (4, 11-12).

Our working hypothesis (for the past 6 years and the years to come) is that the requirements for the improvement of the overall prognosis of metastatic neuroblastoma are:

- an early and good quality remission obtained with chemotherapy and surgery

- an improved detection of residual disease

- the use of HDC (with or without TBI) as soon as remission is obtained

- the use of purged ABMT as hematologic rescue.

The general aim of our study is to improve the actuarial survival and the progression free survival of patient with metastatic neuroblastoma.

To increase the number of patients achieving complete remission (CR) 4 months after diagnosis, we designed a protocol using an unique chemotherapy induction regimen composed of 4 cycles alternating vincristine-cyclophosphamide-doxorubicin (CADO) and a high dose of cisplatinum and etoposide (CDDP-VP16). Collection of data concerning treatment response, treatment related morbidity and surgical procedure is unified, using the criteria of the Forbeck classification (10). Following this induction period, we will study the value of two different early consolidation regimens.

Rationale for a Short Induction Regimen
Alternating High Dose CDDP/VP 16 CADO

The status of induction regimens in our group is as follows:

1. We have reported the effect of an induction regimen made of six cycles alternating cisplatinum (CDDP) (100 mg/m^2 d 1) + VM 26 (160 mg/m^2 d3) with CADO (including VCR 1.5 mg/m^2 D1 and 5, cyclophosphamide 300 mg/m^2 d1 to d5 and doxorubicin 60 mg/m^2 d5) in a group of 35 consecutive unselected patients (4). We observed a 96% response rate with a 68% good partial response

(GPR) rate. These results are similar to that previously reported by Shafford et al (3) using the OPEC regimen - similar regimen without doxorubicin, lower doses of CDDP and VM 26 - and by Hartmann et al (8) with the CADO regimen. We think however that our extended evaluation of the bone marrow does not allow a comparison with the OPEC study. On the other hand it should be noted that 68% of the patients under study reached a disease-free bone marrow status as compared with 40% when using the only CADO regimen. Only one patient progressed under this treatment (3%) as compared to 18% with the CADO regimen (Table 1).

The most disappointing result was that when one considers the remission status (integrating the response induction therapy and the quality of the surgical removal of the primary), only 17% of the patients reached complete remission (4) and we know that the majority of long-term survivors come from this small subgroup of patients. For this reason we tried to set-up new regimens in order to achieve a better CR rate.

2. Results of the association of VP 16 with high dose CDDP: we first reported a high response rate with high dose combined CDDP and VP 16 heavily pretreated children with either relapsed disease or partial remission after a conventional induction regimen (12). Twenty of the patients had received CDDP prior to this trial and the response rate in this subgroup was not different, suggesting a dose effect. We then studied this association of CDDP and VP 16 as first line treatment in order to assess the response rate and the morbidity with two courses of such a regimen (13). According to strictly defined criteria, 70% of the patients showed a partial response and extensive marrow evaluation showed complete clearing from tumor cells in 40% (Table 2).

Table 1. Results of Alternate Versus Repetitive Cycles

	NB83 PE-CADO x 3 n = 37 JL Bernard et al J Clin Oncol 1987	CADO x 6 n = 42 O Hartmann et al J Clin Oncol 1987
Progression	3%	18%
Response Rate on the Primary	96%	77%
Cleared Bone Marrow	68%	40%
Post-Surgical	43%	38%
Remission		
(CR + VGPR*)	(17 + 26)	

Very good partial remission.

**Table 2. Results of 2 Courses of High Dose
Cisplatin and VP-16 as Induction Regimen**

O. Hartmann et al J Clin Oncol 5:1205-11 1987

n = 17 patients	
- More than 50% reduction of the primary site	50 %
- Normal catecholamine excretion	94 %
- Cleared bone marrow	40 %
- Overall response rate	70 %

RESULTS OBTAINED IN TWO MONTHS

These response rates are comparable to those obtained with other CDDP containing chemotherapies like OPEC (3) or PE-CADO (4) or with doxorubicin containing regimens like CADO (8) or MADDOC (5). The striking feature of this high-dose regimen is the rapidity of response with significant reduction of disease after only two courses. Furthermore, audiotoxicity and nephrotoxicity were in the acceptable range (13). This combination has since been tested with an alternating cross resistant regimen with high dose ifosfamide, vincristine and doxorubicin (14). The results showed (in 51 patients) a CR + VGPR rate of 40% comparable with that observed with CADO or PE-CADO but a higher CR rate of 30% (to be compared with 17% after PE/CADO) was obtained within 3 months. However 2 toxic deaths following administration of this combined regimen prompted us to come back to CADO as the non cross resistant regimen of choice (no toxic death with PE-CADO).

Is it Possible to Reduce the Toxicity
of High-Dose CDDP-Containing Regimen?

Our first report on the use of high-dose CDDP and VP 16 in previously untreated patients focused on the possible nephro- and audiotoxicity of this regimen (13). The glomerular filtration rate (GFR) as assessed by creatinine clearance was impaired in 7 of 15 patients. Similarly, formal audiometric assessment of nine children revealed characteristic high tone loss in seven patients. The experience obtained during the ENSG III C trial confirmed our findings and allowed us to estimate that 1/3 of the patients are exposed to a risk of 30% reduction of the GFR (14).

Pharmacokinetic data concerning continuous infusion of CDDP

have been accumulated (15). However clinical results for large groups of patients comparing toxicity and efficacy of CDDP administered in bolus versus continuous infusion are not available. Since preliminary data suggest a possible lower toxicity of continuous infusion of CDDP (16), we designed a randomized trial comparing the renal and audiologic toxicity in patients receiving CDDP in bolus or continuous infusion (Figure 1).

PUSH ADMINISTRATION

	DO	D1	D2	D3	D4	D5	D6

VP 16 100mg/m2

CDDD 40mg/m2

CONTINUOUS ADMINISTRATION

VP 16 100mg/m2

CDDP 200mg/m2

HYPERHYDRATION

Figure 1. High-Dose Cis Platinum and VP 16

We will assess creatinine clearance prior to CDDP therapy and after 1 and 2 courses and compare GPR impairment in both groups. The accrual of 108 patients will be necessary to show a 50% reduction of renal toxicity. We will need two years to include this number of patients. Secondary criteria for the evaluation of the CDDP toxicity will be:

1 – the audiometric study of patients before and after the two courses
2 – the hematological toxicity

Why to Propose Different Consolidation Approaches in the Treatment of Metastatic Neuroblastoma?

It has been shown by J. Pritchard (17) and confirmed since (7, 8, 18) that consolidation regimens are necessary to improve the actuarial survival of patients with metastatic neuroblastoma. However if the radiosensitivity of neuroblastoma has been emphasized (19) and the TBI conditioning regimen used in the different settings (5, 6, 7) the late effects of these regimens remain of concern in these patients who are mainly under 5 years of age at diagnosis. For this reason we think that it is logical to pursue two different approaches of consolidation, one using TBI, the other not.

Similarly we have been working on two different purging methods; one using a cocktail of monoclonal antibodies and magnetic beads and the other Asta-Z. These methods and the rationale for using them will not be discussed here, they are the subject of a presentation by M. Favrot in the same meeting.

In the current study, after a unique induction regimen, standardized surgery and assessment of remission procedure (9, 10) patients depending on the institution where they are treated will receive either the LMCE consolidation or the IGR consolidation regimen. In each of these two approaches, CR and PR deserve a different protocol.

A--The LMCE Consolidation Protocol (Figure 2)

CR patients of the current study will be treated following the intensive therapy regimen for consolidation which has been previously used in the LMCE group since January 1983 (NB 83) regardless if a CR or a PR status was achieved after the induction phase (7). The rationale for this consolidation treatment has been exhaustively described in a previous report (20) and a Phase II study conducted in our group (21) provided the basis for the use of this protocol in NB 83. The 14 CR VGPR patients of NB 83 protocol showed an encouraging probability (44%) of non-progression at 24 months postgraft (32 months post diagnosis) (7).

```
                    POST-INDUCTION STAGING

                CR                        PR

          Vincristine                  BCNU
          MELPHALAN                    VM 26
          Fract. TBI                   CBDCA

                                            2 mo.
      1 CONSOLIDATION               Vincristine
         REGIMEN                    MELPHALAN
                                    Fract. TBI

                                   2 CONSOLIDATIONS
                                       REGIMEN
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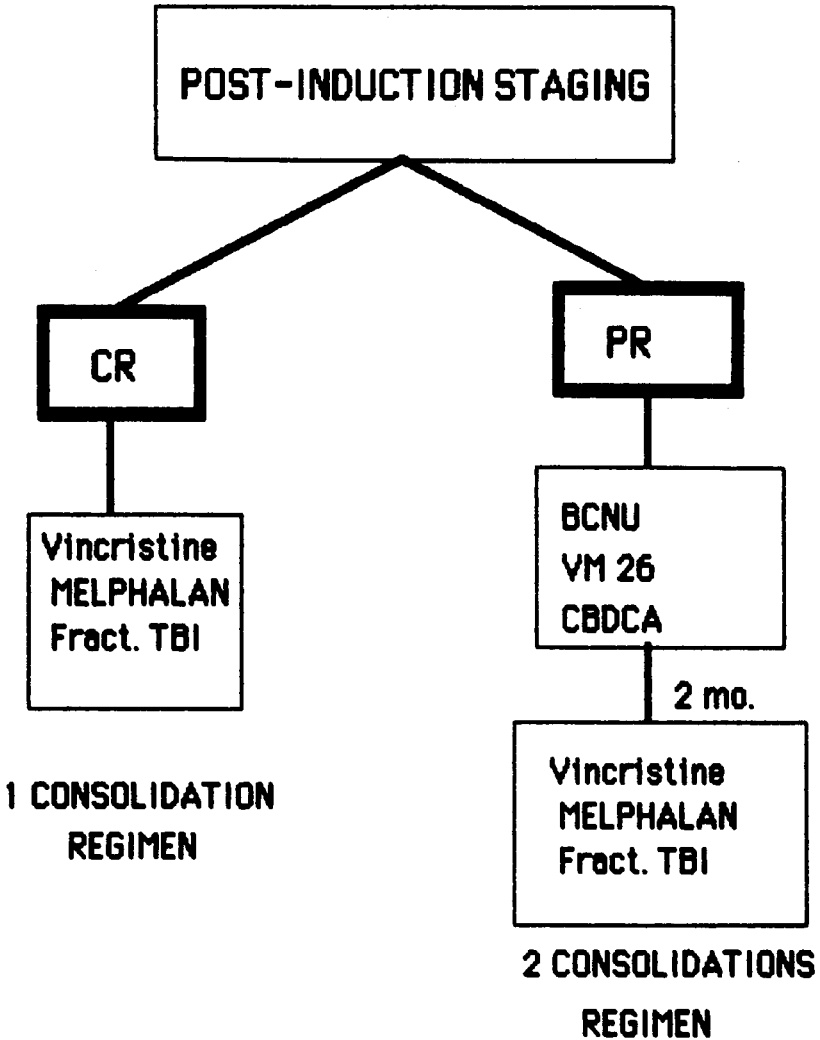
Figure 2. LMCE Consolidation Protocols

In spite of the fact that there was no significant difference between the probability of non-progression at 24 months postgraft in the group of PR compared to CR VGPR patients in the NB 83 protocol (7), we chose to reinforce the consolidation of PR patients in NB 87: PR patients will be treated following an alternate scheme based on 2 successive intensive therapies, 2 months apart, the rationale of which will be detailed elsewhere in this meeting by T. Philip.

B--The IGR Consolidation Regimen (Figure 3)

The use of TBI appears logical according to published results concerning both in vivo and in vitro studies (19). However knowing the delayed complications of TBI especially in children (22), it was chosen to use a multi-drug consolidation regimen excluding TBI. This regimen is based on a likely dose-effect relationship especially in a situation of minimal residual disease. Guidelines for the choice of the drugs combined in a high-dose regimen were:

- preferentially to introduce drugs not already used in the previous conventional chemotherapy: melphalan rather than cyclophosphamide, VM26 rather than VP 16.

- to combine alkylating agents like melphalan and carmustine (BCNU) or busulfan and cyclophosphamide (CTX).
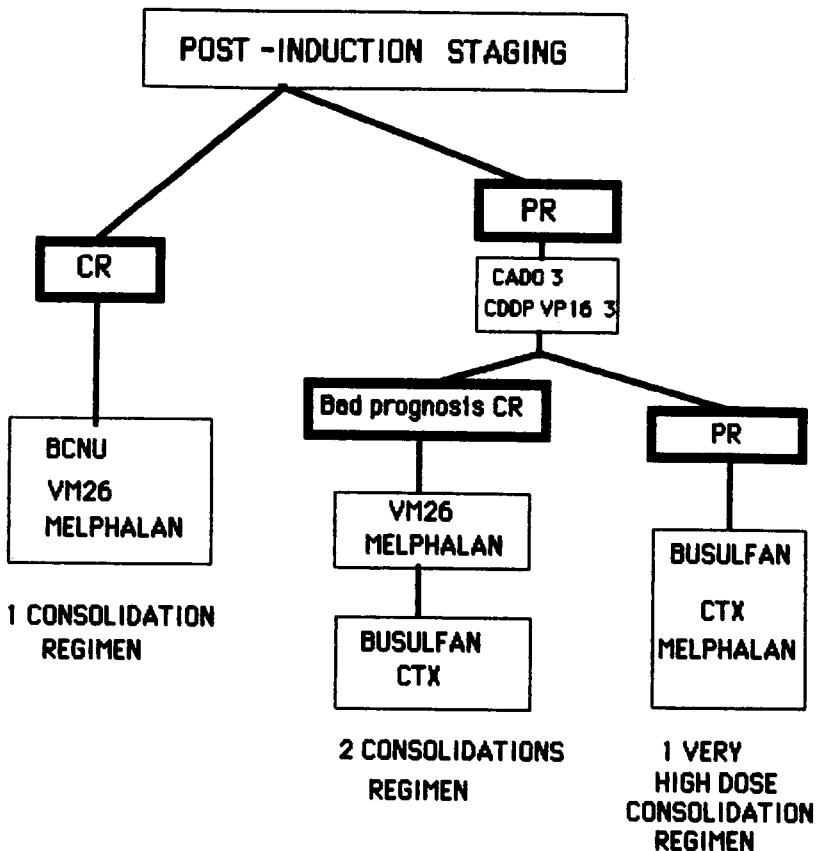
Figure 3. IGR Consolidation Protocol

In our experience with the consolidation regimens for metastatic neuroblastoma:

1) The results of the association of BCNU-VM26-Melphalan repeated twice have been published (8). All patients treated were in CR or VGPR.

   Our results showed that:

   - the actuarial relapse-free survival in grafted patients is 45% 2 years after diagnosis versus 25% in the whole non-selected population.

   - results are better (although not statistically significant) in "good responders" -- patients in CR after a simple 6 CADO cycles regimen -- than in "bad responders" to the first line chemotherapy (needed the use of high-dose CDDP + VP 16 in order to achieve CR).

   - half of the patients received only one intensification regimen because of early relapse, toxic death or heavy toxicity.

   - The superiority of one versus two consecutive regimens has not been proven. Therefore it was thought that one consolidation of this type can be proposed as consolidation regimen for "good responders" who achieve CR after two cycles of CDDP VP 16 and CADO.

2) The association of busulfan and CTX has shown measurable effects in relapsed patients (11). Therefore we think that it is a treatment of choice as part of a new therapeutic approach in the treatment of "poor responder" patients who cannot achieve CR after 2 cycles of CDDP-VP16 and CADO but achieve CR after an additional cycle. These patients, considered as bad prognosis CR, will undergo a double graft program using two non-cross reacting combinations: VM 26+ melphalan (Figure 4) followed by busulfan and CTX after 2 months rest (Figure 5).

3) Patients who do not achieve CR after 3 completed cycles of high dose CDDP-VP16 and CADO have very little chance to be cured with the conditioning regimens described above. The experience of the LMCE group showing encouraging results in such patients using a vincristine, melphalan, TBI conditioning regimen prompted us to try very high dose combinations associating busulfan, CTX and melphalan (Figure 6). This part of the study is aimed at studying the feasibility of this protocol, its efficacy and the duration of the induced response.

D-7 D-6 D-5 D-4 D-3 D-2 D-1 D 0

VM26
250mg/m2

MELPHALAN
180mg/m2

AUTOLOGOUS BONE MARROW RESCUE

HYPERHYDRATION
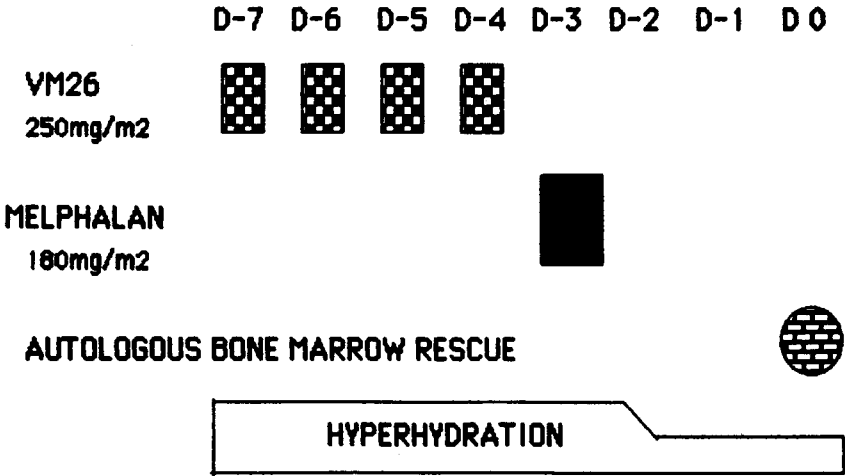
Figure 4. IGR "Poor Responders" Consolidation No. 1

D-8 D-7 D-6 D-5 D-4 D-3 D-2 D-1 D 0

BUSULFAN
150/mg/m2

CTX
2.2 g/m2

UROMITEXAN
2.7g/m2

AUTOLOGOUS BONE MARROW RESCUE

HYPERHYDRATION
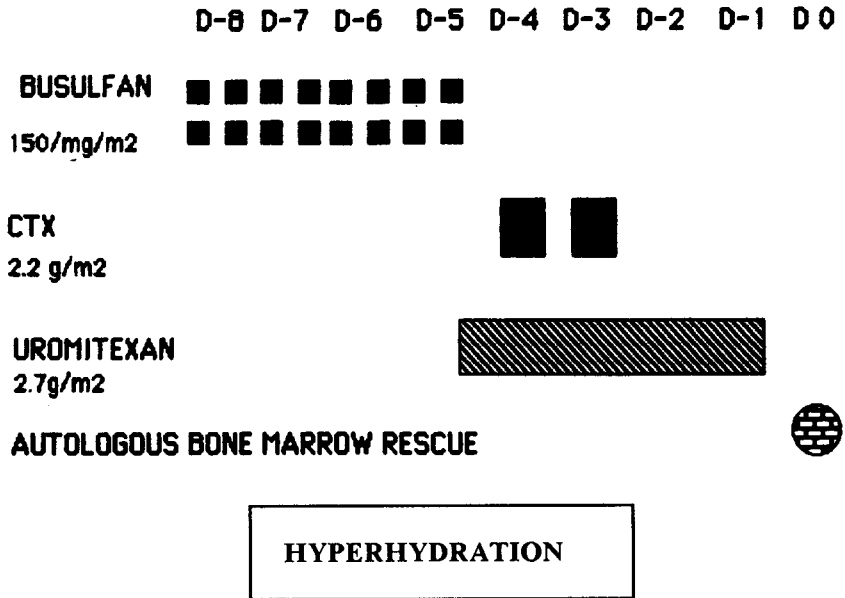
Figure 5. "Poor Responders" IGR Consolidation No. 2
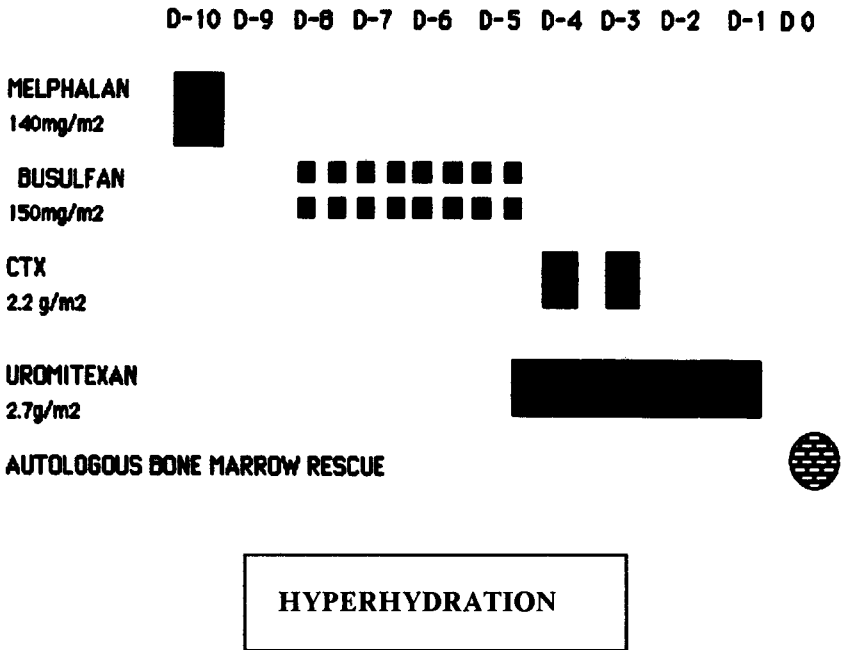
Figure 6. IGR "Consolidation" Regimen for Continuing PR

CONCLUSION

Our aim is to improve the overall progression free survival of children over one year of age diagnosed with metastatic neuroblastoma. We think that this newly designed protocol will help:

1) to explore the efficacy of a regimen using a non-cross resistant multidrug chemotherapy containing high dose CDDP.

2) to discern if audio and nephrotoxicity of CDDP can be reduced by continuous infusion using a randomized trial.

3) to compare in a non-randomized study two comparable cohorts of patients treated with a consolidation regimen with or without TBI.

We recognize, however, that new approaches continue to need development. For this reason, we designed inside the SFOP two phase II trials based either on chemotherapy or on the use of recombinant interleukin 2 and LAK cells. We are currently testing the efficacy of GM-CSF in the double graft program of the LMCE consolidation.

Future studies will allow us to assess the efficacy of monoclonal antibodies and interferons.

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HIGH DOSE CHEMO-RADIOTHERAPY WITH BONE MARROW RESCUE IN STAGE IV NEUROBLASTOMA: EBMT Survey 1988

C. R. Pinkerton, O. Hartmann, G. Dini, and T. Philip

Phase II studies of dose escalation with high dose Melphalan, TBI and other drug combinations demonstrated high response rates in relapsed and refractory disease[1]. Encouraged by these results several groups have investigated the role of this approach as a component of first line therapy for children with metastatic disease. Between 1982 and 1987, 229 children have been registered with the EBMT Solid Tumour Registry, having received megatherapy as consolidation of first line therapy. This is not a complete registry of all European transplant centers but reflects the activity of the majority of pediatric units.

The regimens given are given in Table 1. Patients' ages range from 1 to 21 years, median 3-1/2 years. The initial induction regimens included Cisplatin, Etoposide, Ifosfamide, Cyclophosphamide, Adriamycin and Peptichimio. Megatherapy was given to 119 patients in complete remission or very good partial remission (VGPR). The latter is defined as complete clearing of all metastatic disease with the exception of non-biopsiable positive bone scan sites and less than 10% residual disease at primary site following surgical intervention. Of the 119 patients, 110 were in partial remission, i.e. had shown a greater than 50% reduction at all sites but had significant residual disease excluding them from the VGPR category.

Autologous bone marrow rescue was used in the majority of cases. In 85% of patients the bone marrow was purged using either monoclonal antibodies and magnetic beads or Asta Z. Of these, 62 patients had two high dose procedures using either bone marrow harvested prior to the first procedure, divided in two and cryopreserved or a second harvest following the first high dose procedure. The indications for a double procedure differed between

Table 1.

Megatheray regimens used

    Melphalan - VCR - TBI   84

    Melphalan - BCNU - VM26   89

    Melphalan   12

    Melphalan - VP16 - Carboplatin - VCR   3

    Other 31 (19 included TBI)

Double procedures

    CDDP - VM26 - BCNU   24
                 +
    VCR - Melph - TBI

    Melph - BCNU - VM26   38
        x 2

VCR   = Vincristine
CDDP = Cisplatin
TBI   = Total body irradiation.

centers and were done either as an elective procedure for all patients or only for patients not achieving CR or VGPR following conventional induction[2]. Because of the latter group, this cohort of patients has a high proportion of poor risk patients. The use of total body irradiation was center dependent and was used in 126 patients. In 113, combined chemotherapy alone was given. The decision to use TBI did not reflect initial disease response.

Thirty-eight patients (16.5%) died from treatment related toxicity. One third of these were due to pneumonitis of which 30% were cytomegalovirus. One third suffered from veno-occlusive disease. Neither of these complications showed any clear correlation with the use of total body irradiation.

The overall progression free survival for the 229 patients is shown in Figure 1.

The actuarial survival is 23% with a median observation time of 21 months. Although some centers electively entered all patients into the megatherapy program irrespective of disease response, a number of patients who had persisting bone marrow disease following induction were excluded. This survival curve is therefore not completely representative of the population as a whole. The influence of disease status at the time of high dose therapy is shown in Figure
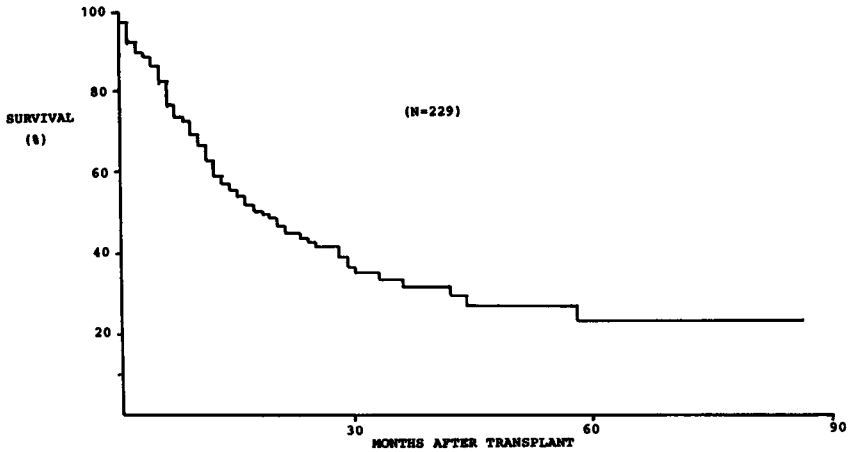
Figure 1. Overall survival of children with metastatic neuroblastoma treated with high dose chemoradiotherapy as consolidation of complete or partial remission.
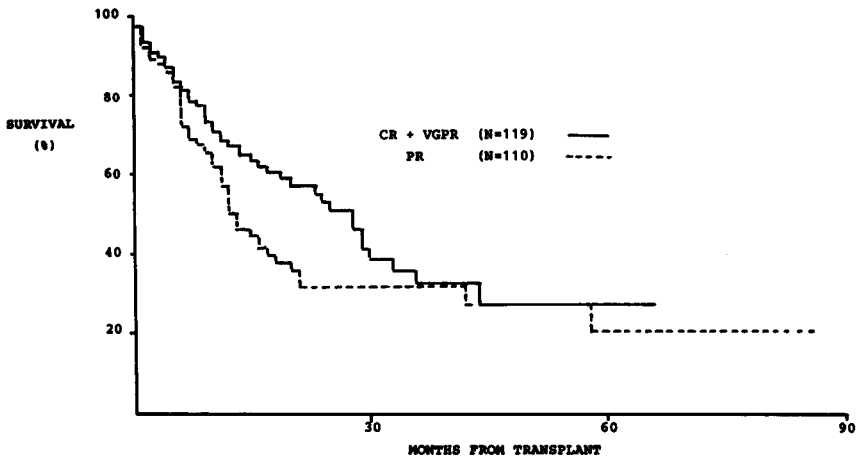
Figure 2. Survival in relation to disease status at the time of high dose therapy.

2. There are no significant differences between the two curves although the median time to relapse for the patients treated in PR was 14 months compared to 28 months for those treated in CR or VGPR.

The influence of inclusion of TBI in the high dose regimen is shown in Figure 3. There is no significant difference between the curves and this applied both to patients in CR/VGPR and PR. The use of double graft procedures appears to have had little impact on progression free survival as shown in Figure 4, although as mentioned earlier the double graft patients were to some extent selected as poor risk patients.

These data from the EBMT Registry represent by far the largest compilation of patients with neuroblastoma treated with megatherapy. Allowing for the inevitable problems of multi-center registration, some conclusions may be drawn.

The overall progression free survival was rather disappointing with median progression free survival post transplant of only 17 months. A plateau appears to be present at around 4-5 years after high dose therapy. The curve seems therefore to have been shifted to the right compared to historical experience where although the median survival was around 12 months, by 30 months over 90% of the patients had relapsed[3,4].

This confirms the observations in the randomized ENSG study of high dose Melphalan versus no treatment[5]. It cannot be concluded from these data whether the addition of agents such as TBI or other drugs adds to the impact of high dose Melphalan alone. The ENSG study was a highly selected group of patients who had cleared all metastatic sites prior to megatherapy whereas the present series includes a high proportion of patients with significant residual disease at the time of megatherapy. A more detailed breakdown of the two
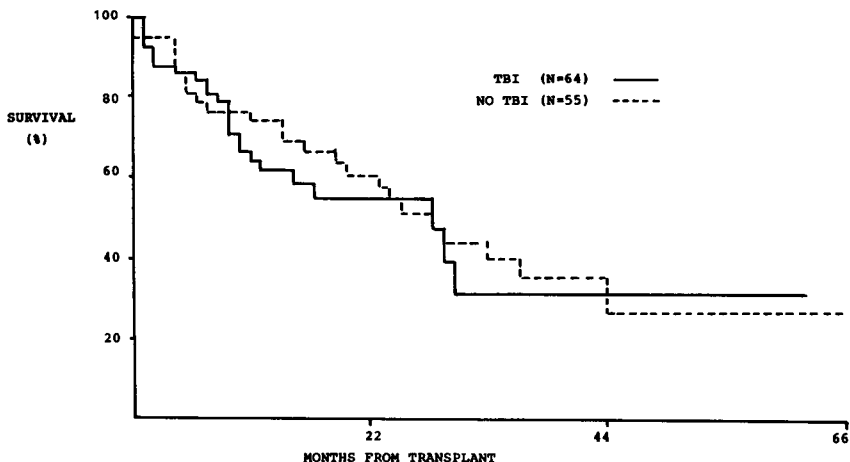
Figure 3. Survival in relation to the inclusion of total body irradiation in the high dose therapy regimen.
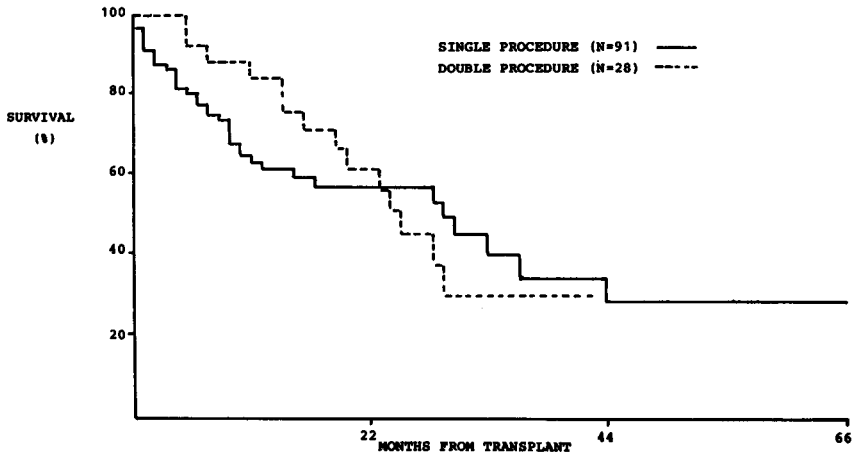
Figure 4. Survival of patients who received a single high dose therapy regimen compared to those in whom a second procedure was used.

study group patients may however allow such a comparison to be drawn. There are clearly major limitations on further advances in the use of this therapeutic approach in neuroblastoma. There are few new drugs available but currently of interest is the inclusion of high dose Carboplatin. Up to 2 grams/m^2 may be combined with Melphalan thus enabling significant dose escalation of the Platinum compound. The substitution of I 131 labelled MIBG for TBI is another approach. This may allow significantly higher doses of irradiation to be delivered to residual tumor sites in combination with high dose chemotherapy but avoiding the additive organ toxicity associated with TBI.

The role of purging cannot be addressed by this review but using current megatherapy regimens, allogeneic procedures appear to have a similar outcome, although patient numbers are small. This suggests that tumor reinfusion may be of secondary importance compared to residual disease at other sites.

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AUTOLOGOUS BONE MARROW TRANSPLANTATION WITH AN IMMUNOMAGNETICALLY PURGED BONE MARROW IN NEUROBLASTOMA: Analysis of 59 Single ABMT and 32 Double ABMT

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More than 90% of children with stage IV neuroblastoma (NB) have bone marrow (BM) involvement at diagnosis. All these patients are transplanted; only 1/3 reach complete remission (CR) with conventional therapy, and 2/3 enter the autologous bone marrow transplantation (ABMT) program in partial remission (PR) with BM persistent micrometastases on the day of BM harvesting (1,2). Purging of the autologous BM thus appeared to be a logical approach. The most commonly used method in NB is the immunomagnetic depletion (3). This method has been modified by several groups, including ours (4,5). We will review here 123 cases of ABMT in NB with an immunomagnetic purged BM and analyze the toxicity of the purging procedure, the hematological and immunological recovery after ABMT, the detection of NB cells in the autograft and their elimination by the purging method. Fifty-nine patients, either in 1st CR (19 patients) or PR (40 patients), entered the LMCE single autograft protocol with the BM harvested and purged after induction therapy, 2 to 3 weeks before high dose chemotherapy. Thirty-two patients had a double ABMT with BM harvested and purged twice (64 cases) (see T. Philip: this conference); the second autograft was harvested and purged between the 2 courses of high dose chemotherapy. Among these 32 patients, 8 were in sensitive relapse (SR); 8 were in PR after induction therapy using the LMCE protocol and 14 were referred from other institutes while in PR after conventional induction.

MATERIALS AND METHODS

BM harvesting and purging have already been described (1,4,5). Briefly, after harvesting BM cells were separated on Ficoll, washed, re-suspended in PBS at 20×10^6 cells/ml, incubated at 4°C for 30 minutes with 5 anti-NB MoAbs kindly provided by J. Kemshead (UJ13A, UJ127.11, H-11, αThy-1, UJ181.4, at appropriate dilution), washed twice and incubated with magnetic immunodisperse 4-5 μm polystyrene microspheres (Dynal ME450) at the concentration of 2 mg/ml and for 10×10^6 BM cells and pushed through a magnetic system. The last two steps (bead incubation and magnetic separation) were repeated twice. Samples were then re-suspended in appropriate medium for the freezing procedure. Toxicity to progenitor cells was evaluated by the clonogeneic efficiency in the CFU-GM method.

Immunological Follow-up

T cell ratio and percentage of NK cells were evaluated by simple immunofluorescence analysis on a microscope. The proliferative index of T cells after 5 days of PHA-stimulation at 0.5/ml was measured by thymidine incorporation. IL2 secretion by PBL after 72 hours of stimulation with PHA was measured by the capacity of the supernatant to induce the proliferation of the IL2-dependent cell line, CTL2, compared to a standard medium. NK function was measured in a ^{51}Cr release assay on K_{562} cell line (40:1 ratio).

BM Micrometastases

BM micrometastases were detected by the analysis of 4 trephine biopsies and 4 aspirates performed on the day of harvest (6). In parallel, residual NB cells in the autograft were detected using a double immunofluorescence (IF) analysis in cell suspension before and after the purging. Briefly, 3 samples of 1×10^6 mononuclear (MN) cells were incubated either with UJ13A, H-11 or 11.14 (J.C. Laurent) (IgG_{2a} anti-NB MoAbs) in combination with an anti-panleucocyte MoAB of IgM isotype (G. Janossy); TRITC antimouse IgM and FITC antimouse IgG were used as second layer. NB cells appeared stained in green whereas normal cells which were specifically stained by the anti-NB MoABS appeared both in green and red (7,8).

RESULTS

As summarized in Table 1, the immunomagnetic purging procedure is very weakly toxic to BM precursors as evaluated by their in vivo clongeneic efficiency ($CFU-GM/2 \times 10^5$ MN cells), but the loss of BN cells is substantial; recovery of both MN cells and total CFU-

Table 1. Total BM Mononuclear Cells and CFU-GM Harvested and Re-Injected in Immunomagnetic Depleted ABMT

| | HARVESTED BM | | | | | | RE-INJECTED BM After Thawing (median range) | | |
| | Before Purging (median range) | | | After Purging (median range) | | | | | |
	MN cells/kg $\times 10^8$	CFU.GM/kg $\times 10^4$	CFU.GM /2×10^5 MN cells	MN cells/kg $\times 10^8$	CFU.GM/kg $\times 10^4$	CFU.GM /12×10^5 MN cells	MN cells/kg $\times 10^8$	CFU.GM/kg $\times 10^4$	CFU.GM /2×10^5 MN cells
LMCE Single Graft (59 pts)	2.82 (0.65-6.96)	28 (0-99)	260 (0-800)	0.71 (0.2-3.15)	5.55 (0-42)	174 (0-600)	0.53 (0.12-2.03)	3.20 (0-17)	160 (0-420)
RECOVERY				24%	21%				
LMCE - Double Graft (8 pts)									
1st graft	2.22 (0.19-4.77)	14.4 (1.14-131)	324 (105-557)	0.85 (0.41-1.70)	5.5 (1.14-10.8)	160 (105-386)	0.41 (0.25-0.82)	2.6 (0-11.7)	208 (0-500)
RECOVERY				38%	38%				
2nd graft	2.34 (1.78-4.13)	28.6 (12-62)	240 (135-400)	0.85 (0.29-1.41)	5 (1.7-9.6)	135 (45-160)	0.6 (0.4-1.32)	1.77 (0.7-3.65)	50 (30-218)
RECOVERY				30%	18%				
Non LMCE (24 pts)									
1st graft	3.16 (0.93-6.3)	31 (3.3-84.6)	240 (24-500)	0.85 (0.10-4)	6 (0.7-13.8)	180 (18-280)	0.48 (0.13-1.88)	3.4 (0-14)	140 (0-500)
RECOVERY				36%	24%				
2nd graft	3.25 (1.4-6.67)	27.4 (4.1-75)	175 (25-450)	0.92 (0.28-4)	6.25 (0.5-28.4)	145 (20-340)	0.70 (0.12-2.22)	2.20 (0.30-14)	78 (12-340)
RECOVERY				33%	23%				

GM ranges from 18% to 38% without any significant difference between the single and the double ABMT, the first and second harvest. In consequence, patients included in one or the other protocol receive very similar total MN cells and CFU-GM. However (Table 2) the delay to hematological recovery of patients after a double ABMT is no longer than after a single ABMT (or after the first one in the double ABMT). If one analyzed individual patients in the three groups, 17 had delay to engraftment (>45 days to recover $0.5x10^9$ PN/1), 5 of the 59 patients after a single ABMT, 12 of the 32 patients after a double ABMT. The number of re-injected CFU-GM and MN cells in those 17 patients was equally distributed below and above the median; 2 had CMV-infection and 3 had hepatitis (A, B, and non A non B); 2 had a veino-occlusive disease. In the single ABMT, the 5 patients had normal BM at the time of harvest (see below) but in the double ABMT 8 of the 12 patients had a pathological marrow. This does not differ from observations in other patients. As previously published, these patients had an excess of CD8+ leu7+ lymphocytes in the blood and the BM. These could have a suppressive effect on hematopoiesis. Seven patients received "in vivo" therapy with anti-CD8 monoclonal antibody for 6 days. Among these, 4 showed PN recovery with 2 weeks after therapy, and platelet recovery within 3 weeks (8).

Immunological recovery is summarized in Table 3. Prior to the transplant, patients already had an important T-cell defect as determined by the proliferative index after PHA stimulation and the decreased IL2 production. The NK function was low, but the B-cell function (immunoglobulin secretion) was normal. After the graft, the profound T-cell defect (nearly undetectable IL2 secretion) persisted, generally for more than one year and up to 3 years in some patients. By contrast, patients had normal B-cell function from the first month post-graft. Interestingly, their NK function recovered already during the first month post-graft, and exceeded normal values until one year post-graft.

On the day of BM harvesting, BM micrometastases (4 trephine biopsies and 4 aspirates) were detected in 18 of the 59 single ABMT patients, in 19 of the 32 patients before the first graft and 10 of the 32 patients after the second graft in the double ABMT. We previously showed that clumps of NB cells were usually detectable on the biopsies whereas aspirates were negative. This can be explained either because malignant cells resist aspiration, or because of difficulties in the cytological examination. In the context of the purging procedure, the questions were, first, whether or not these rare NB cells could be harvested with the normal BM cells and detectable in the autograft and, second, whether they would be eliminated by the purging procedure. In an attempt to answer such questions, we developed a double IF technique using 3 NB monoclonal antibodies in combination with an anti-panleucocyte monoclonal antibody. One of the anti-NB

antibodies (11.14) used for detection was not included in the purging cocktail and it did not cross-modulate with others. In analyzing 3×10^6 total BM cells, this method enabled to accurately detect as few as 10^{-5} residual malignant cells from the harvested autograft. Such analysis has been performed in 55 cases (Table 4). In 27 cases, NB cells were undetectable, neither in the trephine biopsies nor aspirates, nor in the autograft. In 17 cases micrometastases were detectable on the trephine biopsies and/or the aspirates and the autograft contained 10^{-2} to 10^{-5} NB cells; in 3 cases, the cytohistological analysis of the BM was normal but the double IF analysis of the autograft enabled to correct the 3 false negatives by detecting 10^{-3} to 10^{-5} NB cells; finally in 8 cases NB cells were detectable in one of the four biopsies, aspirates were negative and they could not be detected int he graft. The autograft thus contained 10^{-2} to 10^{-5} malignant cells before the purging procedure in 20 of the 54 cases analyzed, in 7 cases of single autograft, in 7 cases of first graft and 6 cases of second graft. After purging, none of the autograft samples contained any residual NB cells that were detectable by immunological analysis; less than 10^{-5} residual NB cells may thus be left after the purging.

Table 2. Hematological Recovery after Immunomagnetic Depleted ABMT

	WBC > 1×10^9/l	PN > 0.5×10^9/l	Platelets > 50×10^9/l
LMCE Single Graft			
(59 patients)	20 (02 - 96)	25 (08 - > 100)	42 (22 - > 188)
LMCE Double Graft			
(8 patients)			
1st Graft	21,5 (14 - 30)	20 (17 - 43)	20,5 (12 - 30)
2nd Graft	23 (14-50)	30 (18- > 90)	51 (30 - 120)
Other than LMCE Double Graft			
(24 patients)			
1st Graft	22 (12-37)	23 (11-57)	21 (14-64)
2nd Graft	27,5 (12-111)	50 (11-111)	75 (20-600)

WBC: white blood count;
PN: granulocytes

Table 3. T, NK and B Cell Functions after an Immunomagnetic Depleted ABMT

	PRE-GRAFT	POST-GRAFT DAY 30	DAY 60 - 90	DAY 90 - 180	> 12 MONTHS
Ratio CD4/CD8 (Normal = 1,5)	1 (16 - 0.2)	0.7 (2 - 0.11)	0.6 (3 - 0.15)	0.75 (3 - 0.33)	1 (4 - 0.5)
Proliferative Index of PHA Stimulation (Normal = 26264)	3400 (139206-179)	2500 (7665-437)	2783 (10016-280)	7000 (19772-348)	5578 (48697-1006)
IL2 Secretion (Normal = 2.68)	0.06 (3.6 - 0)	0 (0 - 0.01)	N.E.	0.04 (0.79 - 0)	0.03 (0.72 - 0)
NKH1 + Cells (Normal = 2%)	2 (30 - 0)	5 (50 - 0)	15 (50 - 5)	6 (28 - 0)	8 (27 - 0)
NK Function (Normal = 10%)	1.6 (18 - 0)	24 (40 - 0)	14 (34 - 0)	15.9 (99 - 0)	19 (66 - 5)
IgM (g/l) (0.6 - 1.5)	0.63 (1.47 - 0.26)	0.43 (0.6 - 0.24)	1.11 (2.78 - 0.67)		
IgG (g/l) (1.7 - 15)	8.50 (13.40 - 5.40)	8.20 (9.8 - 4)	13.5 (13.6 - 9.52)		
IgA (g/l) (1.2 - 2.5)	1.26 (2.74 - 0.84)	0.75 (2.2 - 0.41)	1.36 (3.52 - 0.9)		

Table 4. Comparison Between Cytohistological Detection of BM Metastases and Immunological Detection of NB Cells in the Graft: Analysis of 55 Patients

Cytohistology (4 biopsies + 4 aspirates)		Double Immunofluorscence Analysis of BM Cells in the Autograft	
		Before Purging	After Purging
LMCE SINGLE GRAFT (16 cases)			
+	5 cases	+	−
+	3 cases*	−	−
−	2 cases	+	−
−	6 cases	−	−
DOUBLE GRAFT PROTOCOL			
1st Graft (18 cases)			
+	7 cases	+	−
+	3 cases	−	−
−	8 cases	−	−
2nd Graft (21 cases)			
+	5 cases	+	−
+	2 cases	−	−
−	1 case	+	−
−	13 cases	−	−
		(contamination ranged from 10^{-2} to 10^{-5})	(less than 10^{-5} residual NLB cells)

*In 1 case, biopsies and aspirates were positive in anterior iliac crests and negative in posterior crests and did not contain malignant cells.

DISCUSSION

The immunomagnetic procedure initially described by J. Kemshead has been modified and allows 3 log elimination of malignant cells (4,5). The two clinical questions were then to prove that it was non-toxic, and clinically useful. In this series of 129 ABMT we confirm that the procedure is non-toxic on CFU-GM and results in a substantial loss of MN cells. The latter is, however, comparable to results obtained with other purging methods, and in particular with complement lysis. Many patients present a delay in hematological recovery. For patients included in the double ABMT, the high chemotherapy pressure before the second graft and the damage to the micro-environment are satisfactory explanations. This is unlikely to account for delayed engraftment after single ABMT. The correlation between delayed engraftment and the high level of circulating CD8 ± leu7 + cells is striking and suggests a combination of unfavorable events: involvement of the BM at the time of harvesting, young age of the patient, and TBI (8). The second question was that of the usefulness of the purging procedure. The double IF analysis of the autograft demonstrates that in 20 patients the purging was necessary and efficient. Finally, functional monitoring after BMT showed that these patients recovered very quickly NK function. This exceeds normal levels during the first year. Our finding may be of importance for chemotherapy refractory patients for whom treatments based on different mechanisms of action must be designed. Adoptive immunotherapy with IL2 and in vitro autologous stimulated NK cells appears to be a productive approach.

ACKNOWLEDGMENTS

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MYELOABLATIVE TREATMENT FOR CHILDREN WITH METASTATIC NEUROBLASTOMA SUPPORTED BY BONE MARROW INFUSIONS: Progress and Problems

John Graham-Pole, Terry Pick, James Casper, Gerold Elfenbein, Adrian Gee, Michael Graham, Samuel Gross, Penelope Koch, Rusty Marcus, Nancy Mendenhall, Jonathan Shuster, Wayne Spruce, and Paul Thomas

INTRODUCTION

In spite of progress in our understanding of the molecular biology of human neuroblastoma (NBL), and the development of new treatment regimens, the outlook for children ≥one year with metastatic NBL remains bleak (1,2).

In the past 10 years, several clinical researchers have tried to improve this outcome by using marrow-ablative chemotherapy with or without irradiation, supported by allogeneic or autologous marrow transplantation (BMT) (3-8). The hypothesis, from experimental animal and tissue culture systems (9,10), and from clinical experience with BMT for refractory cancer (11,12), is that higher drugs and radiation doses increase the log cell kill. The dose-limitation in most phase 1 studies is myelosuppression, which is potentially eliminated by BMT.

In our first study of children with metastatic NBL (1976-1978), we used high doses of the alkylating agent melphalan in a dose of 180mg/m^2, with autologous BMT; and some patients had durable remissions (3). Melanoma patients had already been shown to recover

more quickly after 180 mg/m^2 + ABMT than when melphalan was given at 140 mg/m^2 without BMT (13).

Subsequent trials built on these early observations, and several hundred NBL patients have now been treated in Europe and the United States, mostly with melphalan but sometimes other alkylating agents; and mostly combining this with total body irradiation (TBI), but sometimes with local irradiation and other drugs (4-8).

Using autologous BMT to restore hematopoiesis circumvents problems of allogeneic BMT, namely non-availability of HLA-matched donors and graft-host reactions. However, it has its own limitations, particularly the potential for reinfusing residual cancer cells with the marrow, and for permanently reducing the number of hematopoietic cells by either previous treatment of the patient or in vitro treatment of the marrow.

Because most children with NBL have marrow involvement at diagnosis, and because they need intensive induction and consolidation treatments to achieve and maintain clinical remissions, the ideal time for autologous harvesting and freezing is hard to define. Most researchers have adopted the practice of purging the marrow in vitro after harvest, following initial treatment to remove occult neuroblasts (14-16). The purging process could further impair hematopoiesis in the injured marrow, either by disrupting the microenvironment or by eliminating pluripotential cells (17).

The Pediatric Oncology Group (POG) Study

In February 1984, the POG began a pilot trial to study the feasibility, complications, and efficacy of BMT for children with metastatic NBL. This single-arm study used melphalan at the maximal dose previously defined (4), plus fractionated TBI and in appropriate cases irradiation of residual lesions. Figure 1 shows the chemoradiotherapy regimen, the nine participating institutions, and details of patient registration until February 1988. After treating 25 children we increased the total FTBI dose from 9 to 12 Gy, without changing the fractionation or rate.

We excluded one patient because of wrong diagnosis; 54 received BMT in their first complete or partial remission, and 27 after disease progression and attempted reinduction. Seven received allogeneic and 74 autologous BMT. Fifty-seven autologous marrows underwent immunomagnetic purging at University of Florida. The median age at BMT was 4 (range 1-15) years; there were 50 boys and 31 girls; 13 black and 68 white.

Immunomagnetic Purging

This has been described elsewhere (14,15). Briefly, the nucleated cells from the harvested marrow are suspended with a panel of 6

POG:8340: BMT FOR STAGE D NEUROBLASTOMA
2/84 - 2/88
PATIENTS REGISTERED   82   TRANSPLANTED   81
CR/PR # 1   54   CR/PR # 2   27
INSTITUTIONS (9)
BROOKE, CLEVELAND, FLORIDA, FT. WORTH, HOPKINS
McGILL, MIDWEST. OKALHOMA, SAN DIEGO

DAY												
-8	-7	-6	-5	-4	-3	-2	-1	0	+1	+3	+6	+11
		LPAM 60 mg/m²	LPAM 60 mg/m²	LPAM 60 mg/m²					MTX 15 mg/m²	BMT MTX 10 mg/m²	MTX 10 mg/m²	MTX 10 mg/m²
										BMT		
LRT 1.5Gy BID	LRT 1.5Gy BID	LRT 1.5Gy BID	LRT 1.5Gy BID	LRT 1.5Gy BID	FTBI 1.5-2.0 Gy BID	FTBI 1.5-2.0 Gy BID	FTBI 1.5-2.0 Gy BID				PREDNISONE 40mg/m² day +7→+21	

Figure 1. POG 8340: 2/84 - 2/88

antineuroblastoma monoclonal antibodies,* and then with $2-2.8 \times 10^9$ paramagnetic microspheres.** The bead, antibody and cell mixture is drawn through a separation chamber in a controllable magnetic field, where bead- and antibody-coated NBL cells are selectively removed. The remaining marrow is kept in liquid nitrogen until needed, when it is thawed in a 37°C water bath at the bedside for reinfusion.

Supportive Care of the Patients

This has been described elsewhere (7). All patients are isolated while neutropenic receive irradiated blood products, central venous hyperalimentation, and systemic antimicrobials in the event of fever. They are discharged to close outpatient follow-up when clinically stable, off antibiotics and taking adequate oral nutrition. No specific anticancer treatment is given after BMT.

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*Supplied by John Kemshead, Ph.D.; **Supplied by John Ugelstad, Ph.D.

Clinical Results

Figure 1 shows the overall results to date. All patients have been followed since BMT either until death, or subsequent relapse, or for 6 months minimum. Thirty-seven (46%) have relapsed (1 allograft and 36 autografts), a median of 6 (1-21) months after BMT. Twelve (15%) have died from BMT-related complications (3 allografts and 9 autografts) a median of 2 (0-4) months after BMT, 10 from systemic infections. Thirty-two (39%) are alive and free of apparent disease a median of 14 (6-47) months after BMT. Relapse sites have been predominantly the primary lesion and cortical bone, with or without marrow involvement. We have not seen isolated marrow relapse in this series. Figure 2 summarizes the overall outcome.

The overall probability of remaining relapse-free is 31.8% at 21 months after BMT. Nine children remain more than 2 years off treatment. Figure 3 shows the probability of remaining disease-free, using Kaplan-Meier analysis of different factors that might affect this outcome. Our preliminary analysis shows the following relapse-free probabilities at 2 years post-BMT: girls 43%; boys 26%; whites 41%; blacks 10%; stratum 1, 37%; stratum 2, 24%; FTBI 12 Gy 38%; FTBI 9 Gy 20%. These findings suggest that female sex, white race, earlier disease stage, and use of higher TBI dosage are associated with a more favorable prognosis.

OUTCOME

```
                    TOTAL
                   81 (100)
         ┌─────────────┼─────────────┐
   RELAPSED      DIED FROM      CONTINUOUSLY
   37 (46)      COMPLICATIONS   DISEASE-FREE
                   12 (15)         32 (39)
      │              │               │
 MEDIAN TIME     3/7 ALLO.      MEDIAN F/U
  6 (1-21)       9/74 AUTO.      14 (6-47)
   MONTHS                         MONTHS
```
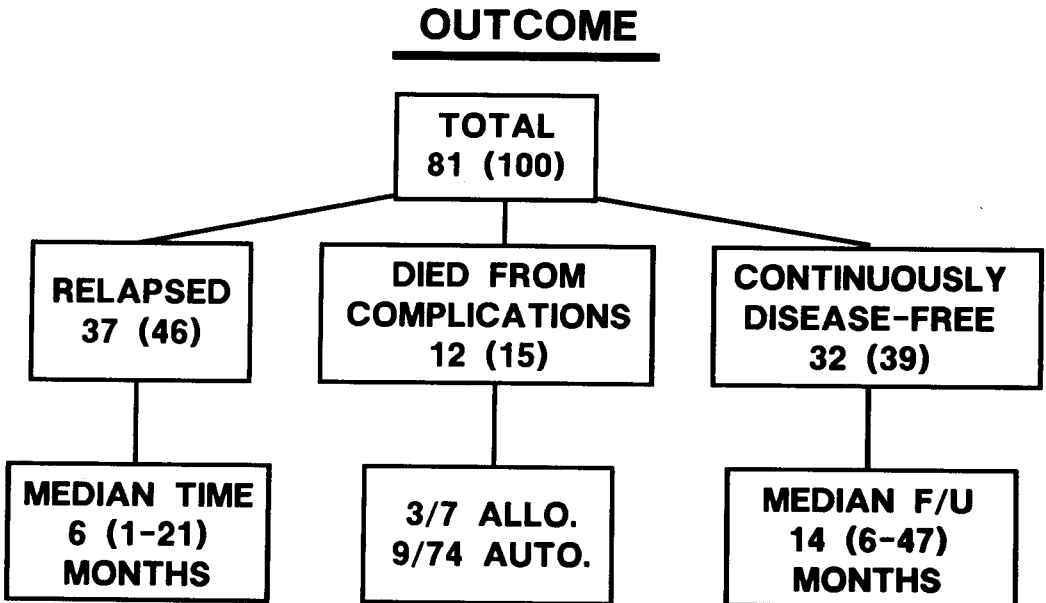
Figure 2. POG 8340: Overall Outcome
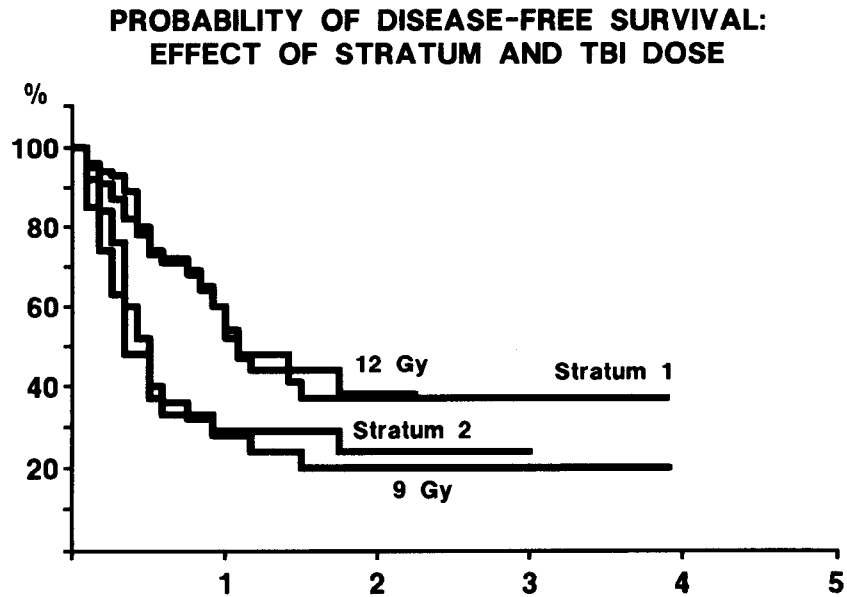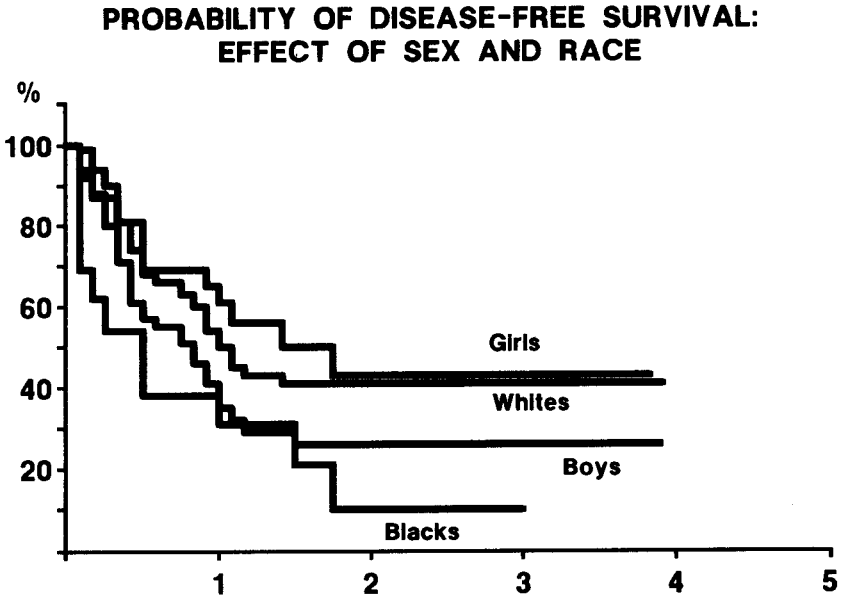
Figure 3. POG 8340: Disease-free survival probability, related to sex, race, stratum and TBI dose

Engraftment after Autologous BMT

Because 74 of 81 children received autologous BMT, most after in vitro purging, and because the rate of hematopoietic recovery varied greatly, we analyzed factors influencing re-engraftment.

Figure 4 shows the cumulative time to achieve a sustained white cell count of ≥1000/cu mm. We performed univariate analyses using both the log rank test (emphasizing longer engraftment times) and the Wilcoxon test (emphasizing shorter engraftment times). We computed 95% confidence intervals (CI) for median engraftment times.

The overall time was 31.3 (CI 26-38) days, with a minimum of 10 and a maximum of 106 days. As seen in Figure 4, 25% had engrafted by 20 days, and 25% took longer than 60 days.
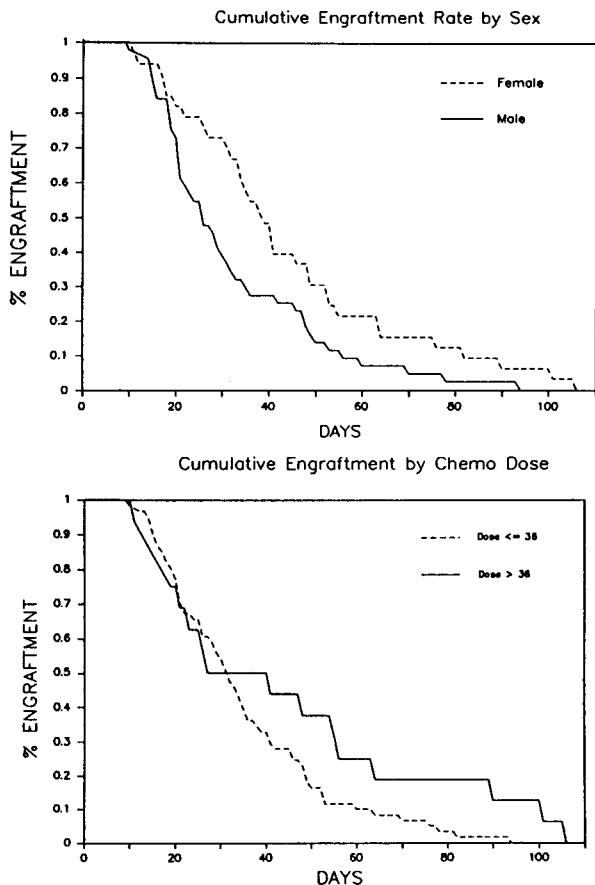
Figure 4. POG 8340: Autologous re-engraftment, related to sex and prior chemotherapy

Univariate analysis showed the following factors to affect engraftment: sex, previous chemotherapy, and time from last chemotherapy to marrow harvest. Girls were significantly slower to engraft than boys (p = .031); a total of more than 36 doses of previous chemotherapy was associated with slower engraftment, compared with less cumulative chemotherapy (p = .028); and an interval ≤ 50 days between the last chemotherapy course and marrow harvesting was associated with slower engraftment than a longer interval (p = .015). Patient stratum, race, number of nucleated cells infused, number of CFU-C infused, use of in vitro purging, and time from harvesting to completing the freezing procedure were not significant factors.

CONCLUSIONS

The potential for myeloablative treatment of children with metastatic NBL is borne out by the early results of the POG study. The combination of melphalan and FTBI is an effective and mostly tolerable one, and the response may be dose-related, leading us to consider higher radiation doses. Patients generally do better if transplanted early but some can be salvaged after disease progression. We have insufficient data to compare allogeneic with autologous BMT.

Unlike others (5), we have not seen relapses to date beyond 21 months, but our follow-up on many patients is short. Almost half have relapsed, stressing the need to improve both initial induction and consolidation and pre-BMT myeloablative regimens. We are exploring alternative chemotherapy as well as FTBI dose escalation for these patients.

The absence of isolated marrow relapse suggest the immunomagnetic purging technology is effective, and it appears safe. Delayed engraftment seems a function of factors other than marrow cell dose and manipulation at the time of harvest.

The effectiveness of purging probably allows us to harvest marrow earlier, perhaps at the first sign of clearing of overt malignant infiltrates, and before cumulative chemotherapy has produced much injury to the marrow microenvironment and stem cell pool.

Ultimately, these questions can only be resolved through multicenter phase 3 clinical trials, as are being undertaken by both North American and European cooperative groups.

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AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR ADVANCED NEUROBLASTOMA AT THE CHILDREN'S HOSPITAL OF PHILADELPHIA: An Update

Charles S. August and Bonnie Auble

INTRODUCTION

In 1982, Pritchard and his colleagues in London reported their initial experience employing high dose melphalan (L-phenylalanine mustard) and autologous marrow rescue in a group of children with Stage IV neuroblastoma (1). Their encouraging results prompted us to combine debulking surgery and local radiation therapy (LRT) with high dose melphalan, lethal total body irradiation (TBI), and conventional doses of VM-6 and adriamycin followed either by either autologous or allogeneic bone marrow transplantation (BMT). Our early experience showed that the combination was remarkably effective in clearing widespread, active, disease (2,3).

In this report, we will describe the evolution of our protocol over the past 4 years and the results in 28 patients who received, at minimum, melphalan in a total dose of 210 mg/m^2, and total body irradiation (TBI) in doses of 999 - 1200 cGy. Adriamycin, VM-26, and, in some cases, Cis-platinum (CPDD), have been also given in conventional doses. The children have been followed from 9 to 90 months.

PATIENTS AND METHODS

Patients

Criteria for selecting or excluding patients, and techniques for harvesting and cryopreserving marrow and the approach to patient

management have been described previously (2,3,4). Between december 1, 1980, and July 1, 1988, we treated a total of 28 patients, 19 boys and 9 girls ages 2 to 30 years. At diagnosis, 22 children had stage IV disease, 5 had stage III disease, and one child had stage II disease. Prior to entering our treatment protocol, 27 children had recurrent or progressive disease or primary tumors which had responded only partially to conventional therapies. Only one patient (with recurrent stage III disease) was thought to be in complete remission. The clinical characteristics of all the patients are presented in Table 1.

We attempted to harvest patients 3-4 weeks after a course of chemotherapy and prior to debulking surgery. Beginning with patient #32 (10/84), marrow showing neuroblastoma was evaluated for the possibility that tumor cells might be purged by the technique of Kemshead employing a panel of monoclonal anti-neuroblastoma antibodies (UJ 13A, UJ223.8, anti Thy 1B, and 1 p14 A) and magnetic microspheres (5). Patients with tumor involving more than 30% of the marrow, or whose tumor cells were not able to be purged, were excluded from the protocol. Thus, 6 of the most recent 12 patients received purged marrows.

TREATMENT PROTOCOL

At the outset, all patients who had operable bulk disease underwent aggressive surgical removal of as much tumor as possible. Local irradiation was then given to any residual tumor and/or the tumor bed and to sites of active bone disease. Thus, 15 patients received both surgery and LRT, 11 patients received LRT alone, 1 patient only surgery, and 1 patient received neither.

Patients were isolated in protected environments until their absolute neutrophil counts exceeded $500/mm^3$ for 3 consecutive days. Eighteen patients have been nursed in laminar air flow rooms (Scimed, Minneapolis, Minn.). Patients discovered to have antibodies to herpes simplex virus were given prophylactic acyclovir. In an attempt to prevent cytomegalovirus (CMV) infection, whenever possible frozen-thawed packed red blood cells were transfused. Since September, 1984, only CMV seronegative platelets have been transfused.

We then treated patients uniformly with VM-26 and adriamycin, and high-dose melphalan (210 mg/m^2, TBI 333 cGy) and BMT (2). In late 1985, after 18 patients had been treated under protocol described above, it was clear that a substantial fraction of our patients were experiencing relapses. We then attempted to intensify our therapy. Thus, in addition to the three drugs, patients #37, 38, 40, and 45 received cis-platinum (100 mg/m^2) 8 days prior to their BMT. Toxicity was not excessive. Patient #43 received no cis-platinum, but

Table 1. Autologous Bone Marrow Transplantation in 28 Children with Advanced Neuroblastoma at the Children's Hospital of Philadelphia

Unique Pt. No.	Age/Sex	Stage at Dx	Prior Treatment	Involvement before ABMT
5	4/M	IV	V,C,D,DOX, VM-26,CPDD	LN, R, acetabulum
7	9/M	IV	V,C,D,CPDD, VM-26	bones x 4
8	6/M	IV	V,C,D,LDTBI	LN x 5 (bulky)
9	4/M	IV	V,D,D,DOX VM-26,CPDD	LN x 2 (bulky)
11	5/M	IV	V,C,D,LRT, DOX	LN
12	19/M	IV	V,C,DOX	Abdomen, bone, BM
13	4/F	IV	V,C,D,DOX VM-26, CPDD	Abdomen, chest wall, bones & BM
15	4/M	III[a]	V,C,D	LN, + spilled tumor in abdomen
16	4/F	IV	V,C,D,DOX VM-26	Skull, retroperitoneum, LN, BM
17	4/M	IV	V,C,D,DOX LRT	Bones x 3
21	5/F	IV	CPDD,VM-26 C,DOX	Retroperitoneum, bone
24	9/M	IV	V,C,D	Retroperitoneum, bone, LN
26	4/M	II[b]	V,C,D,Surg. LRT	Retroperitoneum, LN
28	2/M	IV	V,C,D,LRT	Retroperitoneum, LN
30	2/F	III[3]	V,C,D	Retroperitoneum
31	5/F	III	V,C,D,Surg.	Retroperitoneum
32	6/M	IV	V,C,D,DOX CPDD,VM-26	Bones x 6, BM,
34	12/M	IV	V,C,D,DOX	Paraspinal mass
37	3/F	IV	V,C,D,DOX, CPDD,MELPH	Mediastinum, bone, BM
38	3/M	IV	V,C,D,DOX, CPDD	Adrenal 1˚, LN
40	12/M	IV	C,DOX,CPDD, VP-16	Retroperitoneum, bones x 3
43	9/F	III	C,DOX,CPDD VP-16,VM-26	None
45	30/M	IV	V,C,DOX,5-FU Hydroxyurea	Retroperitoneum, LN, bones x 2
46	9/M	IV	CPDD, VP-16	Bone
47	3/M	IV	V,C,D,CPDD, VP-16	Retroperitoneum, bones x 3
48	2/M	IV	V,C,D,CPDD, VP-16	Retroperitoneum, bones x 11
49	6/F	IV	CPDD,VP-16	Mediastinal mass
52	7/F	III	CPDD,VP-16, V,C,D	bones x 11

rather TBI 1200 cGy) given over 3 days in 6 fractions. (Two other children receiving allogeneic BMT received the same dose of TBI.) Patients #46, 47, 48, and 49 received both cis-platinum and the additional 200 cGy of irradiation. The last 2 patients had lung shielding such that the total dose of irradiation to the mediastinum and lung fields was 100 cGy. In spite of this precaution, both died acute toxic deaths.

No maintenance chemotherapy has been given to any patient after BMT, and no child has received a second infusion of autologous marrow. All relapses have been shown by biopsy or autopsy to be neuroblastoma or to take up 131-IMIBG.

RESULTS

All patients experienced severe mucositis especially of the tongue and buccal mucous membranes. Anorexia persisted for 1 - 2 months, and sometimes as long as 4 - 5 months after mucosal erosions healed. Abdominal pain and bloody diarrhea were common and this complication prolonged the hospitalizations of 5 of the children.

Four children died in the first post-transplant month with the complications described above, plus aspergillus pneumonia (#8), cardio-respiratory arrests of uncertain cause (#11, #17), and hepatic necrosis and veno-occlusive disease of the liver (#48). Patient #49 developed heart and respiratory failure and patient #26 developed interstitial pneumonitis due to cytomegalovirus. Both died in the second post transplant month. It is noteworthy that no patient developed idiopathicinterstitialpneumonitis.

We assessed the early response of the neuroblastoma 3 - 4 months post BMT. Nineteen patients (68%) were found to be in complete remission, and one child had a partial remission (4%). Thus, the overall response rate is 72%. Two children failed to respond. Five of the 6 children who died of acute toxicity had autopsies and neuroblastoma was found in 2.

We attempted to restage all of our patients every 3 - 4 months for the first post transplant year, at 18 months post transplant, and then at the anniversaries of their BMT. As of July 1, 1988, ten children survived in complete remission from 9 to 90 months post transplant (Figure 1). By Kaplan-Meier analysis, the probability of disease-free survival from 20 - 90 months is 0.32.

There were 13 treatment failures. Patient #8 died of acute toxicity with bulky disease. Patient #24 had a partial remission on the basis of the response to surgery and LRT. A residual mass, stopped growing for 6 months but did not regress. Patient #2 experienced no change in either the extent or the growth rate of his tumor. Ten other children relapsed. Figure 2 shows the Kaplan-Meier estimate for the probability of relapse. For the purpose of constructing the curve, the
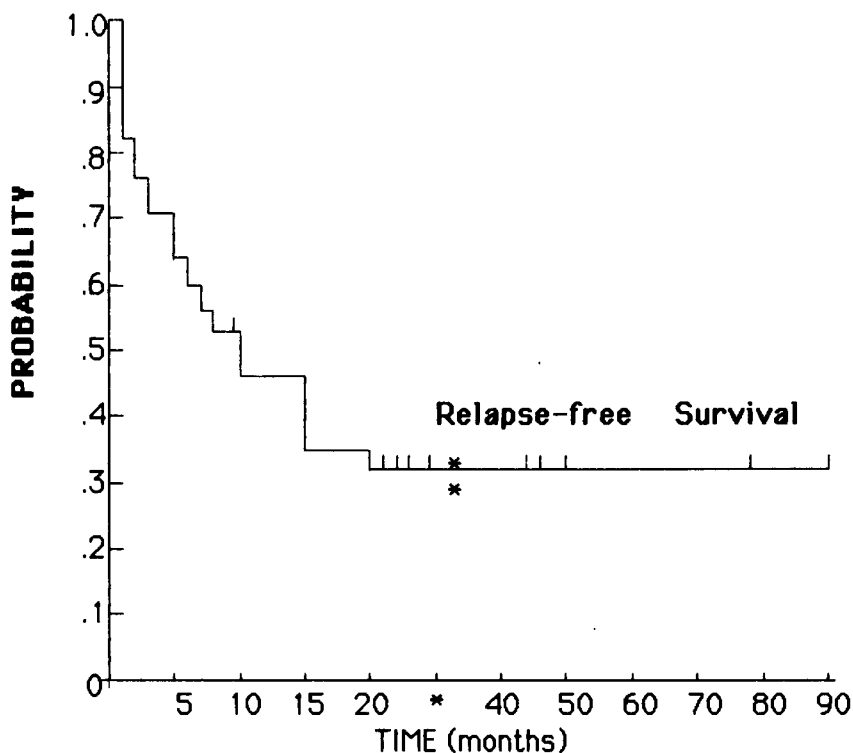
Figure 1. Kaplan-Meier estimate of the probability of disease-free survival in 28 patients undergoing high dose chemotherapy, total body irradiation and autologous bone marrow transplantation for advanced neuroblastoma a the Children's Hospital of Philadelphia, 1980 - 1988.

patients with progressive disease who failed to respond or who died with acute toxicity whose autopsies showed tumor were considered to have relapsed. The last relapse occurred 20 months after BMT and the actuarial risk of relapse from 20 - 90 months is 0.53. In general, relapses have occurred in sites of original disease or at the margins of local radiation fields. No patient has relapsed with military seeding of lungs, liver, spleen, or other reticulo-endothelial organ.

Hematologic recovery has been satisfactory although some children, especially those with purged marrow, experienced incomplete or prolonged recovery.

Late complications of our therapy are numerous. Linear growth has been slow in a significant fraction of the children surviving in CR for more than 20 months. The reasons for this may include hypothyroidism (documented in patient #5), chronic diarrhea (patient
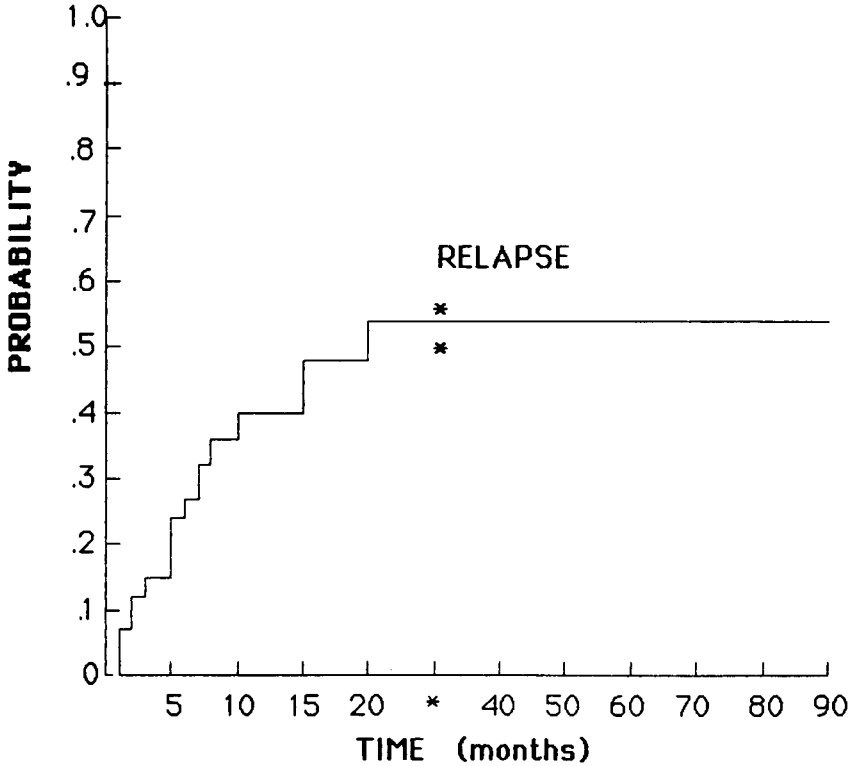
Figure 2. Kaplan-Meier estimate of the probability of progressive neuroblastoma or relapse in 28 patients undergoing high dose chemotherapy, total body irradiation and autologous bone marrow transplantation for advanced neuroblastoma at the Children's Hospital of Philadelphia, 1980 - 1988.

#28), renal insufficiency and/or growth hormone deficiency (pt. #21). Seven of ten patients tested show loss of hearing for high frequency sounds. Most of these children had received cis-platinum before or during their transplants and some had lost hearing prior to BMT.

Second neoplasms have occurred in 2 children. Patient #7 developed a low grade fibrosarcoma of the lung in his 29th post transplant month. It was removed and he survives in CR both from neuroblastoma (>77 months) and the second tumor (>48 months). Patient #34 developed acute myelomonocytic leukemia 8 months post transplant and died with his neuroblastoma in remission 7 months later.

DISCUSSION

The 28 children described herein were given lethal doses of chemotherapy and TBI in an attempt to cure otherwise fatal illnesses.

Intensifying our treatment protocol, attempting to treat patients earlier, and using purged marrow have not produced any dramatic improvements in our results as reported earlier. The two patients who were surviving in CR at the time of our first report (6) continued in cR at >90 and >77 months respectively. Two of the patients surviving in CR at the time of our second report (7) have relapsed and died, but in the past four years overall disease-free survival has not changed (33% in 8/84, 36% at present). Adding the results of 12 allogeneic BMT's to the 28 reported herein does not appreciably change the rates of response, relapse, or toxic deaths (unpublished).

Thus, on the basis of nine years experience with a protocol whose essential features involve debulking with aggressive surgery and LRT followed by high dose melphalan, TBI, and marrow rescue, in patients with advanced disease, we may expect to salvage one third. Approximately one fourth died within three months of the complication of therapy, and one half seem destined to relapse. The time appears ripe for the introduction of either new drugs, biologic response modifiers, or both.

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TREATMENT OF POOR PROGNOSIS NEUROBLASTOMA WITH INTENSIVE THERAPY AND AUTOLOGOUS BONE MARROW TRANSPLANTATION

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INTRODUCTION

While 40% of patients with neuroblastoma have a good chance of survival, the prognosis is poor for 60% of patients. Most patients in the latter group can be identified using prognostic variables which include age, stage, N-*myc* gene copy number, and histopathology. Survival for poor prognosis neuroblastoma patients is generally less than 10% with conventional therapy that includes chemotherapy, local irradiation, and surgery (Breslow and McCann, 1971; Evans, 1980; Hayes and Green, 1983; Shimada et al., 1984; Hann et al., 1985; Seeger et al., 1985; Evans et al., 1987). Recent pilot studies of intensive chemotherapy and total body irradiation followed by allogeneic or autologous bone marrow transplantation (BMT) have given encouraging results (August et al., 1984; Hartmann et al., 1986; Philip et al., 1987; Seeger et al., 1987). The Children's Cancer Study Group (CCSG) has undertaken a limited institution trial of poor prognosis neuroblastoma utilizing BMT with autologous marrow that was purged by sedimentation, filtration, and magnetic immunobeads. Here we report the current data on 31 consecutive patients entered into this study. Although it is as yet too early to reach conclusions about long-term disease-free survival, we have demonstrated that tumor cells can be removed *ex vivo* without impairing subsequent engraftment.

PATIENTS AND METHODS

Patients and Eligibility Criteria

Patients were diagnosed by standard clinical and pathological criteria and were staged as follows: stage II, tumor extending in continuity beyond the organ or structure of origin but not crossing the midline; regional lymph nodes on the homolateral side may be involved; stage III, tumor extending in continuity beyond the midline; regional lymph nodes may be involved bilaterally; stage IV, remote disease involving skeleton, organs, soft tissues, or distant lymph node groups (Evans, 1980).

Eligibility required that patients be over one year of age at diagnosis and have a poor prognosis as defined by one of the following criteria: 1) stage IV; 2) stage III with the primary untreated tumor having genomic amplification of N-*myc* (>10 copies) (Seeger, et al., 1985) or unfavorable histopathology (Shimada, et al., 1984) or with elevation of serum ferritin (>142 ng/ml) (Hann, et al., 1985); 3) stage II with the primary untreated tumor having genomic amplification of N-*myc* (>10 copies).

There were 21 patients with stage IV, three with stage III, and one with stage II neuroblastoma. Written informed consent was obtained for all patients at the time they were entered into this study (CCG-321P3).

Induction Chemotherapy

Twelve of the 31 patients were entered on study at the time of diagnosis and received cyclophosphamide, cisplatin, doxorubicin, and teniposide according to protocol (Green et al., 1986). Other patients were referred after they had received other chemotherapy regimens such as the same four drugs in a different schedule (CCG-321P2) or teniposide/etoposide alternating with cyclophosphamide, vincristine, DTIC (CCG-323P).

Ex Vivo Purging of Autologous Marrow

The median time from diagnosis to marrow harvest was 27 weeks. To plan the purging procedure, numbers of normal and tumor cells in posterior iliac crest marrow were determined three to ten days before the large-scale harvest (Moss et al., 1987). In the large-scale harvest, marrow was obtained from posterior and, if necessary, anterior crests to provide approximately 10^8 marrow cells per kg after *ex vivo* purging.

Details of purging and cryopreservation have been previously described (Wells, et al. 1979; Seeger et al., 1985; Reynolds et al., 1986; Seeger, et al., 1987). Whole marrow was mixed 1:1 with 3% hetastarch

and allowed to sediment, after which supernatant cells were filtered through nylon wool, washed, and mixed with magnetic beads that were coated with a mixture of monoclonal antibodies (390, 459, HSAN 1.2, BA-1) via goat anti-mouse immunoglobulin (1:1 bead to total cell ratio). Following one-half hour of rotation with immunobeads, tumor cells attached to beads were removed with samarium cobalt magnets; the immunobead depletion step was repeated if the pre-harvest marrow contained more than one tumor cell per 10^4 normal cells. Total cell recovery was approximately 66% after sedimentation and filtration and 50% after each magnetic immunobead step; thus, approximately 35% of the initial cells were recovered after sedimentation, filtration, and one cycle of magnetic immunobeads. An aliquot of marrow (10^8 cells/kg) that was treated only by sedimentation and filtration was cryopreserved as a backup in case antibody treated marrow did not engraft.

Autologous marrow was infused only if tumor cells were not detected in an aliquot of the purged specimen by immunoperoxidase staining with anti-cell surface monoclonal antibodies (mixture of antibodies 390, 459, HSAN 1.2, and 126-4) and anti-neuron specific enolase serum; analysis of 3×10^5 bone marrow mononuclear cells gives a 95% probability of detecting one neuroblastoma cell among 10^5 normal cells (Moss et al., 1985; Moss et al., 1987).

Pre-transplant Intensive Chemoradiotherapy

Patients received intensive therapy over nine days before infusion of autologous marrow (Table 1). The median time from diagnosis to BMT was 34 weeks.

Table 1. Pre-transplant Intensive Chemoradiotherapy Regimen (VAMP-TBI)*

day -9:	cisplatin, 90 mg/m^2 IV
day -8:	no therapy
day -7:	teniposide, 150 mg/m^2 IV; doxorubicin, 45 mg/m^2 IV
day -6:	L-phenylalanine mustard, 140 mg/m^2, IV
day -5:	L-phenylalanine mustard, 70 mg/m^2, IV
day -4:	teniposide, 150 mg/m^2, IV
day -3:	TBI, 3.33 Gy, 5-8 cGy/min
day -2:	TBI, 3.33 Gy, 5-8 cGy/min
day -1:	TBI, 3.33 Gy, 5-8 cGy/min

*For patients <2 yrs old or weighing <12 kg, the doses of cisplatin, teniposide, L-phenylalanine mustard, and doxorubicin were calculated according to weight assuming 1 m^2 = 26 kg (eg., cisplatin, 3.5 mg/kg; doxorubicin, 1.7 mg/kg; L-phenylalanine mustard, 5.4 mg/kg and 2.7 mg/kg; teniposide, 5.8 mg/kg).

Marrow Infusion

Recipients of autologous marrow were given a median of 7×10^7 nucleated marrow cells/kg (range $2 - 54 \times 10^7$ cells/kg).

RESULTS

Induction Chemotherapy

Immunocytological analysis of marrows from 16 patients at both diagnosis and marrow harvest showed that marrow tumor content was significantly decreased by induction chemotherapy. At diagnosis, tumor was detected in the marrow for 68% of patients, whereas at harvest it was present in 31%. Even for those patients with detectable tumor at harvest, the percentage of tumor cells in the marrow was decreased an average of 4 logs by induction chemotherapy, with the median tumor content for positive marrows being 50% at diagnosis and 0.008% at harvest.

Ex Vivo Purging of Autologous Marrow

Thirty-one consecutive marrows were purged by sequential sedimentation, filtration, and magnetic immunobeads. Immunocytology demonstrated that detectable tumor was removed from all eleven marrows that were positive, and no tumor was detected after purging in the 31 marrows used for transplantation. Tumor content before purging ranged from 0.001% - 1.8%.

Autologous Bone Marrow Transplantation

Severe oral mucositis and enteritis was observed in all 31 patients after treatment with VAMP-TBI, and total parenteral nutrition via central venous catheter was necessary. Skin desquamation usually was absent or mild in these patients. Four deaths occurred during the first 108 days after transplantation; the causes of these early deaths, which all occurred prior to documented engraftment, were hepato-renal failure and hepatic veno-occlussive disease.

Hematopoiesis was reconstituted completely in all patients who were evaluable; the median time to an absolute neutrophil count of 500 per mm^3 was 25 days.

Thus, purging by sedimentation, filtration, and a mixture of monoclonal antibodies 459, BA-1, 390, and HSAN 1.2 effectively removed tumor cells without removing pluripotent hematopoietic stem cells.
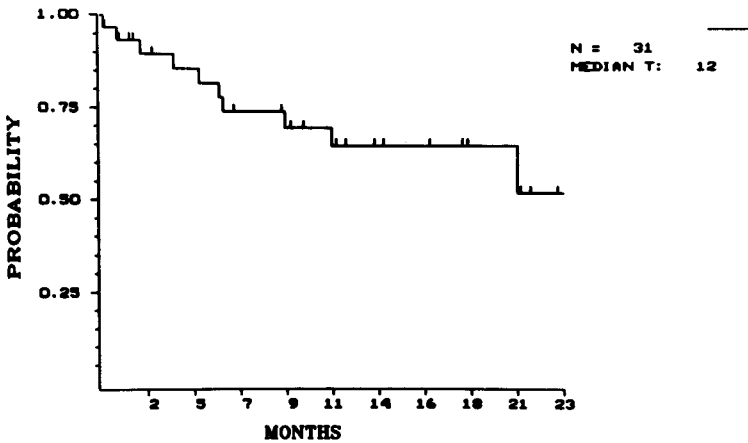
Figure 1. Disease-free survival after autologous BMT. After initially receiving conventional chemotherapy (various regimens), 31 patients received intensive chemoradiotherapy (VAMP-TBI) and then purged autologous marrow. This analysis includes two patients who died from therapy-related complications and five who developed progressive disease. The 19 disease-free survivors have a median time of follow-up of 12 months.

The estimated disease-free survival for these patients after autologous BMT is 52% (Figure 1). The follow-up after BMT for the 19 patients who are disease-free is 1+ to 23+ months with a median of 12+ months. This study is too new to reach conclusions about efficacy; however, considering that transplantation was performed approximately eight months after diagnosis, the outcome is encouraging.

Effect of Purging

To provide some data on the clinical effect of purging neuroblastoma cells from bone marrow used for transplantation, we have compared the survival of patients undergoing autologous transplantation for neuroblastoma without purging. Prior to implementation of our purging regimen, 4 patients who had marrow involvement at diagnosis received autologous transplantation without purging of the marrow. We have compared the survival of the 4 patients who received unpurged marrow to 22 patients who also had metastatic tumor in bone marrow at diagnosis, but received marrow purged by sedimentation, filtration, and magnetic immunobeads. All patients in both groups received VAMP-TBI as the conditioning

regimen. As shown in Figure 2, all 4 patients receiving nonpurged marrow developed progressive disease after BMT and died. By contrast, progression free survival (PFS) of the purged group was significantly higher.

DISCUSSION

Thirty-one consecutive patients with poor prognosis neuroblastoma received autologous marrow transplantation after intensive chemoradiotherapy. Although marrow metastases were detectable by immunocytology in 68% of these patients at diagnosis, induction chemotherapy resulted in a significant reduction in marrow tumor cell content, and remaining detectable metastases were removed *ex vivo* using sedimentation, filtration, and magnetic immunobeads. Thus, the probability of infusing tumor with autologous marrow was minimized by combining aggressive induction chemotherapy with multi-modality purging *ex vivo*, and the combination of these procedures did not compromise marrow engraftment. The results of this study and of our previous one (Seeger, et al., 1987) suggest that long-term disease-free survival for poor prognosis neuroblastoma will be improved by this therapeutic strategy.
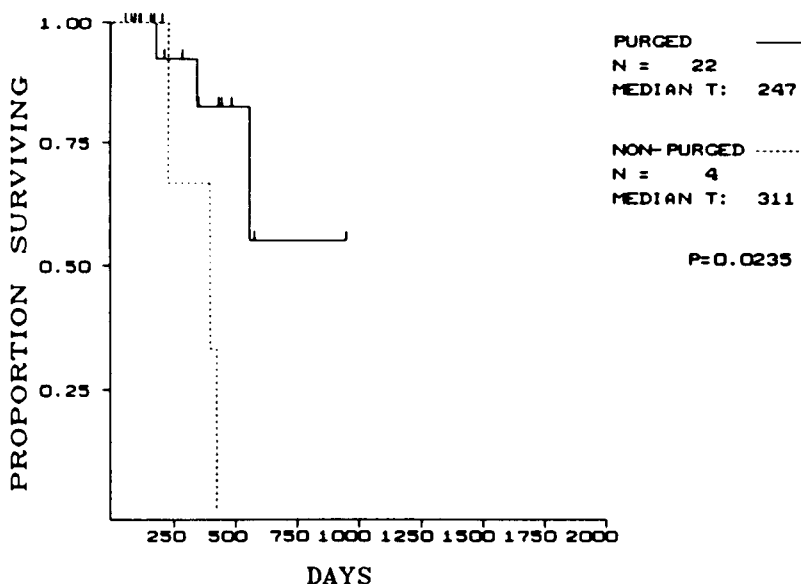
Figure 2. Disease-free survival after autologous BMT comparing patients receiving purged or nonpurged autologous marrow. After initially receiving conventional chemotherapy (various regimens), all patients received intensive chemoradiotherapy (VAMP-TBI) followed by purged autologous marrow (dashed line) or nonpurged marrow (dotted line).

We have compared patients who received purged marrow to patients receiving nonpurged marrow. Definitive proof that purging is beneficial would require a prospective trial. However, as purging does not appear to result in toxic effects for the patient, such a trial is unacceptable to many investigators. Even though the number of patients receiving nonpurged marrow was small, the 100% relapse rate for those patients after BMT suggests that patients who receive autologous marrow without purging are at higher risk for recurrent tumor.

In our initial study (CCG-322P; patient entry January, 1983 to October, 1985), we investigated toxicity and efficacy of teniposide, doxorubicin, L-phenylalanine mustard, cisplatin, and total body irradiation (VAMP-TBI) followed by allogeneic or autologous BMT. Thirty-one patients, who all were diagnosed after one year of age and who had stage IV (n=29) or stage III (n=2) disease, were enrolled in this study. Survival was significantly better for 16 patients (6 allogeneic and 10 autologous) transplanted before disease progression than for 15 (6 allogeneic and 9 autologous) transplanted afterward. Estimated survival was 53% at 54+ months for the former group (6 are tumor-free from 25+ to 54+ months after BMT), whereas it was only 7% at 31+ months for the latter. These data indicated that this intensive chemoradiotherapy regimen (VAMP-TBI) must be given before development of progressive disease in order to increase the likelihood of long-term survival.

In the current study (CCG-321P3), we have attempted to increase disease-free survival by removing detectable tumor cells from autologous marrow and by transplanting patients before they developed resistant tumor (progressive disease). Sensitive detection of marrow metastases, which is possible with immunocytology, is essential for assessing the effect of induction chemotherapy on marrow disease and for planning and evaluating *ex vivo* purging. *In vivo* purging by chemotherapy prior to marrow harvest was highly significant and was a necessary component of preparing marrow for *ex vivo* purging; in some cases, marrow metastases were decreased by as much as five logs. *Ex vivo* purging can remove three or more logs of tumor cells. Tumor in harvested marrow varied prior to purging (0 to 1.8%), and no tumor was detectable afterward. The estimated disease-free survival for all 31 patients is currently 52%, but most are still at risk for relapse since the duration of follow-up after transplantation is short.

A number of changes are planned in an effort to improve the survival rate. Induction chemotherapy will be modified in an attempt to achieve a higher percent of complete responses and the BMT phase will begin by five months after diagnosis. Both of these changes should decrease the risk of a tumor becoming resistant to therapy. Improvement in *ex vivo* purging with additional monoclonal antibodies or other purging methods (such as six-hydroxydopamine) could

further minimize the probability of tumor recurrence (Reynolds, et al., 1982; Reynolds, et al., 1986). Finally, as most failures are due to tumor recurrence after BMT, modifications to the conditioning regimen which should improve tumor cell kill without increased toxicity are being considered.

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Discussion 1 – Session IVA (Neuroblastoma)

Dr. Seeger: Thierry, what is the total hospitalization time roughly for these two transplants?

Dr. Philip: The first one was around 6-8 weeks and the second would be 10 weeks.

Dr. Seeger: How do the patients and the parents deal with 2 grafts, psychologically?

Dr. Philip: It is a very interesting question. The first graft is easy. Sometimes I got problems with the rationale of the second graft because we have no proof that the double graft is better than the single graft. Especially those parents are afraid of the 30% which are in CR after the first graft.

Dr. Sanders: You mentioned a fair amount of toxicity in these patients. Specifically, what were those toxicities and would you count those as regimen related toxicities or others?

Dr. Philip: I think that first the regimen related toxicity is now well known, with vincristine, melphalan and TBI. Since (2 years) we use continuous infusion of heparin to prevent VOD and despite the fact that it is not a randomized study it seems that the number of VOD decreased at least for patients that are transplanted after 3 or 4 years of therapy. That is the first point. The second point is that the majority of toxicity is bad recovery of the marrow. Most of these patients are going very well in the sterile unit, but they stay 40-50 days which is very long and very depressing.

Dr. Sanders: What did you use for infection prophylaxis?

Dr. Philip: We use the classic 3 antibiotic regimen and very quick amphotericin when they got fever.

Dr. Reading: The first question (is) to Marie. Marie, are you sure that the immunomagnetic purging is non-toxic.

Dr. Favrot: I think that I will prove the idea that immunomagnetic purging procedure is non-toxic. That is probably one of the things we have to think about, especially between the groups who are using the purging. There are two possibilities; the first one is that the magnetic purging is toxic and, the second one is that the monoclonals are toxic since we know that monoclonal antibodies against tumor cells react with some normal cells in the bone marrow and especially NK cells.

Dr. Seeger: Does your purging antibody cocktail contain anything that might ablate pre-T cells?

Dr. Favrot: No.

Dr. Seeger: The second is, I would pick an argument with you over your statement that marrow that does no have detectable tumor cells should not be purged. As you pointed out, your level of detection is 1 in 100,000.
    I will just make the point that you could be injecting into a 15 kg child, 20,000 tumor cells without purging and at least in our hands, the purging is not toxic.

Dr. Favrot: I will agree if we are sure that the magnetic purging procedure is non-toxic.

Dr. Seeger: As I say, in our hands, we have not had that.

Dr. Scouros: I presume you measured the NK function in a K562 chromium assay?

Dr. Favrot: Yes.

Dr. Scouros: You were postulating about LAK cell activity as another approach to treatment. Have you looked at LAK cell function?

Dr. Favrot: The patients have normal LAK cell function.

Dr. Pinkerton: A question for Charles August is -- in your experience -- the outcome with allografts comparable to the autografts?

Dr. August: One cannot say that it is totally comparable, because there was an increase in toxicity in the allografts and the conditioning regimen had to be changed. Maybe Bob Seeger is willing to address this.

Dr. Seeger: That is correct. In the initial study we had 50% toxic deaths among the patients transplanted with allogeneic marrow. Of those who survived the toxicity, we have only had 1 relapse. So 5 out of 6 are disease-free survivors in that initial study. And so we lost allogeneic transplants because of toxicity, the autologous we lost because of relapse. In the more recent study where we cut the conditioning regimen for the allogeneics, we are seeing relapses. So, as many other people have seen, the allogeneics do not tolerate conditioning as well as the autologous do.

Dr. Pinkerton: Do you actively look for a potential HLA match?

Dr. Seeger: We still would prefer if somebody has a match. We would do that kind of a transplant, an allogeneic transplant.

Dr. Philip: I want to come back to this issue of bad recovery that was also presented by the Graham-Pole group. What do you think about this problem and obviously the report by the Graham-Pole group and by the Lyon group are the only in the world literature with so many late recovery after BMT and we all use immunomagnetic purge marrow using the Kemshead monoclonal antibody procedure.

Dr. Reynolds: First of all, our recoveries have been fairly good, in our last 15-20 patients the median is about 21 days to recovery of neutrophils greater than 500. And we have even had patients in which we have purged their marrow, shipped it to a remote institution and they have gone home from that institution within 3 weeks of their transplant. So I do not think that recoveries are delayed of -- at least with the procedure that we are using with 2 cycles and the monoclonal antibodies we are using. And this is with patients who have some fairly aggressive induction regimens. So we do not think that this is the explanation. We cannot compare the antibodies you are using with the antibodies we are using, because we never had a chance to look at them.

Dr. Graham-Pole: I think that it is the sort of thing that requires careful analysis and we actually looked at 77 patients which is far too few to really tease out the individual components of what limits or slows engraftment. With that number of patients we have some information, it certainly appears to us it has nothing to do with the handling of the marrow. But it has more to do with biological characteristics. For example, we saw clearly (that) the girls have a significantly slower engraftment than the boys and the amount of prior chemotherapy is an influence. We analyzed at least 10 factors, which I will present next week. In our patient population, we cannot say that purging had an influence on hematopoietic reconstitution.

Dr. Seeger: I want to come back to the issue of VOD. It is a problem that many of us have, not just with kids, but adults, too. And I knew Seattle was using heparin in some very high risk patients. Could we have some anecdotal experience rationale, and so on?

Dr. Philip: We use a low dose 100U/kg per day continuous infusion. We have not seen complications. We are not planning a randomized study.

Dr. Sanders: Yes, we were doing a study looking at the administration of heparin in patients beginning prior to the preparative regimen and carrying them through on a heparin drip to keep their PTT twice normal through the first 30 days post-transplant. The rationale for this study was that Dr. H. Schulman, who is one of the pathologists in our group, found that in his study of veno occlusive disease that there were some deposits in the major venuals that contain factor 8 and the reasoning was that if we could decrease the deposition of these factor 8 containing fibrin deposits, that we could perhaps decrease the incidence and/or severity of veno occlusive disease. I do not know for sure the number of patients that we ended up treating -- about 5 or 6. But we ran into major problems with bleeding and toxicity despite major efforts to keep these patients platelet counts above 40,000. A couple of the patients I know had to have the heparin stopped because of spontaneous hemorrhagic cystitis with platelet counts of over 50,000 that could not be handled or managed while they were continuing on a heparin drip. And a couple of patients who came in were at very high risk for veno occlusive disease with elevated bilirubins an elevated SGOTs actually did get through the first 30 days. So perhaps a lower dose would be able to manage this. But we could not in the doses we were giving.

Dr. Kersey: One of the interesting things that I learned this afternoon was something that John Graham-Pole presented in terms of the relapse rate being independent of whether or not the patient was in 1st CR, 1st PR, 2nd CR, 2nd PR. Is that a general experience?

Because if it is, it is an extremely interesting phenomenon that would suggest that these patients are not resistant to the drugs initially being used and if so you could devise some interesting pharmacologic approaches.

Dr. Pinkerton: I did not show this slide of the ABMT relapsed patients. There were no long-term survivors in patients who were treated in 2nd partial or complete remission.

Dr. August: Our experience was that the relapse rate appears to be higher in those who have been transplanted after progression of disease.

Dr. Philip: In conclusion of neuroblastoma, some conclusions are now very clear. That was not the case at the last meeting. The first conclusion is that 50% of the patients which undergo BMT for stage IV neuroblastoma over 1 year are alive, progression free, 16 months post BMT. This is the first conclusion. This is a clear and statistically significant improvement in this field. The second clear conclusion is that unfortunately, some of these patients which are alive disease-free 2 years, post BMT will relapse. I think nobody now can discuss this point due to short follow-up and we can expect 25 to 30% long-term survival at 5 years and this is also a clear improvement compared with previous world literature. The third conclusion is that patients grafted in PR, or in CR, do as well. The majority of the patients with Stage IV neuroblastoma should be grafted whether they have some residual disease, yes or not. Currently in our group more and more patients in PR are going to the BMT setting. And the last point is that patients who did not receive BMT at front line consolidation, salvage is maybe possible in about 25-30%. I agree with Dr. Kersey that the question raised by Dr. Graham-Pole is a very important issue. I think that everybody will agree that we should graft neuroblastoma stage IV. The question is still open whether we have to graft them in first CR or to wait until they relapse.

Dr. Seeger: I would like to congratulate my co-chairman for a beautiful summary. However, I would like to take issue with one conclusion and that is in both Charles Augusts' and our studies we do have patients with long-term follow-up. The latest relapse that any of us have had is 21 months. So I am not sure that I am quite as pessimistic as you are about a continuing fall on the curve. I agree that we need to have more patients, and more time and more meetings.

Dr. Philip: I want to comment on this point. To my knowledge, the largest follow-up is from the NSG group and they got relapse. The second longest follow-up is in the Villejuif study and

they saw relapses as far as 47 months post BMT. Last week we observed a relapse, 3 years post BMT. I am really, really convinced that, unfortunately, you will observe this late relapse.

Dr. Seeger: We will see two years from now.

SESSION IV - PEDIATRIC TUMORS

B. SARCOMA

AUTOLOGOUS BONE MARROW TRANSPLANTATION IN EWING'S SARCOMA: An Analysis of Phase II Studies from the European Bone Marrow Transplantation Group

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INTRODUCTION

Prognosis of patients with metastatic Ewing's sarcoma (ES) remains poor despite response to conventional chemo- and radiotherapy (1,2). Recent attempts to treat these patients with ablative therapies (AT) have given controversial results (3-11).

To better define the potential role of high-dose chemotherapy, we have analyzed both its effect on measurable lesions and its toxicity in 32 patients tested in several Centers from the European Bone Marrow Transplantation (EBMT) Group.

PATIENTS AND METHODS

Patients reported in this study were included in the EBMT Registry for Solid Tumors (Lyon, France). They had at least one measurable tumor lesion at the time of high-dose chemotherapy. Patient follow-up was updated in July 1988.

Status of the disease at the time of ABMT or response to high-dose chemotherapy was defined as followed:

Complete response (CR)--complete disappearance of all measurable tumor lesion,

Partial response (PR)--when all tumor lesions regressed 50% or more with first-line therapy,

Sensitive Relapse (SR)--when a similar degree of tumor regression had occurred with salvage treatment,

Minor Response (MR)--in the case of a 25-50% reduction of all tumor lesions,

Resistance (R)--when no signifcant change was documented after first-line therapy,

Resistant Relapse (RR)--when no significant change was documented with salvage treatment,

Untreated Relapse (UR)--when no treatment had been given before AT to reduce metastatic lesions.

From June 1979 to March 1988, 32 children aged from 3 years and 2 months to 26 years (median 12 years), received high-dose chemotherapy for Ewing's sarcoma. Patients' characteristics, treatment and follow up are reported in Tables 1, 2 and 3.

Ablative Therapy

Nine patients (4 UR, 3 RR, 2 PD) received L-PAM at 140 - 220 mg/sqm. Three of them received two courses of high-dose chemotherapy (Table 1). Eleven patients (4 PR, 4 RR, 3 PD, 1 R) received an AT including L-PAM or Cyclophosphamide (Cy) in combination with one or two other antiblastic drugs. Three of them received two courses of high-dose chemotherapy (Table 2).
Two patients (including one mentioned above) received a high dose Thiotepa.
Eleven patients (6 RR, 3 PR, 2 SR) received L-PAM at 180 mg/sqm, Vincristine (VCR) in continuous infusion (c.i.) 4 mg/sqm over 5 days and total body irradiation (TBI) 8 Gy in 2 fractions.

RESULTS

Of the 6 patients receiving one course of L-PAM as single agent, 1 achieved CR, 1 PR, 2 MR; one did not respond; one patient is not evaluable for tumor response (infectious death). Of the three patients receiving 2 courses of L-PAM, 1 achieved PR after the first course but developed PD after the second one; 1 achieved MR after the first course but did not improve further after the second course; 1 achieved CR after the first course. Survival was respectively 3 and 6 months for 2 NR patients and ranged between 6 - 17 months (median 8 mos.) for the 7 responders. Of the 8 patients receiving one course of L-PAM or Cy associated with one or two other antiblastic drugs, 2

achieved CR, 3 PR, 1 MR, 1 had PD, 1 died of acute respiratory distress syndrome (ARDS) [at the autopsy no tumor was found]. One of the 4 patients receiving two courses of L-PAM or Cy associated with one or two other antiblastic drugs, 1 achieved PR after both procedures; 1 had MR after the first course but died of aspergillus after the second one; 1 achieved MR after the first course; the lung metastasis was then resected and CR was consolidated with the second AT; 1 achieved PR after the first course and CR after the second one. Survival was 2 months for the NR patient and ranged between 2-65+ months (median 10-1/2 months) for the 10 responder patients.

Of the 11 patients receiving L-PAM associated with VCR and TBI, 1 achieved CR, 3 PR (one of these 3 achieved CR after surgery and radiotherapy) 5 did not respond; 2 died respectively of Gram+ septicemia and pneumonia, and are not evaluable for tumor response. Survival ranged between 1 - 6 months for 5 NR patients (median 3-1/2 mos.) and between 3-54+ months (median 8 mos.) for the 4 responders.

Table 1. Characteristics and Clinical Course of 9 Patients with Ewing's Sarcoma Who Received L-PAM as Single Agent

Pt	Status before AT	Targets of AT	Dose L-PAM (mg/sqm)	Response to AT	Lgth of Response (mo)	Status/ Survival (mo)	Comment
1	RR	lung	180	NR		DWD/3	
2	RR	bone, lung	180	NE		TD/0	pneumonia
3	R	bone	180	MR		DWD/8	
4	UR	lung	200	MR		DWD/7	
5	UR	lung	220	PR	5	DWD/8	
6	UR	lung	220	CR	8	DWD/14	
7A*	R	lung	180	PR	2		
7B*			180	PD		DWD/6	
8A*	RR	bone, lung	140	MR			
8B*			140	NR		DWD/17	
9A*	UR	lung	220	CR			
9B*			150		14	DWD/17	

Abbreviations: L-PAM, Melphalan; AT, ablative therapy; RR, resistant relapse; NR, no response; DWD, dead with disease; NE, not evaluable; TD, toxic death, R, resistant, MR, minor response; UR, untreated relapse; PR, partial response; CR, complete response; PD progressive disease; NR, no response.
* = patients receiving two courses of AT

**Table 2. Characteristics and Clinical Course of 12 Patients with
Ewing's Sarcoma Who Received L-PAM or Cy Associated
with One or Two other Antiblastic Drugs**

Pt	Status Before AT	Targets of AT	AT	Response to AT	Lgth of Response (mo)	Status/ Survival (mo)	Comment
1	PR	bone	BPL	CR	3	DWD/8	
2	PR	bone	BPL	PD		DWD/2	
3	R	bone, lung	Bu,Cy	PR		DWD/15	
4	RR	bone	Bu,Cy	CR #		TD/0	ARDS
5	RR	bone	Bu,Cy	PR		DWD/9	
6	RR	bone	Bu,Cy, L-PAM	PR		DWD/22	
7	RR	lung	Bu, L-PAM	CR	23	DWD/23	
8	R	bone, lung	Thiotepa	MR		DWD/2	
9A*	PR	bone	BPL	PR->PD			
9B*			Bu,Cy	PR		DWD/12	
10A*	PR	lung	BPL	MR			
10B*			Bu,Cy	NE		TD/5	Aspergillus
11A*	RR	lung	BPL	MR			Surgery
11A*	CR		BPL		65+	ACR/65+	After 1st Course
12A*	R	bone, lung	Thiotepa	PR			
12B*			Bu,Cy, L-PAM	CR	4+	ACR/4	

Abbreviations: L-PAM, Melphalan; Cy, Cyclophosphamide; AT, ablative therapy; PR,
partial response; BPL, BCNU-Procarbazine-L-PAM; CR, complete response; DWD,
dead with disease; PD, progressive disease; R, resistant; Bu,Cy, Busulfan-
Cyclophosphamide; RR, resistant relapse, NE, not evaluable; TD, toxic death; ARDS,
acute respiratory distress syndrome; MR, minor response; NE, not evaluable; ACR, alive
complete response.
\# = autoptically documented
* = patients receiving two course of AT

Responses to high-dose chemotherapy in relation to the disease
status were as follows (Tables 4 and 5): of the 7 patients in PR, 2
achieved CR, 3 PR, 1 had PD, 1 died of toxicity; of the 5 resistant
patients, 1 achieved CR, 1 PR, 2 MR, 1 developed PD; of the 4 UR,
2 achieved CR, 1 PR, 1 MR; 2 SR did not respond; of the 14 RR, 3
achieved CR, 3 PR, 3 did not respond, 3 died of toxicity.

Table 3. Characteristics and Clinical Course of 11 Patients with Ewing's Sarcoma Who Received L-PAM Associated with VCR and TB1

Pt	Status Before AT	Targets of AT	AT	Response to AT	Lgth of Response (mo)	Status/ Survival (mo)	Comment
1	PR	bone	VTL	PR		DWD/6	
2	PR	bone	VTL	CR	54+	ACR/54+	
3	PR	leg	VTL	PR	10+	ACR/ 10+	Surgery and RT after ABMT
4	SR	nodes, lung	VTL	NR		DWD/6	
5	RR	nodes, mediastinum	VTL	NR		DWD/2	
6	RR	lung	VTL	NE		TD/0	G+Septicemia
7	RR	bones, lung	VTL	NR		DWD/UK	
8	RR	bones	VTL	NE		TD/0	Pneumonia
9	RR	bones, lung	VTL	PR		DWD/3	
10	RR	bones, lung	VTL	NR		DWD/5	
11	SR	bone	VTL	NR		DWD/1	

Abbreviations: L-PAM, Melphalan; VCR, Vincristine; TBI, total body irradiation; AT, ablative therapy, PR, partial response; VTL, VCR-TBI-L-PAM; DWD, dead with disease; CR, complete response; ACR, alive complete response; RT, radiotherapy; SR, second response; NR, no response; RR, resistant relapse; NE, not evaluable; TD, toxic death; UK, unknown.

DISCUSSION

Efficacy of high dose L-PAM given as a single agent followed by ABMT in Ewing's sarcoma was documented in several studies (3-5). Inclusion of TBI in the conditioning regimen for Ewing's sarcoma was based on Jenkins' observation (9) that 3 out of 11 patients with metastatic disease, treated with a single 300 rad fraction of TBI and primary mass radiation without chemotherapy, remained disease-free more than 10 years. Millburn et al (10), obtained similar results with the same treatment: 2 out of 3 patients who presented localized disease remained disease-free, whereas both patients who presented bone marrow involvement died of disease. Kinsella et al (11) reported on the efficacy of low-dose TBI (15 rad fractions given twice a week for a total of 150 rad) in combination with high-dose chemotherapy in a group of "high-risk patients."

Table 4. Responses to AT According to Status Before ABMT in 25 Patients Receiving 1 Course of AT

Status Before AT	Response to AT						
	CR	PR	MR	NR	PD	TD	Total
PR	2	2			1		5
R		1	2				3
UR	1	1	1				3
SR				2			2
RR	2	3		4		3	12
Total	4	7	3	6	1	3	25

Abbreviations: AT, ablative therapy; CR, complete response; PR, partial response; MR, minor response; NR, no response; PD, progressive disease; TD, toxic death; R, resistant; UR, untreated relapse; SR, second relapse; RR, resistant relapse.

Table 5. Response to AT According to Status Before ABMT in 7 Patients Receiving 2 Courses

Status Before AT	Response to AT					
	CR	PR	NR	PD	TD	Total
PR		1			1	2
R	1			1		2
UR	1					1
RR	1		1			2
Total	3	1	1	1	1	7

Abbreviations: AT, ablative therapy; PR, partial response; CR, complete response; NR, no response; PD, progressive disease; TD, toxic death; R, resistant; UR, untreated relapse; RR, resistant relapse.

More recently Miser designed a consolidation high-dose chemotherapy regimen for the same category of patients giving an AT as consolidation, which included VCR, Doxorubicin, Cyclophosphamide and TBI: long-term survival was around 30% (12). The results obtained in our group of patients treated with high-dose L-PAM as a single agent confirm the efficacy and feasibility of such treatment in patients with a measurable disease: 7/9 patients responded to the drug and less than 9% toxic death were observed.

Response rates obtained in patients who received L-PAM or Cy associated with other antiblastic drugs (11/12) are similar but with a 12% toxic death rate.

Apparently the response rate obtained with L-PAM associated with VCR and TBI is lower (4/11), but the only long-term survivor is in this group.

CONCLUSIONS

By using an aggressive combination of multicycle chemotherapy, the disease-free survival rate for "standard risk" Ewing's sarcoma is around 60%. Unfavorable prognostic factors include the presence of metastasis at diagnosis or their reappearance during the course of the disease, and a primary mass volume > 100 ml. Event free survival for patients with bone marrow metastasis at diagnosis or for patients experiencing a recurrence is 10%; for patients with lung metastasis or a primary mass > 100 ml it is 32-37%.(2) For the former of these two categories it appears justified to propose a high-dose chemotherapy as consolidation in an early phase of the disease. For the second category, a randomized study comparing an aggressive combination to an AT could better define the potential role of the latter.

It is difficult to indicate the best regimen for the high-dose chemotherapy on the basis of the reported data; an association of L-PAM or Cy with one or two other antiblastic drugs could be a reasonable one. The addition of TBI does not seem justified.

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HIGH-DOSE CHEMOTHERAPY WITH AUTOLOGOUS BONE MARROW TRANSPLANTATION IN WILMS' TUMOR:
A Survey of the European Bone Marrow Transplantation Group

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INTRODUCTION

Wilms' tumor is the most frequent malignant neoplasia of the kidney in childhood; it accounts for approximately 6% of all malignant tumors of this age group. Multidisciplinary treatment is highly successful; more than 80% of children attain long-term disease-free survival; present efforts are mostly directed to reduce side effects of treatment (1-2).

In the small group of patients who fail on current treatment strategies, the efficacy of high dose chemotherapy with autologous bone marrow transplantation (ABMT), a promising salvage approach for patients with poor-prognosis chemosensitive malignancies (3-4), is under investigation.

We report herewith the experience of some European Centers in respect to the efficacy and toxicity of this approach in children with poor prognosis Wilms' tumor.

PATIENTS AND METHODS

Data of 20 children with histologically documented Wilms' tumor, who had been submitted to high dose chemotherapy with ABMT in the period February 1985 – April 1988, were collected from 5 Centers contributing to the European Bone Marrow Solid Tumor Registry (EBMSTR). Patients with Wilms' tumor at poor prognosis (unfavourable histology, resistance to first-line chemotherapy, multifocal relapse, disseminated disease at onset) (Table 1) received

Table 1. Clinical Details for 20 Patients with Wilms' Tumor

Case	Stage	Histology	Sites of initial metastases	Initial response	No. of relapses	Sites of of last relapse	Status at ABMT	Conditioning regimen	Outcome (Disease free Survival)
1 GM	IV	F	lungs	CR	1	lungs	CR	VCR L-PAM	DWD (4/10)
2 NF	III	F	--	CR	1	lungs	CR	VCR L-PAM	TD (1/1)
3 PS	I	F	--	CR	1	lungs	CR	VCR L-PAM	DWD (7/24)
4 LL	IV	U	lungs	PM	--	lungs progress.	CR	VCR L-PAM	AFCR (23/27+)
5 CS	I	U	--	CR	1	bone	CR	VCR L-PAM	AFCR (16/26+)
6 QR	I	F	--	CR	1	lungs	CR	VCR L-PAM	ACCR (23+/23+)
7 DE	IV	F	lungs	CR	--	--	CR	BCNU VCR L-PAM	ACCR (24+/24+)
8 AN	IV	F	lungs	CR	--	--	CR	BCNU VCR L-PAM	ACCR (21+/21+)
9 NO	IV	U	nodes, CNS, bone	CR	--	--	CR	BCNU VCR L-PAM	ACCR (25+/25+)

Case	Stage	Histo-logy	Sites of initial metastases	Initial response	No. of relapses	Sites of last of last relapse	Status at ABMT	Conditioning regimen	Outcome (Disease free Survival)
10 CR	II	F	--	CR	2	lungs, CNS	CR	BCNU VCR L-PAM	ACCR (14+/14+)
11 BF	III	U	--	CR	1	lungs, liver, abdomen	NE	BUS CPM	DWD (7/12)
12 SN	IV	U	bone	CR	1	bones	PR	BUS,CPM	DWD (4/7)
13 ME	III	F	--	CR	3	lungs	CR	VCR,L-PAM	TD (1/1)
14 DM	IV	F	lungs	CR	4	lungs	PR	BUS,CPM	DWD (9/16)
15 LN	IV	F	lungs cervical nodes	CR	1	lungs	CR	IFO VP16 L-PAM	DWD (4/12)
16 TA	IV	F	lungs	CR	3	lungs, nodes	CR	IFO VP16,L-PAM	DWD (3/20)
17 TC	II	F	--	CR	1	abdomen	CR	VM26 CDDP L-PAM	AFCR (12/15+)
18 SS	I	F	--	CR	1	lungs	CR	IFO,VP16, DOX	ACCR (3+/3+)
19	IV	F	lungs, nodes	CR	3	lungs	CR	VCR L-PAM	ACCR (30+/30+)
20	I	F	--	CR	3	lungs	CR	VND,CDDP	ACCR (45+/45+)

F, Favourable; U, Unfavorable; CR, Complete Remission; NE, Not Evaluable; VCR, Vincristine; L-PAM, Melphalan; IFO, Ifosfamide; Dox, Doxorubicine; BUX, Busulfan; CPM, Cyclophosphamide; CDDP, Cis-Platinum; VND, Vindesine; TD, Toxic Death; DWD, Dead with Disease; AFCR, Alive further CR; ACCR Alive continuous CR.

a variety of treatment and then underwent high-dose chemotherapy and ABMT on decision of the individual investigator.

Age at diagnosis and at ABMT ranged from 10 months - 15 years (median, 4 years) and from 28 months - 17 years (median, 6 years) respectively. At diagnosis 5 patients had stage I tumor, 2 stage II, 3 stage III, and 10 stage IV. At first surgery, favourable histologic features were detected in 15 cases, unfavourable in 5. One stage IV patient was resistant to conventional treatment, three stage IV cases, all from one Center, received high-dose chemotherapy being in first complete remission (CR). Ten patients had experienced one relapse, 6 had had two or more relapses. Relapses occurred while on therapy in 3 cases, after the discontinuation of the therapy in 13 cases. The interval between diagnosis and ABMT ranged from 6 - 105 months (median, 26 months). At the time of high dose chemotherapy 17 patients were in CR, 2 patients had residual measurable disease, 1 was not evaluable. The harvested bone marrow was cryopreserved without purging in all cases, except one who had had multiple bone metastasis.

High-Dose Chemotherapy Regimens

Seven different schedules were administered; Table 2 gives details on drugs and doses, as well as the number of cases treated and Centers, for each schedule.

RESULTS
Toxicity

All patients experienced profound myelosuppression. Granulocytopenia less than $0.5 \times 10^9/1$ lasted 9-46 days (median 19 days). Thrombocytopenia less than $50 \times 10^9/1$ lasted 14 - 395 days (median 29 days). Seventeen patients developed fever lasting 3 - 28 days (median, 7 days). Two patients died of pneumonitis on day 30 and 45, respectively: both of them had received lung irradiation, 15 days and 6 months before the high dose chemotherapy consisting of Vincristine and Melphalan. Autopsy failed to reveal causative microorganisms. Transient renal failure was documented in two patients. One patient presented severe hemorrhagic cystitis and then developed chronic renal failure with mesangiolysis.

Tumor Response

Of the two patients, who had measurable lesions, respectively in lungs and bones, both obtained CR following high dose chemotherapy.

Table 2. High Dose Chemotherapy Regimens

Regimen	Number of Patients	Center
Vincristine 4 mg/sqm in 5 days	7	Genoa
Melphalan 180 mg/sqm 6th day	1	Marseille
Vincristine 3 mg/sqm in 4 days		
BCNU 300 mg/sqm 1st day	4	Barcelona
Melphalan 180 mg/sqm 5th day		
Busulfan 20 mg/Kg in 4 days	2	Marseille
Cyclophosphamide 120 mg/Kg in 4 days	1	Villejuif
Ifosfamide 4 gr/sqm in 2 days		
VP 16 1 gr/sqm in 5 days	2	Villejuif
Melphalan 140 mg/sqm 6th		
VM 26 1 gr/sqm in 5 days		
Cisplatinum 200 mg/sqm in 5 days	1	Marseille
Melphalan 180 mg/sqm 6th day		
IFO 12 gr/sqm in 5 days		
VP 16 1 gr/sqm in 5 days	1	Genoa
Dox 80 mg/sqm in 2 days		

Clinical Course

The two patients reinduced into CR relapsed after 4 and 9 months and died at 7 and 16 months, respectively. Relapses occurred at the same sites in one case and in a distant site in the other. Of the 18 patients treated in CR, two died of early toxic complications (see above). Relapses occurred in 8 cases at 3-23 months (median, 7 months); five of them died at 10-24 months (median, 12 months), 3 are alive (2 in subsequent CR) at 15, 26 and 27 months from bone marrow transplantation (3, 4, 10 months from relapse). Disease reappeared at the site of previous location in 7 patients, at different sites in 3.

Eight patients persist in complete remission at 3-45 months (median, 23 months).

The overall and event-free survivals projected at 45 months are respectively 48% and 34%.

DISCUSSION

Despite the high survival rates reported for Wilms' tumor, 20% of cases continue to fail on treatment.

The analysis of two large multicenter studies in Europe (5) and in

US (6) have provided valuable indications regarding factors associated with a risk of responding poorly or relapsing.   Unfavourable histology, extension of the disease at onset, size of the tumor, location of relapse have been identified as prognostic factors. For these poor risk patients, a search for more effective treatments appears justified. The efficacy of high-dose chemotherapy with autologous bone marrow rescue has been considered by several investigators and in this report we have tried to summarize preliminary information.

The meaning of the data is somehow reduced by the heterogeneous criteria adopted to elect patients to high dose chemotherapy and by the different ablative regimens employed.

However most investigators independently agreed to use Melphalan (7) in combination with Vincristine or a podophillin derivative. Only few considerations can be made regarding the potential efficacy of high dose chemotherapy in improving the outcome of children with poor-risk Wilms' tumor:

1) Both cases with measurable tumor lesions responded and achieved CR although of short duration.

2) The three stage IV patients treated in first CR continue to be in CR after a follow-up of more than 20 months.

3) Five of the 15 patients who received high dose chemotherapy in a second or further CR maintain CR with a median observation time of 23 months.

4) Overall toxicity encountered in this series of patients appears similar to that in children treated for other malignancies (8).

However the two cases, one of transient impairment of renal function and one of chronic insufficiency observed in this group of nephrectomized patients suggest that the residual kidney may be abnormally susceptible to drug damages.

Finally the two deaths from pneumonitis, both occuring in the group of 9 children who had been previously irradiated to the lungs, confirm that this last treatment modality is associated with increased risk of developing lung complications (9-10).

CONCLUSIONS

High dose chemotherapy regimens are feasible in poor prognosis Wilms' tumor. Special attention has to be given to the possibly increased risk of these patients to develop renal and lung toxicities.

A definite conclusion on the potential role of high-dose chemotherapy in improving the outcome of children with poor risk Wilms' tumor cannot be given on the basis of the few cases treated so

far, of the heterogeneous criteria of eligibility and of the regimens adopted. A well-designed multicenter study appears desirable to clarify this important issue.

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HIGH-DOSE CHEMO/RADIOTHERAPY AND AUTOLOGOUS BONE MARROW TRANSPLANTATION AS CONSOLIDATION THERAPY IN CHILDREN'S METASTATIC EWING'S SARCOMA

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With conventional therapy, prognosis of metastatic Ewing's sarcoma remains generally very poor (1, 2, 3). Recently, encouraging results have been published in patients treated with moderate dose, semi-continuous chemotherapy (4). Taking into account the promising results of high-dose therapy used as consolidation therapy in neuroblastoma (5, 6) this approach has been tested in patients with metastatic Ewing's sarcoma. We report here the results obtained in 24 Ewing's sarcoma patients bearing metastases either at diagnosis or secondarily and who entered complete remission under conventional therapy.

PATIENTS AND METHODS

Patients

Between 1980 and 1987, 24 patients were treated in two French institutions for metastatic Ewing's sarcoma (6 at CLB and 18 at IGR). Their ages at diagnosis ranged from 1 to 26 years with a median of 11 years. There were 12 males and 12 females.

At the time of diagnosis, 20 patients had diffuse metastases involving lungs, bone and bone marrow, or both. Four had localized disease which subsequently relapsed under conventional therapy.

As primary treatment, before high-dose consolidation, patients received conventional chemotherapy and local treatment of the primary tumor.

Conventional chemotherapy was administered for a median duration of six months (range 4 - 24 months). Thirteen patients received alternating courses of Ifosfamide, Vincristine, Actinomycin D (IWA) and Ifosfamide, Vincristine, Adriamycin (IVAd). Eight patients received the same kind of chemotherapy in which Cyclophosphamide was used instead of Ifosfamide (VAC-VAD). Three patients were treated according to the St Jude's protocol (7).

Local treatment of the primary tumor was a combination of surgery and radiotherapy. Surgical excision of the primary tumor was performed when feasible (long bones); dose of local radiotherapy ranged from 45 to 65 Gy, according to the site of the tumor.

As a result of this first line therapy all patients entered complete remission (CR). Six of them subsequently relapsed (1 local, 5 metastases) and were retreated with conventional therapy. Hence, at the time of consolidation therapy, 18 patients were in first CR and 6 were in second CR.

METHODS

Conditioning Regimens

- Six patients were treated with total body irradiation (TBI) (3 fractions of 4 Gy) combined with Vincristine and Melphalan for five of them and with Melphalan alone for the sixth patient (6).

- Eighteen patients were treated with combination of high-dose chemotherapy without TBI. Fourteen received a combination of BCNU, Procarbazine and Melphalan according to the protocol shown in Table 1. Four of these patients received one course of this therapy and ten received two courses 3 to 4 months apart.

- Four other patients were treated with two different courses of high dose chemotherapy, 2 to 3 months apart. The first course was a combination of BCNU and Melphalan; the second course a combination of Busulfan and Cyclophosphamide (see Table 1).

Bone Marrow Procedures

The technique of bone marrow harvesting has been described elsewhere (5). For patients treated with two courses of high-dose chemotherapy, bone marrow cells were harvested once before the first treatment and kept frozen in two parts in order to allow two bone marrow grafts.

Supportive Care

All patients were treated under simple reverse isolation barrier

Table 1. Chemotherapy Regimens

	BCNU - Procarbazine - Melphalan						
	D1	D2	D3	D4	D5	D6	D7
BCNU 300 mg mg/m^2	X						
Procarbazine 400 mg/m^2	X	X	X	X			
Melphalan 180 mg/m^2					X		
ABMT						X	

	BCNU - Melphalan				
	D1	D2	D3	D4	D5
BCNU 300 mg/m^2	X				
Melphalan 180 mg/m^2			X		
ABMT				X	

	Busulfan - Cyclophosphamide									
	D1	D2	D3	D4	D5	D6	D7	D8	D9	D10
Busulfan 4 mg/kg	X	X	X	X						
Cyclophosphamide 50 mg/kg						X	X	X	X	
ABMT									X	
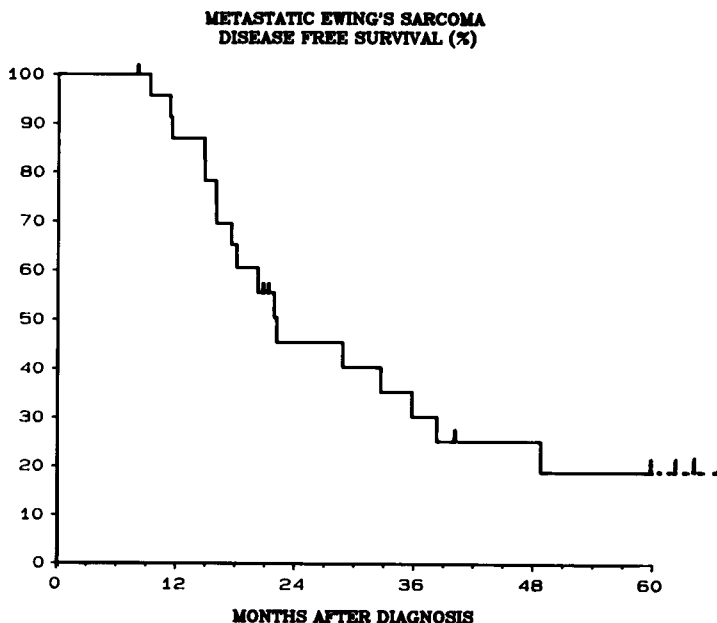
Figure 1. Probability of disease-free survival at 2 years post diagnosis.

conditions. Platelets and red cell concentrates were transfused when indicated. Febrile episodes were promptly treated with broad-spectrum antibiotics.

RESULTS

Survival

- At present, 7 patients are alive in continuous CR with a median follow-up post transplantation of 29 months (range : 3 - 63 months). One was treated with Melphalan - TBI, 3 received two courses of BCNU, Procarbazine, Melphalan, and 3 two courses of BCNU Melphalan, Busulfan, and Cyclophosphamide.

- Fourteen patients relapsed 3 to 22 months after bone marrow transplantation (median 9 months). The majority of these relapses were distant, involving lungs and/or bones. Among these 14 patients, 11 died of the disease shortly after relapse (median 2 months; range 1 - 10 months) and 3 are alive with progressive disease.

- Three complication-related deaths occurred, one following TBI conditioning regimen and two following chemotherapy conditioning regimen.

- Overall, the probability of disease free survival at 2 years post diagnosis is 45 % and 20 % at 4 years post diagnosis (See Figure 1).

Toxicity

All patients experienced profound **myelosuppression**. The median duration of granulopenia <0.5 x 10^9/1 was 21 days (range 5 - 39). Leukopenia <1 x 10^9/1 and thrombopenia <50 x 10/1 lasted for a median of 21 (Range 5 -48) and 24 (Range 4->300) days, respectively. No difference in the duration of these parameters was observed between the different conditioning regimens. Engraftment was delayed in 3 patients in relation to a viral infection.

Gut toxicity was usually mild. Mucositis was the most common complication but severe forms of mucositis requiring pain relief were rare (<20 % of patients).

Severe visceral toxic complications were observed in six patients. Three cases of veno-occlusive disease of the liver were observed. They recovered spontaneously. Three cases of severe hemorrhagic cystitis occurred; they were related to cyclophosphamide or previous pelvic irradiation.

Infectious Complications

- All patients experienced fever and received broad spectrum antibiotics. Two cases of severe sepsis were observed: one was related to gram negative bacillus and led to death with acute renal failure; the second was related to *candida tropicalis* and recovered slowly.

- Two severe viral infections occurred and were lethal. One was the cause of prolonged aplasia with hemorrhagic cystitis and hepatitis; the second was an EBV infection leading to a polyclonal B lymphoma with prolonged aplasia which was lethal.

Complication-Related Deaths

Overall, three patients died of complications. The causes of death were: gram negative sepsis in one case and polyvisceral viral infection in two cases.

DISCUSSION

When compared with conventional chemotherapy, these results

appear encouraging. In our experience, for patients treated by "VAC – VAD" or "IVA – IVAd" the probability of disease free survival of metastatic Ewing's sarcoma was 28% at 2 years and 7% at 4 years post diagnosis. The use of high-dose consolidation therapy is therefore a real improvement in survival for the patients treated with this kind of primary chemotherapy. The duration of CR is prolonged and the rate of long term survivors appears higher.

The numbers of patients treated with the different conditioning regimens are too small to allow any statistically significant comparison between these regimens. However, taking into account the results published by Miser et al. (8), conditioning regimens containing TBI do not appear to be more efficient than combinations of drugs.

Nevertheless, these results of consolidation with high-dose therapy appear poorer than those of conventional semicontinuous conventional chemotherapy (4). Those results published by the St. Jude's hospital's team appear very promising and should be tested in other institutions.

Toxicity of high-dose consolidation therapy was high but acceptable, very close to that observed in other malignancies treated the same way (5, 6). However this therapeutic approach was related to a high morbidity and prolonged intensive care support was necessary to manage the patients.

CONCLUSION

The role of high-dose consolidation therapy in metastatic Ewing's sarcoma remains questionable when the results of this strategy are compared with the semi-continuous conventional chemotherapy. More phase II studies of high-dose therapy are necessary to better define the right place of this strategy in the treatment of these patients.

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HIGH DOSE CHEMO-RADIOTHERAPY WITH AUTOLOGOUS BONE MARROW RESCUE IN PEDIATRIC SOFT TISSUE SARCOMAS

C. R. Pinkerton, T. Philip, O. Hartmann, J. M. Zucker, D. Valteau, and L. Brugiéres

Cure rates are high for the majority of children with non-metastatic rhabdomyosarcoma using VAC or Ifos/VA regimens. There remains however a minority of patients who have metastatic disease, either to lung or bone or unfavorable alveolar histology where long term cure rates are less than 20%. Current investigational regimens for such patients include the addition of platinum or platinum analogues, Etoposide, high dose Epiadriamycin, and dose escalation of Ifosfamide. There is also interest in the use of high dose chemo/radiotherapy with bone marrow rescue once complete remission or good partial remission has been achieved by conventional dose therapy. Moreover, there are also subgroups of patients where radiation therapy is particularly undesirable. For example, in the small child with a head and neck primary high dose irradiation may produce unacceptable abnormalities of bone growth or excessive doses to brain. There are also sites such as the vagina and bladder where in order to preserve organ structure and function dose escalation may be justified as a means of minimizing surgery and either limiting radiotherapy to interstitial irradiation or removing the need for it altogether. In this review the experience of several European groups in treating high risk patients with megatherapy is presented.

Between 1985 and 1987, 31 patients with soft tissue sarcoma treated in first remission were registered with the European Bone Marrow Transplant Group Solid Tumour Registry. Ages range from 3 to 20 years and initial stages included 25 Stage IV, 6 Stage III. Pathological subgroups included 26 Rhabdomyosarcoma, 4 undifferentiated embryonal sarcoma, 1 synovial sarcoma. Initial

chemotherapy and high dose therapy regimens given are shown in Table 1. Only patients who were treated as part of initial therapy are discussed here. Patients treated in relapse or in second remission have been excluded.

Approximately two thirds of patients had achieved complete remission when given megatherapy. The remaining third had some residual disease. Radiotherapy to sites of metastatic disease were not used in the majority of patients. Overall 10 out of 31 patients are alive and event free. One of these, the patient with the synovial sarcoma who was treated in partial remission remains alive but with residual measurable disease. The others are in complete remission. Of those with metastatic rhabdomyosarcoma, 7 of 20 are alive in remission for periods ranging from 12 to 100 months (see Table 2).

Table 1. Initial Chemotherapy and High-Dose Therapy Regimens

Initial Chemotherapy

VAC (Ad)	14
IVA	10
IVA, Ad CDDP	7

Response to Initial Therapy

CR	20
PR	11

High Dose Therapy Regimen

Melphalan			7
Melphalan TBI ± V			9
Melphalan BCNU ± V			7
V BCNU + Melphalan (double procedure)	Procarbazine VP16 Melphalan		8

V = Vincristine, A = Actinomycin D, Ad = Adriamycin, I = Ifosfamide, CDDP = Cisplatin

Table 2. Companion of Treatment Regimens with Patient Survival

Outcome: Survival (months from diagnosis)

31

25 St. IV   6 St. III

20 Rhabdo   5 other   2 NED (18,20)

7 NED   1 NED (30)

(12,18,18,54   1 AWD (11)
60,60,100)
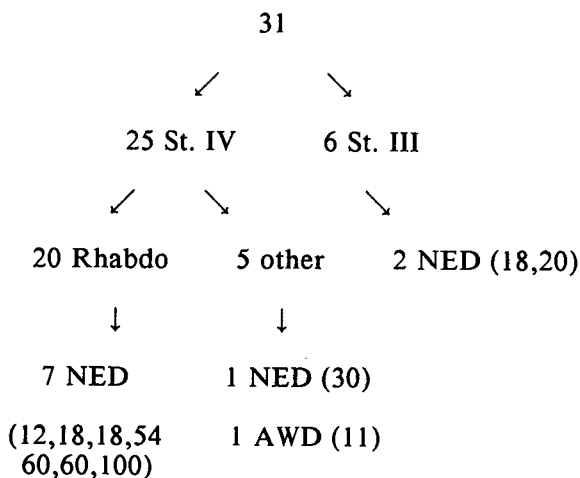
Regimens Used and Survival

Melphalan	2/7
Melphalan + TBI ± VCR	2/9
Melphalan + BCNU ± VCR	2/7
Double Procedure	4/8

There were four toxic deaths (12%), 2 with pneumonitis, 1 aspergillosis, and 1 metabolic derangement.

When considering effectiveness of the high dose therapy regimens used, no clear pattern emerges. Survival appears similar after regimens with and without total body irradiation (TBI). The 50% survival in patients given double procedures compared to around 30% for single procedures must be seen in the context of the very small numbers in each sub-group.

With regard to the next step in the use of megatherapy for high

risk and metastatic sarcomas, the therapeutic options are somewhat limited as few new agents are available to be studied at high dose. The use of combined alkylating agents such as Cyclophosphamide/Busulphan or Melphalan/Busulphan is under evaluation. The addition of high dose Carboplatin (up to 2 g/m^2) to high dose Melphalan is also being studied. Until the current studies have accrued more patients and longer follow up is observed, no firm conclusions can be drawn about the role of high dose therapy. However, with current supportive care techniques the morbidity is improving and this approach remains one of the few new avenues worth investigating in poor risk patients.

Discussion 2 - Session IVB (Sarcoma)

Dr. Philip: Okay. We move now to the two last speakers.

Dr. Seeger: Ross, one gets terribly discouraged about a 15% disease-free survival in Ewings. But remember the initial allogeneic marrow transplant data that we and others have seen for patients transplanted in relapse -- you are dealing with a group of patients who have failed first line chemotherapy. And we are still getting not much better than 15% disease-free survival in allogeneic transplants for acute leukemia when they are transplanted in relapse. So that one "take-home" message, from what you have presented, is not -- though this is terrible and we should not even be doing it -- but rather should we not be moving this earlier in the course of the patient's disease because the regimen is tolerable and it, perhaps, is effective since the major cause of failure is relapse?

Dr. Pinkerton: I probably did not stress enough but, in fact, in that Ewing's series, it was a mixture of patients and I think 1/3 of them were relapse. But, I think, 2/3's were in fact done in first complete or partial remission. It was being put in fairly early on. But I take your point.

Dr. Dicke: Are you recommending a TBI containing program in soft tissue sarcomas or the combined high dose chemotherapy programs?

Dr. Pinkerton: My feeling is that TBI in Ewing's probably is not adding a great deal. It may in fact be preventing an adequate dose being delivered to the local tumor, the primary site. And the trend is away from TBI and the reviews, we have done in neuroblastomas, (show) there has been little evidence that TBI is adding to either response rates in phase II studies or to remission duration. And for both these diseases we are dropping TBI and going for combined alkylating regimens.

Dr. Seeger: These marrows were reinfused by conventional methods that I presume were tumor-free?

Dr. Pinkerton: By conventional methods.

Dr. Seeger: I think one point is that, perhaps, if one begins looking with immunocytology you may find that many of them contain tumor. I think that should be done perhaps in future studies of autotransplant in these tumors.

Dr. August: What conditioning regimen did you use in rhabdomyosarcoma?

Dr. Pinkerton: We are proposing a combination of high dose melphalan with very high dose carboplatin.

Dr. Philip: Dr. Garaventa, could you make a comment about the stage IV patient because I think that the majority of the pediatricians will not accept indication of bone marrow transplantation in first CR. What were the selection criteria for these 3 patients?

Dr. Garaventa: The patients were treated on the basis of the decision of the individual investigator. One of the 3 patients had multiple bone metastasis and CNS and liver metastasis at presentation.

Dr. Philip: I am correct if my conclusion is that you got 15 patients which were after second relapse or subsequent relapse -- which I think the majority of people will agree to consider for bone marrow transplantation -- and 5 of these 15 are still alive.

Dr. Garaventa: Exactly, 2 in second CR and 3 in subsequent CR.

Dr. Philip: I think as a conclusion it is really a new area in which bone marrow transplantation can be considered. Thank you very much and the session is closed.

SESSION V - GROWTH FACTORS

INTERLEUKIN-1 AND HEMATOPOIESIS

Carol L. Epstein

INTRODUCTION

Interleukin 1 (IL-1), which has been demonstrated to be identical to hematopoietin-1 (H-1) (1), effects not only hematopoiesis but other systems as well. This paper will discuss IL-1's effects on hematopoiesis, and implications for use in bone marrow transplantation (BMT).

IL-1 has been shown to consist of two forms, IL-1α and IL-1β, both produced by monocytes. The primary translation products have almost identical molecular weights of approximately 30 Kd. The full length form of IL-1α is active, but the primary biologic activity resides in the approximately 17.5 Kd fragments of each form which occur naturally after proteolysis (2). Although there is only 26% homology between the two IL-1 protein sequences, the two forms compete for binding with the approximately 80 Kd plasma membrane IL-1 receptor (3) and appear to have identical activity profiles, with the exception that IL-1β must be cleaved before it is active (4). To date, the effects of both forms of IL-1 on the hematopoietic system appear to be identical, and therefore this paper will not differentiate between the two.

It has been postulated that the various cytoreductive therapies in BMT may effect the hematopoietic system not only by reducing the number of stem cells, but by the inadequate production of interleukins and colony stimulating factors, or both. Many in vitro and in vivo studies have been conducted in order to investigate these possibilities, to suggest clinical roles for these proteins. These results have been used to put together the schema proposed in Figure 1, showing the effects of various lymphokines and CSFs on bone marrow proliferation and maturation. Some of these studies are discussed below.

LYMPHOKINE ACTIVITY ON THE HEMATOPOIETIC SYSTEM

Pluripotent Stem Cell
IL-1
IL-1, IL-3
IL-1, IL-3
IL-7 ?
IL-3 GM-CSF IL-1 ?
CFU-GEMM
Lymphoid Stem Cell
BFU-E CFU-MEG CFU-GM CFU-Eo
Pre B-Cell
Thymus
IL-3 GM-CSF Epo
IL-3 GM-CSF MMF (MacCSF)
IL-1 IL-3
IL-1
IL-7
IL-7 ?
CFU-E Epo ?
GM-CSF IL-3 IL-5 IL-1
Pre T-Cell
IL-3 GM-CSF Epo
IL-3 GM-CSF M-CSF IL-1
IL-3 GM-CSF G-CSF
IL-3
IL-7 ?
Megakaryocyte
Monocyte
IL-5
B-Cell
T-Cell
Epo
MMF? (MacCSF)
M-CSF GM-CSF
IL-4 IL-2
IL-4 IL-2
Red Blood Cells
Platelets
Macrophage
N
Granulocytes
E
B
Plasma Cell
IL-2
Memory B Cell
T cells
IL-4; IL-2
IL-4 IL-2
IL-2
IL-4; IL-7
Memory T-Cell
Antibody
Effector T-Cell

Figure 1. Effects of various lymphokines and colony stimulating factors (CSFs) on the hematopoietic system. (Courtesy Immunex Corporation)

Early studies by Krumwieh (personal communication) demonstrated cynomolgus monkeys given daily doses of IL-1 developed a marked, but transient, leukocytosis (primarily in the granulocyte population). As IL-1 has been shown to induce the production of a number of CSFs, including GM-CSF (5), it may be this effect, rather than a primary proliferative effect, that results in the granulocytosis. IL-1 is believed to cause the pluripotent stem cell to both replicate and differentiate. IL-1 both potentiates the effects of other CSFs on bone marrow cells and, in culture, causes fibroblasts and endothelial cells to increase production of CSFs. Nemunaitis and co-workers (personal communication) have shown that human stromal cells transformed by SV40 proliferate in response to IL-1 and produce increased amounts of various CSFs. In addition IL-1 may have direct effects on the differentiation of CFU-GEMM, CFU-GM and CFU-Eo, and, in total, could result in the production of BFU-E, CFU-Meg, monocytes, immature neutrophils, eosinophils, immature basophils, and lymphoid stem cells as final products.

In vivo studies have confirmed that, in the mouse, IL-1 supports and enhances hematopoietic activity. A number of experiments by Neta and co-workers have demonstrated that IL-1 given to mice 20 hours before irradiation show more CFU-E, BFU-E, CFU-GM and

CFU-S after 8-12 days. Neta et al (6) have also demonstrated that no significant protection against lethal irradiation was conferred by the administration of GM-CSF, gamma interferon, or IL-2, and they concluded that the protective effects of IL-1 seen in the same model were not solely a result of induction of these cytokines by IL-1. The effect of IL-1 on the survival and myelopoiesis of mice treated with lethal irradiation was confirmed by Castelli and co-workers (7). IL-1 was able to protect mice from lethal and sublethal doses of CTX when givn i.p. either 20 hours prior to CTX as a single dose, as a single dose 48 hours after CTX, or as daily doses following CTX administration. They suggest that IL-1 produces a rapid increase in extramedullary CFU-C activity, with migration of CFU-C from bone marrow to the extramedullary sites of hematopoiesis.

Uckun et al (80 reported that in vitro, preconditioning normal human bone marrow progenitor cells with IL-1 enable both CFU-GM and BFU-E to repair sublethal radiation damage, and renders CFU-GM less sensitive to irradiation. GM-CSF demonstrated similar effects,but G-CSF affected only CFU-GM, not BFU-E. The effect of IL-1 on BFU-E was dependent on the presence of accessory cells, but the effects of IL-1, GM- and G-CSF on CFU-GM, and the effect of GM-CSF on BFU-E appeared to be independent of accessory cells. IL-2 not only did not protect against the effects of irradiation, but appeared to have a sensitizing effect. Morrissey et al (9,10) have confirmed that, in mice, IL-1 therapy given b.i.d. after irradiation increased the GM-CSF responsive colonies and decreased the period of radiation-induced neutropenia. However, these mice appeared to have a delayed recovery of the immune system, as measured by the ability of the spleen cells to respond to T and B cell mitogens when compared to those of the untreated irradiated mice.

They then demonstrated that b.i.d., IL-1 administration to normal mice decreased thymic cellularity, especially in the CD4+/CD8+ population, in a dose-dependent fashion. Cellularity recovered rapidly following cessation of IL-1 therapy. These mice were noted to have increased levels of serum corticosterone. They postulate their results are due to corticosterioid-induced thymocyte cytolysis. The remaining thymocytes appeared to respond significantly better to mitogens than did controls. Therefore, in this setting, the clinical effect of IL-1 on the immune system is not easy to predict.

Moore and his group at Sloan Kettering (personal communication) are studying the effects of IL-1 alone or in combination with either G- or GM-CSF in mice with breast tumors treated with 5-FU. Preliminary data indiate that the combination of IL-1 plus either G- or GM-CSF resulted in significantly higher white blood cell counts than those inthe control group or in any of the groups receiving proteins alone. Neutrophil counts were most significantly increased with IL-1 plus GM-CSF. Warren and Moore (personal communication) also studied the interaction between IL-1 and IL-3,

GM-, G-, and M-CSF on human bone marrow. IL-1 alone did not result in colony formation from purified stem cells, but the combination of IL-1 plus any of the other cytokines resulted in significantly more colonies than were produced by the cytokine alone. Preincubation studies indicated that the early progenitor cells were stimulated maximally by a combination of IL-1 plus IL-3, followed by GM-CSF.

This result supports the current concept of the relative position of action of the various cytokines - IL-1 and IL-3 acting on the earliest progenitor cells, followed by GM-CSF, and finally G- and M-CSF. In addition, the IL-1-induced stimulation of stem cells and their subsequent proliferation is also associated with an increase in the display of cell surface receptors for IL-3, GM- and G-CSF, which could contribute to the synergistic effect of the combination therapy (L. Park, personal communication).

The conclusion that purified stem cells resulting from treatment of human bone marrow with 4-hydroxyperoxycyclophosphamide (4-HC) respond more poorly to "later" acting stimulants such as GM-CSF is supported by data from Blazar et al (personal communication), who studied the use of GM-CSF in autologous bone marow transplant (ABMT) patients whose bone marrow was purged with 4-HC.

Their results differed from those of Appelbaum et al (11) who showed an enhanced rate of engraftment in patients undergoing ABMT with a different method of treatment of the transplanted marrow, and less reduction on the relatively mature progenitor cells. Blazar's patients did not demonstrate a decreased time to engraftment. We propose to study the use of IL-1 in ABMT in settings where the bone marrow has been purged and the percentage of CFU-GM and CFU-GEMM have been significantly reduced.

CONCLUSION

In view of the multiple effects of IL-1 on hematopoiesis in animal model systems and on in vitro bone marrow cultures, we believe that IL-1 has a role in the therapy of bone marrow transplant recipients. However, since several studies in vitro have shown that IL-1 alone does not increase colony formation, it is likely that a combination of CFS and interleukins will be required in order to achieve the optimum stimulation of the various progenitor survivors found in different clinical settings.

In addition, the potential effect on allogeneic transplant recipients must be studied. Determining the exact combination of CSFs and interleukins will require sophisticated in vitro and animal testing, but the definitive studies will be done in the clinic setting.

ACKNOWLEDGMENTS

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USE OF RECOMBINANT HUMAN GRANULOCYTE MACROPHAGE COLONY STIMULATING FACTOR (rhGM-CSF) IN AUTOLOGOUS MARROW TRANSPLANTATION FOR LYMPHOID MALIGNANCIES

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INTRODUCTION

GM-CSF is a regulatory glycoprotein necessary for the survival, proliferation and maturation of myeloid cells.[1] Recombinant cDNA technology[2] has allowed for the production of sufficient quantities of rhGM-CSF to enable clinical trials. Myelopoiesis is enhanced when rhGM-CSF is given to normal monkeys[3] and if given following autologous marrow transplantation, monkeys recovery neutrophil counts more quickly.[4]

In a phase I-II dose escalation study we found that patients with lymphoid malignancies undergoing autologous bone marrow transplantation (ABMT) receiving 60 to 240 $\mu g/m^2$/day of rhGM-CSF (Immunex Corp., Seattle, WA) by two-hour intravenous infusion for 14 days tolerated the drug well, and when compared to historical controls, appeared to recovery neutrophil counts more rapidly, and became platelet transfusion independent sooner (Table 1).[5] Other studies in patients undergoing ABMT demonstrate similar findings at comparable doses.[6]

The present report provides a follow-up of the initial 15 patients given escalating doses of rhGM-CSF[5]. Additionally, observations on 13 subsequent consecutive patients given rhGM-CSF on different schedules are described. In these additional studies, we attempted to address questions concerning the duration of therapy, the infusion schedule and the appropriate timing of infusion.

Table 1. Mean Values of Phase I Study Parameters

GM-CSF Dose[a] $\mu g/m^2/d$	Day ANC > 500*	Day platelet transfusion independent*
0[b] (N[d] = 86)	25 ± 10 (10-60)	38 ± 20 (13-118)
≤ 30 (N = 6)	22 ± 10 (14-41)	30 ± 22 (13-70)
≥ 60[c] (N = 8)	14 ± 2 (12-18)	29 ± 5 (14-31)

[a] GM-CSF administered by 2-hour I.V. infusion from day 0 to 13 post autologous transplant.
[b] Retrospective control group of comparably matched patients with lymphoid malignancy who underwent autologous BMT at FHCRC prior to the start of this study.
[c] Patient 3990 who died on day 8 was unevaluable and is not included.
[d] N = number of patients.
* Mean ± S.D. (range)

As previously described, 5 of 8 evaluable patients (one patient who died of sepsis 8 days after ABMT was not evaluable) receiving from 60 to 240 $\mu g/m^2$/day of rhGM-CSF achieved an absolute neutrophil count (ANC) of greater than 500 cells/mm^3 sooner than day 14, compared to 4 of 86 historical control patients. However, of the 5 patients who reached 500 neutrophils/mm^3 by day 14, 4 temporarily decreased their ANC to below 500/mm^3 within 24 to 72 hours after stopping GM-CSF.[5].

In an attempt to prevent the period of granulocytopenia and maintain ANC's above 500/mm^3 after GM-CSF was stopped, we extended the duration of rhGM-CSF therapy (240 $\mu g/m^2$/day by two-hour infusion) from day 14 to 21 days in an additional six patients with lymphoid malignancies undergoing ABMT. Toxicity was not increased by the extended duration and results (Table 2) show that 2 of 6 patients achieved an ANC of 500/mm^3 sooner than day 14 and neither decreased their ANC to below 500/mm^3 after GM-CSF was stopped. Two other patients achieved an ANC of greater than 500/mm^3 prior to stopping GM-CSF and maintained their ANC above 500/mm^3 after GM-CSF was stopped. However, two patients did not achieve an ANC greater than 500/mm^3 sooner than day 20. These findings suggest that extending the duration of GM-CSF therapy to 21 days induced no additional toxicity and achieved a

similar degree of efficacy compared to those patients receiving $\geq$ 60 μg/m^2/day by two-hour intravenous infusion from day 0 to 13. GM-CSF from day 0 to 20 did appear to prevent reductions in ANC to below 500/mm^3 within 24-72 hours after GM-CSF was stopped.

These six patients, together with the original 8, result in 14 evaluable patients receiving $\geq$ 60 μg/m^2/day of GM-CSF by two-hour infusion. Seven of the 14 reached an ANC of 500/mm^3 sooner than day 14. Four others reached an ANC of 500/mm^3 until day 22, 26 and 27. Animal studies have suggested that GM-CSF might be more effective when given by continuous infusion than by single I.V. injection. Therefore, we studied the effects of continuous infusion GM-CSF (240 μg/m^2/day from day 0 to 20) in three patients (Table 3). The first patient treated with continuous infusion of GM-CSF did not reach an ANC of 500 or a platelet count of 2 x 10^4/mm^3 within 100 days after ABMT. GM-CSF was discontinued in a second patient due to intolerable toxicity. He received 240 μg/m^2/day of GM-CSF by continuous infusion from day 0 to day 12, then again on day 18 post ABMT. The GM-CSF was stopped both times due to intolerable diarrhea and fever with maximum body temperatures (T$_{max}$) of 105°F after amphotericin administration, whereas T$_{max}$ in response to amphotericin while the patient was not receiving continuous infusion of GM-CSF was 102°F. The third patient tolerated muscle cramps and joint pains during GM-CSF infusions and achieved an ANC of 500/mm^3 by day 20. Prolonged severe thrombocytopenia occurred in all three patients (Table 3). Based on the observed toxicity and lack of apparent benefit over a two-hour infusion, we have abandoned using continuous infusions.

Table 2. Day of Engraftment in ABMT Patients Receiving 240 μg/m^2/day of rhGM-CSF from Day 0 to Day 20 post ABMT by 2-Hour Infusion

Unique Patient Number	Diagnosis/ Disease State	Day after marrow infusion ANC >500 cells/mm^3	Day after marrow infusion patient independent of platelet transfusion
3787	NHL[a]/relapse	13	15
4050	ALL[a]/remission	26	100+
4047	NHL/relapse	22	38
4111	ALL/remission	19	16
4038	ALL/relapse	11	54
4113	ALL/remission	27	58

[a] = NHL = Non-Hodgkin's lymphoma; ALL = acute lymphocytic leukemia

**Table 3. Day of Engraftment in ABMT Patients Receiving
240 μg/m²/day of rhGM-CSF from day 0 to day 20
post ABMT by Daily Continuous Infusion**

Unique Patient Number	Diagnosis/ Disease State	Day after marrow infusion ANC >500 cells/mm³	Day after marrow infusion patient independent of platelet transfusion
4107	NHL[a]/remission	100+	100+
4101	ALL[a]/remission	18	--[b]
4100	NHL/relapse	20	64

[a] NHL = NonHodgkin's lymphoma; ALL = acute lymphocytic leukemia
[b] patient 4101 died of GI bleed on day 62 without megakaryocyte engraftment

**Table 4. Day of Engraftment in ABMT Patients Receiving
rhGM-CSF (240 μg/m²/day by 2-hour infusions)
from day 7 to 20 post ABMT)**

Unique Patient Number	Diagnosis/ Disease State	Day after marrow infusion ANC >500 cells/mm³	Day after marrow infusion patient independent of platelet transfusions
4201	NHL[a]/remission	11	11
4222	HD[a]/relapse	19	25
4188	NHL/relapse	22	26
4130	NHL/remission	21	27

[a]NHL Non Hodgkin's Lymphoma;
HD Hodgkin's disease

An additional four patients were treated with two-hour infusions of GM-CSF starting on day 7 post ABMT (Table 4). This approach was taken to avoid stimulating cells with GM-CSF from day 0 to 6 given the possibility that GM-CSF might favor differentiation over self-renewal. We thus wished to test whether allowing a brief period post transplant without the differentiative push of GM-CSF would allow a brief period of self-renewal and therefore a more striking response to the drug later. The observed toxicity of GM-CSF (240 μg/m²/day) given from day 7 to 20 was similar to that in other patients receiving ≥ 60 μg/m²/day by two-hour infusion from day 0 to 13. The first patient achieved an ANC of 500/mm³ and platelet transfusion independence by day 11 post BMT. However, none of the

subsequent three patients reached an ANC of $500/mm^3$ sooner than day 14. Though platelet transfusion independence was earlier than the retrospective control, it was similar to other patients receiving ≥ 60 $\mu g/m^2/day$ of GM-CSF by two-hour infusion. Therefore, withholding GM-CSF from day 0 to 6 did not enhance myeloid engraftment or lessen toxicity compared to patients receiving ≥ 60 $\mu g/m^2/day$ GM-CSF by two-hour infusion.

Toxicity was well-tolerated by all patients who received GM-CSF when a two-hour intravenous infusion schedule was utilized. Two of 28 patients did not complete all scheduled GM-CSF doses, one because of death from sepsis on day 8 post ABMT and another who received continuous infusion of GM-CSF. Cumulative toxicities above expected ABMT toxicities as a percentage of the 27 evaluable patients included nausea (22%); muscle cramps (19%); diarrhea (15%); transient total body rash (15%); joint pains, low grade fever, and headaches (7%). Additionally, two patients developed transient pulmonary infiltrates which resolved with Lasix administration during the GM-CSF infusion course.

Overall, the 22 patients who received GM-CSF ($\geq 60 \mu g/m^2/day$) versus 86 retrospective control patients had earlier discharge dates (day 32 ± 16 versus 41 ± 25), earlier myeloid engraftment (ANC grater than $500/mm^3$ by day 17 ± 5 versus 25 ± 10), earlier platelet transfusion independence (day 28 ± 16 versus 38 ± 20) and less mortality at day 100 (14% versus 44%). There was no difference in relapse rates (18% versus 24%).

In conclusion, compared to a retrospective control group, rhGM-CSF at a dose of 240 $\mu g/m^2/day$ given by two-hour intravenous infusion may induce short-term benefits to ABMT patients by shortening the time to recovery of neutrophils. Transient decreases in ANC to less than $500/mm^3$ after GM-CSF is stopped appear to be prevented by continuing the initial GM-CSF course for 21 days. GM-CSF given at the same concentration by continuous 24-hour daily infusion or (by 2-hour intravenous infusion) from day 7 to 20 post ABMT offered no advantage compared to the two-hour infusion schedule. Decreased septic episodes, earlier discharge times and possibly earlier platelet transfusion independence appear to occur in ABMT patients treated with rhGM-CSF.[5] However, prospective control trials are required to better define toxicity, to more definitively assess the effects of rhGM-CSF on the rate of engraftment, and to assess differences in relapse and survival.

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IL-1 PROTECTS HUMAN COLONY FORMING CELLS, BUT NOT HL-60 AND K562 LEUKEMIC CELL LINES, FROM THE TOXIC EFFECTS OF 4-HYDROPEROXYCYCLOPHOSPHAMIDE

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INTRODUCTION

Recent studies have shown that mice treated with interleukin 1 are protected from the lethal effects of irradiation (1-3). Other investigators have reported that interleukin 1 can enhance recovery of the white blood cell count of mice treated with cyclophosphamide (4) or 5-fluorouracil (5). The mechanism for such protection is unclear and remains to be established.

4-Hydroperoxycylophosphamide (4-HC), a potent derivative of cyclophosphamide, has been used clinically for purging bone marrow of patients undergoing autologous bone marrow transplantation (6,7). In this study, we report the use of an *in vitro* model system which demonstrates the prior incubation with recombinant human IL-1 beta (IL-1β) protects early human hematopoietic progenitor cells from the lethal effects of high doses of 4-HC. On the other hand, IL-1β bone marrow conditioned medium (IL-1 BM-CM) does not protect HL-60 and K562 leukemic cells from a lethal dose of 4-HC.

MATERIALS AND METHODS

Blast Cell Colony Assay

After obtaining informed consent, bone marrow samples were aspirated from the posterior iliac crest of healthy adult volunteers and placed in tissue culture tubes containing 400 U preservative-free heparin. Mononuclear cells were prepared by centrifugation over Ficoll-hypaque (F-H) (S.G. 1.078). Post F-H mononuclear cells (5 x

10^5 cells/ml) were incubated at 37°C in the presence or absence of 100 ng/ml of recombinant human IL-1β (generous gift from Dr. C.A. Dinarello, Boston, MA) for 20 hours. After this time 50 μg/ml 4-HC (generous gift from Dr. M.O. Colvin, Johns Hopkins Oncology Center, Baltimore, MD) was added and the incubation continued for an additional 30 minutes. The cells were then washed 2 times with chilled culture medium to remove 4-HC and cultured in semi-solid medium by the method described by Rowley, et al. (8) and modified by us (9). Cultures consisted of Alpha-MEM supplemented with 1.2% methylcellulose (4000 CP, Fisher), 5×10^{-4} M 2-Mercaptoethanol, 1% bovine serum albumin (Sigma, St. Louis), 30% fetal bovine serum (FBS), (Hyclone, Logan, UT), 10^{-6} M methylprednisolone, 1 U/ml erythropoietin (TcEPO, Amgen) and 5% conditioned medium from the human bladder carcinoma 5637. Four replicate cultures of 5×10^5 cells/ml/plate for each experimental group were maintained in a humidified atmosphere of 5% CO_2 in air at 37°C for up to 6 weeks. Colonies were scored using an inverted microscope at weekly intervals for the first 2 weeks and observed daily thereafter for the appearance of blast cell colonies.

Blast cell colonies were picked from culture plates with a drawn out glass pipette for morphology studies and for replating experiments. One half of a blast cell colony was replated in the same conditions used for primary colony cultures. Secondary colonies were scored on days 7-14 of culture and compared with the type of colonies formed in the primary plate.

Preparation of IL-1β Human Bone Marrow Conditioned Medium

Human bone marrow mononuclear cells were incubated for 20 hours with 100 ng/ml IL-1β. The supernatant was removed by centrifugation and stored frozen at -20°C until used for the experiments with the leukemic cell lines.

Clonal Assay for Leukemic Blast Progenitors

HL-60 and K562 leukemic cell lines were maintained continuously in suspension culture in RPMI 1640 supplemented with 10% FBS. HL-60 or K562 leukemic cells (5×10^5 cells/ml) were incubated with or without IL-1 BM-CM for 20 hours at 37°C in a humidified atmosphere of 5% CO_2 in air. After this time, various amounts of 4-HC were added and the incubation continued for an additional 30 minutes. The cells were then washed 2 times with chilled medium to remove 4-HC and cultured in Alpha-MEM supplemented with 2% methylcellulose and 25% FBS. Four replicate 1 ml cultures with different cell concentrations (10^3, 10^4, 10^5, and 10^6) per plate were prepared for both control and IL-1 BM-CM treated cells. Colonies were scored using an inverted microscope at day 7 of culture.

RESULTS

Table 1 shows that in the absence of prior incubation with recombinant human IL-1β, 50 μg/ml of 4-HC (a predetermined lethal dose for the normal bone marrow used in this experiment) eliminated all but an occasional colony forming cell. In contrast, when the same bone marrow mononuclear cells were incubated with IL-1β for 20 hours prior to exposure to 4-HC, an increased number of large "activated" macrophage appeared at 1 week of culture and after day 14, other single lineage and multilineage colonies also appeared. These included granulocyte, macrophage, erythroid, undifferentiated blast colonies and mixed lineage colonies such as granulocyte/macrophage and granulocyte/macrophage/erythroid/ megakaryocyte colonies. These results demonstrate that IL-1β can protect early hematopoietic progenitors from the effects of 4-HC. *In situ*, the blast cell colonies appeared dispersed and contained round refractile undifferentiated cells, often with variable cell sizes and sometimes with "tail-like" structures. These blast cell colonies appeared at different days of culture and were seen as late as day 28. One half of each blast cell colony was picked and individually replated. The replating results are shown in Table 2 and demonstrate the ability of these blast cells to give rise to single and multilineage secondary colonies. Table 2 also shows that the morphology of the colonies formed int he original and replating dishes are similar.

The results of the experiments with HL-60 and K562 leukemic cell lines are summarized in Tables 3 and 4. Table 3 shows a dose response for 4-HC on the plating efficiency of HL-60 and K562 leukemic cells. Incubation of HL-60 cells with 25 μg/ml 4-HC completely abolished colony formation; whereas 50 μ/ml 4-HC was required to eliminate colony formation by K562 cells. Table 4 shows that preincubation of HL-60 or K562 cells with IL-1 BM-CM did not protect these leukemic cells from the effects of 50 μg/ml 4-HC even when increased numbers of cells (up to 10^6 cells/plate) were plated.

DISCUSSION

In this report, we have described an *in vitro* assay system which demonstrates that prior incubation of human bone marrow recombinant human IL-1β protects early hematopoietic progenitors from a lethal dose of 4-HC. These early progenitors yield different types of colonies, including the undifferentiated blast cell colony characterized by its ability to give rise to secondary single and multiple lineage colonies. Secondary blast cell colonies were also observed. Thus, we have developed an assay for the blast cell colony which resembles the blast cell colonies described by Rowley, et al. (8), Ogawa, et al. (10), and others (11).

Table 1. Effects of 20 Hours Preincubation with Recombinant IL-1β on the Recovery of Hematopoietic Progenitor Cells Following Treatment with 50 μg/ml 4-HC

Type of Colonies[1]	With IL-1 Days of Culture		Without IL-1 Days of Culture	
	14	28	14	28
M	4 ± 1.8^2 (2-6)[3]	7±1.4 (5-8)	0	0.25±0.5 (0-1)
G	0	0.5±0.58 (0-1)	0	0
GM	0.5±01.0 (0-2)	1.25±1.9 (0-4)	0	0
Bl	0.5±0.58 (0-1)	1.5±1.3 (0-3)	0	0
E	0	2.75±5.5 (0-11)	0	0
MIX	0	0.5±0.58 (0-1)	0	0

[1]Abbreviations for colony types were: GM, Granulocyte/macrophage; G, Granulocyte; M, macrophage; GE, granulocyte/erythroid; E, erythroid; MIX, mixed colony containing erythroid, granulocyte, macrophage, and megakaryocyte lineages; B1, blast cell colony.

[2]Results are expressed as mean ±1 S.D. of 4 replicate cultures.

[3]Values in parenthesis = range of colonies found in 4 replicate cultures.

The mechanism by which IL-1 confers protection remains unknown. It is not known whether IL-1β acts directly to protect the early progenitors or via another mediator whose synthesis is induced by accessory cells stimulated by IL-1β. Because of this, we have used IL-1 BM-CM in order to investigate its effect on the HL-60 and K562 leukemic cell lines. We have demonstrated that prior incubation with IL-1 BM-CM did not protect HL-60 or K562 leukemic cells from the lethal effects of 4-HC.

Better purging methods are needed in order to improve the results of autologous bone marrow transplantation in terms of disease-free survival for patients undergoing treatment for acute leukemia. Therefore, the results of this study may have significant clinical

Table 2. Replating Results of Blast Cell Colonies Derived from Cultures of Bone Marrow Pretreated with Recombinant IL-1β and 50 μg/ml 4-HC

Primary Colo- nies[1]	Day of Har- vest	No. of Cells Replated	Lineage Expressed In Secondary Colonies						No. of Secondary Colonies	Replating Efficiency %
			M	G	GM	E	B1	MIX		
GE	18	55	1		2	37			40	73
GM	22	54	3		15				18	28
GM	24	59	9	2	12				23	39
GE	28	50	3			39			42	84
E	28	100				50			50	50
GM	28	100	5		13				18	18
GM	28	28	1		8				9	32
MIX	28	143	4		21	221		1	247	173
MIX	28	70	4	5	10	5	2	1	27	39

[1]Abbreviations for colony types are given in Table 1.

Table 3. Dose Response Effect of 4-HC on HL-60 and K562 Leukemic Cell Lines

4-HC Dose (μg/ml)	Plating Efficiency	
	HL-60	K562
0	25±3[1]	35±7
12.5	4±1	13±2
25	0	3±2
50	0	0
100	0	0

[1]Results are expressed as mean ±1 S.D. of 4 replicate cultures

**Table 4. Effects of 20 Hours Preincubation with IL-1 BM-CM
on HL-60 and K562 Leukemic Cell Lines
Treated with 50 µg/ml 4-HC**

Treatment	Cells/Plate	Plating Efficiency	
		HL-60	K562
Without 4-HC			
Normal	10^3	25 ± 3^1	35 ± 7
With 4-HC			
+ IL-1 BM-CM	10^3-10^6	0	0
- IL-1 BM-CM	10^3-10^6	0	0

[1]Results are expressed as mean ±S.D. of 4 replicate culture.

implications. First, the use of IL-1β is known to have tumoricidal activity against certain leukemic and solid tumor cell lines (12-14), and may thus provide a complementary antitumor effect to enhance that achieved by 4-HC alone.

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PRIMATE STUDIES WITH COMBINED
GROWTH FACTOR THERAPIES

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The effect of recombinant human GM-CSF in preclinical studies using primates has been well documented. The infusion of GM-CSF into normal monkeys has led to an increase in peripheral blood white cells as well as an increase in platelets (1-4). The increase in neutrophils and monocytes has been correlated to a concomitant increase in the bone marrow progenitor cell activity (4). In other rhesus monkey models, GM-CSF treatment has been shown to improve neutrophil and platelet recovery after autologous bone marrow transplantation (5,6) and after radiation induced bone marrow suppression (7). The timing of GM-CSF administration in the radiation model was shown to be critical for hematopoietic regeneration (8).

Until recently, IL-1 has been shown to be involved in inflammation without any effect on hematopoiesis (9). Currently, IL-1 has been shown to induce the production of colony stimulating factors *in vivo* (10) and to lead to a synergistic hematopoietic response when combined with other hematopoietic growth factors *in vito* (11,12). *In vivo* treatment with IL-1 or in combination with G-CSF has been shown to significantly decrease the recovery time of neutrophils in 5-fluorouracil treated mice (13).

In this paper, the efficacy of combined IL-1 and GM-CSF treatment to enhance hematopoietic regeneration and decrease the period of neutropenia after ABMT is evaluated using a primate model.

MATERIALS AND METHODS

Domestic-born male rhesus monkeys, *Macaca mulata*, weighing 4 to 8 kg, were housed and cared for as previously described (6).

Before handling, monkeys were sedated with ketamine hydrochloride (10mg/kg, i.m.).   All research was conducted according to the principles enunciated in the "Guide for the Care and Use of Laboratory Animals," prepared by the Institute of Laboratory Animal Resources, National Research Council.

GM-CSF with a specific activity of 1 x 10^7 units/mg was provided by Genetics Institute.   Il-1α with a specific activity of 2.5 x 10^9 units/mg was provided by Hoffmann-La Roche and IL-1β was provided by Dr. Dinarello.   Il-1 was administered by subcutaneous injections, twice a day.   GM-CSF was administered either subcutaneously as IL-1 or in a continuous delivery mode using an Alzet mini-osmotic pump.   Pump implantation has been fully described elsewhere (6,7).

Eight normal monkeys were used in a seven-day study to evaluate the effects of sequential factor therapy (IL-1α, 1 μg/kg/d, s.c., bid, days 0 and 1; and GM-CSF, 12,500 U/kg/d,s.c. bid, days 2 though 6) on hematopoiesis. ABMT utilized an existing model in which 9.0 Gy total body irradiation was followed by the infusion of 5.0 x 10^7 low density bone marrow cells harvested prior to irradiation (6,14). Data from two previous ABMT studies in which each factor was evaluated separately were used for the combined factor regimen in this study (6,14).   Briefly, IL-1 (α = 10μg/kg/d; β = 5μg/kg/d) was injected subcutaneously, twice daily, days 1 though 5; the mini-pump containing GM-CSF, delivering 50,400 units/kg/d, was implanted on day 4 and removed on day 11. Animals were administered supportive care with antibiotics, fluids and platelets.

RESULTS

The results of sequential factor treatment using IL-1 and GM-CSF in three groups of normal monkeys are presented in Table 1. IL-1α (1μg/kg/d) was used as the initial factor and led to a neutrophilia after 2 days increasing the ANC 3 to 4 fold (Groups A&C). The ANC returned to normal by day 4 in Group A monkeys. However, the ANC remained elevated on day 4 and reached a peak (>4-fold baseline) on day 7 in monkeys further treated with GM-CSF, Group C. In contrast, the ANC in monkeys not previously exposed to IL-1 but treated with GM-CSF (Group B) after 2 days of saline were elevated only 2 to 3 fold on days 4 and 7, with a peak ANC on day 4. Platelet counts increased after all treatment protocols but the time of the peak level and the amplitude of the level varied with each group. Two days of IL-1 treatment (Group A) led to an ˜1.5 fold increase in platelets by day 7; however, GM-CSF treatment alone (Group B) led to an ˜1.8 fold increase but not until day 11. Sequential treatment (Group C) led to a greater increase in the platelet counts (2.2-2.3 fold) and these counts remained elevated from days 7 though 11.

Table 1. Hematological Changes after Sequential Combined IL-1α and GM-CSF Treatment in Normal Patients

	NEUTROPHILS X 10^9/1[a]			PLATELETS X 10^9/1		
TREATMENT*:	[A]	[B]	[C]	[A]	[B]	[C]
DAY						
0	4.9	3.1	4.3	338	343	370
2	18.0	2.5	13.0	330	317	333
4	5.1	10.0	10.0	385	271	421
7	3.8	6.5	19.0	502	426	807
9	6.4	2.0	7.2	368	495	863
11	6.9	4.5	7.3	360	630	848

*Treatments:

A: IL-1 (1μg/kg/d; days 0,1) then saline (days 2-6) (N=2).
B: Saline (days 0,1) then GM-CSF (12,500 U/kg/d; days 2-6) (N=3)
C: IL-1 (1μg/kg/d; days 0,1) then GM-CSF (12,500 U/kg/d; days 2-6) (N=3).
a. Mean values are presented.

Combined therapy as a treatment regimen was proposed to further reduce the period of neutropenia after ABMT in comparison to monkeys transplanted and treated with only GM-CSF(6). The results (Table 2) show that combined factor therapy in comparison to GM-CSF treatment did not significantly decrease the period of neutropenia, reaching an ANC of >1 x 10^9/1 on days 15-16 for IL-1β and GM-CSF or day 17 for IL-1α and GM-CSF treated monkeys versus days 16-17 for GM-CSF alone treated monkeys. However, monkeys treated with IL-1α and GM-CSF had an enhanced neutrophil response with an ANC of > two-fold on day 28 in comparison to other treatment protocols.

Combined factor therapy after ABMT had a major effect on the platelet count (Table 3). The administration of factors led to a rapid decrease in platelet counts to <50 x 10^9/1 by days 5-7 versus day 7-9 and 10-11 for GM-CSF treated and control monkeys, respectively. However, the time of platelet recovery was shortened with combined factor therapy over that of control monkeys, maintaining levels >50 x 10^9/1 by days 15-16 and above 100 x 10^9/1 by day 17. The time of platelet recovery was similar to the monkeys treated with GM-CSF alone. Platelet counts of monkeys treated with combined IL-1α and GM-CSF increased above normal by day 24. In contrast, GM-CSF alone and IL-1β and GM-CSF treated monkeys reached and maintained a plateau platelet level at 50% and 70% of baseline, respectively.

Table 2. Peripheral Blood Absolute Neutrophil Counts (ANC) After ABMT and Combined IL-1 and GM-CSF Factor Therapy

Factor Treatment

	IL-1α +[a] GM-CSF	IL-1β +[b] GM-CSF	CONTROL[c]	GM-CSF[d]
DAY				
0	2.79+0.56	1.50+0.35	4.72+1.40	3.04+0.33
7	0.13+0.01	0.10+0.06	0.20+0.06	0.05+0.01
13	0.68+0.30	0.56+0.09	0.17+0.06	0.40+0.09
17	0.99+0.03	1.90+0.40	0.74+0.13	1.38+0.32
20	3.11+0.66	2.10+0.02	1.64+0.47	2.57+0.68
24	5.63+1.04	2.96+0.25	2.63+0.77	3.35+0.37
28	6.93+2.84	2.76+0.77	2.77+0.67	2.72+0.37

Values are mean + SEM ANC x 10^9/L.
a. Monkeys (N=4) were treated with IL-1α (10μg/kg/d) for 5 days starting on day 1 and also with GM-CSF (50,400 U/kg/d) for 7 days starting on day 4 after ABMT.
b. Monkeys (N=2) were treated with IL-1β (5μg/kg/d) for 5 days starting on day 1 and also with GM-CSF (50,400 U/kg/d) for 7 days starting on day 4 after ABMT.
c. Values are means of control monkeys (N=4) previously reported (6,14).
d. Values are means of GM-CSF (50,400 U/kg/d) treated monkeys previously reported (6).

Table 3. Peripheral Blood Platelet Counts after ABMT and Combined IL-1 and GM-CSF Factor Therapy

Factor Treatment

	IL-1α+[a] GM-CSF	IL-1β+[b] GM-CSF	CONTROL[c]	GM-CSF[d]
DAY				
0	267+33	280+16	404+38	336+26
7	32+11	37+19	289+21	55+15
13	58+18	35+14	55+8	45+9
17	106+27	116+53	59+15	95+16
20	160+56	144+45	69+13	126+17
24	334+33	198+2	65+5	170+18
28	363+35	204+30	67+10	205+29

Values are mean + SEM platelet counts x 10^9/L.
a. Monkeys (N=4) were treated with IL-1α (10μg/kg/d) for 5 days starting on day 1 and also with GM-CSF (50,400 U/kg/d) for 7 days starting on day 4 after ABMT.
b. Monkeys (N=2) were treated with IL-1β (5μg/kg/d) for 5 days starting on day 1 and also with GM-CSF (50,400 U/kg/d) for 7 days starting on day 4 after ABMT.
c. Values are means of control monkeys (N=4) previously reported (6,14).
d. Values are means of GM-CSF (50,400 U/kg/d) treated monkeys previously reported (6).

The combined factor therapy protocol used in this study led to a capillary leakage. Periorbital swelling was first observed within 24 to 48 hours after the beginning of GM-CSF infusion and decreased after the next 48-72 hours. Additional swelling in the lower extremities was observed on days 11 to 13 and lasted without clinical intervention for 5-7 days. In one case, pulmonary edema was so severe on day 9 that the animal was euthanized.

In conclusion, the sequential factor study with normal monkeys demonstrated that combined factor treatment increased and prolonged the elevation of both platelet and neutrophil counts. This suggested that both factors had an effect on increasing the counts, but that each response had different kinetics. Furthermore, the combined effects of the two factors were additive. In comparison to GM-CSF, combined factor therapy after ABMT did not significantly improve neutrophil or platelet recovery times. Thus, combined factor therapy did not change the kinetics of early stem cell regeneration. The combined factor therapy was however complicated by the capillary leak syndrome. Current research efforts suggest that lower doses of IL-1 are more effective and less toxic. Other possible combination of factor therapies to improve hematopoietic recovery are IL-3 and GM-CSF as was reported for normal monkeys (14) or IL-6, IL-3 and GM-CSF. Finally, the isolation of purified stem cells for transplantation studies (16) has further enhanced our capability to evaluate the effect of factors on directing hematopoietic regeneration.

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DIFFERENCES IN CD19 FUNCTION BETWEEN NORMAL AND LEUKEMIC HUMAN B-CELL PRECURSORS

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INTRODUCTION

Human B-lymphocyte development is a complex multistep process which is accompanied by a coordinated acquisition and loss of B-lineage lymphoid differentiation antigens (1), several of which have been well characterized through international workshops (2). While it is generally agreed that the earliest committed B-cell precursors express the nuclear enzyme terminal transferase (TdT) but not surface or cytoplasmic immunoglobulin, nor the T-lineage differentiation antigens CD1, CD2, CD3, CD4, CD5, CD7, and CD8, the sequence of acquisition/expression for the surface antigens CD10, CD19, CD20, CD21, and CD22 during B-cell ontogeny is still controversial. The immunologically distinct types of B-lineage acute lymphoblastic leukemia (ALL) appear to reflect sequential stages of early B-lymphocyte development and provide an opportunity to study subpopulations of BCPs (1, 2). Current models of human B-cell ontogeny are largely based on studies performed on leukemic B-cell precursors (BCPs) from B-lineage ALL patients (1-4). Hokland et al. first described the purification and immunophenotypic characterization of CD 10+ BCPs from fetal hematopoietic tissues (5) as well as normal human bone marrow (BM) (6). These studies revealed striking similarities between the surface antigens expressed by putative normal BCPs and leukemic BCPs, supporting the hypothetical models of B-lineage differentiation derived from leukemia studies. More recent studies by Ryan et al. (7) and Loken et al. (8) employed multiparameter flow cytometry (FCM) to define the sequential stages of human B-lymphocyte development. In both studies, the B-lineage specific antigen CD19 was found on virtually all CD10+ BM lymphoid cells, indicating that all cells in human BM expressing high levels of CD10 are B-lineage affiliated. However, no clear evidence was presented for either CD10 or CD19 to precede the

other during B-lymphocyte development. This is in contrast to the conclusion by Nadler and coworkers from their previous studies on leukemic BCPs (2,4) that CD19 expression precedes the acquisition of CD10 in B-cell ontogeny since CD10 is expressed in fewer BCP leukemias than CD19. With the exception of these controversial reports about the immunological surface marker profiles of normal and leukemic BCPs, critical information regarding the immunobiologic features of BCPs, mechanisms of growth regulation during early stages of human B-lymphocyte development, or the differences between distinct populations of normal versus leukemic BCPs has not been published.

In this study, we used multiparameter FCM and fluorescence activated cell sorting (FACS) to identify as well as to isolate distinct subpopulations of normal human BCPs from fetal livers and leukemic BCPs from B-lineage ALL BM samples. BCP colony assays were performed to elucidate and compare the immunobiologic features of purified subpopulations of normal and leukemic BCPs. Because of accumulating evidence that the B-lineage specific CD19 receptor has an important function in regulation of activation and proliferation of B-lineage cells at multiple stages of development, normal and leukemic BCPs were also compared relative to the functional properties of their CD19 receptors. Our findings suggest that CD10 precedes CD19 expression in early B-cell differentiation. Furthermore, CD10+CD19- normal BCPs in proliferating colonies acquired CD19 antigen, indicating that CD19 expression is developmentally programmed in B-cell ontogeny. Proliferation of normal BCPs from FL was inhibited by cross linking of CD19 in a manner similar to normal mature B-cells. In contrast, proliferation of leukemic BCPs was stimulated by CD19 cross linking.

The obligatory expression of CD19 on BCP ALLs and the differences in CD19 receptor function on normal and leukemic BCPs suggest that the CD19 receptor may be involved in leukemogenesis of BCP ALLs.

RESULTS AND DISCUSSION

CD10 and CD19 antigens are expressed very early in human B-cell ontogeny. thus, the vast majority of leukemic and normal BM BCP co-express these differentiation antigens. To study which of these antigens is acquired first during B-lymphocyte development, we performed two-color/four parameter (PE, FITC, forward-angle light scatter, and right-angle light scatter) immunofluorescence analyses on lymphoid cells from freshly obtained FLs of different gestational ages (9). FL lymphoid cells were identified among the bulk population of low-density Ficoll-Hypaque separated FL cells by their characteristic forward-angle and right angle light scattering properties

(Figure 1). Three immunologically distinct normal BCP subpopulations were evident among the lymphoid cells from human FLs based on correlated expression of CD10 and CD19 antigens. Both CD10+CD19+ or CD10+CD19-BCPs responded to LMW-BCGF and formed BCP colonies in vitro. Notably, CD10+CD19-BCPs were found in larger numbers than CD10+CD19+ or CD10-CD19+BCPs, they acquired CD19 antigen in vitro, and their day 7 progeny in BCP colonies displayed a more immature immunophenotype than the progeny of CD10+CD19+BCPs (9). These findings indicate that CD10 antigen expression precedes the acquisition of CD19 antigen during normal B lymphocyte development. Unlike LMW-BCGF, HMW-BCGF was unable to stimulate normal BCP colony formation (9). We used the data on the surface antigen profiles of CD10+ FL lymphoid cell populations and their progeny in BCGF stimulated cultures to develop a new hypothetical model of early B-lineage lymphoid differentiation, which is illustrated in Figure 2. Significantly, B43/CD19 MoAb (at 1 µg/ml) inhibited LMW-BCGF stimulated proliferation of CD10+ CD19+ Stage II FL BCPs but did not affect CD10+CD19-Stage I BCPs.
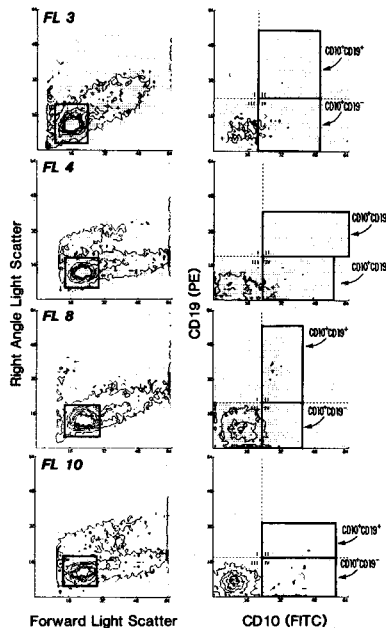
Figure 1. Multiparameter FCM Analysis of FL Cells 2-color stained with 24.1/CD10-FITC & B43/CD19-PE. The forward angle & right angle light scattering contour plots as well the correlations between CD10 & CD19 expression for cells within the indicated light scattering window are shown for 4 FL samples. Boxes in the light scattering contour plots identify the gates used to reanalyze the list mode data for determining the correlations between CD10 & CD19 expression on lymphoid cells. Boxes on the FACS correlated 2-color displays of gated FL lymphoid cells identify the windows used for sorting the CD10+CD19- & CD10+CD19+ BCP populations. The quadrants on the FACS correlated 2-color displays were set using appropriate negative controls.

B-Lineage Differentiation and Leukemogenesis
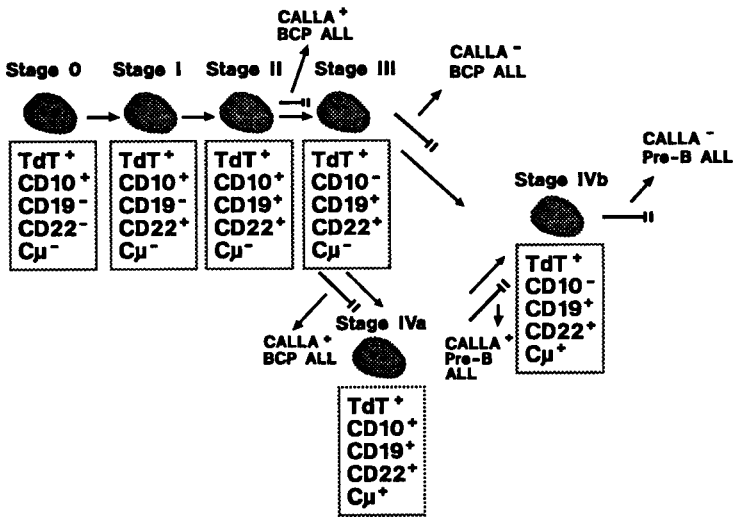
Figure 2. A New Hypothetical Model for B-Lineage Differentiation and Leukemogenesis. the most immature BCPs (Stage 0) express nuclear TdT and surface CD10 but lack the B-lineage specific antigens CD19, CD22, and cytoplasmic/surface Ig. During the subsequent stage of B-lymphocyte development, TdT+CD10+ BCPs become committed to B-lineage differentiation and express CD22 in their cytoplasm (Stage I). The acquisition of CD19 identifies the next stage of differentiation (Stage II) within the BCP pathway. TdT+CD10+CD19+CD22c+ BCPs subsequently lose CD10 yielding TdT+CD10-CD19+CD22c+ Stage III BCPs. Stage II as well as Stage III BCPs can differentiate into pre-B cells (Stages IVa and IVb) which are identified by expression of μ heavy chains in their cytoplasm. Uncoupling of proliferation and differentiation can occur at multiple stages of B-cell ontogeny and may represent the underlying mechanism for the stabilization and expansion of transitory immature cellular phenotypes leading to BCP ALL.

In parallel experiments, we assessed the proliferative responses of leukemic BCP populations to hematopoietic GF. Specifically, CD10+CD19+, CD10+CD19-, and CD10-CD19+ FACS sorted ALL blasts from 4 BCP ALL patients were assayed for leukemic BCP colony formation in the presence of LMW-BCGF or HMW-BCGF. LMW-BCGF as well as HMW-BCGF stimulated colony formation by CD10+CD19+ as well as CD10-CD19+ blasts, but they failed to stimulate CD10+CD19- blasts. Hence, CD10+CD19- leukemic BCPs appear to have different GF responses as compared to CD10+CD19- normal BCPs. This difference may be a consequence of altered/amplified HMW-BCGF receptor gene expression in leukemic BCPs. Synergistic interactions between LMW-BCGF and HMW-BCGF (17) may contribute to a rapid clonal expansion of leukemic BCP populations.

Leukemic B-Cell Precursor   Normal B-Cell Precursor
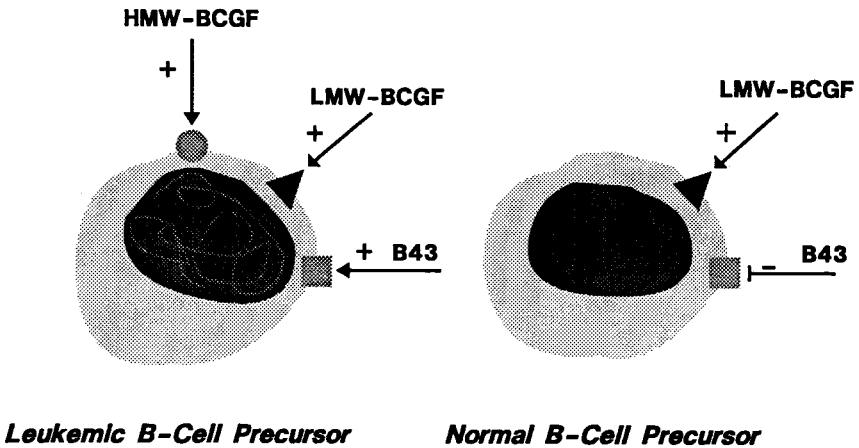
Figure 3. Immunobiologic Differences Between Normal and Leukemic BCPs. The function of the CD19 antigen and BCGF receptors on normal versus leukemic BCPs was analyzed in colony assays (9). Leukemic BCPs are stimulated by both HMW-BCGF and LMW-BCGF whereas normal BCPs respond only to LMW-BCGF. Furthermore, CD19 ligation mediates a stimulatory signal for leukemic BCPs but an inhibitory signal for normal BCPs.

Notably, B43/CD19 MoAb augmented the proliferative response of leukemic BCP populations to LMW-BCGF. There is now considerable evidence that the CD19 receptor is an important functional regulator of normal and malignant B-cell proliferation: 1) CD19 receptor is expressed in all BCP leukemias, and B-lineage leukemic progenitor cells in BCP ALL patients are CD19+(10-12) 2) the density of CD19 expression correlates with the cloning efficiency and with DNA synthesis activity of leukemic BCPs (13); 3) CD19 receptor density on leukemic BCPs is augmented during growth stimulation by LMW-BCGF (10); 4) Cross linking CD19 with a Mo Ab induces increases in cytoplasmic free calcium in leukemic BCPs and mediates a positive signal for proliferation (14). The observation that CD19 molecule mediates stimulatory signals for leukemic BCPs (9,13) and inhibitory signals for normal BCPs (9) suggests that the function of this B-lineage specific receptor may be altered during malignant transformation. Intriguingly, recent cDNA cloning of CD19 by Ivan Stamenkovic and Brian Seed has shown a significant homology in the cytoplasmic domain of CD19 with the INT-1 oncogene and to an Epstein-Barr virus protein (15). The differences in HMW-BCGF responses and CD19 function between normal versus leukemic BCPs, which are summarized in Figure 3, taken together with the obligatory expression of CD19 on BCP ALLs (10) prompt the hypothesis that the signal transmission pathways linked to these B-cell specific receptors may be altered during leukemogenesis in human BCP leukemias.

ACKNOWLEDGMENTS

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THE USE OF RECOMBINANT HUMAN GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR IN AUTOLOGOUS BONE MARROW TRANSPLANTATION

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INTRODUCTION

Intensive chemotherapy programs, particularly those which require marrow transplantation are associated with severe myelosuppression. In the autologous bone marrow transplant (ABMT) setting at the Duke University Medical Center, the three most frequent causes of treatment related death are infection, hemorrhage and major organ toxicity. Others have shown that the incidence and severity of bacterial and fungal infection is directly related to the duration and severity of neutropenia[1,2]. Blood product transfusions (RBC, platelets and granulocytes) can augment, but will not restore impaired hematopoietic function. Bone marrow transplantation from syngeneic, autologous and allogeneic sources will shorten but not eliminate the myelosuppression following chemoradiation therapy[3]. Although the ability to dose intensify has been associated with curative results in some settings[4], infections and the multiple organ failure syndrome associated with prolonged myelosuppression remain major problems. We have found that patients receiving high dose combination chemotherapy and autologous bone marrow support have increasing major organ system toxicity with increasing duration of granulocytopenia[5]. Shortening the period of myelosuppression would have the potential of reducing the toxicity of autologous bone marrow transplantation, and perhaps of other intensive chemotherapy programs.

Over the last decade it has become apparent through *in vitro* studies that the proliferation, maturation and some functional activities of hematopoietic cells are controlled by a series of specific regulatory glycoproteins called hematopoietic growth factors[6,7]. When tested *in vitro*, these factors stimulate hematopoietic colony growth in a dose dependent manner[8]. Four such factors affecting myelocytic precursors have now been molecularly cloned: interleukin-3[9,10,11] granulocyte-macrophage colony stimulating factor (GM-CSF)[12,13], macrophage colony stimulating factor (M-CSF, CSF-1)[14], and granulocyte colony stimulating factor (G-CSF)[15,16].

The hematologic reconstitution following high dose CPA/cDDP/BCNU and autologous bone marrow support is very typical and lends itself to an evaluation of the impact of a systemically infused growth factor on hematopoietic recovery and function. Our previous studies have demonstrated that the time to recovery of a WBC> $1000/mm^3$ is 17.4 ± 2.7 days. Our current trials with human recombinant granulocyte macrophage colony stimulating factor (rHµGM-CSF) provide an opportunity to study hematopoiesis in rHµGM-CSF treated patients and controls to (1) delineate the "normal" pattern of hematopoietic reconstitution after ABMT, (2) to determine the toxicity and efficacy of rHµGM-CSF when administered in the setting of high dose combination chemotherapy and autologous marrow transplantation, and (3) to determine the effects of rHµGM-CSF on the functional capacity of bone marrow and granulocytes maturing during rHµGM-CSF administration.

METHODS

Patient Population

Forty-eight patients with histologically confirmed breast cancer or melanoma were studied during phase I and II trials of rHµGM-CSF administration. The details of patient selection, pretreatment characteristics and experimental design are or will be provided elsewhere[17]. All twenty-four prior consecutive patients treated with an identical high-dose combination chemotherapy regimen from January 1985 to December 1986 served as a control population for comparison; ten additional contemporaneous patients treated with the same chemotherapy program but not receiving rHµGM-CSF had evaluation of granulocyte function.

Administration of Chemotherapy and rHµGM-CSF

All patients studied received the same chemotherapy program which consisted of cyclophosphamide (1875 mg/m², days -6, -5, -4), cisplatin (165 mg/m² as a 72-hour continuous intravenous infusion

days -6 to -3), and carmustine (600 mg/m^2; day -3). Recombinant HuGM-CSF was begun three hours after infusion of autologous bone marrow at the end of day 0. Bone marrow aspiration and biopsy was performed prior to therapy and at five day intervals following bone marrow infusion. The production and preparation of rHμGM-CSF is described elsewhere[17]. Briefly, complementary DNA encoding human GM-CSF was cloned and expressed by Genetics Institute, Inc. (Cambridge, Mass.), and rHμGM-CSF was formulated as a lyophilized powder by Sandoz Pharmaceuticals (Basel, Switzerland). It was reconstituted in sterile water and added to 500 ml of 5% dextrose with 0.45% saline before administration. Nineteen patients received rHμGM-CSF by continuous intravenous infusion for 14 days at doses ranging from 1.2 to 19.2 mcg/kg/day of aglycosylated rHμGM-CSF protein; twelve patients received 21 day continuous infusions of either 4.8 (five patients) or 9.6 mcg/kg/day (seven patients) aglycosylated rHμGM-CSF infusion; seventeen patients received 4.8 mcg/kg/day (eight patients), 9.6 mcg/kg/day (eight patients) or 19.2 mcg/kg/day aglycosylated rHμGM-CSF as a daily four hour infusion for a planned 21 days. The use of rHμGM-CSF as described was approved by the Duke University Institutional Review Board.

RESULTS

Toxicity

The use of rHμGM-CSF was in general well tolerated. When used as a continuous infusion at doses from 1.2 to 9.6 mcg/kg/day, agylcosylated protein, side effects were not dose-limiting and included dependent myalgias, rash, hypotension, fever, weight gain, and pleural and pericardial effusions. With the exception of the dependent myalgias, fever, weight gain and hypotension, the frequency of these side effects was not clearly different from other patients not receiving rHμGM-CSF. At 9.6 - 19.2 mcg/kg/day of continuous infusion rHμGM-CSF 10/22 (45%) of patients developed hypotension (MAP < 70). Of these 10 patients, 80% had pleural effusions and peripheral edema. The hypotension began between days 7-10 (8/10 patients). Measurement of total blood volume by radiolabeled I^{125} albumin showed an increase in blood volume during rHμGM-CSF infusion from 4607 ± 912 to 6223 ml ± 950. Cardiac evaluation by MUGA or ECHO of 4 patients showed normal left ventricular function. Swan-Ganz monitoring in two hypotensive, but non-septic patients showed an increased cardiac index and a low systemic vascular resistance index with a normal pulmonary artery occlusion pressure[18].

Despite the appearance of toxicity related to rHμGM-CSF at high doses, the administration of rHμGM-CSF was associated with reduced

clinical morbidity during the GM-CSF administration. During the administration of rHμGM-CSF, there was objective evidence of clinical benefit with a reduced bacteremia rate, reduced treatment related mortality, and reduced hepatic and renal dysfunction compared to historical controls.

Effects of rHμGM-CSF on Bone Marrow Morphology

Serial analysis of bone marrow biopsies from patients receiving rHμGM-CSF demonstrated that by the end of the infusion (day 15 after marrow reinfusion), 69% of patients had a bone marrow cellularity of 20% or greater. In contrast, none of thirteen controls (12 contemporaneous, pre-historical) had this degree of marrow cellularity by day 15. There was considerable variation in the response of individual rHμGM-CSF patients, with 17% showing recovery of marrow cellularity to 40% or greater, within the normal range for healthy subjects, by day 15. Marrow biopsies also showed a variable degree of granulocyte hyperplasia compared with controls. Megakaryocyte recovery showed a tendency to appear earlier in rHμGM-CSF patients (97.8% by day 20) than in controls (61.5% by day 20; p = 0.00147 by Fisher's Exact Test), while recovery of erythroid elements was not significantly different between the two groups[19]. These results will require confirmation by a prospective, comparative trial.

Effects of rHμGM-CSF on Circulating Counts

Patients receiving rHμGM-CSF showed a dose dependent increase in circulating leukocytes at the end of the infusion compared to historical controls[17]. The pattern of peripheral leukocyte recovery after a 14-day continuous intravenous infusion of rHμGM-CSF was remarkable for four features (Figure 1): (1) the appearance of the initial rise of counts was not different in patients treated with rHμGM-CSF and historical controls; (2) once cells began to appear, there was an acceleration of the rate of leukocyte recovery compared to historical controls reaching an equivalent peripheral leukocyte count to controls about 10 days faster at the end of the infusion; (3) a rapid fall in leukocyte counts following discontinuation of the rHμGM-CSF; and (4) hematopoietic recovery following discontinuation of rHμGM-CSF proceeding in a manner comparable to controls.

Effects of rHμGM-CSF on Granulocyte
Margination and Migration

The administration of rHμGM-CSF results in elevated peripheral leukocyte and granulocyte counts. However, cessation of continuous
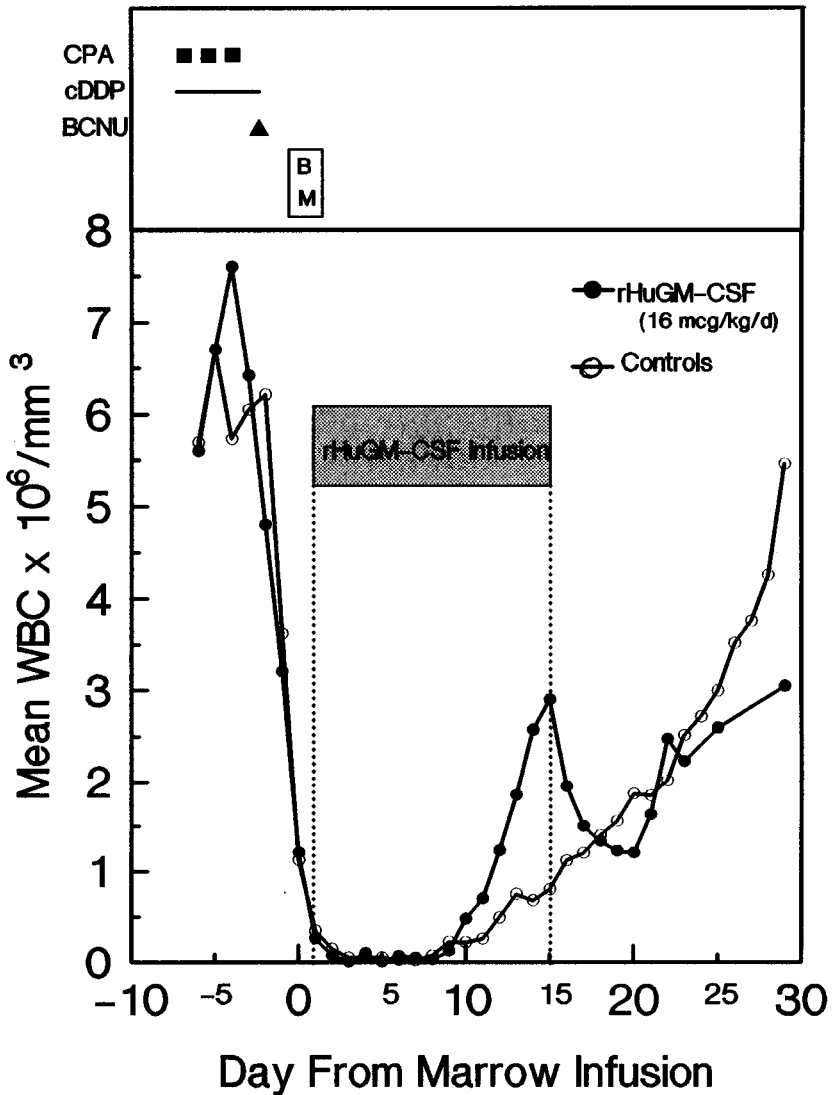
Figure 1. Mean leukocyte counts in 4 patients treated with 9.2 mcg of rHμGM-CSF (agyclosylated protein) per kilogram per Day (solid circles) and 24 historical controls (open circles). Chemotherapy was administered as described in the text. CPA denotes cyclophosphamide (1875 mg per square meter) given intravenously over one hour and repeated daily for three doses; cDDP cisplatin (165 mg per square meter) given by continuous intravenous infusion over 72 hours; BCNU (carmustine) (600 mg/square meter) given intravenously over 2 hours; and BM denotes bone marrow infusion. The duration of rHμGM-CSF infusion is indicated by the hatched lines. Reproduced with permission New England Journal of Medicine.

rHμGM-CSF infusion resulted in a rapid decline in peripheral leukocyte counts in normal non-human primates[20], patients with the acquired immunodeficiency syndrome[21] and myelodysplasia[22], and in the autologous bone marrow transplantation setting in both non-human primates[23] and man[17]. The rapidity of the fall in peripheral granulocytes suggests that mechanisms other than production may be operative. Effects of rHμGM-CSF on leukocyte margination or migration might contribute to rapid changes in the peripheral leukocyte counts. Further, rHμGM-CSF has been demonstrated to be identical to neutrophil migration inhibition factor (NIF-T)24. This inhibitory effect on neutrophil migration may have important clinical implications where granulocyte mobility is relevant for host defense.

To analyze the effect of rHμGM-CSF on leukocyte margination, we labelled purified autologous granulocytes with [111]-Indium and administered these labelled granulocytes to four patients with metastatic breast cancer or melanoma receiving high dose alkylating agent chemotherapy and autologous bone marrow support. Patients were studied before initiation of chemotherapy and again after 14-21 days of continuous intravenous rHμGM-CSF infusion at a time when peripheral blood counts were similar to baseline. Granulocyte half-life was calculated from the disappearance of cell associated [111]-Indium from the circulation. Epinephrine (0.3 mg sc) was administered to produce temporary leukocyte demargination and the marginating granulocyte fraction calculated. Margination of granulocytes was similar prior to (21.5% ± 13.4%) and during infusion (23.3% ± 9.6%) of rHμGM-CSF.

The ability of granulocytes to migrate to a peripheral sterile, inflammatory site was measured using a standardized skin chamber assay[25,26] in 15 patients before chemotherapy and autologous bone marrow transplantation (baseline). Ten of these patients were again studied during continuous infusion of rHμGM-CSF at a time when the peripheral leukocyte count was comparable to baseline; three patients treated with high dose chemotherapy and autologous bone marrow support but who did not receive rHμGM-CSF were also studied at the time of hematopoietic recovery as controls. Migration of granulocytes to a sterile inflammatory site was markedly reduced during continuous rHμGM-CSF infusion (1.2 ± 0.9 WBC/cm^2/24 hours) compared to baseline (39.6 ± 17.7 WBC/cm^2/24 hours; $p < 0.0008$)[27], compared to contemporaneous control patients studied after high dose chemotherapy and reconstitution but not receiving rHμGM-CSF (20.1 ± 7.8).

Effect of rHμGM-CSF on Phagocytosis and Oxidative Burst

We next analyzed the ability of the granulocytes maturing during rHμGM-CSF infusion to phagocytize ^{35}S-labelled *Cryptococcus*

neoformans. The serum dependent ingestion of *Cryptococcus neoformans* was enhanced during administration of rHμGM-CSF compared to patients prior to chemotherapy and rHμGM-CSF administration and similar to patients not receiving rHμGM-CSF but the same high dose chemotherapy and bone marrow support[28].

Granulocytes exert a major portion of their killing function through the generation of reactive oxygen reduction products. This coordinated sequence of biochemical reactions, known as the "oxidative burst", results in the one-electron reduction of oxygen to superoxide anions (O_2-) which is subsequently converted to hydrogen peroxide (H_2O_2) by either spontaneous or enzyme-mediated dismutation. We measured the basal and phorbol myristate acetate (PMA) stimulated production of hydrogen peroxide in patients prior to and during administration of rHμGM-CSF. These experiments demonstrated that hydrogen peroxide production in response to PMA is similar in granulocytes obtained from patients before and during rHμGM-CSF treatment[28].

DISCUSSION

Recombinant granulocyte-macrophage colony stimulating factor has been demonstrated in these studies to accelerate hematopoietic recovery in patients receiving high dose combination alkylating agents and autologous bone marrow support. Further, compared to a historical control population, there were fewer bacteremias, and fewer treatment related complications in patients receiving rHμGM-CSF. While these results require confirmation in a prospective, randomized clinical trial, they provide incentive for continued study. However, the data also demonstrate that rHμGM-CSF administration to man has effects on multiple stages of myeloid maturation. These effects are summarized in Figure 2.

Toxicity associated with the use of this agent is generally mild, although high doses of rHμGM-CSF are associated with the development of systemic toxicity. Whether this toxicity is a direct effect of rHμGM-CSF or mediated through the induction of other molecules, such as tumor necrosis factor (TNF) or IL-1, is as yet undetermined. Further experience with alternative modes of administration may provide methods for predicting or ameliorating the toxicity when it occurs.

The pattern of hematologic recovery during rHμGM-CSF administration suggests that the effect of this agent is on a committed progenitor and that this glycoprotein has little detectable effect on the stem cell population. The failure of peripheral counts to rise before that seen in the historical population suggests that there is a requisite time period of marrow "stem-cell" proliferation before commitment to differentiation occurs or that there is a definite period of marrow

Effect of rHuGM-CSF Infusion on Neutrophil Function

Steps in Neutrophil Function

Steps in Neutrophil Function	Function Normal Pre	Function Normal Post
Oxidative Burst	+	+
Ingestion	+	+
Migration	+	−
Margination	+	+
Circulation	+	++
Bone Marrow Production	+	++

Figure 2. Effects of rHμGM-CSF Infusion on Neutrophil Function. + = normal function; + + = enhanced function or numbers (see text); - = defective function.

maturation before rHμGM-CSF can exert its effect. Other data not presented here on marrow progenitors obtained during rHμGM-CSF infusion is consistent with this result and further suggests that the infused marrow may represent a mixture of stem-cells and a more committed population capable of being affected by rHμGM-CSF.

It is important to remember that the measurement of peripheral leukocytes by conventional means may well underestimate host defense capacity. A common clinical experience is that severely neutropenic patients begin to clinically improve often several days prior to the appearance of circulating leukocytes suggesting that the peripheral leukocyte count may not be a complete measure of host defense. In the end, only a randomized, comparative trial will establish the utility of these growth factors as clinical adjuncts in severely myelosuppressed patients.

The reduced granulocyte mobilization to a sterile inflammatory site seen during continuous rHμGM-CSF infusion is consistent with known biological properties of this agent. Granulocyte macrophage colony stimulating factor has been shown to possess concentration dependent chemotaxis properties[28]. Further, GM-CSF has been shown to be equivalent to neutrophil inhibition factor (NIF-T) which results in decreased granulocyte migration in agar. Hence, continuous intravenous infusion of rHμGM-CSF would be expected to attract granulocytes to the peripheral blood because of the concentration

gradient, but limit the migration to tissues due to NIF-T like activity. The partial recovery of granulocyte migration after discontinuation of rHμGM-CSF is consistent with this interpretation.

The clinical data of reduced bacteremias during rHμGM-CSF infusion is consistent with the presence of activated granmulocytes in the peripheral blood. Fortunately, soft tissue infections in these patients are rare and might be expected to be increased if rHμGM-CSF paralysis of granulocyte migration detected by this assay is relevant to this type of infection. Other mechanisms, however, may be operative. It may be that rHμGM-CSF has effects on cells other than marrow and circulating granulocytes, such as tissue macrophages, and that stimulation of these cells would lead to enhanced host defense. Further detailed investigation will be required.

Nonetheless, the data caution that the peripheral leukocyte count alone should not be considered a sole determinant of the capacity of host defense and that the administration of pharmacologic doses of recombinant growth factors may have unexpected effects on the functional capacity of effector cells.

Relevant to the findings presented here is the potential difference between recombinant growth factors. However, to date, no comparative trial of two hematopoietic growth factors has been undertaken in patients in the same clinical setting. Clearly, this type of study will be of critical value in evaluating the role of these compounds in future clinical development.

ACKNOWLEDGMENTS

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Discussion 1 - Session V (Growth Factors)

Dr. Kersey: I have a comment for the whole group. It has to do with Busulfan. As you know, Andy, we are quite enthusiastic about Busulfan in Minneapolis as well. But we are concerned about the oral form of Busulfan because in collaborative studies with your group as you know, we have shown that, particularly in children, blood levels are not what you would expect. I think that oral Busulfan is suboptimal. And I wonder if we as a group collectively can convince one of the pharmaceutical companies or the NCI to provide us with an intravenous form of Busulfan. It would be a major accomplishment that would help us all.

Dr. Kaiser: It goes to amplify John Kersey's remark. Interestingly enough, Dean Buckner and I were talking last evening about the fact that in the animal studies that have been done and the studies with dimethylmyelaran that were done at Seattle. They simply went ahead and got FDA approval to mix the drug with DMSO -- that is possible with Busulfan, as well. As a matter of fact, we have used Busulfan in the animal model parenterally by mixing the drug with DMSO. That may be a very quick way to go to get an intravenous preparation that we do not even need to have drug company intervention for.

Dr. Hagenbeek: Andy, was the incidence of VOD related to pre-existing liver function disturbances?

Dr. Yeager: The serum level of Busulfan, the pre-existence of abnormal liver functions at time of transplant were determining factors for VOD.

Dr. Frei: I think the interest in Busulfan is major because of its selective toxicity and selective antitumor effect. And that is probably true for chlorambucil in a somewhat different direction and for some of the alkylating agents as well. It might be good some time to

document all of that and see if we can't use that to put drugs together in a somewhat more scientific way. In answer to the question about getting Busulfan into solution. You can do it with liposomes. And unfortunately, the drug houses are not very interested in it because it is a low volume type thing. But it could be done with some orphan approach say from the NCI. My other question has to do with your new study, your phase I study, I believe it is Busulfan, etoposide and cyclophosphamide. I wonder why you have chosen that sequence? Intuitively, if etoposide is inhibiting DNA synthesis you would want to do that at the same time, if not before the exhibition of alkylating agents. And I wonder just what your thinking was in assembling that sequence?

Dr. Yeager: We just thought about a sandwich of something in between two agents that we had pretty predictable and pretty good experience with, nothing more than that.

Dr. Frei: Andy, you used your model very elegantly. That is a rat model -- I guess for Busulfan plus cyclophosphamide? Did you look at the etoposides sequence in that setting?

Dr. Yeager: No.

Dr. Dicke: Andy, what is known about immune reconstitution after Bu/Cy and autografting?

Dr. Yeager: Limited analyses, by Dr. Albert Donnenberg in the transplant group indicate that there is probably not much difference in immunologic reconstitution between Bu/Cy and cyclo TBI.

Dr. Lowenberg: With regards to the question whether relapses after auto: BMT originate from the host or from the graft and whether emphasis should be on conditioning or purging, I think there were data in the paper of Ton Hagenbeek, which may clarify some of the discussion. He showed in the rat model that 5 leukemic cells produced 13% relapse, 25 cells 50% and 50 cells 75% relapse. Thus, a 1: log cell difference really determined the difference between 13 and 75% relapse rate. If a similarly steep relationship exists between cell number surviving the conditioning regimen and relapse rate, it really may explain a lot of our discussion. That is, if we all apply suboptimal treatment we should find considerable variations in outcome from one study to the other. Secondly, it may explain that GVL and the more intensive conditioning regimens applied in allografting will give better results. Finally, we get better results regardless of purging. Finally, it may be useful to intensify the conditioning, maybe by only 1 or 2: logs and really achieve something significantly in terms of clinical outcome.

Dr. Gale: There is supposedly a very low relapse rate with busulfan and cyclophosphamide in allogeneic transplants in AML in first remission. And the implication is that it is a very good antileukemic regimen. Based on that you would guess that the twin relapse rate ought to be low. There are very few twins alone in the AML CR1, approximately 7. Surprisingly, the twin relapse rate with busulfan and cyclophosphamide is 50%, which is similar to every other regimen. That suggests that if Busulfan and cyclophosphamide is really a better regimen, there must be something else which determines the relapse rate. I brought it up because it is substantiated by the fact that the autotransplant relapse rate is 50% which is precisely the same as with every other regimen. My other comment is a more general one. We are following allogeneic transplants and so the concept Bob Lowenberg brings up -- getting a better conditioning regimen -- might do the trick. We have to remember, as Dean Buckner pointed out, unfortunately that we have not gone anywhere in 15 years in conditioning regimens that reduce the relapse risk in patients with AML, particularly patients without GVHD. That is the patient population we have to look at. In patients getting allogeneic transplants that do not develop GVHD, any conditioning regimen existing with a lower relapse rate. You should get high odds on your money if you are betting on this.

Dr. Peterson: We have done several phase I studies escalating the dose of TBI and have finished two of those studies indicating that if TBI is given from an unshielded cobalt source, maximum tolerated dose was 1440 with unshielded cobalt irradiation in patients receiving standard dose cytoxan. The schedule of TBI is 120 rad x 3 daily for 4 day. I have one question for Dr. Yeager. Because of the increase in use of Busulfan, cytoxan, are there any results regarding the long-term side effects of that regimen?

Dr. Yeager: We are just in the process of undertaking really comprehensive late follow-ups in patients who are years and years out from it, mostly allogeneic transplants. There are no apparently unexpected side effects besides gonadal, ovarian, and testicular failure. We do not appear to have an increased risk of restrictive lung disease.

Dr. Herzig: We did a dose escalation study in autografting with 120/kilo of cytoxan and an escalating TBI doses of 200 rad fractions twice a day. The dose rates were between 10 and 25 rad a minute, mostly linear accelerator. The maximum tolerated dose is 1400 rad with a sharp increase in interstitial pneumonitis at 1600 rad. So it is fairly similar to Seattle.

Discussion 2 - Session V (Growth Factors)

Dr. Buckner: We heard from Bob Lowenberg this morning that a significant number of patients, if entered prospectively, can achieve remission but not actually come to marrow storage. What is the percentage?

Dr. Lowenberg: About 25 to 30%, I think.

Dr. Buckner: Is that true of the French study?

Dr. Buckner: Of the 9 patients who did not have a second graft, based on hematopoietic difficulties, were they harvested or were they just kind of left?

Dr. Goldstone: In the early 3 or 4 years, they were all left. Now if you have a slow graft, we will later on harvest the marrow as soon as the neutrophils and platelets are up.

Dr. Burnett: There are 12 patients who, if they followed Dean Buckner's strategy, you may be able to persuade -- or you may consider it worth the risk of doing them very early in relapse rather than just leaving them alone.

Dr. Goldstone: Yes, we now elected to do both those we have (been) left alone and those of whom we have marrows stored in first relapse. Also we have grafted the first patient who relapsed from a double graft but managed to get a second CR. He has had a purged autograft in second CR. So he has had his third autotransplant. And this patient is a 100 days out with about 2000 white cells recovering from what we think is a drug induced pneumonitis and is bleeding from his gut. It is not a pretty situation to be in.

Dr. Frei: Just one point of information on your paper. The interval between the first and second transplant was 2 months. Is there an increase in marrow toxicity in terms of time to recovery after the second transplant?

Dr. Goldstone: About a week in granulocytes and about 10 days in platelets.

Dr. Frei: But the real question -- I think -- that relates to what Dr. Dicke said is whether we can really do 2 transplants depends more on non-myelosuppressive toxicity. Did you see more toxicity after the second than after the first?

Dr. Goldstone: Of the 19 patients, we have lost no patients in the second transplants. No procedure related deaths. Now we have got these four significant pneumonitis all of which recovered. In terms of other organ toxicity, it does not appear significantly greater after the second transplant as far as we can see.

SESSION VI - PERIPHERAL BLOOD STEM CELLS

THE IN VIVO EFFECT OF GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR ON CIRCULATING PROGENITOR CELLS IN MAN

Mark A. Socinski, Stephen A. Cannistra, Anthony Elias,
Karen H. Antman, Lowell Schnipper, and James D. Griffin

INTRODUCTION

Hematopoietic stem cells capable of autologous reconstitution following marrow ablation circulate in the peripheral blood (PB) of man (1). The total number of circulating granulocyte-macrophage colony-forming units (CFU-GM) and erythroid burst-forming units (BFU-E) is one-tenth to one-fifteenth that of bone marrow (BM) thereby limiting efficient procurement of these progenitors from the PB (2). The absolute number of CFU-GM in the PB appears to increase (3-20 fold) during the return of the white blood cell count following myelosuppressive chemotherapy in the majority of patients with solid tumors (3,4). In vitro, granulocyte-macrophage colony stimulating factor (GM-CSF) exerts a proliferative effect on both the CFU-GM and BFU-E (5). As part of a clinical trial to assess the ability of GM-CSF to reduce chemotherapy-related myelosuppression in sarcoma patients, we evaluated the effects of in vivo administration of GM-CSF, given alone or after chemotherapy, on BM and PB hematopoietic progenitor cells.

MATERIALS AND METHODS

Nineteen adult patients (pts) with localized non-resectable or metastatic sarcoma receiving GM-CSF on a protocol assessing the effect of this factor on chemotherapy-induced myelosuppression were studied. The details, clinical results, and toxicity of this study have

been reported elsewhere (6,7). Briefly, all patients had normal hematologic parameters and bone marrow (BM) aspirates and biopsies. None had prior chemotherapy. Patients initially received GM-CSF (Sandoz Pharmaceuticals, Bazel, Switzerland) at doses of 4 (3 pts), 8 (4 pts), 16 (3 pts), 8 (4 pts), 16 (3 pts), 32 (6 pts) or 64 (3 pts) ug/kg/day as a continuous infusion (CI) for 3-7 days (average duration 5.6 days). Following GM-CSF, a one-week rest period was given prior to initiation of therapy with doxorubicin, ifosphamide, dacarbazine by CI over 3 days. Mesna was given by CI over 4 days. After completion, GM-CSF was re-instituted on day 5 and continued through day 15. Patients then received a second cycle of chemotherapy following which GM-CSF was not administered.

Mononuclear cells (MNC) obtained from either peripheral blood or BM were assayed for CFU-GM and BFU-E as previously described (6). The percentage of BM CFU-GM in S phase of the cell cycle was determined utilizing a cytosine arabinoside (Ara-C) technique (6). Bone marrow was assayed prior to and on the final day of GM-CSF, and one week later. In 5 patients, PB CFU-GM were determined throughout the two courses of chemotherapy (with and without GM-CSF).

RESULTS

Effect of GM-CSF on BM CFU-GM and BFU-E

The effect of GM-CSF on the concentration of BM CFU-GM and BFU-E is shown in Table 1. The concentration of both CFU-GM and BFU-E tended to decrease however this did not achieve statistical significance. The percentage of BM CFU-GM in S phase as determined by an ARA-C suicide technique increased significantly (Table 1) suggesting an in vivo proliferative effect of GM-CSF. Three of the 6 patients had BM assayed one week after cessation of GM-CSF and all had return of the percentage of S phase BM CFU-GM to their baseline.

Effect of GM-CSF on PB CFU-GM and BFU-E

The effect of GM-CSF on the absolute number of PB CFU-GM and BFU-E is shown in Figure 1. Administration of GM-CSF significantly increased the number of PB CFU-GM (37 ± 10 CFU-GM/ml PB pre-GM-CSF vs 698 ± 208 CFU-GM/ml PB post-GM-CSF, p =0.005). Individually, 14 of the 18 patients (78%) had a significant (p < 0.01) increase in the absolute number of PB CFU-GM on the final day of GM-CSF treatment. The median fold increase was 18 (range 2-200). Additionally, although the number of PB CFU-GM decreased significantly one week after cessation of GM-

Table 1. Effect of GM–CSF on BM CFU–GM and BFU–E

	Pre–GM CSF[1]	Post GM–CSF[2]
CFU–GM/10^5MNC (n = 14)	33.3±7.5	20.2±4.0
	p = 0.16	
BFU–E/10^5MNC (n = 10)	11.2±1.8	8.4±2.1
	p = 0.4	
% Ara–C sensitive CFU–GM (n = 6)	29.2±2.8	53.8±6.9
	p = 0.035	

1. Pre–GM–CSF refers to BM samples obtained prior to GM–CSF.
2. Post–GM–CSF refers to BM samples obtained on the final day of GM–CSF.
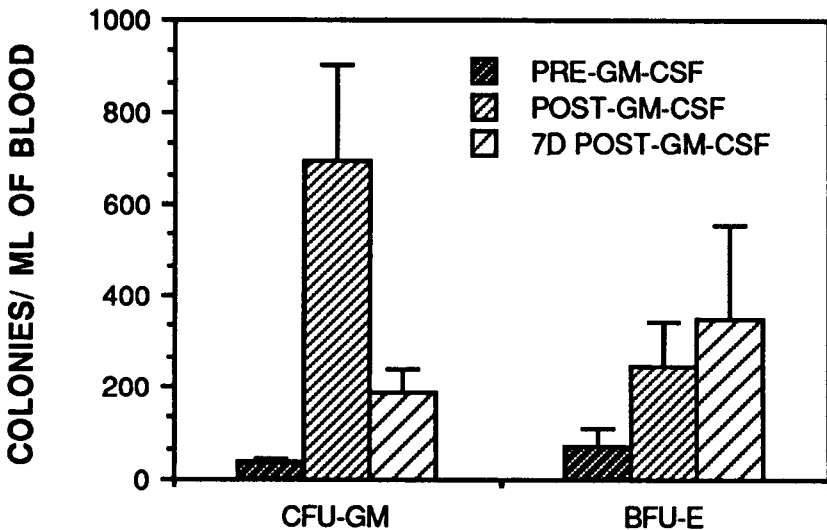
Figure 1. In vivo effect of GM–CSF on peripheral blood CFU–GM and BFU–E. GM–CSF was administered as a single agent. Post GM–CSF refers to the number of peripheral blood progenitor cells on the final day of GM–CSF. 7D post–GM–CSF refers to the number of peripheral blood progenitor cells one week following cessation of GM–CSF.

CSF, the level remained significantly elevated compared to the pretreatment baseline (187 ± 50 CFU-GM/ml PB 7d post-GM-CSF vs 37 ± 10 CFU-GM/ml PB pre-GM-CSF, p = 0.009). Ten of 14 patients (71% assayed one week later continued to have significantly elevated PB CFU-GM compared to their pretreatment baseline with a medium fold increase of 4 (range 0 - 40).

The absolute number of PB BFU-E also increase (68 ± 40 BFU-E/ml PB pre-GM-CSF vs 242 ± 103 BFU-E/ml PB post-GM-CSF, p - 0.08). Four of 12 patients (33%) showed significant (p < 0.05) increases in the number of PB BFU-E with a median fold increase of 8 (range 5-26). When assayed one week after cessation of GM-CSF, there was no significant difference as a group in the number of PB BFU-E (68 ± 40 BFU-E/ml PB pre-GM-CSF vs 351 ± 203 BFU-E/ml PB 7d post-GM-CSF, p = 0.13). However, 7 of the 9 patients assayed had levels of PB BFU-E significantly (p < 0.05) higher than their own pre-GM-CSF baseline.

There was no apparent correlation between the dose or duration of GM-CSF and the response of PB progenitor cells. There was also no correlation between the change in BM progenitor cells and the response of PB progenitor cells.

Effect of GM-CSF on PB Progenitor Cells Following Chemotherapy

Five patients were studied while receiving chemotherapy with and without GM-CSF (Table 2). Immediately following chemotherapy, no circulating CFU-GM were detected in the PB. Coincident with the post-nadir leukocyte recovery, the PB-CFU-GM peak was significantly higher (p > 0.01) in 4 of 5 patients and occurred earlier in the cycle. Similar results were obtained in 2 patients with regard to the PB BFU-E (data not shown). A representative patient is shown in Figure 2. In the 4 patients in whom chemotherapy followed by GM-CSF led to a significantly higher peak than chemotherapy without GM-CSF, the level was 60 fold higher than the pre-GM-CSF baseline and 9-15 fold higher than the peak count on the final day of GM-CSF prior to chemotherapy.

DISCUSSION

In this study, we have shown that the administration of GM-CSF as a single agent increases the number of circulating PB CFU-GM and BFU-E. Furthermore, GM-CSF used as an adjunct to chemotherapy increased the absolute number of circulating PB CFU-GM during the post-chemotherapy period in 4 of 5 patients compared to chemotherapy without GM-CSF. This was the most dramatic finding with levels of PB CFU-GM 60-fold higher than the pre-GM-CSF baseline. This effect of GM-CSF has obvious implications with

**Table 2. Effect of GM-CSF on Return of PB
CFU-GM Following Chemotherapy**

Pt #	+GM-CSF[1]	CFU-GM/ml PB Peak Day[2]	-GM-CSF	Peak Day
1	2912 ± 175	16	83 ± 9	19
2	2838 ± 81	16	57 ± 10	27
3	1004 ± 651	16	83 ± 6	22
4	2553 ± 244	16	3002 ± 481	27
5	717 ± 77	16	150 ± 18	23

1. +GM-CSF refers to the cycle of chemotherapy following which GM-CSF was administered. -GM-CSF refers to the cycle of chemotherapy following which GM-CSF was not administered.
2. Peak day refers to the day of the chemotherapy cycle upon which the level of PB CFU-GM was highest.

regard to the collection of PB progenitor cells for use in autotransplantation.

The mechanism of the increase in circulating progenitor cells caused by GM-CSF is unclear. GM-CSF did increase the proliferative state of the BM as evidenced by an increase in the percentage of BM CFU-GM in active cell cycle (Table 1). The concentration of BM CFU-GM was not statistically effected by GM-CSF however it is difficult to quantify the total number of progenitor cells in man. In the murine system (8), GM-CSF appears to increase the number of CFU-S when given after either sublethal or lethal irradiation followed by suboptimal doses of BM cells. A potential effect of GM-CSF in man is a redistributional effect mobilizing BM progenitors into the PB. However, other possible mechanisms include a reduced exit from the PB or proliferation of progenitor cells within the vascular system.

In man, PB progenitor cells have been shown to be fully capable of autologous hematopoietic reconstitution following marrow ablative chemotherapy (1, 9-11). In these studies, PB progenitor cells have usually been collected during the recovery period following chemotherapy. Kessinger et al (11) recently reported on 9 patients in whom PB progenitor cells were collected without the benefit of chemotherapy-induced increases in circulating levels. These patients generally underwent 32 hours of pheresis for collection of a median of 8.4 x 10^8 mononuclear cells kg of patient weight that contained a
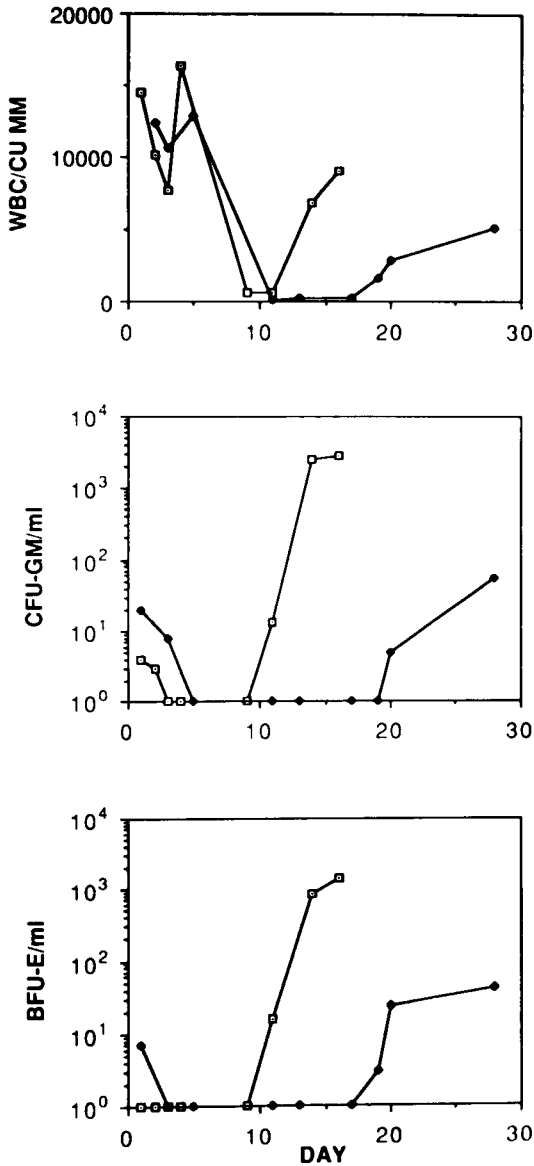
Figure 2. Effect of GM-CSF on the recovery of the white blood cell (WBC) count (top panel), CFU-GM PB (middle panel) and BFU-E (bottom panel) following chemotherapy in a representative patient. The open squares represent the cycle of chemotherapy following which GM-CSF was administered while the closed squares represent the cycle of chemotherapy following which GM-CSF was not administered (Reprinted with permission from the Lancet 1988;1:1194).

median of 8 x 10^4 CFU-GM/kg after thawing. This dose of PB CFU-GM led to prompt and sustained engraftment in all patients. Since GM-CSF increases the absolute number of circulating progenitor cells when administered as a single agent or following chemotherapy, it may significantly decrease the amount of leukopheresis time required to collect sufficient progenitor cells. It should be noted that what number defines a sufficient level of PB progenitor cells for use in autotransplantation is as yet undefined. Also, a theoretical concern is what effect GM-CSF may have on the balance between the pluripotent stem cell and the more committed progenitor cells such as the CFU-GM and BFU-E. Since the true pluripotent stem cell count cannot as of yet be defined in man, these theoretical concerns and the ability of GM-CSF-stimulated PB progenitor cells to restore hematopoiesis following marrow ablation may only be answered in the context of clinical autografting studies.

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CULTURE OF HEMATOPOIETIC PROGENITOR CELLS
FROM HUMAN PERIPHERAL BLOOD

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INTRODUCTION

Human peripheral blood (PB) contains hematopoietic stem cells (HSC). Autologous transplantation of PB HSC has reconstituted hematopoiesis after bone marrow ablative therapy for breast cancer, lymphoma, and leukemia (1-4). Recently, Kessinger et al reported the first successful allogeneic transplant of T cell depleted PB HSC (5). After collection by leukapheresis, PB HSC may undergo several processing steps, including ficoll-metrizoate density gradient fractionation, T cell depletion, and cryopreservation. An estimate of HSC recovery would be beneficial, as each step contributes to loss of HSC. Most laboratories rely on the granulocyte-monocyte progenitor cell (CFU-GM) assay since pluripotent HSC cannot be determined directly, although the number of transplanted CFU-GM may or may not be predicative of engraftment (6-8). We previously reported that growth of PB CFU-GM is strongly influenced by the separation of mononuclear cell (MNC) populations (9). Changes in CFU-GM cloning efficiency are due to shifts in the absolute and relative number of monocytes (MO) and T cells. The purpose of the present study was to further characterize the modulation of progenitor cell growth by MO and T cells.

MATERIALS AND METHODS

Peripheral blood was collected from random healthy donors with informed consent (American Red Cross Blood Services, Washington D.C. Region). MNC were isolated by centrifugation of buffy coats on ficoll-metrizoate (Ficoll-Paque, Pharmacia). Large quantities (> 3 x 10^9) of MNC were obtained by ficoll-metrizoate separation of

plateletapheresis residues (provided by American Red Cross Blood Services, Chesapeake Region) in the Haemonetics V5O (10). MNC or non-adherent MNC were rosetted with aminoethylisothiouronium bromide (AET; Sigma) treated sheep red blood cells (sRBC; M.A. Bioproducts). E-rosette positive cells (T cells) and other high density cells were separated from hematopoietic cells on 4-step Percoll (Pharmacia) density gradients (11). The low-density fraction ($\leq$ 1.0718 g/ml), enriched for hematopoietic cells and depleted of T cells, was subjected to a second adherence step to remove residual MO resulting in a population termed "B/Null" (9,12). In some experiments, MO were removed by lysis with phenylalanine methyl ester (PME, Sigma) (13). CFU-GM and BFUe were not affected by PME treatment (14). T cells (> 90% CD3 positive) were recovered from the high density Percoll fraction, and the sRBC were lysed with NH_4Cl B/Null cells contained 81 $\pm$ 2% lymphocytes, 9 $\pm$ 3% MO and 10 $\pm$ 3% basophils and eosinophils (Wright stained cytocentrifuged preparations). By indirect immunofluorescence, 16 $\pm$ 3% of the B/Null cells were T cells (CD3 positive), 7 $\pm$ 1% were B cells (CD19 positive), 10 $\pm$ 2% were MO, and 33 $\pm$ 4% were natural killer cells.

CFU-GM were grown in 1 ml in IMDM (Gibco) containing O.35% agarose (FMC Corp) with 20% pre-screened fetal bovine serum (FBS; Hyclone), penicillin + streptomycin (M.A. Bioproduct), and 5 x 10^{-5} M mercaptoethanol (Sigma) (11). Colony stimulating activity (CSA) was provided by mixed donor MNC conditioned medium (LCM) (15), CM from the human bladder carcinoma cell line 5637 (5637CM) (16), or CM from giant cell tumor (GCT; Gibco) (17). LCM and 5637CM were concentrated 5x by Amicon filters, and were used at optimal concentrations determined by preliminary experiments. Colonies of more than 40 cells were scored after 14 days in culture. BFUe were grown in 1 ml 0.35% agarose in IMDM with 30% FBS, penicillin + streptomycin, 1% bovine serum albumin, 10^{-4} M hemin (Sigma), 1.5 units erythropoietin (Connaught Lab), and 5637CM as a source of burst promoting activity (BPA) (16). Red colonies with >40 cells were counted as BFUe after 14 days of incubation. Autologous T cells were added at 2.5 x 10^5/ml to either CFU-GM or BFUe cultures. In some experiments, the T cells were supplemented in a 0.5% agarose underlayer. Colonies were identified in whole agar discs by triple staining (18): alpha naphthol acetate esterase (Sigma), naphthol AS-D-chloroacetate esterase (Sigma) and Luxol Fast Blue (MCB Reagents) were used to identify MO, neutrophils and eosinophils, respectively.

Mean $\pm$ SEM were calculated unless otherwise stated. Statistical significance was determined by Student's t-test.

RESULTS AND DISCUSSION

In previous experiments we demonstrated that addition of autologous T cells stimulated CFU-GM growth (9). In this study, we
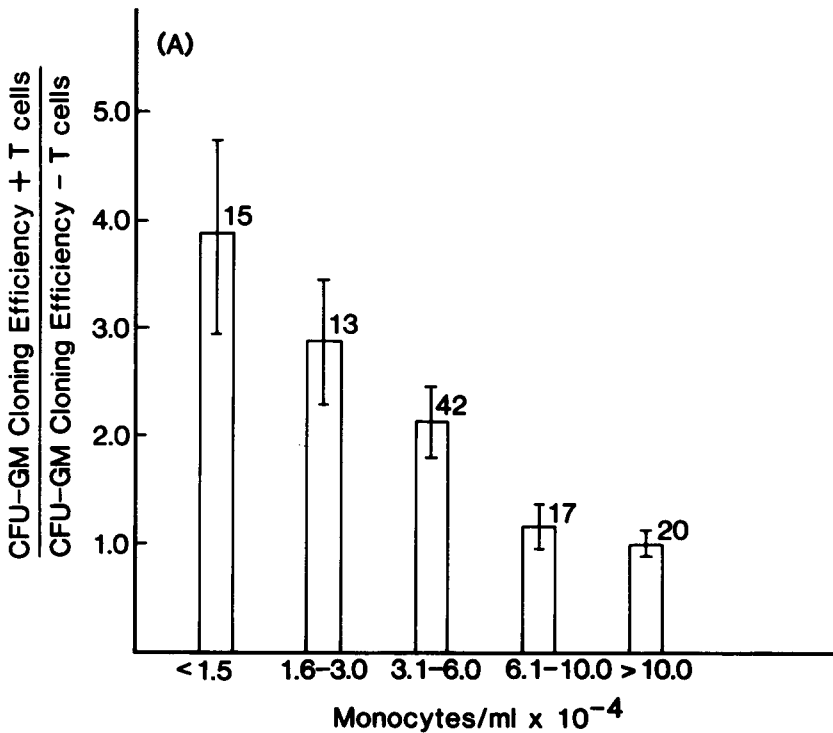
Figure 1. Effect of Monocytes on T-Cell Stimulation of Peripheral Blood CFU-GM. MNC, non-adherent MNC, low-density E-rosette negative MNC (at $1-2 \times 10^5$ per 35 mm dish) or B/Null cells (at $5-10 \times 10^4$/dish) were cultured for CFU-GM with or without autologous T-Cells ($2.5-10 \times 10^5$/dish) in 1 or 4 ml of medium. LCM was used as a source of CSA. The extent to which autologous T-cells stimulated CFU-GM was plotted against the concentration of monocyte in the culture system. Monocyte content was determined from Wright's stained cytocentrifuged preparations. In some experiments where high monocyte levels were needed, known numbers of autologous monocytes purified by centrifugal elutriation were added to the cultres. The number of independent determinations for each concentration range was given beside the bar.

ask how T cell stimulation is affected by MO concentration. Low density T cell-depleted MNC or B/Null cells were cultured in the presence or absence of autologous T cells. In some experiments, known numbers of autologous MO (purified separately by centrifugal elutriation) were added to the dishes. The degree of T cell stimulation of CFU-GM growth was plotted against MO concentration in Fig 1. Cloning efficiency of CFU-GM increased 4-fold when T cells were added at low MO concentrations ($2-15 \times 10^3$/ml). Between $31-60 \times 10^3$ MO/ml, stimulation was significantly reduced ($p < 0.05$), and no increase was found at higher MO concentration. CFU-GM colony

formation was completely inhibited when MO exceeded 7 x 10^5/ml (9). Other laboratories have observed the stimulatory effects of T cells (12) and the inhibitory influence of MO (19-21). This study shows that the ratio of T cells to monocytes (as well as the absolute concentrations) strongly affects CFU-GM cloning efficiency.

T cell stimulation was initially demonstrated with LCM as a source of CSA (9). In this study, comparable stimulation by T cells was seen with 2 other CSA sources, GCT and 5637CM (Table 1A). T cells enhanced progenitor growth to similar extents with all 3 CM. In each case, the CM was used at its optimal concentration. LCM and 5637CM supported CFU-GM formation better than GCT (p < 0.05), regardless of whether T cells were present in the culture system. Mixing 2 different CM at their optimal concentrations did not yield as many CFU-GM as did 1 CM supplemented with T cells (Table 1B). The number of CFU-GM in 2 CM were not different from those cultured

Table 1A. Stimulation of CFU-GM Growth by Autologous T-Cells in 1 or 2 CM[a]

B/Null Cells Cultured in a Single CM

	LCM	5637CM	GCT
B/Null + T cells	1.00	0.84±0.08(10)	0.72±0.05[b](9)
B/Null	0.44±0.10[c](12)	0.38±0.07[c](12)	0.22±0.07[c](10)

Table 1B. Stimulation of CFU-GM Growth by Autologous T Cells in 1 or 2 CM[a]

B/Null Cells Cultured with Combinations of 2 CM

	- T Cells	+ T Cells
LCM & GCT	0.32±0.10 (9)	0.86±0.10 (8)
LCM & 5637CM	0.43±0.11 (12)	1.13±0.12 (12)
5637CM & GCT	0.38±0.09 (9)	0.81±0.12 (8)

[a]Relative cloning efficiency of CFU-GM grown with T cells to that without T cells is shown in the table. The number of CFU-GM in LCM with added autologous T cells was arbitrarily defined as 1.0 for each donor to facilitate comparison. An average of 36.5 ± 20.3 (12) CFU-GM were observed in 5 x 10^4 B/Null cells. The number of independent experiments is given in brackets.

[b]Significantly different (p < 0.05) from the LCM value with T cells.

[c]Significantly different (p < 0.05) from the corresponding values in the same CM without T cells.

in a single CM. The inability to generate more CFU-GM in 2 CM was not the result of nutrient dilution as both LCM and 5637CM were concentrated 5-fold. Nor was it due to an increase in the level of inhibitory factor(s), as CFU-GM growth increased when T cells were added to the dishes with 2 CM. T cells cultured alone did not produce any CFU-GM in all 3 CM.

When T cells and B/Null cells were cultured in separate agarose layers, the degree of stimulation was the same as when T cells and B/Null cells were cultured together (Table 2). Thus, it appears that T cells secrete a stimulatory factor into the surrounding growth medium.

We asked whether T cells specifically enhanced a particular class of CFU-GM (Table 3). The distribution of colony types varied with the CM. Eosinophilic colonies predominated (51% of all colonies) in LCM, while 5637CM promoted more neutrophilic colonies (51%). GCT stimulated neutrophilic and eosinophilic colonies to similar extents (36% and 38% respectively). The colony distribution data agree with other published reports (17,22). Adding T cells to the cultures increased the number of CFU-GM in all 3 CM, however the percentages of the different colony types remained the same ($p > 0.1$) as those in the absence of T cells. When LCM and GCT were used in combination, there was a small shift towards eosinophilic colonies.

Table 2. T Cell Stimulation of CFU-GM in Single Layer and Double Layer Agarose Culture[a]

Culture Conditions		Relative Cloning Efficiency
B/Null + T + LCM	Single Layer	1.0
B/Null + LCM	Single Layer	0.63 ± 0.10[b] (9)
[B/Null + LCM] / [T + LCM][c]	Upper Layer / Lower Layer	1.18 ± 0.21 (9)
[B/Null + LCM] / [T][c]	Upper Layer / Lower Layer	1.15 ± 0.20 (9)

[a]B/Null cells were cultured with or without autologous T cells in single or double layer agarose. The cloning efficiency in a single layer with LCM and T cells was defined as 1.0. An average of 88.2 ± 56.8 CFU-GM were found in 5×10^4 B/Null cells when T cells were added to the same agarose layer. The number of independent experiments is given in brackets.

[b]Significantly different ($p < 0.05$) from the value with T cells.

[c]T cells with or without LCM in 0.5% agarose were put in the bottom of the culture dish. B/Null cells in 0.3% agarose were layered on top of the T cells. CFU-GM were enumerated after 14 days in culture as described.

**Table 3. Colony Differential of CFU–GM
with and Without T Cells in 1 or 2 CM[a]**

	N	M	N/M	Eos	Eos/N
LCM					
-T (7)	27.6±6.5	7.1±3.0	6.1±2.8	50.9±8.8	6.4±3.0
+T (10)	19.6±3.7	10.2±2.9	5.8±2.2	52.9±5.0	10.8±2.4
5637CM					
-T (6)	51.3±9.2	1.7±1.1	4.0±2.0	38.2±8.9	2.5±1.2
+T (9)	56.8±9.4	2.4±1.1	3.1±1.8	34.3±8.9	2.5±1.1
GCT					
-T (3)	35.7±4.7	10.1±7.6	2.2±1.6	37.6±8.5	0.0±0.0
+T (5)	51.1±10.3	1.5±0.9	1.2±0.8	35.3±10.2	1.8±0.8
LCM &					
-T (3)	26.7±11.5	6.3±3.5	4.3±1.2	44.6±11.2	3.9±1.9
GCT					
+T (6)	19.7±5.6	13.3±4.1	6.5±2.3	45.0±9.8	7.1±2.3
GCT &					
-T (3)	43.5±4.6	7.5±3.2	5.0±0.0	32.5±3.2	2.5±1.8
5637CM					
+T (5)	51.0±11.9	1.1±0.6	0.6±0.5	42.0±10.5	3.2±1.2
LCM &					
-T (6)	13.4±4.4	18.3±5.9	5.2±3.9	55.7±2.4	3.6±2.4
5637CM					
+T (9)	11.5±2.6	12.7±2.9	4.4±1.0	65.7±2.2	4.0±1.5

[a]The CFU–GM colonies were classified into neutrophilic (N), monocytic (M), eosinophilic (Eos), and mixed colonies with neutrophils and monocytes (N/M), and eosonophils and neutrophils (Eos/N).

The change was not statistically significant, as the percentages of eosinophil colonies in LCM and GCT differed by less than 15%. However, when 5637CM and LCM were mixed together, the majority of colonies became eosinophilic ($p < 0.05$), regardless of whether T cells were present. The colony distribution pattern resembled that of LCM but not 5637CM.

When B/Null cells were cultured with T cells in the absence of CM, no CFU–GM colony formation was detected. Thus, T cells require CM to release the stimulating factor(s), or the factor(s) by itself lacks CSA and functions synergistically. Experiments with saturating concentrations of recombinant colony stimulating factors (CSF) and/or with appropriate antibodies against known CSF may help define the nature of the T cell factor. Recent studies in this laboratory showed that the CFU–GM culture medium was only slightly mitogenic to T cells (23). Furthermore, the capacity of T cells to stimulate CFU–GM was not diminished by levels of transforming growth factor–beta 1 (TGF–beta) which inhibited T cell proliferation (submitted for publication). Those data indicated that relatively little T cell proliferation is needed for the production and release of the

stimulating factor(s). Whether or not T cells require activation to secrete the factor is unclear. Attempts to make CM from quiescent T cells have been unsuccessful.

BFUe formation was also stimulated by autologous T cells, in agreement with other published reports (12). An average of 46.0 ± 26.3 BFUe were grown from 5 x 10^4 B/Null cells in 3 independent experiments, but 135.3 ± 47.7 were formed when 2.5 x 10^5 T cells were added, an increase of almost 3 fold. CFU-GM colony formation in the BFUe growth medium also increased by 2.3 fold when autologous T cells were present. T cells cultured alone in 5637CM did not produce any BFUe.

CONCLUSIONS

Cell separation techniques which alter the concentrations of MO and T cells profoundly affect the sensitivity of peripheral blood progenitor cell assays. T lymphocytes produce diffusible factor(s) that increase the cloning efficiency of peripheral blood CFU-GM in 3 different CM. The factor stimulates BFUe and all CFU-GM classes to a similar extent. The factor may have synergistic activity with other CSA, requires little T cell proliferation for its synthesis, enhances CFU-GM growth in the presence of TGF-beta, but loses its stimulatory effect when the MO concentration > 6 x 10^4/ml. To accurately quantitate clonogenic progenitors, with or without cell separation, close attention must be paid to the number and ratio of accessory cells in the culture system.

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ARE OCCULT TUMOR CELLS PRESENT IN PERIPHERAL STEM CELL HARVESTS OF CANDIDATES FOR AUTOLOGOUS TRANSPLANTATION?

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INTRODUCTION

The number of autologous hematopoietic stem cell transplantation procedures is increasing rapidly (1). Recently, peripheral blood stem cells obtained by apheresis have been employed to reconstitute aplastic patients (2). This procedure has some advantages over the use of bone marrow for transplantation, including the option of high dose therapy for patients whose marrow is involved by disease. The procedure also increases the pool of patients who are eligible for autologous hematopoietic stem cell rescue.

Our concern in all of these transplantation procedures is that occult malignant cells may contaminate the hematopoietic cells used to reconstitute the recipient, and may lead to recurrence of the disease. We have reported previously that culture techniques permit the detection of occult tumor cells in histologically normal bone marrow from patients with lymphomas and solid tumors, particularly breast cancer (3,4).

In this report we present the preliminary results of our efforts to detect tumor cells in the peripheral blood stem cell harvests of patients with histologically confirmed malignancy in their bone marrow or who were otherwise ineligible for autologous bone marrow transplantation.

METHODS

Peripheral blood stem cells were harvested from candidates for autologous transplantation and normal donors as described previously (2). Aliquot of these harvests were cultured in a medium consisting of Iscove's modified Dulbecco's medium with 12.5% fetal calf serum, 12.5% horse serum, folic acid (2×10^{-6}M), myoinositol (5×10^{-4}M), hydrocortisone (10-6M), 2-mercaptoethanol (10^{-5}M), penicillin (100 U/ml) and streptomycin (100 ug/ml) and Iglutamine (2mM) as described by Douay et al. (3). For comparison, cells scraped from the screens used to filter harvested bone marrow were cultured as described previously (4,5). Cytospin preparations were made from the cultures on a weekly basis, blind coded, and examined by the pathologist for morphologically-abnormal cells.

RESULTS AND DISCUSSION

We have examined the peripheral stem cell harvests of 11 patients with either non-Hodgkin's lymphoma (NHL), Hodgkin's disease (HD) or breast cancer (BC). These patients had peripheral blood stem cells harvested because histologically demonstrable tumor was present in their marrow (nine patients), prior significant pelvic radiation had been given precluding a marrow harvest (one patient) or they had an unsuccessful marrow harvest (one patient). For purposes of comparison, we have also examined normal apheresis products (12 volunteers) and bone marrow (13 prospective allogeneic donors) as well as the bone marrow of 11 patients with histological involvement of their marrow by tumor. These results are presented in Table 1. No morphologically-abnormal cells were observed in cultures of the apheresis products or the bone marrow of normal donors. Tumor cells were detected in all cultures from marrow histologically-involved by tumor even after freezing and thawing of the harvest. In contrast, only two of the patient apheresis products had suspected malignant cells in the cultures. When the culture results were uncoded, we noted that tumor cells had been detected cytologically in the peripheral blood smear of one NHL patient at the time of the first apheresis. Consequently, the patient's apheresis had been terminated.

These data are too preliminary to permit a statistical analysis for each disease category. If the data are pooled, abnormal cells were detected significantly less frequently in the aphereis products than in histologically involved bone marrow (2/11 versus 11/1, $p \leq 0.001$ Fisher's exact test). This suggests that the application of culture techniques to peripheral blood apheresis products as a means of detecting tumor cells may be less sensitive than when applied to cultured marrow. Alternatively, there may be few or no circulating tumor cells in most apheresis harvests. We are currently trying to

**Table 1. Detection of Morphologically Abnormal Cells
in Apheresis and Bone Marrow Harvests of Normal Donors
and Autologous Transplantation Candidates with
Histologically Confirmed Bone Marrow Metastases**

| Population Examined[a] | Marrow Biopsy Positive at | | Abnormal | (%) |
	Diagnosis	Apheresis	Total	
N- marrow	NA[b]	NA	0/13	(0%)
NHL - marrow+	6	NA	6/6	(100%)
HD - marrow+	1	NA	1/1	(100%)
BC - marrow+	4	NA	4/4	(100%)
All tumors marrow+	11	NA	11/11	(100%)
N - apheresis	NA	NA	0[c]/12	(0%)
NHL - aspheresis	6	3[d]	1[e]/6	(17%)
HD - aspheresis	4	0[d]	1/4	(25%)
BC - aspheresis	0	0[f]	0/1	(0%)
All tumors aspheresis	10	3[d]	2/11	(18%)

a. N = normal donor; NHL = non-Hodgkin's lymphoma; HD = Hodgkin's lymphoma; BC = breast cancer; + = histologically positive.

b. Not applicable.

c. Some normal donor cultures contained large lymphoblastoid cells suspected to be Epstein-Barr virus infected lymphocytes.

d. Some patients received additional treatment between diagnosis and apheresis.

e. Retrospective analysis indicated this patient had cytological evidence of circulating tumor cells detected at the 1st apheresis.

f. Marrow biopsy negative; lumbar metastases present on bone scan.

resolve this dilemma by estimating the efficiency of tumor cell detection using titrated numbers of known malignant cells added to uncultured and cultured apheresis products from normal donors. Molecular biologic and cytogenetic studies will be necessary to confirm that the morphologically-abnormal cells are indeed tumor cells related to the primary malignancy.

Our observations confirm the report of Douay et al. (3) that human peripheral stem cell harvests can be cultured long term. Although other investigators have sampled peripheral blood from patients with non-hematogenous malignancies for the detection of tumor cells, no data were presented on the outcome (6). The increasing application of peripheral blood stem cell transplantation in patients with cancer, particularly those with bone marrow metastases, will require a careful assessment of the frequency of occult tumor cells in apheresis products.

CONCLUSIONS

These results indicate that the application of culture techniques to the peripheral blood stem cell harvests of cancer patients whose marrow is known to be involved by tumor leads to the detection of tumor cells much less frequently than when applied to the involved marrow. Whether this is because the culture technique is less sensitive when applied to peripheral blood stem cell harvests than to bone marrow or because there are few or no circulating tumor cells is an important question yet to be answered.

ACKNOWLEDGMENTS

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PERIPHERAL BLOOD STEM CELL
COLLECTION AND TOXICITY

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INTRODUCTION

Procedures for the collection of stem cells from peripheral blood contrast sharply with those for the collection of bone marrow stem cells. A bone marrow harvest is a surgical procedure performed under general anesthesia by a full operating room team. Peripheral blood stem cell (PBSC) collection is performed by apheresis in an outpatient setting, by blood center personnel. Peripheral stem cell collection, however, requires multiple procedures and the volume of products reinfused is much greater than for bone marrow stem cells. In collecting PBSC from 75 patients and performing 50 PSC transplants, we have studied several protocols for the collection of PBSC and reviewed the morbidity associated with reinfusion.

METHODS

Standard Collection of Peripheral Stem Cells

Mononuclear cells were collected using the Haemonetics V-50 apheresis machine using a modification of the platelet/granulocyte protocol. Blood was drawn at 60-80 ml/min until the platelet band began to discharge. The rate was slowed to 20 ml/min and collection was begun manually when half the platelet band had passed and continued until two minutes after the red cell layer began to discharge. No hydroxyethyl starch was used.

Cryopreservation

The apheresis product was centrifuged and plasma removed until either the hematocrit is 50% or the nucleated cell count was 1×10^8/ml. Sodium heparin was added (10 units/ml). The specimen was transferred to a cryopreservation bag (Stericon, Broadview, IL) and cold freezing media containing 40% dimethylsulfoxide (DMSO) and 6 μg/mg of DNA'se I (Sigma Chemical, Chicago, IL) was added at a ratio of 3 parts specimen to 1 part media. The bag was placed in an aluminum cassette and transferred to the controlled rate freezer until the temperature reached -85°C and then transferred for storage to the vapor phase of liquid nitrogen.

Ficoll-Hypaque

This procedure was performed as described by Law, et al. The apheresis product collected by the standard procedure was loaded into the bowl at 80 ml/min, then Ficoll-Hypaque was added at 20 ml/min until the interface was slightly below the shoulder of the bowl and then the pump was stopped. After 10 minutes the MNC layer had formed and the pump was restarted at 20 ml/min. Collection continued from slight before the MNC layer until 100 ml after the MNC layer began to discharge. The product was washed twice by centrifugation and resuspended in Hanks balanced salt solution with 20% autologous serum.

Lymphocyte Surge

The "lymphocyte surge" protocol was performed as described in the V-50 manual. Blood was drawn into the bowl at 60 ml/min until the optics detected the RBC layer at the shoulder of the bowl. Then the patient draw was stopped and the already collected plasma was pumped quickly through the bowl. The mononuclear cell collection was begun after the machine optics had detected a declining platelet band and was continued for 60 ml. The procedure was repeated on each pass for four hours.

Modified Lymphocyte (Nebraska) Surge

In contrast to the above procedure, this modified procedure was not used to collect the apheresis product but was used to process the product which had been collected by the standard apheresis protocol. After a 4-hour collection, the product was loaded back into the bowl and the apheresis machine programmed for one cycle of "lymphocyte surge" using a 300 ml surge volume (this requires a modification of the program chip by the manufacturer). After the bowl was reloaded,

blood was gain drawn from the patient. The collection was begun manually at the beginning of the platelet band.

RESULTS

Table 1 lists the protocols we have studied. The first protocol was used to collect PBSC from 10 patients. Each patient had 8 four-hour apheresis procedures. The MNC dose ranged from 4.9 to 15.8×10^8/kg (median = 9.0×10^8/kg) and the CFU-GM dose from 2.3 - 98.6×10^4/kg (median = 6.6×10^4/kg). No correlation was found between engraftment and CFU-GM dose. Since three patients transplanted with less than 7.0×10^8 MNC/kg had all engrafted, in subsequent protocols collection were continued until this dose was achieved. Studies in our laboratory on normal apheresis donors had shown a decline in the CFU-GEMM numbers as the duration of the apheresis continued. We therefore limited the next series of patients to seven passes per apheresis. This increased the number of apheresis procedures and subsequent culture data did not confirm this CFU-GEMM drop in patients[2], therefore we returned to the four-hour procedure.

A review of the toxicity of PBSC infusion in the first 25 transplant (Table 2) showed that 12 patients developed fluid overload as demonstrated by weight gain and 3 developed pulmonary edema. One patient was clinically suspected of having a pulmonary embolism which was temporally related to the reinfusion. Macroscopic hemoglobinuria was almost universal and 3 patients developed renal

Table 1. Stem Cell Collection Protocols

1. 8 Four-Hour Apheresis.

2. Seven Passes per Apheresis until 7×10^8 MNC/kg.

3. Four-Hour Apheresis until 7×10^8 MNC/kg (Ficoll-Hypaque).

4. Four-Hour Apheresis until 7×10^8 MNC/kg (lymphocyte surge every other product).

5. Four-Hour Apheresis until 7×10^8 MNC/kg (modified lymphocyte surge every other product).

6. Four-Hour Apheresis until 7×10^8 MNC/kg (modified lymphocyte surge all products).

Table 2. Complications of Peripheral Stem Cell Infusion

Pts	Vol. ml	Hemoglo-binuria	Fluid* Overload	Pulmonary Edema	Pulmonary Embolism	Renal Dys-function
25	1206	24	12	3	1	3

*Wt gain > 1 kg within 24 hrs of infusion

dysfunction. (A rise in creatinine > 2 mg/dl within 72 hours of infusion.) Since these complications were likely to be related to the volume, DMSO content and hemolysate content of the products we attempted to decrease the RBC content and the volume of the products.

A Ficoll-Hypaque procedure was next used to isolate MNC; however, it added approximately 1-1/2 hours to the procedure and processing and required more *in vitro* manipulation. Studies in normal donors indicated that the "lymphocyte surge" protocol would yield a product with significantly less RBC contamination.

Table 3 shows the results when the lymphocyte surge protocol was used alternately with the standard protocol to collect PBSC[3]. In patients, RBC contamination was higher than it had been in normal donators and MNC yield was yield was less than that using the standard protocol. There was no overall advantage to using the lymphocyte surge protocol.

Our modification of the lymphocyte surge protocol, by increasing the surge volume and collecting the platelet band to increase the MNC yield and using only one cycle to process the final product rather than collect the product, has greatly improved the RBC depletion (Table 4)[4]. The procedures both gave excellent yields of MNC and hematopoietic progenitors. The Ficoll-Hypaque procedure does remove significantly more RBC, however, both procedures remove enough RBC that the hematocrit is no longer the limiting factor in the concentration of the specimen.

CONCLUSION

Many questions still remain with regard to how to assess the dosage of peripheral blood stem cells, however, we have been successful in basing our collections on the mononuclear cell dose of 7×10^8/kg. The resulting product is large in volume and RBC content which has resulted in some morbidity in our patients. Therefore,

Table 3. Collection of Peripheral Blood Stem Cells by a Standard Platelet/Granulocyte Protocol and by a Lymphocyte Surge Protocol

	RBC (ml)	MNC $(\times 10^8/kg)$	RBC/MNC $(ml/10^9)$	CFU-GM $(\times 10^3/kg)$	BFU-e $(\times 10^3/kg)$	CFU-GEMM $(\times 10^3/kg)$
Standard	65.9	0.76	5.41	1.63	5.94	8.74
Surge	39.1*	0.61*	4.15	1.87	9.54	12.05

*p <.001

Table 4. A Comparison of Cell Recoveries Following Processing of Peripheral Stem Cell Apheresis Products by a Ficoll-Hypaque Density Gradient Method and a "Surge" Method

	WBC %	MNC %	GFU-GEMM %	Plts %	RBC %
Surge*	70 ± 2	91 ± 2	104 ± 7	87 ± 3	25 ± 2
Ficoll	73 ± 2	95 ± 2	123 ± 12	77 ± 4[+]	5 ± 1[a]

*Mean ± SEM   [+]p <.05   [a]p <.001

methods to decrease the RBC content and volume of the final product are important. A modification of the lymphocyte surge protocol using the Haemonetics V-50 apheresis machine is a possible solution to the problem.

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RESPONSE OF PATIENTS WITH REFRACTORY LYMPHOMA TO HIGH DOSE THERAPY AND AUTOLOGOUS PERIPHERAL STEM CELL TRANSPLANTATION

Anne Kessinger and James O. Armitage

INTRODUCTION

Autologous peripheral stem cell transplantation (PSCT) following marrow ablative therapy has successfully restored marrow function.[1-4] PSCT offers certain advantages when compared with marrow transplantation, specifically: general anesthesia can be avoided, the cells can be collected in an outpatient setting, the risk of tumor contamination may be less[5], and sufficient marrow at customary harvest sites is not necessary. At the University of Nebraska Medical Center, we have used autologous PSCT following high dose therapy for patients with refractory lymphomas who were candidates for bone marrow transplantation except that: (1) histopathologic evidence of marrow metastases was present, (2) prior pelvic irradiation had been given, (3) an attempt to collect autologous marrow resulted in a hypocellular product, or (4) histopathologic evidence of marrow metastases had been documented earlier in the disease course.

PATIENTS AND METHODS

Forty patients, 24 with Hodgkin's disease (HD) and 16 with non-Hodgkin's lymphoma (NHL), were treated with high dose therapy and autologous PSCT prior to June 15, 1988. Twenty one patients, 13 with HD and 8 with NHL and histopathologic evidence of marrow metastases at the time of study entry. Six patients, 2 with HD, had had documented marrow metastases in the past. Ten patients, 7 with HD, had received prior pelvic irradiation and three patients, 2 with HD, had had an inadequate collection following an attempted marrow

harvest. The patients' ages ranged from 12-57 years (median = 34 years) and 28 were males. The median time of PSCT after diagnosis of malignancy for the HD patients was 66 months and for the NHL patients, 12 months. The HD patients had received 1-7 trials of combination chemotherapy prior to stem cell collection and 20 had also received radiation therapy. The NHL patients had received 1-3 trials of chemotherapy and 7 had received radiation therapy.

Peripheral stem cells were collected with a four-hour apheresis procedure repeated until approximately 7×10^8 mononuclear cells/kg patient weight were collected. No manipulations were performed to increase the number of circulating stem cells except for one patient who received oral prednisone.

For each patient, aliquots of each collection were frozen, thawed, pooled and cultured for progenitors. Thirty-three patients' collections assayed for BFU-E contained a median of 0.40×10^4 BFU-E/kg (range 0-10.5 $\times 10^4$/kg). Thirty-seven patients' cells contained a median of $.51 \times 10^4$ CFU-GEMM/kg (range 0 - 34.3). Thirty-five patients' cells were assayed for CFU-GM using a feeder layer for colony stimulating factors and contained a median of $.60 \times 10^4$ CFU-GM/kg (range of .0 - 98.6).

The preparatory regimens used for the patients varied according to the lymphoma treated. All but 2 HD patients received a single regimen; carmustine 300 mg/M^2, etoposide 125-150 mg/M^2 x 6 and cyclophosphamide 1.5 gm/M^2 x 4. One patient had received this therapy earlier in her disease course followed by an autologous bone marrow transplant and disease recurrence, so she was treated with a combination of thiotepa, cyclophosphamide, and etoposide as described by Herzig et al[6]. One HD patient and four NHL patients received carmustine 300 mg/M, cyclophosphamide 35 mg/kg x 4, etoposide 100 mg/M^2 every 12 hours x 8 plus or minus cytarabine 100 mg/M^2 every 12 hours x 8. Six NHL patients received cyclophosphamide 60 mg/M^2 x 2 followed by total body irradiation fractionated into 5 doses. One patient received cytarabine 18 gm/M^2 in 6 divided doses, cyclophosphamide 90 mg/M^2, and 9 Gray TBI fractionated in 5 doses. Five NHL patients received carmustine 300 mg/M^2, cyclophosphamide 2.5 gm/M^2 x 2, hydroxyurea 1.5 gm/M^2 every 6 hours x 12 etoposide 150 mg/M^2 every 12 hours x 6. Following therapy, the autologous cells were thawed at the bedside and immediately transfused.

RESULTS

A median of 7.03×10^8 mononuclear cells/kg patient weight (range 4.61 - 16.36 $\times 10^8$/kg) were transplanted. One patient died on day +2 before evidence of stem cell engraftment. For 39 patients, the first white cell appeared at a median of seven days following PSCT (range
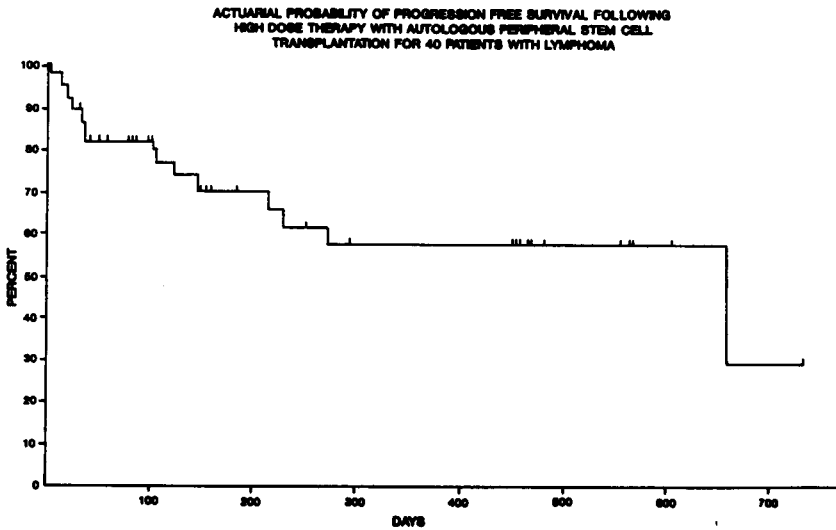
Figure 1.

3 - 15 days). Five patients died 2-35 days after PSCT of complications of sepsis or hemorrhage before .5 x 10^9/l granulocytes were in the circulating blood. Two patients have not recovered .5 x 10^9/l granulocytes 50 and 70 days after PSCT but are otherwise well. Thirty-three patients had 0.5 x 10^9/l granulocytes in the circulating blood at a median of 25 days after PSCT (range 11 - 87 days). The last red cell transfusion for 29 patients occurred at a median of 27 days following PSCT (range 7 - 159 days). Two patients remain red cell transfusion dependent 28 and 50 days following PSCT. The last platelet transfusion for 29 patients occurred at a median of 25 days after PSCT (range 8 - 116 days). Nine patients died of hemorrhage, sepsis or disease progression while platelet and red cell transfusion dependent 2 - 146 days following PSCT. Two patients remain platelet transfusion dependent 28 and 50 days following PSCT.

For these 40 patients, a clinical complete response was documented in 18, a continued complete response in 1, a partial response in 12, and failure to respond in 1. Six patients died before they were restaged 2 - 38 days after transplant, and 1 patient has not yet been restaged 31 days from transplant. At present, 26 patients survive and 18 of them remain free of disease progression for 31 - 374 days. The actuarial 2-year progression free survival for these 40 patients is 25% and the actuarial 2-year survival is 29%.

DISCUSSION

The patients initially entered in this study were candidates for high dose therapy because they had otherwise incurable lymphomas, but were not candidates for autologous bone marrow transplantation because their marrows contained tumor cells or were hypocellular. When we learned that istopathologically normal marrow contained occult malignant cells in 20% of NHL patients studied[7], we included lymphoma patients with a past history of marrow involvement even though the marrow as histopathologically normal at the time of peripheral stem cell harvest.

The patients had received multiple therapies over an extended period of time -- the HD patients had been diagnosed for a median of 66 months -- which could suggest that their disease was less sensitive to high dose therapy than patients who are treated with such therapy earlier in their disease course.   An actuarial 2-year progression free survival for these 40 patients is encouraging but the prolonged survival might be higher if patients were treated earlier in their disease course, at the first indication of refractoriness to conventional therapy.

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AUTOLOGOUS PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN SOLID TUMORS

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The use of peripheral blood stem cells (PBSC) offers the possibility of high-dose therapy followed by autologous rescue in patients with prior pelvic radiation where marrow cannot be harvested (1). Patients with tumors such as neuroblastoma, lung carcinoma and breast carcinoma where clinical or subclinical involvement of bone marrow with tumor is frequent would also be candidates for PBSC rescue assuming blood cells can be harvested free of detectable tumor. To date, 19 patients have had PBSC rescue for solid tumors including 17 published and two additional unpublished patients from our institution (2-6). The primary diagnoses of these patents have been breast cancer, small cell carcinoma of the lung and neuroblastoma (Table 1). In general, recovery time following PBSC has been the same or somewhat sooner than patients prepared in a similar fashion and rescued with marrow, although Korbling, et al did report "delayed or incomplete" engraftment in their two patients with sarcomas who had extensive pretreatment with alkylating agents (5).

PATIENTS AND METHODS

We have harvested peripheral blood stem cells in six patients with neuroblastoma and one patient with Wilms' tumor who had biopsy or aspirate evidence of microscopic and/or immunohistologic involvement of iliac crest bone marrow at the time of harvest (n = 5) or within 6 weeks prior to harvest (n = 1) (Table 2). The peripheral blood cell collections from the patients with neuroblastoma were examined for the presence of tumor cells by immunohistologic techniques with a sensitivity to detect 1 in 10^5 tumor cells (7). The

**Table 1. Reports of Autologous PBSC Rescue in Solid Tumors
Other Than Lymphoma or Hodgkins Disease**

Tumor	N	Preparative Regimen	Cells x 10^8 infused/kg	Recovery vis-a-vis marrow
Breast	4	P(125) C(120) TBI(11.12.5)		
			8 (median)	similar
Breast	2	P(125) C(120) E(900)		
Lung	4	B(900)		
			3.8 (mean)	similar
Lung	3	B(900) C(4000) E(500) P(100)		
PNET	1	M(120) TBI (12)	not given	delayed or
RMS	1	M(200)	not given	incomplete
NBLIV	3	C(150) E(3000) P(150)	7.4, 4.4., 2.8	faster
NBLIV	1	C(150) E(3000) P(150) B(300)	3.9	similar

P = cisplatin (total dose, mg/m^2 B = carmustine (total dose, mg/m^2)
C = cyclophosphamide (mg/kg total dose)   E = etoposide (total dose, mg/m^2)
TBI=total body irradiation (total dose, Gy) M = melphalan (total dose, mg/m^2)
NBL IV=neuroblastoma stage IV   RMS = rhabdomyosarcoma
PNET=primitive neuroectodermal tumor

**Table 2. Results of Peripheral Blood Stem Cell Collections
in Patients with Metastatic Tumor in the Bone Marrow**

UPN	Diagnosis	Age	Cells x 10^8/kg per collection mean ± sem	Tumor detection results number of collections			
				pos	neg	unk	total
788	NBL IV	3	1.73±0.22	2	3	0	5
834	NBL IV	23	0.55±0.05	0	8	0	8
841	NBL IV	4	0.48±0.10	0	3	3	6
N006	Wilms	10	1.38±0.22	0	4	0	4
881	NBL IV	13	0.55±0.11	0	6	0	6
N007	NBL III	3	1.45±0.20	0	2	2	4
	group mean±st dev		1.02±0.56 total	2	26	5	33

UPN = unique patient number, N = patient not transplanted, NBL = neuroblastoma,
stage at diagnosis listed, unk = unknown, test not done

collections from the patient with Wilms' tumor were evaluated for
cells showing immunoreactivity for cytokeratin and vimentin which
are expressed by the cells of this tumor (8). This patient had bilateral
iliac crest aspirates positive for tumor with a large pelvic mass that
did not extend into the iliac bones on CT scan.

Three of the patients were young children weighing between 13 and 15 Kg, demonstrating the safety of apheresis in this setting (6).

RESULTS

Of a total of 28 collections examined in six patients, only two collections from one patient contained small numbers of detectable tumor cells (8 and 1 per 10^5; Table 2). These collections were obtained 21 and 25 days after a bone marrow examination that contained 500 per 10^5 tumor cells on aspiration. A third collection taken 2 days after the last positive collection, was negative for tumor as were two collections obtained three weeks later, after a course of chemotherapy. None of the other neuroblastoma patients whose bone marrow aspirates had <10 tumor cells per 10^5 tumor cells and/or occasional residual tumor clumps on bone marrow biopsy had detectable tumor in the PBSC collections.

Four of these patients have been successfully rescued with PBSC following high dose chemotherapy, three with regimen II (total etoposide dose 3000 mg/m^2) and one with regimen III (total carmustine dose 300 mg/m^2; Table 3). We have not rescued any patients with PBSC who received regimen I, which utilizes high dose chemotherapy and total body irradiation, as this regimen is no longer in use at our institution due to fatal pulmonary toxicity with total doses of etoposide greater than 1500 mg/m^2. The time to recovery of blood counts in the three patients given regimen II and rescued with

Table 3. University of Minnesota BMT Program Preparative Regimens for Solid Tumors

Day	Regimen I	Regimen II	Regimen III
-7		C P E (1000)	
-6	TBI 8.5 Gy Single fraction	C P E (1000)	
-5	P E (250-750)	C P E (600-1000)	C P E (1000)
-4	P E (250-750)	C P E (600-1000)	B (150-225)
-3	P E (250-750)	C P E (600-1000)	B (150-225)
-2	C	rest	
-1	C	rest	
0	BMT	BMT	

C = cyclophosphamide 50 mg/kg IV over 2 hours
P = cisplatin 50 mg/m^2 as continuous infusion
B = carmustine (BCNU) mg/m^2/day), IV over 1 hour divided q 8 hours
E = etoposide (mg/m^2) as continuous infusion
Maximal tolerated daily etoposide dose = 500 mg/m^2 with TBI and 1000 mg/m^2 without TBI

**Table 4. Comparison of Recovery Times for Patients
Receiving Peripheral Blood Stem Cells or Marrow
Following Cyclophosphamide, Cisplatin,
and Etoposide (Regimen II)**

UPN	Etoposide total dose mg/m^2	Cells x10^8/kg	Days from PBSC/BM rescue to:			
			CFU-GM x10^4/kg	WBC> 1000/μl	ANC> 500/μl	Platelets> 150k/μl
638	1800	3.1	0.34	16	17	22
655	1800	1.5	0.58	25	27	28
745	2400	1.0	4.0	26	37	22
763	3000	1.5	0.82	23	24	28+
	BM (mean)	1.8	1.4	23	26	25
788	3000	7.4	6.7	14	19	19
834	3000	4.4	8.7	20	20	NA
841	3000	2.8	1.6	19	28	24
	PBSC (mean)	4.9	5.7	18	22	22

UPN = unique patient number
PBSC = peripheral blood stem cells, BM = bone marrow
ANC = absolute neutrophil count, NA = not available

PBSC is comparable and perhaps somewhat shorter than four patients who received bone marrow, although three of the four received reduced doses of etoposide, making a strict comparison not possible (Table 4).

DISCUSSION

Although it has not been definitively demonstrated to be necessary in humans, bone marrow specimens with tumor involvement are usually purged with chemotherapy, anti-tumor antibodies or a combination thereof prior to reinfusion. Tumor cells must circulate in the blood or lymphatics to metastasize. It is not clear that the circulation of tumor cells in the blood occurs on a continuous basis precluding the use of unpurged peripheral blood stem cells as a substitute for purged bone marrow in patients with solid tumors metastatic to the bone marrow. Large reviews have found concomitant blood and marrow involvement to occur in only 2% of cases of adult tumors with no descriptions in pediatric tumors (9).

Circulation tumor cells is usually and rarely described as a terminal event or following surgical manipulation of a primary tumor (10,11). By routine light microscopy, infrequent neuroblastoma cells were demonstrated in the blood in 3 of 53 patients with stage IV neuroblastoma with bone marrow involvement, seen at the University of Minnesota from 1960 to 1983 (12). Additional patients studied at

UCLA had evidence of circulating neuroblasts by immunohistology in five of 14 cases, 2 during progressive disease and 2 just prior to progressive disease (13). It is possible that peripheral blood stem cells and purged bone marrow, for that matter, may contain a small amount of contaminating tumor cells below the limits of detection of even the most sensitive assays. It is not known if "low-level contamination" (ie. less than 1 tumor cell in 10^5 cells) of bone marrow leads to tumor recurrence as most patients with solid tumors recur at sites of prior bulk disease suggesting residual tumor in the patient is far more critical than residual tumor in the bone marrow or peripheral blood stem cell infusion.

CONCLUSIONS

Peripheral blood stem cells offer an alternative to purged autologous bone marrow for patients with neuroblastoma and possibly other tumors with "low-level" metastasis to the bone marrow at the time of pheresis. Examination of the collected material for contaminating tumors cells should be undertaken prior to their use. Following additional pilot studies, a randomized comparison of peripheral blood stem cells to purged bone marrow in neuroblastoma and other diseases should be contemplated.

ACKNOWLEDGMENTS

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AUTOLOGOUS BLOOD STEM CELL TRANSPLANTATION

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INTRODUCTION

High dose chemotherapy with or without total body irradiation followed by autologous stem cell rescue is an increasingly popular treatment for hematologic malignancies and selected solid tumors. Using blood stem cells as an alternative to bone marrow stem cells in selected cases has been proposed. Potential advantages of the use of blood stem cells over bone marrow stem cells are the decreased likelihood of contamination with malignant cells, the avoidance of general anesthesia and the infusion of immuno competent cells which might hasten immunorecovery in the autologous setting. We are here reporting about the use of blood stem cell transplantation in the treatment of five patients with Hodgkin's disease and malignant lymphoma.

MATERIALS AND METHODS

Using the IBM 2997 cell separator and the Fenwal CS 3000 separator, we collected 8-9 leukaphereses from each patient over a three- to four-hour period each. Most of the procedures were performed using a Vas-cath central venous catheter (Gambro, Lincolnshire, Illinois) as access for both the withdrawal and return lines. Flow rates and centrifuge speeds were selected for optimum peripheral blood mononuclear cell collection. The resulting cell suspensions were adjusted to ten units per ml with heparin before further processing. Buffy coat was cryopreserved at the concentration of 10^7 nucleated cells/ml using 10% dimethylsulfoxide and 20% autologous plasma from which cryoprecipitate was previously removed

(1). Samples from each separate leukapheresis were analyzed for granulocyte macrophage colony units (CFU-GM) and burst forming units-erythroid (BFU-E) prior to freezing and after thawing (2). Blood samples were analyzed for lymphocyte surface markers in a standard way at 3-month intervals after autologous stem cell rescue (3). For every patient, autologous bone marrow was also harvested to serve as a backup for hematopoietic reconstitution.

PATIENTS

Table 1 describes the five patients who underwent this procedure. Four patients had Hodgkin's disease and one had non-Hodgkin's lymphoma of intermediate grade. Two patients had bone marrow involvement at diagnosis (#2 and #4) and one patient at time of relapse (#1). One patient had received extensive radiation to the pelvis.

Conditioning Treatment

The four patients with Hodgkin's received the CBV protocol consisting of Cytoxan 1.5 gram/m^2 on three consecutive days, BCNU 300 mg/m on two consecutive days and VP-16 100 mg/m^2 IV q 12 hours x 6. The patient with the non-Hodgkin's lymphoma received Busulfan 4 mg/kg p.o. on four consecutive days, Cytoxan 60 mg/kg IV on two consecutive days and VP-16 15 mg/kg IV.

RESULTS

Transfusion

The mean nucleated cell count infused per kilogram body weight was 7.3 x 10^8 (3.4-15.6 x 10^8), mean CFU-C count per kilogram: 7.8 x 10^3 (range: 4-12.1 x 10^3), mean BFU-E per kilogram: 21 x 10^3 (range: 2.4-33.6 x 10^3).

Engraftment

Three patients reached granulocyte counts of more than 500/microlitre and platelets of more than 20,000/microlitre in less than one month (12,20,16 days and 9,24,33 days). Two patients had incomplete engraftment by day 31 and day 36 and therefore received infusions of back up autologous bone marrow which led to hematologic reconstitution at days 24 and 87. The number of peripheral blood mononuclear cells infused for these two patients was 3.4 and 4.6 x 10 per kilogram body weight.

Table 1. PB Stem Cell Transplant Patient Population

Number	Age	Sex	Dx	Previous Treatment	Bone Marrow at Diagnosis	Bone Marrow at Transplant	Previous XRT	Resistant Disease at Time of BMT
#1	20	M	H.D.-N.S. (1983)	MOPP-ABVD Mantle XRT CCNU, Vp16, Mtx MOPP-ABVD Velban DHAP	-	+	+	Lung, lymphode
#2	46	M	N.H.L. int. grade (mixed nodular and diffuse small and large cell) (1986)	M-BACOD	+	- ?	-	Small bowel
#3	40	F	H.D.-M.C. (1980)	TNI + XRT lung MOPP-ABVD C.V.P.	-	-	+	Pleural effusion lymphnode
#4	29	M	H.D.-N.S. (1987)	MOPP ABVD	+	- ? Necrosis and fibrosis, inaspirable	-	Lymphnode
#5	31	F	H.D.-N.S. (1976)	MOPP Mantle XRT MOPP Inverted Y XRT	-	-	+	Lymphnode

Response

Four patients had a complete response. One patient had partial response of two months duration and died six months later in relapse. One patient with CR relapsed after two months and is now alive off of chemotherapy. One patient died four months post transplant in complete remission from an Ecoli sepsis while receiving corticosteroids for asthma. Two patients are alive and disease-free; the patient with non-Hodgkin's lymphoma at 17+ months and the patient with Hodgkin's disease at 7+ months.

Infectious Complications During the Acute Transplant Period

All patients developed fever of unknown origin. There was no sepsis or pneumonia. One patient developed a herpes zoster infection in the early post transplant period.

Prediction of Engraftment

There was a significant correlation with the number of nucleated cells infused per kilogram body weight with days to reach a granulocyte level of 500/microlitre and platelets of 20,000/microlitre ($p < .05$). There was no correlation between CFU-C and BFU-E and days of hemopoietic recovery.

Immune Recovery

A steep early increase in T-lymphocytes (T_3) and subsets (T_4 and T_8) was observed during the first 90 days followed by a decline and second increase from day 270 on. B_1-carrying cells increased after day 270.

DISCUSSION

It appears from the presented data that rescue with cryopreserved blood stem cells is feasible with a cell number of 5-7 x 10^8/kg body weight. These results are similar to the observation of Kessinger et al.(5). At this point it is unresolved what parameters predict for consistent engraftment. Korbling, Juttner and Reiffers did not find a correlation with the number of nucleated cells infused and rapidity of engraftment (6,7,8). Korbling and Juttner found a positive correlation with the number of GM-CFC infused, Kessinger and Reiffers did not. Timing of the cell collections may be important, too. Juttner et al. reported three patients whose blood stem cells were harvested very early in remission after induction for acute myeloid leukemia at which time an overshoot of circulating precursor cells was

noted. In all three patients prompt initial engraftment was followed by a fall in counts by day 16 and a secondary but incomplete rise (9).

At this point it appears that blood stem cell autografting is feasible and might be as effective as autografting with bone marrow stem cells. Possible advantages like faster hemopoietic and immune recovery and decreased contamination with tumor cells have not yet been established.

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TUMOR RESPONSE AFTER AUTOLOGOUS BLOOD STEM CELL TRANSPLANTATION IN LEUKEMIC PATIENTS

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INTRODUCTION

Autologous blood stem cell transplantation (ABSCT) has proved to reconstitute hematopoiesis after supralethal therapy and may be used as an alternative to autologous bone marrow transplantation (ABMT)(1,2). In leukemic patients, hematopoietic reconstitution is often more rapid after ABSCT than in ABMT patients (3). Another advantage of ABSCT over ABMT may be that peripheral blood stem cells are less contaminated by residual leukemic cells than bone marrow. This latter advantage is still hypothetical (4) and could be demonstrated by comparing the clinical results of ABSCT and ABMT in similar situations. In order to answer this question, we retrospectively reviewed the data concerning 55 patients who underwent ABSCT for acute nonlymphoblastic (ANLL=30) or lymphoblastic (ALL=25) leukemias in first or second complete remission during a four-year period (1984-1987).

MATERIALS AND METHODS

As described elsewhere (3), peripheral blood stem cells were collected by means of several leukaphereses performed during hematologic recovery after induction or consolidation chemotherapy. For ABSCT, a median number of 5.1 x 10^8/kg nucleated cells were infused (0.9 - 26.1) corresponding to a median number of 11.6 x 10^4/kg CFU-GM (0-155)(3).

ANLL in First CR

Twenty-one patients underwent ABSCT. One patient died early (infection) thus 20 patients were evaluable for response. The median age of patients was 31.5 years (8-50). Three patients had a preleukemic phase before overt leukemia and 17 patients had "de novo" ANLL. Nine patients were prepared for ABSCT with Busulfan (4 mg/kg/day, orally, 4 days) associated with Cyclophosphamide (50 mg/kg/day, IV, 4 days) (3 patients) or with Melphalan (140 mg/m^2, IV) (6 patients). Eleven other patients were prepared with Total Body Irradiation (TBI) (1000 - 1200 rads) either preceded by Cyclophosphamide (60 mg/kg/day, IV, 2 days) (8 patients), or associated with high dose Aracytine and Melphalan (3 patients) (TAM protocol). All patients were transplanted within 4 months following obtainment of CR.

ALL in First CR

Twelve adult patients (more than 20 years) underwent ABSCT, nine were evaluable. Two patients had Burkitt ALL. Before ABSCT all patients received TBI associated with either Cyclophosphamide (7 patients) or with the BEAM protocol (2 patients with Burkitt ALL). Four children underwent ABSCT for ALL in first CR because of initial bad prognostic factors such as hyperleukocytosis or Ph1 chromosome. They were prepared with TBI associated with Cyclophosphamide (2 patients) or high dose Aracytine (2 patients).

Acute Leukemias in Second CR

Eighteen patients (adults = 14, children = 4) were transplanted, sixteen patients were evaluable. Twelve patients were prepared with TBI associated with either Cyclophosphamide (7 patients) or with high dose Aracytine and Melphalan (TAM protocol) (5 patients). Four patients were prepared with Bulsulfan and Melphalan.

RESULTS

Haematopoietic Recovery

Results concerning hematopoietic recovery have previously been reported (3,5). Six of the 55 patients died early from infection or bleeding (2 patients who had incomplete engraftment), veno-occlusive disease (2 patients) or interstitial pneumonitis (2 patients). Forty-nine patients were thus evaluable for survival. Of the 20 ANLL patients transplanted in first CR, three patients with secondary leukemia relapsed 3 to 8 months after ABSCT. Of the 17 remaining patients,

eight had a relapse 4 to 13 months (median = 4.5 months) after ABSCT while nine other patients are in continuous complete remission (CCR) 3 to 41 months after ABSCT (median = 13 months). The estimated chance of surviving without disease for 24 months was 46%. The risk of relapse and survival were not influenced by the FAB subtype or the type of conditioning regimen (with or without TBI).

Six of the nine adult patients transplanted in first CR relapsed 2 to 15 months after ABSCT (median = 5 months) and three patients are still alive in CCR 13, 19 and 34 months after ABSCT. One of these patients had Burkitt ALL with CNS involvement at presentation. Only one of the 4 children transplanted for ALL in first CR did not relapse (26 months follow-up). Among the sixteen patients transplanted in second CR, twelve had leukemic relapse within one year following ABSCT (median = 4 months) and only four patients are in CCR 6, 14, 22 and 33 months after ABSCT. In one of these latter patients, the duration of second CR was longer then the duration of the first remission.

DISCUSSION

These results confirm that ABSCT may be safely performed in leukemic patients but does not allow us to draw any definitive conclusion on the place in the treatment of acute leukemias. In ANLL or ALL patients transplanted in second CR, the results were poor (4 of the 16 patients transplanted survived) but did not differ significantly to that observed after autologous purged or unpurged bone marrow transplantation (8). The results observed for ALL in first CR are difficult to interpret since most patients were assigned to receive ABSCT because of initial bad risk factors such as Ph1 chromosome (2 patients), Burkitt ALL (2 patients) or hyperleukocytosis (4 patients).

A significant number of patients of this present series was transplanted for ANLL in first CR. Nine of the 17 patients with "de novo" ANLL did not relapse and are alive 3 to 41 months after transplantation, with a median follow-up of 13 months. Korbling et al reported that eleven of the 16 ANLL patients who underwent ABSCT in first remission did not relapse but their follow-up was very short (5 months).

Since these overall results did not differ significantly from that usually observed after ABMT (6), we therefore conclude that it remains unknown if the results of ABSCT are inferior, equivalent or superior to those of ABMT for leukemic patients. Prospective studies are needed to answer this question in patients in first remission.

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VERY RAPID AND COMPLETE HAEMOPOIETIC RECONSTITUTION FOLLOWING MYELOABLATIVE TREATMENTS: The Role of Circulating Stem Cells Harvested after High-Dose Cyclophosphamide and GM-CSF

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High dose chemo-radiotherapy followed by autologous bone marrow transplantation is an effective therapeutic approach for patients with haematological malignancies and selected solid tumors (1). Although substantial progress in supportive measures has rendered the procedure much safer in recent years, morbidity and mortality are still high during the two to three week period preceding marrow recovery. Shortening the phase of marrow suppression that follows high-dose chemotherapy and radiation will substantially reduce the risks of bleeding and infections, and improve the therapeutic index of the procedure. This goal has been recently achieved by several research groups (for a review, see 2) using peripheral blood mononuclear cells as the sole source of stem cells. While the majority of patients experienced a very rapid myeloid recovery, others showed incomplete and/or temporary engrafment (3,4).

We have recently reported (5) that when autologous bone marrow cells are supplemented with a small number of peripheral blood nucleated cells collected after high-dose cyclophosphamide (7 g/m^2 I.V.), the haematopoietic recovery following a fully myeloablative chemo-radiotherapeutic regimen is significantly accelerated and therapy-related toxicity reduced. We report here that the administration of recombinant human colony-stimulating factor (GM-CSF) further increases the number of progenitor cells appearing in the peripheral blood after cyclophosphamide treatment. Upon use of these

circulating stem cells in combination with marrow stem cells, complete haematological recovery is prompt.

PATIENTS AND METHODS
Patients

Control subjects were 14 previously untreated, consecutive patients (5 males, 9 females, mean age 41.5, range 22-55) with high-grade, poor prognosis non-Hodgkin's lymphoma (4 patients), inflammatory breast cancer (7 patients) or small cell lung carcinoma (3 patients) GM-CSF was administered to 5 patients (1 male, 4 females, mean age 45, range 39-53) with high-grade non-Hodgkin's lymphoma (1 patient) or inflammatory breast cancer (4 patients).

Treatment

All patients received high-dose chemo-radiotherapy consisting of the sequential administration of (a) cyclophosphamide (7 g/m^2) on day 0; (b) vincristine (1.4 mg/m^2), methotrexate (8 g/m^2) plus leucovorin rescue, cisplatin (120 mg/m^2) on days +21-25); (c) total body irradiation (12.5 Gy total) plus melphalan ($120\text{-}180 \text{ mg/m}^2$) on days +42-45 or melphalan alone (200 mg/m^2) for inflammatory breast cancer patients. Bone marrow with peripheral blood stem cells was returned the day after melphalan infusion. GM-CSF (Sandoz Pharmaceuticals, Basel, Switzerland) was given as a continuous infusion via a central catheter at 8 μg/kg/day for 14 days (3 patients) or 10 days (2 patients) starting from 24 h after cyclophosphamide infusion (7 g/m^2). Bone marrow harvesting, leukapheresis, cryopreservation of stem cells and patient care following high-dose chemo- and chemo-radiotherapy has already been described (5).

CFU Assay

Blood samples were obtained prior to treatment and every 2-3 days following cyclophosphamide infusion. CFU-GM technique has been reported (6).

RESULTS
Haematological Toxicity of High-dose Cyclophosphamide and Effect of GM-CSF

As already reported by others (7), autologous bone marrow rescue is unnecessary after cyclophosphamide, even at this very high dosage (7 g/m^2 I.V.). Haematological toxicity (see Figure 1) was predictable
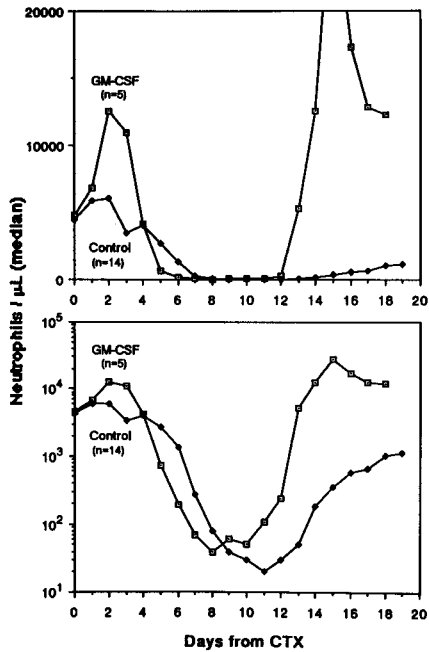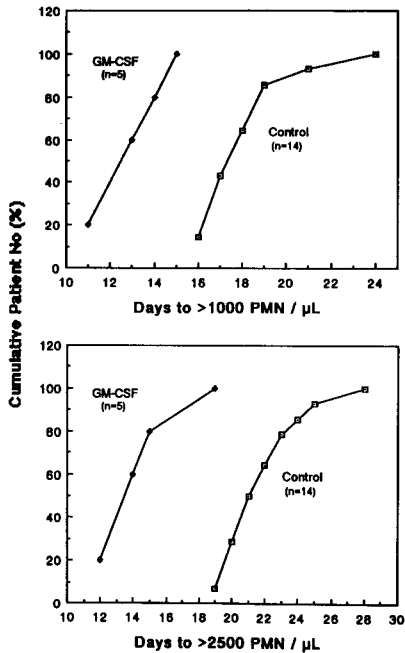
Figure 1. Granulocyte numbers (median values) following high-dose cyclophosphamide (7 g/m^2 on day 0) in 14 control patients (■) and in 5 patients treated with GM-CSF (♦) (8 μg/kg/day from day 1 to day 10 or to day 14). Upper panel: linear scale; lower panel: log scale.

and uniform, with severe leukopenia (< 500 neutrophils/μL) occurring in all patients by the eighth day. Neutrophils reached more than 1000/μL in 17-24 days (median 18) and more than 2500/μL in 19-28 days (median 21.5). In the majority of patients platelet counts fell below 50,000/μL, but severe thrombocytopenia (<20,000 /μL) requiring platelet transfusions developed in only 3 cases (19%).

The administration of GM-CSF starting 24 h from the end of cyclophosphamide infusion and continuing for 10 days (two patients) or 14 days (three patients), resulted in a dramatic change of the recovery pattern (Figure 1). As compared with controls, neutrophil counts rise above the starting level for 2 to 3 days although the nadir occurred 1-2 days earlier; the recovery phase was associated with marked leukocytosis (up to 25,000 leukocytes/ μL) that gradually resolved over 3-4 days after GM-CSF discontinuation. Neutrophils reached more than 1000/μL in 11-15 days (median 13) (p< 0.001, Mann-Whitney U test) and more than 2500/μL in 12-19 days (median 14) (p< 0.001) (Figure 2). No differences in platelet counts or haemoglobin levels were documented between GM-CSF-treated patients and controls.

Figure 2. Distribution of neutropenia duration measured from the end of treatment (cyclophosphamide, 7 g/m^2 on day 0) in 14 control patients (■) and in 5 patients treated with GM-CSF (♦). Curves represent the cumulative proportions of patients reaching, within the indicated interval, more than 1000 neutrophils (upper panel) and more than 2500 neutrophils, respectively (lower panel).

Effect of GM-CSF on Circulating Haemopoietic Progenitor Cells Following Therapy with Cyclophosphamide

Under the experimental conditions adopted (6), the number of circulating colony forming units in unperturbed conditions is approximately 40 CFU-GM/10^6 mononuclear cells and 40-60 CFU GM/ml of peripheral blood. Following cyclophosphamide treatment, we observed an approximately 100-fold higher peak value in both the proportion (CFU-GM/mononuclear cells) and the absolute number (CFU-GM/ml of peripheral blood) of colony forming units (Figure 3). Upon addition of GM-CSF (8 μg/kg/day from day 1 to day 10 following cyclophosphamide), there was an additional 3-fold increase in CFU-GM concentration and 2.5-fold increase in absolute number of CFU-GM. Of note, these peak values obtained after cyclophosphamide persisted several days both in controlled and GM-CSF treated patients. Thus, as compared with normal subjects, the total number of CFU-GM circulating in the peripheral blood during
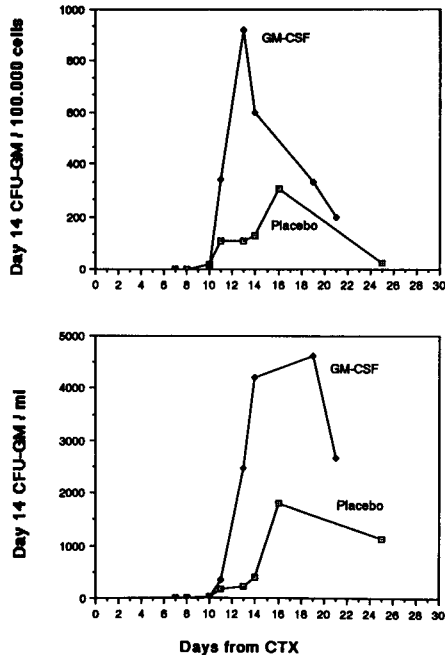
Figure 3. Effect of GM-CSF (♦) or placebo infusion (■) on circulating CFU-GM proportion (upper panel) and blood concentration (lower panel). GM-CSF was continuously infused for 10 days, starting on day 1 following cyclophosphamide $(7g/m^2)$.

the days of stem cell expansion (area under the curve) was approximately 30-fold higher following cyclophosphamide and 90-fold higher following cyclophosphamide plus GM-CSF. A further increase can be anticipated following optimal length of GM-CSF infusion.

Effect of Circulating Stem Cells Reinfusion on Haematopoietic Reconstitution After High-dose Chemo-radiotherapy and Autologous Bone Marrow Transplantation

We have recently reported (5) that autografting of peripheral blood mononuclear cells in combination with marrow-derived stem cells, results in a very rapid haematological recovery involving the myeloid and, to a lesser extent, the megakaryocytic and erythroid lineages. As a part of a study to assess the ability of recombinant GM-CSF to
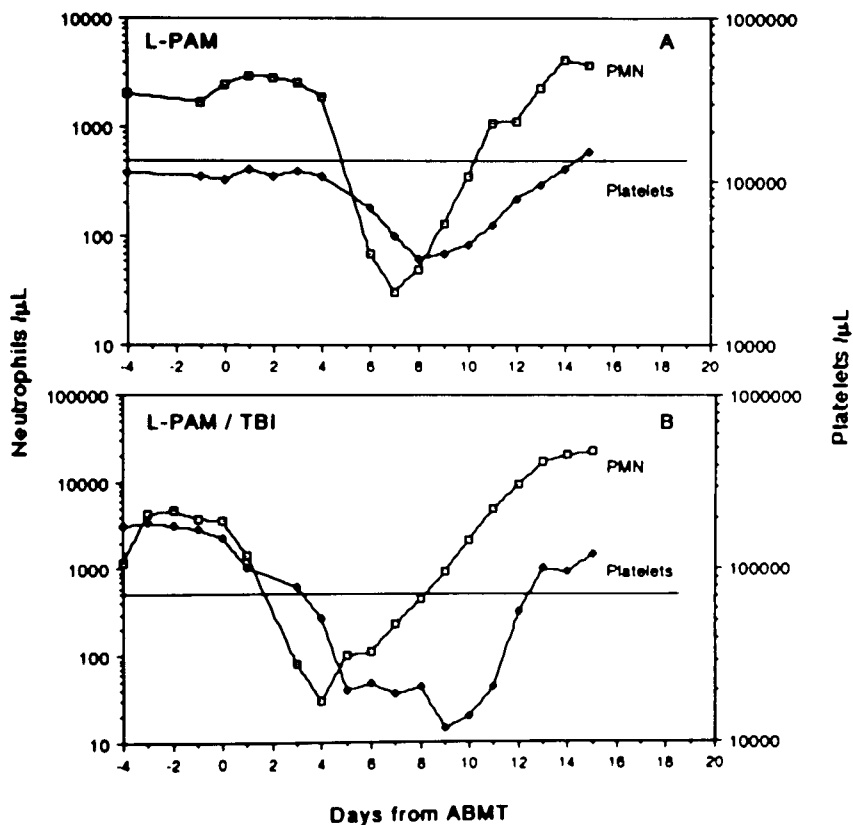
Figure 4. Granulocyte (■) and platelet (♦) counts in two patients following myeloablative treatment and autografting of bone marrow plus peripheral blood stem cells. Patient A received melphalan alone (200 mg/m^2); patient B total body irradiation (12.5 Gy, fractionated over 3 days) plus melphalan (180 mg/m^2). Circulating stem cells were harvested twice for each patient, following prior high-dose cyclophosphamide and GM-CSF infusion (see Methods).

reduce severe myelosuppression following high-dose single-agent chemotherapy, we observed a dramatic increase in white cell counts (Figure 1) and in circulating haematopoietic progenitors (Figure 3). These circulating precursors have been harvested and infused, in combination with bone marrow cells, in two patients following myeloablative regimens. The results are shown in Figure 4. Patient B had high-grade non-Hodgkin's lymphoma and received 4.7x10^8/kg bone marrow cells plus 8.8x10^8/kg peripheral blood mononuclear cells 24h after completion of high-dose chemotherapy (12.5 Gy fractionated total body irradiation and 160 mg/m^2 melphalan I.V.). On day 3 the granulocyte count fell below 500/μL. Starting from day 5, the patient showed a steady increase in white cell counts, reaching >500 neutrophils/μL on day 8, >1000/μL on day 10 and more than

10,000/μL on day 12. On a log scale (see Figure 4), daily counts lie on a straight line, as expected for an exponential increase of the maturing granulocytes. The patient's platelet count fell below 20,000/μL on day 9, requiring transfusion of one unit of platelets. Platelet counts increased to >50,000/μL on day 12 and to >100,000/μL on day 13, respectively. Patient A received high-dose melphalan (200 mg/m^2) followed by infusion of 2x10^8/kg bone marrow cells and 1.8x10^8/kg circulating mononuclear cells. Although she received a lower number of bone marrow and blood derived mononuclear cells, haematological toxicity followed a similar pattern. On day 6, the granulocyte count fell to less than 500/μL, remaining below this threshold for 5 days only. Platelet count never fell below 30,000/μL and no transfusions were required. Platelet count increased to 79,000/μL on day 12 and to 120,000/μL on day 14 without transfusion.

DISCUSSION

It is well known that a positive correlation exists between dose and response to treatment with antitumor agents (8,9). However, the role of high-dose chemotherapy or chemo-radiotherapy, particularly in the treatment of solid tumors, remains controversial, mainly as a consequence of the substantial morbidity and mortality that follows high-dose regimens.

A major cause of treatment-related complications and death is duration and severity of myelosuppression. The use of autologous bone marrow transplantation overcomes irreversible myelotoxicity, but patients have a substantial risk of bleeding and infection during the 2 to 4 weeks preceding marrow recovery. The ability to accelerate haematopoietic recovery after bone marrow transplantation is expected to substantially increase the therapeutic index of the procedure.

Recently, several groups (2) have reported that very rapid myeloid recovery can be achieved upon autografting peripheral blood mononuclear cells harvested during the phase of circulating stem cells expansion following chemotherapy. Unfortunately, the ability of circulating stem cells to sustain a life-long haemopoiesis is presently unknown (2). This prompted us to combine the use of marrow-derived and blood-derived stem cells as a means to avoid the risks of incomplete and/or temporary engraftment. As previously reported (5), when autologous bone marrow cells are supplemented with a small number of peripheral blood nucleated cells collected after high-dose cyclophosphamide, all patients experienced a very rapid and complete marrow recovery.

More recently, GM-CSF has been used to accelerate haematopoietic recovery in 19 patients undergoing high-dose combination chemotherapy (cyclophosphamide, cisplatin and

carmustine (10)). Leukocyte and granulocyte counts were significantly higher at the end of GM-CSF infusion (day 15), whereas the time required to achieve a leukocyte count of 1000/μL and a granulocyte count of 500/μL did not differ significantly. These observations suggest that, besides accelerating *in vitro* proliferation and differentiation of marrow stem cells, GM-CSF probably has important *in vivo* effects on marrow release and distribution of leukocytes and granulocytes. Moreover, no effect on platelet counts was observed.

As a part of a study on the ability of GM-CSF to accelerate haematopoietic recovery following high-dose single-agent chemotherapy, we have determined the effects of this growth factor on the number of peripheral blood progenitor cells. As compared with control patients treated in a similar manner, GM-CSF infusion following high-dose cyclophosphamide treatment, resulted in a three-fold increase in CFU-GM. Similar results have been recently reported by Socinski et al (11), who documented in 3 patients a dramatic increase in the number of circulating CFU-GM when GM-CSF was administered following combination chemotherapy. Circulating mononuclear cells have been harvested twice by continuous flow centrifugation during the rebound period (between day 14 and 17) following cyclophosphamide, and infused in combination with bone marrow cells. Although both patients received fully myeloablative regimens, including total body irradiation in one case, they experienced an exceedingly rapid haematopoietic recovery, involving both granulocytes and platelets. The effect on erythropoiesis is somewhat more difficult to assess due to the frequency of flebotomy for laboratory monitoring during the immediate post-transplant period.

In the present study, we have not addressed the issue of whether the very rapid recovery was simply due to a 'dose' effect or to qualitative differences between blood-derived and marrow-derived stem cells. Experiments in progress suggest that the observed effect is possibly the result of infusing large doses of committed precursors at different developmental stages, generating mature cells *in vivo* quicker than the more primitive bone marrow stem cells.

Whatever the explanation, we confirm that manipulations of the haematopoietic system with appropriate combinations of drugs and growth factors, can shorten the period of marrow failure following high-dose chemo-radiotherapy. The use of GM-CSF after transplantation of marrow-derived plus blood-derived stem cells could further shorten duration and severity of myelosuppression.

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Discussion 1 - Session VI
(Peripheral Blood Stem Cells)

Dr. Dicke: I want to ask both speakers and especially Dr. Socinski. I saw a discrepancy of the number of GM-CFCs between the Omaha and the Adelaide results. Dr. To, on your slide, it was 78 x 10^4 GM-CFCs /kg b.w. vs. Anne Kessinger at around 8 x 10^4. If we now take Dr. Socinski's stimulation into account of the GM-CSF -- if we assume that an 8 x 10^4 GM-CFC is necessary -- then you only need to collect 700cc's of peripheral blood vs. 7 liters. Also, did you take into account the plating efficiency of the two GM-CFC assays?

Dr. To: I think the question of measuring GM-CFC in blood is an important issue. I think all the groups doing it will realize that because if we do not standardize re-agents, the plating numbers will be significantly different. So I can't be sure that the 78 are directly comparable to the 8. That is less important an issue than the actual rate of recovery. As clinicians looking after patients, I think the rate of recovery is what we are interested in.

Dr. Dicke: I do not understand it. When you both collect in steady state -- that there is a 2-fold difference in number of cells collected where the technology, which has the lowest cell yield, has the highest GM-CFC recovery.

Dr. To: Maybe I did not explain it clearly, but it said on the slide that in Adelaide the collection is done during time of recovery. The level is very high, 20 to 100 times higher than the steady state level.

Dr. Dicke: Great, that explains it.

Dr. Socinski: We do not have any collection data yet, we have noted the absolute number changes. I think the important thing is that there may be a potentially drastic reduction in the amount of time that a patient has to undergo apheresis.

Dr. Dicke: What I really liked in your slide was that you expressed it /ml, peripheral blood. First of all you can see the difference and you can calculate the number of CFCs necessary for engraftment.

Dr. To: I think I forgot to mention that in patients receiving CM-GSF following the chemotherapy, if you compare their peak level with their base line level, it is approximately 65-fold greater at that time. So therefore, that may allow for a drastic reduction, compared to the steady state which is a long time of apheresis.

Dr. Spitzer: Could I ask Dr. To, if you analyzed your GM data a little bit differently and actually determined if there is a direct relationship between the GM-CFC number infused and the rapidity of white cell recovery rather than these kind of two cut-off points which don't tell us much whether GM can predict for recovery -- except if you collect super blood you do O.K. If you collect terrible blood, you do bad.

Dr. To: I think I have been capable in saying that the number of CFU-GM is not the only criteria and there is a frame of time that is also important. I have not finished the analysis and that is why I have not shown the data. It is important, for the time, to reach a 150 platelets and I am quite sure when I finish my analysis -- when I go back -- it will have a correlation between a GM dose. But what is important to realize is that they all have rapid recovery, even the lower GM-CFC dose have rapid recovery but the problem is that they do not reach normal levels.

Dr. Kessinger: I have a question for Dr. Socinski. You gave GM-CSF for 7 days. Have you any information regarding what time it took to achieve maximal circulating CFU-GM values?

Dr. Socinski: We do not have good data on that. Basically, the points that we looked at were pre-, and on-the-end, and 7 days later. There were a selective number of patients were the increase occurred very quickly within 2 or 3 days and lasted. There were a few patients who remained very stable during the 7 days after GM-CSF. Most patients went down. Unfortunately, we could not follow that out any further than a week.

Dr. Kessinger: I mean -- during the first week, not after. How long did it take you to achieve the maximal effect?

Dr. Socinski: The maximal effect that we saw in the majority

of patients occurred on day 7. Some people achieved that effect and remained stable after 3 days, meaning that from day 3 to day 7, they did not change. Others took 7 days to increase.

Dr. Sharp: One of our concerns in collecting peripheral cells in the period of rebound is that you may, relatively, enrich for progenitors and so you may give a relatively fewer number of primitive cells. I would like to ask Dr. To, therefore, if he could tell us about the hematological status of his patients about 1 year or 2 years out after transplantation. Are they hematologically normal later on?

Dr. To: I certainly agree with you and in fact the clinical data would suggest that there is a relative enrichment of the progenitor cells compared to the pluripotent stem cells. That would explain why we need to give a much higher dose of CFU-CM when we use peripheral blood compared to bone marrow. We have followed patients out more than 1 year and a lot of other groups have done that as well, and once they reach a normal, they stay normal and do not have a depletion.

Dr. Gorin: Dr. Kessinger asked me to report very shortly on our experience with Folinic acid to expand the CFC-GM pool. I know it is very curious to speak of acid after GM-CSF which is the drug to date. But I would like to say shortly what happened. We started doing peripheral blood stem cell autografting in patients with acute leukemia, collecting peripheral blood stem cells immediately after induction.
As some people know here, in the audience, we were unlucky. The first 3 patients relapsed very soon. So we decided that maybe there was not enough consolidation and we wanted to collect the stem cells later on, after 1 or 2 courses. But if you do that, as mentioned by the speakers, the CFU-GM yield goes down. So we took 5 patients, 4 with AML and 1 with ALL, and we decided to collect the peripheral blood stem cells either after induction or after consolidation. The first set of leukepheresis were done without any acid and the second was with acid. I think that it is interesting to see that if you give acid starting on day 15, after the first day of chemotherapy -- it is a conventional regimen, in fact it is 3+7, or 9+3. The increase of the recovery of CFU-GM is 11-fold. The increase in BFU-E is 3.4-fold. At the moment we have no correlation in terms of engraftment as compared to the CFU-GM recovery but we know that acid increases very much the yield CFU-GM. I think it compares favorably at least for this part with GM-CSF. However, there would be one question I wanted to ask to both of you. Did you study, by long-term cultures, the primitive stem cells?

Dr. Kessinger: Karen, can you answer that? Were any long term cultures done after the GM-CSF? The answer is no, I think.

Dr. Gale: This is for the first speaker. I would like to suggest an alternative hypothesis to explain your data which is perhaps simple minded. But I assume your intention was to try to collect the same number of stem cells or process the same amount of blood from every donor. So if you have 3 people who give you a low number and 10 who give you a high number, the one explanation is that the 3 people have worse bone marrow than the other ten. Now the early phase of recovery after conditioning is probably dictated by the cells you put in. But the late phase of recovery probably has to do with the recovery of endogenous hematopoiesis. Certainly the doses of drugs and radiation we give do not abrogate all stem cells. We know for example after Chernobyl, that you can recover after 12 Gy of whole body irradiation without a transplant. So I would suggest that your observations are a self-fulfilling hypothesis. It is people who give you poor numbers of stem cells have poor bone marrow and poor stem cells. The fact that you did not see a correlation in the early period suggests that, in fact, the dose of CFU-GMs is unimportant and (the) reason that you saw a late effect is because those 3 people have poor bone marrow. Is that possible?

Dr. To: I think the question of endogenous recovery is an issue which we always have to consider in all types of bone marrow transplantation. At the myeloablative therapy, we use TBI and cyclo in three patients and use Bu Cy in the standard dose for the other 10.

Dr. Gale: I do not want you to misunderstand me. I am not questioning whether the early recovery after an autotransplant comes from the infused cells, but one does not destroy a patients own bone marrow no matter what dose of chemotherapy you give. So the later recovery must be at least part of his own hematopoiesis. There is no reason why those cells should not recover.

Dr. To: If the late recovery is solely a function of endogenous recovery I would expect the same type pattern of recovery no matter what CFU-GM dose I gave.

Dr. Gorin: A question for Dr. Sharp. I would like to know if you did long-term cultures peripheral blood stem cells obtained from patients with leukemia? We tried that but we never saw any leukemic cells. Did you try?

Dr. Sharp: No, we have done essentially nothing with leukemia and we do not really see a lot of leukemia patients. So we have no information.

Dr. Kessinger: If there are no other questions then we will move right along to Dr. Korbling from Heidelberg.

Dr. Gorin: A question for you, Martin. Your first or second slide, I saw it 2 or 3 months before. I think it is very interesting, but I would like you to comment on that. In your first or second slide, you show that after cyclophosphamide you have an increase in CFU–GM, and you say that "in some model" there is no modification in the pluripotent stem cell level. Can you comment on the model?

Dr. Korbling: That is a mathematical model and it is based on dog data, on CFU-GM, and on the calculation of the marrow cellularity. This is a design which goes back more than 10 years and it shows that from those calculations the pluripotent stem cell pool is unharmed.

Dr. Goldstone: I would like to ask you, Dr. Korbling, about the AML first remission patients who were autografted with peripheral blood stem cells. You described 18. First of all, were they 18 consecutive patients? Were there any in whom it was impossible to get sufficient stem cells and what happened to those patients. Secondly -- I am not clear -- after which course of chemotherapy did these people get their stem cells harvested? If it was always after the induction course with no consolidation, might that account for what might be a trend toward rather poor results?

Dr. Korbling: Most patients had induction treatment with what we call in Germany, double TAD regimen. The consolidation treatment consisted of a third cycle of TAD. We usually harvested stem cells after one consolidation course. You are right. In 1 patient, we have a very poor CFU-GM yield but this patient got, as consolidation treatment, the HAM treatment and that is very toxic and that wipes out probably almost all cells.

Dr. Goldstone: Were the historical control group who got bone marrow, were they getting more chemotherapy than those 3 courses before they got their autograft?

Dr. Korbling: No, these patients come all from the same national protocol. They also got TAD, but some of those patients, the control patients got only one cycle of TAD, and not 2 TAD because these patients were grafted some years ago. That is why.

Dr. Goldstone: Was there a difference in interval between onset of CR and transplant between the marrow group and the peripheral stem cell group?

Dr. Korbling: No, the blood stem cell transplantation was done even earlier than the bone marrow transplantations. That may explain the rather unfavorable data.

Dr. Dicke: I have one question for Dr. Gianni. First of all, I think we have to congratulate him with this excellent piece of work. This is the first time that I see a real advantage of the combination peripheral blood cells and bone marrow cells over bone marrow cells alone. I think that the reduction of hospitalization time and the absence of myelosuppression is impressive. Now the question I have for you is, when you collect the bone marrow, what is the cell yield of the bone marrow cells in the non-GM-CSF treated and in the GM-CSF treated patients?

Dr. Gianni: Dr. Socinski presented data that there is a very high increase in the peripheral blood circulating stem cells but a steady state as far as the bone marrow is concerned. So, we are almost in the same situation.

Dr. Dicke: The number of GM-CFCs in the bone marrow?

Dr. Gianni: More in the peripheral blood than in the bone marrow.

Dr. Burnett: Martin and also to Dr. To, you both have patients that did rather poorly in the AML groups. Is there any evidence at all to suggest that people who give poorly are the ones who relapse?

Dr. Korbling: From our experience we do not have that evidence.

Dr. To: The first part of our studies, we had 20 patients collected and observed without maintenance and in those we looked at the cell dose and the time to relapse. There is no correlation between clinical outcome and yield of collection.

Dr. Korbling: I think it is interesting to follow some of the white cell and platelet counts in those patients who have poor prognosis and I think -- as you might know -- the platelet count goes down. In some patients where we got low counts and low cell numbers, they nicely reconstitute for the first 2 and 2 1/2 weeks but then they go over into an oscillation of the peripheral platelet count. That is probably due to an insufficient number of stem cells transfused. That is my explanation.

Dr. Goldstone: I would like to address a comment to Dr. Kessinger and ask her a question. In relation to lymphoma patients

with previous bone marrow involvement, the AMBT data -- for what is worth -- shows that for intermediate and high grade NHL and for Hodgkin's disease, patients who have had previously marrow involvement but do not have it at the time of the autograft, actually are multivariant analysis, do no worse or no better than anybody else. So I would not agree that that was specifically an indication to take peripheral blood stem cells. My question is specifically about those patients with lymphoma who had marrow involvement at the time of the stem cell autograft. It was not clear from the data how that particular group were faring and how many of those you appeared to be giving a significant disease-free interval.

Dr. Kessinger: The only way I could answer your question is to guess. It is the best thing for me to do is to write you. I do not have the data at hand.

Dr. Gorin: My question is for Anne Kessinger. If I did not miss the slide, your median number of CFU-GM/kg administered is $6 \times 10^{4\cdot}$ Is that right?

Dr. Kessinger: That is correct.

Dr. Gorin: In the presentation of Dr. To, he showed us a relationship of the kinetics in recovery with a cut-off point at 5×10^5 CFU-GM/kg and I would confirm that because in our own institution we use 1×10^5. So I would like to have your comment on this discrepancy. You believe that the dose could be lower because your patients have different diagnoses?

Dr. Kessinger: It is my understanding that Dr. To's CFU-GM data is fresh data on the fresh product. Our data is on frozen and thawed product, number one. Number two, it is not clear to me that a biologic assay like CFU-C is reproducible from one center to another. We use an entirely different culture technique than he does and so I am not clear on how we can compare our data to his. Dr. To collects peripheral stem cells during chemotherapy overshoot. Our patients are heavily pretreated and these patients will not overshoot. We pay little attention to the number of CFU-Cs that we finally collect and we emphasize the number of mononuclear cells collected much the same way as when bone marrow harvests are done, the number of cells collected is what is emphasized. I am sure that explains why his recovery data is quicker than ours, because he collects more progenitors than we do. But my hope is that we collect the same number of pluripotent stem cells as he does, or perhaps more. But heaven knows, I do not know how to prove that. We just have not had that much trouble, as long as we got 7×10^8/kg mononuclear cells.

Dr. Philip: I have one question for Dr. Bostrum. I want to know what you call the minimal marrow involvement in your 4 patients with neuroblastoma. They were biopsy minimal involvement or aspirate.

Dr. Bostrum: Two of the patients had a biopsy minimal involvement and 2 had aspirates and the specimens had less than 10 per 10^5 tumor cells.

Dr. Philip: O.K. Just to comment, it is a very common situation to have minimal residual disease and you know that the majority of these patients got a normal bag of marrow. Your data is interesting but I think the most interesting thing would be to try this kind of collection in highly involved patients with more than 10% at aspirate. This one is the one that we cannot cure with bone marrow transplantation.

Dr. Bostrum: The one patient that did have high involvement, at least in 2 of 5 collections did have small numbers of tumor cells detectable.

Dr. Philip: O.K. that is important information.

Dr. August: We always worry about the possibility that as tumor involves the marrow, hematopoietic stem cells and hematopoietic cells are reduced in number to the point where even if you could purge marrow of the tumor cells, you would not have enough hematopoietic stem cells left over to affect marrow engraftment successfully. Is there any evidence that when the marrow is involved with tumor, the stem cells are displaced preferentially in the peripheral blood at any level of contamination or any level of tumor involvement, low or high?

Dr. Kessinger: No.

SESSION VII - SUPPORTIVE CARE

TREATMENT OF CYTOMEGALOVIRUS PNEUMONIA IN MARROW TRANSPLANT PATIENTS WITH GANCICLOVIR AND INTRAVENOUS CYTOMEGALOVIRUS IMMUNOGLOBULIN

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INTRODUCTION

Cytomegalovirus (CMV) pneumonia occurs in approximately 15% of allogeneic marrow transplant recipients and has a case fatality rate of 85% (1). Although CMV pneumonia occurs less frequently in autologous transplant patients, the case fatality is the same as in allogeneic patients (2). Previous therapeutic trials of antiviral agents, including ganciclovir (GCV) (3,4) and foscarnet (5), which have proven *in vitro* and *in vivo* activity against CMV, have failed to improve survival in marrow transplant patients with CMV pneumonia. Although Blacklock *et al* reported survival in 9 of 18 or 50% of marrow transplant patients with CMV pneumonia treated with intravenous cytomegalovirus immunoglobulin (CMV-IG) (6), a second study using similar doses of a different intravenous CMV-IG reported survival in only 3 of 14 patients (7). The poor clinical results of previous clinical trials led us to treat 25 allogeneic marrow transplant patients with proven CMV pneumonia with the combination of GCV and intravenous CMV-IG in an uncontrolled trial.

METHODS

All patients were allogeneic marrow recipients with interstitial infiltrates on chest radiograph and CMV virus isolate from lung tissue or broncholaveolar lavage fluid. Patients dependent on mechanical ventilation prior to diagnostic procedure, having a serum creatinine of 2 mg/dl or greater or a second pulmonary pathogen were excluded from the study.

During induction, GCV (Burroughs Wellcome Co., Research Triangle Park, NC and Syntex Research, Palo Alto, CA) was given as a one hour infusion at the dosage of 2.5 mg/kg every 8 hours for the first 14 days. CMV-IG (CMV IGIV, pH 4.25; Cutter Laboratories, Berkeley, CA) was given at the dosage of 400 mg/kg on days 1, 2, 7 and at 200 mg/kg on day 14. At the end of 14 days those patients who were asymptomatic and able to be discharge from the hospital received no further treatment. Patients who were stable or improved but not asymptomatic after 14 days of treatment were given maintenance treatment, which was GCV at 5 mg/kg every 24 hours on days 14-28 and CMV-IG at 200 mg/kg on day 21. Patients who had clinically deteriorated during the first two weeks of treatment were continued on induction doses of GCV and weekly CMV-IG at the dosage of 200 mg/kg until improvement or death. Urine, blood and throat specimens were cultured 3 times a week.

RESULTS

Clinical Outcome

Pneumonia onset, defined as the first day an interstitial infiltrate was recognized on chest radiograph, occurred a median of 60 days (range 44-118 days) after transplant and treatment was started a median of 3 days (range 1-10) later. Patients received a median of 14 days (range 3-18) of induction therapy and 8 patients continued on maintenance therapy for a median of 11 days (range 2-18). Twenty-one of the 25 patients were seropositive for antibody to CMV at the start of treatment. There were no differences in age, time of pneumonia onset, time to treatment or seropositivity between those patients diagnosed by open biopsy or lavage.

Thirteen of 25 patients survived the first episode of pneumonia. Six of those patients stopped treatment after 14 days of induction treatment, while 7 patients were given a median of 11 additional days (range 3-18) of maintenance therapy.

Four of the 13 survivors had proven or possible recurrent CMV pneumonia. Two of these patients had CMV isolated from lavage and lung autopsy specimens 11 and 89 days after treatment was stopped, respectively. Two other patients, both rehospitalized for brain abscesses, developed recurrent interstitial infiltrates 29 and 33 days after stopping therapy. Those patients died before the infiltrates could be diagnosed.

Twelve of the 25 patients died after a median of 12 days after treatment (range 3-18). When age, time of pneumonia onset, time to treatment, initial leukocyte and neutrophil counts, and diagnostic procedure was compared between survivors of the initial episode of pneumonia and those who died, the only significant difference was

age. The survivors were younger with a mean age of 25 years while those patients who died had a mean age of 37 years (p=0.02, Student's T-test).

Virology

Twenty-four of the 25 patients had CMV recovered from throat, urine or blood. Cultures became negative for virus after a median of 6 days of treatment. Among the 23 patients who received 96 hours or more of treatment, CMV was persistently isolated during treatment in 6 patients. Three of these patients survived the first episode of pneumonia. Of the 9 survivors who stopped excreting virus on treatment, 5 patients had virologic relapse 4 to 50 days after treatment ended and 3 of those had recurrent pneumonia. No recurrent viral excretion was documented in patients on maintenance therapy.

Side Effects

Seven of 16 or 44% of patients who were treated for at least 14 days had a 50% or greater decrease in neutrophil counts. Three patients had induction therapy stopped because neutrophil counts were less than $500/mm^3$ and 6 of 8 patients who continued on maintenance therapy developed neutropenia. Neutrophil counts returned to pretreatment levels after therapy was discontinued. No other toxicity was noted.

DISCUSSION

Treatment of CMV pneumonia with GCV and CMV-IG resulted in survival of the first episode of pneumonia in 13/25 (52%) patients. Survival was significantly better (p=0.001) than in nine previous trials of antiviral agents, done at the same institution in comparable patients with biopsy proven CMV pneumonia (3,4,7-13). Survival from the first episode of pneumonia in those previous trials which included therapy with GCV and CMV-IG as single agents, ranged from 10-22%.

Pneumonia recurred in as many as 31% of patients, and in view of the rapid virologic relapse after stopping treatment and the absence of both virologic and disease relapse on reduced doses of ganciclovir, maintenance therapy for all patients seems appropriate.

However, the increased incidence of neutropenia after 14 days of treatment may limit our ability to give maintenance therapy for extended periods of time.

CONCLUSION

The use of GCV and GCV-IG for treatment of CMV pneumonia in marrow transplant recipients resulted in a 52% survival from the first episode of pneumonia. This is significantly better than survival with other antiviral agents used at the same institution in similar patients. Further studies aimed at minimizing the problems of neutropenia and marrow toxicity are needed.

ACKNOWLEDGMENTS

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VIRAL INFECTION IN PATIENTS UNDERGOING AUTOLOGOUS BONE MARROW TRANSPLANTATION

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William H. Burns, George W. Santos, and Rein Saral

Viral infections are a major complication of bone marrow transplantation (BMT); in particular, herpes viruses may impose substantial morbidity and mortality following BMT. Detailed analysis of herpes virus infections after autologous BMT (ABMT) have recently been published and will be discussed. The role which acyclovir may play as possible prophylactic therapy and as treatment will be reviewed.

Patients undergoing BMT or induction therapy for leukemia who are seropositive for herpes simplex virus (HSV) are at high risk for reactivation of the virus (1,2). In a prospective, randomized study, the prophylactic use of intravenously administered acyclovir in patients undergoing allogeneic BMT completely suppressed reactivation of the virus during drug administration. There was no adverse effect on hematopoietic recovery nor increased toxicity to liver and kidneys with acyclovir administration(1). Following discontinuation of therapy, 7 of 10 patients treated with acyclovir had mild clinical infections or viral shedding, confirming that patients remained at high risk when prophylaxis was discontinued until reconstitution of an effective immune response against HSV was complete. Another study was performed using intravenous, followed by oral, acyclovir to provide extended prophylaxis of HSV (3). Results of this analysis showed that extended acyclovir prophylaxis significantly delayed the time to the first positive culture, however the total number of HSV recurrences was not significantly different between the two groups. Repeated therapeutic administration of acyclovir remains an alternative to extended prophylaxis for management of recurrent HSV

infections in patients undergoing BMT. Because of the similarities of patients who undergo ABMT and allogeneic BMT and similar reactivation rates in patients with leukemia undergoing cytoreductive therapy less intensive then regimens used in ABMT (2), we continue to routinely use prophylactic acyclovir after ABMT in seropositive patients to maximize antiviral efficacy and to minimize occurrence of resistant viral strains. Since HSV infections after the first month have not resulted in the emergence of resistant virus and resolve with or without acyclovir as patients become more immunocompetant, we have not initiated extended prophylactic therapy in patients undergoing BMT.

Our experience with cytomegalovirus (CMV) infection in 143 consecutive patients undergoing ABMT over a 9-year period has recently been reviewed (4,6). Pretransplant CMV serology was assayed in 134 patients, 94 (70%) were seropositive prior to transplant. Evidence for CMV infection was detected in 65 patients (45%). The actuarial incidence of virus excretion during the first 50 days after ABMT was 18%: 6% in seronegative patients and 28% in seropositive patients (p<.01). Seroconversion was defined as a four-fold or greater rise in antibody titer to CMV in both seronegative and seropositive patients. Seroconversion in either initially seronegative or seropositive patients did not correlate well with CMV excretion or severe CMV disease. The median onset of CMV excretion and viremia was 14 and 49 days, respectively. CMV pneumonitis developed in 3 patients 15, 38, and 38 days after BMT; there were no cases of CMV retinitis or enteritis.

The effect of CMV infection on hematopoietic recovery was also examined (Table 1) (4). Because of the relatively small number of seronegative patients, the effect of a primary CMV infection could not be evaluated. However, among seropositive patients, those with CMV infection had significantly slower platelet recovery. CMV-infected seropositive patients had slower neutrophil recovery than noninfected patients.

A detailed comparison between CMV infection in patients undergoing both autologous BMT and allogeneic BMT has been reviewed in a previous symposium (5). There were no differences in the rates of virus excretion or viremia between autologous and allogeneic transplants during the first 50 days, however there was clearly a significantly greater risk for CMV pneumonitis after allogeneic BMT (12% vs 2%, p=.0002). The CMV excretion rate among allogeneic BMT patients was not affected by the occurrence of acute GVHD - the incidence of CMV excretion at 1 year was 36% in patients without GVHD and 41% in those with GVHD. The actuarial incidence of CMV pneumonitis in allogeneic BMT with acute GVHD (23%) was significantly greater than either the incidence of CMV pneumonitis in those without acute GVHD (6%, p<.001) or in autologous BMT patients (2%. p<.001)(6).

**Table 1. Number of Days Until Platelet and Neutrophil
Recovery in Seropositive Patients (N=94) Comparing
Patients with or without Virus Excretion,
Seroconversion, or Total Infection**

Groups Compared	Median Day to Platelet Recovery*	P Value (Wilcoxon)
Excretion/non-excretion	132/49	.03
Seroconversion/ non-seroconversion	76/43	.06
Total infection/ noninfection	97/35	.003
	Median Day to Neutrophil Recovery[+]	
Excretion/non-excretion	33/25	.08
Seroconversion/ non-seroconversion	29/36	.06
Total infection/ noninfection	31/24	.02

A review of the cases of CMV pneumonitis in patients undergoing ABMT in Seattle is similar to our results (7). Of 70 patients with acute leukemia or malignant lymphoma who received ABMT. 3(4.3%) patients developed CMV pneumonitis. This incidence of CMV pneumonitis was significantly lower than in allogeneic narrow recipients (16%, p<0.05), but was not significantly different from syngeneic transplants (0%, p>0.05).

Attempts to reduce the incidence of life-threatening CMV infections in patients undergoing allogeneic BMT with the use of CMV seronegative blood products and/or CMV immunoglobulin (8), and the use of high-dose acyclovir for seropositive patients(9) has been studied. However, since severe CMV infections in patients undergoing ABMT is uncommon, the use of these agents in this setting does not seem appropriate at this time. However, whether one of these preventive strategies might be of use in speeding platelet recovery needs to be assessed.

The frequency of reactivation of varicella zoster virus (VZV) and the severity of infection after ABMT is currently being investigated. Previous reports indicate that 30 to 40% of allogeneic patients develop

VZV during the first year after transplant (10,11). Since VZV is less susceptible to acyclovir than HSV, higher blood levels are needed to inhibit the virus which generally require the use of intravenous acyclovir in severe infections (12,13). Accordingly, the use of oral administration of acyclovir in routine doses should be discouraged in patients at risk for dissemination. The use of oral acyclovir given in higher doses as prophylaxis against VZV has been examined. In a Swedish study where six months of oral acyclovir was used to prevent HSV infections in BMT patients (intravenous acyclovir 250mg/m^2 twice daily 4-6 weeks, followed by oral acyclovir 400mg three times daily thereafter) VZV was also controlled (11). However, after acyclovir discontinuation, patients who received prophylaxis developed similar incidences of VZV as the non-treated group, although episodes were delayed. Other studies caution against the use of long term acyclovir administration to prevent VZV infections because of the reduction of subclinical reactivation during ayclovir administration which may result in diminished subsequent protection (14). Decreased humoral responses to HSV (15) and decreased cell-mediated responses to HSV (14) following acyclovir prophylaxis for HSV in patients following BMT have been noted. Whether these phenomena will have significant clinical impact has yet to be determined. Because of these potential problems with prolonged use of acyclovir, an immunoprophylactic approach with an appropriate vaccine may need to be considered.

In summary, viral infections continue to be a major problem in patients undergoing BMT. However, the use of acyclovir has controlled reactivation of HSV during the early post-transplant period; prolonged use of acyclovir to provide extended prophylaxis needs further study. The occurrence of CMV infection is similar in patients undergoing either autologous or allogeneic BMT, but life-threatening CMV infections are uncommon in autologous transplants and, therefore, the use of CMV seronegative blood products and CMV immunoglobulin may not be justified for patients undergoing ABMT. Because of delayed platelet recovery associated with CMV reactivation, however, strategies to prevent reactivation, such as prophylaxis with high-dose acyclovir or intravenous immunoglobulin with high anti-CMV titers, should be examined. The occurrence of VZV in ABMT is currently under study. At present, there is no effective prophylactic therapy other than the possible prolonged use of high-dose oral acyclovir which may present additional problems to the patient, because of delayed or impaired long-term immunity.

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INTRAVENOUS AND/OR ORAL IMMUNOGLOBULIN
FOR AUTOLOGOUS MARROW TRANSPLANTATION:
An Interim Report

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INTRODUCTION

High-dose preparative regimens designed to eliminate large tumor burdens often require reinfusion of autologous bone marrow to allow timely recovery of normal hematopoietic function. Associated with this aggressive therapy is the increased morbidity and mortality which frequently is related to infectious complications (1). Although engraftment of bone marrow cells usually results in adequate neutrophil counts by the fourth week, phagocyte dysfunction can continue for months after transplant (2). Lymphocyte proliferation to mitogen stimulation is diminished, IL-2 production is decreased (3), and hypogammaglobulinemia and anergy may persist (4). In our experience, 50 to 60% of all transplant patients develop bacteremia some time during their course of observation, and an additional 25 to 40% develop fevers of undetermined origin. Although acyclovir has been effective in treating herpes simplex virus infections (5), antiviral therapy for other viral agents has been inadequate.

Several years ago, we completed a randomized study of intravenous immune globulin (IVIg) in 34 patients receiving autologous marrow transplants (unpublished data). In that study, those designated to receive IVIg received Gamimune (Cutter Biologicals, Emeryville, CA), 250 mg/kg, with the first dose on the ninth day prior to bone marrow reinfusion and subsequent doses every three weeks until 100 days after the transplant. Treatment was postponed or modified depending on the patient's clinical condition. Because the majority of our patient population has a cytomegalovirus (CMV) titre of at least 1:64, all patients were included despite previous CMV exposure. The

incidence of interstitial pneumonitis in that study was 9%, and particularly of cytomegalovirus pneumonitis, 3%. CMV enteritis (6) was not a problem in this study group. In addition, there was no effect on time to recovery, episodes of bacterial or fungal sepsis, or utilization of blood products. This study was hampered in addition by low numbers of patients and poor compliance.

Our current study uses Gammagard (Baxter Healthcare Corporation, Glendale CA) and has three goals. The first is to determine whether IVIg, tested in a much larger number of patients, could be shown to affect the rate of infections and gastrointestinal complications. Also, since locally produced antiviral Ig appears to be critical protection from local and systemic infections (7), the second objective is to see if orally administered Ig, with a broad spectrum of specificities and with capability of neutralizing enteric organisms (8), may substitute for deficient intestinal antibodies. Finally, the tolerability of an oral preparation of antibodies is being observed. Hence the study has three arms: IV alone, IV plus oral, or no Gammagard.

The rationale for including a study arm containing oral immunoglobulins was based on observations by Tutschka and his collaborators (personal communication). Due to compromised digestive function after cytoreductive therapy, antigenically intact IgG appeared in stool specimens of their patients during all 28 days of oral administration. No enteropathic viral agents were isolated, no patient developed diarrhea, none of the six patients studied developed septicemia, and three of these did not require any therapeutic antibiotics.

The results here are for the first 100 treatment courses of IV and/or oral immunoglobulin.

METHODS

All patients receiving autologous bone marrow transplantations at UTMDACC were eligible, though patients with a history of hypersensitivity to plasma products were excluded. All registrants had baseline stool viral cultures and cytomegalovirus titres and cultures, which were repeated as symptoms warranted. Patients were stratified by disease, treatment, and location inside or outside the Protected Environment (sterile laminar air flow rooms).

Patients were randomized into one of three arms: 1) no immune globulin treatment, 2) Gammagard 500 mg/kg IV every 14 days from two days prior to transplant until post-transplant day 96, and 3) the above, plus Gammagard 50 mg/kg orally in three divided doses from post transplant day 1 to 30, or until recovery of 1000 granulocytes/ul. The lyophilized powder was reconstituted in the supplied diluent and mixed with grape or apple juice if desired. Compliance to the

regimen was monitored strictly; patients refusing or missing a dose of intravenous IVIg while hospitalized were excluded from the study, though missing oral doses was allowed but recorded.

All patients received Bactrim DS and ketoconazole 200 mg every 8 hours, and all received acyclovir 250 mg/kg IV every 8 hours post-transplant days 1 to 10 as viral prophylaxis. Patients were observed daily for fever >38.3°C and for mucositis/esophagitis and enteritis/diarrhea; grading of the latter was 0-absent, 1-mild, 2-moderate, 3-severe, and 4-life threatening.

RESULTS

One hundred courses of therapy were assigned to 80 patients. Distribution of diagnoses was similar in the three groups, with roughly half the patients having solid tumors and half having hematologic malignancies (leukemia, lymphoma, or multiple myeloma). The percent of patients who were CMV positive and the median titres of those positive were also similar in all groups. Three patients received additional doses of IVIg to combat refractoriness to platelets late in their hospital courses; they are included in the analysis. Of the patients assigned to take oral medication, 38% were fully compliant, 44% received at least one dose on at least half of the days on study, and the remaining 18% were judged noncompliant, but were included in the analysis. Reasons for missing doses included severe mucositis, vomiting, or patient refusal. Occasional fever or chills with infusion were controlled readily with addition of steroids and/or antipyretics.

Major complications are shown in Table 1; the difference is not significant. Table 2 shows the incidence of bacteremia by organism; the large number of infections caused by alpha-hemolytic streptococci is distressing and unexplained. The average number of days of moderate to severe diarrhea and mucositis and fever are shown in Table 3, and the average total use of antibiotics and length of hospitalization are shown in Table 4.

Table 1. Major Complications

	No IG	IV Only	IV + PO
Early death	1	2	1
Cytomegalovirus	0	2	1[1]
Herpes virus	1	3	1[1]
Septicemia	17	16	15

[1] - pneumonitis

Table 2. Positive Blood Cultures

	No IG	IV Only	IV + PO
Staph epi	6	4	1
Staph aureus	2	0	0
Alpha strep	4	8	7
Other Gram pos	1	2	2
Gram negative	3	1	1
Other bacteria	1	0	2
Fungi	0	1	2

Abbreviations: Staph, staphylococcus; epi, epidermidis; Alpha, alpha hemolytic; Strep, streptococcus; pos, positive.

Table 3. Average Days of Moderate to Severe Toxicities

	No IG	IV Only	IV + PO
Stomatitis			
All	3.3	3.8	2.6
Heme	6.0	1.3	0.8
Diarrhea			
All	3.8	3.4	5.2
Heme	6.2	4.3	2.2
Fever >38.3° C			
All	4.9	5.7	7.3
Heme	5.0	8.3	6.5

CONCLUSIONS

Overall, tolerance of the regimen was good, although a number of patients cannot take all the oral doses. Nevertheless, even with 100 treatment courses analyzed, there is not a significant difference in the amount of fever, infections, stomatitis, or enteritis/diarrhea in the different patient groups, and needs for hospitalization and antibiotics is similar for all. A difference appears in the group of hematologic

malignancies (leukemia, lymphoma, multiple myeloma) with respect to a lower incidence of mucositis in the treated patients; however, these same patients also, on the average, received more antiviral and antifungal therapy than those not receiving IVIg. Viral pneumonia did occur in two patients, both late in the course of treatment and neither prevented by the patients' having received immunoglobulins.

The most frequent and most often fatal complication of autologous transplantation, septicemia, appeared not to be affected by the administration of immunoglobulins; this is in spite of a report in allogeneic transplantation wherein systemic infections were reduced in patients receiving IVIg, particularly in those patients in laminar air flow rooms receiving prophylactic antibiotics (9). The current study does not as yet have enough patients treated inside the Protected Environment to corroborate this finding, and its design was based on antiviral applications; dosing and schedule for antibacterial affects may be different.

Table 4. Average Days of Use

	No IG	IV Only	IV + PO
Hospital days			
All	25.5	24.3	29.2
Heme	27.5	35.4	32.6
Antibacterials			
All	16.9	17.1	16.8
Heme	17.4	22.5	18.3
Antifungals			
All	3.1	4.4	3.8
Heme	3.4	6.2	6.5
% use	33.3	46.8	35.7
Antivirals (excluding prophylaxis)			
All	1.0	2.5	2.5
Heme	0.5	6.0	4.0
% use	13.8	28.5	21.4

Approaches for the future include: 1) concentrating investigation of IVIg in hematologic malignancies, where a positive trend is appearing, and 2) restudy the use of IVIg in preventing bacterial infections. Use of immunoglobulins in autologous transplantation still must be considered investigational.

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Discussion 1 - Session VII (Supportive Care)

Dr. Dicke: Well, first of all, I would like to thank both of the speakers because I think this is a very important problem which we face after bone marrow transplantation. Not so much any more in, my opinion, the herpes simplex -- which I think can be extremely well prophylacted by acyclovir. Controversial is the CMV infection. How do we do the prophylaxis? Dr. Reed, what would you recommend?

Dr. Reed: I think that this is a controversial area. In seropositive patients I just do not think that all of the data is in. Of course I think the main two tasks at this time have been the prophylaxis with immunoglobulin whether high doses of high titers of CMV antibody or not, or the use of high doses of acyclovir and that experience came out of Seattle. As I say, I do not think that question is answerable at this time. I do suggest that the availability of antiviral agents that have in vivo activity against CMV, offer us a third choice and, perhaps, with the use of phoscarnate which does not have the marrow suppressive effect or the combination of acyclovir used for a longer term...plus or perhaps...a follow-up with phoscarnate may be possibilities.

Dr. Dicke: Would you agree that if you found a definite effective treatment as you presented, such as the combination of DHPG and an immunoglobulin, to give that as a prophylaxis -- provided that you give the DHPG, maybe in a pulse dose, in order to avoid myelosuppression.

Dr. Reed: I think that during the early post transplant period it is unlikely that we would be able to give DHPG and have a good and timely engraftment. CMV pneumonia occurs at a median day of 60 but you do see seroconversion and so forth earlier. It is true that you could start prophylaxis at about day 30 to 40 and possibly get

away with that. I think studies may be going on at UCLA and Sloan-Kettering that could better address that.

Dr. Peterson: A question to Dr. Yeager. I think that there are now several studies that convincingly show that giving CMV negative blood products to CMV negative patients -- getting a marrow from CMV negative donors -- protects these patients against CMV pneumonitis. Even though the incidence is low for those autologous patients getting CMV pneumonia it is a catastrophic event in many cases. I wonder... since there is such an effective treatment or prevention, why withhold that prevention from autologous patients?

Dr. Yeager: Dr. Peterson brings up an important point. If we could do it and support both our allogeneic and our autologous patients with CMV negative blood products we would do that. Right now we have to, it is just a matter of product availability for us.

Dr. Milpied: I would just add a comment to the issue raised by Dr. Dicke about the prophylactic cures of potent antiviral agent against CMV; and, just to say, that we started to try to use DHPG and acyclovir. By now 12 patients received the drug at the dose mentioned 7.5mg/kg for 20 days and no one died of any tissue infection, pneumonitis, gut or liver infection. Only one patient had to have the treatment stopped because of neutropenia.

Dr. Seeger: I have a question for Dr. Yeager. It is an interesting observation -- the relationship between CMV positivity and delayed platelet recovery -- is that an association or do you think it is causal?

Dr. Yeager: Right now, it is an association. There have been other studies as you know that have made the association between delayed platelet recovery and CMV infection. We have not pursued in vitro studies.

SESSION VIII - FURTHER DEVELOPMENTS IN PURGING

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MEROCYANINE 540 AS A MARROW PURGING AGENT:
Interactions of Merocyanine 540 with
Normal and Neoplastic Cells

Fritz Sieber

Merocyanine 540 (MC 540) is a lipophilic plasma membrane probe (1). In the presence of serum or certain serum components (2), the dye binds preferentially to electrically excitable cells, leukemia and lymphoma cells, and certain types of normal hematopoietic progenitor cells (3, 4). Not much is known about cellular dye "binding sites" except that they are lipophilic and bind MC 540 in a non-covalent fashion. The dye is readily extracted from cells by polar solvents (3, 4), and spectrophotometric analyses show that membrane bound dye is in a hydrophobic environment (3, 5). Fluorescence quenching and fluorescence resonance energy transfer experiments with anthracene-labelled fatty acids localize the dye deep in the lipid bilayer of the plasma membrane of leukemia cells (5). MC 540 binds preferentially to small liposomes (i.e. liposomes with a high radius of curvature) (6), to liposomes that contain high amounts of unsaturated fatty acids and low amounts of cholesterol (7), and to red cells whose typical asymmetrical distribution of membrane lipids has been disrupted (8).

   If cells with a high affinity for MC 540 are simultaneously exposed to the photosensitizer, oxygen, and light of a suitable wavelength, the cells are killed (4). Singlet molecular oxygen is probably an important mediator of the cytotoxic effect (9). The rate of cell kill is directly related to the dye concentration and the fluence, and inversely related to the serum concentration (2, 4, 10). Whether a cell is killed rapidly or slowly under a given set of conditions depends primarily on the amount of dye it binds. To a lesser extent, it depends on the cell's intrinsic sensitivity to toxic photo products. For instance, mutant L1210 leukemia cells with elevated levels of intracellular glutathione are less sensitive to MC 540-mediated photosensitization than their

wild type counterparts (12). Furthermore, inhibition of glutathione biosynthesis or inhibition of glutathione peroxidase activity increase a cell's sensitivity to MC 540-mediated photosensitization (5).

MEROCYANINE 540 AS A MARROW PURGING AGENT

Our decision to evaluate MC 540 as a marrow purging agent was based on two observations. 1) Valinsky and collaborators had shown that peripheral blood leukocytes from patients with acute or chronic leukemia or lymphoma in leukemic phase consistently display a high affinity for MC 540 (4). 2) A comparison of the relative photosensitivities of normal human and murine hematopoietic progenitor cells and several tumor cell lines showed that leukemia, lymphoma, and neuroblastoma cells are killed much more rapidly than primitive normal progenitor cells (10-19; Table 1). The experimental tumors included drug resistant mutants and cell lines that had been isolated from patients with refractory disease. The preclinical evaluation also showed that MC 540 has a low acute systemic toxicity (18), is not mutagenic (20), is quite insensitive to fluctuations of cell concentrations (11), and, because it absorbs light in the visible range, is not affected much by contaminating red cells. The already significant therapeutic index may be further enhanced by adding the photosensitizer in split doses instead of one full dose at the beginning of the irradiation period (21).

Transplantation experiments with simulated autologous remission marrow grafts (i.e. defined mixtures of normal mouse bone marrow, cells and L1210, P388, or P388/ADR leukemia or Neuro 2a or NB41A3 neuroblastoma cells) confirmed that MC 540-mediated photosensitization is capable of eliminating substantial numbers of tumor cells without abrogating the graft's capacity to rescue lethally irradiated syngeneic hosts (14-16, 18).

Based on these preclinical data, a phase I clinical trial was initiated at the Milwaukee County Medical Complex and the Johns Hopkins Oncology Center (22). The trial has two objectives, 1) to determine the maximally tolerated dose of dye-mediated photosensitization, and 2) to determine if the small amounts of dye that remain associated with photosensitized marrow grafts cause systemic toxicity in the recipient. As of this writing, 16 patients with acute lymphocytic or non-lymphocytic leukemia, Hodgkin's disease, or non-Hodgkin's lymphoma have received autologous remission marrow grafts that were treated with MC 540 and light at three different dose levels. Calibration runs with K562 cells showed that tumor cells were reduced by >6 log at all three dose levels. Linear extrapolations of survival curves indicated reductions of 12, 15, and 18 log for dose levels I, II, and III, respectively.

Table 1. Merocyanine 540-mediated Photolysis of Normal Human Hematopoietic Progenitor Cells and Experimental Tumor Cells Treated Under Identical Experimental Conditions

Cell Type	Origin	Reduction Factor*
CFU-Mix	normal marrow	2
HL-60	promyelocytic leukemia	100,000**
K562	CML in blast crisis	100,000***
Raji	Burkitt's lymphoma	100,000
Daudi	Burkitt's lymphoma	50,000
OCI-LY13-1	non-Hodgkin's lymphoma	100,000
OCI-LY13-2	non-Hodgkin's lymphoma	100,000
OCI-LY9	non-Hodgkin's lymphoma	10,000
HUT-102	cutaneous T-cell lymphoma	10,000
IMR-32	neuroblastoma (epithelioma)	50,000
SK-N-MC	neuroblastoma	10,000
SK-N-SH	neuroblastoma	500
A 204	rhabdomyosarcoma	200
RD 136	rhabdomyosarcoma	130
SK-MES-1	lung carcinoma	110
SK-LU-1	lung adenosarcoma	20
A 549	lung carcinoma	4
G 361	malignant melanoma	6
HT 144	malignant melanoma	3

*   As defined by their capacity to form colonies in vitro.
**   Extrapolation: 100,000,000.
***   Extrapolation: 10,000,000.

So far, we have noticed no adverse reaction to the infusion of photosensitized marrow grafts. Definitive conclusions about the effects of the merocyanine treatment on engraftment are not yet possible because the number of evaluable patients is still too small.

Also, it is not clear to what extent delays in engraftment are attributable to decreased numbers of stem cells in marrow grafts from heavily pretreated patients and to what extent they are attributable to the purging procedure. However, the fact that one patient engrafted only after infusion of the untreated backup marrow indicates that MC 540-mediated photosensitization causes sole stem cell toxicity and suggests that we may have already reached or will soon reach the maximally tolerated dose.

CONCLUSIONS

Preclinical data suggest that MC 540 is effective against a broad range of hematopoietic/lymphopoietic tumors and a few solid tumors. Early clinical data indicate that the agent can be used safely in humans. Since its mechanism of action is different from that of most other marrow purging agents, MC 540 is a promising candidate for purging protocols that combine two or more purging principles (23). A better understanding of structure-function relationships, of the nature of cellular dye binding sites, and of toxic photo products and their cellular targets will lead to the development of better photosensitizers and provide guidelines for the design of more effective purging protocols.

ACKNOWLEDGMENTS

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STABLE IN VITRO ACTIVE ALDOPHOSPHAMIDE ACETALS FOR BONE MARROW PURGING

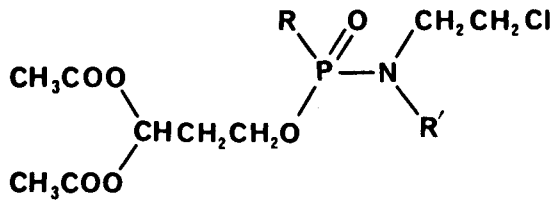*Borje S. Andersson, Yui-Qui Wang, and David Farquhar*

In vitro chemotherapy with the aim of selectively eliminating occult tumor cells from remission bone marrow prior to autologous bone marrow transplantation is currently investigated at a number of centers (1,2). The most widely used agents are the "activated" cyclophosphamide derivatives 4-Hydroperoxycyclophosphamide (4-HC) and Mafosfamide. Both of these drugs are unstable in vitro. Their spontaneous conversion to 4-Hydroxycyclophosphamide followed by ring-opening to aldophosphamide is, however, necessary to yield the active cytotoxic moiety, phosphorodiamidic mustard (PM). Aldophosphamide can also be degraded to the non-toxic metabolite carboxyphosphamide through the action of cellular aldehyde dehydrogenase (3). Since 4-HC and Mafosfamide share mechanistic pathways and give rise to same cytotoxic metabolite, PM, they may be expected to have similar antitumor efficacy with possibly only minor differences in potency. We wished to explore different ways of improving the anti-leukemic selectivity of this class of in vitro active alkylating agents. This work has generated a new class of stable open-chain bis(acyloxy)acetals of aldophosphamide and aldoiphosphamide, that are activated by carboxylic esterases. The structures of these compounds are shown in Figure 1. Their preparation and physicochemical properties are described elsewhere (4-6). This communication reports in vitro cytotoxic data on two of the most promising of these new compounds, acetaldophosphamide (I) and acetaldoiphosphamide (II). For comparison, 4-HC is included in some experiments.

MATERIALS AND METHODS

Drugs

The acetaldophosphamide and acetaldoifosphamide were synthesized in laboratory (6). When dissolved in Dulbecco's

FIG 1

$$CH_3COO \diagdown \atop CH_3COO \diagup CHCH_2CH_2O - P \underset{O}{\overset{R}{\diagup}} N \diagup{CH_2CH_2Cl} \diagdown R'$$

	R	R'
I	NH₂	CH₂CH₂Cl
II	NHCH₂CH₂Cl	H

	R	R'
I	NH_2	CH_2CH_2Cl
II	$NHCH_2CH_2Cl$	H

Figure 1. Chemical structure of the aldophosphamide acetals; I=acetaldophosphamide, II=acetaldoiphosphamide.

phosphate-buffered saline (PBS, pH 7.4) at room temperature, their half-lives are approximately 50 and 80 hours respectively. 4-HC was obtained from Dr. M. Colvin at the Oncology Center of the Johns Hopkins University School of Medicine. 4-HC was weighed and dissolved in PBS immediately prior to each experiment. The acetaldoiphosphamide and acetaldophosphamide were dissolved in PBS at 265 μM as a stock solution and stored at -80°C; aliquots were thawed immediately prior to use.

Bone Marrow

Bone marrow was obtained from different normal volunteers and suspended in Heparin-containing PBS. The mononuclear cell fraction was isolated on a Ficoll-Diatrizoate gradient (density 1.08 g/cm³).

Cell Lines

The human myeloid cell line KBM-3 was established in our laboratory from a patient with acute myeloid leukemia in 1983. Its in vitro growth characteristics have been described elsewhere (Andersson B.S. et al. submitted for publication). Sublines of this cell line that are 100-130 fold resistant to m-AMSA (KBM-3/AMSA) and Doxorubicin (KBM-3/DOXO), assessed with clonogenic assay, have been

established. KBM-7 is a human myeloid cell line established in our laboratory from a patient with near-haploid myeloid blastic crisis of chronic myeloid leukemia (7). HL-60 was obtained from the laboratory of Dr. R. Gallo at the National Cancer Institute (8).

Assay for Granulocyte-Macrophage Colony Forming Cells (GM-CFC)

Bone marrow cells were resuspended in Iscove's modified Dulbecco medium (IMDM) supplemented with 20% fetal calf serum (FCS) and 0.3% agar. One mL fractions containing 1×10^5 mononuclear cells were then layed on a "feeder" layer of IMDM with 20% FCS, 0.5% agar and as a colony-stimulating factor human placenta conditioned medium (HPCM) (9), in 35 mm Petri dishes. The cultures were incubated at 37°C for 8 days in a humidified atmosphere of 5% CO_2 in air. Colonies of 50 or more cells were counted under a phase contrast microscope. Leukemic CFC were cultured in a single layer system without addition of HPCM since they grow without addition of exogenous colony-stimulating factor. After counting the colonies on day eight, survival curves were constructed and IC_{50} values (the concentration that eliminates 50% of the clonogenic cells) were calculated.

Drug Toxicity to Normal and Leukemic Clonogenic Cells

Mononuclear bone marrow cells or leukemic cells in enriched PBS (PBS supplemented with Ca^{++}, glucose and 5% FCS were incubated with the respective drug over a range of concentrations for 60 min. Cells incubated in parallel in drug-free enriched PBS served as controls. After incubation, the cells were washed twice in PBS, resuspended in IMDM and used for in vitro cultures.

Long-Term Suspension Cultures

A two-step technique was employed as previously described (10). Briefly, 1×10^7 mononuclear bone marrow cells were suspended in IMDM with 12.5% FCS, 12.5% horse serum and 5×10^{-6} M Hydrocortisone (HyC). After 10 days an adherent fibroblast layer had been established that would support hemopoiesis. The non-adherent cells were washed off and the feeder layers were irradiated with 3.0 Cy to prevent proliferation of GM-CFC from the first inoculum. A second marrow sample was obtained from the same donor and mononuclear marrow cells were isolated. After incubation with drug or in plain PBS as above, the cells were seeded onto the feeder layers at 1×10^6 per mL, in IMDM with 10% FCS and 5×10^{-6} M HyC. Eight-five percent of the media was replaced twice weekly and all

cultures were incubated for a total of five weeks in an atmosphere of 5% CO_2, 12% O_2 balanced with N_2. Immediately after drug exposure and at weekly intervals, one mL was withdrawn and assayed for GM-CFC.

Results

Exposure of bone marrow cells to compounds I, II and 4-HC in increasing concentrations resulted in a concentration-dependent inhibition of GM-CFC proliferation with the surviving being exponentially related to the drug concentration (Figure 2). The inhibition of leukemic CFC proliferation showed a similar concentration dependence; the mode of cell kill is consistent with the proposed alkylating and cell-cycle independent action. On a molar basis, the new compounds were about 8-10 times more potent than 4-HC against normal GM-CFC. When the ratio

$$\frac{IC_{50} \text{ GM-CFC}}{IC_{50} \text{ leukemic CFC}}$$

was calculated as an in vitro "therapeutic index," the ratios were identical for compound I and 4-HC, that share the final cytotoxic metabolite, phosphorodiamidic mustard. In contrast, compound II whose cytotoxic metabolite is iphosphoramide mustard, appeared approximately five times more selective than the aforementioned agents (Table 1). Similar results were obtained when the activity of the new compounds were compared to that of 4-HC on the human myeloid cell lines KBM-7 and HL-60 (data not displayed).

Regeneration Kinetics of GM-CFC in Long-Term Suspension Cultures (LTSC)

After exposure of mononuclear bone marrow cells to $\geq$ IC_{99} of compounds I, II and 4-HC the recovery of GM-CFC proliferation in LTSC showed a biphasic pattern, with rapid recovery to control level in the first 1-2 weeks. During the final three weeks of culture the GM-CFC in drug-exposed cultures paralleled that of the control cultures (Figure 3). Beyond the first week, no GM-CFC could be recovered from the cultures containing only irradiated feeder layers.

At the end of the five-week culture period, the stromal cell layers were trypsinized and the resulting cell suspension was assayed for GM-CFC production. At this time, about half of the total GM-CFC of the control cultures and cultures exposed to 4-HC, compound I and compound II, in concentrations of less than IC_{100}, were found in the
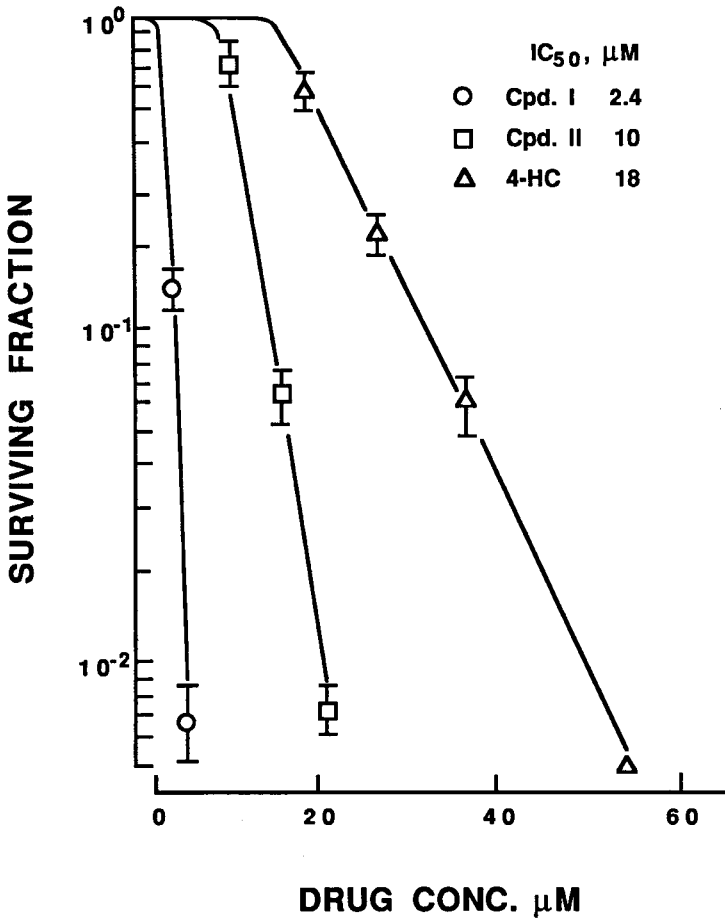
Figure 2. Comparative cytotoxicity of acetaldophosphamide (o), acetaldoiphosphamide (□), and 4-HC (△) to normal bone marrow GM-CFC from three different donors. The cells from each marrow were exposed to the three compounds in parallel for 60 min at 37°C. Each point is the mean ±S.D. of triplicate cultures.

adherent layer. There was no difference between cultures exposed to the different compounds and the control cultures.

To simulate remission and leukemic bone marrow, 5% and 30% leukemic cells (cell line KBM-3) were mixed with normal bone marrow prior to exposure to compound II at a concentration of 39.6 μM (IC$_{99}$). The rate of recovery of GM-CFC production in these cultures paralleled that of marrow alone (Figure 4). Furthermore, leukemic regrowth was observed in only one of the cultures exposed
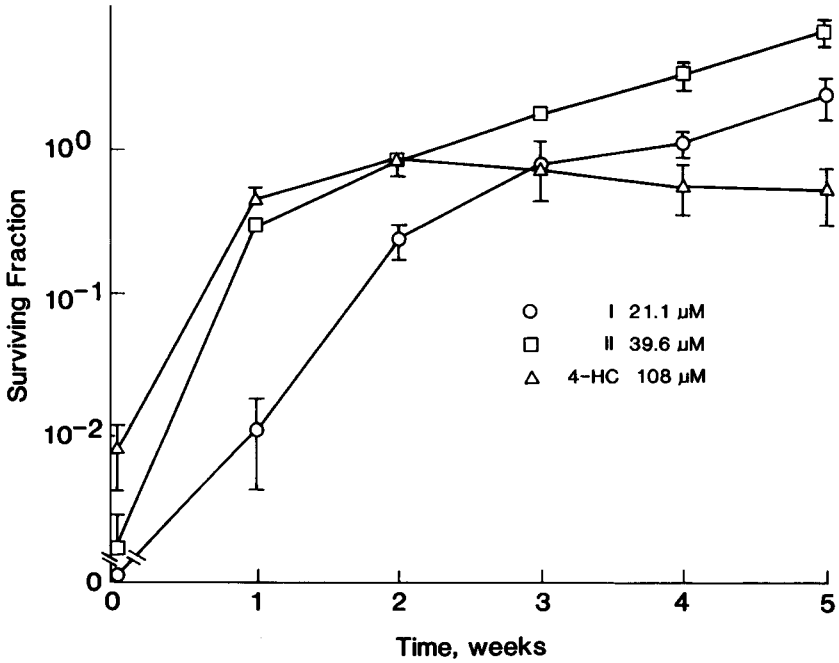
Figure 3. Comparative regrowth of GM-CFC in long-term suspension cultures of normal bone marrow after exposure to acetaldophosphamide (o), acetaldoiphosphamide (□), and 4-HC (Δ) at concentrations that abolished ≥ 99% of GM-CFC. Cells incubated in PBS only served as controls. 5×10^6 cells (total volume 2 ml) were exposed to drug for 60 min at 37°C and, after washing, seeded in suspension at 10^6 cells/ml. Each point is the mean of triplicate GM-CFC determinations from two separate cultures.

Table 1. Sensitivity of GM-CFC and Leukemic CFC to Aldophosphamide Analogs

Cell Type		IC_{50} μM (60 min. exposure)		
		4-HC	Cpd. I	Cpd. II
GM-CFC		18	2.4	9.8
KBM-3		2.4	0.30	0.26
Ratio	$\dfrac{IC_{50} \text{ GM-CFC}}{IC_{50} \text{ Leuk. CFC}}$	7.5	8.0	38

Table 2. Recovery of Hemopoiesis in Long-Term Suspension Cultures of Human Bone Marrow

Cells	Drug	Maximum recovery of GM-CFC; % of control*	No. of cultures free from Leuk CFC at the end of 5 weeks	
NBM	4-HC	75-90	--	
NBM+5% KBM-3	4-HC	90	1/4	
NBM+30% KMB-3	4-HC	80-90	0/5	1/9
NBM	Cpd II	>100	--	
NBM+5% KBM-3	Cpd II	>100	4/5	
NBM+30% KBM-3	Cpd II	100	5/6	9/11

*Average of three separate experiments.

to compound II whereas return of leukemic CFC proliferation was observed in nine of the eleven cultures exposed in parallel to 4-HC at equipotent concentration (Table 2).

CONCLUSIONS

This novel class of stable open-chain acetal analogs of aldophosphamide and aldoiphosphamide are unique in their
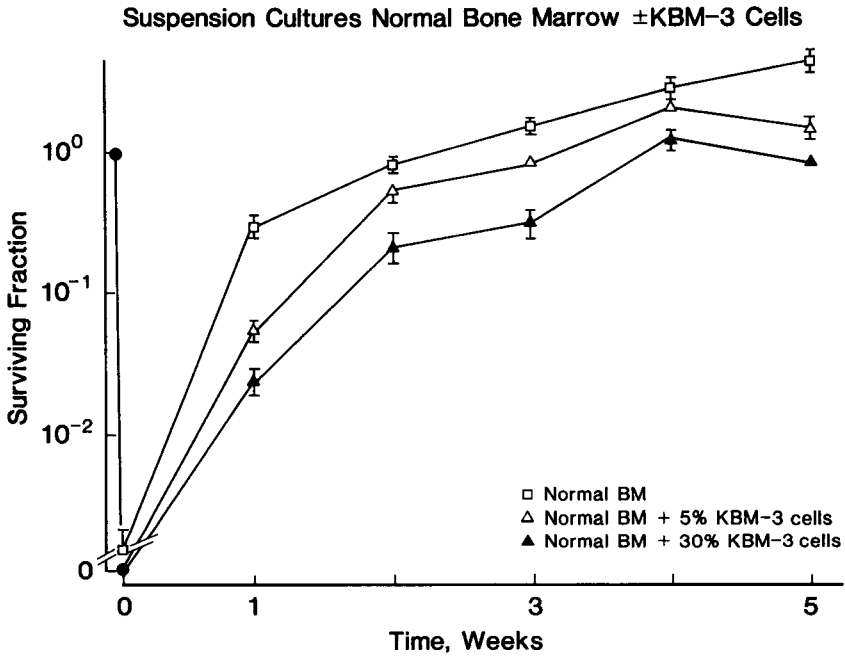
Figure 4. Recovery of GM-CFC production in long-term cultures of human bone marrow along ($\square$), or contaminated with leukemic cells (cell line KBM-3) at 5% ($\triangle$; a total of 5×10^5 cells per culture), or 30% ($\blacktriangle$; a total of 4.2×10^6 cells per culture) prior to exposure to acetaldophiosphamide at a concentration of 39.6 μM (IC_{99}) as described in Materials and Methods.

requirement for enzymatic activation. They are highly active, having a molar potency that appears 7-10 times higher than that of 4-HC against leukemic cells in vitro. The aldoiphosphamide analog in particular, holds promise of significantly higher anti-leukemic selectivity than 4-HC. The LTSC results indicate that

acetaldoiphosphamide has a similar sparing of immature hemopoietic stem cells as 4-HC. We conclude that acetaldoiphosphamide may be a valuable agent for in vitro chemotherapy of bone marrow in a clinical setting. Studies to determine the optimal conditions for large scale "purging" of bone marrow is underway in our laboratory.

ACKNOWLEDGMENTS

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Discussion 1 - Session VIII
(Further Developments in Purging)

Dr. McGlave: Dr. Sieber, you have a third patient surviving at day 139. That patient apparently received a back-up marrow or peripheral blood at day 47. Did the patient have graft failure?

Dr. Sieber: He did graft very slowly around day 95.

Dr. McGlave: So there was no relapse in that patient at any point?

Dr. Sieber: No.

Dr. Dicke: I have two technical questions, Fritz. Does the binding of merocyanine to the HL60 cell change when you drive the HL60 cell into differentiation?

Dr. Seiber: I think Hoffman once looked at that and it failed to do so. There is however, one system I am very familiar with and that is the BN leukemia. If you drive that to differentiate, the moment it nucleates it looses its merocyanine binding sites. In the HL60, it is not the case. It remains sensitive.

Dr. Dicke: The other question I have is related to the glutathione that is involved in protection from merocyanine toxicity. Have you done anything to manipulate the glutathione levels?

Dr. Seiber: Yes, and it is very predictable. You give them DMSO, that interferes with glutathione synthesis and the cells get more sensitive. When you eliminate DMSO it bounces back and it gets more resistant. You can further manipulate the system for instance to grow them into selenium deficient medium, which also increases the sensitivity. It is not much -- I mean on the total scale. You start

out with a cell line; under certain conditions, you kill 5-logs and you get it up to 6-logs, you squeeze out an additional log.

Dr. Goldstone: What are your criteria to take it to a first remission program, because I can't see that you will ever have any more data of efficacy at this level? You have shown the kill of the cells in the lab and all these end-stage patients will not produce any better results, whatever you do.

Dr. Seiber: In order to get this trial we had to get an IND from the FDA. There is a lot of bureaucacy involved and they want you to start out with the worst possible patient category; I think at this point --where we can prove that we probably do no harm--we have a possibility to move into a better patient category.

Dr. Favrot: Just one more question. You do not have any toxicity on progenitor cells? Do you also look for the toxicity on T, B and NK cells and what is your immunological reconstitution?

Dr. Seiber: We have not looked at progenitor cells of the lymphoid series. We have looked at CFU-E, BFU-E, CFU-megakaryocytes, CFU-GM. The CFU-Es are the most sensitive cells. They get wiped out. BFU-E and megakaryocyte progenitors are the most sensitive. CFU-GMs hold up quite well. When we take a marrow, in the clinical trial and look before and after purging, we have lost about 60% of the CFU-GMs. We do not find any CFU-Es and we have lost about 80% of the BFU-Es. There is a hint that progenitors, from heavily pretreated patients, might be a bit more sensitive than those from normal donors where we did the initial evaluation.

Discussion 2 - Session VIII
(Further Developments in Purging)

Dr. Peterson: My question is for Drs. Mills, Yeager and Gorin. What was the percentage of patients who at the time of marrow aspiration did not have purging done because of insufficient cell numbers? If so, can you comment on how to interpret the results as far as disease-free outcome?

Dr. Gorin: All our patients have marrow purge. If the marrow harvest is not sufficient, we do a second marrow collection.

Dr. Mills: That is true at Dartmouth as well. We have had 3 or 4 patients in whom we have done 2 marrow collections. In addition, in 2 patients we have done peripheral blood stem cell collections. We had to use the back-up marrow in several patients because of the persistent thrombocytopenia and those patients never wanted their back-up infused. So we did take the marrow out of the freezer, treated it with MCAB and infused it. To this date, it did not seem to make any difference on the outcome of the thrombocytopenia.

Dr. Yeager: The patients that were excluded were patients who came to us not in remission but in relapse. Marrow purge was done in every patient. We did not do second harvests. I had mentioned 2 patients who did not have satisfactory engraftment. All of the 88 patients were transplanted with first harvest.

Dr. Gale: I will get a last say at the end of the meeting. But I just have a general comment for anyone who wants to. I will take one example, AML in first remission. There are about 45 or 47 twins transplanted in first remission now. Their relapse rate is about 60%. So it means that relapses occur due to persistent leukemic cells in the patient escaping the conditioning regimen -- I call that relapse in the patient instead of relapse by the transplant. Now, all of the data that

I have heard so far this morning suggest that unpurged BM has a relapse rate of about 40%. So, I would assume that this means that all of the relapses that are occurring in patients in first remission can't be accounted for by relapses in the patient. One need not. It is not to say that the marrow could not make it worse. However, when we compare it with the twin data, the relapse rate is so high that we already can account those relapses after auto transplant to the relapses in the patient. So just based on that, one would not expect to be able to show any effect of marrow purging. Now we have heard some data that suggests that marrow purging can reduce the relapse rate to 50% or even 40%. This would seem to be impossible, there is no real sensible explanation of how it can go lower than the twins. So I would just like to throw it (out): How is it that there seems to be no problem proving that marrow purging is effective when you would say that all of the relapses can be accounted for by relapses in patients?

Dr. Gorin: It is a real possibility that syngeneic transplantation may be different from autologous transplantation. One reason may be that the marrow we infuse in a patient who received an ABMT is his own marrow, in which NK cells and other cells are present, which react against leukemia. This situation may be completely different in the syngeneic transplantation setting.

Dr. Reading: I have a question for Dr. Porcillini. We do a lot of limiting dilution assays and we use K562s. We can find 1 remaining cell in a million of plated cells. So it was not clear from your presentation as to whether or not you have an absolute frequency of remaining cells that you can tell us how efficient your purging actually is? In other words, do you have no wells at 10^4 cells/well plated which are coming up positive after the mixture so you can say that you have got rid of 5% down to less than 1 in 10^4 or, can you give us the real frequency?

Dr. Porcillini: Are you talking about the sensitivity of the test? I mean our sensitivity is 1 out of 10^4 cells. I did not understand the meaning of your question.

Dr. Reading: When you do the combination, you have no remaining tumor cells when you plate 10^4?

Dr. Porcillini: Yes. Measurable with our test.

Dr. Lowenberg: I assume, Adolfo, that these experiments are done to extrapolate to clinical utility. Now you express positive wells using these cell lines as residual clonogenic cells surviving the purging. How do you plan to do these experiments in human AML? I would

see tremendous problems there because there is no relationship at all between clonogenic cells and DNA synthesis. Positivity is difficult to express in terms of numerical means. Could you shed some light on that?

Dr. Porcillini: Bob, this is what I stated at the end of my presentation. These are cells in exponential growth and you cannot compare these with fresh AML cells. I think that our assay is useful to test the efficacy of this drug to kill cells. We tried fresh human leukemia but could not get a cut-off because our assay hinges on the possibility of a clear cut-off between responders and non-responders.

Dr. Janossy: Is it possible that these syngeneic grafts being collected throughout a fairly long period of time including early cases which have not been treated optimally.

Dr. Gale: The question is if the syngeneic grafts somehow are a different group. First of all, the vast majority of cases I reviewed, got cyclophosphamide and TBI, except for 3. So they got the regimen that I guess has been identified as the most effective in the autotransplant setting. The other question is were they transplanted at a different interval, post-transplant? Their median interval from remission to transplantation is short if anything. So I do not know.

Dr. Gale: Is there any specificity of your monoclonals against the leukemia as opposed to normal? In other words, do you do the same damage to the normal cell as you do to the leukemia?

Dr. Mills: There is a differential effect, we have tested extensively the antibodies against normal CFU-GM, BFU-E and there is minimal effect on the colony numbers.

Dr. Gulati: I just wanted to comment about the syngeneic experience. The influence of the leukemic cell population in the harvested marrow may be minimal. The marrow is frozen in DMSO.

It is very well possible that the comparable, in relapse rate after autologous marrow, may be due to the fact that the few remaining leukemic cells go through differentiation or have a lower survival rate after freezing.

Discussion 3 - Session VIII
(Further Developments in Purging)

Dr. Favrot: I would like to ask Dr. Gordon Phillips to speak on liquid cultures. (*directed to Dr. Dicke*) Do you have any specific questions?

Dr. Dicke: I have one, Marie. Gordon, what was the analysis of the chromosomes on day 10 of the liquid cultures?

Dr. Phillips: We generally did not do the day 10 and that is a controversial point. But the reason for doing them at day 4 and, at some circumstances, (at) 5 weeks was that we just felt that reflected the situation of probably both malignant and normal stem cells at several weeks earlier, which is arguably day 10. We have not done the day 10.

Dr. Dicke: But you expect them to be positive on day 10?

Dr. Phillips: What one sees, if one does frequent samples, is a drop off in pseudo linear fashion.

Dr. Dicke: So, that means that you may have infused Philadelphia positive cells and that is a very interesting biological phenomenon -- that there is preferential growth most likely of the Philadelphia negative cell population after BMT.

Dr. Phillips: I am sure you are aware of the case that John Goldman has reported recently a patient with stable phase CML reinfused marrow two years later by every parameter hematopoiesis was normal without any purging technique.

Dr. Keating: Karel anticipated my question. We have looked at about 6 patients in long-term culture and it is interesting to note that at day 10 a fairly significant portion of them will have clonogenic Philadelphia chromosome positive cells. This however declines by week 4 so that at day 10 one may well have a fairly high proportion

of Philadelphia chromosome positive clonogenic cells. These are CFU mix or BFU-E. The other point of interest, which I think needs to be explored further, is that there might be a chimeric presentation in so far as the majority of supernatant progenitors may be Philadelphia chromosome positive whereas the progenitors in the adherent layer are negative. So it is a toss up between determining how long to wait -- because of the problem with a decline in normal progenitors as a function of time in long-term culture (and) the problem with the presence of Philadelphia positive cells earlier on.

Dr. Dicke: Just a technical question. Gordon, is there any reason why you are not cryopreserving these? (Is it) just to make life a little easier? I think that one would be on "tender hooks" having committed oneself to treating the patient. But the cultures are still in progress and there is always the concern that they might come down with contamination.

Dr. Phillips: The most pragmatic answer is that there has been a harvest of non-treated peripheral blood stem cells before this was started. We were just concerned about adding another variable to what was already pretty much of a black box.

Dr. Gorin: I wanted to know, are you using the conditioned medium similar to the one used by Dexter?

Dr. Phillips: There are some differences. I suppose the most striking of which is our enzymatic treatment of the adherent layer where he uses a physical method -- a rubber policeman or something like that. Then there are some slight modifications of the amount of horse serum.

Dr. Gorin: Have you studied the growth pattern of the marrow in terms of CFU-GM quantity before and after long-term culture? Were you able to do these?

Dr. Phillips: The results more or less mirror what I have shown earlier in the assays -- that those cells do grow. They are a fairly low number at that time. But they are not strikingly different than what one sees in a normal situations. Not more than an order of magnitude.

Dr. Stoppa: Just a comment. We reinfuse marrow harvested during refractory relapse in AML after long-term marrow culture (7-day marrow culture) and cryopreservation, and the recovery was nice. We did the same thing in CML patients harvested in CR and cryopreserved the marrow. The damage was substantial in terms of nucleated cells and GM-CFC. We reinfused this marrow and there was no good recovery.

Dr. Gorin: I would like to add a comment. We have tried to cryopreserve this kind of material in patients with AML where we have pushed the cultures for up to 1 month. The cryopreservation efficiency is awful and, in fact, if you do that you may have very bad recovery. In addition to what Dr. Stoppa said, we had in the past very bad recoveries with CML patients. So, I would never do that -- I mean -- in fact I would use fresh material. Now, I would like to ask you why didn't you push your culture longer than 10 days? I mean -- if, at 10 days, you see Philadelphia positive cells, they may disappear, I understand that. But on the other hand, you may wish to push the culture longer and infuse material which is totally deprived of Philadelphia positive cells.

Dr. Phillips: I would just say -- "you walk before you run." That certainly would be an alteration. One can think of many ways to modify this. For instance, what would the effect of interferon be? I mean that is a very obvious point. Elongating the culture, etc. I must say that we were concerned about doing this. If one looks back at the published material, which is Dexter's material, those patients had extremely long recoveries -- long enough that one could argue that those marrows did not really take and it was just endogenous repopulation. We were concerned about this. So, in a sense, we tried to duplicate his results but, certainly, we are exploring that area, Claude. We just decided not to start with that.

Dr. Keating: I just wanted to add a comment in defense of Gordon's approach -- that there is a very dramatic decrease in progenitors in both the adherent layer and in the supernatant, in our experience, at about 4 weeks. There are patients whose cultures do eliminate the Philadelphia chromosome at day 10. So, I think, as a first step, that it is not an unreasonable approach to attempt to insure engraftment.

Dr. Favrot: I will ask Dr. Kulkarni to speak on multi-drug resistance for purging.

Dr. Reading: If you have a cell line that resistant, it is MRK16 positive. If you do not select that -- continuously with drug -- do you now get cells that are no longer expressed in the glycoprotein? And, the second part of that is in the patient that is expressing it. What percentage of the tumor cells are expressing it and do they also lose it without continuous selection?

Dr. Kulkarni: The cell line that we have used, the p-glycoprotein expression is maintained for about 10 months without the addition of additional drugs. Dr. Dalton could make a comment please.

Dr. Dalton: I would be happy to address that particular question. The line is very stable outside of drug. We have kept it out of drug for approximately 1 year. It continues to express p-glycoprotein at the same level, with the same level of resistance being maintained. What is the level? The level of resistance is approximately 100-fold. The level of expression in terms of messenger RNA is also about that level and, in terms of the dilution, it can go to 16-fold dilution of RNA and still be detected.

Dr. Reading: But the question really relates to the specific cell level. In other words, if you have 5% of the cells that lose this marker, you will not see any change in drug resistance when you grow them up and you will not see any change in RNA. But if you start cloning cells that are not eliminated by this technique, you will see them right away. Both in cell lines and in patients, is that going to be a problem? Are you going to get revertants, meaning without selective pressure on a loss of glycoprotein?

Dr. Dalton: Yes, that is possible. I can say we have a technique now to look at the single cell, and the amount of p-glycoprotein expressed on that single cell. We do that by immunohistochemical staining and then using a computerized analysis system which measures optical density. There is an amount of variation within the cell line, a co-efficient of variations of about 13%. But if you look at a cell line that is being maintained in the presence of drug and compare to one that has been out of drug for a year, the level of intensity per cell is about the same within a coefficient of variation of about 13%.

Dr. Reading: Another technical question?

Dr. Dalton: I have been asked to comment about untreated myeloma patients. In untreated myeloma patients which we have looked for p-glycoprotein, we have not found it. On the otherhand we have looked at about 14 patients with drug refractory disease and these are patients that are refractory to VAD and have seen a whole host of other drugs. Six out of the 14 have had p-glycoprotein at a level comparable to a 10-fold resistant cell line. So we are finding p-glycoprotein in drug resistant patients. We have also looked at the function in terms of drug uptake in those patients and found that the amount of drug uptake being vincristine or daunorubicin is decreased, relatively, to a sensitive line.

Dr. Favrot: I will ask Dr. Andersson to speak on purging with aldoifosphamide derivatives.

Dr. Yeager: I wanted to get an idea of how stable these compounds are.

Dr. Andersson: Yes, indeed. I do have an idea of how stable they are. The ifosphamide acetyl is very stable at neutral pH in PBS. The half-life at room temperature is clearly in excess of 80 hours, somewhere in the order of 80 to 85 hours. The aldoifosphamide acetyl is almost as stable but the half-life is around 60 hours.

Dr. Yeager: With 4-HC, the problem is that it has to be shipped in dry ice, and we keep it in the freezer most of the time, and the aqueous solution has a short half-life.

Dr. Andersson: Yes. I do not want to try to downgrade the efficacy of 4-HC but you are right. Technically, there may be some problems with the handling. That is one of the minor reasons for attempting to manipulate this type of compound. When we have new drugs in powder form, they are totally stable as far as we can judge. In PBS, at room temperature, they will be stable unless you are mixing the drug in serum where you have esterases provided by the serum.

Dr. Reading: I would like to ask, Dr. Seiber, the merocyanine is a fluorescent dye and is it possible to first of all look for minimal residual diseases. Is there enough of a differential in binding, to certain leukemia cell types, that you can pick up high binders that way? Secondly, have you looked at this question (that) I eluded to in the introduction -- about -- what is going on in CR? If you go to second or third CR patients, where you know that at presentation or relapse they were high binders, are those cells apparently normal in terms of their binding?

Dr. Seiber: I have not done these experiments myself, but Jay Valenski has done a series of experiments that addresses your question. If you follow a leukemia patient from remission induction to remission, in the periphery you cannot use a drug as a diagnostic tool in marrow because there are immature erythroid cells that give you a positve response. If you look at staining properties of leukocytes, you will find that, without exceptions, in untreated disease or in relapse disease, leukocytes bind a lot of merocyanine. Now if remission induction is successful, the percentage of positive cells drops preciptiously. Then you see two patterns. In some patients, it stays flat at the base line. These patients go into prolonged remission. It is the second group where within 2 to 3 weeks the count creeps back up and reaches pre-treatment level. These patients look okay to the hematologist. They are in remission. But up to 15 weeks later, they relapse. Now, if you use a cell sorter and fish out these merocyanine positive cells, they look normal. I mean you may actually find polys in it that look perfectly okay.

Dr. Reading: That is a predictor for relapse?

Dr. Seiber: It has only been used on 12 patients and I think it is difficult to advertise the method as a reliable method to predict relapse. But in these 12 patients it has panned out. There was one incidence of a fake positive which however, could clinically sorted out and that was a transient little "blip" of positive cells. The patient had a fever. One knows now, if, for instance, you take peripheral blood cells from a patient without cancer but somebody who has, for instance, mononucleosis, you get a fake positive response. But this can be sorted out. I think it has some potential, but it is not extremely sensitive if you do mixing experiments with leukemia cells and normal. You can pick it up. The cut off point is about 1 in 1,000. So we have seen more sensitive methods yesterday.

Note: Although the manuscripts of Dr. Sulabha Kulkarni and Dr. Gordon Phillips on "In Vitro Long-Term Cultures" do not appear in this publication, it was felt important to include the discussions which related to their presentations.

SESSION IX - INTERNATIONAL PROTOCOLS

PARMA INTERNATIONAL PROTOCOL: Pilot Study of DHAP and of Involved Field Radiotherapy and BEAC with Autologous Bone Transplantation

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Patients with advanced diffuse non-Hodgkin's lymphoma are rarely cured of their disease after conventional chemotherapy fails. However, experience gained in Burkitt's lymphoma has shown that these lymphomas are still sensitive to intensive chemoradiotherapy after failing treatments with conventional dosage (1-6). With bone marrow transplantation providing hematologic support, it has been possible to administer intensive chemoradiotherapy and to cure some children or adults with disseminated, aggressive non-Hodgkin's lymphoma (7-21). Many questions about such a program remain unanswered. The many prognostic variables in adults and the absence of long-term follow-up in many of the studies of first-line conventional therapeutic regimens (22-28) have led to confusion in analyzing the world experience. The appropriate place for high-dose chemoradiotherapy and bone marrow transplantation has not yet been determined, despite encouraging results in numerous pilot studies. For this reason, several groups of investigators active in autologous bone marrow transplantation have suggested that a response to preceding therapy may have prognostic importance in the outcome of autologous bone marrow transplantation in patients who have relapsed (11, 12) and confirmation of these findings was the major achievement of the 100 patients retrospective analysis reported in 1987 in the New England Journal of Medicine (29). Since 1987, an international group called PARMA was set up among various Bone Marrow Transplant Centers and the objective of this group was reviewed in detail in the 1987 Houston meeting (30).

The purpose of this report is to review the pilot study of the PARMA protocol which was done in 1986 and 1987. The objective of this study was to confirm the response rate of the DHAP regimen and to pilot the conditioning regimen of involved field radiotherapy and BEAC (30).

PATIENTS AND METHODS
Patients

Fifty patients, 16 to 60 years old, at first relapse of intermediate (30) or high grade (20) Non Hodgkin's lymphomas were included in the study from 19 centers in the world. They all had achieved a previous first CR under an adriamycin containing regimen. Fourteen were female and 36 male. They also had normal marrow at relapse, no CNS relapses and all give an inform consent according to the PARMA protocol and each institution's rules. All patients but one were evaluable for response to the rescue regimen.

Methods

All patients were submitted to the DHAP regimen as reported by Velasquez (31) and as shown in Figure 1.

Among responding patients, 20 were submitted to the involved field radiotherapy and BEAC protocol as shown in Figure 2 (2 patients previously irradiated at site of bulky disease did not received radiotherapy according to the PARMA protocol). In addition 2 patients were grafted with cyclophosphamide and TBI by individual center decision and 7 were not grafted.

Autologous bone marrow transplantation was performed as previously reported (29). Bone marrow collection was done 21 to 28 days after DHAP 1.

Agent	Dose	Day	Route	Time
Dexamethasone	40 mg	1-4	I.V.	15 min.
Cisplatin	100 mg/m^2	1	I.V.-C.I.	24 hr.
Cytarabine (ARA-C)	4 g/m^2	2	I.V.	2 g/m^2 in 200 ml normal saline 3 hrs q12 hrsx2

Figure 1. DHAP Regimen

Wk	Mon		Tues	Wed	Thurs	Fri	Sat	Sun
1*	IFXRT	X	X	X	X	X	--	--
		X	X	X	X	X	--	--
2		X	X	X	X	X	--	--
		X	X	X	X	X	--	--
3	BCNU		VP-16	VP-16	VP-16	VP-16	--	--
	**		Ara-C	Ara-C	Ara-C	Ara-C		
			CYT	CYT	CYT	CYT		
4	BMT							

*: Day 20 post DHAP 2
**: Day 34 post DHAP 2

Figure 2. Radiotherapy and BEAC Protocol Schedule

RESULTS

Response to DHAP Regimen

Forty-eight patients are evaluable. One was in complete remission before DHAP (complete surgical excision) and one was lost to follow up. Of the remaining 46 patients, 28 were responders (58%) including 7 CR (25% of responders) and 21 PR. No difference was observed between intermediate grade and high grade patients (11/26 PR, 5/26 CR intermediate grade; 18/22 PR, 2/22 CR high grade).

Toxicity to DHAP

Severe neutropenia was observed in 22 courses (22/50 = 44%). Thrombopenia with less than 50,000 platelets was recorded in 19 (34%). Severe renal impairment was found in 7 (14%), severe gastro-intestinal in 6 (12%), cytosine related neurologic disorders in 2 (4%) and sepsis in 8 (16%). One reversible myocardiopathy was also observed.

Two toxic deaths occurred. One after DHAP 1 in Madrid (sepsis) and one after DHAP 2 in Lyon (hemolytic and uremic syndrome). Both were observed in responding patients.

Treatment of Sensitive Relapse Patients after DHAP 2

Twenty of the 29 patients (28 responding and the one already in CR) had received as scheduled the involved field and BEAC protocol as shown in Figure 2. Twelve were still with evaluable disease (PR to DHAP). Among them 11/12 were converted in CR after ABMT (91%

response rate to BEAC). Median time to reach 1,000 white cells was 15 days, 500 polynuclears 15 days, 200 polynuclears 13 days and 50,000 platelets 25 days. Two toxic deaths were recorded (hepatic and renal early failure and pulmonary bleeding). One early relapse was also observed and 17 were in CR after ABMT. Six relapses and 9/20 are still alive disease free with a median follow-up of 16 months as shown in Figure 3.

Two patients were grafted with a classic cyclophosphamide and TBI regimen. They both are alive disease free with 6 months and 11 months of follow up. A total of 12/22 of the grafted patients are alive disease free (45% overall survival disease free).

The 7 other responders were not grafted because of toxic death (2) or individual center decision (5). One refused treatment and died. Four had received 4 to 6 additional courses of DHAP. None of the 7 are alive disease free at the present time.

COMMENTS

The 58% observed response rate is a clear confirmation of Velasquez data and allowed us to conclude that DHAP is a very active salvage regimen for relapsed lymphomas. The 25% CR rate is a little bit low, but as expected from M.D. Anderson reports (30%). The absence of difference in response rate between intermediate and high
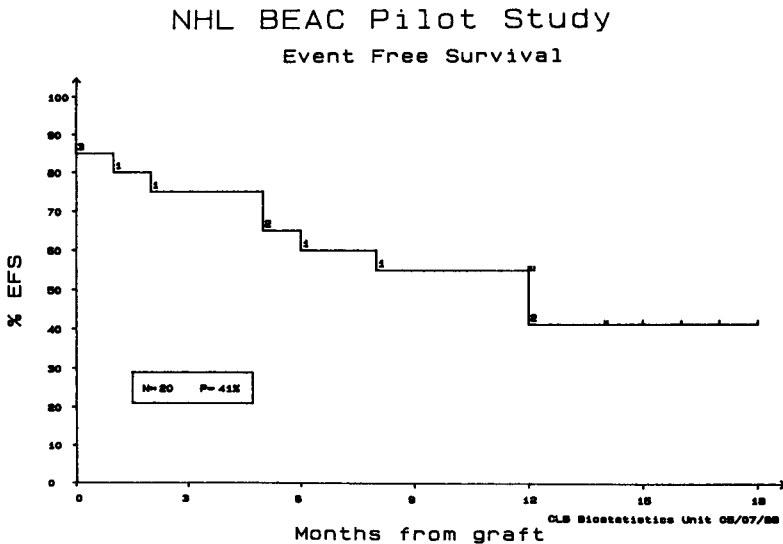

Figure 3. NHL BEAC Pilot Study. Event Free Survival.

grade lymphomas is also a major finding from this pilot study. These findings are as expected for this prospective PARMA study and confirms the relevance of our approach.

The reported toxicity from 19 different centers in the world is comparable to the Velasquez report. This toxicity is acceptable in a cooperative group. However, despite its low incidence (14% PARMA, 20% Velasquez), the renal toxicity was a major problem just before ABMT and this is certainly the major risk in using this protocol.

The 41% long term overall event free survival with BEAC and the 44% long term overall disease-free survival for the 22 grafted patients are major confirmations of our previous reports (29). This showed that a group of sensitive relapses NHL from 19 centers, entered consecutively in the same rescue protocol, can lead to 40-45% survivors with ABMT as reported previously from various centers and as a retrospective finding (29).

The major conclusion of this pilot study is that DHAP is active and of tolerable toxicity. In addition, this study confirms that 40 to 45% of sensitive relapse patients can be long term survivors after ABMT with not more than 10% toxic death. The ongoing prospective and randomized PARMA study is based on these very encouraging results.

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A PILOT STUDY OF DOUBLE ABMT IN ADVANCED NEUROBLASTOMA (32 Patients)

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Stage IV Neuroblastoma in children over one year of age at diagnosis is the most common childhood malignancy before the age of five. Considerable progress has been observed since 1982 in this disease (1–7). A variety of combination therapy regimens have achieved initial response rates up to 90%. Our group has reported in 72 consecutive patents with stage IV neuroblastoma over 1 year of age at diagnosis (4,8 and unpublished). Consolidation with Vincristine, Melphalan and total body irradiation followed by purged autologous bone marrow transplantation (ABMT) has had a significant impact in the progression-free survival at 2 years (45% versus 8% previously, p < 0.005) (8). However, relapses are still observed after 2 years and only 25 to 30% of the grafted patients are projected to be alive disease-free at 5 years. Despite this clear improvement the long-term outcome remains questionable for 75% of the patients in whom the initial response rate is high enough to consider intensification. Our initial report of the LMCE first prospective study (8) showed no relapse between ABMT and day 120. The majority of relapses were observed between 4 and 12 months post-ABMT and relapses were reported as late as 27 months post-graft. Our objective was to test whether double conditioning regimen and double harvesting (i.e. in vivo purging) were able to decrease early relapses and to avoid late relapses after ABMT.

PATIENTS AND METHODS
Patients

Thirty-two patients were included in this pilot study from 30/04/85 to 01/02/85. Twenty were male and 12 were female; median age was 25 months. Among the 8 patients selected as still sensitive to VP16 and CDDP after relapse (5), 0 were relapses post-ABMT. At time of ABMT, 4/8 had bone marrow involvement, 6/8 bone involvement at MIBG scanning, 5/8 had residual tumor after surgery and 6/8 had active metabolites in urines. Twenty-four patients were stage IV disease over one year of age at diagnosis. Eight were part of the LMCE prospective study and had received VP16 and CDDP for minor response to conventional induction therapy (5). Sixteen were not part of the LMCE prospective study and were referred to Lyon for ABMT in partial remission after conventional induction. Among these 24 patients, 15 (62.5%) had bone marrow involvement at time of ABMT, 16 (16%) had bone involvement at MIBG scanning, 14 (58%) had residual local tumor after surgery and 11 (46%) had catecholamine secretion in urines.

Methods

Following inclusion in this protocol, two courses of VP16 and CDDP were given in an attempt to achieve a further response, as previously reported (5). The parents of all the children involved in this study gave informed consent and understood the experimental nature of the protocols and their probable toxicity.

As shown in Figure 1, a first harvest was scheduled 4 weeks after the last chemotherapy. Bone marrow was immunomagnetically purged as previously reported (8,9). The first conditioning regimen, as shown in Figure 2, was a combination of BM26, BCNU and CDDP or VM26, BCNU and CBDCA as previously reported (10). A second harvest, as shown in Figure 1, was performed 60 to 90 days post-ABMT 1 and a second immunomagnetic purge was undertaken at this stage. The second conditioning regimen was a combination of Vincristine, Melphalan and total body irradiation as previously reported in detail (7,8).

Event-free survival curves were used and day 0 was defined as the date of ABMT 1. Toxic deaths were taken into account and were recorded as events comparable to relapses.

RESULTS
First Harvest and First ABMT

The median number of nucleated cells harvested was 3.2×10^8/kg (0.19 to 6.8×10^8). The median number of GM.CFU harvested was

PR after induction 24

Relapse 08

-      VP16 CDDP x 2 (Phillip et al. J. Clin. Oncol.,

1987, 5, 941-950)

-->   Harvesting 1 + Purge

BCNU   -----------
VM26   ABMT 1
CDDP or CBDCA   -----------

-->   Harvesting 2 + Purge

VCR   ABMT 2
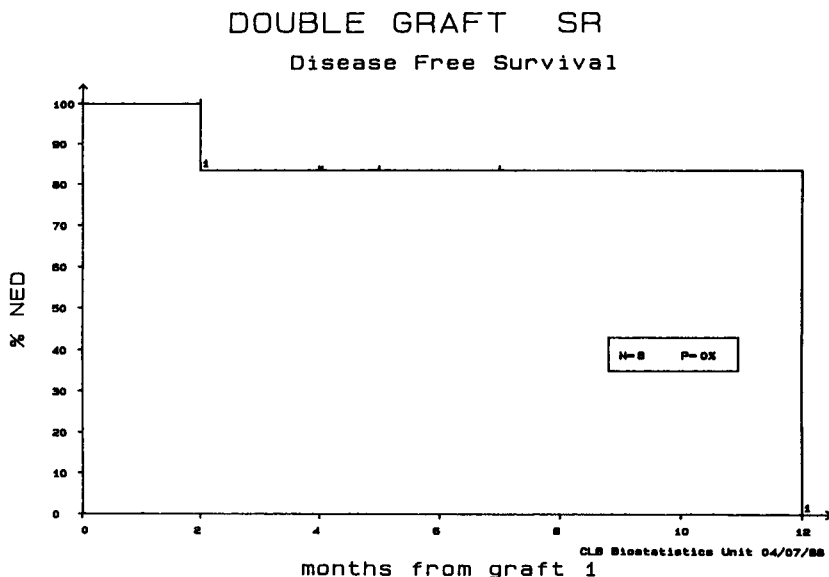TBI   -----------

Figure 1. Double ABMT in Advanced Neuroblastoma

Figure 2. Double graft. SR. Disease-free Survival

$40.5x10^4$/kg (1.14 to $131x10^4$). A median of $0.57x10^8$ nucleated cells/kg were grafted at ABMT 1 (0.25 to $1.8x10^8$/kg) and a median of $5.6x10^4$ GM.CFU/kg were grafted (0.2 to $14x10^8$/kg). Twenty-five patients were evaluable for response to BCNU, VM26 and CDDP or CBDCA (3-non-evaluable and 4 too recently included). Seven (28%) were in complete remission 2 months after this therapy and 15 improved by 50% or more (partial response). A total of 22/25 (88%) were responders to the conditioning regimen, 2 were stable and only 1 had progressive disease. The median time to reach 1,000 white cells after ABMT was 21 days (12-37) and 26 days (11-57) to reach 500 polynuclear, 20 days (10-37) to reach 200 polynuclear and 28 days (12-64) to reach 50,000 platelets. One toxic death was observed (sepsis) and 3 patients had significant morbidity (2 sepsis, 1 allergy). Median time to return home was 30 days post-ABMT.

Second Harvest and Second ABMT

It was not significantly worse than first harvest and was generally performed 60 to 90 days post-ABMT 1. The median number of nucleated cells harvested was $3.24x10^8$/kg (1.3 to $7.2x10^8$/kg) and the median number of GM.CFU harvested was $32.5x10^4$/kg (4.1 to $7.5x10^4$/kg). The median numbers of nucleated cells and GM.CFU grafted per kg were respectively $0.5x10^8$ (0.21 to $1.8x10^8$) and $2.2x10^4$ (0.3 to $6.7x10^4$). Twenty-three patients were evaluable for response after the second conditioning regimen. Sixteen (69.5%) were in complete remission 2 months after ABMT 2 and 3 (13%) in partial response. The response rate after ABMT 2 is 83%. Only 2 had progressive disease. Median time from ABMT 1 to ABMT 2 was 3 months. The duration of aplasia for the second ABMT was longer. Median time to return home was 40 days, with 32 days median to reach 1,000 white cells (12-111), 36 days median to 500 polynuclear (11-111), 30 days median to reach 200 polynuclear (10-111) and 84 days median to reach 50,000 platelets (16-600). Six toxic deaths (19%) were recorded (2 CMV, 2 VOD, 1 pneumocystis and 1 non documented interstitial pneumonitis). Four patients experienced significant toxicity (sepsis) and mucositis, diarrhea and pain were generally observed as previously reported with this particular conditioning regimen (8).

Survival

As shown in Figures 2 and 3, survival was good considering the very bad prognosis group of patients who entered this phase II study. Among the 8 sensitive relapse patients, 3 are still alive with short follow up and it is too early to draw definitive conclusions (Figure 2). Among the 24 patients consolidated with this double graft program after initial partial response to conventional therapy, 10 are still alive,

progression-free, 9 to 36 months post-ABMT. No relapse was observed after 12 months post-graft 2, but only 6 patients are progression-free with more than one year follow-up (Figure 3).

COMMENTS

The major objective of this study was to show whether these 2 regimens were able to produce good response rates without major toxicity and to conclude on the feasibility of two successive harvests with two immunomagnetic purges of the harvested marrow in the setting of highly pre-treated neuroblastoma. The first conditioning regimen was not toxic (i.e. 1 toxic death and 30 days median to return home) and quite effective with a response rate of 88%. However, the CR rate of only 28% is not good enough to rely only on this intensification regimen to increase the survival of the disease. The second harvest was not a problem in terms of nucleated cells harvested ($3.2x10^8$/kg versus $3.2x10^8$/kg at first harvest) even for extremes (minimum $0.2x10^8$/kg at first harvest and $1.3x10^8$/kg at second). The number of GM.CFU harvested was also very similar at first and second harvests. The number of cells lost in the purging process was not different from one harvest to the other and the grafts were also similar. However, the time to return home (40 versus 30, and extreme 35 to 120), the time to reach 500 polynuclear (36 versus 26, and extreme 57 to 111) and the platelet recovery (84 versus 28, extreme 64 to 600) were significantly worse at second ABMT.
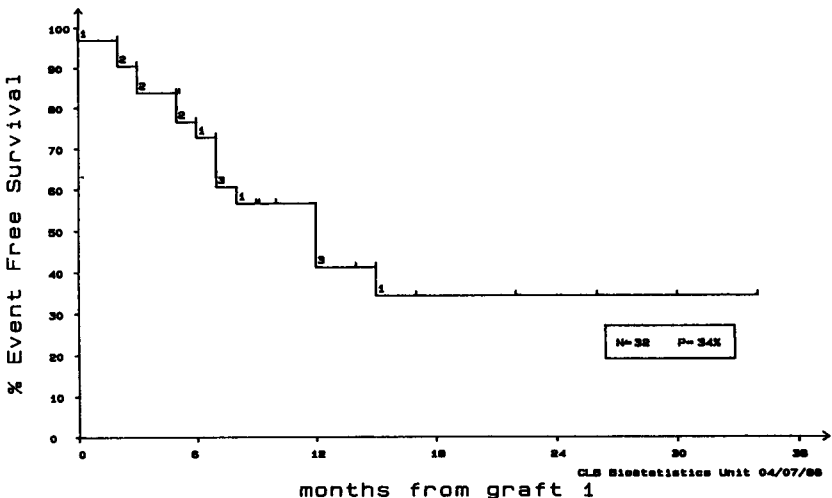

Figure 3. Double graft. Event-free Survival

The role of the micro-environment damage after first ABMT and the host marrow inability to accept a graft are putative explanations and should be reviewed in detail in future double graft programs. Some of the grafts also had a very low recovery because of an excess of CD8-leu7 cells and anti-CD8 monoclonal antibody was sometimes effective, as previously reported (11). The second conditioning regimen is very effective as previously reported (7,8). The high CR rate is encouraging and could be responsible for an improvement in cure rate. However, as previously shown by various teams, efficacy and toxicity and not separable and the cost of CR in terms of toxic death is still very high (19% at second ABMT) and remains the major obstacle to widely disseminate the LMCE regimen.

Comparison of this group of 32 patients and even of the 24 partial responders with the prospective trial of the LMCE (8) is not possible because of selection criteria. The number of patients with bone marrow, bone or local involvement is very different in this group of clearly worse prognosis compared with the LMCE patients (8). The median follow up in this group (18 months) is also too short to allow definitive conclusions. However, the number of relapses between 4 and 12 months post-ABMT 1 is still very similar to previous reports indicating few chances for this regimen to improve the number of patients alive progression-free at one year. The absence of late relapses in this group is not yet significant with only 6 survivors over 12 months post bone marrow transplantation and will be the major interest of further follow-up in this group.

ACKNOWLEDGMENTS

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General Discussion

Dr. Kaizer: This is not directed at any particular speaker or tumor, but a general challenge I think to the entire group. And that is if you assume that the intent of ABMT is curative, I would like to know what we learn in phase II data by reporting PRs? And if the answer is nothing, let's stop it.

Dr. Philip: I want to comment on this point. It is very, very clear from the lymphoma model that when you get a 75% response rate with 90% PR, you will never reach cure with this kind of regimen. However, when you got a 50% response rate with half of the patients in CR, cures will be obtained. So from my point of view, response rate means nothing. That is why the Boston study in breast carcinoma was so enlightening when they observed CRs. That is why I think breast carcinoma is a very exciting field.

Dr. Roger Herzig: There are 2 problems with that, Herb. One is that for these patients that are undergoing this therapy, this represents the best available therapy. The fact that you have biologic activity gives you some idea of where to go next. For example when you have a 20% response rate with DTIC in advanced melanoma it is not surprising that you do not have any good adjuvant data. In that respect it is important to report that kind of data. Number two is that there are some patients, a significant minority of the patients, that have only a PR because of our definition. Probably they have a better than a PR response but they do not undergo surgery again. For example in our phase II thiotepa with colon cancer we have 8 out of 10 were PRs. However, the median duration of those responses was 8 months and there is still somebody out past 2 years. So that person probably has a better than PR but there is something different about that patient.

Dr. Kaiser: Roger, your last point simply says that you ought

to pathologically stage your responses I think. The question I had was what do you learn?

Dr. Frei: I think that responses have to be qualified. We learned 30 years ago that PRs, for example, in ALL did not significantly effect survival. We have to be concerned, I think, about generalizing from some of the hematologic malignancy models because they generally told us that when you combine agents at full or nearly full doses -- agents which generally produce just partial responses -- that you would get a high complete response rate. Now, Roger, you are telling us that that is probably not true for melanoma and maybe breast cancer is intermediate. So (what) we may be facing here a biological characterization of the disease rather than a pharmacologic principle. If you would argue that in melanoma 10 to 20% of the cells are pliatropically resistant to all alkylating agents that would explain in fact what you observed. That presumably does not happen in Hodgkin's disease, non-Hodgkin's, maybe testes, probably acute leukemia. That has to be subjected to experimental study. That would mean that the proposed Milan study might not work if there is pliatropic resistance. But it is obviously an important thing to test, particularly in breast cancer.

Dr. Gianni: A brief comment. In fact in Milano we are not using only alkylating agents. In all of our protocols we are using a variety, looking for no cross-resistance and we are using phase specific and non-phase specific agents in the combination in which you try to exploit the advantage of putting the cell into cycle.

Dr. Frei: I appreciate that and that is very important. It turns out, however, that there maybe less cross-resistance among alkylating agents than there is, for example, in the multidrug resistance arena that is etoposide vs. an anthracycline, etc.

Dr. Jeff Herzig: In terms of the question about combinations, I have been interested in this for a long time. One of the things is there has not been a careful examination of most of the drugs in combination. There are a lot of combinations especially in leukemia and lymphoma protocols. But very few has there been an actual phase I effort to establish the maximum tolerated dose of the agents in combination. So these are the drugs that we have seen to have some activity as single agents at high dose in melanoma. I listed them in the order that they seem to be active as single drugs. You are thinking of putting them together. The melphalan produces mucosal toxicity and an unexpected problem was that it attentiated BCNU lung toxicity even though melphalan has no lung toxicity of its own -- even at maximum tolerated doses -- so that limited the ability to put BCNU and melphalan together, which looks superficially like they should be

a very good combination because the dose limiting toxicity of BCNU was lung. It also has some liver and some CNS toxicity. For thiotepa, CNS toxicity was dose limiting and it also produces major mucosal toxicity. So clearly it is not going to be a very good drug to put together either with BCNU because we expect that we will see serious interaction of CNS toxicity. It is not a good drug to put together with melphalan because of the mucosal toxicity that is so prominent with either of them. Nitrogen mustard also produces dose limiting CNS toxicity in the marrow autograft setting and is not a good drug to combine with either of those two. Of the drugs that have been looked at in combination in leukemia and lymphoma, cytoxan is the one that you can usually put together with the other agents and not sacrifice dose. Dose limiting toxicity for cytoxan as you know, is carditis and it produces only modest other toxicities. It may potentiate the lung toxicity of TBI to a modest degree and it has some mucosal toxicity but that is about it. It turned out though that with BCNU there probably is a significant additive toxicity that again is more than you might have suspected in terms of pulmonary complications especially and with thiotepa there seems to be a potentiation of thiotepa, CNS toxicity even though the cytoxan does not have any noticeable CNS toxicity at all. So I think that we run into problems. The final point is that we do not have any data about cytoxan as a single agent in melanoma. So I would say that we ought to find out first what cytoxan does as a single agent. There is not much hope for even 2 drug combinations of the active drugs that we now know for melanoma and that is why I said that I think that if I were going to try to do something in melanoma, that the best way we could go with our current data would be multiple courses with the drug at the maximum tolerated dose.

Dr. Frei: I think those are very important points. Another point is that we have only got 3 or 4 drugs that you indicate, and that kind of limits our range. There are other alkylating agents like chlorambucil, others agents as well. I think that we have to examine more of those up into the high dose ABMT range to see if we can identify ones that are ideal for combination therapy. I think we have no more questioners, so it is time to break for coffee.

SUMMARY

CURRENT STATUS OF AUTOTRANSPLANTS
IN ACUTE LEUKEMIA

Robert Peter Gale

This meeting reviewed considerable data of autotransplants in acute leukemia. In this summary, I consider three issues: (1) are there convincing data that results of autotransplants are superior to those achieved with chemotherapy alone; (2) are there convincing data of a role *in vitro* bone marrow treatment in preventing relapse; and (3) what are reasonable future directions?

To analyze whether autotransplants are effective, it is necessary to consider results achieved with chemotherapy alone in acute leukemia. Two concepts are important in this regard: patient selection and time-censoring. Patient selection is an important determinant of treatment outcome in acute leukemia; one example is age. Most autotransplant studies involve persons <40-50 years; median age is about 20 years and <5% of recipients are >40 years. Thus, autotransplants in acute myelogenous leukemia (AML), for example, should be compared to chemotherapy in a comparable age cohort. If long-term outcome of chemotherapy is about 50% in children <16 and about 30% in adolescents and adults <40 then the combined chemotherapy outcome should be about 40%.

Time censoring is a second important concept. Most autotransplant studies involve persons in remission for periods of 1 month to 1 year; median is about 6 months. This means that persons relapsing before receiving an autotransplant are excluded from these studies. Figure 1 shows that the impact of time-censoring on a chemotherapy outcome in which the uncensored 4-year, leukemia-free survival is 40%. If persons relapsing at 3, 6 months or 1 year were excluded, the outcome would appear to be substantially better, 45%, 50%, and 60% respectively. The impact of time-censoring is greatest in situations where the initial relapse rate is high, such as AML in 2nd remission.
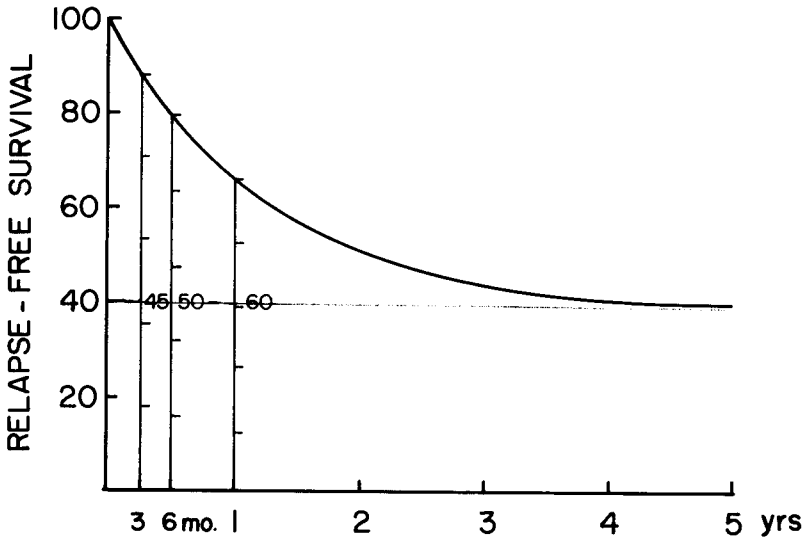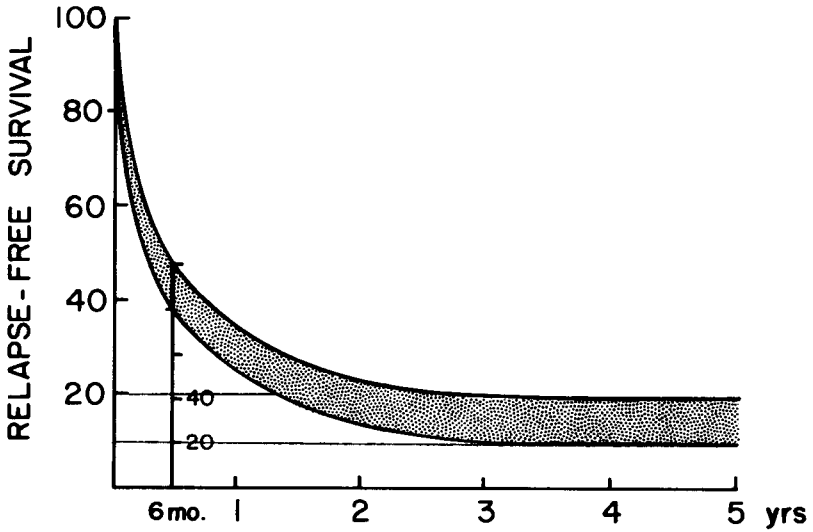
Figure 1.

Figure 2.

Here the 10-20% reported long-term survival would increase to 20-40% with censoring (Figure 2).

Another approach to analyzing results of autotransplants is to determine whether the remission duration achieved with an autotransplant exceeds the length of the preceding chemotherapy induced remission. A longer 2nd remission is presumed to indicate efficacy of the autotransplant. The scientific basis of this approach is unclear since relatively few data are reported regarding remission "inversion" in persons treated with chemotherapy alone. Although there is likely to be an overall correlation between the length of 1st versus 2nd remission, some persons may have longer 2nd remissions following rescue chemotherapy. This is evident in the 10-20% of long-term survivors of AML who previously relapsed.

If one compares results of autotransplants in acute leukemia with those achieved with chemotherapy in an age and time-censoring matched cohort, it is evident that there are no substantial differences in AML or acute lymphoblastic leukemia (ALL) in 1st remission. Slight, but not significant, trends exist for these diseases in 2nd remission and in more advanced disease. These data are summarized in Table 1.

The second issue to consider is whether *in vitro* treatment is effective in preventing relapse following autotransplantation. To answer this question, it is necessary to analyze transplant outcome in recipients of transplants from genetically-identical twins or from HLA-identical siblings without GvHD. These data are summarized in Table 2.

Table 1. Autotransplants vs. Chemotherapy in Acute Leukemia[1]

Disease	Remission	Relapse-Free Survival	
		Autotransplants	Chemotherapy
AML[2]	1	40	40
	2	30	20
ALL	1[3]	50	50
	2	20	0-40[4]

[1] Literature review and this meeting
[2] Corrected for ages 0-40 years and 6-month time censoring
[3] High-risk children and adults
[4] Results depend on whether relapse occurred < > 18 m of remission

Table 2. Autotransplants

Disease	Remission	Relapse Autotransplants	Control[1]
AML	1	60	60
	2	70	80
ALL	1	50	50
	2	80	90

[1]Twin or HLA-identical sibling without GvHD

As indicated, relapse rates in autotransplants in AML and ALL in 1st remission are similar to those observed in twins who receive normal bone marrow from their donor. This suggests that relapse in the recipient can account for the leukemia recurrences observed after autotransplants without the need to implicate the reinfused bone marrow. This observation should not be taken to mean that the reinfused bone marrow is not the cause of relapse but rather that persons with leukemia in the cryopreserved bone marrow aliquot are precisely those most likely to have leukemia cells in the bone marrow remaining in them. Thus, there is competition in these persons between two potential sources of relapse. Nevertheless, all these individuals will relapse from one or the other source. To alter this scenario, it is necessary that leukemia be completely removed both from the recipients bone marrow and from the cryopreserved bone marrow. It seems unlikely that either is currently achievable. Consequently, relapse from the infused bone marrow, if it occurs, is presently of little import in determining relapse.

Because of these considerations, it is also impossible to critically evaluate its efficacy; effective or ineffective treatments would produce identical relapse rates in this scenario. A similar conclusion applies to persons without residual leukemia prior to their autotransplant. They have no likelihood of relapse and their cryopreserved bone marrow contains no leukemia cells. Consequently, effective and ineffective *in vitro* treatments would produce equivalent results. The problem for the investigator is that it is not possible to identify cured and non-cured person *a priori*. A benefit of *in vitro* treatment can only be observed when it is useful in those rare (if any) persons cured of leukemia but with leukemia contamination of the cryopreserved bone marrow.

A further consideration is that with current conditioning regimens the impact of effective *in vitro* treatment will be small, about 5-10%. Consequently it would require hundreds of subjects in randomized trials to determine if *in vitro* treatment is effective. Because of these considerations it seems premature to probably perform clinical trials of *in vitro* treatment presently.

At this meeting data from a recent survey showing a relationship between the interval from remission to autotransplant and relapse rate was reported. It was suggested that the lower relapse rate associated with longer delays reflects the efficacy of *in vivo* cytoreduction of leukemia cells. Another suggestion was that these data are indirect evidence of the efficacy of *in vitro* treatment. The reasoning is that continued chemotherapy reduces the likelihood of leukemia cells being harvested in the cryopreserved bone marrow and that *in vitro* treatment is most effective when the number of leukemia cells is low. However, the impact of transplant delay is best explained by the effect of time-censoring and there are little or no data to indicate that these other mechanisms operate.

In one recent analysis from the EBMTG, *in vitro* treatment with mafosphamide was associated with a reduced risk of relapse in AML in 1st remission. These data were interpreted as showing a benefit of *in vitro* treatment. However examination of these data indicate that the relapse rate in untreated recipients is similar to twin allograft recipients. This suggests that if the *in vitro* treatment is effective, the mechanism must be via reducing relapse in the recipient rather than by removing leukemia cells from the cryopreserved bone marrow.

In summary, data presented at the conference do not convincingly prove either that bone marrow autotransplants are superior to chemotherapy or that *in vitro* treatment is presently useful. What should be done to resolve this problem? First, the high rate of relapse in the recipient must be reduced by developing more effective conditioning regimens. Until this is accomplished, it is unreasonable to expect progress. Whether this can be achieved is unknown. Evaluation of the efficacy of *in vitro* treatment remains difficult under these circumstances and it is premature to reach conclusions. Whether *in vitro* treatment should continue to be done when results are unevaluable is an important question.

A final encouraging observation is the proportedly lower risk of leukemia relapse following autotransplants in which the bone marrow was treated *in vitro* with mafosphamide. If true, this suggests that autotransplantation might work by a possible immunotherapeutic mechanism. This approach might then be applicable to other persons with acute leukemia and whether or not they receive autotransplants.

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