Autologous Bone Marrow Transplantation

Proceedings of the Fifth International Symposium

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August 22-25, 1990
Omaha, Nebraska

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Publication of these proceedings is supported
by an educational grant from the Oncology Division
of Bristol-Myers.

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Printed in the United States of America.

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PREFACE

The Fifth International Symposium on Autologous Bone Marrow Transplantation was a real success! The title of the Proceedings, "Autologous Bone Marrow Transplantation", does not reflect the significance of peripheral blood as an alternate source of stem cells. Peripheral stem cell transplantation was one of the highlights of this symposium. Another highlight was the session on breast cancer. The rapid improvement of results of high dose chemotherapy with stem cell support in this disease may have caused a headache for the insurance carriers but not for the clinical investigators and certainly not for our patients! In the laboratory new strategies are under development which may have reached the clinic by the time of the next symposium. Last but not least, explosive progress with hematopoietic growth factors introduces a new era in the field of high dose therapy and stem cell transplantation.

Over 4000 autotransplants were performed in 1989 and in 1990, a marked difference compared with 10 transplants in 1976! This expanding field needs cohesiveness of investigators to enhance effective progress. This symposium has been, is and will be, one of the main instruments to achieve this. Therefore, plans are underway for the next symposium to be held here in July 1992. Again, welcome to Omaha!

ACKNOWLEDGEMENTS

The organizers thanks are due to the Oncology Division of Bristol-Myers and to the Administration of the University Hospital for their generous grant in support of this symposium.

The administrative staff, Mrs. Shirley Yeich and Mrs. Deanna Hansen, were instrumental to the success of this symposium. Their patience, endurance and energy is greatly appreciated.

The meticulous editing and the compilation of the proceedings were done by Mrs. Frances McEntee, for which the organizers are very thankful.

EVALUATION OF AUTOLOGOUS BONE MARROW TRANSPLANTATION IN ACUTE MYELOID LEUKEMIA: A PROGRESS REPORT ON THE MRC TENTH AML TRIAL

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INTRODUCTION

Several single centre series suggest that autologous bone marrow transplantation may be the best method of maintaining first remission of acute myeloid leukemia (1-5). Indeed the experience is not apparently inferior to the results achieved by allogeneic transplantation. A major limitation in accepting the single series or registry results is the concern that the patients included have been, to a greater or lesser extent, selected by "time censoring". That is, that they are patients who have survived in remission - usually for 4-6 months - until the autograft was done, and therefore were at a lower risk of relapse anyway (6, 7). If series involving chemotherapy alone are similarly time censored, the 5 year outcome is not substantially inferior to the autograft results. Since the outcome of receiving an autograft at second remission is not significantly inferior to a graft in first remission, it might be more appropriate to reserve the use of autograft only for those patients who relapse and achieve a second remission.

While delaying the autograft would have the advantage of avoiding unnecessary transplants for patients who were already cured, it does have some potential pitfalls. First, the prospects of achieving a second remission depend largely on the length of first remission, and are probably about 20-30% for patients relapsing within a year. Second, the rate of relapse in second remission, as in first remission, is most marked in the first six months, so a further time censoring bias is introduced to the second remission data. to compare the efficacy, in patients who have all received 4 intensive courses of chemotherapy, of (1) allogeneic versus autologous BMT versus no further treatment in first remission, (2) autologous BMT in first remission versus no further treatment (STOP) with late autograft in those who relapse and achieve a second remission. A subsidiary objective is to compare "DAT 3+10"

(Daunorubicin, Ara-C, Thiogmanine) with "ADE 10+3+5" (Ara-C, Daunorubicin, and Etoposide) as remission induction treatment.

MATERIALS AND METHODS

Rationale of Trial Design

The rationale of the MRC AML 10 Trial design (figure) has some specific points. It is intended that all entrants, who must be under 55 years of age, should have identical chemotherapy pre-transplant comprising four courses. The evaluation of transplantation (allogeneic or autologous) will be based on its potential additional value compared with no further chemotherapy. The induction and consolidation phases of chemotherapy are designed to achieve a high rate of remission, and subsequently be of adequate intensity (a) to achieve the maximum leukaemic cytoreduction as a means of "in vivo purging" of the harvested marrow, (b) to minimize the risk of relapse during the period of pre-transplant remission and (c) to provide as good a prospect as possible of curing patients without recourse to BMT which will be seen in the STOP arm. On the other hand it was hoped that the chemotherapy would not be sufficiently severe to prevent patients' progression through the protocol, or to damage the regenerative potential of the graft or increase the toxicity of a subsequent transplant.

The myeloablative protocol will be cyclophosphamide/TBI in first remission but will be Busulphan/Cyclophosphamide in second remission because uncontrolled data suggest that it may be superior to TBI in this context (8).

PROGRESS REPORT

The study opened in summer 1988. At June 1990, 494 patients were on study, including 109 children. Of 373 evaluable, 299 (80%) have entered remission (226/292 adults 77%; 73/81 children 90%). Of those who remitted, 71% achieved CR after course 1, and 24% after course 2, so about three quarters of the patients will receive 3 courses of post-remission induction therapy. Important haematological toxicity occurred after courses 3 and 4. The median times taken to regenerate neutrophils to 1.0 x 10^9/l, were 17, 18, 24 and 31 days after the four courses, respectively. Similar prolongation of thrombocytopenia was noted. Regeneration of platelets to 50 X 10^9/l being achieved respectively in 16, 19, 24 and 25 days.

Ten patients have relapsed during the time scale of the post-induction chemotherapy, which is an actuarial relapse of 7-8% in the pre-transplant interval, indicating that few patients are being lost to the transplant option, which was the main criticism of the autograft data. Ten patients have died in remission following course 3 or 4. Although this is not an unacceptable rate (10 out of 360 courses administered 3%), it is, in part, a reflection of the haematological toxicity of the protocol combined with the traditional approach of attempting to give post remission therapy on an increasingly outpatient basis. While the number of days in hospital did not reflect this (27, 22, 25, 25 days

after course 1-4); we have been able to show that the risk of death in these patients was related to the duration of hospital stay after treatment.

Of the 299 patients in remission, 52 have not yet reached the second randomization point and 22 are excluded because of early relapse (10 cases) or death in CR (12 cases). Sixty-three patients have an HLA matched sibling and have been allocated to an allograft. One hundred and fourteen patients have been randomized to early autograft (n=57) or STOP treatment with a potential late autograft (n=57). An additional 7 patients elected to be autografted, and 41 patients for various reasons of their own, or their physicians' choice, were not submitted to randomization but have usually collected all four courses of treatment. The reasons for election to stop have not yet been examined in detail.

Of the 63 patients intended for allograft, 5 will not receive it because of intercurrent relapse (3 cases) or death (2 cases). Nineteen are pending or have not yet been reported, and 39 transplants have been completed, of whom 29 are alive 2 to 21 months post-transplant. There have been 8 procedural deaths and 2 post-transplant relapses. Sixty-four patients were intended to receive an autograft but 2 died pre-transplant (1 relapse). Twenty-four have not yet been reported. Amongst the 38 cases transplanted thus far, there have been 4 procedural related deaths.

Fifty-seven patients have been randomized to STOP treatment and are candidates for late autografts, 2 have died in remission. No information on the scheduling or outcome of second remission autografts is yet available. Several of these patients will however be off protocol because the relapse occurred within 6 months of the bone marrow harvest.

Observations on Trial Progress

Recruitment and achievement of complete remission has so far been very good, particularly in children. The intensity of post remission courses three and four, has been reflected in important haematological toxicity which is consistent with its objective, namely to induce minimal residual disease. The disadvantage of this has been the occurrence of a few deaths in complete remission, but these have, in several cases, been related to the early discharge from hospital of these patients before recovery of peripheral blood counts. It has also contributed to refusal of the second randomization, by physician or patient. One of the major biases in the reported autograft series is the CR to ABMT delay, during which patients will relapse at a rate of around 5% per month. The total actuarial relapse rate during this delay in this trial is low at around 8%, thus vindicating the use of intensive post remission chemotherapy. It will be some time before it is know whether this will result in less relapses post autograft (and indeed post allograft) than usually seen.

Subsidiary objectives of this trial include efforts to study the quality of remission at the time of bone marrow harvest, by cytogenetic analysis, molecular-genetic studies of clonality, growth in long-term bone marrow culture, and the exploitation of inappropriate co-expression of antigens on

individual's leukaemic cells. The relevance of these features, together with the more usual prognostic factor analysis, will emerge in due course.

ACKNOWLEDGEMENT

The Trial co-ordinators are grateful to the 170 Haematologists in 115 centres in the United Kingdom, Eire and New Zealand, who are participating in this study.

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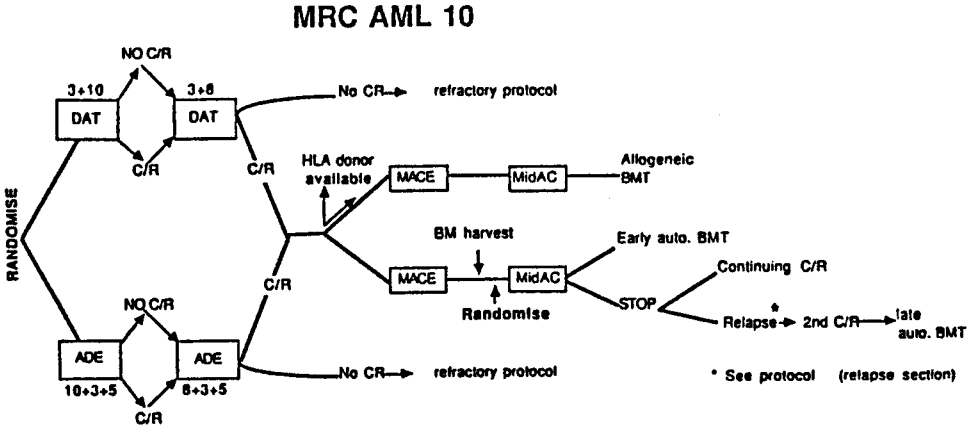
FIGURE 1



3 + 10 DAT	Daunorubicin 50 mg/m^2 IV push days 1,3,5 Ara-C 100 mg/m^2 12-hourly IV push days 1-10 days Inclusive 6-Thioguanine 100 mg/m^2 12-hourly orally days 1-10 Inclusive
3 + 8 DAT	Daunorubicin 50 mg/m^2 IV push days 1,3,5 Ara-C 100 mg/m^2 IV push days 1-8 Inclusive 6-Thioguanine 100 mg/m^2 12-hourly orally days 1-8 Inclusive
ADE 10+3+5	Ara-C 100 mg/m^2 12-hourly IV push days 1-10 Inclusive Daunorubicin 50 mg/m^2 IV push days 1,3,5 Etoposide (VP-16) 100 mg/m^2 IV daily 1-5 Inclusive
ADE 8+3+5	Ara-C 100 mg/m^2 12-hourly IV push days 1-8 Inclusive Daunorubicin 50 mg/m^2 IV push days 1,3,5 Etoposide (VP-16) 100 mg/m^2 IV daily 1-5 Inclusive
MACE	m-amsa 100 mg/m^2 IV days 1-5 Ara-C 200 mg/m^2 IV continuous infusion days 1-5 Inclusive Etoposide 100 mg/m^2 days 1-5
MidAc	Mitozantrone 10 mg/m^2 IV days 1-5 Ara-C 1.0 g/m^2 12-hourly days 1-3

AUTOLOGOUS BONE MARROW TRANSPLANTATION VERSUS CHEMOTHERAPY FOR ADULT ACUTE MYELOBLASTIC LEUKEMIA IN FIRST REMISSION: PRELIMINARY RESULTS OF A MULTICENTER RANDOMIZED STUDY

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INTRODUCTION

Complete remission is now expected in the majority of previously untreated adults with de novo acute myeloid leukemia (AML). Patients under age 60 enter complete remission in 70-75% of cases (1). Treatment strategies are directed on maintaining these remissions by intensification chemotherapy (2) or very intensive chemotherapy or chemo-radiotherapy followed by autologous or allogeneic bone marrow transplantation.

To evaluate the relative value of each approach, the GOELAM group decided, in November 1987, to initiate a prospective randomized study comparing early autologous BMT to a second intensification chemotherapy for all patients less than 50 years old. Patients less than 40 with an HLA identical sibling were randomized at diagnosis and received allogeneic BMT after achieving CR without consolidation or intensification therapy.

PATIENTS AND PROTOCOLS

All patients aged less than 50 years old with untreated de novo AML were randomized at diagnosis between two induction therapies: regimen A consisting of idarubicin (8 mg/m^2/d x 5 days) and cytarabine (200 mg/m^2/d x 7 days) or regimen B consisting of zorubicin (200 mg/m^2/d x 4 days) and cytarabine (same dose than regimen A).

Patients under age 40 who had an HLA identical sibling donor proceeded to allogeneic bone marrow transplantation without other intensification therapy. Conditioning regimen and graft-versus-host disease prophylaxis were chosen according to each center's protocol.

The other patients attaining complete remission received a first intensification with high-dose Ara-C (3 g/m^2 q 12 h from D1 to D4) followed

by the same anthracycline (randomized at induction): idarubicin (10 mg/m^2/d D5 and D6) or zorubicin (200 mg/m^2/d D5 and D6).

After hematological reconstitution, unpurged marrow was harvested in each patient. A second intensification was then randomized consisting of either a five-day chemotherapy regimen (m-Amsa : 150 mg/m^2/d x 5 days; VP 16 : 100 mg/m^2/d x 5 days) or autologous bone marrow transplantation. Conditioning regimen prior to autologous BMT was Busulfan and Cyclophosphamide according to the Johns Hopkins group (3) with Busulfan (4 mg/kg/d x 4 days) and anticonvulsivant therapy followed by Cyclophosphamide (30 mg/kg/d x 4 days) associated with Mesna.

Delay between first intensification and marrow harvest was fixed at 75 days, after which patients were no longer eligible for the second intensification.

PRELIMINARY RESULTS

As of June 1990, 197 patients have entered the study and 169 are evaluable for induction therapy : 130/169 patients (76.9%) achieved complete remission ($p=0.72$ between the two regimens). Fourteen patients (8.2%) died during induction therapy and 25 (14.8%) had a resistant disease. Thirty-one patients with HLA identical sibling received an allogeneic BMT and eighty patients received their first intensification. Four patients (5%) died from the procedure or relapse and, at time of analysis, 55/80 were randomized for the second intensification.

Twenty-nine received autologous bone marrow transplantation with a mean follow-up of 370 days since the first randomization and 256 days since marrow harvest. At time of analysis, 22/29 (75.9%) are alive; 21 in continuous complete remission (72.4 %). Out of the 29 patients, 7 died: 5 from relapse and 2 from toxicities.

Twenty-six patients received a second intensification with m-Amsa and VP 16 with a mean follow-up of 342 days since the first randomization and 220 days since marrow harvest. Twenty-four are alive at time of analysis; 20 are in continuous complete remission (76.9%) and 4 in relapse. Two patients died after relapse.

Thirty-one patients have been allografted: 20 are alive, 18 remain in continuous complete remission (58%). Eleven patients died: 5 from GVHD, 1 from relapse and 5 from other causes.

DISCUSSION

It appears from this preliminary analysis that such a cooperative study comparing intensive chemotherapy and autologous bone marrow transplantation may be feasible with completion of the randomized treatment not appearing to be a major problem. Only 7 out of 80 patients (8.7%) were not randomized following medical decision or patient refusal, which seems acceptable.

Due to improvements in intensification chemotherapy results, we chose to compare this approach with autologous bone marrow transplantation for AML in first remission.

In order to eliminate the heterogeneity in the ABMT arm, the Busulfan-Cytoxan regimen was chosen, thus avoiding the bias of different total body irradiation protocols as well as limiting the delay on waiting lists in the different transplant units.

Without definite proof of purging efficacy (4), it is difficult to homogeneize between several groups (standard versus adjusted dose of mafosfamide), thus, we decided to use unpurged marrow after the first intensification. Furthermore, in our previous experience (P. Herve et al., unpublished), we found that chemical purging after high-dose Ara-C was not always feasible.

Preliminary results of this randomized study do not allow us to draw any conclusions because of the limited number of included patients and the short follow-up. The study is expected to be closed to inclusion at the end of 1991, with at least 50 evaluable patients in each arm.

The analysis will compare planned treatments, performed or not, as well as comparison of groups who effectively completed their treatments.

Due to the absence of intensification post-induction, the allogeneic BMT evaluation will be biased in this study: this approach was chosen in order to lower cumulative toxicities. However, another important parameter to be evaluated in this study will be the impact of bone marrow transplantation on an unselected population of adult AML, all included at diagnosis.

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LONG TERM FOLLOW-UP OF ACUTE MYELOGENOUS LEUKEMIA PATIENTS AFTER TREATMENT WITH DOUBLE INTENSIFICATION AND AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT)

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ABSTRACT

To determine if a second intensification with ABMT would effect long term disease-free survival (DFS), data was analyzed from 18 AML patients (pts) in 1st remission 4-6 years after treatment. This pt group was induced with AMSA-OAP, intensified with AMSA and HD Ara-C followed by cyclophosphamide 6g/m^2, BCNU 300 mg/m^2 and VP-16 750 mg/m^2 + ABMT ensued at 6 months from CR (range 5-9). Bone marrow (BM) was stored after AMSA-HIDAC. ABMT was followed by 6 cycles of AD-OAP. There were no treatment-related deaths. Presently pts remain in CR (median 52+ mo; range 46-64+); 8 pts have relapsed (median 18+mo; 7-24). The 5 yr DFS is 56%. AMSA-HIDAC prior to BM harvest may decrease the leukemic load of the infused marrow (in vivo purge). Of the 12 pts with favorable prognostic factors, 8 are still in CR (66%) compared to 2 out of 6 pts (33% p=0.07) with unfavorable factors. These data are superior but not significantly better than the results of a concomitantly treated chemotherapy group. Interpretation of results is limited due to small patient numbers and patient selection. A study has been designed in which patients are stratified according to prognostic factors and in which the role of auto-BMT can be evaluated.

INTRODUCTION

The treatment of acute myelogenous leukemia (AML) with conventional chemotherapy protocols resulted in a 15-25% disease free survival rate.[1] In recent years, factors predicting outcome of treatment have been described of which age, cytogenetics, WBC count at time of diagnosis and number of courses needed to achieve complete remission are most significant.[2] Selection on the basis of those factors resulted in the identification of patient subpopulations with a long term disease free survival (DFS) rate of 40%. In the past years, advances have been made in post remission induction treatment; the development of high dose (HD) Ara-C programs as early intensification

after induction treatment has contributed significantly to the long term DFS rate.[3,4] On the basis of the principle of intensification of remission, we initiated 8 years ago a post remission induction treatment in which in addition to the early HD Ara-C intensification, a subsequent intensification program was included. This consisted of high dose Cyclophosphamide, BCNU and VP-16 (CBV) in conjunction with autologous bone marrow transplantation (auto-BMT). In this paper, we report a follow-up of the results and we describe our current study.

MATERIALS AND METHODS

Patient Population

Patients under age 60, diagnosed with AML using the FAB criteria were entered in protocol 82-86 approved by the Institutional Review Board (IRB). Schema 1 presents a schematic design of this protocol. A minimum of $1x10^8$ marrow cells/kg body weight was collected after the first second or third maintenance course after HD Ara-C treatment. The methods of marrow storage and preparation procedures of the marrow cells for transplantation have been described previously.[5] The CBV regimen has been documented in Schema 2.

RESULTS WITH PROTOCOL 82-86

Of the 63 patients achieving CR, 18 were treated with CBV and BMT as second intensification (Schema 3). Of the 18 patients, 10 are in continuous complete remission (CCR) with a median follow-up of over 5 years (Table 1). Eight patients relapsed after a median remission duration of 19 months (Table 2). Of the 18 patients, 12 had favorable prognostic factors according to Keating's model[2], 8 (67%) are in CCR. Of the 6 patients with unfavorable prognosis, only 2 are still in remission (Table 3). There is no transplantation related mortality.

As can be noted in Schema 3, 45 of 63 patients who achieved remission were not transplanted. One of the major reasons of not being entered into the transplantation program is relapse of leukemia occurring within 6 months after remission induction; over 20% of the patients relapsed before reaching the transplantation phase in the protocol (Schema 3). The majority of the relapses is after the course of maintenance immediately after CR induction and after the second and third course of maintenance after HD Ara-C intensification (Schema 1).

CURRENT AML PROGRAM (#273-90)

Based on our experience with protocol 82-86, two major changes have been introduced in the new protocol. In the first place the maintenance treatment after induction has been deleted and the time interval between HD Ara-C treatment and transplant has been shortened; only one maintenance course has been included. The marrow for transplantation is harvested

immediately after HD Ara-C intensification. In the second place, the patients are stratified according to prognosis. Stratification is done after HD Ara-C intensification. Patients with a favorable prognosis will be treated with the CBV program and unmanipulated marrow. In patients with unfavorable prognostic factors, the cytoreduction of the second intensification has been increased. The BCNU in the CBV program has been replaced by 1020 cGy of total body irradiation (TBI). In addition, the bone marrow cells harvested for bone marrow transplantation are incubated with 4 hydroperoxy cyclophosphamide (4HC) and vincristine. The prognostic factors used in protocol 273-90 have been listed in Table 4. Protocol 273-90 has been outlined in Schema 4 and Schema 5.

DISCUSSION

The interpretation of the results obtained with protocol 82-86 is limited. Patient selection and time census may explain the favorable results. When the results of this patient population are compared with those of a group of patients concurrently treated with the same protocol except for transplantation and matched for prognostic factors, the results of the BMT treated patients are better. However, the level of significance has not been reached due to the small numbers of patients in both treatment arms.

It seems that the patients with poor prognostic factors have a higher relapse rate than those with favorable indicators (67% vs. 33%, Table 3). An increase in cytoreduction for the poor prognostic group seems therefore indicated. Reoccurrence leukemia after auto-BMT is originated either from the leukemic cell population present in the bone marrow transplant or from leukemic cells surviving the conditioning regimen. Therefore, in the poor prognostic group besides the in vivo purge, also ex vivo treatment of the bone marrow cells is used for leukemic cell reduction. In order to minimize the leukemic cell population escaping the conditioning regimen, we increased its cytoreductive potential. We selected the VP-16-cyclophosphamide-TBI regimen used for allogeneic bone marrow transplantation (Table 5). Although in the allogeneic population, no treatment regimen related deaths were recorded, it needs to be seen if in the autologous setting similarly favorable results can be obtained. This program is the first program in which high dose treatment with BMT is tailored according to prognosis.

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TABLE 1

CR DURATION
RELAPSE FREE PATIENTS

NUMBER:	10/18 (56%)
MEDIAN DURATION:	64+ MONTHS

76+, 68+, 68+, 68+,
67+, 62+, 59+, 58+,
56+, 55+

TABLE 2

CR DURATION
RELAPSED PATIENTS

NUMBER:	8/18 (44%)
MEDIAN DURATION:	19 MONTHS

24, 22, 29, 29
18, 13, 9, 7

TABLE 3

PROGNOSTIC FACTORS AND OUTCOME AFTER CBV

Prognosis	No. of Pts	CCR	Median CR Duration (mos)	P
Good	12	8 (67%)	64+	0.069
Bad	6	2 (33%)	19	

Prognostic Model: PCR1, Keating, et al. (Ref.2)

TABLE 4

FRONT-LINE THERAPY FOR ADULT AML RISK GROUPS

- Good prognosis:
  Karyotypes: inv 16, + (15;17), + (8;21), diploid 45 X-Y

and

Achievement of CR in one cycle

- Poor prognosis:
  CD 34 (+) blasts > 70%
  Any karyotype other than above
  Above karyotypes if > 1 cycle to CR

TABLE 5

Increase of Leukemic Cell Kill in Bad Prognosis

1. Marrow Harvest: 4HC in vitro treatment
2. Recipient conditioning regimen:

VP-16	:1500 mg/m^2 day -7
CTX	: 60 mg/kg days -6,-5
TBI	: 1.7 Gy bid days -3,-2,-1

FIGURE 1. Schema 1

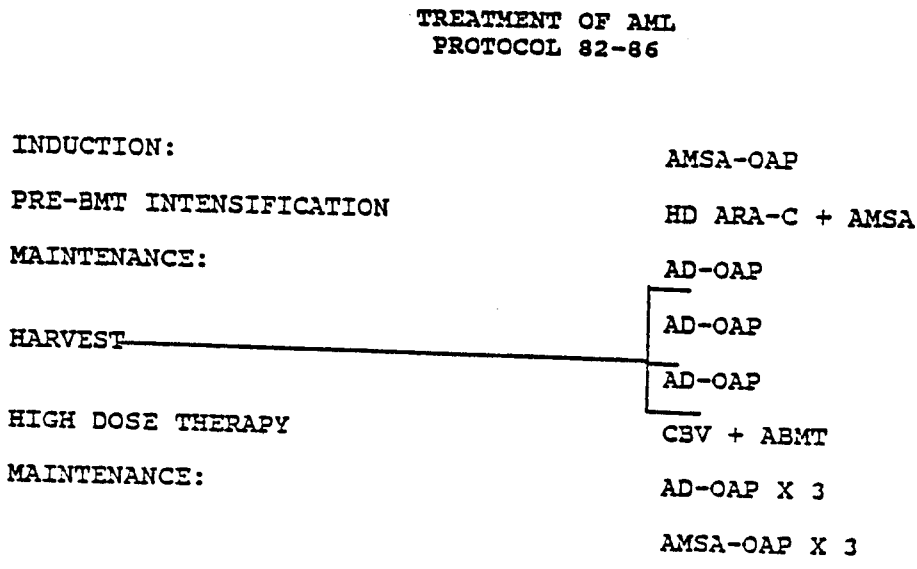


FIGURE 2. Schema 2

THE CBV REGIMEN

	Total Dose	
Cyclophosphamide	6 gr/m^2	day 1-4
BCNU	300 mg/m^2	day 1
VP-16	750 mg/m^2	day 1-3

BMT day 7

FIGURE 3. Schema 3

TREATMENT DISTRIBUTION OF AML PATIENTS IN PROTOCOL 82-86

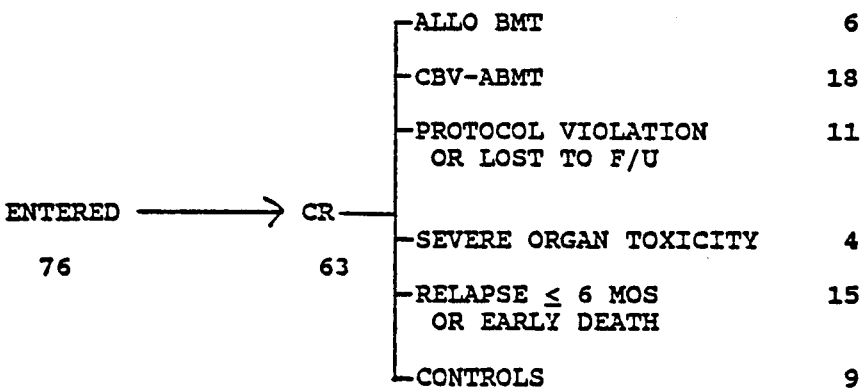


FIGURE 4. Schema 4

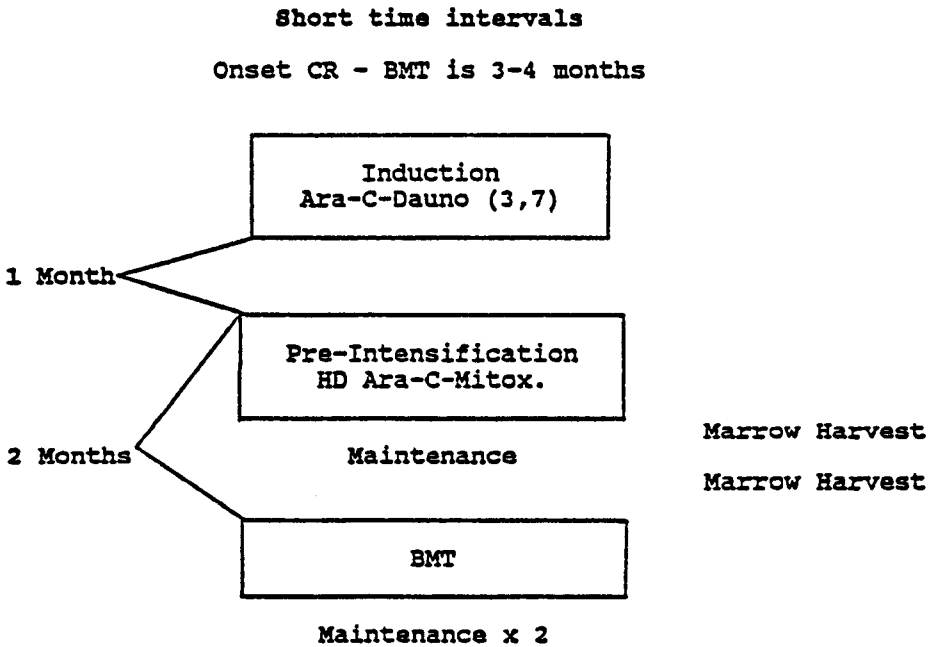
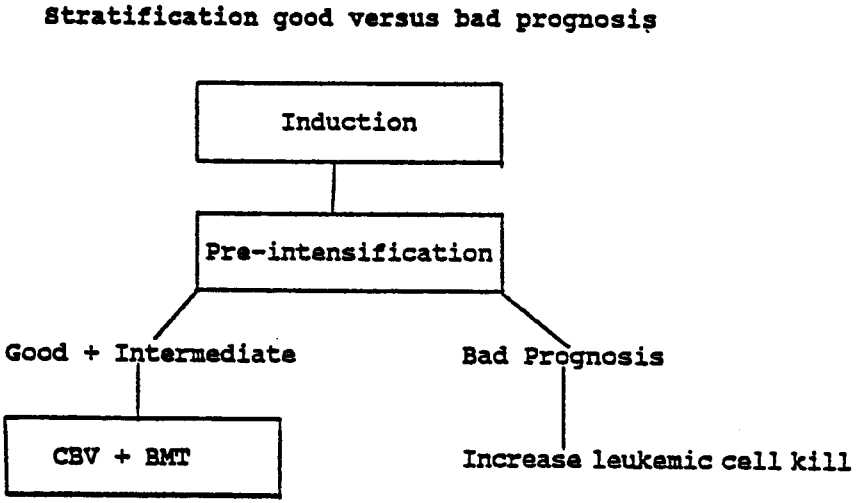


FIGURE 5. Schema 5



AUTOLOGOUS BONE MARROW TRANSPLANTATION IN ACUTE LEUKEMIA: THE ROLE OF MARROW PURGING

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INTRODUCTION

Allogeneic bone marrow transplantation (BMT) has improved the prognosis of acute leukemia in patients with HLA identical siblings. As an alternative, in the past decade, several teams including ours, have developed autologous bone marrow transplantation (ABMT) to allow high dose consolidation therapy in an effort to offer a similar chance to most patients with no available donor. Results of ABMT from various institutions and the European Registry indicate that this goal may have been reached and, further, the possibility to extend ABMT even to patients who are candidates for allo BMT is being considered.

Despite the clear demonstration in numerous animal models of the efficacy of marrow purging in reducing leukemic cell contamination in marrow collected in complete remission (CR) or in artificial marrow tumor cell mixtures, resulting in cure of leukemia by ABMT in these models, the important question of the efficacy of marrow purging in the human situation has remained unanswered for several years. This situation has dramatically changed in 1988 when for the first time the analysis of the European registry demonstrated the value of marrow purging with mafosfamide in acute myelocytic leukemia (AML) autografted in first remission (CR1) (1). Later, this demonstration was confirmed by the 1989 (2) survey, and further extended by the last EBMT analysis presented in May 1990 in the Hague. We will summarize these data in a first part. In a second part, we wish to report on the present status of our own trial in Hopital Saint Antoine Paris in 100 patients with AML and ALL receiving marrow purged with mafosfamide at a level individually adjusted, defined as the CFUGM LD 95 which spares 5 ± 5 % CFUGM. We found that the relapse rate was significantly related to the residual amount of CFUGM progenitors in the marrow after purging. We interpreted these data as indirect evidence that the outcome of patients autografted is indeed dependent of the quality of the marrow infused (3).

PATIENTS AND METHODS

Marrow Purging with Mafosfamide is Effective for AML Autografted in First CR: The European Surveys

a) The 1988 European survey, after several years of frustration, was the first to show clear evidence in favor of marrow purging in AML CR1. As a consequence of a first analysis, a specific second study was independently conducted by 2 distinct statistical teams (1).

Several steps were taken to improve the quality of the data and to structure the analysis:

1) Before the study, all teams received a printout of their own previous reports for verification and corrections.

2) Although we studied both the probability of relapse and the leukemia-free survival, we felt that the first parameter, which censors all causes of death not directly related to leukemia, was more appropriate to establish the possible antileukemic effect of purging.

3) We decided to focus within the study on a relatively homogeneous subpopulation of patients without high risk criteria, autografted in CR1 after TBI. Indeed, in the past there has been difficulty in studying patients with AML treated by ABMT because of the variety of pretransplant regimens used, in contrast to allogeneic BMT, where TBI is the standard modality.

4) It had been suggested that marrow purging might not bring any additional and/or detectable beneficial tumor cytoreduction in a situation where chemotherapy previously given to the patient would achieve a sufficient degree of so called "in vivo purge". Therefore, we analyzed purging versus no purging, by intervals from CR to ABMT, postulating that the effect of purging might be more easily detectable, or alternatively, purging might be more effective, in patients transplanted earlier after fewer courses of consolidation chemotherapy following the induction of CR. This analysis produced some interesting results.

5) A careful comparison of the two populations, purge versus no purge, did not detect any selection bias. This study included an analysis of all chemotherapy induction and consolidation regimens given in the pretransplant period with a special attention to high doses Ara-C, a comparison of pretransplant intervals, and a search for a possible center effect.

We analyzed data from 263 patients with acute myelocytic leukemia (AML) autografted in first remission (CR) during the period from January, 1982 to January, 1987 at one of 34 centers in the European Bone Marrow Transplant Group. The median age of patients was 30 years (range, 1 to 65). The median interval between achieving CR and autografting was 5 months (range, 1 to 23). Of the 263 patients, 131 patients received cytoreductive regimens that included total body irradiation (TBI); the remainder received various combinations of cytotoxic drugs. Sixty-nine patients received autologous marrow purged in vitro with mafosfamide, and 194 received unpurged marrow. The median follow-up was 24 months (range, 12 to 97). For patients with standard risk AML in CR1 autografted after TBI (n = 107),

the leukemia-free survival (LFS) was higher, and the probability of relapse was lower in recipients of purged than of unpurged marrow (63% versus 34%, p = .05 and 23% versus 55%, relative risk 0.34, p = .005, respectively). The superior results of purging were most obvious in patients autografted within 6 months of achieving CR (probability of relapse, 20% versus 61%, p = .01). Patients with longer intervals between CR and autografting had higher LFS and lower probability of relapse than those autografted early in CR (intervals greater than 9 months, 7 to 9 months, 4 to 7 months, and < 3 months : LFS = 56%, 40%, 35%, 27%, p = .007, probability of relapse = 25%, 56%, 59%, 67%, p = .005; respectively). We concluded that marrow purging with mafosfamide was valuable for patients autografted early in first CR.

b) The 1990 survey confirmed the efficacy of marrow purging in AML CR1, not only after TBI, but even when considering the whole population of patients autografted in CR1 whatever the pretransplant regimen. It further extended the observation that the efficacy of marrow purging was easier to detect in patients more likely to have persisting residual tumor at time of ABMT, with the finding of a statistically significant advantage for purging in patients achieving CR1 with a delay from initial to CR1 > 40 days, and not in those with a delay < 40 days. For the 1990 EBMT survey, 62 European teams (see appendix) reported 1688 autografts for consolidation of acute leukemia, as of December 31, 1989. The distribution for bone marrow transplant (ABMT) was the following : AML : status CR1: 671; CR2: 196 - pretransplant regimens : total body irradiation (TBI) : 456, Busulfan + Cyclophosphamide (BU-CY) 174. Marrow purging with mafosfamide 269 corresponding to 26% of the patients in CR1 and 41% in CR2. ALL status : CR1 312, CR2 259 pretransplant regimens : TBI 537, BU-CY 52. Marrow purging with mafosfamide 256, with monoclonal antibodies 175 corresponding to 61% purged in CR1 and 75% purged in CR2. The overall results were the following : for patients autografted in CR1, the leukemia free survival and relapse rates at 7 years were 48 $\pm$ 2% and 41 $\pm$ 3% for AML (fig 1) and 44 $\pm$ 5% and 45 $\pm$ 5% in ALL (fig 2). In CR2 the figures were LFS 34 +/- 4% and relapse rate 54 $\pm$ 5% for AML and 32 $\pm$ 3% and 62 $\pm$ 4% respectively for ALL. Patients not relapsing at 1 year post ABMT had a probability of being cured at 7 years of 86% and 71% if autografted in CR1 and CR2 for AML (fig 3), and 81% and 59% for ALL (fig 4). Multivariate analysis of relapse rates in several subpopulations confirmed the efficacy of marrow purging in AML CR1: in patients transplanted prior to January, 1988 (minimum follow up 2 years), the relapse rate with purged marrow was 35 $\pm$ 5% vs 47 $\pm$ 3% (p = 0.005) (fig 5). In patients autografted after TBI only, it was 29 $\pm$ 5% vs 50 $\pm$ 4% (p < 0.0001) and further 16 $\pm$ 6% vs 60 (p = 0.001) in those autografted within 6 months from induction of CR (fig 6). In patients autografted after TBI who reached CR1 with a delay from diagnosis to CR1 > 40 days, the figures were 23 $\pm$ 8% vs 56 $\pm$ 6% (p = 0.001) in favor of purging while relapse rates with and without purging were similar in those who initially reached CR1 within 40 days. Results were also significant in multivariate analysis though at lower levels when considering all patients autografted until December, 1989. Finally,

it was found that patients with AML CR1 autografted post TBI not relapsing at 1 year had a 91% probability of cure with purged marrow versus 80% with non-purged marrow ($p = 0.1$) (fig 7). Relapse patterns were different in that the plateau for persisting remission started at 18 months with purged marrow versus 30 months with unpurged marrow. Multivariate analysis similarly performed for ALL failed to show any advantage for marrow purging whatever the technique used (fig 8). These results on 671 AML CR1 patients confirmed the initial finding, that marrow purging is effective and easier to detect in situations where residual tumor is more likely to persist (initial diagnosis to CR1 > 40 days, CR1 to ABMT < 6 months).

In contrast to AML, the 1990 EBMT survey did not show any beneficial effect for purging, either with mafosfamide, or monoclonal antibodies, or even all techniques combined, in ALL. This absence of beneficial effect of purging in ALL may alternatively mean that in vitro purging has no major impact in the absence of sufficient reduction of body tumor load or that present means for purging in AML are inadequate.

Marrow Purging With Mafosfamide at the Individually CFUGM LD 95 Adjusted Level

Design of the study. In the late seventies, our team decided to design a program of high dose consolidation therapy containing total body irradiation (TBI), followed by ABMT with purged marrow for patients with acute myelocytic (AML) and acute lymphocytic leukemia (ALL) in remission (4). In designing this program, we tried to define an optimal technique for purging which would achieve a maximum antileukemic activity without jeopardizing marrow engraftment. As a consequence of the initial report by Sharkis and Santos in the Brown Norway myelocytic (BNML) rat leukemia model (5), showing that the efficacy of cyclophosphamide derivatives for marrow purging existed only above a certain threshold of dose, and also as a result of our own preliminary in vitro studies showing wide variations in the individual sensitivity of progenitor cells including clonogenic leukemic cells (CFUL) from patient to patient, we selected mafosfamide for purging at the highest tolerable dose which we defined as the CFUGM LD 95 sparing, $5 \pm 5\%$ CFUGM.

Previously, we have published several aspects of our purging procedure as well as our first clinical results in a small series of 23 patients. We are reporting here on our global experience in 100 patients autografted since January 1983. In addition, to producing leukemia free survival (LFS) which we believe compare favorably to allo BMT, we also found interesting correlations of LFS and relapse rate to several characteristics of the marrow transplanted. We consider that some of these may be indirect evidence in favor of marrow purging at levels individually adjusted.

One hundred patients (55 AML, 45 ALL) entered the study. The sex distribution was 61 males and 39 females. The median age was 36 years (range, 6 to 56) for AML and 25 years (range, 5 to 55) for ALL. Six out of the 55 AML and twelve out of the 45 ALL patients were children.

The distribution of the patients was the following : AML in first complete remission with standard prognosis factors (AML CR1 SR) : 32, AML in first CR with high risk factors (AML CR1 HR) : 13, AML in second remission (AML CR2) : 10, ALL in first CR with standard prognosis factors (ALL CR1 SR) : 9, ALL in first CR with high risk factors (ALL CR1 HR) : 24 and ALL in second CR (ALL CR2) : 12.

Each marrow collection was preceded by the preincubation test (PIT) realized on a 10 ml marrow sample taken 15 days before, in order to determine the CFU-GM LD 95 dose of mafosfamide to be used for in vitro purging. Buffy coat from the part of marrow to be treated was collected on a haemonetics H30 cell separator and adjusted to a final cell concentration of 2.10^7 cells per milliliter with TC 199 medium and a final hematocrit of 5 g. The suspension was finally incubated with mafosfamide at the concentration previously established from the PIT for 30 minutes in a water bath at 37C, with gentle shaking. Following incubation, the BM suspension was cooled, centrifuged, washed, resuspended in irradiated autologous plasma and cryopreserved. All patients received the standard pretransplant regimen consisting of cyclophosphamide 60mg/kg/day x 2 and TBI. Thawed marrow was infused on day 0. Following ABMT, no maintenance chemotherapy was used.

RESULTS

Clinical Results

In the population of 45 AML CR1 patients the probability of persisting remission, relapse rate and leukemia free survival at 6 years were respectively 70% $\pm$ 7, 30% $\pm$ 7, 56% $\pm$ 7. When comparing AML CR1 SR to AML CR1 HR, the difference was not statistically significant both in terms of probabilities of relapse and LFS.

Of the 10 patients autografted in CR2, only 3 are alive and well including 2 for more than 2 years post-transplant, in whom the duration of their CR post ABMT has presently reached 2 times (25 months vs 13) and 9 times (54 mo vs 6) the duration of their first remission (inversion).

In the 33 ALL patients autografted in CR1, the figures for probability of remission, relapse rate and LFS at 5 years were 67% $\pm$ 9, 33% $\pm$ 9 and 60% $\pm$ 9. Interestingly, of 2 patients with a Philadelphia chromosome at initial diagnosis, 1 relapsed at 12 months and 1 remains in CR at 42 months. A patient with the t (4, 21) translocation is still in CR 28 months post transplant. There was no statistically significant difference in terms of disease free probability and leukemia free survival when comparing the standard risk and high risk subgroups (50% $\pm$ 18 vs 73 $\pm$ 9; 44% $\pm$ 17 vs 67 $\pm$ 10, p = NS). In the 12 patients autografted in CR2, one patient died from sepsis while in persisting remission 12 months post ABMT; 8 patients (62%) relapsed at a median of 6 months (2-15). The figures for probability of remission, relapse rate and LFS at 15 mo were respectively 31 $\pm$ 14%, 69 $\pm$ 14% and 25 $\pm$ 12%.

Purging and Relapse Rate

We studied the relapse rate in relation to marrow purging. We found no relation to the dose of mafosfamide used, but a statistically significant relation to the residual amount of CFUGM progenitors in the marrow after in vitro treatment, expressed in percentage of the initial value pretreatment : all patients combined, the relapse rate was 29 $\pm$ 8% vs 54 $\pm$ 7% in those receiving marrow containing less more than 3% residual CFUGM ($p < 0.05$) figure 9. The figures were 27 $\pm$ 9% vs 39 $\pm$ 8% in patients autografted in CR1 and 38 $\pm$ 21% vs 66 $\pm$ 13% in those autografted in CR2 ($p < 0.01$). Relapse rates were not correlated in a similar way to the residual amount of BFUE progenitors.

These results are somewhat similar to those of Rowley et al of the Hopkins group (6), who observed in their own series of AML patients that CFUGM recovery of less than 1 g resulted in actuarial LFS of 36% compared with 16% for those with a recovery of more than 1%.

CONCLUSION

Presently available data indicate that marrow purging with CY derivatives reduces the relapse rate in patients autografted for AML in first remission. Although there is a suggestion that purging is similarly effective for AML in second remission, a longer follow up and a further patient accrual are necessary to validate (or dispute) this hypothesis. While these conclusions result from the analysis of the European registry, a few individual studies such as those conducted at Johns Hopkins University in Baltimore, in Parma (7), as well as our own in Hopital Saint Antoine, further indicate that the relapse rate indeed in AML is linked to the way marrow has been treated (mafosfamide constant dose vs adjusted dose, or correlation with the number of residual progenitors in the treated marrow). In contrast there is yet no indication whatsoever suggesting a possible beneficial effect of purging for ABMT in AML. The significance of this is unclear; it may be of course that marrow purging results in no detectable improvement in the absence of a sufficient tumor load reduction obtained with the pretransplant regimens in use. Alternatively, however, it should be considered that presently available methods of purging in ALL may be inadequate.

ACKNOWLEDGEMENTS

For the Department of Hematology and Formation Associee Claude Bernard "Unite de Recherches sur les Greffes de Cellules Souches Hematopoietiques", Hopital Saint Antoine, Paris, France and on behalf of the European Cooperative Group for Bone Marrow Transplantation (EBMT).

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TABLE 1

AUTOLOGOUS BONE MARROW TRANSPLANTATION
FOR ACUTE LEUKEMIA (December 1989)
LIST OF INSTITUTIONS REPORTING DATA

TEAM	COORDINATOR	NUMBER OF PATIENTS
ROMA, ITALY	MELONI	158
PARIS, ST ANTOINE, FRANCE	GORIN	124
HEIDELBERG, WEST GERMANY	KORBLING	108
UCH, LONDON, UK	GOLDSTONE	91
BESANCON, FRANCE	HERVE	80
GENOVA, ITALY	CARELLA	69
LEIPZIG, DDR	HELDIG	48
ROYAL FREE HOSP., LONDON, UK	PRENTICE	47
UPPSALA, SWEDEN	SIMONSSON	46
PARMA, ITALY	RIZZOLI	44
TOURS, FRANCE	COLOMBAT	41
GLASGOW, UK	BURNETT	39
TORINO, ITALY	AGLIETTA	35
MILANO, ITALY	POLLI	34
BRUXELLE, BELGIUM	FERRANT	34
BORDEAUX, FRANCE	REIFFERS	31
LYON, FRANCE	SOUILLET	30
BARCELONA, SPAIN	BRUNET MAURI	30
SANTANDER, SPAIN	IRIONDO	30
UTRECHT, HOLLAND	VERDONCK	28
BARCELONA, SPAIN	ORTEGA	28
BOLZANO, ITALY	COSER	26
HUDDINGE, SWEDEN	BJORKSTRAND	26
PAVIA, ITALY	ALESSANDRINO	25
ROTTERDAM, HOLLAND	LOWENBERG	22
NIJMEGEN, HOLLAND	DE WITTE	21
PESARO, ITALY	PORCELLINI	19
BIRMINGHAM, UK	FRANKLIN	19
NANTES, FRANCE	HAROUSSEAU	19
PADOVA, ITALY	COLLESELLI	19

TABLE 2

AUTOLOGOUS BONE MARROW TRANSPLANTATION
FOR ACUTE LEUKEMIA (December 1989)
LIST OF INSTITUTIONS REPORTING DATA

NANCY, FRANCE	WITZ	19
WESTMINSTER, UK	BARRET-POYNTON	18
NEWCASTLE, UK	PROCTOR	18
PESCARA, ITALY	TORLONTANO	16
FIRENZE, ITALY	FEURINI ROSSI	16
LEIDEN, HOLLAND	WILLEMZE	15
ULM, WEST GERMANY	WIESNETH	15
ROMA, ITALY	DE LAURENZI	15
BOLOGNA, ITALY	VISANI-TURA	13
ROTONDO, ITALY	GRECO	13
ZAGREB, YUGOSLAVIA	NEMET-LABAR	13
TRIESTE, ITALY	ANDOLINA	12
BERN, SWITZERLAND	BRUN DEL RE	12
BIRMINGHAM, UK	MILLIGAN	12
HOTEL DIEU, PARIS, FRANCE	ZITTOUN	11
AMSTERDAM, HOLLAND	VAAN LEEWEN	10
NICE, FRANCE	GRATECOS	10
TIANGIEN, CHINA	YAN WENWEI	10
LUND, SWEDEN	BERGASSY	9
GENEVE, SWITZERLAND	CHAPUIS	8
WIEN, AUSTRIA	HINTERBERGER	7
INNSBRUCK, AUSTRIA	HUBER	7
LAS PALMAS, SPAIN	HERNANDEZ	7
LA PITIE PARIS, FRANCE	LEBLOND	6
HENRI-MONDOR, CRETEIL, FRANCE	VERNANT	6
ST ETIENNE, FRANCE	FREYCON	6
GEMELLI, ROMA, ITALY	LEONE	4
LONDON, UK	SAMSON	4
MILANO, ITALY	DE CATALDO	3
PALERMO, ITALY	MAIOLINO	3
COCHIN, PARIS, FRANCE	BELANGER	2
BEIJING, CHINA	CAO LU XLN	2
TOTAL :		1688

FIGURE 1

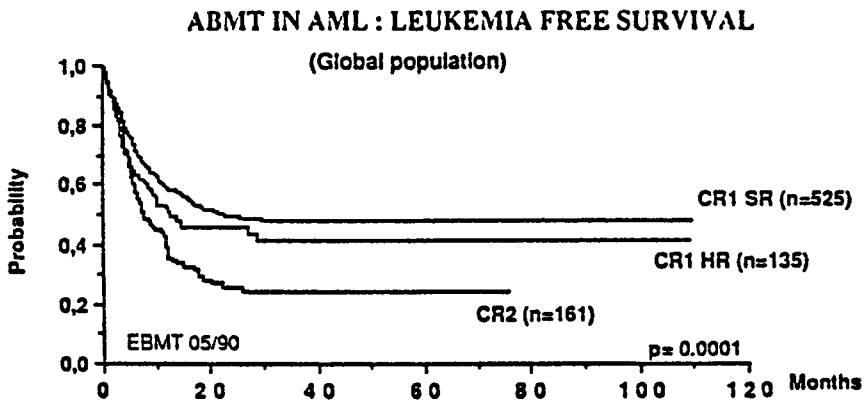


FIGURE 2

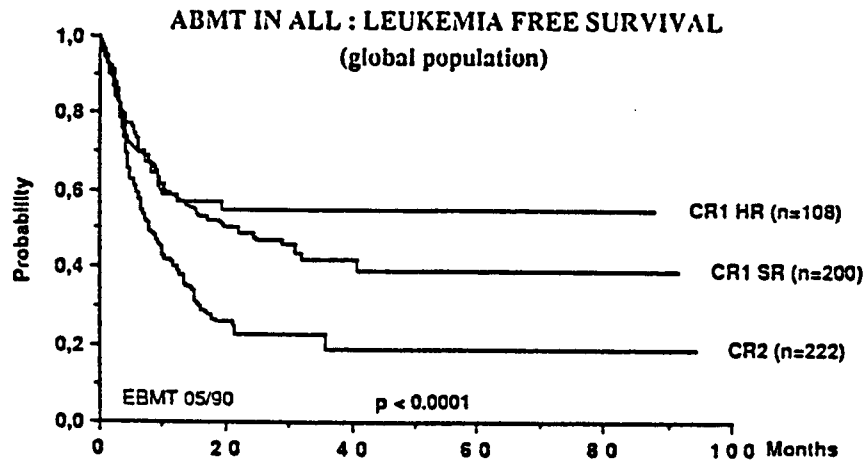


FIGURE 3

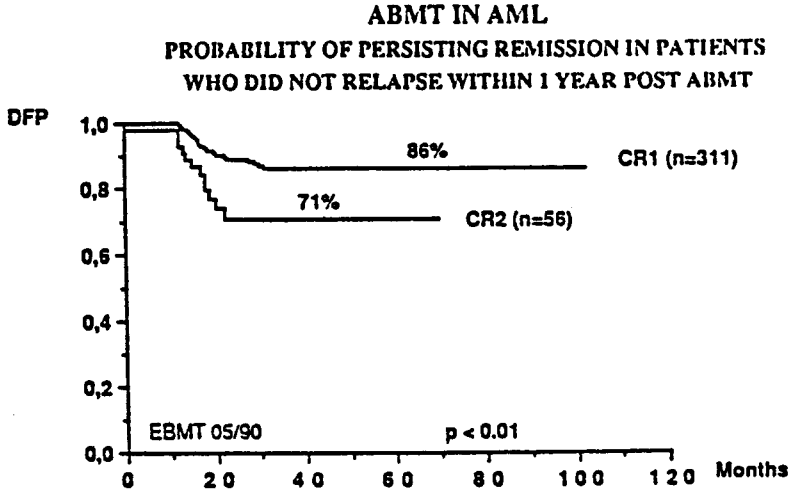


FIGURE 4

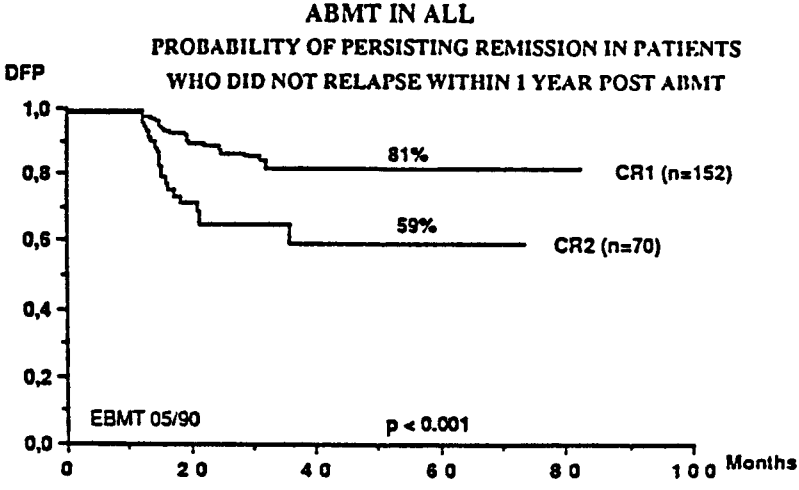


FIGURE 5

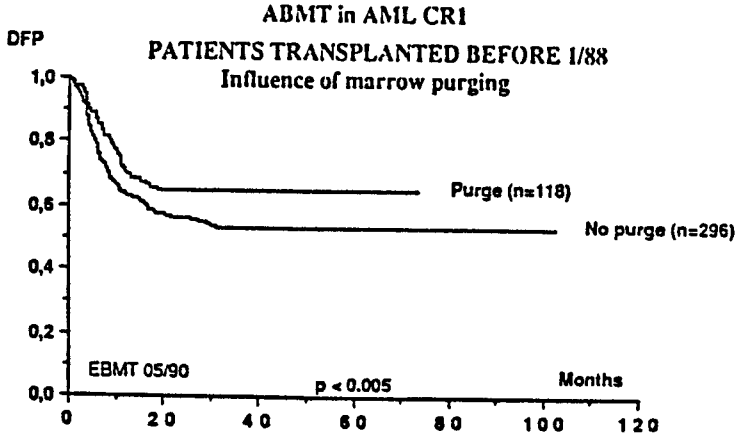


FIGURE 6

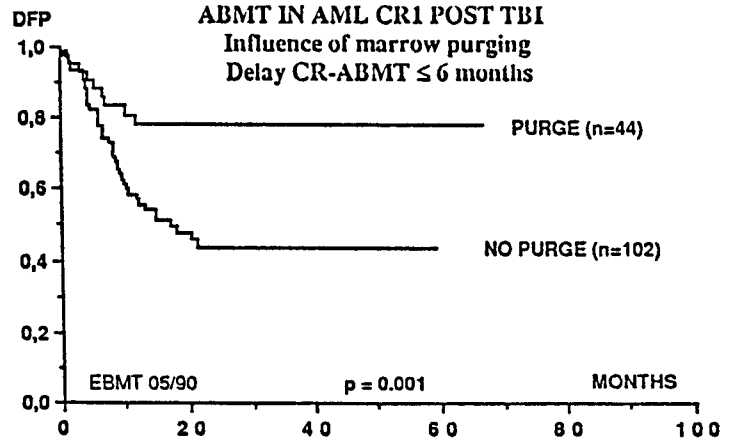


FIGURE 7

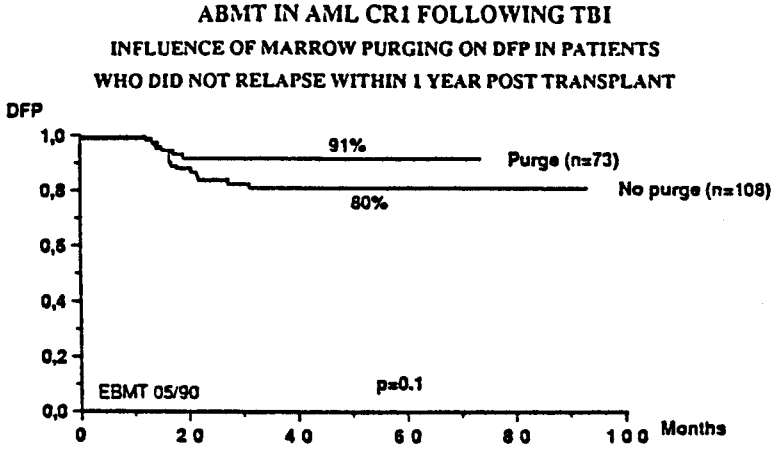


FIGURE 8

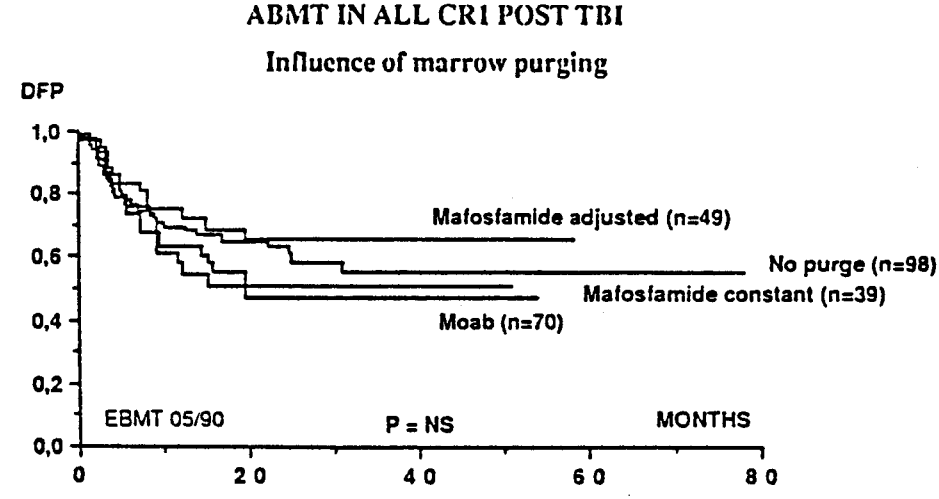
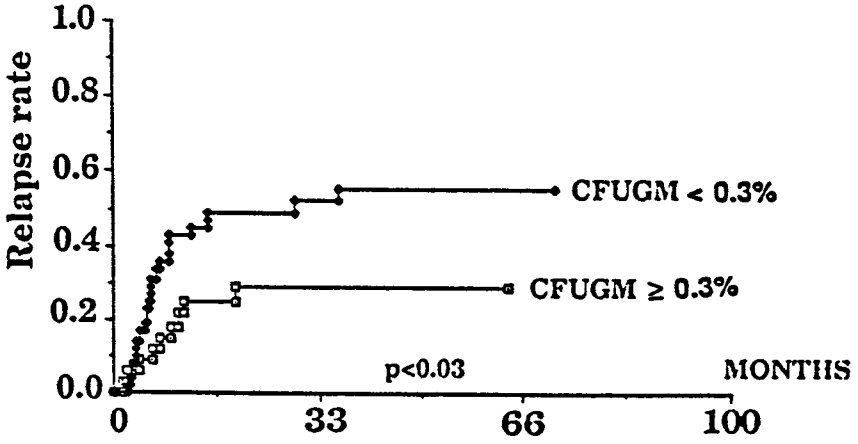


FIGURE 9

Relapse rate after autologous bone marrow transplantation for consolidation of acute leukemia in complete remission : relation to recovery of CFUGM in the marrow infused, following purging with mafosfamide.



AUTOLOGOUS BONE MARROW TRANSPLANTATION IN ACUTE MYELOID LEUKEMIA IN FIRST REMISSION: AN EASTERN COOPERATIVE ONCOLOGY GROUP PILOT STUDY

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INTRODUCTION

Induction chemotherapy in adults with acute myeloid leukemia (AML) regularly yields complete remission (CR) rates of 65-70%. The optimal approach to post-remission therapy that will prolong remission duration and improve the cure rate remains conjectural. Previous studies of the Eastern Cooperative Oncology Group (ECOG) have established that maintenance chemotherapy improves remission duration and survival compared to no further therapy (1). Moreover, ECOG demonstrated in a randomized trial that a single course of intensive post-remission chemotherapy prolonged complete remission and survival compared to patients receiving maintenance chemotherapy (2). Other pilot studies confirm that after intensive post-remission therapy, long-term, disease-free survival occurs in >30% of patients (3-5). These results suggest that intensification of post remission therapy, even by means of a single course of treatment, improves the outcome. Further dosage escalation, however, requires bone marrow rescue, either using allogeneic bone marrow transplantation (ALLOBMT) or autologous bone marrow transplantation (AUTOBMT). ALLOBMT in first CR provides long-term, disease-free survival in approximately 50% of patients (6). Similarly, AUTOBMT, using purged or unpurged marrow, effectively salvages a proportion of patients with AML in second or third remission (7,8) and yields long term survival of 38-61% in first CR (8,9). Critical analysis of the value of AUTOBMT in AML in first remission in a number of studies is hampered because patients received varying induction therapy regimens, underwent AUTOBMT at widely disparate times after CR, and received differing schedules of conventional chemotherapy post-remission prior to AUTOBMT. This ECOG study provides data on AUTOBMT in patients with AML in first CR who are uniformly treated to

induce CR, receive no other therapy before AUTOBMT, and undergo AUTOBMT in a fixed time frame shortly after CR.

We describe below the preliminary analysis of this study, ECOG P-C486, which closed in April, 1990.

PATIENTS AND METHODS

After obtaining written informed consent, patients with de novo, previously untreated AML, aged 15-55 years, were eligible for this study. All patients' diagnoses and FAB type were confirmed by centralized histologic review. Induction chemotherapy consisted of IV daunorubicin, 60mg/m^2/d on days 1-3, plus cytarabine, 25mg/m^2 by IV push, followed by continuous IV infusion of 200mg/m^2/d on days 1-5 and 6-thioguanine, 100 mg/m^2 orally every 12 hours on days 1-5. Patients failing to obtain complete remission after one to two courses of induction therapy were taken off study. Patients in CR who were < 41 years old and had a histocompatible sibling were offered ALLOBMT. The balance of patients in CR underwent AUTOBMT one to three months after CR, providing that they were free of infection, had no overt or occult central nervous system leukemia, and had adequate pulmonary, cardiac, hepatic and renal function. The treatment plan for AUTOBMT was as previously developed and described by the Johns Hopkins group (7). In brief, bone marrow was harvested under general or spinal anesthesia from the iliac crests. The buffy coat was separated and residual hematocrit adjusted to approximately 7%. The buffy coat cells were exposed to 4-hydroxyperoxycyclophosphamide (4-HC) at a final concentration of 100 mcg/ml for 30 minutes at 37C. Residual 4-HC in the supernatant was removed by centrifugation, the cells were resuspended and cryopreserved. The treatment regimen consisted of busulfan, 1 mg/kg orally every six hours x 16 doses (days 1-4), followed by IV cyclophosphamide, 50 mg/kg/d on days 5-8. Twenty-four to 36 hours after completing chemotherapy, the cryopreserved cells were thawed at the bedside and reinfused through a central venous catheter. During the period of busulfan therapy, patients received phenytoin therapy to prevent seizures. Subsequent supportive care of patients adhered to established ECOG guidelines for bone marrow transplant patients.

RESULTS

The CR rate for the 106 eligible, currently evaluable, patients receiving induction therapy was 74%. Of the 78 patients achieving CR, data are available at the time of this report on 32 patients undergoing AUTOBMT and 15 patients undergoing ALLOBMT. Patient undergoing AUTOBMT had a median age of 36 years (range, 18-50), a median time to CR of 46 days (range, 22-126) and underwent BMT at a median of eight weeks post CR (range, 2-24). Patients receiving ALLOBMT had a median age of 33 years (range, 17-40), a median time to CR of 47 days (range, 19-103) and underwent BMT at a median ten weeks post CR (range, 1-24).

Patients undergoing AUTOBMT were reinfused with a median 2.6 x 10^8 nucleated cells/kg (range, 1.4-5.4). Starting from this day of bone marrow reinfusion, the median time for patients to recover > 500 granulocytes/mm was 31 days (range, 17-58), and the median time to self-sustaining platelet counts was 46 days (range, 26-151). The number of infused nucleated bone marrow cells correlated inversely with the time to granulocyte recovery ($p = .001$) and to platelet recovery ($p = .0006$). It is noteworthy that the time for patients to be platelet transfusion-free was >2 months in 25% of patients and >3 months in 15% of patients. Three patients (10%) died of complications of AUTOBMT, including one case each of venoocclusive disease of the liver, sepsis and lung toxicity. With a median follow-up of 11 months, six patients (20%) have relapsed; one before transplant at three months and five after transplant at 4, 10, 11, 21 and 21 months. Currently 23/32 (72%) of patients are alive and in continuous CR at a range of 2+-31+ months, and 24/32 (75%) of patients are alive.

Of patients undergoing ALLOBMT, four died of transplant-related complications, including one case each of sepsis, acute graft versus host disease, venoocclusive disease of the liver and cytomegalovirus pneumonia. Three patients relapsed, including two relapses prior to transplantation at one and two months. One of these patients is currently receiving reinduction therapy and the other was reinduced into second CR followed by ALLOBMT. This latter patient is alive and in CR at 14+ months. One patient relapsed after ALLOBMT at seven months. This patient was reinduced into second CR and underwent a second ALLOBMT. This patient is alive in CR at 21 + months. With a median follow-up of nine months, 8/15 (53%) of patients are alive in continuous CR at 1+-28+ months, 10/15 (67%) are currently in CR and 11/15 (73%) are alive. The actuarial survival cures for patients undergoing ALLOBMT and AUTOBMT are shown in Figure 1.

DISCUSSION

The preliminary results of this cooperative group study indicate that the survival from ALLOBMT and AUTOBMT in adults with AML in first CR are comparable. As expected, the proportion of deaths from therapy-related complications and relapse of leukemia differ between ALLOBMT and AUTOBMT. Patients failing therapy with AUTOBMT are more likely to die of recurrence of their leukemia than from the therapy, whereas the converse is true for ALLOBMT. With longer follow-up, one would anticipate additional relapses in the AUTOBMT group and additional deaths from complications of graft versus host disease and immunosuppression in the ALLOBMT group. The results of AUTOBMT are better than might be expected because of the absence of the benefit of the graft versus leukemia effect that accompanies graft versus host disease in ALLOBMT. It may be, however, that in the setting of recovery from AUTOBMT, there is enhanced generation of a population of autologous lymphocytes that are cytocidal for the patient's own residual leukemic cells (10,11). This study employed 4-HC marrow purging to eliminate clonigenic

occult leukemic cells in the harvested marrow. Purging in this multi-institutional, cooperative group, setting provided results similar to those reported by John Hopkins(7), with a low mortality rate (<10%). Nevertheless, the necessity and value of marrow purging remains uncertain (12). A definitive conclusion on the relative value of intensive conventional chemotherapy, AUTOBMT, and ALLOBMT in first remission AML awaits the results of the randomized study (ECOG EST 3489) recently initiated by ECOG in conjunction with the Southwest Oncology Group.

ACKNOWLEDGEMENT

Supported by NCI grant CA 21115 and individual cooperating institutional NCI grants.

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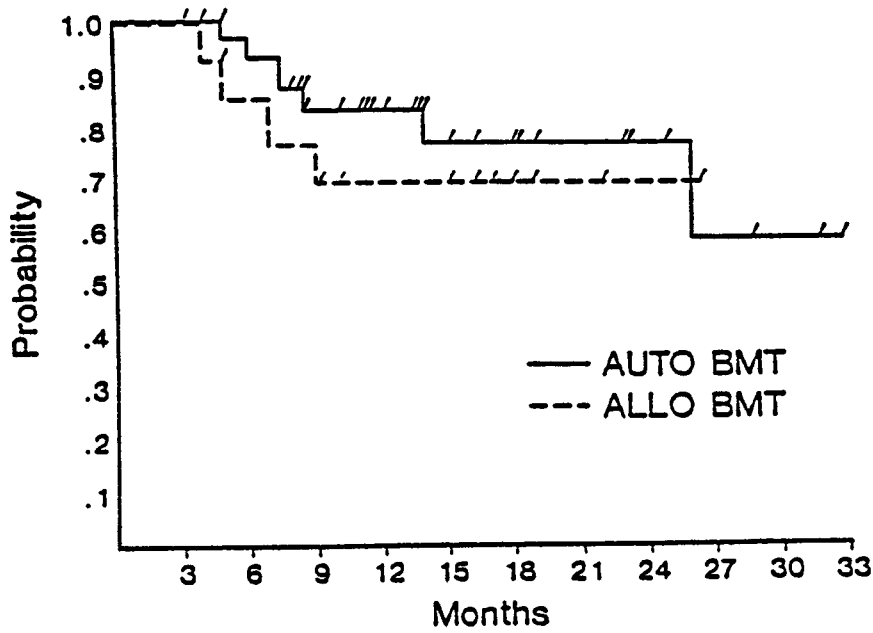
FIGURE 1.

Actuarial survival duration of patients in CR. The hash marks indicate surviving patients at the time of this data analysis.



AUTOLOGOUS BONE MARROW TRANSPLANTATION (ABMT) IN ACUTE NON-LYMPHOID LEUKEMIA (ANLL) IN FIRST REMISSION: EFFECT OF MAFOSFAMIDE PURGING WITH STANDARD VERSUS ADJUSTED DOSE

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INTRODUCTION

In the last three years several papers reported the role of autologous bone marrow transplantation (ABMT) as a technical approach to maintain in complete remission (CR) acute leukemia.

Data on leukemia free survival (LFS) obtained with allogeneic bone marrow transplantation seem to be very close to those obtained with ABMT. The relapse rate after transplant is more significant in ABMT, but the incidence of toxic effects is higher with the allogeneic technique. At present, it is not clear which of these two therapeutic approaches is preferable (1-3). A major concern with autograft is the possibility that reinfused cells might contain malignant clones. Several methods, i.e., monoclonal antibodies, chemical agents, long-term cultures, have been proposed for "ex vivo" purging in order to eliminate minimal residual disease (MRD), but the relevance of these techniques is still uncertain (4-9).

In the present study, we report the clinical results obtained in 101 patients with acute non lymphoid leukemia (ANLL) grafted in first complete remission (CR1) with unpurged or purged marrow. As purging agent the cyclophosphamide derivative mafosfamide was used. In the last 4 years, in order to improve the efficacy of the purging procedure, the dose of mafosfamide has been individually evaluated in each patient by means of a "programmed method", based on the sensitivity of blast colony-forming cells (Blast-CFC) to the drug.

One of the most attractive approaches to control the MRD "in vivo" is the manipulation of immunological reactivity of the host (10). We report "in vitro" data concerning the modification of immunophenotype induced by mafosfamide on the hemopoietic cells.

MATERIALS AND METHODS

Patients

We evaluated 101 patients with ANLL in CR1. The subgroups were as follows: 50 patients grafted with unpurged marrow, 33 with marrow purged with mafosfamide at standard dose and 13 with mafosfamide at programmed dose. Of the 101 patients, 65 were male and 46 female. The median age was 27 years (range 5 to 54 years): 12% were children (less than 14 years of age); 48% were between 15 and 30 years old; 40% were between 40 years old and 54 years old. The FAB subtypes of the patients are reported In Table 1. According to EBMT Group, we classified as "high risk" the patients presenting one of these characteristics: 1) leucocyte count > 10^9/l at presentation; 2) secondary ANLL; 3) blastic diffusion to central nervous system (CNS); 4) presence of cytogenetic abnormalities. The majority of the patients (n = 84) were classified at standard risk and 17 were defined at high risk.

All patients received the same "7+3" induction regimen [daunorubicin (DNR) 45 mg/sq m days 1 to 3 and ARA-C 200 mg/sq m, days 1 to 7 continuous iv infusion]. As consolidation protocols the patients obtaining CR were treated with 2 courses of chemotherapy with intermediate doses of ARA-C plus AMSA or high dose ARA-C plus DNR.

Pretransplant Regimens

The pretransplant regimens were: Cyclophosphamide (CY) 50mg/kg x 4 days or 60 mg/kg x 2 days, plus Total Body Irradiation (TBI: 10 Gy single dose or 12 Gy fractionated dose); this protocol was performed in 48% of the patients. The remainder received Busulfan (BU) 4 mg/kg x 4 days followed by CY 60 mg/kg x 4 days. None of the patients received chemotherapy after ABMT.

Bone Marrow Purging with Mafosfamide

Harvested marrow was centrifuged (2,500 rpm, 20 min) and the bully-coat was collected and resuspended ($2x10^7$ cells/ml) in TC199 medium (80%, v/v) and autologous plasma (20%, v/v). The hematocrit of the cell suspension was always <5%. Mafosfamide (ASTA Werke, Bielfeld, FRG) was provided as lyophilized compound and reconstituted with saline at 10 mg/ml. Marrow cells were incubated with the drug (30 min, 37C) with gentle agitation every 5 min, then the reaction was stopped by immersion in ice-cold water (4C). Following centrifugation (2,800 rpm, 15 min), marrow cells were resuspended ($4x10^7$/mi) in irradiated autologous plasma (55%, v/v), TC 199 (35%, v/v) and DMSO (10%, v/v). When the standard purging technique was used, mafosfamide concentration was 80-100 ug/ml. When the programmed purging method was used, the "optimal dose" of mafosfamide was evaluated in each patient 10-15 days before marrow harvest. Marrow sensitivity to increasing doses (50-200 ug/ml) of the drug was tested on a feederlayer colony assay (11,12). This method allows the growth of undifferentiated (type I) as well as differentiated (type II-III) clonogenic cells. The dose of mafosfamide

used for purging was that able to spare 50% undifferentiated colonies, the so--called Blast-CFC. For cryopreservation of marrow cells, a programmed biological freezer Nicool 416 was used.

After marrow infusion all patients were hospitalized in laminar air flow room, supported with irradiated platelets and packed red cells. A broad spectrum of antibiotic, antimycotic, antiviral drugs and immunoglobulin were administered until the patients are free of fever and the total leucocyte count rose above $1x10^9$/L, with an absolute number of neutrophils more than $0.5x10^9$/L.

The disease free survival (DFS) was calculated by Kaplan & Meier method. Differences between groups were determined by means of the Logrank test.

Immunological Analysis

Following mafosfamide treatment, marrow cells were cultured in suspension at 37C for 7 days. The percentage of natural killer and IL-2 receptor-positive cells was evaluated using the monoclonal antibodies B73.1 (gently provided by Dr. B. Perussia, Wistar Institute, Philadelphia, USA) and IL2-Rl (Coulter, Hialeah, USA). Unpurged marrow calls were used as control.

RESULTS

Table 2 shows the hemopoietic reconstitution in patients autografted with unpurged, standard dose and adjusted-dose purged marrow. The kinetic of engraftment was not significantly different when the two purging techniques were compared.

As shown in Figure 1, clinical results comparing standard versus adjusted-dose purging versus unpurged marrow demonstrated a LFS of 48% [median follow-up (mFU): 13 mos., range: 2-64 mos.], 76% (mFU: 15 mos., range: 1-31 mos.) and 37% (mFU: 10 mos., range: 1-96 mos.), respectively. A significant statistical difference was observed between the adjusted-dose purged group versus the unpurged and standard-dose purged group ($p < 0.01$). The probability of relapse rate was: 49% for standard-purged patients, 24% for patients grafted with adjusted-dose of mafosfamide, and 61% for untreated patients ($p < 0.01$) (Figure 2). When the interval CR1-ABMT was less than 4 months, the results of marrow purging were as follow: a) LFS: 44% for purged marrow and 37% for unpurged marrow, (p = not significant) (Figure 3); b) probability of relapse: 60% for unpurged patients and 52% for purged patients, ($p < 0.05$) (Figure 4).

When the mafosfamide concentrations inducing 50% inhibition of Blast-CFC growth (Blast-CFC ID_{50}) were analyzed In a group of 17 patients, it was observed that 35% of the patients required < 100 ug/ml to achieve 50% growth inhibition, whereas 65% of the patients required > 100 ug/ml. In particular, 90% of the patients in the latter group required 100-130 ug/ml of mafosfamide to achieve a Blast-CFC ID_{50}.

To investigate the immunological modifications induced by mafosfamide, expression of B73.1 and IL-2 receptor was studied (n = 8) in mafosfamide-treated marrow cells cultured in suspension for seven days (Figure 5). The percentage (mean+/-SEM) of B73.1 positive cells was 5±2 and 24±3 in unpurged and purged samples, respectively. Similarly, following mafosfamide treatment IL-2 receptor expression was increased from 10±3 to 28±5.

DISCUSSION

The aim of the present study was to evaluate the possibility of using an experimental "in vitro" approach for marrow purging to decrease the high rate of relapse in patients with ANLL in CR1 grafted with autologous marrow. The patients entered into this study were very homogeneous in terms of induction therapy, consolidation regimens and prognostic factors.

The conditioning regimens (CY-TBI and BU-CY) did not show significant differences in term of LFS. Analysis of patients undergoing ABMT <4 months after achieving CR did not demonstrate a significant difference between the standard purging group and the unpurged group in terms of LFS. However, a significant advantage of purging In terms of probability of relapse was observed (Figure 4).

The analysis of clinical data on patients treated with individual purging measuring the Blast-CFC ID_{50}, demonstrated impressive results (76% of LFS at 36 months), showing a different sensitivity to the drug in each patient which reflects the efficacy of the technical approach. The kinetic of engrafment in this group was not significantly delayed when compared to that observed in ABMT using standard doses of mafosfamide, thus confirming that the Blast-CFC evaluation is an effective method to measure the more immature hemopoietic compartment.

The increasing number of B73.1-positive cells In ANLL marrow mafosfamide treatment suggests an immunological-mediated mechanism of the drug active in the control of MRD "in vivo" and determining a decreased probability of relapse after ABMT. Recently, Skorski et al (10) demonstrated that post graft appearance of natural killer (NK) cells and macrophages are prolonged in mafosfamide-treated mice and suggested that this phenomenon is probably due to the lack of a feedback inhibition of hemopoiesis, mediated by accessory cells destroyed by mafosfamide. The enhanced expression of IL2 receptors on hemopoietic precursors induced by the drug may suggest that mafosfamide could increase the LAK precursors cells.

The efficacy of 4-hydroperoxycyclophosphamide (4-HC) and mafosfamide in a rat model (Brown Norwey myelocytic leukemia) (13,14). The clinical results reported in our study and the increasing percentage of cells expressing the NK-cells marker as well as the IL2 receptor, allow us to conclude that the chemical purging with cyclophosphamide derivatives with optimized techniques determines a reduction of tumor load "in vitro" and an enhancement of immunological control of MRD "in vivo".

ACKNOWLEDGMENTS

This work was supported in part by grants from Consiglio Nazionale delle Ricerche (nos. 88.01907.04 and 88.00847.44), and by Ministers Pubblica Istruzione (40%-60%, 1989). Authors' affiliations: (1) Department of Hematology, Bone Marrow Transplantation Unit, University of Parma Cattedra di Ematologia, Via Gramsci, 14, 43100 Parma, Italy; (2) Division of Hematology, Ospedale S. Martino, Genova, Italy; (3) Division of Hematology, Ospedale Civile, Bolzano, Italy; (4) Division of Hematology, Ospedale Civile, Pescara, Italy; (5) Division of Hematology, Firenze, Italy.

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TABLE 1. Patient Characteristics

No. of Patients	101
Median Age (range) (yr)	27 (5 - 54)
Male	54.4%
Female	45.6 %
FAB Classification	
M1	17.2 %
M2	22.1 %
M3	25.0 %
M4	22.3 %
M5	13.4 %
Pretransplant Regimens	
Purged Group	
Cy + TBI	26 %
Bu + CY	74 %
Unpurged Group	
Cy + TBI	67 %
Bu + CY	33 %
Unpurged Patients	52 %
Standard-Dose Purged Patients	36 %
Adjusted-Dose Purged Patients	13 %

TABLE 2

Hematologic Recovery

Landmark	UNPURGED	PURGED	
		Standard Dose	Adjusted Dose
Leukocytes 1 x 10^9/L	12 - 36 *	15 - 39	16 - 49
Neutrophils 0.5 x 10^9/L	14 - 40	18 - 42	19 - 55
Platelets 50 x 10^9/L	18 - 190	22 - 145	19 - 230

* Range in days

FIGURE 1

Leukemia free survival (LFS) after ABMT in ANLL in CR1. Patients were grafted with either unpurged (NP) or mafosfamide-purged marrow, using standard (P) or adjusted (ADJ) doses of the drug.

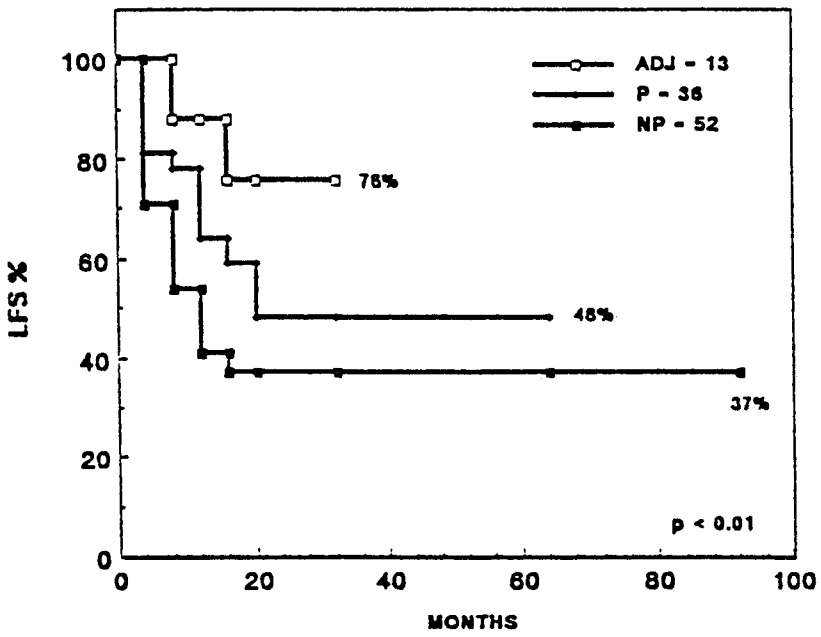


FIGURE 2

Probability of relapse for patients with ANLL in 1st CR following ABMT with either unpurged (NP) or mafosfamide-purged marrow, using standard (P) or adjusted (ADJ) doses of the drug.

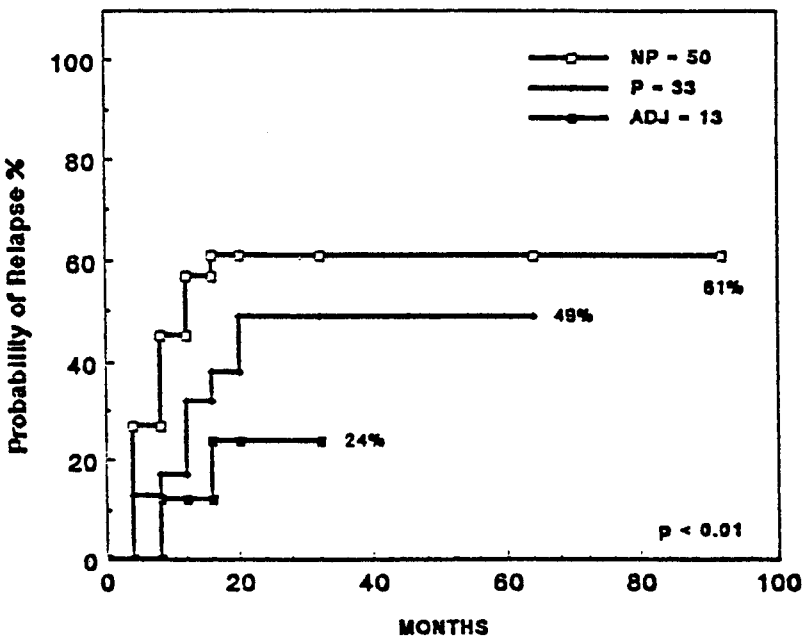


FIGURE 3

LFS following ABMT in ANLL in relation to a CR-ABMT interval < 4 months. Patients autografted with unpurged (NP) or standard dose (P Std) purged marrow are compared.

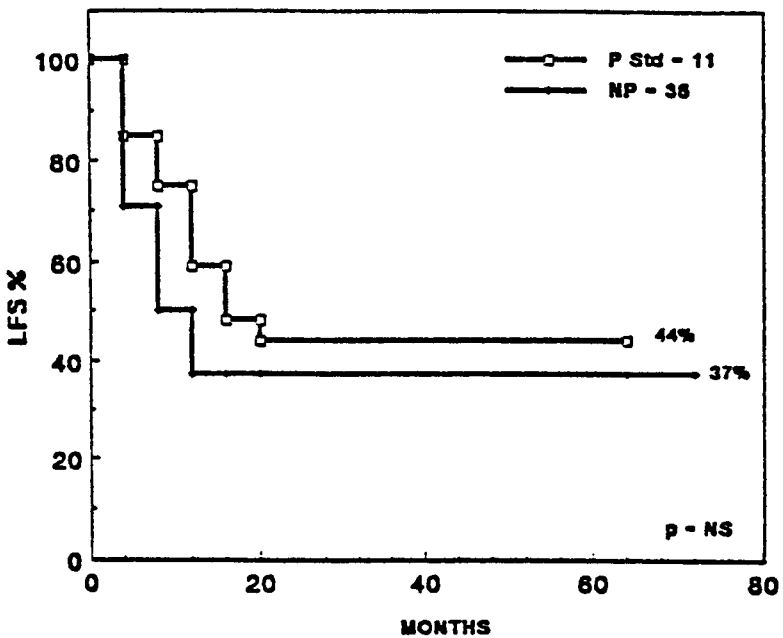


FIGURE 4

Probability of relapse following ABMT in ANLL in relation to a CR-ABMT interval <4 months. Patients autografted with unpurged (NP) or standard dose (P Std) purged marrow are compared.

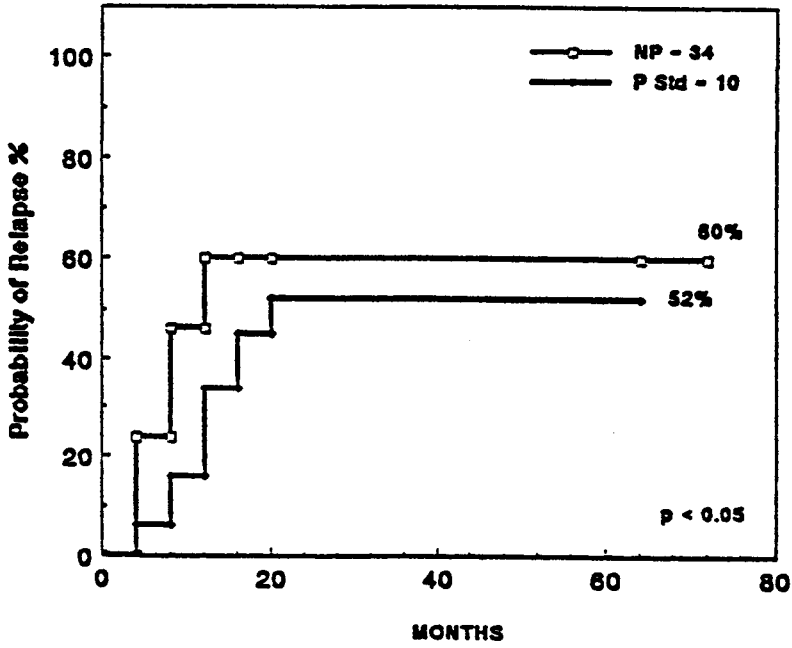
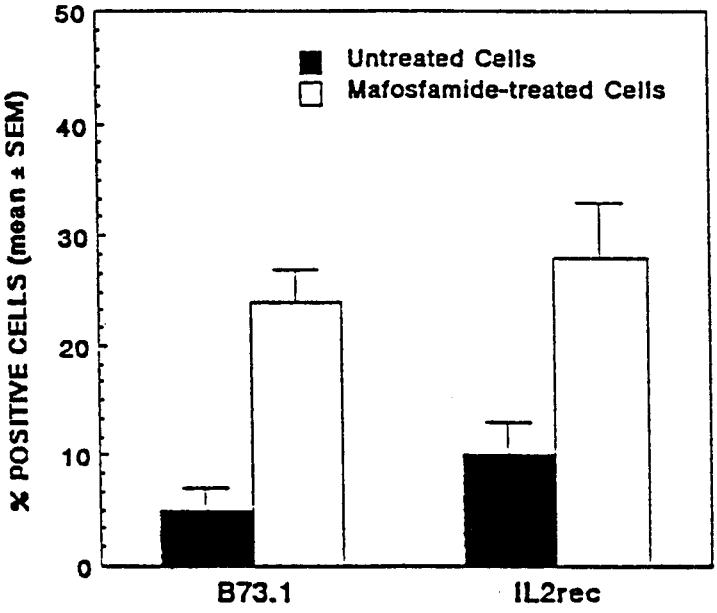


FIGURE 5

B73.1 and IL2rec expression in mafosfamide-treated cells from ANLL patients (n=8).



AUTOLOGOUS MARROW TRANSPLANTATION FOR PATIENTS WITH ACUTE MYELOGENOUS LEUKEMIA BEYOND FIRST REMISSION

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INTRODUCTION

Most patients with acute myelogenous leukemia (AML) who relapse after receiving intensive remission induction regimens are rarely cured by further conventional chemotherapy. High dose chemotherapy or chemoradiotherapy followed by allogeneic marrow transplantation has been used to successfully treat some of these patients (1-3). Since only 25-30% of patients have an HLA compatible family member donor available, the use of autologous marrow transplants (AMT) is being explored. Most marrow transplant centers restrict AMT to patients with AML in first or second remission (4-6). This report details the Seattle experience with AMT for patients with AML in first untreated relapse or in second remission.

METHODS

From September 1979 through December 1989, 56 consecutive patients with AML beyond first remission were treated with high dose chemotherapy or chemoradiotherapy and an infusion of cryopreserved autologous remission marrow. Table 1 shows the pre-transplant characteristics. Fifty-five patients had relapsed in marrow and one patient had a large chloroma without marrow involvement. Thirty patients were transplanted in untreated first relapse with cryopreserved marrow stored while in first remission. Two marrows were incubated in vitro with the anti-CD33 monoclonal antibody L4F3 and 13 were incubated with 4-hydroperoxycyclophosphamide (4-HC), 2 at a dose of 100 ug/ml 4-HC and 11 at 60 ug/ml (4). The 26 patients transplanted in second remission had remission status determined by bilateral iliac crest aspirates within 2 weeks of transplant. Two of the 26 patients had marrow stored while

in first remission and 24 while in second remission. One patient had marrow purged with L4F3, 6 with 100 ug/ml of 4-HC and 5 with 60 ug/ml of 4-HC.

Transplant preparative regimens consisted of cyclophosphamide (CY) and total body irradiation (TBI) given as a single exposure of 10.0 Gy or 12.0-15.75 Gy over 4-7 days, or busulfan 16 mg/kg plus CY 120 mg/kg, or busulfan 8 mg/kg plus CY 60 mg/kg plus 12.0 Gy TBI (Table 2). Cryopreserved marrow was thawed rapidly and immediately infused following the last dose of TBI or 36 hours after the last dose of CY (7). 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, 1990.

Kaplan-Meier estimates were used to determine probabilities of engraftment, non-leukemic mortality, relapse and disease-free survival for each group (8). Engraftment is defined as peripheral blood granulocyte level $>1000/mm^3$ and platelet level $>50,000/mm^3$. Logrank statistics were used to test for differences. The following parameters were entered into multivariate analyses: duration of first remission, months between marrow storage and relapse or months between relapse and storage, marrow cell dose $x10^8$/kg, in vitro marrow purging, transplant preparative regimen X 10^8/kg, in vitro marrow purging, transplant preparative regimen and phase of disease at transplant. Endpoints evaluated included engraftment, relapse and survival. The relative risk of an event in this proportional hazards model was the instantaneous risk in comparison to those patients free of the event when adjusted for other variables (9).

RESULTS

First Untreated Relapse

Six of 30 patients transplanted in first untreated relapse survive relapse free from 289-2802 days (Table 2). The probability of disease-free survival is 20% at one year and 10% at 3 years (Figure 1). Five of the six patients have complete recovery of marrow function, but one survives >1.5 years with persistent thrombocytopenia.

Recurrent leukemia occurred in 10 patients between day 28 and day 233. Figure 1 illustrates the probabilities of relapse for patients receiving purged (55%) or unpurged (63%) marrow ($p=0.20$).

Twenty of the 30 patients had incomplete recovery of marrow function following transplant. Five of these 20 received a median of 4.2 x 10^8/kg (1.1-6.1) nucleated marrow cells, but died of infection before day 20 without recovery of any peripheral blood counts. The remaining 15 patients received a median of 3.0 x 10^8/kg (1.1 - 8.6) nucleated marrow cells and 9 of the 15 died of non-leukemic causes. Four of the 9 never had recovery of any peripheral blood counts and died before day 100. Four of 5 patients had only recovery of granulocyte levels (500-1,000/mm^3) and died of bleeding or infection, and one patient survives >1.5 years without platelet recovery. The probability of non-relapse mortality before day 100 was 43%. The 10 patients with complete

hematopoietic recovery had a median aspirated marrow cell dose of 4.6 x 10^8/kg (2.8-5.1). For these 10 patients, granulocyte levels (>1,000/mm^3 recovered at a median of day 42 (33-81) and (>50,000/mm^3) platelets recovered at a median of day 66 (45-160).

Second Remission

Seven of 26 patients transplanted in second marrow remission survive leukemia-free from 300-1185 days post-transplant (Table 2). Figure 2 illustrates the probability of disease-free survival (26%). Five patients have complete recovery of marrow function and 2 survive 300-720 days with platelet levels of 20,000 and 50,000/mm^3.

Eleven patients relapsed between 45 and 198 days after transplant. The probability of relapse for the 12 patients who received purged marrow was 37% and for the 14 patients given non-purged marrow was 79% (P=0.23).

Incomplete recovery of hematopoiesis occurred in 18 patients. Among the 8 patients with complete marrow reconstitution, the median aspirated marrow cell dose was 3.0 x 10^8/kg (1.0 - 9.1). Circulating granulocytes recovered to >1000/mm^3 at a median of day 44 (22 and 115) and platelets recovered to >50,000 by a median of day 117 (36-337). Among the patients with poor engraftment, 5 received a median of 2.8 x 10^8/kg (1.8-5.6) nucleated marrow cells and died of infections before day 20. Thirteen received a median marrow cell dose of 3.0 x 10^8/kg (0.8-6.3). Three of these 13 patients died of transplant related complications, 8 of recurrent leukemia and 2 survive with platelet counts between 20,000 and 50,000/mm^4. The probability of non-relapse mortality was 328 at 100 days.

When all 56 patients were considered in a multivariate analysis, none of the factors tested were significantly associated with engraftment. The number of months between storage and relapse or months between relapse and storage was the only factor associated with relapse. When evaluated as a continuous variable, the greater the number of months between storage and relapse the less the risk of relapse (Relative Risk (RR) 0.88) (P=0.05). Patients who had less than 5 months between storage and relapse had a relapse rate of 54% whereas those more than 5 months had a relapse rate of 22%. Survival was significantly related to the length of first remission (RR = 0.96, P=O.05). None of the patients whose first remission was less than 7 months survive. Among patients whose first remission was 7-12 months 15% survive compared to survival of 32% for patients whose first remission was >12 months.

DISCUSSION

We have previously reported that patients with AML who have an HLA matched sibling do not benefit from attempts at induction into second remission before transplantation (1,10,11). In the current study, the disease-free survival of patients in first untreated relapse was similar to that of patients transplanted while in second remission. These results are similar to those achieved following matched allogeneic transplants for patients with AML at the same

phase of disease. The two groups of autografted patients however are not directly comparable since most patients who had marrow stored in first remission could proceed to transplant whereas patients transplanted in second remission could only be transplanted if they achieved a second remission which probably occurred in 30-40% of relapsed patients. Transplantation in untreated first relapse increases the total number of patients who could benefit from a transplant and transplantation in second remission would need to be twice as effective to be the preferred time for a transplant. It is, therefore, reasonable to consider marrow storage for all patients in first remission who do not have a suitably matched family member donor with the intent to perform an autologous transplant at first sign of relapse.

The majority of autologous marrow transplants performed for patients with AML utilize some form of marrow purging in an attempt to deplete the marrow of residual leukemic cells (4). The present study included patients who received purged and unpurged marrow, but was not a randomized study. For the more recent patients, purging has been restricted to patients where more than 4.0×10^8/kg total nucleated cells were collected and patients yielding less than this number received non-purged marrow. Thus, conclusions about the effectiveness of purging cannot be made. However, there were no statistically significant differences observed in relapse rates between purged and unpurged marrow and the probabilities of relapse were similar to those seen following allografts in similar patients. Improved purging methods are unlikely to result in a significant decrease in relapse rates. More effective pre-transplant or post-transplant treatment regimens are more likely to effect overall outcome.

The major reason for treatment failure in these patients was poor engraftment with an accompanying high non-relapse mortality. However, the reason for the high proportion of patients with poor engraftment is not clear and these results may differ from those reported by others (6,12). The apparent differences may reflect patient selection, differences in purging or freezing techniques or the collection of larger numbers of marrow cells. It cannot be concluded from this study that 4-HC purging increases the graft failure rate since there were no differences in the frequency of graft failures between the recipients of purged and non-purged marrow. The more likely explanation for poor engraftment is a quantitative or a qualitative defect in hematopoietic stem cells. There was a suggestion in this study that patients receiving a high cell dose had less graft failure suggesting a quantitative defect, however this trend was not statistically significant. Attempts to aspirate more marrow cells or augment the marrow harvest with peripheral blood stem cells could be of potential benefit (13). Whether or not normal hematopoiesis can be enhanced by growth factors remains to be determined. In addition, the use of more aggressive supportive care techniques could decrease mortality and allow time for engraftment and result in improved survival (14).

These data demonstrate that for the patient with AML beyond first remission, autologous marrow transplantation in first untreated relapse or in second remission results in small but probably significant fraction of long term survivors. The major problems which need to be addressed relate to

improvements in complete engraftment rate in order to decrease non-relapse mortality. In addition, improved methods to prevent relapse are also required.

ACKNOWLEDGEMENT

This investigation was supported by CCA 8510/019 U.S./Spain Joint Committee for Scientific & Technological Cooperation, and grants CA 47748, CA 18029, CA 18221, CA 15704 awarded by the National Cancer Institute, DHHS.

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TABLE 1

Patient Characteristics

	First Untreated Relapse	Second Remission
Number Patients	30	26
Age, years: median (range)	26 (3-56)	31 (6-50)
FAB Group		
M 1 - M 2	11	11
M 3	1	1
M 4	6	8
M 5	2	1
M 6	2	1
Unknown	8	4
Length First Remission months: median (range)	12 (3-35)	18 (3-40)
Months CR 1 to Storage median (range)	7 (3-35)	18 (6-44)
Number Purged Marrow	15	12
% Marrow Blasts at Transplant median (range)	46 (5-95)	0

* One patient was in first marrow remission but had an extramedullary (chloroma) relapse

TABLE 2

Transplant Results

	First Untreated Relapse	Second Remission
Number Patients	30	26
Preparative Regimen		
CY + 10.0 Gy TBI	1	3
CY + 12 - 15.75 Gy TBI	19	8
BU + CY + 12.0 Gy TBI	3	8
BU + CY	7	7
Engraftment:		
No Graft	7	4
Partial Graft	8	9
Complete Graft	10	9
Not Evaluable*	5	5
Relapse:		
Marrow Purged	4	4
Marrow Not Purged	6	7
Cause of Death		
Infection	9	3
Bleeding	4	2
ARDS/IIP	1	2
VOD	0	1
Relapse	10	11
Survival	6	7

*Death from infection before day 20.

FIGURE 1

Kaplan Meier probability of disease-free survival (10%), and Kaplan-Meier probability of relapse for 15 patients whose marrow was not purged (NO BM Rx) and 15 patients whose marrow was purged (4-HC) (P=0.20). Tic marks signify patients surviving in remission after autologous transplant in first untreated relapse.

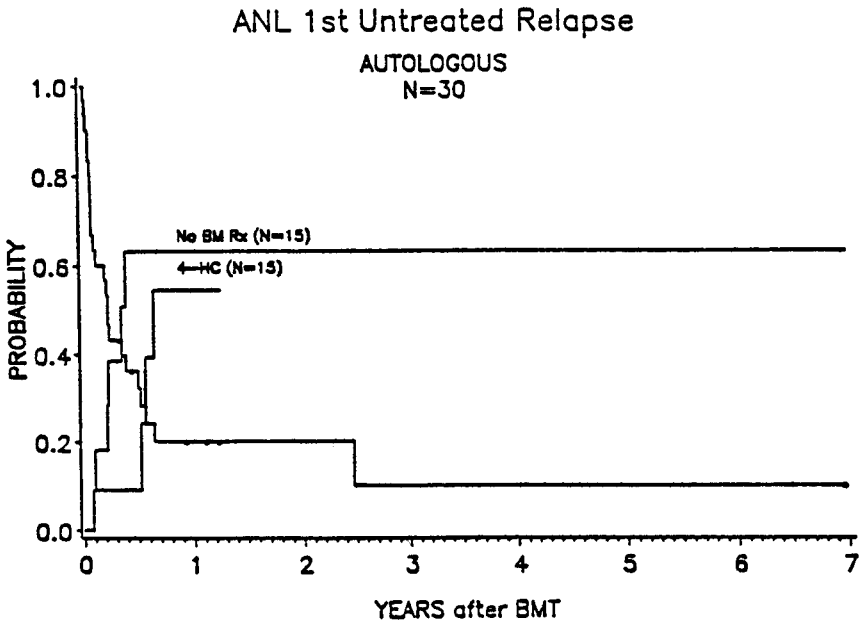
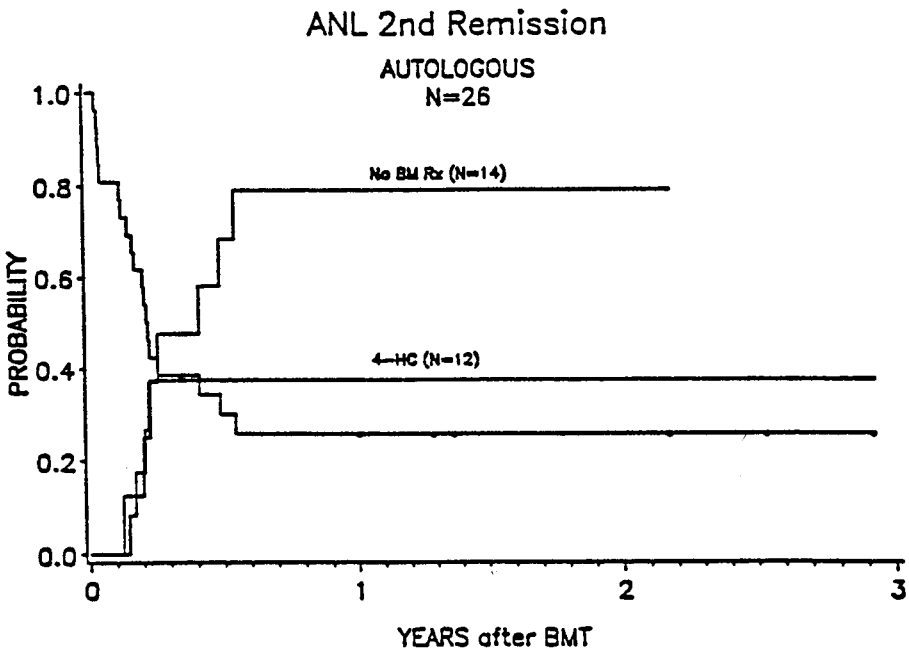


FIGURE 2

Kaplan-Meier probability of disease-free survival (26%) and Kaplan-Meier probability of relapse for 14 patients whose marrow was not purged (NO BM Rx) and 12 patients whose marrow was purged (4-HC) (P=0.23). Tic marks signify patients surviving in remission after autologous transplant in second remission.



HIGH DOSE CHEMOTHERAPY AND UNPURGED AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR ACUTE LEUKEMIA IN SECOND OR SUBSEQUENT REMISSION

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INTRODUCTION

Treatment of acute leukemia (AL) in adults frequently achieves complete remissions (CR); however, most patients suffer relapses, and long-term leukemia-free survival (LFS) is achieved in only a minority of patients. Once relapse occurs, salvage chemotherapy regimens can be attempted[1-4], but long-term LFS is very rarely achieved.

Drug-sensitive tumors (such as leukemia) show a dose-response curve in animal tumor systems.[5] High doses of cytosine arabinoside (Ara-C) can produce CR in patients refractory to lower doses[6]; addition of an Ara-C intensification to front line therapy seems to improve long-term DFS when compared with low dose maintenance therapy.[7] For many drugs, dose escalation is limited by bone marrow toxicity, but increased cytoreduction is possible if myeloablative regimens are followed by bone marrow transplantation (BMT). The value of dose escalation in acute leukemia is confirmed by the syngeneic BMT data.[8]

We have shown that high-dose therapy and ABMT produced only transient remissions for patients with relapsed acute leukemia.[9,10] Patients who receive transplants while still responding to chemotherapy have a lower tumor load and less resistant disease, and high-dose therapy with autologous bone marrow transplantation (ABMT) has been used to prolong second remission in acute leukemia.[11-15] We treated 22 adult patients with acute leukemia in second or subsequent remission with high dose chemotherapy and unmanipulated ABMT; three patients obtained long-term remissions that were longer than any of their prior remissions ("inversions"). Since results of salvage regimens are difficult to compare because of patient selection biases, we propose the adoption of a standard way of reporting results of salvage therapy that would allow for more accurate comparison of different treatments.

PATIENTS AND METHODS

Patient Population

All patients gave informed consent; the protocol was approved by the Institutional Review Board of the M. D. Anderson Cancer Center. Twenty-two patients were treated (Table 1). The median age was 31.5 years (range, 21 to 55 years). Seven patients had ALL and 15 had AML; 13 were in second remission, eight in third remission, and one in fourth remission. The median duration of first remission was 18 months (range, 2 to 29 months). For the patients receiving transplants in third remission, the median duration of the second remission was 10 months (range, 1 to 93 months). Other details of patient characteristics are given in Table 1, and prior therapies and duration of prior remissions are detailed in Table 2.

Bone Marrow Collection

Bone marrow was collected during remission. A median of 1.65×10^8 nucleated cells/kg (range, 1.0 to 5.0) was harvested and cryopreserved in liquid nitrogen. Eight patients had bone marrow collected in the same remission in which they were transplanted. For these patients, the median time from CR to BMT was 3 months (range, 1 to 6 months), and median time from harvest to BMT was 1 month (range, 1 to 2 months). Fourteen patients were harvested in a remission previous to the one when they had ABMT; of them, ten were harvested in first remission, three in second remission, and one in third remission (Table 1). In this group, the median time from harvest to relapse was 10 months (range, 2 to 22 months), and the median time between remission and ABMT was 2 months (range, 1 to 4 months).

High-Dose Therapy

On day -6, patients received cyclophosphamide, 1.5 g/m^2 intravenously (IV) daily x 4 days; BCNU (carmustine) 300 mg/m^2 iv x 1 dose, and VP-16 (etoposide) 100 to 150 mg/m^2 IV every 12 hours x 6 doses (CBV).[10,16] On day 0, all the stored bone marrow was thawed at 37C and infused through a central venous catheter. The median interval between remission and BMT was 2 months (range, 1 to 6 months). The first two patients received only three days of cyclophosphamide; the first 15 patients received 600 mg/m^2 of VP-16, and the rest received 750 mg/m^2 except for patient 18, who received 900 mg/m^2. No further chemotherapy was given unless patients had a relapse.

Statistical Methods

Relapse was defined as the presence of $>5\%$ blasts in two consecutive bone marrow differentials or any evidence of extramedullary leukemia. Blood count recovery times were measured from day of BMT to the day when the reported value was reached; patients who relapsed before full recovery were censored at the time of relapse.

Remission duration was measured from date of BMT to date of relapse, death from any cause, or last follow-up. The method of Kaplan and Meier [17] was used to plot remission duration curves. The duration of remission in distinct patient groups was compared using the generalized Wilcoxon test.[18] All reported P values are two-sided.

RESULTS

Patients 2, 16, and 18 (two transplanted in third remission and one in second remission) are still disease-free at 87+, 51+, and 49+ months after transplant, respectively. These remission durations surpassed the length of first remission for all three patients and that of the second remission for the two patients transplanted in third remission (Table 3). Seventeen patients have relapsed at a median time of 5 months (range, 4 to 7), and all but one have died of their disease (Fig. 1). Two patients had treatment-related deaths (see below). The long-term DFS rate is 14% (95% CI, 0% to 25%).

Prognostic Factors

The following factors were evaluated to determine their relationship with remission duration: age, sex, diagnosis, number of relapses before BMT, history of CNS involvement, dose of cyclophosphamide, dose of VP-16, duration of first remission, timing of bone marrow collection (same CR as transplant or not), remission in which bone marrow was collected, and interval between CR and transplantation. For patients whose marrow was harvested in first remission, the interval between bone marrow collection and first relapse was also evaluated. Cytogenetics were analyzed in AML patients, who were grouped as good risk (inv 16), intermediate risk (diploid, t(8;21), and poor risk (all others).[19] No significant differences were observed between different patient groups, a finding that may be due to the small number of patients studied.

Toxicity

All patients had fever while neutropenic, and ten patients had culture-proved sepsis. Patients 9, 10, 13, and 18 had pneumonia (three with concomitant sepsis). Patient 20, who had received anthracyclines in the past, had cardiogenic shock on day 21 and was discharged with Grade II congestive heart failure that resolved after 5 months. Patients 7 and 13 experienced seizures during the administration of chemotherapy; in patient 7, the seizures were preceded by an extrapyramidal reaction to antiemetics. Neither patient had a history of CNS involvement, and both had normal findings in cerebrospinal fluid examination and computed tomography of the brain. Patients 6 and 13 had gross hemorrhagic cystitis which subsided with bladder irrigation.

There were two early deaths: patient 9 died on day 19 after transplant of *Pseudomonas aeruginosa* bronchopneumonia and probable CNS bleeding (coma, anisocoria); patient 13 had pneumonia, sepsis, gastrointestinal bleeding, and status epilepticus of unclear cause, and died on day 12.

Hematopoietic Recovery

The two patients who died early after transplantation were not evaluable. The median times (ranges) from BMT to absolute granulocyte count of $0.5x10^9/1$ and to platelet count of $50x10^9/1$ were 22 days (14 to 55+ days) and 29 days (13 to 76 days) respectively. There was no significant difference in recovery times if bone marrow was harvested in first remission as opposed to bone marrow harvested in subsequent remissions.

DISCUSSION

Three of our patients have achieved inversions and remain disease-free 87+, 51+, and 49+ months after CBV + ABMT, respectively. The major question is whether these long-term remissions are due to the high-dose regimen. Patient 2, who had an 11-month first CR with doxorubicin, Ara-C, vincristine, and prednisone (Ad-OAP), obtained his pre-BMT remission with amsacrine (AMSA). It is unlikely that single-agent AMSA is curative for patients with relapsed AML; although several large phase-II evaluations of AMSA for relapsed or refractory AML showed 41 CRs in 245 patients, none of the remissions lasted more than 59 weeks.[20-26] Patient 16 was in third remission after high-dose Ara-C, a therapy that had failed to cure him in the past. It is very unlikely that repeating the same regimen would have achieved prolonged DFS in the second relapse. Finally, patient 18 had achieved a long first CR of ALL, but the second CR was very short. In spite of the long first remission, the poor outcome of the first salvage therapy indicates a very low likelihood of prolonged DFS with the third line regimen. Consequently, we believe that the high-dose treatment, and not the regimens used to induce remission before it, was responsible for the good outcome in these three patients.

Comparisons of efficacy of salvage treatments for relapsed leukemia are difficult because of heterogeneity of patient populations. The natural history of acute leukemia shows progressive shortening of remission duration after each relapse; remissions that last longer than the preceding ones are unusual, if adequate treatment is given in every occasion. We have proposed the use of the term "inversion" for evaluation of results of salvage therapies. [27] Inversion is defined as a remission that lasts longer than prior one, and has a clinically significant duration (i.e., 12 months or greater). In this concept, each patient serves as his own control, thus eliminating selection bias related to length of previous remission and allowing comparison of results between different groups.

Conventional dose salvage therapy produces long-term DFS almost exclusively in patients with prolonged first remissions.[2,4,28,29] A better prognosis is seen with first remissions greater than 12 months [1,2] or 18 months.[2,4,29] We did not find a correlation between duration of first remission and remission duration after salvage in our patients, suggesting that dose escalation may be equally effective in late and may be an artifact due to the Inversion rates are difficult but seem low. A series of 80 salvage chemotherapy[1] had ten received

BMT (five allogeneic term DFS rate of chemotherapy early relapses, but this finding small number of patients treated. to estimate from published data, AML patients who achieved a CR with long-term survivors, but seven and two autologous), for a longterm DFS rate of chemotherapy of less than 5%. Another series of 41 AML patients in second CR had three inversions.[3] In ALL, a series of pediatric patients with initial CR lasting 18 months or longer had a 3-year DFS of 54% (95% confidence interval, 39%-69%), whereas none of those with shorter first remissions reached 3 years free of disease.[4] Second remissions were longer than the first ones in ten of 31 patients. Overall, conventional dose chemotherapy is unlikely to produce inversions in a large percentage of adult patients with acute leukemia in second or greater remission, and results are very poor in patients with short first remissions.

Results of ABMT in second or subsequent remission are variable, because of marked heterogeneity of treatments before BMT, conditioning regimens, and length of follow-up among diverse patient groups; most trials have small numbers of patients, and the confidence limits of the reported long-term LFS rates are wide. Four groups have reported long-term LFS rates of 18%, 20%, 43%, and 52% in groups of 20 or more patients [11-14]; their respective inversion rates were 21%; unknown, [12] 22%, [13] and 52%;[14] an earlier publication from the second group [15] described an inversion rate of 30%.

The failure of high-dose therapy to cure relapsed leukemia may be due to the regimen utilized or to infused leukemic cells. With allogeneic transplantation, which eliminates the risk of infusing leukemic cells, only about 25% to 30% of AML patients transplanted in first relapse or second CR with cyclophosphamide and total-body irradiation (TBI) are cured, [31,32] whereas 35% to 55% suffer relapses. Clearly, better cytoreductive regimens are needed; this need is greater in autologous BMT, where the graftversus-leukemia effect is absent. The encouraging preliminary results with the BAVC regimen[14] need to be confirmed with larger numbers of patients. Since the marrow infused with ABMT may contain residual clonogenic leukemic cells and induce relapse, many investigators have tried to eliminate these cells ex vivo [11-13] (purging). The efficacy of purging is unclear because there is no reliable method to monitor the efficacy of cell removal, and because of the heterogeneity of patient groups discussed above. The European Bone Marrow Transplantation Group (EBMTG) found no difference between purged and unpurged bone marrow in second remission,[30] and other reported results with unpurged bone [14] marrow seem equal or superior to those of purging. More data are needed to fully ascertain the role of ABMT and the need of purging; this report provides additional information about ABMT with unpurged bone marrow.

Although three (14%) of our patients are long-term survivors and appear to have been cured by CBV with ABMT, the inversion rate is not better than that of conventional-dose chemotherapy, and seems worse than that of some ABMT trials. We do not believe that this poor outcome is solely due to the unpurged bone marrow, because these results are not worse than those obtained with CBV and marrow purged with vincristine and 4-HC.[27] It seems

that CBV is not effective enough, and a better cytoreductive regimen will be needed to obtain a larger cure fraction.

ACKNOWLEDGEMENTS

Supported in part by the National Cancer Institute Grant CA-28513. We thank Maggie Maher for expert help.

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TABLE 1

Patient Characteristics

Pt. No.	Age	Sex	DX	Cytogenetics	Remission No. at Storage	BMT (mo)
1	24	M	ALL	Diploid	2	2
2	32	M	AML	Diploid	2	2
3	31	M	AML	Ph(+)	2	3
4	25	M	AML	t(8;21),+8	1	2
5	32	M	AML	+8	1	2
6	21	F	ALL	Iso 7q	3	3
7	22	F	ALL	No metaphases	2	2
8	51	M	AML	Diploid	3	3
9	55	M	AML	Diploid	1	2
10	43	M	AML	t(8;21), 45X-Y	1	2
11	28	M	ALL	Diploid	3	3
12	50	M	AML	Diploid	1	2
13	32	M	AML	Diploid	1	2
14	27	F	ALL	Diploid	1	2
15	21	F	AML	Inv12	1	2
16	40	M	AML	Inv16	2	3
17	48	F	AML	Inv16, +8	1	2
18	37	M	ALL	Unknown	1	3
19	24	M	AML	Inv16	2	2
20	32	M	ALL	Ph(+)	3	3
21	28	M	AML	Inv16	2	3
22	27	M	AML	Inv16	3	4

DX: diagnosis; CR: complete remission; BMT: bone marrow transplantation; AML: acute myelogenous leukemia; ALL: acute lymphoblastic leukemia.

TABLE 2

Prior Therapy

Patient No.	DX	Rx1	Duration CR1	Rx 2	Duration CR2	Rx 3	Duration CR3
1	ALL	Ad, V, Ara-C, P	2	C, R, V, P, L, M	4*		
2	AML	Ad, V, Ara-C, P	11	AMSA	6*		
3	AML	Ad, Ara-C	9	Ara-C, T, Dx, V, P, HU	4	HD Ara-C	1*
4	AML	D, Ara-C, T	9	AMSA, Pl	1*		
5	AML	Ad, V, Ara-C, P	29	Ad, V, Ara-C, P	2*		
6	ALL	Ad, V, Ara-C, P, MP	23	Ad, V, Ara-C, P	10	Ad, V, Ara-C, P	2*
7	ALL	Ad, V, Ara-C, P, M, AMSA	17	Ad, V, Ara-C, P	1*		
8	AML	V, Ara-C, P, M, MP	25	Ad, V, Ara-C, P, M, MP	93	Ad, V, Ara-C, P	4*
9	AML	Ad, V, Ara-C, P, AMSA	15	HD Ara-C	1*		
10	AML	B, Ara-C, T	9	D, Ara-C, T	4*		
11	ALL	D, Ara-C, T, V, P	17	D, Ara-C, T	4	Ara-C, T, M, L	3*
12	AML	Ad, V, Ara-C, P	18	Ad, Ara-C	1*		
13	AML	Ad, V, Ara-C, P	18	Ad, V, Ara-C, P	2*		
14	ALL	V, P, Ad, Ara-C, L, M	18	M, L, V, Ad, Dx	2*		
15	AML	Ad, Ara-C, T	25	Ara-C, T, M, E	2*		
16	AML	AMSA, Ad, V, Ara-C, P	21	HD Ara-C	24	HD Ara-C	2*
17	AML	AMSA, V, Ara-C, P	20	HD Ara-C	1*		
18	ALL	D, Ara-C, V, P, T	24	D, Ara-C, T, M, L	1.5	V, Ad, Dx	1*
19	AML	AMSA, V, Ara-C, P	4	HD Ara-C	3*		
20	ALL	V, Ad, Dx, M, L	3	Ad, HD Ara-C, D, M, P, V	3	Ad, Ara-C, M, D, P, V	2*
21	AML	Ad, V, Ara-C, P	27	Ad, V, Ara-C, P	20	Mit, HD Ara-C	2*
22	AML	Ad, V, Ara-C, P	23	Ad, V, Ara-C, P	12	Ad, V, Ara-C, P	22[1]

Ad: doxorubicin; AMSA: amsacrine; C: cyclophosphamide; D: daunorubicin; Dx: dexamethasone; E: etoposide; G: thioguanine; H: homoharringtonine; HD Ara-C: High-Dose Ara-C; HU: hydroxyurea; L: L-asparaginase; M: methotrexate; Mit: mitoxantrone; MP: mercaptopurine; P: prednisone; Pl: cisplatinum; R: rubidazone; V: vincristine

* Remission in which CBV-ABMT was given (time CR to BMT).

[1] Achieved CR4 with HI, HD Ara-C and received BMT 2 months later.

TABLE 3

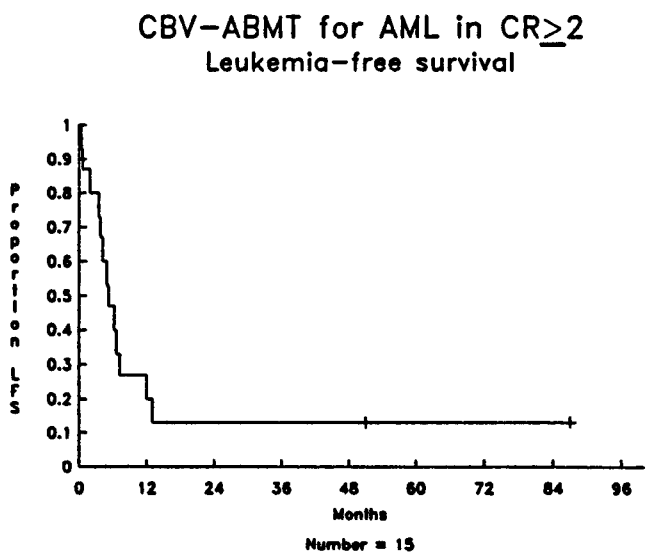
Post-Transplantation Course

Pt. No.	Remission duration (mo)	Severe toxicities
1	3.5	Mucositis 3+
2	87.0+	--
3	1.9	Sepsis, delayed platelet recovery
4	3.8	--
5	4.9	--
6	5.8	Cystitis
7	7.5	Sepsis, jaundice, Seizure
8	6.6	--
9	0.6	Sepsis, pneumonia, CNS bleeding, jaundice, died day 19
10	5.2	Pneumonia
11	8.5	Sepsis
12	6.2	--
13	0.4	Sepsis, pneumonia, mucositis 3+, GI bleeding, seizures, cystitis, died day 12
14	1.1	--
15	13.0	--
16	51.0+	Sepsis
17	3.5	--
18	49.0+	Sepsis, pneumonia
19	4.2	Sepsis, perirectal cellulitis
20	2.8	CHF
21	7.2	Sepsis
22	12.0	Sepsis

Pt: patient; RD: remission duration; CHF: congestive heart failure.

FIGURE 1

CR duration, measured from day of transplantation. Tic marks indicate patients alive and in continuous remission.



BAVC FOLLOWED BY UNPURGED MARROW IN II CR AML PATIENTS

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INTRODUCTION

Autologous bone marrow transplantation (ABMT) is a promising therapeutical strategy which offers an alternate approach to post-remissional management of patients with acute myelogenous leukemia (AML) (1-2). Its role is still to be demonstrated in first remission AML patients and various pluricentric controlled trials are ongoing in Europe and the USA to verify the value of ABMT versus either intensive postremissional chemotherapy or allogeneic BMT.

The situation is different in second remission AML. So far results from chemotherapy programs are very poor and few patients, if any, can be cured without bone marrow transplantation. ABMT represents an alternative approach to allogeneic BMT and a 25% of disease free survival (DFS) is reported at 7 years in the 1990 EBMT survey (3).

From this survey, positive impact of marrow purging has been established in patients treated with regimens including total body irradiation (TBI) for the first time. Another important answer which has been drawn from this analysis concerns the timing of marrow collection; in fact no difference has been observed in the DFS between patients transplanted with marrow collected in 1st or in 2nd CR.

In our experience we have transplanted AML patients in 2nd CR with a high dose conditioning regimen BAVC (BCNU, AMSA, VP-16, ARA-C) (4) utilizing unpurged marrow collected during first or second remission.

From October 1984 and June 1990, 31 consecutive AML patients, without HLA compatible donor, suitable for aggressive chemotherapy entered this study. Median age was 22 years (range 1-49), 12 were female, according to FAB classification, the patients were allocated as M1(5) M2(8) M3(4) M4(11) M5(2) M6(1). Median first CR duration was 14 months (range 1-44). ABMT was performed after a median of two months (range 1-13) from reaching 2nd CR. The majority of patients were induced in 2nd CR with high or

intermediate doses of ARA-C plus either AMSA or Mitoxentrone. After complete remission a consolidation with the same drugs employed for induction was performed in 22 cases while the others underwent directly ABMT. Twenty-five patients underwent marrow collection immediately prior the starting of pretransplant chemotherapy and 6 were transplanted with marrow harvested during 1st CR. The techniques of marrow collection, cryopreservation and reinfusion have been previously described (5). Patients were nursed in single or double rooms, bowel decontamination was performed with either norfloxacine or cotrimoxazolo. Broad spectrum antibiotics were given for fever during aplasia adding amphotericin 8 when a persistent fever or a documented fungal infection occurred. All patients at risk for the recurrence of herpes virus infection received prophylactic intravenous acyclovir. Conditioning regimen was well tolerated and only various degrees of nausea and vomiting were observed during administration of chemotherapy. Five patients developed severe oral mucositis which was generally resolved at the time of bone marrow engraftment. Episodes of severe hemorrhage were not observed. Twenty-one had fever during aplasia: in 11/21 (52%) fever was associated with positive culture for bacteria (10) or fungus (1 patient). One patient died in aplasia on day + 31 without evidence of engraftment. In the remaining 30 patients the median time required to attain an absolute neutrophil count in excess of 0.5 x 10^9/l was 18 days (range 11-40). A platelet count exceeding 50 x 10^9/l was observed after a median of 30 days (range 13-180). No correlation was observed between the number of nucleated bone marrow cells infused and the rate of hematological recovery.

As of June 1990, 19 of 30 evaluable patients are in CCR with a median follow-up of 35 months (range 1-66). Ten patients relapsed in the bone marrow and 1 patient had an isolated meningeal relapse. Median time to relapse was 6 months (range 2-18).

The duration of the second CR has exceeded the duration of the 1st CR in 13 patients. Among patients relapsing after ABMT, a 2nd CR duration greater than the first one was observed only in the case with isolated CNS relapse. Projected probability of DFS in all 31 transplanted patients is 56% at 66 months (Figure 1). A relationship between probability of CCR and length of first CR, FAB classification, time of bone marrow collection sex, age and consolidation therapy was not observed.

From November 1989 in the attempt to eradicate minimal residual disease we devised a plan of post transplant therapy utilizing IL-2 (6). IL-2 was used as consolidation after complete hematological recovery by continuous infusion for 5 days with daily escalating doses from 100 mcg/m^2/day, up to a maximum tolerated dose. Maintenance treatment consisted of low dose IL-2 (100-200 mg/m^2) given daily 5 days per month for 6 months on an outpatient basis over a 6 hours infusion period (7).

So far, 3 patients (Table 1) have been treated without major side effects and are in CCR at 8, 8 and 6 months from transplant respectively. In two of these patients the duration of the second CR has exceeded the duration of the first one.

In our experience BAVC regimen was well tolerated with less then 3% transplant related deaths. It is worth noting that, despite the fact that the marrow was not purged ex vivo, in 13/30 (43%) evaluable patients duration of 2nd CR has exceeded the duration of first CR and that an EFS of 56% is projected at more than 5 years after a median follow up of 3 years.

It would be very important that these results will be confirmed by several other groups on a larger series of patients to verify the feasibility and the antileukemic efficacy of BAVC regimen. The value of IL-2 as post-transplant therapy is under investigation.

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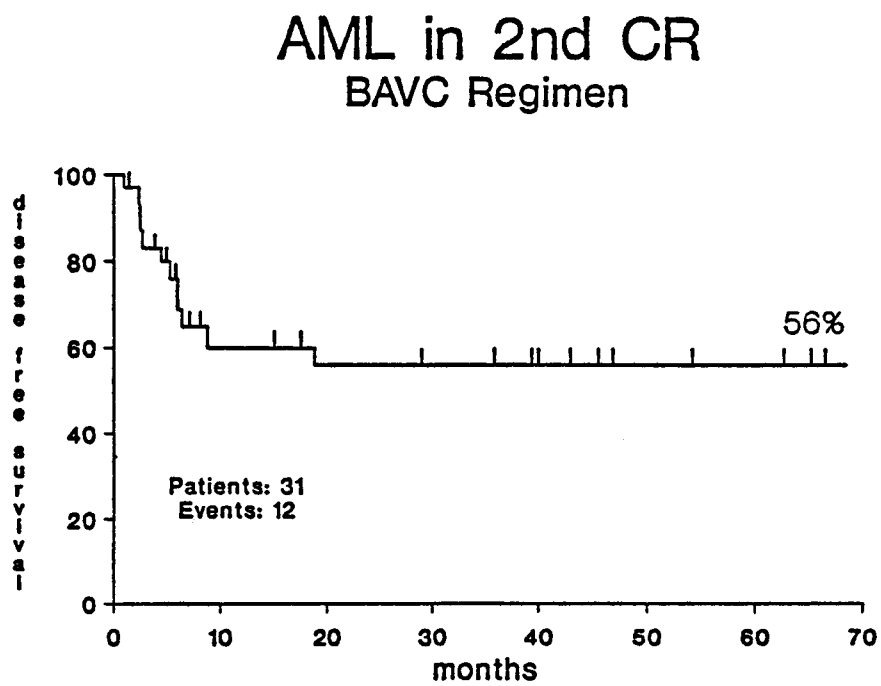
TABLE 1

			IL-2 TREATMENT			
Pts	*Sex/Age*	*FAb*	*I CR (months)*	*II CR --> ABMT (months)*	*ABMT --> IL-2 (days)*	*Total II CR (months)*
1	*F / 5*	*M7*	*3*	*1.5*	*58*	*7.5*
2	*F / 11*	*M4*	*9*	*2*	*84*	*10*
3	*F / 24*	*M4*	*17*	*3.5*	*103*	*11.5*

FIGURE 1



AUTOLOGOUS BONE MARROW TRANSPLANTATION WITH UNPURGED MARROW IN ACUTE NON-LYMPHOBLASTIC LEUKEMIA

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INTRODUCTION

During the last 10 to 15 years a number of transplant centres have reported their results of successfully treating patients with acute non-lymphoblastic leukemia (ANLL) in relapse [1,5] or complete remission [2,3] with high-dose chemo/radiotherapy followed by autologous bone marrow transplantation (ABMT). Attempts to further increase the efficacy of ABMT in ANLL have been made, e.g. by double sequential autografting [4,5] or ex vivo marrow purging, mainly with cytostatic drugs [6,7]. Randomized prospective trials comparing ABMT to other treatment modalities have recently been published, where ABMT in first complete remission ANLL was shown to be superior to standard chemotherapy [8] but less effective than allogeneic BMT [8,9] to prevent leukemic relapse and improve patient survival. As far as ex vivo purging is concerned, no controlled prospective trials comparing purged and unpurged ABMT have been undertaken. Thus, the value of purging is still uncertain, although in one uncontrolled trial of ANLL autografted in first complete remission (CR1) after marrow purging with mafosfamide, the leukemia free survival was significantly better when compared to an "unpurged" control group [10].

The aim of the present study was to evaluate the efficacy and toxicity of high dose cytoreductive treatment followed by ABMT for ANLL in different stages of disease. For patients in second or subsequent remission the very poor long term survival with conventional antileukemic therapy justified the uncontrolled use of ABMT. The CR1 patients were compared to a historical control group of individuals in the same stage of disease, who had received conventional chemotherapy in the two centres in charge of this study during the seven years prior to the start of the Huddinge University Hospital ABMT-program for ANLL in CR1.

PATIENTS AND METHODS

Patients

Twenty-six adults, between 15 and 60 years of age, with ANLL underwent unpurged ABMT at Huddinge University Hospital from 1985 to April 1990. Thirteen patients were in first complete remission (CRl), nine were in second and one was in third complete remission (CR2-3) and three were in first relapse. The diagnosis of ANLL was preceded by a six month phase of myelodysplastic syndrome - MDS - (refractory anemia with excess of blasts) in one of the CR1 patients and one of the patients transplanted in relapse. Twenty-three of the 26 patients (including 12 of the 13 patients transplanted in CR1) received their primary induction and consolidation treatment in the University Hospitals of Huddinge or Linkoping. The 13 CR1 patients were compared to a historical control group (n = 26), consisting of all the patients who were in the same age group, were treated with conventional chemotherapy in the two centres from 1981 to 1987, and who reached CR1. These control patients also lacked HLA-matched sibling donor and therefore would have been eligible for ABMT. Pre-transplant characteristics and induction chemotherapy for the ABMT-group and the control group were similar (table 1, table 2). Four additional patients (three in CR1 and one in CR2) were planned for ABMT, but were never transplanted because of leukemic relapse or infectious death shortly before transplantation; these patients are not included in the analyzes.

The bone marrow harvest was performed within two months prior to ABMT in all patients who were autografted in remission. Patients transplanted in relapse were autografted with marrow cryopreserved in CR1. One marrow harvest was sufficient in 22 patients, but due to low cell numbers marrow was harvested twice in three patients and three times in one patient. Bone marrow was obtained by aspirations from the iliac crests. The processing of the marrow then consisted of density gradient separation on IBM 2991 cell separator, controlled rate freezing to -80Celsius with dimethylsulfoxide and autologous plasma and cryopreservation in liquid nitrogen at -190Celsius. At transplantation, the marrow was thawed in water bath at +40Celsius and then was immediately infused into the patient through a central venous line.

The conditioning regimens used were cyclophosphamide 120mg/kg + total body irradiation 10 Gy (Cy/TBI) in 15 cases, busulfan 16 mg/kg + cyclophosphamide 120 mg/kg (Bu/Cy) in nine, busulfan 16 mg/kg + melphalan 140 mg/sqm (Bu/Mel) in one and busulfan 16 mg/kg (Bu) in one case. All patients received oral antimicrobial prophylaxis with ciprofloxacin or norfloxacin and amphotericin B through the neutropenic period. Prophylactic acyclovir was given to patients seropositive for Herpes simplex virus. Complete information on the transplanted patients is presented in table 3.

Statistical Methods

For the CR1 patients, a series of pretransplant variables were studied (table 1) and analyzed using Mann-Whitney's test or Fischer's exact test.

Kaplan-Meier curves for the CR1 patients (figure 1) were calculated and compared using the log rank method.

RESULTS

Results of all patients are summarized in table 3.

CR1

Of the patients transplanted in CR1, eight of 13 (62%) are in continuous complete remission (CCR) at a median time of 21 (range 10-36) months after achieving CR and at median 16 (range 4-32) months after ABMT. Three patients have relapsed at one, three and six months after ABMT (three, six and ten months after CR), and of these two have died. Two patients died in therapy related complications (gastrointestinal bleeding; veno-occlusive disease of the liver plus CMV-pneumonitis). For the historical control group, the corresponding figures are five of 26 (19%) of the patients surviving in CCR at 29, 32, 52, 65 and 85 months. Twenty patients relapsed after a median remission duration of 8.5 (2-36) months, and one died of infection while in CR. Fourteen of the relapsed patients died from leukemia; the remaining six patients underwent ABMT in second or subsequent remission, and are accounted for below. Disease free survival at 24 months was 62% for the ABMT-group and 23% for the control group ($p = 0.1$) - see figure 1.

CR2-3

Five of 10 (50%) of the patients transplanted in CR2-3 are in CCR with a duration of seven, 27, 32, 44 and 63 months respectively (i.e. five, 25, 26, 34 and 49 months post ABMT); all five have inverted (CR2-3 is longer than CR1) - see figure 2. Five have died: two in leukemic relapse after three and five months, and three due to transplant related complications (two infectious deaths, one multi-organ failure). One of the relapsed patients had an incomplete conditioning therapy, because he interrupted the planned Bu/Cy-conditioning regimen due to psychological reasons after only receiving busulfan (Bu).

Relapse

Of the three patients transplanted in relapse, one is in CR four months after ABMT, and two have died from transplant related infection (aspergillosis) and relapse shortly after ABMT, respectively.

The two patients with ANLL preceded by MDS are both among the relapsed patients.

Kinetics of Engraftment

The median time to reach granulocyte counts of $>0.5 \times 10^9$/L was 27 days (16-86), and that for platelets $>50 \times 10^9$/L 175 days (106-591), without significant difference between patients in CR1 and CR2-3. See table 3.

Toxicity

In total, there were five treatment related deaths: two of 13 patients in CR1, three of 10 in CR2-3 and one of three relapse patients. The causes of death have been accounted for previously in the text. All 26 patients had fever necessitating the use of intravenous antibiotics. In the majority of cases, the fever was due to gram-positive bacterial septicemia, mainly with Staph.epidermidis or alpha hemolytic streptococci, or of unknown origin. No gram-negative infections were seen. Five patients had CMV disease (three with fever only, two with pneumonitis of which one was fatal and one resolved on antiviral treatment). There were three cases of deep fungal infection, with one death. Most patients had oropharyngeal mucositis of mild to moderate severity. Veno-occlusive disease of the liver occurred in two patients, and was fatal in one.

DISCUSSION

We conclude that ablative chemoradiotherapy followed by ABMT with unpurged marrow is effective as consolidation treatment for ANLL in complete remission, and that it might induce sustained CR in relapsed ANLL. For patients transplanted in CR1, disease free survival was superior to that of a historical control group of patients of similar characteristics, but who had conventional antileukemic treatment. The control patients were consecutive and treated in only two hematological centres. Although the difference between the two groups of CR1 patients is not statistically significant, mainly due to the limited number of patients, there is a strong trend towards it. The efficacy of ABMT is, despite the small number of patients, further supported by the fact that afl five patients in continuous CR2 have second remissions exceeding the duration of their first remissions. The two patients with ANLL preceded by MDS had no or short post-transplant remissions, which supports the results of a previous study that ABMT is not effective in this group of patients [11]. Transplant related toxicity was acceptable, but seems to increase with more advanced stages of leukemia. Our results are consistent with those of similar trials [2-5, 8-9], and comparable with those of ABMT with "purged" bone marrow [6-7, 10].

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TABLE 1

Characteristics of CR1 Patients:
ABMT Group and Chemotherapy Control Group

	ABMT		Controls		
Number of patients	13		26		
Median age at diagnosis (years)	49	(18-59)	46.5	(16-60)	N.S.
Sex					
Males	5	(38 %)	17	(65 %)	*
Females	8	(62 %)	9	(35 %)	*
FAB-classification					
M1	1	(8 %)	4	(15 %)	*
M2	4	(31 %)	8	(31 %)	*
M3	0	(0 %)	2	(8 %)	*
M4	5	(38 %)	6	(23 %)	*
M5	2	(15 %)	5	(19 %)	*
M6	1	(8 %)	1	(4 %)	*
M7	0	(0 %)	0	(0 %)	*
Induction treatment modality (see table 2)					
TAD	9	(69 %)	10	(38 %)	*
POCAL	1	(8 %)	5	(19 %)	*
POCALdna	0	(0 %)	4	(15 %)	*
Ara/dauno	1	(8 %)	2	(8 %)	*
Mitox/HiDAC	1	(8 %)	2	(8 %)	*
AraC/Mitox	0	(0 %)	3	(12 %)	*
HiDAC/Amsa	1	(8 %)	0	(0 %)	*
Mean n:o of induction courses to achieve CR	1.4	(1-3)	1.6	(1-3)	*
Median time from diagnosis to CR (days)	33	(25-110)	48.5	(21-252)	*
Mean n:o of consolidation treatments	2.5	(2-3)	2.3	(1-3)	*
Maintenance treatment					
N:o of patients	-		10	(38 %)	
Median duration (months)	-		11	(2-28)	

TABLE 2

TAD

AraC 100 mg/m^2 b.i.d. i.v. bolus day 1-7; Thioguanin 100 mg/m^2 b.i.d. orally day 1-7; Daunonibicin 60 mg/m^2/day i.v. day 5-7.

POCAL/POCALdna*

AraC 100mg/m^2/day cont. i.v. inf. day 1-7; Thioguanin 50 mg/m^2 b.i.d. orally day 1-7; Prednisone 30 mg/m^2 b.i.d. orally day 1-7; Doxorubicin/Doxorubicin-DNA complex 30 mg/m^2/day i.v. day 4-5; Vincristin 2 mg i.v. day 1 + day 5.

Ara/Dauno

AraC 100 mg/m^2/day cont. i.v. inf. day 1-10; Daunorubicin 50 mg/m^2/day i.v. day 1-3.

Mitox/HiDAC

Mitoxantron 12 mg/m^2/day i.v. day 1-5; AraC 1 g/m^2 bi.d. i.v. day 1-3.

AraC/Mitox

AraC 100 mg/m^2/day cont. i.v. inf. day 1-7; Mitoxantron 12 mg/m^2/day i.v. day 1-3.

HiDAC/Amsa

AraC 3 g/m^2 b.i.d. i.v. day 1-5; Amsacrine 75 mg/m^2/day cont. i.v inf. day 2-4.

*During the time period Huddinge University Hospital participated in a randomized prospective multicentre trial of the Leukemia Group of Middle Sweden (LGMS), comparing the two variants of the POCAL regimen.

TABLE 3

Characteristics of All ABMT Patients

	CR1	CR2-3	Relapse	Total
N:o of patients transplanted	13	10	3	26
Median age at ABMT	50 (19-60)	28 (23-53)	43 (26-59)	41 (19-60)
Median time CR to ABMT (months)	4.1 (2.9-6.6)	4.4 (0.5-15.1)	-	4.2 (0.5-15.1)
Continuous CR (CCR)				
N:o of patients	8 (62 %)	5 (50 %)	1 (33 %)	14 (54 %)
Median duration (months)	21 (10-36)	32 (7-63)	4	25.5 (4-63)
Median duration of CR1, for CR2-3 patients (months)	-	19 (4-36)	-	-
N:o of relapses post ABMT	3 (23 %)	2 (20 %)	1 (33 %)	6 (23 %)
N:o of transpl related deahts	2 (15 %)	3 (30 %)	1 (33 %)	6 (23 %)
Conditioning regimen				
Cy/TBI	10 (77 %)	5 (50 %)	0 (0 %)	15 (58 %)
Bu/Cy	3 (23 %)	3 (30 %)	3 (100 %)	9 (34 %)
Bu/Mel	0 (0 %)	1 (10 %)	0 (0 %)	1 (4 %)
Bu	0 (0 %)	1 (10 %)	0 (0 %)	1 (4 %)
Granulocyte count $<0.5x10^9/L$ (days)	27 (16-69)	25 (18-86)	20 (20)	27 (16-86)
Platelet count $<50x10^9/L$ (days)	175 (106-182)	148 (67-591)	N.R.	175 (106-591)

FIGURE 1

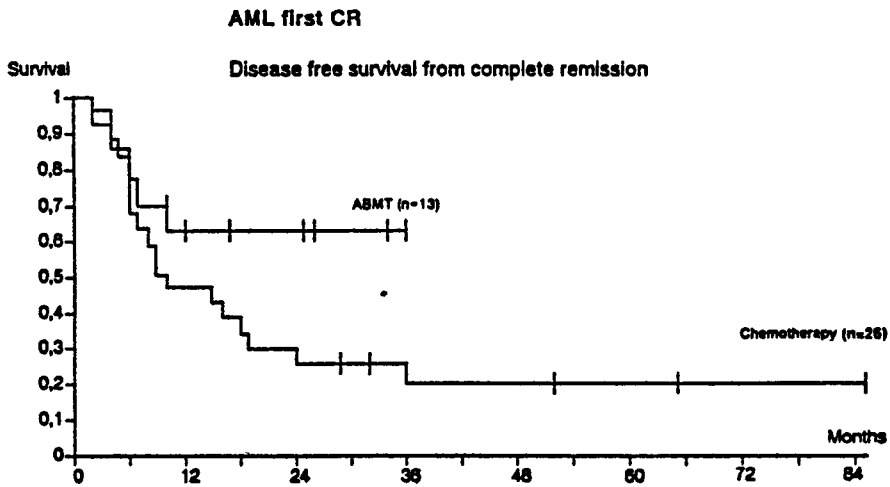
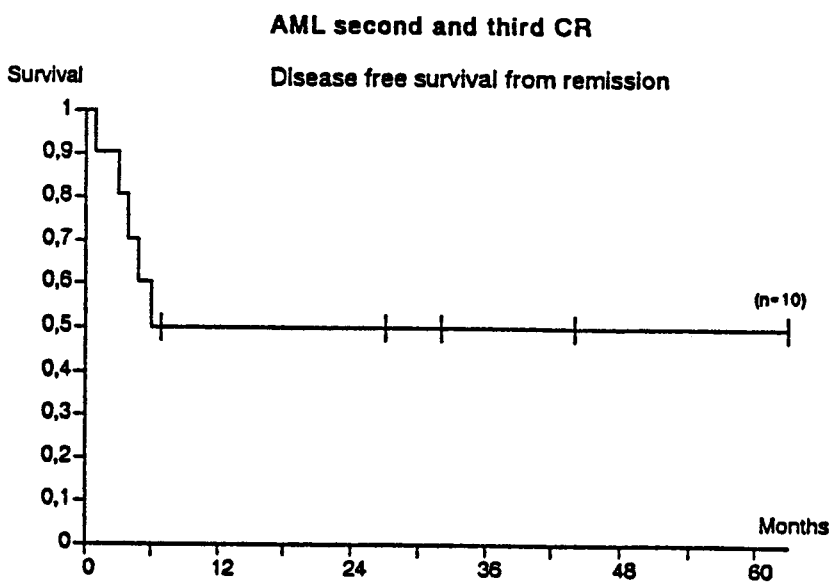


FIGURE 2



AUTOLOGOUS TRANSPLANTATION WITH CHEMOPURGED BONE MARROW IN PATIENTS WITH ACUTE MYELOCYTIC LEUKEMIA IN SECOND AND THIRD REMISSION

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INTRODUCTION

Autologous bone marrow transplantation (ABMT) may be an alternative to allogeneic BMT for the treatment of acute myeloid leukemia (AML) in remission. We have previously described the preliminary results of autografting using marrow treated ex vivo with 4-hydroperoxycyclophosphamide (4HC), an active alkylating agent in aqueous solution, in patients with AML in second or third remission (CR2 or CR3) (1,2). In this report we present an update and extension of these studies with a description of autografting with chemopurged marrow in 88 patients with second- or third-remission AML.

METHODS OF STUDY

Patients

Eighty-eight patients with AML (37 females, 51 males) were included in this study. The median age of these patients was 25 years (range, 2 to 56). Seventy-three patients were in CR2 and 15 were in CR3 at the time of marrow collection and ABMT. The distribution of FAB morphological subtypes was: M1, 29; M2, 12; M3, 8; M4, 32; M5, 6; M7, 1. The median duration of CR1 was 14 months (range, 2 to 96); in patients transplanted in CR3 the median duration of CR2 was five months (range, 1 to 25). At the time of marrow collection and ABMT, the median duration of CR2 or CR3 was 2.5+ months (range, 1.5+ to 6+). All protocols for ABMT 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 3-4 x 10^8 nucleated marrow cells/kg from the iliac crests. Approximately 70 to 75% of the collected marrow was incubated with 4HC ex vivo and the remainder was cryopreserved without treatment to be used as a reserve or "backup" marrow in the event of failure of engraftment with the 4HC-treated portion. In 75 patients, the autologous marrow cell suspension was centrifuged in transfer packs, the buffy-coat was extracted, and the hematocrit was adjusted to 5 to 7%. The buffy-coat cells were then incubated ex vivo for 30 min with 4HC at a final concentration of 60 ug/mL (1 patient), 80 ug/mL (2 patients), or 100 ug/mL (72 patients) (1). Since aldehyde dehydrogenase in erythrocytes can inactivate 4HC and its levels may vary from patient to patient (3), a technique to obtain erythrocyte-free marrow mononuclear cells by density-gradient centrifugation was used in 13 patients. Briefly, the marrow buffy-coat cells were layered onto Ficoll-diatrizoate in a COBE Model 2991 cell washer and centrifuged at 2000 rpm; the light-density (sp. gr. < 1.077 g/mL) mononuclear cells were collected, washed and incubated for 30 min with 60 ug/mL of 4HC (4). After incubation, the marrow cells were recentrifuged, 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 37C water bath and injected intravenously at a rate of 10 to 15 mL/min.

Preparative Regimens

Eighty-six patients received high-dose busulfan (BU; 1 mg/kg/day p.o. every six hours for 16 doses) and cyclophosphamide (CY; 50 mg/kg/day i.v. for four days). Two patients with a history of central nervous system leukemic involvement were given CY (50 mg/kg/day i.v. for four days) followed by total body irradiation (3.0 Gy/day for four days, with lungs shielded after 9.0 Gy).

RESULTS

Transplant-Related Mortality

Eighteen patients died from non-leukemic causes after ABMT. Eleven patients (eight in CR2 and two in CR3) died with documented or presumptive bacterial or fungal sepsis during aplasia or early hematologic recovery, eight to 35 days after ABMT. One patient with AML in CR2 had nonfatal sepsis with Klebsiella pneumoniae at the time of marrow infusion, had persistent marrow hypoplasia, and died with Gram-negative sepsis five months after BMT. Two patients (one in CR2, one in CR3) died with interstitial pneumonitis three and eight months, respectively, after BMT; one of these cases was attributable to cytomegalovirus and one was idiopathic. Three patients (one in CR2, two in CR3) died with hepatic veno-occlusive disease five to seven weeks after autografting. One patient died with idiopathic hyperammonemia syndrome 43 days after BMT.

Hematologic Reconstitution

Satisfactory hematologic reconstitution occurred in 75 of 77 evaluable patients (the 11 patients who died with ABMT-related complications during aplasia were excluded). The neutrophil count exceeded 0.5 x 10^9/L at a median of 39 days in recipients of buffy-coat marrow and 45 days in patients given light-density marrow mononuclear cells (range, 12 to 99 days; p=0.09). The platelet count exceeded 50 x 10^9/L at a median of 66 days after buffy coat ABMT and 86 days following infusion of light-density marrow cells (range, 23 to 259 days; p=0.64). Two patients in CR2 did not have hematologic reconstitution after autografting with marrow buffy-coat cells incubated with 100 ug/mL of 4HC. One of these patients did not have a reserve marrow available for infusion and, as indicated above, died with persistent marrow hypoplasia and gram-negative bacterial sepsis five months after ABMT. The other patient had satisfactory hematologic reconstitution after infusion of untreated reserve marrow 38 days after ABMT and is currently in unmaintained CR2 45 months later. The granulocyte-macrophage colony-forming cell (GM-CFC) content (mean +/- standard deviation) of these marrow grafts after 4HC treatment was 4.5 $\pm$ 8.8 x 10^5/kg in buffy-coat recipients and 1.9 $\pm$ 4.4 x 10^5/kg in patients given light-density marrow mononuclear cells (range, 0.0 to 71.3; p=0.05). The logarithm of GM-CFC content of the 4HC-treated marrow graft could be correlated with the time to hematologic recovery (5).

Leukemic relapses and disease-free survival. Thirty-five patients (30, CR2; 5, CR3) had leukemic relapses at a median of 6.1 months (range, 1.8 to 23.2) after ABMT; all of these were hematologic relapses, and no extramedullary relapses were observed. The actuarial relapse rates are 57% in CR2 and 61% in CR3, but these differences are not statistically significant (Figure 1). Thirty-five patients (31, CR2; 4, CR3) are in unmaintained remission at a median of 26.5+ months (range, 0.5+ to 105+) after ABMT with 4HC-treated marrow. The actuarial disease-free survival is 36% in CR2 and 23% in CR3 (Figure 2); although the period of observation is still limited in these patients, there is a trend towards a lower relapse rate and a higher disease-free survival in recipients of erythrocyte-free marrow mononuclear cell suspensions versus buffy-coat suspensions. In 22 of the 35 patients in unmaintained CR after ABMT, the duration of post-transplant remission exceeds that of CR1 ("inversions"); in contrast, inversions were observed in only two of the 35 patients who relapsed after ABMT for second- or third-remission AML.

DISCUSSION

Intensive antileukemic therapy followed by autologous BMT with 4HC-treated marrow may be associated with long-term disease-free survival in patients with AML 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 compares favorably to that observed after allogeneic BMT in similar groups of patients. In this series, one problem is the

high transplant-related mortality in patients autografted in CR3; indeed, although the actuarial relapse rates are virtually indistinguishable in patients transplanted in CR2 and CR3, the nonleukemic deaths in third-remission patients are well over twice that in second-remission patients (six of 15, versus 12 of 73). Whether this mortality rate can be improved upon is debatable; the deaths from hepatic veno-occlusive disease could not be predicted before ABMT by abnormalities in hepatic function chemistries (6), and fatal CMV pneumonitis is decidedly rare in recipients of marrow autografts (7). Furthermore, although the median age was somewhat greater in patients transplanted in CR3 versus CR2 (33 versus 21 years), the range of ages was similar in both groups. Perhaps one recommendation, given this higher procedure-related mortality when ABMT is carried out for AML in CR3, is to proceed with autografting in CR2, since there is no proof of cures of AML in second or subsequent relapse with conventional chemotherapy alone.

Our previous studies with 4HC-treated marrow buffy-coat cell suspensions have demonstrated that the effectiveness of ABMT in AML, as shown both by lower relapse rates and increased disease-free survival, can be correlated with a greater degree of reduction in GM-CFC (i.e. $<1\%$ of pre-incubation levels) after ex vivo incubation with 4HC (8). Presumably this assay, which measures sensitivity of a normal committed hematopoietic progenitor cell to the drug, indirectly determines the sensitivity to 4HC treatment of the leukemic cells. More recently, using a clonogenic assay system, Miller and colleagues have detected growth of residual occult AML in remission marrows and have correlated the sensitivity to 4HC of these leukemic cells with the effectiveness of ABMT with 4HC-treated marrow in these patients (9). In addition, the studies of Gorin et al. (10,11) strongly suggest that marrow "purging" is indeed a factor that influences relapse rates and disease-free survival in patients undergoing ABMT for AML.

Until recently, no studies demonstrated long-term relapse-free survival after autografting with unpurged marrow in relapsed AML. However, the Rome group has reported an impressive disease-free survival (59%) and actuarial relapse rate (45%) in patients with second-remission AML who received ABMT with unpurged marrow after a polychemotherapeutic preparative regimen (12). If others can reproduce these results, then a more critical appraisal of the need for ex vivo autologous marrow treatment would be warranted. Even more controversial is the role of ABMT and the need for autograft purging in first-remission AML. Here, too, studies from the European ABMT Group indicate that ex vivo marrow treatment is beneficial (10,11). To address these issues, several prospective multicenter randomized collaborative trials are in progress that compare outcomes in both adults and children with first-remission AML given either chemotherapy alone or autologous transplantation with 4HC-treated marrow.

Finally, the substantial differences in relapse rates between recipients of allogeneic and syngeneic or autologous BMT underscore the potential role of an allogeneic graft-versus-leukemia (GVL) effect, similar to but dissectable from the graft-versus-host (GVH) reaction (13,14), in eliminating residual

leukemia in vivo. One approach to decrease the high relapse rates after autologous or syngeneic BMT for the acute leukemias might therefore involve post-BMT immunomodulation by providing a GVL effect. Preclinical studies have shown that rodents given short-course cyclosporine after syngeneic or autologous BMT develop a syndrome that mimics allogeneic GVH disease (15,16), with which is associated the appearance of cytotoxic lymphocytes with polyclonal anti-Ia specificity (16). In an Ia-bearing rat myeloma model, this induced syngeneic GVH disease also has an antitumor effect (17). In patients with refractory lymphoma undergoing ABMT with unpurged marrow (18) and AML patients transplanted with 4HC-treated marrow (Yeager AM, unpublished observations), histopathologically documented cutaneous GVH reactions can be induced with post-ABMT low-dose cyclosporine (1 mg/kg/day for 14 to 28 days), although the onset of these changes is later in AML recipients of chemopurged marrow (median 28 days, versus 11 in lymphoma patients). Studies are currently in progress to determine whether induction of this GVH-like effect conveys an additional antileukemic advantage after ABMT with 4HC-treated marrow in patients with AML.

ACKNOWLEDGEMENT

This work was supported in part by grant nos. PO1 CA15396 (G.W.S.) and RO1 CA40282 (A.M.Y.) from the National Institutes of Health, Bethesda, MD, by a gift from the W.W. Smith Charitable Trust (S.D.R., J.M.D.), and by a grant for the Children's Cancer Foundation, Inc., Baltimore, MD (A.M.Y.).

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Figure 1

Probability of remaining in remission after ABMT with 4HC-treated marrow in 73 patients with AML in CR2 (solid line) and 15 patients with AML in CR3 (dashed line).

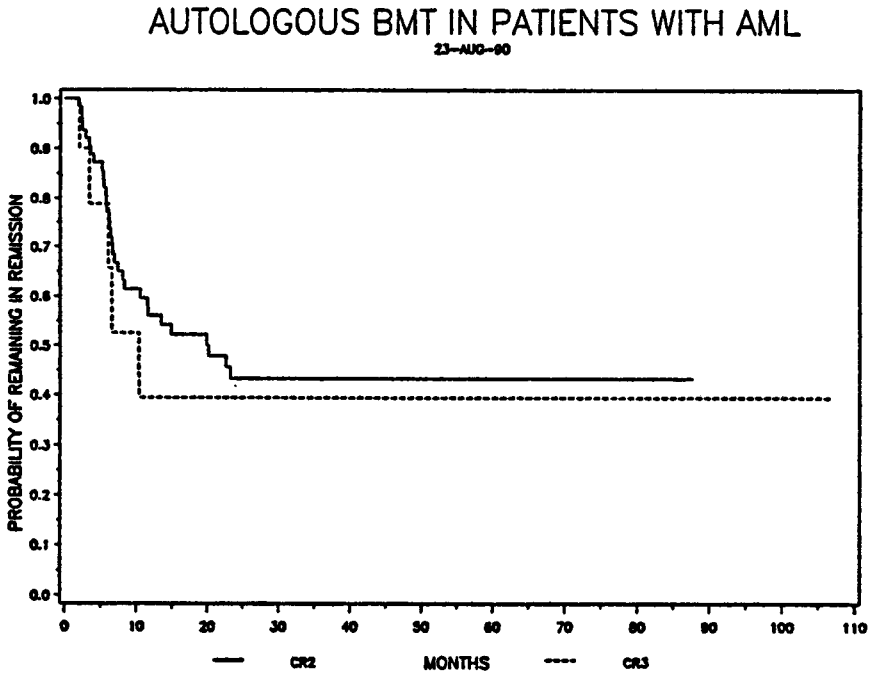
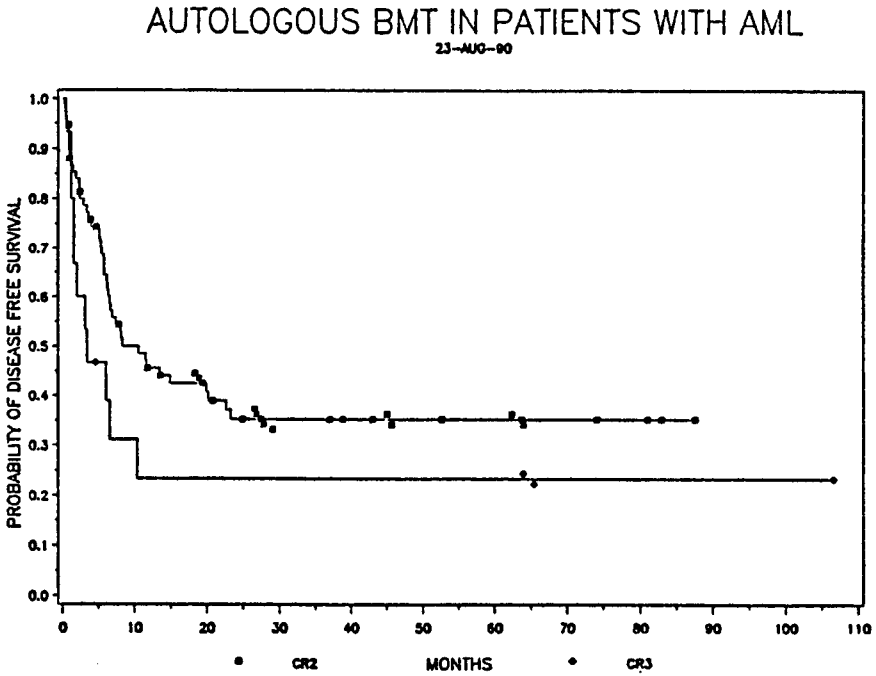


Figure 2

Probability of disease-free survival after ABMT with 4HC-treated marrow in 73 patients with AML in CR2 (squares) and 15 patients with AML in CR3 (diamonds). Individual living patients are indicated by the symbols.



4-HYDROPEROXYCYCLOPHOSPHAMIDE (4-HC) PURGING OF AUTOLOGOUS BONE MARROW IN SECOND AND LATER REMISSION AML: CLINICAL RESULTS

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INTRODUCTION

Allogeneic bone marrow transplantation (BMT) appears to be the best available treatment for acute myelogenous leukemia (AML), because the risk of relapse is greater and the disease-free survival shorter in patients who receive intensive chemotherapy, compared to patients who undergo BMT (1). Unfortunately, approximately 70% of patients otherwise appropriate for transplantation have neither an HLA-identical donor nor an identical twin (2). An autologous bone marrow transplantation (ABMT) is their only treatment option.

While ABMT avoids the universal immunosuppression and frequent graft-versus-host disease which occur with allogeneic transplants, an obvious limitation is the significant possibility that the marrow will be contaminated by malignant cells at the time of collection. Rather than reinfuse contaminated marrow, it would appear logical to deplete the marrow selectively of neoplastic cells, leaving the normal cells relatively unscathed. The technique which has seen the greatest use, in the United States and worldwide, has been pharmacological purging of the contaminated bone marrow by brief incubation with small amounts of 4-hydroperoxycyclophosphamide, an activated form of cyclophosphamide. This method depletes the marrow of tumor cells, while relatively sparing the normal hematopoietic elements (3-6).

What follows is a discussion of data which have been collected for 201 patients with a diagnosis of AML in second or later complete remission whose autologous marrows were treated with 4-HC and subsequently reinfused.

PATIENTS AND METHODS

Patients were selected from an Oracle-based clinical database, collected and audited by Nova Pharmaceutical Corporation, containing information concerning all patients known to have received a 4-HC-treated ABMT between January 1980 and March 1989 in the United States. The patients reported here

had a diagnosis of AML and a clinical status of second or later complete remission at the time of transplant.

Patients' bone marrows were harvested, and a buffy coat of the marrow was incubated for 30 minutes at 37C in a 4-HC solution containing 2.0 x 10^7 nucleated cells/ml and 5-log red blood cells (hematocrit). The marrows were then washed and cryopreserved. Patients were subsequently treated with a marrow-ablative regimen of cyclophosphamide and total body irradiation or cyclophosphamide and busulfan, after which their treated marrows were reinfused.

The primary endpoints considered in this study are total survival and disease-free survival. Total survival is defined as the time in weeks from bone marrow transplantation until death. Since the study was closed for data collection on March 1, 1989, patients alive at that time had censored survival times. Disease-free survival is defined to be the time in weeks from bone marrow transplantation until relapse. If death occurred prior to relapse or if the patient was alive and disease-free at the study termination date, his or her disease-free survival time was censored. Treatment of deaths without evidence of relapse as censored data can cause estimated median disease-free survival time to be greater than median total survival time. Engraftment times were also determined as the number of days between ABMT and attaining a total granulocyte count of at least 1000/ul.

Using the product limit method in the SAS statistical software package and SAS/GRAPH, survival functions were estimated and curves drawn both for disease-free survival and for total survival. From these functions, estimates of median disease-free survival times and median total survival times were reported. Censoring is indicated by asterisks on the survival curves.

RESULTS

Two-hundred, one (201) patients were treated at 24 centers: 168 were in CR2, 30 were in CR3, and 3 were in later complete remissions at the time of ABMT. The median age of the patients was 30 years, with a range of 1 to 66 years. There were 115 male and 86 female patients.

Fourteen patients received preparative regimens of cyclophosphamide and total body irradiation, while 111 patients were prepared with cyclophosphamide and busulfan. The remaining 76 patients had marrow ablative regimens containing other combinations of chemotherapeutic agents with or without irradiation.

The concentration of 4-HC used in the purging process ranged from 20 ug/ml (1 patient) to 140 ug/ml (1 patient), with most patients receiving 100 ug/ml (176 patients). Twelve patients received 4-HC in combination with other agents.

For all patients, the median disease free survival was greater than 72 weeks, with 52% in complete remission beyond 72 weeks, and for as long as 423 weeks post-ABMT. For patients in CR2, median disease free survival was greater than 71 weeks, with 55% in complete remission beyond 71 weeks.

When the patients were divided according to the duration of their first remissions (<12 months, 12-18 months, and >18 months), there were no differences in disease free survival. See Figure 1.

For all patients, median total survival was 44 weeks, with 36% of patients living beyond 96 weeks. For CR2 patients, median total survival was 54 weeks, with 42% living beyond 96 weeks. When the patients were grouped by duration of CR1 (as above), total survivals were not different. See Figure 2.

The median interval between ABMT and engraftment was 29 days, with a range from 9 days to 130 days for 168 patients who engrafted. A total of 28 patients (14%) failed to engraft, but 21 of these died within 30 days of ABMT, leaving less than a 4% failure-to-engraft rate among AML patients surviving at least one month. Engraftment data were not available for 5 patients.

DISCUSSION

These results confirm that long term disease free survivals are possible for AML patients in second and later complete remissions who receive autologous bone marrow transplantations treated with 4-HC. Both disease free survivals and total survivals reported here exceed those reported for patients who are treated with chemotherapy alone, only 5-10% of whom are expected to enjoy long term survival (7). Although comparative data have not been presented here for unpurged autologous transplants, recent reports support the necessity and superiority of purged ABMT compared to untreated ABMT (8, 9).

Furthermore, although times to engraftment are somewhat greater than those encountered with allogeneic transplantations, the actual engraftment rates are as good as those achieved with allogeneic transplants (1). The avoidance of graft-versus-host disease and the lower risk of immunosuppression and interstitial pneumonitis faced with autologous transplantation may, in fact, make 4-HC-purged ABMT a lower morbidity therapy than allogeneic transplantation.

No randomized, controlled trial has been conducted to address the question of the necessity of purging autologous bone marrow. Given the extensive experience with and widespread use of 4-HC in AML, it is questionable whether such a trial could ever be completed. Nevertheless, it is clear that 4-HC can be used safely to attain long term disease free survivals in certain patients who undergo autologous bone marrow transplantations.

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FIGURE 1

Disease-Free survival. Patients are divided according to their durations of first complete remissions as labelled: <12 months, 12-18 months, or >18 months.

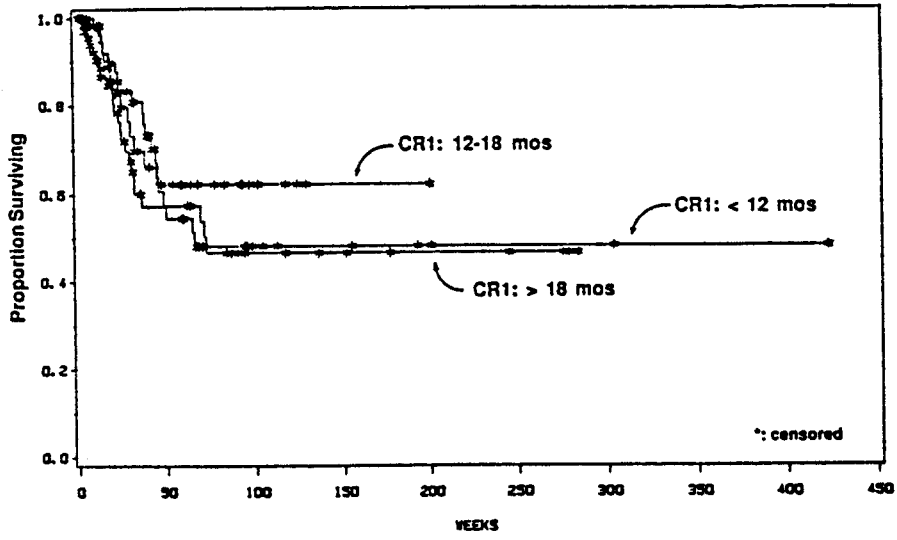
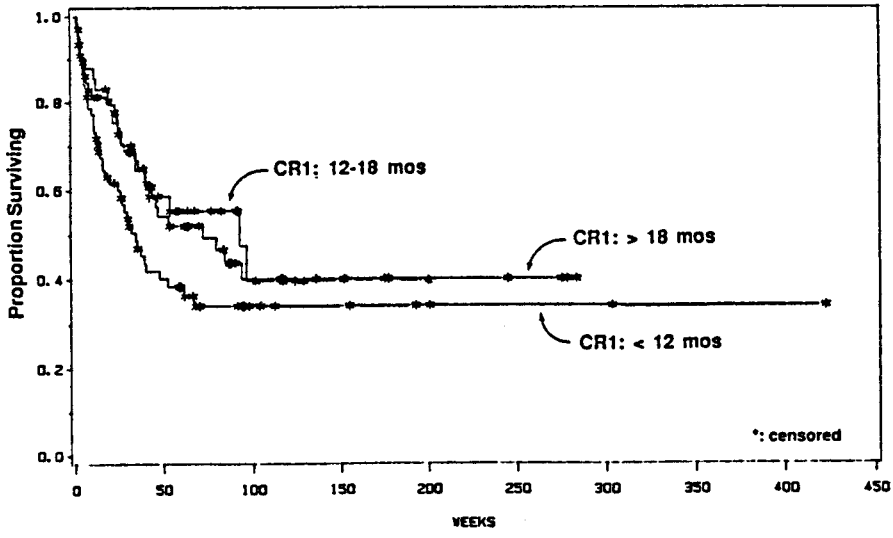


FIGURE 2

Total Survival. Patients are divided according to their durations of first complete remissions as labelled: <12 months, 12-18 months, or >18 months.



AUTOLOGOUS BONE MARROW TRANSPLANTATION WITH 4-HYDROPEROXYCYCLOPHOSPHAMIDE PURGED MARROWS FOR CHILDREN WITH ACUTE NON LYMPHOBLASTIC LEUKEMIA IN SECOND REMISSION

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INTRODUCTION

The primary therapy for children with acute non-lymphoblastic leukemia (ANLL) has dramatically improved over the last decade. Approximately 70% of patients can be induced into remission (1,2). Patients in first remission who receive an allogeneic bone marrow transplantation (BMT) from a histocompatible sibling donor have a 60-65% chance for prolonged disease free survival (3,4). Patients who receive maintenance chemotherapy rather than BMT in first remission have a 40-45% chance for long term remission. However, once a patient with ANLL on maintenance chemotherapy suffers a relapse, there is little hope for prolonged survival without BMT (5). One alternative for the patient with ANLL who relapses is to receive an ABMT.

ABMT may be successful if residual clonogenic leukemia cells are eliminated from the graft. One approach to the removal of residual ANLL cells is the incubation of the harvested marrow cells with 4-hydroperoxycyclophosphamide (4HC) (6-8).

In this report we describe our results with ABMT for thirteen pediatric patients with ANLL in second remission. One innovation in our process was the removal of red blood cells (RBC) from the purging environment. RBC contain aldehyde dehydrogenase, an enzyme which inactivates 4HC ex vivo (9). Factors that inactivate 4HC may reduce the efficiency of the leukemic cell purge. Therefore, our methodology included the reduction of the RBC concentration ($<0.5\%$) in the marrow/4HC mixture.

PATIENTS AND METHODS

Patients

Thirteen patients with ANLL in second remission were entered on this study. The median age was 5 years (range 1 to 21). Marrow was harvested from 11 patients in second remission; 2 patients had marrow harvested during first remission. The median duration of first remission was 12 months (range 2-60). The median duration from second remission to transplant was 1 month (range 1-12). Informed consent was obtained for all patients.

Marrow Processing

Step 1. Marrow was collected from the posterior iliac crests according to the methods of Thomas and Storb (10). A median of 3.3 X 10^8 nucleated marrow cells/kg (range 1.8-6.5) of recipient body weight were obtained for 4HC purging; additional marrow was obtained and not purged for backup use in case of non-engraftment.

Step 2. Separation: Five cc of ficoll-hypaque (Pharmacia, Uppsala, Sweden) was overlayed on 10 cc of 72% percoll (Sigma Chemical, St. Louis, MO) in 50 cc centrifuge tubes. After the addition of magnesium-free, calcium-free, Hanks Balanced Salt Solution (HBSS) (Irvine Scientific, Santa Ana, CA) to the packed bone marrow to return the marrow to original volume, 30-35 cc of the marrow was overlayed onto each ficoll/percoll gradient, which was then centrifuged at 2000 rpm for 30 minutes. The interface cells were removed and washed twice with HBSS. In our laboratory, the addition of the ficoll/percoll gradient results in a mean recovery of >80% of the nucleated marrow cells and a mean recovery of >95% of the mononuclear cells.

Step 3. 4HC Treatment: The cells were adjusted to a concentration of 2 X 10^7/ml with RPMI 1640 medium (Irvine Scientific, Santa Ana, CA) and 10-15% autologous plasma. The hematocrit was always < 0.5%. The cells were incubated with 4HC (100 micrograms/ml) (M. Colvin, Johns Hopkins University, Baltimore, MD) at 37C for 30 minutes, then cooled rapidly on ice for 10 minutes, centrifuged at 2500 rpm for 10 minutes and resuspended in autologous plasma at a concentration of 50 X 10^8 cells/ml.

Preparative Regimen and Marrow Infusion

All patients received a transplant preparative regimen of busulfan, 1 mg/kg orally every six hours on Day -9, -8, -7 and -6 followed by cyclophosphamide, 50 mg/kg intravenously on Day -5, -4, -3 and -2. Intrathecal therapy was not administered. No patient received diphenylhydantoin. Bone marrow was infused on Day 0. A median of 1.2 X 10^8 (range 0.4-3.5 X 10^8) mononuclear cells per kilogram were infused.

RESULTS

Engraftment and Post-Transplant Complications

All but one patient (#8) attained an absolute neutrophil count (ANC) greater than 500/mm^3 by a median of 40 days post transplantation (range 25-70) (Table 1). All but two patients (#8, #13) attained a platelet count greater than 50,000/mm^3 by a median of 58 days post transplantation (range 22- 100 days). The two patients who did not achieve hematologic reconstitution relapsed at 60 and 120 days. There were no treatment related deaths. All patients developed mild to moderate mucositis. Twelve patients developed symptomatic veno-occlusive disease of the liver.

Progenitor Cell Assays

Results of 14 day and 30 day progenitor cell colony assays are summarized in Table 2. There was minimal recovery of colony forming units after 14 days. There was a substantial increase in the percent recovery of all colony types in the 30 day assays, with a median 20% recovery of BFU-E, 90% recovery of CFU-GM and 35% recovery of CFU-GEMM.

Survival

Seven of thirteen patients are disease free survivors (median 34 months, range 22-39 months) at the time of analysis. For six of the seven disease free survivors, the duration of the post transplant remission has exceeded the duration of their first remission. Six patients relapsed in bone marrow at 2, 4, 5, 6, and 19 months post-transplant, including 1 patient (#11) whose post transplant remission was longer than his first remission. The projected disease free survival is 53%.

DISCUSSION

Since most pediatric patients with ANLL who have a histocompatible sibling donor are transplanted in first remission, there is usually no successful therapy for children with ANLL who attain a second remission. The results of the present study indicate that ABMT with 4HC purged bone marrow is a safe and efficacious therapy for children with ANLL in second remission. The projected disease free survival for our patients is 53%, with a median follow up of 34 months. Although the number of patients in the present study is relatively small, the number of patients reported here represents more children with ANLL in second remission receiving ABMT with a longer median follow up than three previously published studies combined (7,11,12). It is unlikely that significant numbers of additional children with ANLL in second remission will become eligible for an ongoing study, since 4HC purged ABMT are now being conducted in children with ANLL in first remission who lack a matched donor.

Our results suggest that the disease free survival for pediatric patients may be superior to that seen in adults. There were no toxic deaths in our series, whereas there were five early deaths among the first 25 patients reported by the Baltimore group. In addition, our method for the separation of marrow and for purging with 4HC, i.e., the elimination of exogenous RBC from marrow/4HC mixture, may lead to a more effective purge of leukemic cells from the harvested bone marrow. Jones et al. examined the effects of graded concentrations of RBC on the ex vivo activity of 4HC against normal human bone marrow and against the K562 myeloid leukemia cell line (13). They also studied the effects of the incubation concentration of RBC during clinical purging with 4HC. They found that increasing the concentration of RBC resulted in less toxicity to marrow progenitor cells as well as less effective leukemic cell purge. Their observations may be explained by the inactivation of 4HC ex vivo by the red cell enzyme, aldehyde dehydrogenase. Therefore, a higher RBC concentration during clinical purging would be expected to hasten hematologic recovery following ABMT, and reduce the overall morbidity and mortality of the procedure. Thus, other investigators have maintained the RBC concentration of the purging medium >5% (11). Unfortunately, a concentration of RBC sufficient to reduce 4HC activity against normal marrow cells is likely to be associated with a decreased leukemia cell kill, a factor which might increase the chance for leukemic relapse following BMT. In fact, recent data suggests a correlation between increasing RBC concentration and relapse following 4HC purged ABMT (14).

The RBC concentration of the marrow/4HC mixture in our laboratory was maintained < 0.5%. The removal of RBC is enhanced by our method of mononuclear cell recovery. Traditional methods for mononuclear cell recovery involve the use of buffy coat removal (by cell washer or manual methods) or separation on a ficoll gradient. In our laboratory ficoll gradient separation yields a 50-60% recovery of the mononuclear cells. The addition of a percoll layer to the ficoll gradient results in a nearly 100% recovery of the mononuclear cell fraction. Since the hematopoietic progenitor cells are distributed throughout the mononuclear cell compartment, the total recovery of progenitor cells is enhanced. Additionally, the separation of nucleated marrow cells on the ficoll/percoll gradient results in an efficient removal of RBC. Recently, Korbling et al reported successful engraftment in all evaluable patients receiving ABMT transplants with bone marrow that was incubated with mafosfamide in a medium depleted of RBC (12).

Our results with 14 day assays for hematopoietic progenitor cells showed almost no recovery of CFU-GM, BFU-E or CFU-GEMM from the 4HC purged bone marrow. Rowley et al have reported a correlation between the CFU-GM content of 4HC purged bone marrow and time to engraftment (15). However, no such correlation was present for our patients due to the low numbers of progenitor cell colonies counted at 14 days. Since our clinical observation was that it required 2-3 weeks before the earliest signs of engraftment, we have recently extended the analysis of colony forming ability to include the 30 day assay. We have found a significant increase in the

detection of progenitor cells from 4HC treated marrow in the 30 day assay as compared to the 14 day assay although no correlation exists between the rate of engraftment and the number of progenitor cells assayed at 30 days.

In summary, high dose chemotherapy followed by infusion of autologous bone marrow purged with 4HC can result in long term disease free survival for some children with ANLL in second remission. Studies comparing ABMT and allogeneic BMT for children with ANLL in first remission are in progress.

ACKNOWLEDGEMENT

We thank the dedicated nurses of our bone marrow transplant program; our fellows and housestaff; and Ms. Juanita Rogers for typing this manuscript.

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TABLE 1

Patient Characteristics and Results

Age/Sex Yrs[1]	Length of 1st Remission (months)	# mono-nuclear cells infused x 10^8/Kg	Days to ANC > 500/mm³	Relapse (months)	DFS[2] (months)
1. 4/F	8	1.2	45	--	39
2. 1/M	2	1.8	34	--	35
3. 21/M	60	0.5	39	--	34
4. 1/M	4	1.1	25	--	38
5. 7/F	16	1.4	51	--	30
6. 5/F	17	2.7	38	--	30
7. 19/F	24	0.4	28	6	--
8. 12/M	48	0.5	--	2	--
9. 2/F	6	1.0	40	--	22
10. 6/F	6	1.5	45	6	--
11. 16/M	3	0.5	70	5	--
12. 5/F	24	1.6	46	19	--
13. 4/M	12	3.5	40	4	--

1. Patients #7 and #12 had marrow harvested in 1st remission.

2. Disease Free Survival

TABLE 2

Progenitor Cell Colony Assay:
Comparison of 14 Day and 30 Day Cultures

Percent Recovery (post 4HC/pre 4HC x 100)

	14 Day	30 Day
BFU-E	.06 (0-3)[1]	20 (3-60)
CFU-GM	0 (0-3)	90 (0-100)
CFU-GEMM	0 (0-21)	35 (5-66)

1. Median (range)

NEW APPROACHES TO AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR ACUTE MYELOID LEUKEMIA USING MONOCLONAL ANTIBODY-PURGED BONE MARROW

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ABSTRACT

We have been exploring the use of anti-myeloid monoclonal antibodies (mAb) in the treatment of acute myeloid leukemia (AML) to purge marrow for autologous bone marrow transplantation (ABMT). Forty patients with AML in either 1st CR (n=4) or 2nd and 3rd CR (n=33), or early 1st relapse (n=3) were treated from August, 1984 until May, 1990 using highdose chemotherapy and using monoclonal antibody (mAb) and complement (C') treated bone marrow. Seven patients in 2nd or 3rd CR survive disease-free from 11 to 71 months post-ABMT. Six of these seven long-term survivors showed "inversions", where their post ABMT remission lasted longer than any previous one. Actuarial two and three year disease-free and overall survival of patients in 2nd and 3rd CR was 27% (± 9%) and 20% (± 9%), and 33% (± 9%)and 27% (± 9%), respectively. Three patients were transplanted at 1st relapse with marrow previously harvested in 1st CR; two survive at six and 15 months in 2nd CR. We also discuss two new approaches to the problem of recurrent disease: 1) the use of neuraminidase to increase mAb plus complement killing of AML cells and 2) the use of cellular cytotoxicity mediated by bispecific antibodies.

INTRODUCTION

Treatment of AML with chemotherapy can induce a complete remission (CR) in 50-80% of patients (1). However, most patients relapse and ultimately die of their disease. Bone marrow transplantation (BMT) offers the promise of complete elimination of occult leukemia cells during CR (2-4). Encouraging results have been reported with allogeneic BMT as consolidation therapy in 1st CR. In 2nd and 3rd CR, allogeneic BMT can also be curative, although relapse-free survival is lower than in 1st CR. However, since the majority of patients with AML either do not have an HLA matched-donor or are considered too old for an allogeneic BMT, autologous BMT (ABMT) is a viable alterative

(5-7). Methods of purging autologous marrow using monoclonal antibodies (mAb) (5) or cytotoxic drugs (6,7) are under study.

We are using two mAbs, PM-81 and AML-2-23, that bind to leukemia cells from >95% of AML patients (8- 10). In the presence of complement (C') they can lyse leukemia cells from almost all patients with AML, including their progenitor cells (11). As of May, 1990 we have performed ABMT in 40 patients in 1st-3rd CR using mAb plus C'purged marrow. This report updates our experience with this approach to ABMT in AML and describes two novel adaptations of immunotherapy using anti-myeloid mAb.

MATERIALS AND METHODS

Clinical Trial

Patients under the age of 60 years with good performance status were eligible. Leukemia blast cells, obtained at diagnosis or at relapse, when available, were required to express the antigens reactive with PM-81 (CD 15) and/or AML-2-23 (CD 14) on >20% of cells.

A mean of 7.28×10^8 nucleated bone marrow cells/kg were harvested. After concentrating mononuclear cells by Ficoll-Hypaque gradient centrifugation, there was a mean recovery of 20.6% cells available for treatment. A mean of 9.97×10^7 cells/kg were treated with mAb plus C'on the Haemonetics cell processor as described (5) and from that there was a mean recovery of 39%. An average of 3.60×10^7 cells/kg was used for the transplant.

Thirty-one (31) patients were treated with cyclophosphamide (CY) (60 mg/kg x 2 days) (days -5 to -3) and fractionated total body irradiation (fTBI) (200 cGy twice daily for three days, total dose of 1200 cGy) (days -2 to 0). Eight patients (in 2nd CR) was treated with busulfan (16mg/kg/day for four days) (days -9 to -6) and cyclophosphamide (60mg/kg/day for two days) (days -5 and -4). One patient (in 3rd CR) was treated with busulfan (16 mg/kg/day for four days) (days -7 to -4) and VP-16 (60mg/kg) (day -3).

Neuraminidase Treatment of Bone Marrow

Based on our findings that the enzyme, neuraminidase, increases the binding of PM-81 to the CD15 antigen on AML blasts, we treated one patient in this series with bone marrow that was treated with neuraminidase prior to purging with mAb plus C'(12). Details of this case can be obtained from a published report (12).

Bispecific Antibody

Bispecific antibodies were prepared using an anti-FcγR I mAb, 32.2 (an IgG1), and the anti-myeloid cell mAb, PM-81, reactive with the CD 15 antigen, for studies of antibody-dependent cellular cytotoxicity (ADCC). Conjugates were made by cross-linking sulfhydryl groups of Fab fragments of mAb 32 and sulfhydryl groups added to intact PM-81 (an IgM) using N-succinimdyl-acetyl-S-thioacetate (SATA). The ability of the bispecific antibody to mediate

attachment of human monocytes to tumor target cells was confirmed in a microtiter well assay of binding using MTT-labeled U937 cells (FcγRI-bearing) to SKBR-3 (breast carcinoma) target cells.

RESULTS

Forty patients with AML ranging in age from 11 to 53 years were transplanted between August, 1984 and May 1, 1990 at the DHMC (Table 1). All but one patient had de novo AML at the time of initial diagnosis. This patient had a myelodysplastic syndrome prior to diagnosis of AML. With two exceptions, the patients met the specified criteria for cardiac, pulmonary, renal, and hepatic function. Exceptions were a patient with elevated hepatic enzymes, presumably due to non-A, non-B hepatitis and one with an abnormal left ventricular ejection fraction. The median time between the current remission and ABMT was 60 days, with a range of four days to 15 months. The transplants in the four patients in 1st CR were performed at a median time of 10 months (range 6-14 months) after achieving CR.

All cases were > than 20% positive for PM-81 and 37% of cases were positive for AML-223 binding. On average, 78.5% of leukemia cells were positive for binding to mAb PM-81 (range 31-100%, median 81%).

Toxicity

Four patients died within two months of ABMT: one each from overwhelming fungal sepsis, hepatic veno-occlusive disease, hemorrhage and from cardiac failure. Three additional patients died later from non-leukemic causes related to ABMT: two from intracerebral hemorrhages and one of overwhelming pseudomonal sepsis at three months while the bone marrow was hypocellular.

Colony-Forming Units and Engraftment

The median recovery of CFU-GM was 36% (range 22-150) for the 1st CR group and 47% (range 17-156) for the 2nd/3rd CR group. Median recovery of BFU-E was 38% (range 34-93) for the 1st CR group and 68% (range 13-1003) for the 2nd/3rd CR group. Median recovery of CFU-MIX was 46% (range 29-96) for the 1st CR group and 37% (range 0-226) for the 2nd/3rd CR group. Median numbers of 1.02×10^4 and 0.8×10^4 CFU-GM/kg body weight were infused in each 1st and 2nd/3rd CR patient, respectively, at the time of ABMT. A median number of 2.5×10^7 cells/kg body weight (range 1.7 to 8.2×10^7) were infused into each 1st CR patient. The median number of cells transfused into the 2nd/3rd CR group was 2.8×10^7/per kg (range 0.8 to 7.4×10^7).

Median recovery times for neutrophils to 500 cells/ul were 27 and 32 days for the 1st and 2nd/3rd CR patients, respectively. Median times to reach platelet counts of >20,000 and >50,000ul were 38 and 66 days (1st CR) and 46 and 82 days (2nd/3rd CR). Days to achieve engraftment was negatively correlated with weight-adjusted CFU-GM, BFU-E, CFU-MIX, and the number

of cells infused. This means engraftment was faster in those infused with larger numbers of CFU.

Relapse

One 1st CR patient relapsed 11 months post-ABMT. The pre-ABMT CR durations of the other three 1st CR patients surviving relapse-free after the transplant were 6, 9, and 11 months. Ten 2nd/3rd CR patients relapsed at times ranging from three to 32 months post-ABMT. Median time to relapse for 2nd/3rd CR patients was 11.4 months post-ABMT and 19.5 months post-CR.

Survival

The survival from transplant of all patients as of April 26, 1990 are shown in Figure 1 by CR group. The median relapse-free survival time of the CR1 group has not been reached, since half of 1st CR patients had not relapsed as of this analysis; however, it is at least 28 months post-ABMT and 34 months post-CR. Actuarial two year survival and relapse-free survival post-ABMT are both 75% ($\pm$21%). Three of the four patients are surviving at 33, 35, and 45 months post-ABMT. Actuarial two-and three-year survival of the patients in the 2nd/3rd CR group is 33% ($\pm$9%) and 27% ($\pm$ 9%) from ABMT. Median survival of this group is 6.4 months from the date of ABMT and 8.1 months from documentation of the current remission; median relapse-free survival post-ABMT is 5.2 months. Seven patients survive disease-free at 11, 17, 26, 42, 51, 65, and 71 months post-ABMT. Of the patients transplanted in 2nd and 3rd CR, six were shown to have "inversions" where the duration of their post-AMBT CR2 or CR3 exceeded the duration of the CR1 or CR2 by 12, 13, 18, 37, 39, and 39 months. Five of these are in the group of seven survivors. In the analysis of the 1st 30 patients, the FAB subclass correlated with relapse-free survival in the 2nd/3rd CR group (5). Patients with FAB subclasses M4 and M5 had better disease-free survival than other subclasses. Their rate of mortality or relapse was on average 15% (95% confidence interval, 3% to 85%; $p=.03$) that of the other subclasses.

Neuraminidase Treatment

One patient was treated by ABMT with marrow that had been exposed first to the enzyme neuraminidase to enhance CD15 expression. The remarkable aspect of this case was that the marrow engrafted very rapidly thus showing that cryptic CD 15 is not found on the pluripotent stem cell and that neuraminidase may have actually increased homing of stem cells into the bone marrow, or, alteratively, decreased non-specific uptake in extra-medullary sites.

Bispecific Antibody Killing

The ability of the bispecific antibody to mediate killing of HL-60 promyelocytic leukemia cells was studied using a 6 hr chromium-51 release assay. Effector cells were monocytes obtained by cytopheresis and cultured for 18 hr with gamma interferon (100 units/ml). Monocytes alone caused minimal killing (5-20%), monocytes plus bispecific antibody caused moderate killing

(20-50%), and monocytes plus bispecific antibody plus human serum resulted in maximal killing (50-80%) (Figure 2). Thus, this bispecific antibody possessed the ability to lyse tumor cell targets by two different mechanisms, complement and cellular-mediated lysis.

DISCUSSION

Multi-center and uncontrolled studies in Europe have demonstrated that AML patients transplanted within the 1st six months of their 1st CR show a relapse-free survival benefit using marrow that had been treated with mafosfamide (7). Though no randomized studies directly comparing ABMT with and without marrow purging have been reported, long-term survival has been noted for AML patients in 2nd and 3rd CR after ABMT using various methods for removing occult leukemia cells (5-7). Our study has demonstrated three-year overall survival in 27% of patients in 2nd and 3rd CR and relapse-free survival of 20%. Interestingly, we found a survival benefit with FAB M4, M5 subclasses. This contrasts with many studies that have shown that FAB M4 and M5 cases have had lower survival rates.

Overall, these results are encouraging. The number of long-term survivors in 2nd and 3rd CR is similar to that reported with allogeneic BMT and from ABMT using alternative forms of marrow purging (13). Long-term disease free survival in patients with AML in 2nd or 3rd CR after chemotherapy is unusual. Recently, the M.D. Anderson Hospital reported that patients with 1st CR greater than 18 months have a better prognosis than those who relapse sooner. In this report, there were several long-term survivors (14). These data do raise questions about the relative efficacy of ABMT in patients with AML after 1st CR. Although the numbers in our study are small, we looked at the proportion of patients transplanted in 2nd and 3rd CR who had 1st CR of less than 12 months, 12 to 18 months, and greater than 18 months of which there were eight, seven, and eight patients, respectively. There were three relapses in the 1st group, two in the 2nd, and three in the 3rd group and there were one, two, and three long-term survivors in the respective groups. The only difference in the outcomes in the groups was a greater number of toxic deaths in the group with the shortest 1st CRs. Another criticism of marrow transplantation data has been that there is an inherent selection bias due to the time required to get to transplant (17). The median time to ABMT for patients in 2nd and 3rd CR in this study was 60 days. Thus, because some patients treated with chemotherapy might have already relapsed by two months, we may have selected somewhat for a population at lower risk for relapse. However, as discussed above, the duration of 1st CR, which has considerable predictive value for subsequent relapse potential, did not obviously correlate with outcome in our patients.

The major questions for investigators in ABMT seem to be the following: when to transplant, who to transplant, what preparative regimen is optimal, whether to purge the marrow, and how to purge marrow (assuming one believes the marrow should be purged). The issue of when to transplant

is clearly difficult. We favor the idea of transplant in 1st CR, but in the context of a clinical trial with a control arm using chemotherapy only. Who to transplant could perhaps be phrased by saying who shouldn't be transplanted. Perhaps patients with clearly defined good risk factors should be spared the potential toxicity of ABMT. For example, patients with chromosomal abnormalities including translocations of 8;21 and 15;17 or an inverted 16th chromosome, who have been shown by several groups to have a favorable survival, should be excluded from 1st CR transplant. Whether to purge residual disease is an important technical issue that can only be resolved in a randomized study. However, potential problems with such a study are that both physicians and patients may have strong feelings against using unpurged marrow and thus not participate in the study. As long as purging is safe, arguments against the need for purging are reduced to discussions of economics. Similarly, whether any of the available purging methods is superior is not clear from the data available. However, an argument for a randomized study comparing two different purging methods is stronger because there is no known difference between any technique presently in use. The difficulty of any of the above mentioned studies is that AML is a relatively rare disease and thus enrolling sufficient numbers of patients is a barrier to successful completion of studies in a timely manner.

In addition, it appears that more attention needs to be given to post-transplant therapy in order to lower relapse rates. We have described in this report the creation of a novel immunotherapeutic agent, a bispecific antibody comprised of an anti-CD 15 mAb and a mAb to the monocyte-associated Fc receptor. This antibody is able to mediate ADCC as well as complementdependent lysis using human C' and could be used post-transplant to treat residual disease. We have infused this antibody into three patients and found it to be well-tolerated. We are exploring the possibility of using the antibody post-transplant in a randomized fashion. Finally, we wish to call attention to an intriguing observation that a patient whose marrow was treated with neuraminidase prior to mAb plus C'purging had a remarkably rapid engraftment. This raises the question of whether the pluripotent stem cell can be manipulated to enter the marrow cavity faster by biochemical changes and accelerate hematopoiesis. Since the morbidity and mortality of ABMT is related in large part to myelosuppression, the benefit of shortening recovery times is obvious.

ACKNOWLEDGEMENTS

Supported in part by grants CA31888 and CA23108 awarded by the National Cancer Institute, DHHS. Dr. Ball is a Scholar of the Leukemia Society of America.

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TABLE 1

Clinical characteristics of patients.

				FAB SUBCLASS		
CR	N	Ages (Median)	Male:Female	M1/M2	M3	M4/M5
1st	4	34-46 (43)	3:1	1	2	1
2nd	26	11-53 (38)	16:10	13	3	10
3rd	7	29-57 (44)	6:1	4	1	2
1st Relapse	3	18-36 (26)	1:2	1	0	2
All	40	11-57 (42)	26:14	19	6	15

FIGURE 1

Overall survival of 37 patients who have undergone ABMT with mAB and C'-purged bone marrow. Solid line represents patients in 1st CR (four patients) and the dashed line represents patients in 2nd and 3rd CR combined (33 patients).

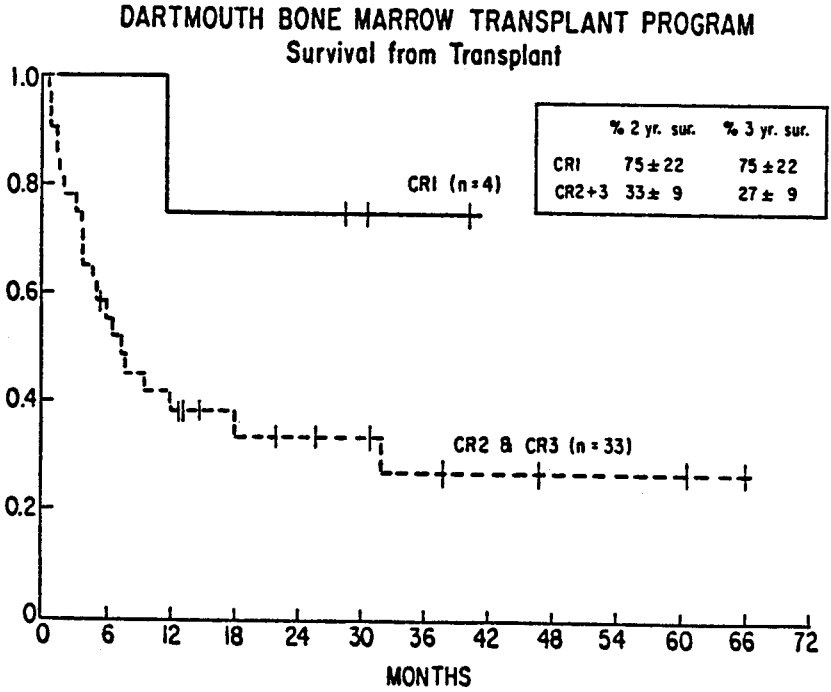
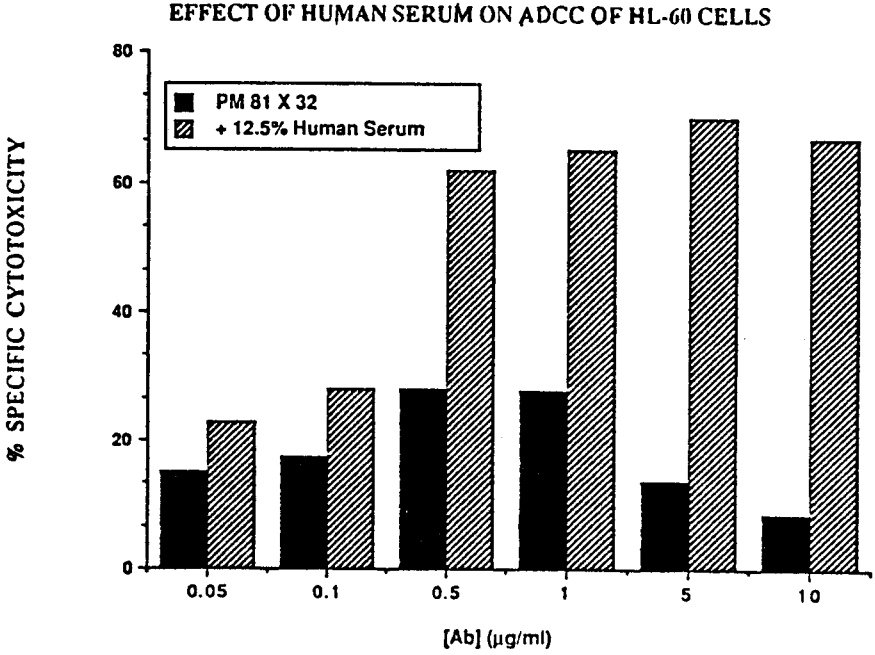


FIGURE 2

Ability of bispecific antibody to kill HL60 target cells with and without human complement.



HEMATOLOGICAL RECOVERY AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION IN ACUTE LEUKEMIA: PROGNOSTIC FACTORS

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INTRODUCTION

Kinetics of hemopoietic reconstitution after autologous bone marrow transplantation (ABMT) can be influenced by many different factors, such as diagnosis, disease status, chemotherapy before harvesting and ex vivo bone marrow manipulation (fractionation, purging and cryopreservation procedures). The relationship between the patterns of hematological recovery and the amount of marrow cells or hemopoietic precursors transfused has been extensively examined with controversial results [1-7]. Inadequate engraftment after ABMT has been more frequently reported in patients with acute leukemia (AL). The aim of this study is to identify the main factors influencing hematological recovery in these patients. With this information we will be able to recognize cases with high risk of prolonged aplasia, and to select candidates to post-transplant support with cytokines or peripheral stem cells to accelerate recovery.

PATIENTS AND METHODS

Patient Selection and Characteristics

Seventy successive AL patients (32 ALL and 38 ANLL) who underwent ABMT were retrospectively studied. Ages ranged from 7 months to 50 years (median 17 years). There were 39 males and 31 females. Patient characteristics are shown in Table 1.

METHODS

Bone Marrow Processing

Two cell separation methods have been employed. Forty eight patients had been autografted with marrow buffy-coat (BC) obtained by automatic procedure (IBM 2991) and 22 with marrow mononuclear cells (MNC) obtained

by density gradient on Ficoll (Lymphoprep). This second group included 19 "in vitro" treated marrows (3 ASTA Z and 16 MoAb treatments).

Median number of cryopreserved cells was 3.2 (0.6-5.5) E8NC/Kg for the first group (BC), and 1.8 (0.14-8.8) E7MNC/Kg for the second one. Median numbers of cryopreserved CFU-GM were 4.9 (0.4-19.3) E4/Kg and 1.3 (0.2-4.3) E4/Kg, respectively.

Purging, cryopreservation and thawing were performed following standard methods.

Hematologic Recovery Evaluation

After bone marrow infusion, hematologic reconstitution was evaluated through peripheral blood counts and sequential marrow examinations. Leucocyte, granulocyte, reticulocyte and platelet recoveries were monitored.

Post-Transplant Outcome

The median follow up has been 7 months (4-58m). Thirty-eight patients are alive, with a median survival time after ABMT of 7 months. Thirty-four were in maintained post-transplant CR. Median disease free survival (DFS) was 6 months (0.5-58m), with an actuarial probability of DFS of 42% at two years. Four patients had relapsed but were alive on new chemotherapy. Seven early deaths had occurred and 24 patients had died after relapse at 1.5-24 months after ABMT (median 4m).

Statistical Analysis

Days to reach peripheral counts over 500 granulocytes/mm^3, 20,000 platelets/mm^3 and 50,000 platelets/mm^3 were the three engraftment parameters analyzed. Two groups of variables were studied. The first group included all the patient characteristics exposed in Table 1. In ANLL patients chemotherapy before marrow collection was also analyzed. The second group of variables included the following marrow processing parameters: cell separation methods, marrow purging, number of cryopreserved cells (two independent groups according to fractionation method) and number of cryopreserved CFU-GM (data not available in 19 cases). As marrow MNC fractionation was a variable strongly correlated with "in vitro" treatment, it could not be independently studied.

The influence of these variables on engraftment parameters was evaluated using univariate and multivariate statistical analysis (test for comparison survival curves proposed by Peto & Peto [8], and Cox regression models [9]). Correlation tests have also been performed (Spearman).

RESULTS

Granulocyte Recovery

Hematological recovery was not evaluable in 6 patients, as early death had occurred due to transplant related complications. Four out of 64 patients failed to engraft (6.25%), and 60 patients showed a successful granulocyte

recovery within a median time of 24 days (10-67d; mean 28). Engraftment data are summarized in Table 2.

Univariate analysis led to the following results:

1. Higher amounts of cryopreserved CFU-GM (>4E4/Kg) accelerated granulocyte recovery ($p<0.001$). See Figure 1. The Spearman rank correlation test showed a low but statistically significant correlation between this two parameters: $rs=-0.4878$ ($p<0.006$).

2. "In vitro" marrow treatment adversely affected granulocyte recovery ($p<0.0007$). See Fig 2.

3. Patients conditioned with CY+single dose TBI showed a faster recovery ($p<0.006$).

4. High AraC doses (>12gr/m^2) prior to marrow collection prolonged severe neutropenia after ABMT ($p<0.002$). See Figure 3. A correlation was found between these two variables in the Spearman test: $rs=0.6815$ ($p<0.001$).

5. We have not observed differences in the kinetics of granulocyte recovery between ALL and ANLL.

6. Finally, we failed to identify any other variable influencing this engraftment parameter.

The following variables were selected for the multivariate analysis: amount of CFU-GM, purged vs non purged marrow, total AraC dose prior marrow collection and conditioning regimens. The first three variables maintained their prognostic value for granulocyte recovery ($p<0.01$, $p<0.01$ and $p<0.002$ respectively). Conditioning regimen was not identified as a prognostic factor, probably due to the reduced number of patients in the single-dose TBI group.

Platelet Recovery

Time to reach maintained peripheral blood counts over 20,000 and 50,000 platelets/mm^3 were the two parameters employed to evaluate the kinetics of post-transplant platelet recovery.

The first parameter, evaluable in 60 cases, was not achieved on 9 occasions (15%). Seven of these patients relapsed in a short period of time. Fifty one patients reached this platelet level, within a median time of 40 days (17-97d; mean 43d).

Time to reach 50.000 platelets/mm^3 was evaluable in 53 patients, with a median recovering time of 57 days (18-250 d; mean 72d). Thirteen cases failed to achieve this level (24%), with further evidence of disease progression in 9 of them.

At the univariate analysis the following results were observed:

1. Younger patients (<18 years) showed a faster platelet recovery ($p<0.002$ and 0.0003 for the 2 points respectively). See Fig 4.

2. Purging adversely affected platelet recovery, leading to a prolonged time to reach 20,000 platelets/mm^3. The second parameter analyzed was not influenced by this variable.

3. Diagnosis was not identified as a factor with predictive value for platelet recovery. However, considering patients grafted with unpurged

marrows, ALL diagnosis was a good prognostic factor ($p<0.03$ and $p<0.005$ respectively).

4. Single-dose TBI conditioning regimen was associated with a shorter time to reach 20,000 platelets/mm^3 ($p<0.01$) but did not modified further platelet recovery.

5. There was a trend for a prolonged thrombocytopenia in patients that had received higher AraC doses before marrow harvesting, but without a statistically significant difference.

6. None of the other factors influenced these parameters.

Age was the only variable showing influence on platelet recovery at the multivariate analysis ($p<0.006$).

DISCUSSION

We have analyzed the kinetics of hemopoietic reconstitution in patients undergoing ABMT for acute leukemia. The incidence of failed or delayed engraftment following autotransplantation ranges from 5 to 38% in different published studies. In our series, lack of engraftment has been observed in 6% of the evaluable cases. Furthermore, up to 24% of the patients failed to achieve peripheral platelet counts over 50,000/mm^3. As previous reports have already pointed out [4], we have observed that early post-transplant relapse represents a relatively frequent cause of graft failure in these patients.

Several authors have described a linear correlation between the number of CFU-GM grafted and the kinetics of hematologic recovery [1]. Others have reported an exponential correlation of the marrow CFU-GM content and post-transplant engraftment [3]. Finally, some studies have shown a CFU-GM threshold in respect to the rate of recovery [2]. However, as these observations have not been confirm by other authors, the usefulness of CFU-GM assays in predicting engraftment remains controversial. In our opinion, one of the reasons for the absence of consistent results is the heterogeneity of the groups studied. In our series we have found that patients grafted with more than $4x10^4$ CFU-GM/KG have a significantly faster granulocyte recovery. Furthermore, a linear correlation between CFU-GM and neutropenia period could be established. On the other hand, this parameter did not correlate with the kinetics of platelet recovery. This finding, consistent with other reported data, reflects the lineage commitment of these precursor cells.

It has been postulated that the number of nucleated marrow cells transfused was predictive, to some extent, of hematological recovery [6]. However, this correlation could not be established in our study.

As previously exposed, we have observed a delayed hemopoietic reconstitution in patients autografted with purged marrows. Incubation with ASTA Z to eradicate residual leukemia cells is known to delay neutrophil and platelet recovery [10-11]. Similar results have been observed in some cases after marrow purging with MoAb. However, other studies did not confirm this finding. As we have studied a limited number of cases autografted with purged marrow, the influence of different "in vitro" treatments could not be evaluated.

Several studies have described a more severely altered engraftment in ANLL than in ALL [12-13]. Considering the overall group, we failed to confirm this observation. However, excluding patients autografted with marrow purging, a faster platelet recovery has been observed in ALL patients.

Extensive chemotherapy before harvesting is known to reduce the stem cell potential of the marrow, being one of the factors delaying post-transplant hematological recovery [14-15]. As ANLL patients had all received similar protocols, we have been able to evaluate the influence of chemotherapy in this set of patients. Our results clearly shows that granulocyte recovery is more delayed in patients previously treated with higher AraC doses. We have also found that platelet recovery was influenced, to a lesser extent, by this factor.

Age has been identify as one of the main factors influencing platelet recovery in our patients. Previous reports had already described a faster recovery in younger patients [16].

We have observed that patients receiving conditioning regimens involving unfractionated TBI showed a fast hematologic recovery. This finding may reflect a less microenvironmental damage in this subgroup, or a possible endogenous marrow recovery.

In summary, our study shows that marrow graft CFU-GM content, "in vitro" purging and chemotherapy before harvesting (AraC) are the main variables conditioning granulocyte recovery after ABMT in AL patients. In a similar way, the kinetics of platelet recovery is essentially affected by age, diagnosis and, to some extent, by marrow purging.

ACKNOWLEDGEMENTS

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TABLE 1

PATIENT CHARACTERISTICS

Diagnosis	32 ALL / 38 ANLL
Age (median-range)	17 years (7 months-50 y)
Sex (males/females)	39 / 11
Disease status at BMC :	CR1 36 CR2 29 +CR2 5
Time in CR before BMC:	4 months (2-16)
Interval chemotherapy- BMC:	1.5 months (1-17)
Interval from BMC to ABMT:	2 months (0.3-22)
Disease status at ABMT:	CR1 28 CR2 33 +CR2 9
Conditioning regimens:	
CY + fractionated TBI (1200cGy)	56
CY + single dose TBI (1000cGy)	6
Chemotherapy protocols	8

CR: Complete remission
Cy: Cyclophosphamide
BMC: Bone marrow collection
TBI: Total body irradiation

TABLE 2

ENGRAFTMENT DATA

GRANULOCYTES $> 500/mm^3$:

- Six non evaluable patients (early deaths)

- Evaluable patients:

  - 4 graft failures (6%)
    - 1 relapse (6th week)
    - 3 back-up marrows (on days +23, +28,+40)

  - 60 succesful granulocyte recovery:

    MEDIAN: 24 days (10-67d)

PLATELETS $> 20.000/mm^3$:

- Ten cases could not be evaluated

- Sixty evaluable patients:

  - 9 did not reached this platelet level (15%)
    - 7 early relapses
    - 2 back-up marrows (+90,+180)

  - 51 cases reached this end point:

    MEDIAN: 40 days (17-97d)

PLATELETS $> 50.000/mm^3$

-Fifty three evaluable patients:

- -13 did not achieved this platelet level (24%)
  - 9 disease progression
  - 4 maintained thrombocytopenia

- 40 satisfactory platelet recovery:

  MEDIAN: 57 days (18-250)

FIGURE 1

Cumulative probability of granulocyte recovery after ABMT depending on CFU-GM transfused.

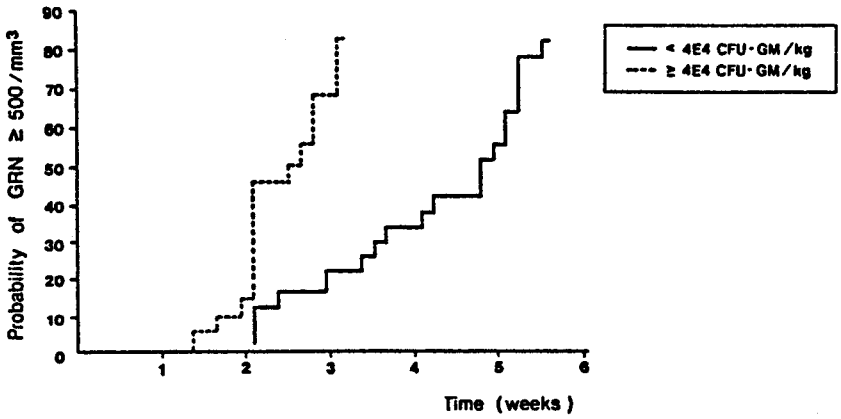


FIGURE 2

Cumulative probability of granulocyte recovery after ABMT for patients autografted with purged or unpurged marrows.

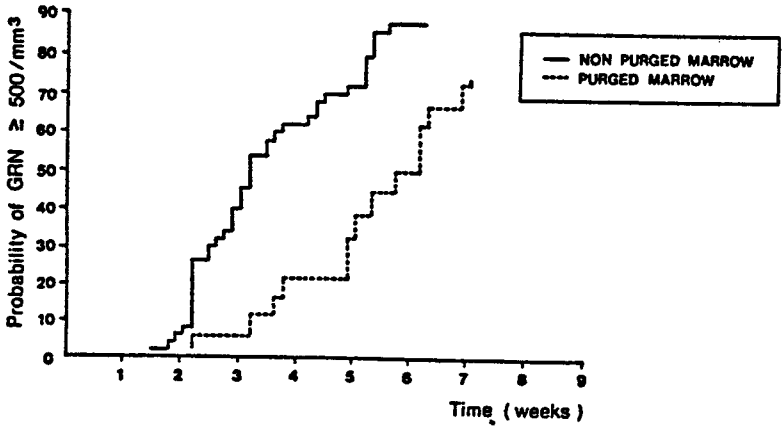


FIGURE 3

Cumulative probability of granulocyte recovery after autotransplant depending on AraC doses received before marrow collection.

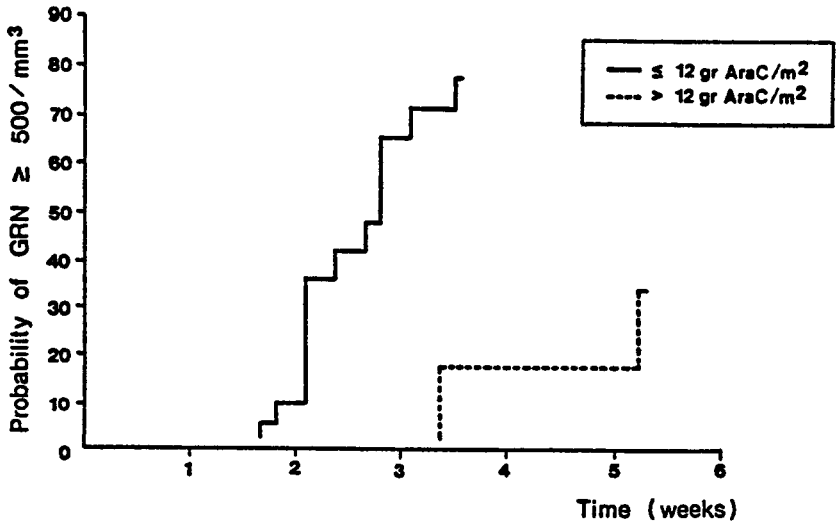
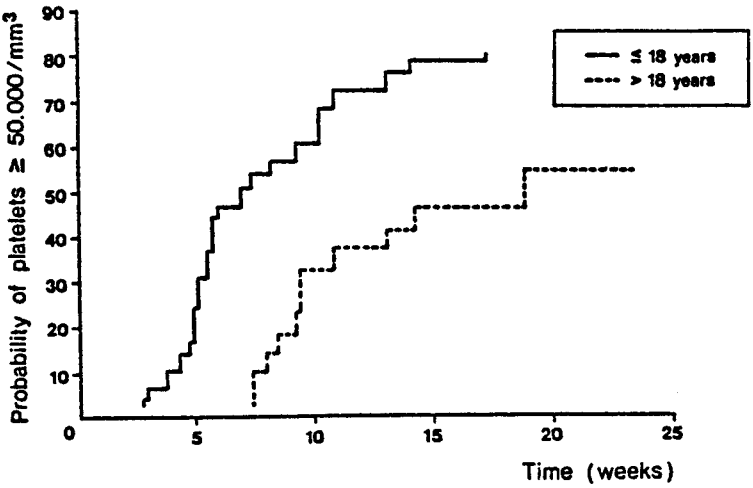


FIGURE 4

Cumulative probability of achieving peripheral blood counts over 50,000 platelets/mm^3 after ABMT for patients younger or older than 18 years.



"IN VITRO" CELL PROLIFERATION STUDIES ON ASTA Z 7654 (AZ) TREATED BONE MARROW (BM): PRELIMINARY RESULTS

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INTRODUCTION

Autologous Bone Marrow Transplantation (ABMT) is a promising therapeutical approach for an increasing number of malignancies (1). Nevertheless, its efficacy can be impaired by the reinfusion of neoplastic cells remaining in the cryopreserved marrow.

Several methods for removing residual neoplastic cells from BM have been proposed, being immunological and chemical methods are the most widely employed (2,3,4). Immunological methods are usually based on the recognition of specific antigens by appropriate monoclonal antibodies for its subsequent complement mediated lysis or separation by physical methods (5,6,7). Chemical "ex vivo" BM treatment is usually based on the utilization of cytotoxic agents from which cyclophosphamide derivatives are the most frequently employed (8,9).

Mafosfamide-lysine (ASTA-Z 7654) is a cytotoxic and phase independent cyclophosphamide-derived aliquoting agent. In theory, its cytotoxic effect is more effective on highly proliferating cells and less effective on undifferentiated cells (normal stem cells?) (10, 11) in which aldehyde dehydrogenase content (AZ inactivating enzyme) is lower (12).

These characteristics confer some specificity to this compound, making it useful for the "ex vivo" treatment of eventually neoplastic cells contaminating BM, which has already demonstrated its effectiveness on AML marrow purging and to a lesser extend in ALL and CML (13).

In order to increase AZ clinical effectiveness in preserving normal hemopoiesis potential, adjusted doses, according to CFU-GM growth inhibition, have been proposed (14). In preliminary analysis of the European Bone Marrow Transplantation group, autologous transplants performed with AZ adjusted dose have shown longer disease free survival (15).

Since CFU-GM inhibition studies used for adjusting AZ doses, are tedious, expensive and time consuming, alternative methods should be explored.

With this purpose, we have initiated a series of experiments combining CFU-GM and Long Term Marrow cultures (LTMC) and cell cycle (CC)

analysis by flow cytometry. In this paper we expose our preliminary results obtained after BM treatment with an AZ single dose.

MATERIAL AND METHODS

Eleven BM samples from patients suffering from different malignant hematological diseases (ANLL, HD, NHL and Neuroblastoma) and one healthy donor have been treated "in vitro" with AZ.

BM mononuclear cells (MNC), obtained by density centrifugation with Ficoll Diatriazoate (hematocrit <1%), at a 10^7 CMN/ml, have been incubated with AZ 7654 (100 uM concentration), at 37C during 30 min, according to the protocol proposed by NC Gorin.

Cytotoxic effect has been assessed by CFU-GM cultures, LTMC and CC analysis. CFU-GM cultures were performed according to Pike and Robinson (16) technique. Briefly, 10^5 marrow cells were cultured on soft agar 0.3% in McKoys supplemented medium over a feeder layer constituted by 10^6 peripheral on 0.5% agar in McKoys supplemented medium.

LTMC were performed according to Gartner and Kaplan technique (17).Briefly, 2 x 10^7 MNC were suspended in alpha medium supplemented with 20% fetal calf serum, horse serum and hydrocortisone. Cultures were incubated at 37C in 5% CO_2 atmosphere, and maintained by weekly demi-population with addition of fresh medium. The cell suspension obtained was assayed for CFU-GM cultures.

Cell cycle proliferative phase assessment ($S+G_2M$) has been performed by cell nuclei isolation by means of treatment with triton-100 (0.1%) and sodium citrate (0.1%), incubated in solution of RNase (0.5% in phosphate buffer, pH 7.4) for 30 min. at 37C to prevent RNA staining. After centrifugation, the cells were stained for DNA with propidium iodide (0.02 mg/ml in phosphate buffer, pH 7.4). The nuclei were analyzed on an EPICS V cell sorter, tuned at 488nm., and the fraction of cells in phases $S+G_2M$ was estimated by using the PARA-1 program of parametric analysis of the MDADS software (Coulter Cientifica). All these analyses have been performed before, immediately after marrow treatment and on days 2,14,21,28 and 35 of LTMC.

Statistical Analysis

Statistical analysis has been performed by means of Wilcoxon Signed Rank test, to avoid disturbances due to the non normal distribution of the results.

RESULTS

CFU-GM Cultures

As expected, BM incubation with AZ induces an immediate CFU-GM growth inhibition of 99.66% (99.93%-90%) with respect to untreated BM. After 48 hours in LTMC, a progressive increase of CFU-GM growth has been

observed until reaching a maximum on day 7, followed by a rapid and progressive reduction (Fig 1).

One out of the eleven cases did not follow this kinetic pattern, showing no CFU-GM growth inhibition after treatment and a progressive and non reversible growth reduction from day 2 (Figure 2) .

Flow Cytometry Analysis of Cell Cycle

After treatment, an immediate reduction of 77.896 (51.16%-97%) of cells in S+G2M has been observed, followed by an increase of proliferating cells from 48h of LTMC, reaching statistically significant differences ($p<0.02$) on day 14, with respect to its control. After day 21 a progressive decrease of cells in phase S+G2M was observed (Fig.3). A linear correlation between the number of proliferating cells before treatment and AZ cytotoxicity has been observed ($cc= 0.8414$, $p<0.05$).

Relationship Between CFU-GM Growth and Cell Cycle

From 48 hours until 14 days of culture, a linear correlation was found between the proliferative fraction and number of CFU-GM obtained ($cc=0.8032$, $p<0.05$) (Fig 4).

DISCUSSION

For effective ABMT, the ideal "in vitro" chemotherapy should be selective, with a specific killing effect on clonogenic leukemic cells, while simultaneously sparing the normal hematopoietic stem cells. Previous preclinical studies as well as the present study have demonstrated that the "in vitro" cyclophosphamide treatment of BM strongly inhibits the proliferation of myeloid committed precursors, preserving normal "stem cell" ability for long term growth (11).

As it has been demonstrated, "in vitro" adjusting of chemotherapeutic agents dose correlates with a better early progenitor cell preservation and more efficient ABMT results. With the aim of determining the relationship between CFU-GM, LTMC and flow cytometry analysis we have performed a series of experiments utilizing AZ at a single dose (100 mill). Our results confirm the expected immediate cytotoxic effect of this agent over myeloid committed precursors followed by a progressive recovery of the CFU-GM content of LTMC (10). Even the slightly lower total CFU-GM recovery, compared with untreated samples, reflects some damage extended to normal progenitors, this committed cell growth recovery suggests an adequate preservation of normal stem cells.

Flow cytometry analysis generally confirms this finding, showing a progressive increase of the proliferating fraction which prolonged beyond CFU-GM growth. This cell cycle behavior may reflect the addition of the differentiation and proliferation from stem to committed cells and from committed cells to the late myeloid differentiation steps (Fig 5). Moreover, the CC and CFU-GM proliferation kinetics of treated samples also suggest a

feedback effect, promoting a simultaneous pass through the committed status of the majority of resting stem cells. As expected, it has been demonstrated that the BM fraction with high proliferative potential is more sensitive to the cytotoxic effect of AZ.

Currently CFU-GM cultures are the only way to determine the adjusted dose of cyclophosphamide derivatives for ABMT, thus, it is worth consideration to use the flow cytometry analysis as an alternative to evaluate the response of bone marrow granulocyte-macrophage colony forming cell progenitors to AZ as demonstrated before by the correlation of number of CFU-GM and the proliferative fractions that contain cells in $S+G_2M$ phase in the first weeks, however after two week time the number of CFU-GM decreases for the cell maturation, meanwhile the proliferative fraction is still increasing until day 21.

The preliminary results described suggest that the use of flow cytometry may be useful to determine the effect of different pharmacological agents on hemopoietic precursors.

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FIGURE 1

Comparison of the number of CFU-GM between treated and non-treated bone marrow. The treated ones show an immediate growth inhibition of 99.44% after ASTA-Z treatment.

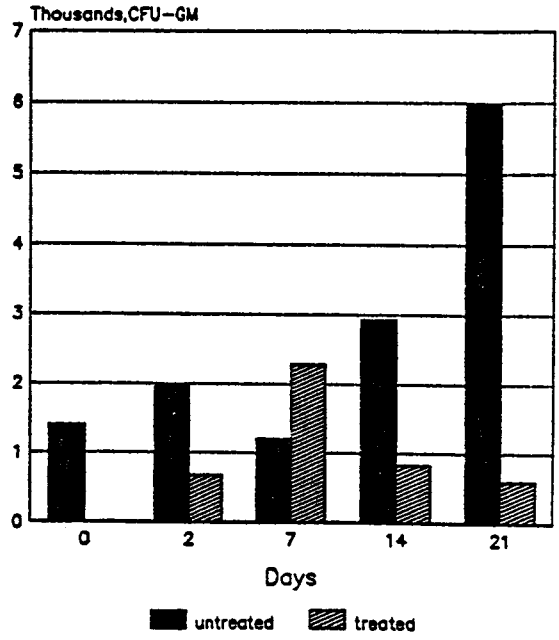


FIGURE 2

Different cases compared with the others analyzed (see Fig 5).

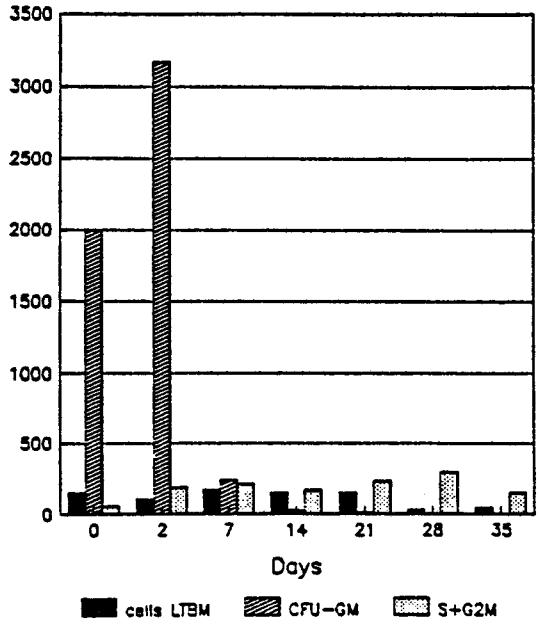


FIGURE 3

Cellular growth kinetics in treated and non-treated bone marrows determined by flow cytometry.

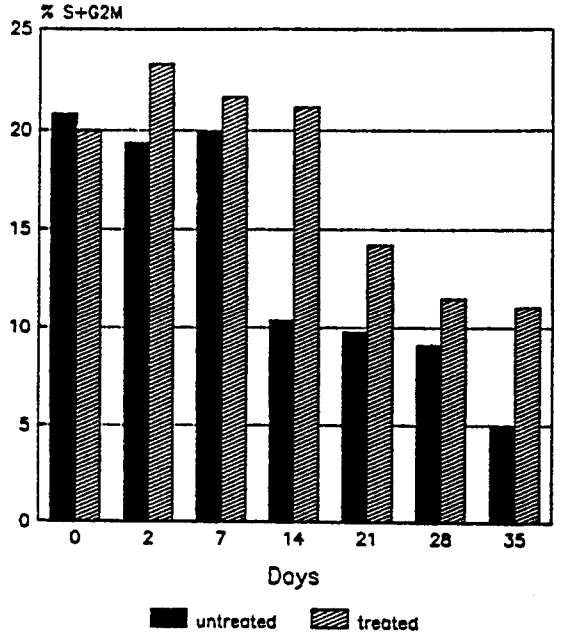


FIGURE 4

Correlation between the proliferative fraction and number of CFU-GM determinates by flow cytometry, before and after treatment.

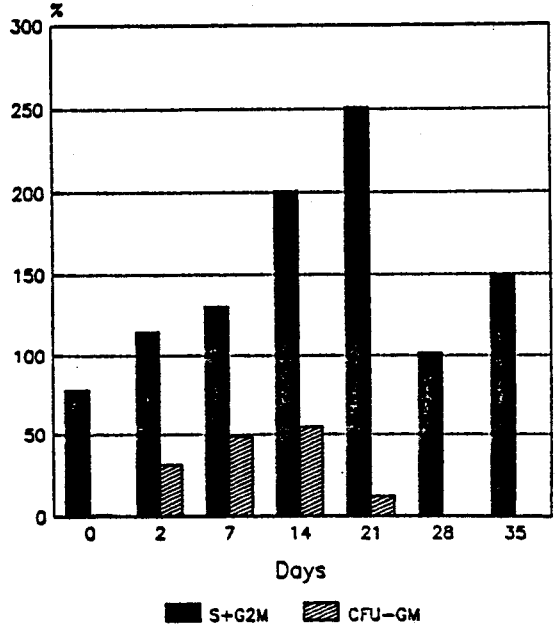
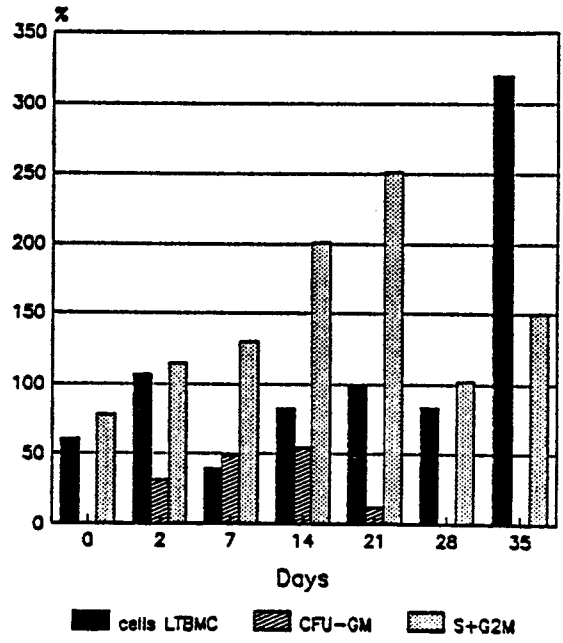


FIGURE 5

Effect of ASTA-Z on the CFU-GM recovery and cellular kinetics of LTBMC.



DETECTION OF MINIMAL RESIDUAL DISEASE IN HEMATOPOIETIC MALIGNANCIES

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ABSTRACT

A major problem in the effective treatment of cancer is the high frequency of recurrence of disease most likely from clinically undetectable minimal residual disease. Molecular technology has provided us with means to demonstrate minimal residual disease by identifying non-random genetic rearrangement patterns in malignant cells and by identifying genes whose expression contribute to the malignant characteristics of these cells.

Minimal residual disease in hematopoietic malignancies has been detected by the use of two sensitive techniques, polymerase chain reaction (PCR) and in situ hybridization (ISH). Restrictions in the application of PCR to disease in which a) the chromosomal breakpoint is variable, b) quantitation of tumor load is of significance and c) phenotypic data is valuable emphasizes the advantages offered by the use of ISH. We have explored the use of ISH to monitor purging of bone marrow in acute myelogenous leukemia (AML). Using aberrant MYC expression as a marker we find that small numbers of tumor cells are present in first remission AML patients and that these cells are partially removed by purging based on the phenotype of the leukemic cell population at presentation. We conclude that both PCR and ISH analyses for minimal residual disease are important, but that the choice of technique used is seldom interchangeable and should rest on the information necessary for interpretable results.

MATERIALS AND METHODS

Cells

Fresh human normal bone marrow cells were obtained from hematologically normal donors. Leukemia marrow samples were obtained from patients in first complete remission whose marrow was being purged for future transplantation.

RNA-In Situ Hybridization

A very sensitive and rapid RNA-in situ hybridization procedure was performed as described earlier (1). Briefly cells to be analyzed 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 52C, followed by extensive washes in 1X SSC, 0.5X SSC and finally 0.1X SSC containing RNase. The slides were coverslipped 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 (1) and then labeled with Photobiotin™ (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 a parameter of expression levels in these studies. Several samples were further analyzed using a laser-based image analysis system (Meridian ACAS 570). 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. In these experiments overexpression 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

It is unclear at this time if the purging of bone marrow from leukemic cells is beneficial to the long term survival of AML patients undergoing autologous bone marrow transplantation (ABMT). The biological significance of such procedures will depend in part on the role these re-infused leukemic cells play in the recurrence of disease after transplantation. The fewer leukemic cells to escape the transplantation conditioning regimen, the more significant the leukemic cell population in the graft will be. A significant drawback in the answering of this question has been the inability to monitor the assumed removal of these residual leukemic cells.

When testing leukemic cell separation techniques on untreated or relapsed leukemic patients, it is possible to monitor the separations with little difficulty using standard techniques such as morphology, cytochemistry, cytogenetics and the in vitro colony formation assays. None of these assays are

specific and sensitive enough to detect the small numbers of leukemic cells which may be present in remission marrow. Since the purpose of the leukemic cell separation techniques is to be used for remission marrow, such detection methods leave us to do these separations blindly. For the past decade considerable effort has been made to use molecular technology to resolve these problems.

Since these tumor cells are part of a heterogeneous population of cells, the use of Southern and/or Northern blotting techniques have not enabled us to detect fewer than 1% contaminating tumor cells unless pre-selection is used through which our sensitivity increases to 0.1% at best. From estimates of the leukemic cell population in remission marrow, the frequency of the leukemic cell population is one leukemic cell in 20,000 cells, or 0.005%, which is far below the detection limits of the techniques discussed above. PCR does have the sensitivity to detect rare events in a cell population, but does not lend itself to quantitation of relatively small changes in the population (less than 10-fold) and in disease where there are considerable variations in chromosomal abnormalities.

Our approach to this problem has been the identification of an abnormality which is detectable at the single cell level. We have reported the development of an extremely sensitive and rapid RNA-in situ technology which permits the detection of specific mRNAs within individual cells (1). This technology enables us to identify cells with the abnormal expression of any marker gene at a level of 1/50,000 cells. Using this RNA-in situ hybridization methodology, we have identified the abnormally high expression of MYC which occur greater than 90% of untreated and relapsed AML patients (2). The high levels of MYC mRNA found in these cells cannot simply be attributed to the proliferative capacity of these cells or to the presence of normal, immature hematopoietic cells. Bone marrow cells from over fifteen solid tumor patients have now been examined in a longitudinal study choosing several time points before transplantation and at two time points (1 month and 2.5 months) after BMT when the hematopoietic cells are in a highly proliferative state. With the exception of two patients who appeared to have a subpopulation of cells with high MYC expression before ABMT, in no case were any cells found which express MYC at the high levels found in the leukemic cell population (Dr. Spencer, personal communication).

As confirmation that the cells we were examining actually belonged to the leukemic cell compartment, comparisons were made with the percentage of blast cells determined morphologically in these AML patients. We found that the percentage of cells overexpressing MYC at least equals, and often exceeds, the number of blast cells present in the marrow. We have also examined bone marrow cells of 10 AML patients who are long term survivors after BMT. 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 similar to that found in AML short term remission patients, does not occur in this patient group. It is unclear at this time if the purging of bone marrow of leukemic cells is

beneficial to the long term survival of AML patients undergoing ABMT. As discussed earlier, a significant drawback in the answering of this question has been the inability to monitor the assumed removal of these residual leukemic cells. We have begun to apply our RNA-in situ hybridization technique to this problem. Since we find such high levels of MYC to be present in AML patient bone marrow, we were interested to see if these cells are removed during a purging procedure using monoclonal antibodies directed against the leukemic cell population. Early results indicate that there is indeed a decrease, although not a complete elimination, of this MYC overexpressing subpopulation of cells after purging (Table 1). Although we have not yet attempted to quantify the efficiency with which these abnormal cells are removed, this test does appear to be a promising means by which to assess leukemic cell removal.

DISCUSSION

The effective treatment of cancer is diminished by the high frequency of recurrence of disease. Since a combination of cytogenetic and molecular markers can often identify the cells present in recurrent disease as similar if not identical to the original tumor cell population, the recurrence of disease most likely stems from clinically undetectable minimal residual disease. Molecular technology has been helpful in demonstrating the presence of low numbers of tumor cells in patients clinically in remission for as long as 10 years.

Two sensitive techniques which have been used to detect minimal residual disease in hematopoietic malignancies have been polymerase chain reaction (PCR) and in situ hybridization (ISH). PCR analysis is complimentary to such techniques as Southern blotting and chromosomal banding since it provides an increased sensitivity of detection, but does not offer any additional information over the more standard assays.

We have explored the use of RNA-ISH to monitor purging of bone marrow in acute myelogenous leukemia. Using aberrant MYC as a marker we found that small numbers of tumor cells are present in first remission AML patients and that these cells are removed to a variable degree by purging based on the phenotype of the leukemic cell population at presentation.

In situ hybridization offers an important advantage in permitting genetic analyses on single cells where results can also be related to cell morphology, phenotype and protein expression. In addition, the frequency of cells carrying an abnormality can
readily be assessed.

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TABLE 1

IMMUNOPURGE

Patient		Percent Positive MYC	SIS	Log Removal
1	before	73	69	
	after	71	64	0
2	before	45	30	
	after	>0.13	0?	2
3	before	39	0?	
	after	>0.05	0?	3
4	before	22	0?	
	after	>0.2	0?	2
ND	before	0?	0?	
	after	0?	0?	NA

*At least 5,000 cells examined per slide

HIGH DOSE CHEMOTHERAPY AND ABMT FOR ADULT ACUTE LYMPHOBLASTIC LEUKEMIA IN FIRST REMISSION

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INTRODUCTION

Current treatment regimens for adult ALL achieve CR rates of 70-80%.[1,2] Cyclic intensive chemotherapy produces long term leukemia free survival (LFS) rates of 20% to 40%.[3-12] In an effort to improve this outcome, autologous bone marrow transplantation (ABMT) has been used, allowing dose escalation unlimited by hemopoietic toxicity. Several groups have reported long term LFS rates of 38-65%.[13-17] We present our experience with 26 adult patients with ALL who received high-dose cyclophosphamide, BCNU, and VP-16 (CBV) + ABMT, with a long term leukemia-free survival (LFS) of 54%.

PATIENTS AND METHODS

Patient Population

Eighty-eight out of 105 adult ALL patients who received induction chemotherapy with VAD (vincristine/doxorubicin/dexamethasone) followed by CVAD (VAD + cyclophosphamide) achieved CR (67 with VAD, 21 with CVAD). After CVAD, patients received three sequential maintenance chemotherapy combinations, as described in reference 5, followed by late intensification with high-dose CBV + ABMT. After recovery from CBV-ABMT, patients repeated all non-BMT cycles once. Eligibility for ABMT was restricted to patients aged <60 years, with performance status <2 in the Zubrod scale, and normal renal, hepatic, cardiac and pulmonary function, who were not eligible for allogeneic BMT. Twenty-six patients received ABMT at a median of 34 weeks from CR (range, 24-50). Nineteen patients who received the same program, except for the CBV intensification, and who were alive and in CR at the point when CBV was to be given, are used as controls. Patient characteristics are described in Table 1. The rest of the CR patients are accounted for as follows: 9 patients received allogeneic BMT, 11 had treatment-related deaths, 2 were $>$ 60 years old, and 21 relapsed prior to scheduled BMT.

High-Dose Therapy

All patients gave informed consent; the protocol was approved by the Institutional Review Board of the M.D. Anderson Cancer Center. On day -6, patients received cyclophosphamide, 1.5/m^2 intravenously (IV) daily x 4 days; BCNU (carmustine) 300 mg/m^2 IV x 1 dose, and VP-16 (etoposide) 125 mg/m^2 IV every 12 hours x 6 doses (CBV).[18] On day 0, a median of 1.6x10^8 nucleated cells/kg (range, 1.0 to 4.3) was thawed at 37C and infused through a central venous catheter.

Statistical Methods

Relapse was defined as the presence of > 5% blasts in two consecutive bone marrow differentials or any evidence of extramedullary leukemia. Low and high risk patients were defined according to the criteria used in the German cooperative studies3, to which B cell phenotype and presence of the Philadelphia chromosome were added.[4,5] Blood count recovery times were measured from day of BMT to the day when the reported value was reached. Leukemia-free survival was measured from date of BMT to date of relapse, death from any cause, or last follow-up. The method of Kaplan and Meier[19] was used to plot remission duration curves. The duration of remission in distinct patient groups was compared using the generalized Wilcoxon test.[20] All reported P values are two-sided.

RESULTS

BMT Patients

With a median follow-up of 156 weeks, 14 patients were still alive and in CR at 86 to 243 weeks (median LFS, not reached), and 10 patients had relapse of leukemia at a median of 82 weeks (range, 45-126). One patient died of CNS bleeding in the early posttransplantation period, and another patient died in CR during a cycle of intensive maintenance chemotherapy 18 weeks after BMT. The four-year LFS rate was 54% (Fig. 1).

Control Patients

The four-year LFS rate was 35%, and the median LFS was 112 weeks (Fig. 1). Eight patients remain alive and NED at a median of 185 weeks (range, 154-314); 10 patients have suffered relapses at a median of 89 weeks (range, 53-221), and one patient died of sepsis at 111 weeks while still in CR.

The risk factors: age, WBC count, cycles to CR, B cell leukemia and Philadelphia(+) ALL were equally distributed between control add ABMT patients (Table 1). When control and ABMT patients were considered together, there were 16 good risk and 29 poor risk patients. There was a trend for improved LFS in good risk patients (P= .107) (Fig. 2). Among BMT patients, there were 8 good risk and 18 poor risk patients. There were two relapses at 47 and 121 weeks among the former, with six patients alive and well (LFS = 75%), whereas there were 8 relapses and two deaths in remission in the latter

group, with 8 patients alive and well (LFS = 44%; P= .164). Among the control patients, there were 8 good risk and 11 poor risk patients; in the former group, there were 3 relapses and 1 death in remission, with 4 patients still alive and well (LFS = 50%); among the poor risk patients, 7 relapsed, and 4 are alive NED (LFS = 23%; P= .38).

There was no significant difference between BMT and control patients in either the good risk groups (P= .353) or the poor prognosis patients (P= .84) (Fig. 3a and 3b, respectively).

Toxicity of CBP-ABMT

There was one treatment-related death (CNS bleeding in a patient with platelet alloimmunization). Three patients had grade 3 mucositis; 5 patients had sepsis, and one patient had reversible pericarditis of unclear etiology.

Hemopoietic recovery was evaluable in 25 patients (the early death is excluded). The median times (ranges) from BMT to absolute granulocyte count of $0.5x10^9/1$ and to platelet count of $50x10^9/1$ were 29 days (14 to 39 days) and 29 days (12 to 50 days), respectively. All patients but one were able to receive the planned maintenance therapy.

DISCUSSION

This group of ABMT patients had a long term LFS of 54%, with a low treatment related mortality (1/26). Other trials with similar number of patients show longer term LFS rates of 40-65%; [13-15] cooperative group reports [16,17] show lower LSF rates (Table 2). Although it is difficult to compare results of groups that are heterogeneous in composition, treatment regimens, and interval between CR and BMT, the results reported here are certainly not worse than the average, with the added advantage of a very tolerable morbidity and low mortality.

Risk groups are well defined in pediatric ALL, and current treatment protocols assign different regimens according to risk factors. In adult ALL, recent large studies have identified a small population of good prognosis patients, with long term LFS rates of 50%-70%, and a larger population of poor prognosis patients with LFS of 20-30%.[3-5] In this report, there was a trend favoring the good prognosis patients that did not reach statistical significance. This may be because most poor prognosis patients had suffered relapses by the time they were eligible for CBV, and thus only the best patients in that subgroup were included in this analysis.

Although results of ABMT were not significantly better from those of control patients, the sample size may be too small to detect differences. When only good prognosis patients were analyzed, a trend favoring BMT over controls was seen. Very large numbers of patients will be required to discern whether ABMT benefits patients with good prognostic features; however, the low morbidity and mortality of this therapy make it attractive as an alternative, possibly non-cross-resistant regimen. Since CBV did not prevent administration of subsequent intensive chemotherapy, it is an excellent regimen to integrate in

ALL treatment strategies, where maintenance chemotherapy is very important. Maintenance chemotherapy after BMT for ALL is probably necessary, as indicated by the decreased relapse rate of allogeneic transplant patients who received methotrexate for GVHD prophylaxis.[21]

Disappointingly, although the majority of adult ALL patients belong in the poor prognosis group, we did not see an advantage for CBV in this population. In these patients, CBV only seems to delay relapses, and probably is not cytoreductive enough to be curative. Alternatively, it may be that the high dose chemotherapy was given too late (median, 8 months after CR), allowing for the development of resistant cells in these patients heavily exposed to agents that induce the mdr gene (vincristine, anthracyclines) or other mechanisms of resistance (methotrexate). Our experience with CBV-ABMT suggests that ABMT should be given earlier, and that the poor prognosis patients should receive a more intensive cytoreduction than CBV. These hypotheses will be tested in our current front line regimen.

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Table 1

Characteristics	CBV-ABMT (no. = 26)	Control pts. (no. = 19)
Median age (range)	32 (15-49)	24 (15-59)
Pts. > 35 years	8	6
Median WBC (range)[1]	4 (1-428)	5 (1-358)
Pts. > 30[1]	4	4
Cycles to CR > 1	7	5
B-cell leukemia	1	0
Philadelphia (+)	1	1

[1] Expressed as WBC x 10^9/l

Table 2

RESULTS OF ABMT FOR ALL IN CR1

Group	Pts.	Regimen	Long-term LFS	
Seattle[13]	14	Cy-TBI	5	4 %
Uppsala[14]	21	Cy-TBI + other	6	5 %
Marseille[15]	22	Cy/Mel-TBI	40%	
This report	26	CBV	5	4 %
ISG[16]	37	Various	3	8 %
EBMTG[17]	233	Various	41%	

Abbreviations: Pts: patients; ISG: Italian Study Group; EBMTB: European Bone Marrow Transplantation Group; Cy- TBI: cyclophosphamide and total body irradiation; Mel: Melphalan.

FIGURE 1

Leukemia-free survival (LFS) by treatment modality.

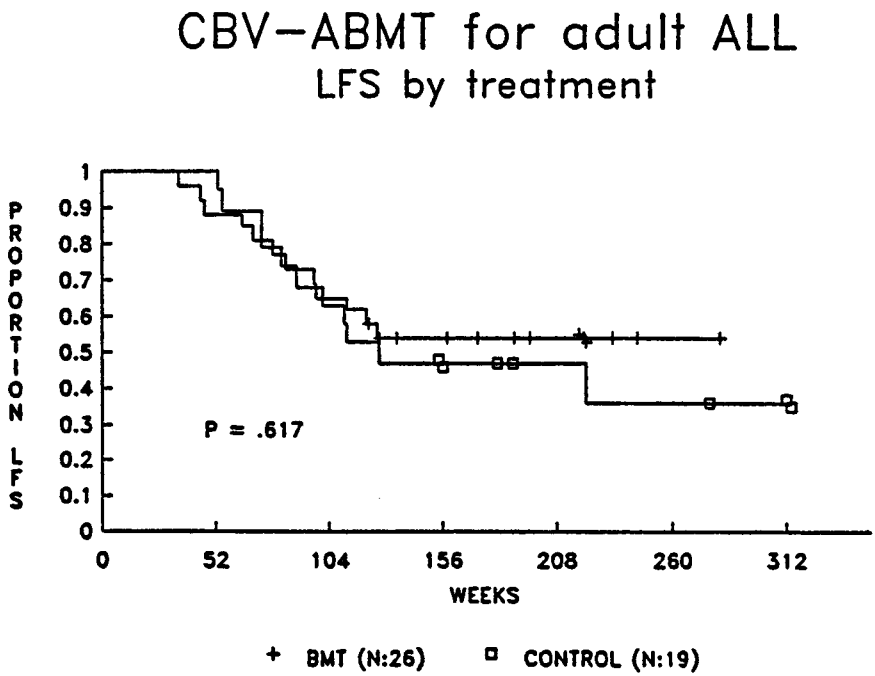


FIGURE 2

Leukemia-free survival (LFS) by risk groups.

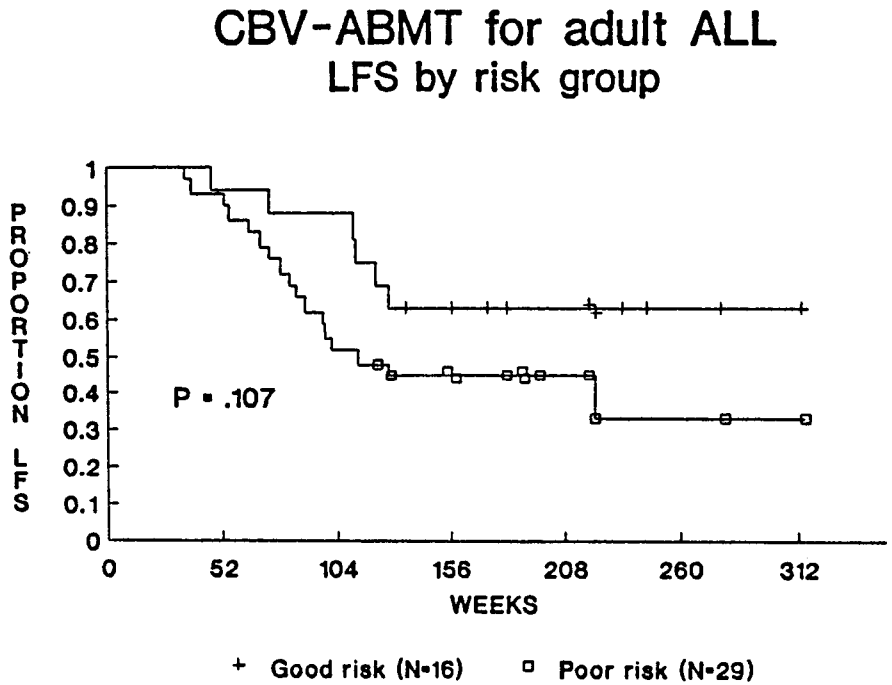


FIGURE 3A

Good risk patients (as defined in text) : LFS by treatment modality

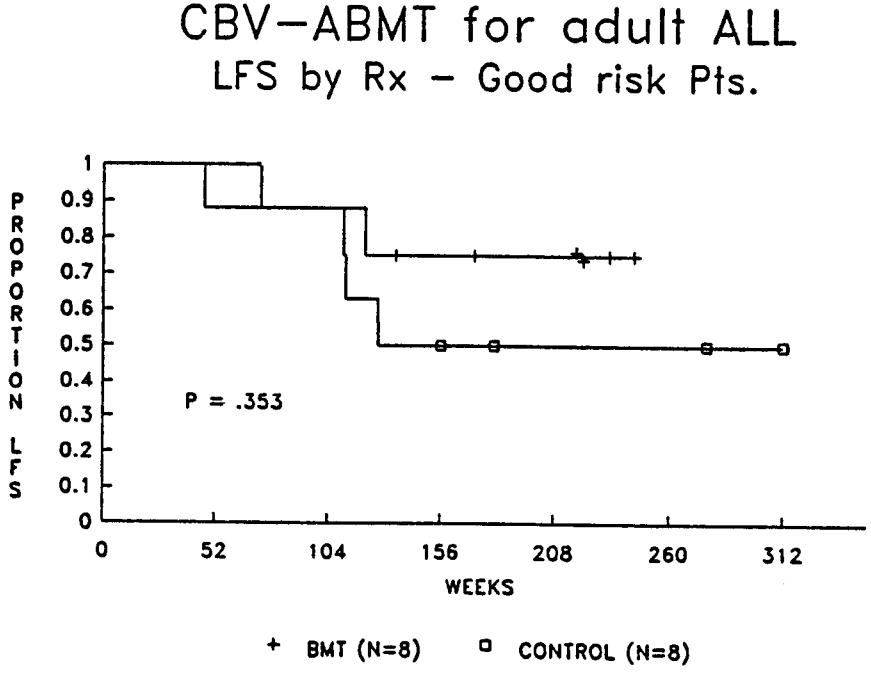
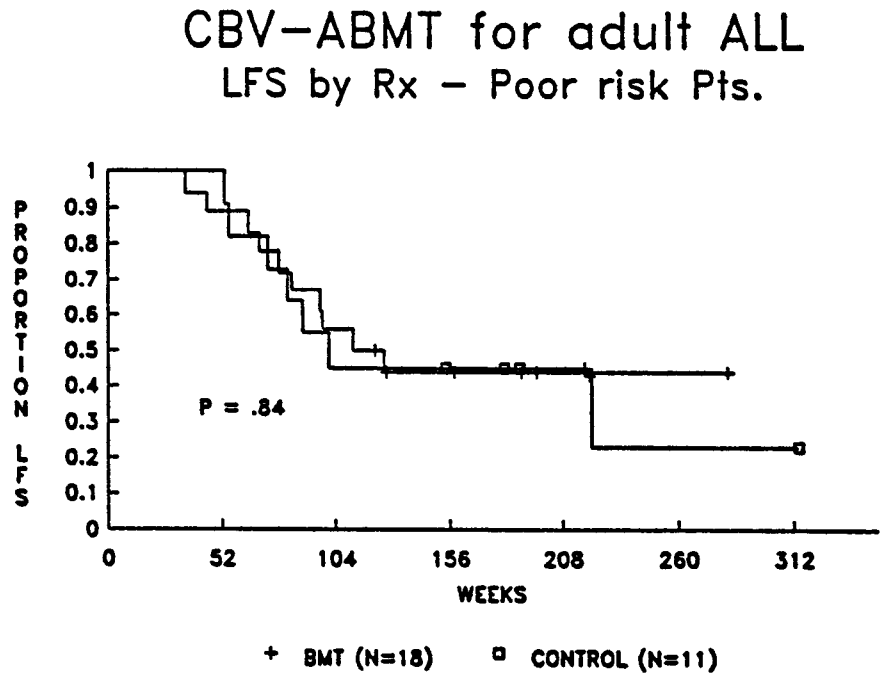


FIGURE 3B

Poor risk patients (as defined in text) : LFS by treatment modality.



AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR ACUTE LYMPHOBLASTIC LEUKEMIA IN CHILDREN: ISOLATED RELAPSE AS GOOD PROGNOSTIC FACTOR

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INTRODUCTION

The substantial advantages of autologous bone marrow transplantation (ABMT) are the absence of GVHD related complications and the shorter recovery time. On the contrary the disadvantaged are the higher relapse rate due to absent GVL effect and perhaps the transfer of leukemic cells with the graft.

In this paper we studied 45 children affected by acute lymphoblastic leukemia (ALL) treated with ABMT.

PATIENTS AND METHODS

We considered 45 children transplanted for ALL, 28 males and 17 females, with a median age of 10.7 years. Four children were treated in 1st complete remission because of resistance to primary induction therapy; 25 children were transplanted in 2nd CR; 16 in > 2nd CR. The mean of 1st remission duration was 25.8 months for patients transplanted in 2nd CR, 35.1 months for those transplanted in > 2nd CR. Analysis of the sites of relapse before transplantation showed that 29 patients had bone marrow relapse, 12 patients had CNS and/or testicular isolated relapse only.

Twenty-nine harvests were purged in vitro by 1 mc gr/ml Vincristine and 30 mc gr/ml Prednisone; 14 by 100 mc gr/ml mafosfamide; 2 by monoclonal antibody incubation. Twenty-one patients received high dose Vincristine (4 mgr/M^2), TBI (1200 cGy), Cyclophosphamide (3600 mgr/m^2) as conditioning regimen before transplant.

Thirteen patients received high dose Aracytin (24 gr/m^2) or Cyclophosphamide (3600 mgr/m^2) and TBI. Three children received Busulphan (16 mg/Kg) and Cyclophosphamide (200 mgr/Kg).

RESULTS

Of the 45 children, 23 relapsed from 1 to 9 months after ABMT. Four toxicity related deaths were observed for sepsis (2), VOD (1), or lung embolism (1). Seventeen children remained in continuous complete remission from 1 to 47 months after transplantation. The probability of EFS at 4 years was 30% (figure 1).

High dose Vincristine did not ameliorate the EFS of this group (figure 2). On the contrary when the EFS was related to the site of relapse, isolated or systemic, the difference was statistically significant (P = 0.05) (figure 3).

DISCUSSION

The EFS of 30% at 4 years may be acceptable but not good. The global results do not seem to be influenced by toxic deaths, less then 10%, but by the high relapse rate. In fact only 5 children relapsed off therapy, 4 children in 1st CR were affected by poor prognosis disease and the remaining 36 children relapsed on therapy. High dose Vincristine added to TBI and cyclophosphamide did not increase the leukemia free survival nor the toxicity rate.

The better EFS of isolated relapse group (57% versus 24%) indicates the eligibility of these patients for autologous marrow transplantation.

ACKNOWLEDGEMENTS

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FIGURE 1

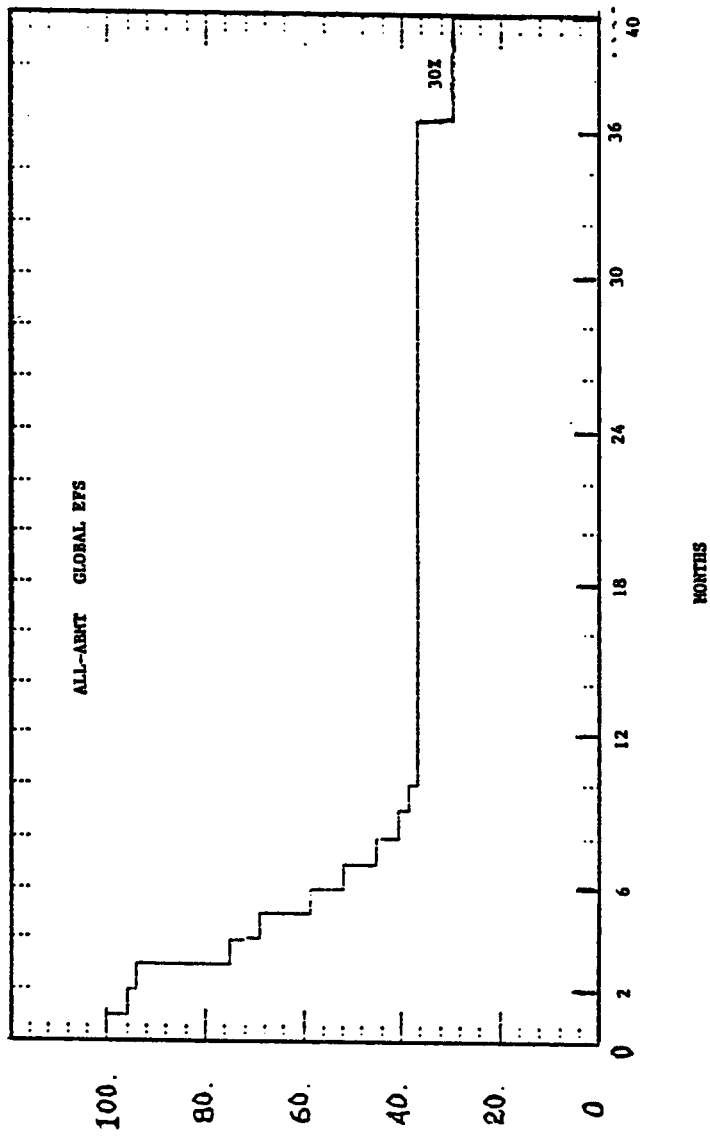

ALL-ABMT GLOBAL EFS
30%
100.
80.
60.
40.
20.
0
0
2
6
12
18
24
30
36
40
MONTHS

FIGURE 2

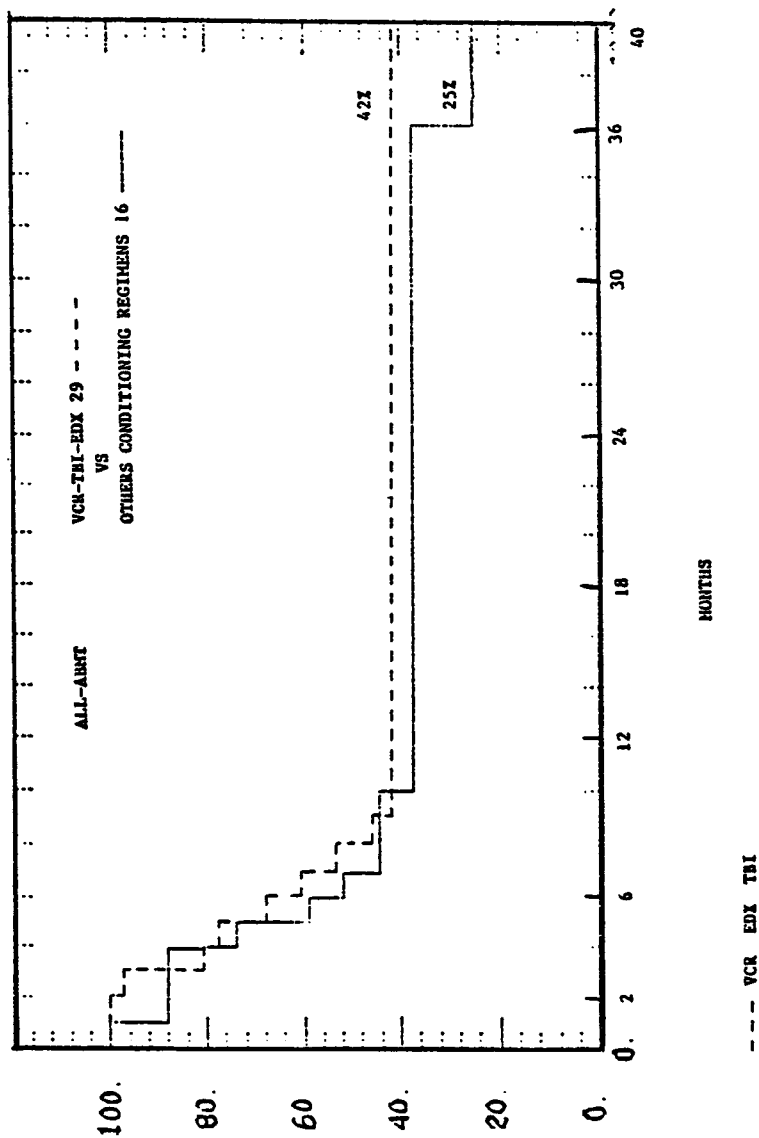
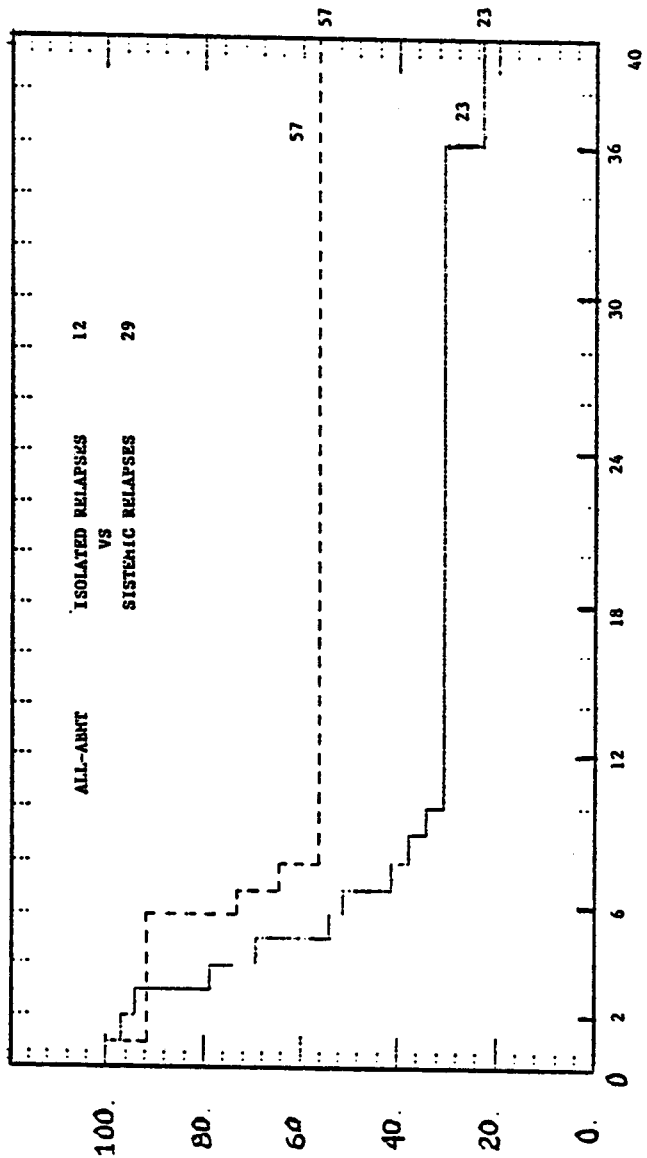

ALL-ABMT
VCR-TBI-EDX 29 - - - -
VS
OTHERS CONDITIONING REGIMENS 16 ——
42%
25%
100.
80.
60.
40.
20.
0.
0.
2
6
12
18
24
30
36
40
MONTHS
- - - VCR EDX TBI

FIGURE 3


ALL-ABMT
ISOLATED RELAPSES 12
VS
SISTEMIC RELAPSES 29
57
23
57
23
100.
80.
60.
40.
20.
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2
6
12
18
24
30
36
40
MONTHS
--- RECIDIVA ISOLATA

AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR ACUTE LYMPHOBLASTIC LEUKEMIA IN SECOND OR SUBSEQUENT COMPLETE REMISSION: TEN YEARS EXPERIENCE AT DANA FARBER CANCER INSTITUTE

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INTRODUCTION

During the past 20 years, significant strides have been made in the treatment of patients with acute lymphoblastic leukemia (ALL). The minority of children and some adults presenting with the disease are cured with combination chemotherapy (1,2). For those patients who relapse after remission induction, however, the prognosis is generally poor, particularly in adults (2-4). Salvage combination chemotherapy regimens can induce second and third remissions but only produce sustained disease-free intervals in a small number of individuals with extended initial remissions (4,5). Allogeneic bone marrowtransplantation (BMT) has achieved promising results for patients in > 2nd complete remission with 25-50% of patients remaining alive and free of disease at 3-4 years (6-10). Results in children have generally been more successful than in adults. Allogeneic transplantation, however, does have its limitations. Only 30-40% of eligible patients will have an HLA compatible sibling donor (11). The use of closely matched related and unrelated donors has expanded the number of potential candidates for allogeneic transplantation to some degree (12). The use of such alternative donors, however, carries with it an increased risk of graft-versus-host disease (GVHD) and graft rejection (13). Moreover, a large number of patients still will remain without available donors.

An alternative approach involves the use of autologous marrow following high dose chemoradiotherapy. Autologous BMT has several theoretical advantages. It allows all patients who can achieve a remission the opportunity to undergo transplantation whether or not an HLA matched donor is available. Also, it virtually eliminates the risks of GVHD and graft failure and reduces the risk of interstitial pneumonitis. Potential disadvantages to autologous BMT include the use of marrow from individuals previously treated with chemotherapy, the probable loss of graft-versus-leukemia activity

associated with allogeneic grafts, and the possible contamination of infused marrow with leukemia cells. A variety of techniques designed to eliminate residual leukemia in harvested marrow have been developed and employed in clinical trials (10,14-16).

In this report, we describe our current experience in 80 patients with ALL in 2nd or subsequent remission who underwent BMT using autologous marrow treated ex vivo with monoclonal antibodies (J2, J5) which recognize the CD9 and CD10 antigens on pre-B ALL cells. We have previously demonstrated the feasibility of this approach and demonstrated that stable hematologic engraftment can be attained with marrow treated in this fashion (14,17). A recently published report on the initial 44 children who underwent transplantation with J2/J5-purged autologous marrow at our institution revealed that approximately 30% of patients were alive, free of disease at 4 years post-BMT (18). In this update, we find that autologous BMT can produce prolonged disease free intervals in greater than one-third of both pediatric and adult patients who have previously failed conventional dose therapy.

PATIENTS AND METHODS

Patients with a history of relapsed ALL who did not have an HLA matched sibling donor were eligible for transplantation. Immunophenotypic analysis of leukemic cells at the time of diagnosis or relapse revealed the presence of the CD9 (gp26) or CD10 (CALLA) antigen on the cell surface in all patients. The combination of the J2 antibody (IgM reacting with CD9) and the J5 antibody (IgG2a reacting with CD10) allows recognition of cells from greater than 90% of patients with pre-B ALL (19). All patients had achieved a complete clinical remission with salvage chemotherapy and had an adequate performance status (ECOG 0-2) at the time of marrow harvest and BMT. Marrow treatment with J2 and J5 antibody plus complement was performed as previously described (18). Marrow was cryopreserved in liquid nitrogen in medium containing 10% dimethylsulfoxide and 90% autologous serum.

Over the past 9 years, a number of adjustments have been made in the ablative regimen. All patients received cyclophosphamide (1800 mg/m^2/day x 2d in children and 60 mg/kg/day x 2d in adults) and total body irradiation (TBI). Cytosine arabinoside was administered prior 10 the cyclophosphamide in all pediatric patients and 9/17 adult patients. VM-26 was used at 150-200mg/m^2 x 2 doses in 33 pediatric and 3 adult patients. TBI (1200-1400 cGy total dose) was given as 175-200 cGy fractions twice daily in the last 67 consecutive patients. The initial 13 patients had been treated with single fraction TBI. Following the last dose of radiation, the antibody-treated marrow was rapidly thawed, diluted in media containing 25 U/ml DNA-ase to prevent clumping, and infused intravenously into the patient. Patients were hospitalized at Children's Hospital, Dana-Farber Cancer Institute, or Brigham and Women's Hospital in single non-laminar air flow rooms observing strict reverse isolation precautions.

RESULTS

Characteristics of the 80 patients in this study are listed in Table 1. There were 63 pediatric and 17 adult patients; 73% of patients were male. The median age of the children was 8.5 years (range, 3-18 yrs) while the median age in adults was 25 years (range 18-55 years). Sixty percent (60%) of patients were in a 2nd complete remission (CR) and 38% in 3rd or greater CR at the time of transplantation Two patients transplanted in 1st remission had been considered induction failures because of the prolonged times required to attain CR. A history of extramedullary leukemic involvement (CNS,testes) was present at some point in 41 % of patients prior to BMT. Of the 80 patients, 63% had at least one remission exceeding 2 years. Forty percent (40%) of the study population had relapsed while receiving chemotherapy. The median interval between achieving a CR after relapse and BMT was 3 months (range 0.5-5 months).

The event-free survival (EFS) of the patients in this study is displayed in Figure 1. At 4 years post-BMT, the EFS of the pediatric population is 36% $\pm$ 7%. In the adults, EFS is 34% $\pm$ 13% at 4 years. One pediatric patient relapsed late, 6 years post- BMT. However, immunophenotypic analysis revealed that the leukemic blasts were of myeloid origin, suggesting that this event represented either a secondary leukemia or a de novo process. A summary of our clinical results is shown in Table 2. Thirty-five of 80 patients remain in continuous complete remission with a median follow-up of 9 months. Twenty-nine patients relapsed, 53-887 days post-BMT. Sixteen patients died while still in clinical remission. Toxic deaths occurred within 3 months of BMT in all cases. Fungal sepsis accounted for 7 deaths, hemorrhage for 3, interstitial pneumonitis for 2, cardiac necrosis for 1, and VOD for 1. The etiologies of 2 deaths could not be determined. The minority of fatal events occurred early in the development of our transplant program. Of the 29 patients transplanted before 1985, 11 (38%) developed fatal complications. In contrast, only 5 of 51 patients (9.8%) treated since January 1985 have suffered treatment related mortality.

We analyzed several pre-transplant characteristics to determine if they exerted any influence on ultimate outcome. Relapse post-BMT did not appear to be related to sex, age, WBC at diagnosis, antibodies used in marrow treatment, remission number, or presence of extramedullary involvement in either the adult or pediatric patients. Confirming our earlier observation, a pre-BMT remission lasting more than 24 months correlated with prolonged survival in transplanted children (Figure 2a). Among the 17 adults in our series, initial remission duration did not appear to have the same strong prognostic significance as in the children (Figure 2b).

Of the patients evaluable for hematologic engraftment, the median number of days to achieve a granulocyte count greater than 0.5 x 10^9/1 was 23 days (range 10-161 days). Platelet counts exceeding 20 x 10^9/1 on successive days without transfusion were achieved a median of 26 days post-BMT (range 12-110 days). No correlation between the number of marrow cells infused and

hematologic engraftment was found. The rate of engraftment in these patients is similar to that observed in recipients of purged autologous transplants for non-Hodgkin's lymphoma and multiple myeloma at our institution (data not shown).

DISCUSSION

Our experience over the past decade suggests that autologous bone marrow transplantation can help to consolidate the remissions of patients with ALL who have previously experienced a clinical relapse. Given that the vast minority of patients with relapsed ALL do not experience prolonged disease-free intervals following standard combination chemotherapy, our 4 year EFS of 36% in children and 34% in adults is encouraging. Moreover, with the introduction of modifications in the ablative regimen and improvements in supportive care during the past 5 years, the procedure has become safer and the toxic death rate has dropped considerably. Transplant related mortality has decreased from 38% to less than 10% since January 1985.

Our overall results are relatively similar to those from several large series on allogeneic bone marrow transplantation for ALL in 2nd CR, in which 25-50% of patients are long term survivors (6-10). A recent study from the University of Minnesota comparing allogeneic and autologous BMT for ALL found a slight advantage for patients undergoing allogeneic transplantation (10). It is important to note that the 3 year EFS for patients undergoing auto-BMT in that series was only 20%. Our data, however, suggest that autologous BMT is a very reasonable alternative for eligible patients with ALL who do not have a suitable sibling donor. Under such circumstances, it would seem preferable to perform an autologous transplant rather than pursue a time consuming and costly search to locate a closely matched unrelated donor, particularly in light of the high incidence of GVHD associated with transplantation of unrelated marrow.

While there has been a minor reduction in the transplant related mortality which we have observed over the past 5 years, relapse post-BMT remains a significant problem. This is particularly evident in children with short initial durations of remission. There are several potential ways to approach this difficult problem. These include alteration of the ablative regimen, administration of additional pre- or post- transplant chemotherapy, or possibly manipulation of the immune system post-BMT to induce "graft-versus-leukemia" activity. We are currently attempting to address these issues at our institution. High risk pediatric patients (those with brief initial remissions) are being treated with an alternative conditioning regimen and schedule. Adults are being entered onto a phase I clinical trial using low doses of recombinant interleukin-2 designed to augment the number of activated natural killer (NK) cells. Preliminary data suggest that non-toxic doses of rIL-2 can successfully expand the NK population in vivo in patients after autologous transplantation. Further work is needed to evaluate these and other methods to reduce the rate of relapse post-BMT.

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TABLE 1

PATIENT CHARACTERISTICS

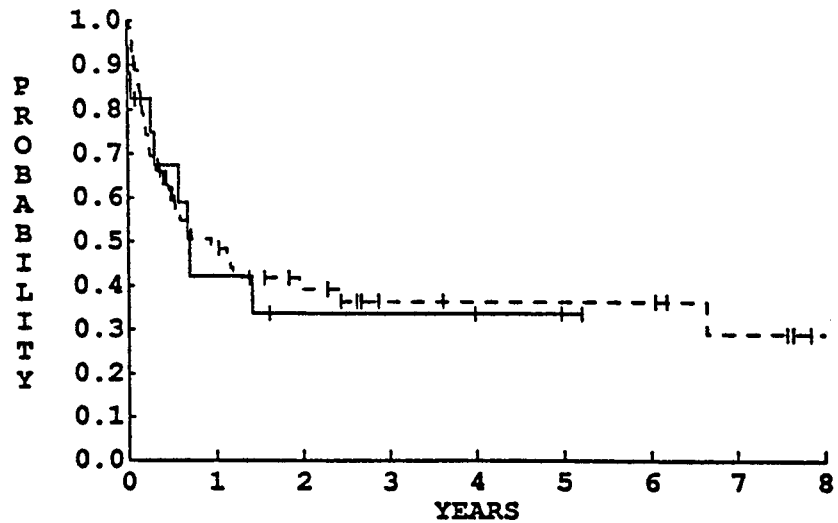
	Pediatric	Adult
Number	63	17
Sex	45M, 18F	13M, 4F
Age at BMT (3-18yr) (18-55yr)	8.5 yr	25 yr
Remission #1		
1	1	1
2	39	9
>3	23	7
Duration of Longest Remission		
<2 yr	20	9
>2 yr	43	7
Extramedullary Involvement		
yes	29	4
no	33	13
Interval from Last CR to BMT Months (median)	3	3

TABLE 2

SUMMARY OF RESULTS

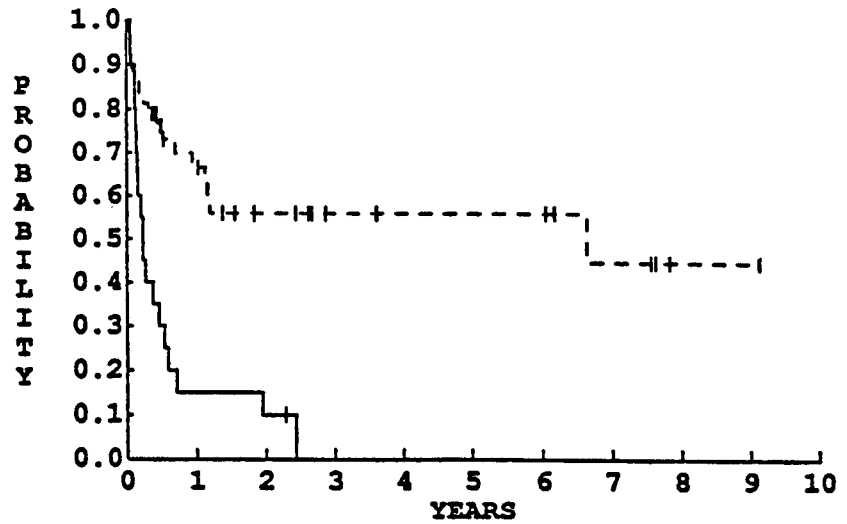
	Pediatric (n=63)	Adult (n=17)
Alive (CCR)	27	8
Relapse	24	5
Died in Remission	12	4

FIGURE 1



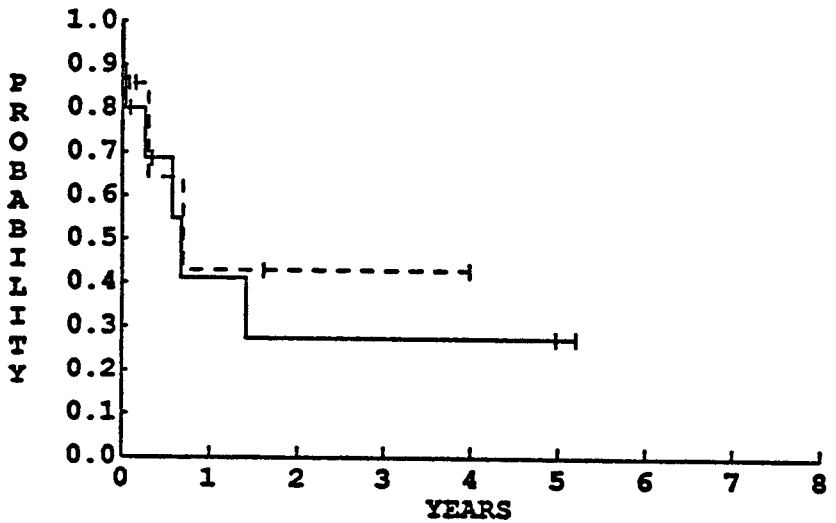
POPULATION	EFS	FAIL	TOTAL	MEDIAN
——— ADULT	8	9	17	0.7
- - - - PEDIATRIC	27	36	63	0.9

FIGURE 2a



LENGTH-MONTHS	EFS	FAIL	TOTAL	MEDIAN
——— < 24	1	19	20	0.2
- - - - > 24	26	17	43	6.6

FIGURE 2b



REMISSION	EFS	FAIL	TOTAL	MEDIAN
—— < 2 YRS	4	6	10	0.7
- - - - ≥ 2 YRS	4	3	7	0.7

BONE MARROW PURGING AND TRANSPLANTATION IN ACUTE LYMPHOBLASTIC LEUKEMIA : BIOLOGICAL AND CLINICAL FEATURES

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INTRODUCTION

Autologous Bone Marrow Transplantation (ABMT) is a promising therapeutical approach for treatment of some of these patients lacking histocompatible donors (1,2). The main problem of such transplants is, in some cases, the high relapse rate due to the persistence of residual neoplastic cells, either in patients or in reinfused bone marrows, resistant to the induction and consolidation regimens.

Theoretically, patient persistent cells can be eradicate using more aggressive eradicative therapies, while bone marrow residual cells can be eliminated by means of different techniques: biological, chemical, physical and immunological (36).

Monoclonal antibodies and Complement (C') cytolytic treatments of bone marrow collected in complete remission permit, in theory, the BM decontamination of neoplastic cells based on the MoAb ability for binding to the specific antigen, activating the classical C' pathway and producing final cell lysis (7-9). From those, the most commonly used for "in vitro" BM cell purging include CD2, CD5 and CD7 (10,11) for "T" malignancy and CD9, CD10, CD19 and CD24 (12-14) for "B" malignancy treatments. The aim of the present work is to describe our experience on marrow purging of 56 patients suffering from ALL using MoAb specific for "T" cells: 18 treatments (CD2,3,4,5,6,8 and 28), and for "B" cells: CD10 (16 treatments), CD20 (3 treatments) and CD10, 19, 20 (17 treatments), and 1 treatment with 7 MnAbs pool (6 anti T MoAbs + anti CALLA). Thirty-two patients transplanted with such treated marrows have been clinically monitored paying special attention to the hematological reconstitution and disease free survival.

MATERIAL AND METHODS

Patients

Fifty-six poor prognosis ALL patients have been selected for marrow purging followed by ABMT. Selection criteria for marrow purging was the

existence of a complete phenotypical study of neoplastic cells. Patients lacking such determinations have constituted a control group. Patient ages, sex, diagnosis and status at marrow harvesting and at ABMT are shown in Table I.

"In Vitro" Bone Marrow Treatment

Bone marrow was obtained in complete remission periods, excepting 3 (two in 1st PR and one in 3rd Rel), by means of standard technique and divided in two parts: one for "in vitro" treatment (> 2.5 x 10E8 cells /Kg) and the other as "back up" marrow (> 1.5 x 10E8 cells/Kg).

Density centrifugation technique was used for mononuclear cell (MNC) separation of treatment part and automatic buffy coat separation with IBM 2991 was employed on "back up" marrows. The low density fraction (MNC) was incubated once with specific MnAb during 30 min at 40C to avoid antigenic modulation. After washing with phosphate buffer activated with 0. 5% v/v human serum albumin 20% (PBA), MNC have been treated twice successively with new born rabbit serum (Peel Freez), as complement source, and DNAsa during 60 min at 37C at 1 x 10E8 cell/ml concentration on every cycle. After treatment both BM fractions were cryopreserved with 10% DMSO using standard techniques.

Monoclonal Antibodies

Mouse MnAb used in this study (table II), gently donated by Prof. P.J. Martin from Fred Hutchinson Cancer Research Center (Seattle, USA), were utilized in purified form at saturating concentrations.

"In Vitro" Monitoring

Bone marrow treatment monitoring has been performed by means of cell counts, CFU-GM and Long Term Bone Marrow Cultures (LTBMC), cell viability and indirect immunofluorescence (using EPICS V flow cytometer) on the following steps: in fresh bone marrow, after fractionation, after MnAb incubation and after every C' incubation.

Hemopoietic Cultures

CFU-GM cultures have been performed according to Pike and Robinson technique (15), using feeder layer as conditioning medium. LTBMC have been performed using a modification of Gartner and Kaplan technique (16).

Cell Viability Studies

Cell viability has been assessed by means of double staining with Fluorescein Dyacetate (FDA) and Propidium Iodide (IP) (Sigma) followed by flow cytometry (FC) analysis (EPICS V, Coulter Cientifica, Spain).

Biparametrics histograms: forward angle light scattering (FALS) - green fluorescence logarithm (LIGFL) / FALS- red fluorescence logarithm (LIRFL) define alive cells (green fluorescence), dead cells (red fluorescence) and

intermediate status by simultaneous measuring of cell size and fluorescence (17).

Undirect Immunofluorescence Studies

Undirect immunofluorescence studies have been performed to assess the positive cell percentage in each treatment step. Cell samples (5 x 10E5 cells) with bounded primary MnAb have been incubated, as second antibody, with 50ul (1:25 v/v) fluoresceinated goat anti-mouse antibody (GAM-FITC)(Becton Dickinson) at 49C over 30 min.

The determination of the amount of positive cell among all the process has been performed by biparametric histograms: FALS/LIGFL.

BM Treatment Yield Assessment

Due to the our current lack of sensitive tests for determining very small concentrations of true neoplastic cells (i.e. $< 1:1000$) we have been monitoring the processes by means of all positive cell quantification, preliminarily assuming that neoplastic cells behavior during cytolytic treatments correlates with normal ones.

Thus, specific, cell depletion yielding has been determined by quantifying positive alive cells in non treated sample and after C' treatments, applying the following formula:

$$\text{Depletion Log} = \text{Log}\left(\frac{\%\ \text{initial (+) cells} \times \%\ \text{pre treatment alive cells}}{\%\ \text{post treatment (+) cells} \times \%\ \text{post treatment alive cells}}\right)$$

Patients Autografted

Thirty-two BM from patients whose features are shown (table I), were treated with anti "T" MnAb pool, six were treated with anti CD10 MnAb (four with 26.2 MnAb and two with 24.1 MnAb), one were treated with B1 MnAb and twelve were treated with anti "B" MnAb pool.

All patients received similar eradicative regimen : 1200 to 1400 fractionated total body irradiation and Cyclophosphamide 120 mg/Kg.

Hematological Recovery Assessment

Hematological parameters used for monitoring hematological recovery after transplant were: time, necessary (days) to reach 1000 leukocytes, 500 granulocytes, 20000 and 50000 platelets per pl and BM cytological studies considering first appearance of erythroid, myeloid and megakaryocytic precursors.

Statistical Methods

To avoid disturbances produced by results without a normal distribution, we have employed non parametric statistical tests: Spearman correlation, Wilcoxon Rank sum tests for two groups and Wilcoxon signed rank sum test. Actuarial survival curves were compared by means of Logrank test (18).

RESULTS

Bone Marrow "In Vitro" Treatment

Overall "in vitro" treatment results of 56 BM are shown in Table III. After some preliminary experiments (data not shown) we defined 0.1% as a safety limit for FC cell positive detection. Pretreatment positive cell percentage before treatment, assessed by undirect immunofluorescence and FC, depends on MnAb utilized, ranging from 3.91 to 45.88% (median 13.59%) on "T" treatments and from 0.30 to 39.79% (median 7.79%) in "B" treatments.

Globally, median depletions ranged from 1.25 to 1.61 Logs, without showing any statistically significant difference between the different treatments employed (Table III).

CFU-GM recovery after "in vitro" treatments has been variable: 60.15 (17.7 to 173.0) on "T" treatments, 110.65% (26.5 to 302.0) on CD10 treatments, 24.20% (20.0 to 51.80) on CD20 treatments and 72.95% (35.6 to 547.7) on "B" pool treatments.

Bone Marrow Infusion and Hematological Reconstitution

Thirty-two patients were transplanted with such treated BM, receiving a median cell dose of 1.32 x 10E7 mononucleated cells/Kg (0.14-6.8). Mean CFU-GM infused was 1.6O x 10E4/Kg (0.115.2).

Seven patients were reinfused with back up marrows because of hematological recovery lack 21 days after ABMT. Median cell (buffy coat) and CFU-GM dose administered in these second infusions respectively were 3.33 x 10E7 /Kg (0.59-13.60) and 0.79 x 10E4 /Kg (0.07-4.1). Two patients died early at days +26 and +33 post ABMT showing some signs of hematopoietic reconstitution.

Median time to reach 1000 leukocytes, 500 granulocytes, 20000 and 50000 platelets/ul respectively was 36 (12-91), 38 (12-94), 57 (20-114) and 46 (20-292) days, which strongly contrast ($P<0.05$) with a control group autografted with non treated marrows whose median time to reach 500 granulocytes, 20000 and 50000 platelets/ul respectively was 18 (10-37), 28 (12-48) and 41 (18-75) days (Table IV).

Cell dose and number of CFU-GM infused doesn't correlate nor define any special criteria for hematological recovery prognosis. Only LTBMC shows some predictive power on hematological (19).

Clinical Outcome

Nineteen patients remain alive and well in CR from day +46 to +660 (median +265) after transplant. Ten patients relapsed between days 50 and 365 (median 91). Three patients died from transplant complications, pneumonitis (Table IV).

Actuarial disease free survival is better in patients transplanted with treated marrows, compared to the above mentioned control group, however, it is not statistically significant (see fig 1).

DISCUSSION

Bone marrow purging with MnAb and C' is a widely employed technique in the ABMT setting when BM is impaired. Since today many teams have been performing such procedures, following the logical approach of reaching the maximal neoplastic cell reduction in autologous grafts.

Our team has been performing ABMTs in ALL during the last four years using "ex vivo" treated BM following the above mentioned techniques. According to the assumption of the existence of some correlation degree between normal and neoplastic cell depletion we have analyzed the overall purging behavior as well as the eventual toxicity over normal hemopoietic precursors.

In our hands, the 1.44 logs specific cell depletion median, can be considered as quite low in comparison to the results previously reported in preclinical models (14). This fact can be explained by the high cellular heterogeneity, by an anticomplementary effect in human bone marrow or/and by the existence of low antigenic density cell populations.

In our cases, the observation of the low MnAb labeling of the positive, after treatment, residual cells (data not shown) support this last theory. In agreement with other authors we have observed better depletion results after two complement courses than after a single one (20) and a moderate toxicity over hemopoietic committed precursors. Paying attention to the clinical patients' behavior, in our series we observed a peripheral cell counts recovery delay (500 granulocytes and 20000 platelets/ul recovery) which does not differ from that reported by other authors.

According to data already published, we couldn't find any predictive value of the cell number nor CFU-GM infused. Contrarily, our preliminary LTMC data suggest some correlation between its results and hematological reconstitution. In our patients we are not able to statistically demonstrate any positive clinical effect of marrow purging, which is a common finding in other series. Nevertheless, our data showing a better but not statistically significant DFS in patients grafted with purged marrow and in patients receiving marrows more effectively purged (more than 1.5 logs.) suggest some positive effect of this procedure. Probably, the high relapse rate of ALL after ABMT is the consequence of two different factors: the ineffectiveness of eradicative therapies delivered as conditioning regimen and the persisting neoplastic cells in the graft.

Solutions to overcome this two main problems should consider the development of new eradicative regimens and more effective purging procedures.

ACKNOWLEDGMENTS

Work supported by grant of the Comite Conjunto Hispano-Norteamericano para la Cooperacion Cientifica y Tecnologica CCA8510/019 and by the Comision Asesora de Investigacion Cientifica C.A.I.C.Y.T : PA 85/0244. Authors' affiliations: H. Clinic i H. Infantil RSSS Vall d'Hebro H. de Ntra. Sra. de la Esperanza, Barcelona, Spain.

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TABLE 1

Description of the patients studied

* PURGED	HARVEST	ABMT
Number	56	32
Age median (range)	16.5 (4-48)	16 (4-40)
Sex		
Male	35	18
Female	21	14
Diagnosis		
T-ALL	19	13
B-ALL	36	18
null-ALL	1	1
Disease status		
1st CR	29	10
1st PR	2	2
2nd CR	20	16
3rd CR	4	3
3rd Rel	1	-
4th CR	-	1
* UNPURGED		
Number		14
Age median (range)		12.5 (3-22)
Sex		
Male		9
Female		5
Diagnosis		
ALL		14
Disease status		
1st CR	4	2
2nd CR	6	6
3rd CR	2	4
4th CR	-	1
Rel	-	1

TABLE 2
Characteristics of the Monoclonal Antibodies Used in This Study

CELLULAR SPECIFICITY	NAME	IG CLASS	ANTIGEN DESIGNATION	MW AG (KD)
Pool anti T	35.1	IgG2a	CD2	50
	10.2	IgG2a	CD5	67
	38.1	IgM	CD3	19
	64.1	IgG2a	CD3	19
	12.1	IgG2a	CD6	100
	9.3	IgG2a	CD28	44
	66.1	IgM	CD4	55
	51.1	IgG2a	CD8	32
Anti CALLa	24.1	IgG3a	CD10	100
	26.2	IgM	CD10	100
Anti B1	IF5	IgG2a	CD20	35
Pool anti B	J149	IgM	CD10	100
	4-35	IgM	CD19	95
	IF5	IgG2a	CD20	35

TABLE 3
Summary of the Results of the "In Vitro" Treatments: Specific Cell Depletion and CFU-GM Cultures

	% cells (+) depletion		Log.	% CFU - GM recovery
	1st C'	2nd C'		
T pool (N=18)	94.63 (47.9 - 99.5)	96.50 (68.3 - 99.6)	1.45 (0.49 - 2.45)	60.15 (17.7 - 173.0)
T pool + CD 10	94.38	95.95	1.39	N.D
CD 10 (N=16)	93.85 (11.3 - 99.7)	97.59 (42.2 - 99.9)	1.61 (0.23 - 3.37)	110.65 (26.5 - 302.0)
CD 20 (N=3)	88.88 (80.3 - 99.2)	93.50 (94.5 - 99.7)	1.25 (1.18 - 2.57)	24.20 (20.0 - 51.8)
B pool (N 17)	94.96 (78.6 - 95.9)	96.97 (87.9 - 96.9)	1.55 (0.91 - 2.24)	72.95 (35.6 - 547.7)
TOTAL (N=56)	94.22 (11.3 - 99.7)	96.41 (42.2 - 99.9)	1.44 (0.23 - 3.37)	70.10 (17.7 - 547.7)

TABLE 4
Summary of the Results of Hematological Recovery and Disease Outcome of Patients Autografted

	Leukocytes >1000	Granulocytes >500	Platelets >20000	Platelets >50000
Purged N=32	36 (12 - 91) N=29	38 (12 - 94) N=29	57 (20 - 114) N=25	46 (20 - 292) N=18
Unpurged N=14		18 (10 - 37) N=13	28 (12 - 48) N=13	41 (18 - 75) N=13

	Relapse (days)	D.F.S (days)	Died (days)
ALL-T N=13	53 (50 - 63) N=3	+503 (75 - 660) N=8	86 (33 - 138) N=2
ALL-B N=18	165 (58 - 365) N=7	+123 (46 - 396) N=10	26 N=1
null-ALL N=1	-	+210 N=1	-
Total purged N=32	91 (50 - 365) N=10	+265 (46 - 660) N=19	33 (26 - 133) N=3
Total unpurged N=14	150 (60 - 720) N=10	+360 (180 - 983) N=3	45 N=1

FIGURE 1
Disease-free survival of acute lymphoblastic leukemia patients: influence of marrow purging.

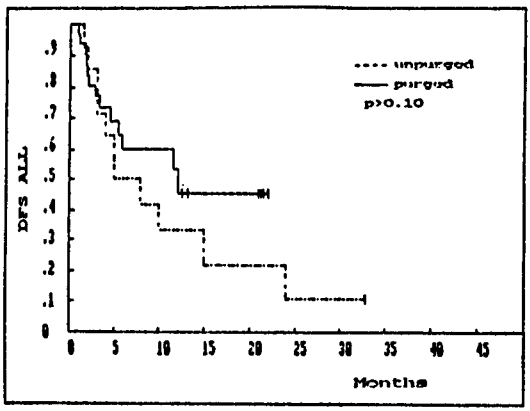


FIGURE 2
Disease free survival of patients with acute lymphoblastic leukemia who received purged autografts: anti "T" vs. anti "B" treatments.

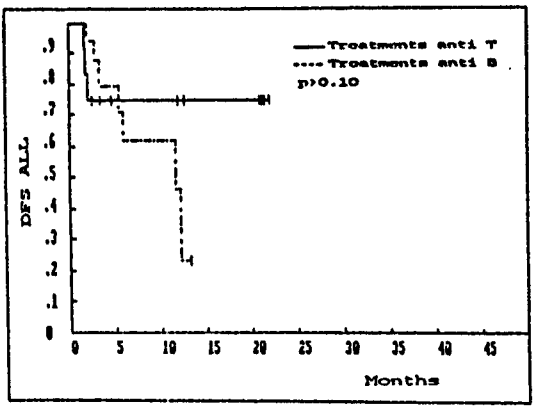
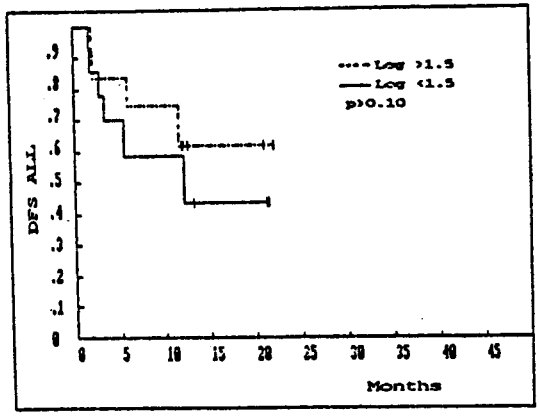


FIGURE 3
Disease-free survival of patients grafted with purged marrow: influence of the effectiveness in the purging.



REGIMEN-RELATED TOXICITY AFTER MARROW TRANSPLANTATION FOR ACUTE LEUKEMIA

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INTRODUCTION

Bone marrow transplantation is the treatment of choice for many hematologic malignancies. The morbidity and mortality of marrow transplantation may be considerable, as a consequence of the high-dose chemotherapy or chemoradiotherapy used to prepare patients for transplant. Toxicities which are attributed to the preparative therapy are referred to as regimen-related toxicity (RRT). Assessment of regimen-related toxicity is useful in comparing large disparate groups of patients receiving the same or different preparative regimens (1), as well as in determining maximum tolerated doses for phase I studies (2). This report will discuss the grading criteria for RRT, factors associated with severe RRT, and some potential modalities to prevent or ameliorate severe RRT.

PATIENTS AND METHODS

Toxicity Grading

RRT was graded using an empirically designed system to assess morbidity in the heart, bladder, kidneys, lungs, liver, central nervous system, oral mucosa, and gastrointestinal tract (1, 3) due to the marrow transplant preparative regimen (Table 1). Data attesting to RRT were obtained documenting all transplant related toxicities on a daily basis. RRT was graded on the day of transplant (day 0) and on days 7, 14, and 28 post-transplant. Because lung RRT may develop after day 28 post-transplant, the lungs were graded again at day 100. Patients who died after day 28 were graded with the highest RRT score achieved by day 28. Data attesting to RRT were obtained from chart reviews documenting all toxicities on a daily basis. Toxicity which could be attributed to infection, bleeding, medications, or graft versus host disease were excluded from RRT. Whenever possible, biopsy or autopsy data were used to corroborate RRT. Each patient's maximum RRT score was the

highest score achieved in any single organ. All chart reviews were done by a single reviewer.

Patient Selection

Between July 1, 1988 and June 30, 1989, 348 consecutive patients underwent a first marrow transplantation for malignancy at the Fred Hutchinson Cancer Research Center or Swedish Hospital Medical Center. Of these, 155 patients were transplanted for acute leukemia and are included in this analysis. Eighty-three patients were male and 72 were female. The median age for all patients was 24 (range 0.7 to 54) years. Patient characteristics are shown in Table 2. Preparative regimens for transplant included cyclophosphamide (CY, 120 mg/kg) plus fractionated or hyperfractionated total body irradiation (TBI, 12-15.75 Gy); Busulfan (Bu, 14-16 mg/kg) plus CY (120 mg/kg); Bu (6.7 mg/kg) plus CY (49 mg/kg) plus TBI (12 Gy); VP16 (52 mg/kg) plus CY (103 mg/kg) plus TBI (12 Gy); Carmustine (BCNU, 300-600 Mg/M^2) plus VPI6 (2400 Mg/M^2) plus CY (7.2 g/M^2) ; or hyperfractionated TBI (13.2-14.4 Gy) plus CY (120 mg/kg). With the exception of autologous or syngeneic marrow recipients, all patients received one of several immunosuppressive regimens postgrafting as prophylaxis for acute graft versus host disease (GVHD).

Statistical Methods

Kaplan-Meier probability estimates were used to display survival, with logrank statistics used to test for differences in survival. The two-sided Fisher's exact test or the chi-square test was used to determine the difference in proportions of grade 3 or 4 RRT between discrete groups. Multivariate analysis for development of grade 3 or 4 RRT was performed using the stepwise logistic regression procedure.

RESULTS

Regimen-Related Toxicity

Twenty-seven patients (17%) developed grade III or IV RRT in one or more organs (Table 3). The incidence of grade 3 or 4 RRT was not influenced by diagnosis or type of transplant. Grade 3 or 4 RRT occurred less commonly in younger patients and in patients early in their disease process (first remission or first untreated relapse or as primary therapy for acute leukemia evolving from myelodysplastic syndrome, 8/70, 11%) compared with patients transplanted beyond untreated first untreated relapse (19/85, 22%), $p=0.090$. Grade 3 or 4 RRT was less frequent in patients who were prepared for transplant with cyclophosphamide and 12 Gy of fractionated TBI than for patients prepared with most other regimens but the numbers are small in each regimen category. Organs most severely affected were the lungs, liver, kidneys, and CNS (Table 4). The distribution of severe RRT among each of the organs graded was not influenced by diagnosis or type of transplant. A multivariate analysis was performed using the variables age (< 18 vs ≥ 18), preparative regimen (Cy/12 Gy TBI vs others), GVHD prophylaxis

(cyclosporine + methotrexate vs others), type of transplant, diagnosis, and disease status to predict development of grade 3 or 4 RRT. Both age $\geq$ 18 and poor risk status (transplantation in second remission or beyond) were independent significant predictors for the development of grade 3 or 4 RRT. The relative risks were 2.77 (95% confidence intervals 1.01, 7.55) for age > 18 and 2.70 (95% confidence intervals 1.07, 6.80) for poor risk disease status.

Survival

The Kaplan-Meier probability estimate for survival at 100 days post-transplant was 63% for all patients. The probability of surviving 100 days post-transplant was not influenced by diagnosis or by the type of transplant (data not shown). Patients who developed grade 3 RRT had a significantly greater probability of dying prior to day 100 posttransplant than did patients whose maximum RRT was grade 1 or 2 (Figure 1), despite the fact that the majority of patients who developed grade 3 RRT did not die of RRT (data not shown).

DISCUSSION

Almost one-fifth of patients undergoing marrow transplantation for acute leukemia will develop life-threatening (grade 3) or fatal (grade 4) regimen-related toxicity. Most patients developing grade 3 RRT will die prior to post-transplant day 100.

Older patients and those with more advanced disease appear to be at greatest risk. This is in keeping with prior studies illustrating that patients with advanced disease tolerate the rigors of transplantation less well than patients with less advanced disease (1,3).

Recipients of autologous marrow developed just as much grade 3 or 4 RRT as recipients of allogeneic marrow. We previously reported that autologous marrow recipients may be protected from severe RRT (1). In that study, less than 25% of patients who received autologous marrow were transplanted in relapse, suggesting that the apparent protective effect may have been due to the good risk status of those patients rather than the type of transplant. More recent reports also show that autologous transplantation is not free of life-threatening or fatal RRT (1, 4, 5).

Prevention of severe regimen-related toxicity after transplantation has several potential benefits. Superior early transplant survival would probably result if severe RRT could be prevented. Though the majority of patients who develop grade 3 RRT died of causes other than RRT, this study and previous studies show that grade 3 RRT appears to predispose patients to develop additional life-threatening transplant-related complications.

Prevention of severe RRT may permit delivery of potentially higher doses of chemoradiotherapy and, in theory, result in lower relapse rates and improved disease-free survival. Clift et al. recently reported lower relapse rates for patients with AML in first complete remission (6) and for patients with CML in first chronic phase (7) who were randomized to receive cyclophosphamide plus 15.75 Gy TBI versus cyclophosphamide plus 12 Gy TBI. However,

preparation with 15.75 Gy TBI did not result in improved survival because mortality from causes other than relapse was increased. Clift et al. also showed that patients transplanted for AML in first remission with the higher dose of TBI were unable to receive full doses of immunosuppression post-transplant and developed more acute GVHD (4). It is possible, though unproven, that amelioration of severe RRT may permit tolerance of the prescribed postgrafting immunosuppressive therapy, thereby resulting in fewer deaths from acute graft versus host disease.

One potential agent to minimize regimen-related toxicity is prostaglandin E_1, a vasodilatory prostaglandin with antiplatelet and fibrinolytic activity. Gluckman and colleagues treated 50 patients undergoing marrow transplantation for leukemia with PGE_1 as prophylaxis against veno-occlusive disease of the liver (8). They found that fewer patients treated with PGE_1 developed VOD when compared with patients who did not receive PGE_1. They also showed that patients with a prior history of hepatitis, a documented risk factor for VOD, developed less VOD if treated with PGE_1 versus no PGE_1.

A second agent which may have activity in preventing regimenrelated toxicity is Pentoxyfylline (PTX), a hemorrheologic agent approved for the treatment of claudication. PTX has been shown to modulate inflammation by its effect on deformability of neutrophils and red blood cells (9, 10), thereby improving blood flow. PTX also promotes chemotaxis and reduces superoxide production. It also affects production by the vascular endothelium of prostaglandins I2 and E2 (11) which enhance local-regional blood flow and thrombolysis (12). PTX has also been shown to modulate the inflammatory monokine tumor necrosis factor alpha (TNFa). Plasma levels of TNFa correlate with development and severity of GVHD, VOD, and non-infectious pneumonia after marrow transplant (13). In animal models of sepsis, ARDS, and radiation-induced lung injury, PTX significantly reduced levels of TNFa and improved survival of treated animals when compared to untreated controls (14, 15). Both PTX and prostaglandin E_1 are under investigation at our institution.

One final agent of note is tissue plasminogen activator (tPA). tPA is a potent thrombolytic agent used for the treatment of coronary thromboses and peripheral venous and arterial thromboses. Because the pathogenesis of VOD is, in part, due to deposition of clotting factors in the subendothelial zone of affected hepatic venules leading to congestion and ischemia from reduced sinusoidal blood flow, thrombolytic agents may have potential efficacy in reversing the manifestations of VOD. Baglin et al. recently reported on the use of tPA in a patient with VOD after autologous marrow transplantation for multiple myeloma (16). Administration of tPA resulted in prompt resolution of encephalopathy and ascites with improvement in liver function. Severe VOD after marrow transplantation has a mortality rate in excess of 80% (17). Therefore, intervention in the setting of severe VOD must be considered, even when the potential toxicity of that intervention (eg. thrombolytic agents) is substantial.

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TABLE 1

Criteria for Regimen-Related Toxicity (RRT)

(Grade 4 is fatal; toxicities caused by infection, hemorrhage, GVHD; non-transplant mediations are excluded.)

	Grade 1	Grade 2	Grade 3
Cardiac RRT	ECG changes not requiring treatment; cardiomegaly with or without symptoms	ECG changes requiring therapy continuous monitoring; CHF responding to digoxin or diuretics	Severe ECG changes minimally or not responsive to therapy; voltage decrease of $\geq$ 50%; cardiogenic shock
Bladder RRT	Painless macroscopic hematuria lasting 2 days	Painless macroscopic hematuria lasting 7 days ; painful hematuria	Frank hemorrhagic cystitis requiring local invasive therapy
Kidney RRT	Creatinine < 2 x baseline	Creatinine $\geq$ 2 x baseline	Renal failure requiring dialysis
Lung RRT	Dyspnea without CXR changes or isolated infiltrate/interstitial changes not caused by congestive heart failure (CHF)	Extensive infiltrate or moderate interstitial changes with dyspnea; decrease in pO_2 > 10% from baseline not requiring >50% O_2 by mask or intubation	Idiopathic pneumonia requiring more than 50% O_2 by mask or mechanical ventilation
Liver RRT	2.0 $\leq$ bilirubin < 6.0; weight gain 2.5 -5% from baseline; SGOT 2-5 x baseline	6.0 $\leq$ bilirubin < 20.0; SGOT $\geq$ 5 x baseline; ascites; weight gain > 5% from baseline	Bilirubin $\geq$ 20.0; ascites compromising respiration; hepatic encephalopathy
CNS RRT	Somnolent but arousable	Somnolent but confused when aroused; other signs/symptoms	Seizure or coma
Mucosal RRT	Mild pain or ulceration	Mucositis requiring IV narcotic analgesia	Mucositis requiring prophylactic intubation or causing aspiration
GI Tract RRT	Watery stools 0.5-2.0 L /day	Water stools >2 L/day; partial ileus; bleeding without hypotension	Ileus requiring suction; hemorrhagic enterocolitis causing hypotension

TABLE 2

Patient Characteristics

	AML		ALL	
	No.	%	No.	%
Number of patients	84		71	
Median age, in years (range)	31	(2-54)	18	(.7-52)
Disease status				
First remission	24[a]	29	7	10
First untreated relapse	28	33	10	14
Second remission	10	12	7	10
Other	22	26	47	66
Source of marrow				
Autologous	14	17	13[b]	18
Family donor	60	71	47	66
Unrelated volunteer donor	10	12	11	15
Preparative Regimen				
Cy/12 Gy TBI	12	14	0	
Cy/13.2 - 14.4 Gy TBI	21	25	39	55
Cy/15.75 Gy TBI	20	24	8	11
13.2-14.4 Gy TBI/Cy	0		14	19
Bu/Cy	14	17	0	
Bu/Cy/TBI	15	18	0	
BCNU/Cy/VP16	0		6	8
VP16/Cy/TBI	2	2	4	6
GVHD Prophylaxis				
Cyclosporine	7	8	17	24
Methotrexate	11	13	1	1
Cyclosporine + Methotrexate	49	58	37	52
Other	3	4	3	4
None	14	17	13	18

[a] includes 2 patients transplanted as initial therapy for AML after myelodysplastic syndrome; [b] includes 3 patients who received syngeneic transplants.

TABLE 3

Incidence of Grade 3 or 4 RRT

Overall incidence		**27/155**	**17%**	
Diagnosis	**AML**	**15/84**	**18%**	**p=1.000**
	ALL	**12/71**	**16%**	
Type of Transplant				
	Autologous	**4/27**	**15%**	
	Family donor	**18/107**	**17%**	**p=0.707**
	Unrelated donor	**5/21**	**24%**	
Patient Age	**< 18**	**6/56**	**11%**	**p=0.124**
	≥ 18	**21/99**	**22%**	
Preparative Regimen				
	Cy/12 Gy TBI	**0/12**	**0%**	
	Cy/13.2-14.4 Gy TBI	**12/60**	**20%**	
	Cy/15.75 Gy TBI	**5/28**	**18%**	
	13.2-14.4 Gy TBI/Cy	**3/14**	**21%**	
	Bu/Cy	**1/14**	**7%**	
	Bu/Cy/TBI	**4/15**	**27%**	
	BCNU/Cy/VP16	**2/6**	**33%**	
	VP16/Cy/TBI	**0/6**	**0%**	
GVHD Prophylaxis				
	Cyclosporine + MTX	**18/85**	**21%**	**p=0.228**
	Others	**5/43**	**12%**	

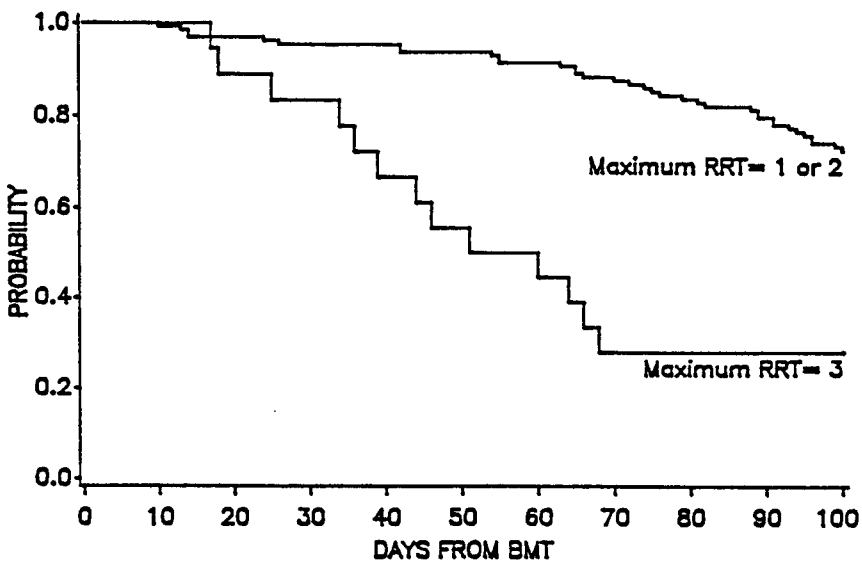
TABLE 4

Grade 3 or 4 RRT According to Organ System

Organ	AML No.	AML %	ALL No.	ALL %	p
Heart	**0**		**0**		
Bladder	**0**		**0**		
Kidneys	**5**	**6**	**4**	**6**	**1.000**
Lungs	**2**	**2**	**6**	**8**	**0.143**
Liver	**6**	**7**	**5**	**7**	**1.000**
CNS	**1**	**1**	**3**	**4**	**0.333**
Oral mucosa	**3**	**4**	**1**	**1**	**0.625**
GI Tract	**0**		**0**		

FIGURE 1

Survival at day 100 post-transplant for patients whose maximum RRT in any organ was 1 or 2 versus patients whose maximum RRT was grade 3 (p=0.0001).



BUSULFAN AND CYCLOPHOSPHAMIDE AS CONDITIONING REGIMEN FOR AUTOLOGOUS BONE MARROW TRANSPLANT IN ACUTE LYMPHOBLASTIC LEUKEMIA

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INTRODUCTION

Allogeneic bone marrow transplantation (BMT) for acute lymphoblastic leukemia (ALL) in complete remission (CR) yields 5 year disease-free survival approaching 30% (1). Unfortunately, less than a third of patients have a suitable donor. Autologous bone marrow transplantation (ABMT) has therefore become increasingly used.

Although the majority of patients with ALL ultimately relapse after ABMT, long-term survival is similar to the outcome after BMT (2). The high relapse rate associated with ABMT for ALL is due to the persistence of leukemic cells in vivo and perhaps to their presence in the autograft although the relative contribution of the two sources to relapse remains to be established.

As an approach to reducing relapse, we and others (3,4) have explored conditioning regimens other than the cyclophosphamide-total body irradiation (CY-TBI) protocol developed by the Seattle Group (5).

An attractive alternative is busulfan and cyclophosphamide (BU-CY) (6) which appears to be effective in acute myeloid leukemia (AML), as shown by Yeager et al (7). Results with autografting AML patients in second and third remission using this regimen are comparable to those obtained with allogeneic transplantation (5,6). We found that in contrast to CY-TBI, BU-CY was capable of eradicating thalassemic marrow (8). Moreover, it was successfully employed in a second transplant for chronic myeloid leukemia in blast crisis in a patient who relapsed after an initial transplant in which CY-TBI was the conditioning regimen (9).

Here, we report our results obtained in 30 consecutive ALL patients autografted in CR after BU-CY conditioning regimen. The results are presented in Tables I, II and III. The patients autografted in CR I (Table I) were all adults except for two children who underwent ABMT because they both met the criteria of high risk ALL. Of the 5 patients autografted in CR III, 3 survive disease-free at 17+, 7+ and 3+ months respectively after ABMT.

Sixteen ALL patients were autografted in CR II: 9 died of recurrent leukemia between 9 and 2 months after ABMT and 1 of ARDS, while 6 are disease- free from 60+ to 2+ months after ABMT. Of the 9 patients autografted in CR I, 1 underwent allogeneic BMT at 64 months after ABMT for an ANLL recurrence and died of acute GvHD 2 months later, 5 died of recurrent leukemia and 3 survive disease-free at 33+, 14+ and 8+ months respectively after ABMT.

As shown in Table IV, recovery to 0.5 x 10^9/L neutrophils was similar to results reported by others (mean 20.8 $\pm$ 5.7), while for 3 patients it took up to 52, 71 and 210 days (mean 43.3 $\pm$ 14.7) for the platelets to reach 50 x 10^9/L.

The BU-CY regimen was in general well tolerated with acceptable toxicity similar to previously published reports on the use of BU-CY for transplant in acute non-lymphoblastic leukemia, chronic granulocytic leukemia and thalassemia (7-9).

In most of the patients the main side effect was mucositis. Two patients had serious hemorrhagic cystitis despite the use of MESNA.

Because of the small number of patients treated with the BU-CY regimen and the limited follow-up, we cannot draw definite conclusions. However, the results suggest that the busulfan-cyclophosphamide regimen should be investigated further in ABMT of lymphoid malignancies.

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TABLE 1

BU-CY IN ALL CR I

Pt	Age/Sex	Interval CR/ ABMT (mos)	PURGING	DFS	SURVIVAL	OUTCOME
1	3/M	2	-	+14	+14	A.W.
2	10/F	8	AZ 100	60	66	ANLL-BMT
3	22/M	6	-	+33	+33	A.W.
4	20/M	7	AZ 80	12	13	LEUK.
5	19/F	16	AZ 100	5	8	LEUK.
6	8/M	7	AZ 80	12	24	REL.
7	23/M	9	AZ 80	8	14	LEUK.
8	18/M	6	-	5	+17	REL.
9	20/F	3	AZ 110	+8	+8	A.W.

TABLE 2

BU-CY IN ALL CR II

PT	AGE/SEX	INTER.CR/ ABMT (mos)	PURGING	DFS	SURVIVAL	OUTCOM
1	6/M	4	100	+60	+60	A.W.
2	22/M	5	100	15 d	15 d	ARDS
3	26/M	2	120	6	7	REL.
4	12/F	1	-	3	9	REL.
5	23/M	5	-	+49	+49	A.W.
6	11/M	1	120	+46	+46	A.W.
7	6/M	3	-	16*	+27	C.R.
8	12/M	1	-	2	3	LEUK.
9	8/M	1	120	+25	+25	A.W.
10	12/F	1	120	5	8	LEUK.
11	10/F	2	120	4	6	LEUK.
12	5/M	2	-	4	8	LEUK.
13	54/F	3	120	4	5	LEUK.
14	9/M	3	120	2	2	LEUK.
15	13/M	4	120	2	2	LEUK.
16	11/F	6	120	+2	+2	A.W.

TABLE 3

BU-CY IN ALL CR III

Pt	Age/sex	Interval CR/ ABMT (mos)	DFS	SURVIVAL	OUTCOME
1	7/M▪	3	4	4	LEUKEMIA
2	19/M	2	7	7	RELAPSE
3	13/M	3	•17	•17	A.W.
4	13/F	1	• 7	• 7	A.W.
5	11/M	1	• 3	• 3	A.W.

PURGING ▪ MAFOSFAMIDE 120 • • 80

TABLE 4

BU-CY IN ALL

	DAYS TO REACH	
	PMN › 0.5x10 /l	PLT › 50x10 /l
Mean SD	20.8 5.7	43.3 14.7
range	12 - 35	22 - 210

IDARUBICIN PLUS HIGH OR INTERMEDIATE DOSES OF ARA-C FOLLOWED BY BONE MARROW TRANSPLANTATION IN ADVANCED ALL

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INTRODUCTION

The prognosis of patients with acute lymphoblastic leukemia (ALL) has been improved during the last decade, with a probability of cure in more than 50% in children (1) and 30% in adults (2). An important, still unsolved, problem is what should be done with patients whose initial therapy failed or in whom bone marrow relapse occurred during therapy or shortly thereafter.

In recent years, new drugs and schedules have been employed in many centers in order to increase the complete remission (CR) and disease-free-survival (DFS) rates in these patients. In accordance with current efforts, two consecutive pilot studies were carried out for advanced ALL patients utilizing high or intermediate cytarabine (Ara-C) doses and a new daunorubicin analogue, idarubicin (IDA), as induction treatment, followed by megadose chemo/radiotherapy plus bone marrow transplantation (BMT) as intensive post-remission phase. Reinfusion of hematological progenitor cells, which circumvents the limitation of doses, allows the administration of potentially marrow-lethal doses of cytotoxic therapy (3). For refractory leukemic patients with matched sibling donors, allogeneic BMT offers improved survival (4) when compared with conventional chemotherapy. As only 15% to 25% of patients have a matched donor, autologous bone marrow cells can be utilized as an interesting alternative approach (5), if the marrow in likely to be free of clonogenic tumor cells and can be collected and stored without loss of viability of hematological stem cells.

The first trial LAL-R85 was initiated in August 1985 and closed in April 1989. The second trial LAL-R87 started in March 1987 and is still in progress.

The first induction schedule, LAL-R85 (Table 1), consisted of Ara-C given at a dose of 3 g/m^2 every 12 hours by a 3-hour infusion for a total of 6 doses and IDA, administered at a dosage of 12 mg/m^2/day as a 30-minute infusion at 0, 24 and 48 hours. In the second approach, LAL-R87, Ara-C was reduced to 1 g/m^2 given as continuous infusion for 6 hours daily for 6 days and

IDA to 5 mg/m^2/day for 6 doses as a 30-minute infusion administered 3 hours after the end of Ara-C (6).

The LAL-R87, envisaged for patients in CR, is a consolidation therapy consisting of two weekly doses of Vincristine + Prednisone followed by two L-VAMP (Vincristine, Ara-C, Methotrexate, Prednisone) cycles (Table 2).

Non-B ALL patients in first or subsequent bone marrow (BM) relapse with or without simultaneous extra-hematological involvement and patients refractory to conventional induction regimens were eligible for both studies.

Selective criteria for the LAL-R85 trial included: a) age < 50 years; b) first relapse occurring during post-remission treatment or within 1 year after therapy discontinuation. Criteria for the LAL-R87 trial included: a) age < 55 years; b) remission duration < 24 months for patients in first hematological relapse. Adequate liver, cardiac and pulmonary functions were also required.

Ninety-one (91) ALL patients (31 children and 60 adults) were enrolled in the LAL-R85 trial. CR was achieved in 21/31 children (68%) and 31/60 adults (52%) with an overall CR rate of 57%. Twelve patients relapsed early, 2 patients died in CR. Sixteen patients (4 children and 12 adults) were considered not suited for bone marrow transplantation and 22 (42%) (11 children and 11 adults) underwent BMT: 17 were autografted and 5 patients who had HLA identical siblings received allogeneic BMT. The projected actuarial disease-free-survival (DFS) for all responding patients is 15% at 55 months.

As of June 1990, 78 patients (43 children and 35 adults) were enrolled in the LAL-R87 trial. CR was achieved in 35/43 children (81%) and 19/35 adults (54%) with an overall CR rate of 69%. Ten patients relapsed early, one child died in CR. Ten patients (4 children and 6 adults) were considered not suitable for BMT, 3 patients were too early, and 30 patients (55%) (24 children and 6 adults) underwent BMT: 18 were autografted and 12 received allogeneic BMT. The projected actuarial DFS for all responding patients is 23% at 34 months. In Table 3 are summarized the characteristics of the LAL-R85/LAL-R87 transplanted patients. Thirty-five patients were autografted, median age was 13 years (range 2-55), 28 were male, transplant was performed after a median of two months (range 1-5) from CR. Conditioning employed regimens were the BMVC (BCNU, Mitoxantrone, VP-16, Ara-C)(7), the Busulphan (BU)+ Cytoxan (CY)(8) and the CY+TBI, in 9, 20 and 6 patients respectively.

Seventeen (17) patients received allogeneic BMT, median age was 13 years (range 4-41), 10 were male, transplant was performed after a median of 3 months (range 1-5) from CR. Conditioning regimens were the BU+CY and the CY+TBI in 11 and 6 patients respectively.

Twenty of 35 autografted patients relapsed, 1 in the TBI arm, 6 in the BMVC arm and 13 in the BU+CY arm; median time to relapse was 6, 3 and 3 months respectively. Three patients died in CR in the CY+TBI group and 1 in the BMVC group. Eleven patients are in CCR after a median follow up of 36 months (range 3-49) from ABMT. Projected probability of DFS is 29% at 49 months.

Five of 17 allografted patients relapsed: 2 in the CY+TBI group after 13 and 15 months and 3 in the BU+CY group after 3, 7, and 14 months. Two patients died in CR in the CY+TBI group and 2 in the BU+CY group; 8 patients are in CCR after a median follow up of 14 months (range 1-49) from transplant. Projected probability of DFS is 32% at 49 months.

In transplanted patients no difference was observed in the probability of DFS concerning conditioning regimens, age, sex, induction schedules and transplant procedures.

Because of 22 early relapses, 3 deaths in CR and 3 too early, 78/106 responders were evaluable for ablative programs; among them, 52 were submitted to transplant procedures and 26 were considered not eligible for various reasons and received conventional chemotherapy. No statistically significant difference of DFS was observed between the two groups, although a positive impact on DFS was achieved in the transplanted group (DFS 30% at 49 months vs 12% at 45 months).

In ALL the problem of relapse and drug resistance of leukaemic cells is still unsolved. New remissions can be obtained with conventional chemotherapy, but the length of second and subsequent remissions is usually less than one year with a minimal probability of cure, especially when relapse occurs in therapy or shortly thereafter (9).

New agents and different combinations have been employed in refractory patients with the aim of prolonging remissions by overcoming the problem of drug resistance (10). High-dose ARA-C therapy was utilized in the treatment of relapsed or refractory acute leukemia and beneficial effects were reported (11). Better results were achieved by combining administration of high or intermediate dose Ara-C with an anthracycline.

In our experience, the combination of high or intermediate Ara-C doses and IDA was effective in inducing CR in 52/91 patients (57%) in the LAL-R85 and in 64/78 (69%) in the LAL-R87.

The value of high-dose chemo/radiotherapy followed by reinfusion of hematopoietic stem cells in prolonging DFS in advanced ALL is still unclear, even though recent reports demonstrate the possibility of long-term survival in patients submitted to such an intensive program (12).

A randomized trial will be necessary to elucidate the role of a very aggressive post-remission approach in improving long-term prognosis in patients with advanced ALL.

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FIGURE 1

LAL R 85

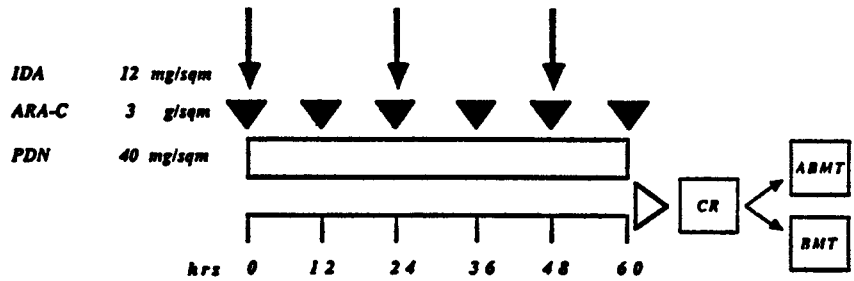

FIGURE 2

LAL R 87

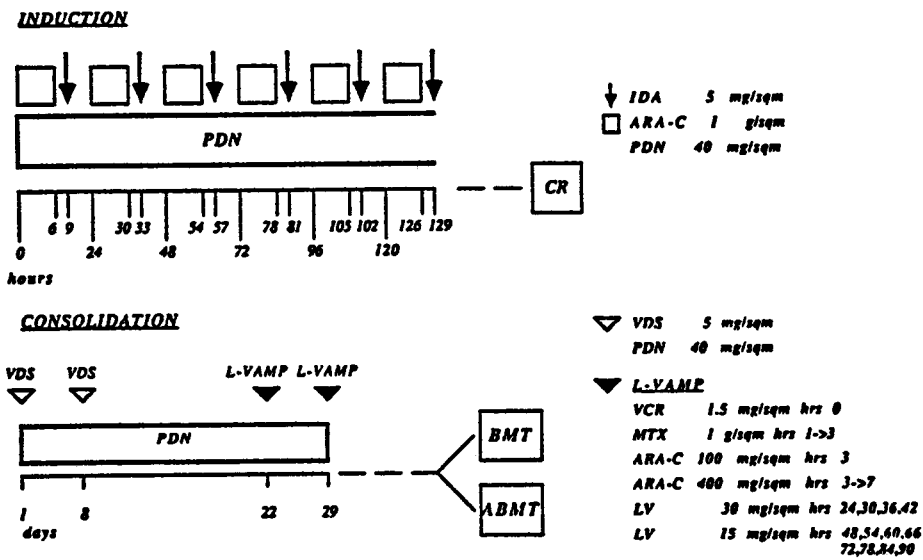

FIGURE 3

LAL R 85 / LAL R 87

PATIENTS DETAILS

	ABMT	*BMT*
Patients	*35*	*17*
Median age(years)	*13*	*13*
range	*(2-55)*	*(4-41)*
Sex M/F	*28/7*	*10/7*
LAL R 85	*17*	*5*
LAL R 87	*18*	*12*
Months to transplant		
median	*2*	*3*
range	*(1-5)*	*(1-5)*
Conditioning regimen		
BMVC	*9*	*-*
Bu + Cy	*20*	*11*
Cy + TBI	*6*	*6*

DETECTION OF MINIMAL RESIDUAL LEUKEMIA IN AUTOLOGOUS REMISSION BONE MARROW GRAFTS OF T-LINEAGE ALL PATIENTS

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INTRODUCTION

A number of BMT centers have investigated the role of high dose radiochemotherapy followed by autologous BMT for the treatment of high risk remission ALL (1-7). A potential limitation of autologous BMT is the likely presence of occult leukemia cells in remission autografts. Therefore, most BMT centers use remission autografts which are purged ex vivo to eliminate the residual leukemic cell contamination (1-10). Most commonly applied purging methods include the use of monoclonal antibodies plus complement, cyclophosphamide congeners, monoclonal antibodies linked to magnetic beads, immunotoxins, and combined immunochemopurging using monoclonal antibodies plus complement or immunotoxins in combination with 4-HC (10). This report summarizes our results on 14 consecutive patients with high risk remission T-lineage ALL who underwent autologous BMT using autografts which were purged ex vivo using an anti-CD5 immunotoxin plus an anti-CD7 immunotoxin combined with 4-HC. We used a new quantitative minimal residual disease (MRD) detection assay system which combines multiparameter FACS with leukemic progenitor cell assays (5) for detection of residual leukemic blasts in autografts before and after purging. Our findings demonstrate the clinical value of this new MRD detection assay in (a) evaluating the efficacy of purging against residual clonogenic leukemic blasts in remission autografts and (b) predicting the outcome of autologous BMT.

MATERIALS AND METHODS

Patients

Between October 1986 and May 1989, 14 consecutive patients with high risk ALL in complete remission were enrolled in this study. Follow-up was complete through September 1990. The pretransplant characteristics are summarized in Table 1. All patients had an immunophenotype consistent with T-lineage ALL. Four patients were in first remission, 5 were in second

remission, and 5 were in third remission. The first remission patients included 3 adult males 2 of whom had high WBC (>50,000/uL) at diagnosis and an adolescent male with a WBC of 17,900/uL and a pseudodiploid karyotype. In the remaining 10 patients, who were in their second or third remission, the median duration of first remission was 10 months and the median time from diagnosis to BMT was 20 months. The remission status of the bone marrow was confirmed in all patients within 10 days prior to the preparative regimen. The percentage of lymphoblasts in the pretransplant bone marrow biopsy specimens ranged from 0% to 3%.

Conditioning and Purging

The first 9 patients were conditioned with single dose TBI (850 cGy delivered in a single fraction at 26 cGy/min) followed by high dose ARA-C (12 doses at a dose of 3g/m^2 and a total dose of 36 g/m^2). The last 5 patients were conditioned with hyperfractionated TBI (1375 cGy total dose in 11 x 125 cGy fractions) followed by cytoxan (60 mg/kg/day x 2 days). Autografts were purged ex vivo with a cocktail of two immunotoxins (T101-Ricin/anti-CD5 and G3.7-Ricin/anti-CD7 each at 500 ng/ml, 2 hrs treatment at 37C in the presence of 200 mM lactose) plus 4-HC (10 ug/ml, 30 min treatment at 37C), as previously described (5).

MRD Detection Assay

Ficoll-Hypaque separated bone marrow mononuclear cells (MNC) from prepurge and postpurge samples of autografts were analyzed by two-color immunofluorescence for the early T-lineage surface antigens CD5 and CD7 using a mixture of FITC labeled 10.2 (anti-CD5) and G3.7 (anti-CD7) MoAb and the late T-lineage surface antigen CD3 using the PE conjugate of G19.4 (anti-CD3) MoAb, as described (11). Sterile cell sorting at 1500 cells/sec was performed to isolate viable CD5,7$^+$CD3$^-$ as well as CD5,7$^+$CD3$^+$ T-lineage cells. CD5,7$^+$CD3$^+$ cells were analyzed since some ALL cases had a CD3$^+$ mature T-cell ALL phenotype. FACS sorted cells were assayed for T-lineage ALL blast colony formation in a leukemic progenitor cell (LPC) assay system, as previously described (8, 11, 12). Colony cells had blast morphology and their immunological marker profiles (TdT$^+$CD2$^+$CD3$^+$CD5$^+$CD7$^+$ CD25$^-$ in the CD3$^+$CD5,7$^+$ FACS sorted fraction and TdT$^+$CD2$^+$CD3$^-$CD5$^+$CD5$^+$CD25$^-$ in the CD3$^-$CD5,7$^+$ FACS sorted fraction) were consistent with T-lineage ALL. The number of CD5,7$^+$ T-lineage LPC per 10^8 MNC in the autografts was calculated from the percentages of CD3$^-$CD5,7$^+$ and CD3$^+$CD5,7$^+$ lymphoid cells and the sum of the number of blast colonies in these fractions, as previously reported.

RESULTS

Residual Leukemic Progenitor Cells in Autografts

The number of CD5,7$^+$ T-lineage LPC per 10^8 MNC in the remission autografts prior to purging showed a pronounced interpatient variation, ranging

from <233 (=<0.0002%) to 214,047 (= 0.2%) (median = 8,568 = 0.009%) (11). When CD5,7$^+$ FACS sorted T-lineage lymphoid cells from postpurge autograft samples were assayed for T-lineage ALL blast colony formation, no blast colonies were observed in 11 of 13 patients. The numbers of T-lineage LPC in the postpurge autografts ranged from < 14/10^8 MNC (=<0.0000 1 %) to 1,488/10^8 MNC (=0.001%). Thus, 82.6% - >99.96% of residual T-lineage LPC were eliminated from autografts by the applied combined immunochemopurging protocol (Table 2).

Bone Marrow Transplantation and Posttransplant Course

The bone marrow cell dose of the autografts ranged from 0.23-0.78 x 10^8 MNC/kg. The number of reinfused T-lineage LPC ranged from <8/kg to 655/kg. Thirteen of 14 patients engrafted, as defined by a WBC> 1,000/uL for 3 consecutive days, at a median of 23 days post BMT. Nine patients relapsed 1.2-17 months (median = 2.2 months) post BMT and 6 have subsequently died of leukemia (11). Two patients are alive disease free at 26 and 28 months post-BMT. The Kaplan-Meier estimates and standard errors of the probability of remaining in remission were 14 $\pm$ 13% for patients who had <0.009% residual LPC in their prepurge autograft samples and 53 $\pm$ 25% for patients who had > 0.009% residual LPC in their prepurge autograft samples (P=0.006) (Table 3). By comparison, the percentage of TdT$^+$ cells or the percentage of lymphoblasts did not correlate with the probability of relapse after BMT. Eight of 9 patients who relapsed did not have any residual LPC detected in their postpurge autografts. In the remaining patient, the residual LPC contamination was only 0.0002% in the purged autograft Thus, no apparent correlation was found between the efficacy of purging/LPC contamination in the purged autograft and the probability of relapse post BMT.

DISCUSSION

Our findings provide initial evidence that in high risk remission T-lineage ALL, high numbers of residual LPC in the remission bone marrow prior to BMT indicate a poor prognosis. This correlation suggests that the LPC assays Rely detect the in vitro counterparts of the in vivo clonogenic T-lineage ALL blasts. Importantly, we found no correlation between the outcome and (a) the efficacy of purging, (b) the number of residual LPC in autografts after purging, or (c) the estimated numbers of reinfused LPC. These preliminary findings suggest that in high risk remission T-lineage ALL, the primary reason for the recurrence of leukemia after autologous BMT is not the reinfusion of leukemic blasts in autografts due to ineffective purging, but rather the inability of the conditioning regimen to eradicate the therapy refractory residual leukemia burden in vivo. The MRD detection assay used in this study may help us to determine (1) the optimal timing for BMT so that high risk remission ALL patients can undergo BMT when their residual disease burden is minimal, and the probability of a given preparative regimen to eradicate their minimal

residual disease is high, and (2) the need for additional therapy to achieve a more substantial remission with minimal residual leukemia burden.

ACKNOWLEDGEMENTS

This work was supported in part by U.S. Public Health Service Grants RO1-CA42633, RO1-CA-51425, RO1-CA25097, RO1-CA-25097, PO1-CA-21737, R29-CA-42111, RO1-CA-31618 and RO1-CA-36725 from the National Cancer Institute, DHHS. Fatih M. Uckun is a Scholar of the Leukemia Society of America. Daniel Vallera was a Scholar of the Leukemia Society of America during parts of this study. John H. Kersey is the Recipient of an Outstanding Investigator Award (CA49721) from the National Cancer Institute. This is publication #60 from the Tumor Immunology Laboratory, University of Minnesota.

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TABLE 1

Patient Characteristics

Immunophenotype	$CD1^{+/-}$ $CD2^{+/-}$ $CD3^{+/-}$ $CD5^{+/-}$ $CD7^{+}$
Diagnosis	T-lineage ALL (n = 14)
Sex	Male: n = 12 Female: n = 2
Age	Median = 14 yr Range = 6-35 yr
WBC at D_X	Median = 164,000/μL Range = 3,600/μL - 800,000/μL
Remission Number	First Remission: n = 4 Second Remission: n = 5 Third Remission: n = 5
% Lymphoblasts in Bone Marrow	Median = 0.4% Range = 0-3.1%
% TdT^{+} Cells in Bone Marrow	Median = 0% Range = 0-6%

TABLE 2

Residual LPC in T-lineage ALL Remission Autografts

LPC/10^8 MNC Median (Range)		% Elimination of LPC Median (Range)
Pre purge	Post purge	
8,568 (≤ 233 - 2114,047)	≤ 60 (≤ 14 - 1,488)	≥ 99.48 (≥ 82.63 - ≥ 99.96

TABLE 3

Bone Marrow Transplantation and Post-Transplant Course

UPN	BM REM Status			Autograft		Post BMT Course	
	% Lymphoblasts	% TdT+ Cells	% LPC	Marrow Cell Dose (x10^8/kg)	LPC/kg	Time to (days)	Time to (days)
643	0	1	0.214	0.78	<69	27	86
654	2	3	0.133	0.56	<175	44	65
665	2	0	0.105	0.51	100	34	37
675	1	0	<0.0003	0.58	<8	23	510
692	1	2	0.004	0.67	<13	62	died of hemorrhage
701	0	1	0.009	0.44	655	30	died of hemorrhage
735	0	0	<0.0002	0.34	<8	–	died of pneumonia
767	3	6	0.018	0.41	<25	18	76
817	0	1	0.0008	0.43	<27	23	NED at 28 months
836	1	0	0.004	0.23	<8	24	NED at 26 months
882	0	0	0.031	0.26	<15	20	45
1051	3	2	0.005	0.59	<30	20	106
1081	0	4	0.023	0.73	<189	18	62
1087	0	0	ND	0.60	ND	19	97

INDUCTION OF PHILADELPHIA-NEGATIVE HEMOPOIESIS AND PROLONGATION OF CHRONIC PHASE IN PATIENTS WITH CHRONIC MYELOID LEUKEMIA TREATED WITH HIGH DOSE CHEMOTHERAPY AND TRANSFUSION OF PERIPHERAL BLOOD STEM CELLS

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ABSTRACT

We treated 16 patients with Ph-chromosome positive chronic myeloid leukemia in chronic phase by autografting with blood-derived hemopoietic stem cells. Thirteen patients were autografted electively and three were autografted after marrow cells from HLA-identical sibling donors had failed to engraft. In 14 patients hemopoiesis recovered; one failed to engraft and died 3 months after autografting; another has remained pancytopenic for 22 months after autografting. Three patients developed late cytopenias; two became pancytopenic and received further stem cell transfusions at 3 and 40 months respectively after first autograft; the third received further stem cells for thrombocytopenia 39 months after a second autograft. Four patients have entered blast crisis and died. Four patients have not required chemotherapy after autograft; in 2 cases hemopoiesis is almost exclusively Ph-negative (48 & 63 months post autograft). The actuarial survival at 5 years post autograft is 63% $\pm$ 19%. We conclude that autografting in chronic phase with peripheral blood stem cells probably delays transformation by reducing the size of the leukemic stem cell population and might in some cases result in cure by inducing permanent damage to the leukemic clone in association with reconstitution of normal hemopoiesis.

INTRODUCTION

We have previously reported the results of autografting patients during the chronic phase of chronic myeloid leukemia (CML) with peripheral blood stem cells. We now update our results in 16 patients who have been followed for a median of 48 months since autograft.

PATIENTS AND METHODS

Patients

Thirteen patients with Ph-positive CML in chronic phase were treated with cytoreductive therapy followed by autografting with peripheral blood buffy-coat cells which had been collected at the time of diagnosis (1,2). Three other patients were autografted as a rescue procedure after an allograft failed (Table 1). There were six women and ten men. Their median age was 39 years (range 29-62). One patient (no.4) had not received any treatment; another patient had only received standard conditioning therapy preceding allogeneic marrow graft. The remaining patients received various combinations of busulphan, 6-thioguanine and/or hydroxyurea. Two patients (nos.14 & 16) had also received alpha-interferon. The median duration of chronic phase before the autograft was 20 months (range 1-67).

Cytoreductive Therapy Before Autografting

Twelve of the patients treated electively received busulphan 4 mg/kg orally on 4 consecutive days followed 24 hours later by melphalan 60 mg/m2 given by IV bolus injection. The remaining patient (no.16) who was autografted electively received cyclophosphamide 60 mg/kg intravenously for two consecutive days instead of melphalan. The autograft was administered 24 hours after the melphalan or cyclophosphamide ceased. In three cases (nos.1-3) the patient received standard conditioning therapy for T-depleted marrow transplants from HLA identical siblings. These patients were autografted when it was clear that the allograft had failed to take.

Autografting

The techniques for collection and cryopreservation of blood-derived hemopoietic stem cells has been described elsewhere (1,2).

Follow-Up

The clinical status of the patients together with blood counts were monitored at regular intervals post autograft. Between 2 and 13 marrows were studied in each patient. Duration of follow-up in surviving patients was calculated to the 1st of August 1990.

Cytogenetic Analyses

Routine cytogenetic analysis was performed on all patients at frequent intervals for the first 12 months and at various intervals thereafter.

PCR Analysis

RNA was prepared from peripheral blood and bone marrow cells from one patient (no.4) and cDNA was prepared using an ABL specific oligonucleotide primer and reverse transcriptase. cDNA was then used for PCR as previously described (3) using nested primers for a second round of PCR.

RESULTS

Toxicity

The chemotherapeutic regimen was relatively uncomplicated. Mucositis was usually severe but transient. There were only one transplant-related death which was attributable to non-engraftment (see below).

Initial Engraftment

There was early evidence of trilineage engraftment in 14 of the 16 patients. One patient (no.14) never engrafted and remained severely cytopenic until he died of hemorrhage 3 months post autograft. Another patient (no.15) remained pancytopenic despite a second infusion of cells 3 months after the first autograft but has gradually increasing cell counts 22 months post autograft.

Graft Failure After Initial Engraftment

Three of the 14 patients with initial engraftment developed cytopenias subsequently. One patient (no.11) had abrupt graft failure at 3 months. She received two further autografts without further cytoreductive therapy but restoration of adequate hemopoiesis on each occasion was only transient. She remained red cell and platelet transfusion dependent for 24 months but has gradually increasing cell counts (WBC 2.2 x 10^9/l and platelets 20 x 10^9/l at last follow-up) 46 months post first autograft. The second patient (no.1) became gradually cytopenic 30 months after autograft. She received a second autograft without preceding chemotherapy 42 months after the first procedure which rapidly restored her leucocyte and platelet counts to normal without progressing to leucocytosis. The third patient had received a second autograft with chemotherapy 14 months after the first procedure. He then developed severe thrombocytopenia (platelets 1-5 x 10^9/l with virtually absent megakaryocytes in the marrow aspirate and trephine 39 months after the second autograft while on a course of alpha interferon. A further infusion of cells resulted in a steady rise in platelet and leucocyte counts which have recently been controlled with hydroxyurea.

Hematologic Evolution of CML After Autografting

In general cytotoxic drugs were restarted when the leucocyte count remained consistently above 30-50 x 10^9/l. Following the autograft no patient required chemotherapy for at least 8 months. The pattern after that in the 14 evaluable patients was variable. Three patients (Group 1, Figure 1) commenced cytotoxic drugs 12-14 months post autograft and have remained in stable chronic phase (follow-up 60-73 months post autograft). Four patients (Group 2) commenced cytotoxic drugs 10-26 months post autograft for chronic phase disease but this transformed within 12 months to blastic phase. All four patients died soon after transformation. Four patients (Group 3) have not required cytotoxic therapy post autograft. Three of these patients are referred to above (nos.1, 11 & 15). The fourth patient (no.4) is mildly neutropenic (1.5-2 x 10^9/l) with normal marrow morphology 63 months after autograft.

Three patients were electively treated with Second autografts with preceding conditioning (group 4) 11, 22 and 26 months after first autograft. Two remain in chronic phase on cytotoxic therapy 62 and 66 months post first autograft respectively. The third patient has not required further treatment and is now 26 months post second autograft.

Cytogenetic Studies

During the first year post autograft Ph-negative metaphases were observed in between 0 and 88% of metaphases studied. After one year post autograft Ph-negative metaphases have been observed in 3 cases (figure 2). One patient (no.15) has 40% Ph-negative metaphases in bone marrow cells 23 months post autograft (previous studies were unsuccessful). The second patient (no.11) has had 100% and 93% Ph-negative metaphases on 2 studies 34 and 40 months post autograft respectively. The third patient (no.4) has had exclusively Ph-negative metaphases at 51, 57 and 61 months post autograft. At 61 months blood and marrow cells were also studied by PCR for evidence of cells containing the leukemia-specific BCR/ABL chimeric transcript. No evidence of residual leukemia was found.

Survival

The median survival of the group has not yet been reached. Eleven of the 16 patients remain alive. The actuarial survival at 5 years is 63% $\pm$ 19%.

DISCUSSION

Sixteen patients with CML in chronic phase were treated by autografting with peripheral blood-derived stem cells. One patient failed to engraft and died in marrow aplasia. Four patients developed blastic transformation and died. Eleven survive with or without evidence of CML in chronic phase. The overall actuarial probability of survival for these 16 patients is 63% $\pm$ 19% at 5 years from the first autograft procedure. This figure is substantially better than the survival that would be expected for a comparable cohort of patients treated by conventional agents (20-30%). This difference may have been achieved by chance but it seems likely that the onset of blastic transformation was delayed at least in some cases by the autograft procedure. If this were so, one may speculate that the high dose chemotherapy or chemoradiotherapy is effective by reducing for a period of years the size of the leukemic stem cell pool susceptible to blastic transformation.

The relatively frequent observation of cytopenia developing early or late after initial engraftment deserves comment. We have speculated previously that CML stem cells collected from the peripheral blood may in some cases have only a finite capacity to maintain leukemic hemopoiesis and further that they may differ in this regard from marrow-derived stem cells. We have no proof of this contention and further studies with other myeloablative regimens and marrow-derived stem cells will be required to confirm or refute it. If, however, one accepts provisionally the limited replicative capacity of the

transfused stem cells, then later hematologic events will depend on the viability of normal and/or leukemic stem cells that have survived the conditioning chemotherapy. Such residual stem cells may have been damaged to varying degrees. The timing of the cytopenias we have observed would thus depend on numbers and replicative capacity of the stem cells transfused and the extent to which leukemic or normal hemopoiesis can take over when the transfused stem cell population is exhausted.

We can use this kinetic theory to interpret the sequence of hematologic events in patients who did not become cytopenic after autografting. There were three distinct patterns (figure 3). First, there may be gradual repopulation of the marrow and blood with leukemic cells which re-establish CP disease and dictate the need for further chemotherapy (line A, seen in 10 cases) ; secondly the leukemic clone may reconstitute hemopoiesis but fails to expand to the point where cytotoxic drugs are required (line B, seen in 3 cases including one after a second autograft) - this suggests impaired proliferative capacity of the leukemic clone; and thirdly, the leukemic clone may be effectively eradicated with re-establishment of normal hemopoiesis (line C, seen in 2 cases). This situation may or may not be tantamount to cure.

The observation that small numbers of Ph-positive metaphases can persist in the marrow for a number of years without other evidence of leukemia cell proliferation is of great theoretical interest. An analogous sequence of events may be seen occasionally after allografting for CML. If the Ph-positive metaphases reflect the persistence of leukemic stem cells, studies designed to define the factors that could restrain their proliferation after autografting or allografting would be rewarding.

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TABLE 1

Clinical details of 16 patients treated by autografting (A/G)

Patient	Sex/age at A/G (yr)	Duration of CP before A/G (months)	Treatment before A/G	Events after (first) A/G: Duration without cytotoxic drugs (months)*	Events after (first) A/G: Further treatment	Events after (first) A/G: Survival (months)*	Survival from diagnosis (months)*
1^	F/36	32	BU	74+	2nd A/G#	74+	106+
2^	M/49	10	HU	14	IFN	60+	70+
3^	M/34	10	Nil	28	HU, Allograft, 2nd A/G, CT$	42	52
4	F/29	1	Nil	63+	Nil	63+	64+
5	M/32	30	HU	13	2nd A/G, Allograft, IFN	73+	103+
6	F/39	25	6TG	26	2nd A/G,BU,IFN	70+	95+
7	M/32	30	BU/6TG	10	2nd A/G, IFN, 3rd A/G	68+	98+
8	F/29	15	HU	8	CT$	14	29
9	M/33	20	HU	19	HU, 2nd A/G	58+	78+
10	M/31	6	HU	14	HU, Allograft	61+	67+
11	F/53	10	HU	48+	2nd A/G#, 3rd A/G#	48+	58+
12	M/51	8	HU	24	HU, IFN, CT$	34	42
13	F/45	44	BU	19	HU, CT$	37	81
14	M/59	12	IFN/HU	3	Nil	3	15
15	M/62	14	HU	22+	2nd A/G#	22+	36+
16	M/41	67	HU/IFN	8+	Nil	8+	75+

CP=chronic phase; HU=hydroxyurea; 6TG=6-thioguanine; IFN=alpha-interferon; BU=busulphan; 2nd or 3rd A/G= second or third autograft.

* + indicates that the patient continues without cytotoxic treatment (column 5) or continues to survive (columns 7 and 8).

^ Patients 1, 2 and 3 were autografted after failure of HLA identical donor cells to engraft.

Autograft performed without preceding cytoreductive therapy.

$ Patients 3, 8, 12 and 13 entered blastic transformation and were treated by combination chemotherapy (CT).

FIGURE 1

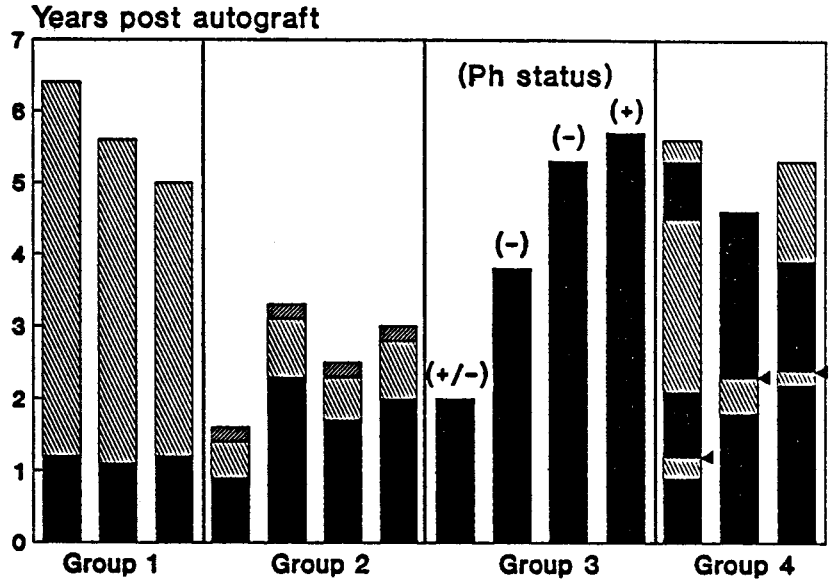


Figure 1: The pattern of CML in 14 patients with more than 12 months follow-up post autograft. Patients are divided into 4 groups (see text). The Ph chromosome status of patients in group 3 is shown in brackets. Patients in the other groups are all Ph-positive.

- ■ Represents the interval in which no cytotoxic therapy was given to the patient.
- ▨ Represents the interval in which cytotoxic therapy was given for chronic phase CML
- ▨ Represents the interval of blastic phase of CML.
- ◀ Indicates second autograft with preceding chemotherapy (same protocol as the first autograft).

FIGURE 2

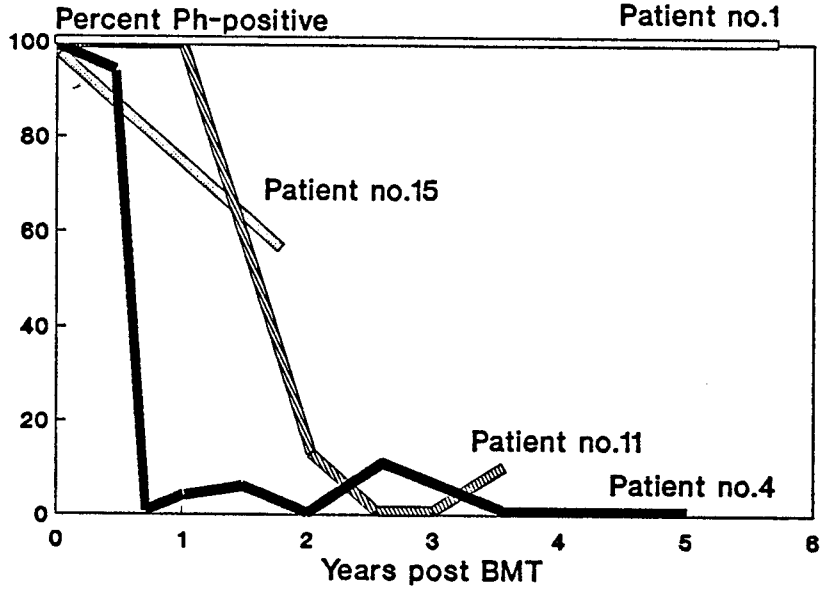
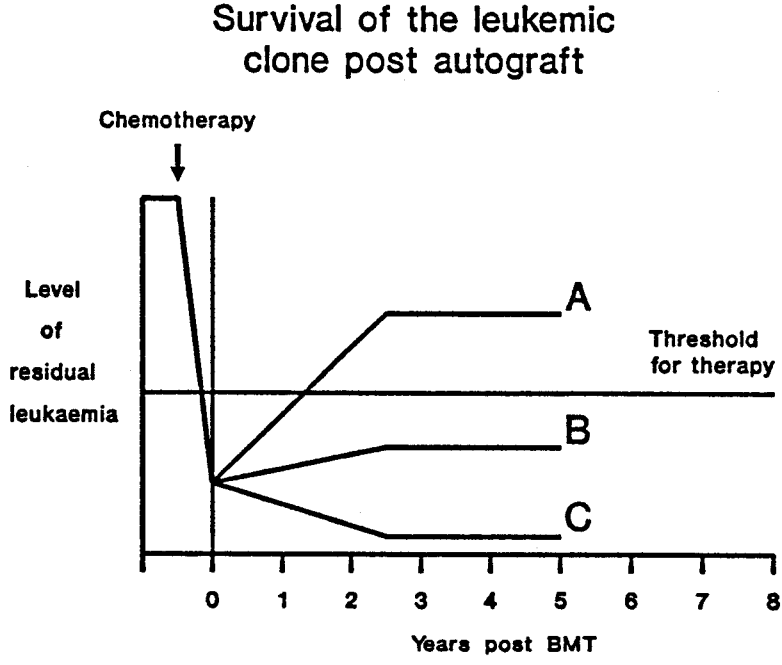


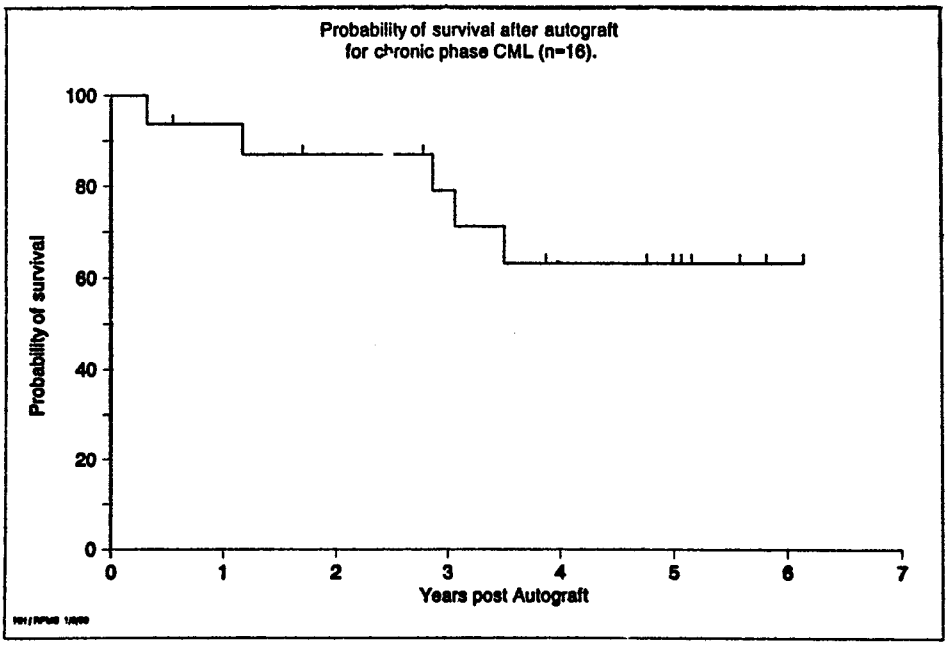
Figure 2: The pattern of cytogenetic results in the 4 patients (Group 3) who have received no further therapy since autograft.
Patient no. 1; Patient no. 11;
Patient no. 15; Patient no. 4

FIGURE 3



Possible fates of the leukemic clone after autograft. Line A represents the gradual return to chronic phase CML with leucocytosis requiring cytotoxic therapy. Line B represents the re-establishment of the leukemic clone as the predominant source of hemopoiesis but without leucocytosis or thrombocytosis. Line C represents failure of the leukemic clone to re-establish Ph-positive hemopoiesis although Ph-positive cells may remain present at a low level.

FIGURE 4


Probability of survival after autograft
for chronic phase CML (n=16).
Probability of survival
100
80
60
40
20
0
0
1
2
3
4
5
6
7
Years post Autograft

INTEGRATION OF MOLECULAR BIOLOGY AND GENETICS WITH BIOLOGICAL AND CHEMOTHERAPEUTIC APPROACHES TO THE REARRANGEMENT OF CHRONIC MYELOGENOUS LEUKEMIA

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INTRODUCTION

Alpha interferon induces durable cytogenetic remissions in 20% of chronic myelogenous leukemia (CML) patients (1). 30% of CML patients are currently eligible for allogeneic bone marrow transplantation which cures half of the patients treated (2). We are developing autologous transplantation for the CML patients who are ineligible for allografts or alpha interferon. Table 1 shows the treatment plan for our program.

The goal of this program is to increase percentage of patients who achieve stable major cytogenetic remissions (Ph+ cells less than 30%) following intensive therapy and autologous bone marrow transplantation. The first step is to use in vivo combination chemotherapy (daunomycin, 120 mg/m^2 dl, high dose cytosine arabinoside, 1.5 gm/m^2 CI qd x 4, and GMCSF, 125 mcg/m^2 s.c. qd d4 to until the granulocyte count is greater than 500/mm^3) to increase the number of Philadelphia chromosome negative cells. The second step is storage of these peripheral blood and marrow autologous cells.

Next, we propose to use in vitro separation, autologous reconstitution, and interferon maintenance to further increase the percent of autologous cells which are diploid following intensive therapy and autologous bone marrow transplantation. The precedent for the use of chemotherapy for the in vivo cytogenetic conversion of Ph+ to Ph- cells is the experience of Dr. H. Kantarjian and his colleagues in the M. D. Anderson Leukemia Section with combination chemotherapy in three settings: (1) DOAP Therapy in previously untreated CML patients, (2) daunomycin and high dose ara-c treatment of interferon resistant previously treated CML patients, and (3) daunomycin, high

dose ara-c and GMCSF treatment of CML blast crisis patients. The summary of this data is that 30-50% of such CML patients exhibit major cytogenetic remissions following this chemotherapy. Although these remissions are brief, they persist for a sufficient period of time to permit collection of autologous hematopoietic progenitor cells which are enriched in diploid cells (3).

We are evaluating two types of systemic preparation for autologous bone marrow transplantation: (1) combination chemotherapy (the CBV regimen: cyclophosphamide 1.5 gm/m^2 qd x 4, BCNU 300 mg/m^2 x 1 day, and VP16 125 mg/m^2 q 12 h x 6) and (2) a combination of TBI (1020 rads), cyclophosphamide 60 mg/kg qd x 2 days and 125 mg/m^2 of VP16 q 12h x 6. We studied these two regimens in late chronic phase or advanced (accelerated phase or blast crisis) CML patients in pilot trials. The marrow was stored at a median of 36 months after diagnosis and the therapy was delivered on the average several months thereafter. In the chemotherapy trial, there were 8 first chronic phase patients, 3 second chronic phase patients, 2 third chronic phase patients, 1 accelerated phase patient, and 1 blast crisis patient. In the TBI VP16 cytoxan trial, there were 5 chronic phase patients, 1 second chronic phase patients, and 2 accelerated phase patients. The time required for hematopoietic recovery to 500 granulocytes/mm^3 was 26 and 36 days for the CBV and TBI regimens, respectively. Treatment deaths occurred at the 12% level with one patient dying of cytomegalovirus pneumonitis and the other from a CNS bleed. Ten out of 15 patients in the combination chemotherapy trial (CBV) and 4 out of 8 patients treated in the TBI, VP16 and cytoxan program exhibited reappearance of diploid hematopoiesis following intensive therapy.

Among the patients enrolled in the CBV program, 5 out of 15 patients developed a complete or major cytogenetic remission with reduction of the Philadelphia chromosome positive cells to less than 30%. The probability of achieving a major cytogenetic remission was 75% in those individuals in whom the percentage of Philadelphia chromosome positive cells was less than 12% at the time of autologous storage. In contrast, the probability of achieving a major cytogenetic remission was less than 30% in those individuals in whom greater than 30% Philadelphia chromosome-positive cells persisted at the time of autologous reconstitution.

The duration of major or minor cytogenetic remissions in those individuals (Ph+ cells greater than 30%) was between one and two months, whereas the duration of major cytogenetic remissions after autologous reconstitution was 18, 12, 4, 3, and 1 months. The trends in this data suggest that CML patients ineligible for allograft or interferon should be induced into cytogenetic remission before autologous storage, and that every attempt should be made to achieve a major or complete cytogenetic remission following transplant in order to produce a durable remission after therapy.

The probability of being sufficiently sensitive to alpha interferon after transplant to be maintained on alpha interferon as a single agent in our series was highest in the 8 first chronic phase CML patients who were treated with combination chemotherapy as a preparative regimen, whereas it was very low in advanced disease (second chronic phase or accelerated and blast crisis).

Similarly, the probability of remaining alive after transplant was directly correlated with being in first chronic phase at the time of treatment.

We will, therefore, attempt to deliver our therapy to patients who are in the first chronic phase, as early as possible in their disease, and with cells that have been collected at a time when major cytogenetic remissions have been induced by chemotherapy. We will also to continue to utilize interferon maintenance in these patients since four patients appeared to have acquired collateral sensitivity to interferon after autologous bone marrow transplantation, and other center have similar findings.

An area of very active investigation is the development of techniques which can be used for in vitro fractionation of normal and Philadelphia chromosome positive cells.

Previous reports indicate that there is an adhesive defect in the late myeloid CML progenitor cells (4). As reported by Guba and Emerson (5), we have found a surface cytoadhesion molecule, LFA3, to be expressed at lower than normal levels in the CML cell late myeloid progenitor cells. This deficiency is correctable by exposure to interferon.

As shown in Figure 1, we are incubating ficoll hypaque fractionated mononuclear cells from CML marrow on pre-irradiated allogeneic feeder layers for two hours. Following this period of incubation, the suspension cells are rinsed and then the cultures are carried along for 3 to 14 days. Colonies grown in methylcellulose from cells derived from the marrow normal cell cultures are evaluated by the polymerase chain reaction (PCR) for Philadelphia chromosome positive cells.

As shown in Figure 2, we are also currently standardizing conditions in serum free medium under which the growth of Philadelphia chromosome positive cells can be inhibited by antisense oligonucleotides to the bcr-abl transcript. We are currently working out the conditions in which both of these manipulations will result in complete eradication of the Philadelphia chromosome positive population.

As shown in Figure 3, we have discovered a cytoplasmic phosphatase, which is increased 50-fold CML myeloid progenitor cells. This phosphatase opposes the action of interferon on the activation of cytoplasmic precursors of nuclear transcriptional regulatory proteins of which bind to interferon inducible Transcriptional Enhancers (6). Were we to find a way of circumventing the effect of this phosphatase in CML cells, we could develop an approach to sensitization of CML cells to the in vivo antiproliferative effects of interferon CML cells. We have already isolated the nuclear proteins which bind to the transcriptional enhancers of interferon inducible genes (7), and we are isolating the phosphatase as well.

We have also been studying ways of predicting interferon sensitivity to alpha interferon (8). This assay measures the sensitivity of cells from CML patients to the inhibitory effects of interferon on reducing the phosphatase induced change of nuclear proteins binding to interferon inducible transcriptional enhancers (8). Eventually, this assay will be used to identify

CML patients who are sensitive or resistant to interferon therapy so as to permit the allocation of CML patients to interferon therapy or to a transplant program.

As shown in Table 1, we are approaching the development of autologous reconstitution by first induction of cytogenetic remission with chemotherapy, collection of autologous stem cells which are enriched in Philadelphia chromosome negative cells due to chemotherapy induced cytogenetic remissions, in vitro fractionation to further enrich the autologous cells in Philadelphia chromosome negative cells, bone marrow transplantation with these autologous cells, and interferon maintenance. Hopefully, the combination of these approaches will lead to more durable remissions in a greater percentage of interferon resistant CML patients who have no allograft donors.

ACKNOWLEDGMENTS

We recognize support to Albert Deisseroth, M.D., Ph.D. from the NCI (POl CA-49639-01), the American Cancer Society (IM-580), the Sid Richardson Foundation, the Gillson Longenbaugh Foundation, and the Kleberg Foundation. We are grateful for the editorial assistance and typing of Janet Rae-Skaggs and Rose Lauzon.

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Table 1

USE OF AUTOLOGOUS BONE MARROW TRANSPLANTATION IN CML

1. INDUCTION OF CYTOGENETIC REMISSION
   - DAUNO
   - HD ARA-C
   - GMCSF
2. STORAGE OF PBMC WHEN WBC = 1000 DURING RECOVERY PHASE
3. STORAGE OF BONE MARROW
4. TBI/VP-16/CYTOXAN
5. AUTOLOGOUS TRANSPLANTATION WITH MARROW AND PBMC SELECTED IN VITRO AND IN VIVO FOR PH- CELLS
6. INTERFERON MAINTENANCE THERAPY

FIGURE 1

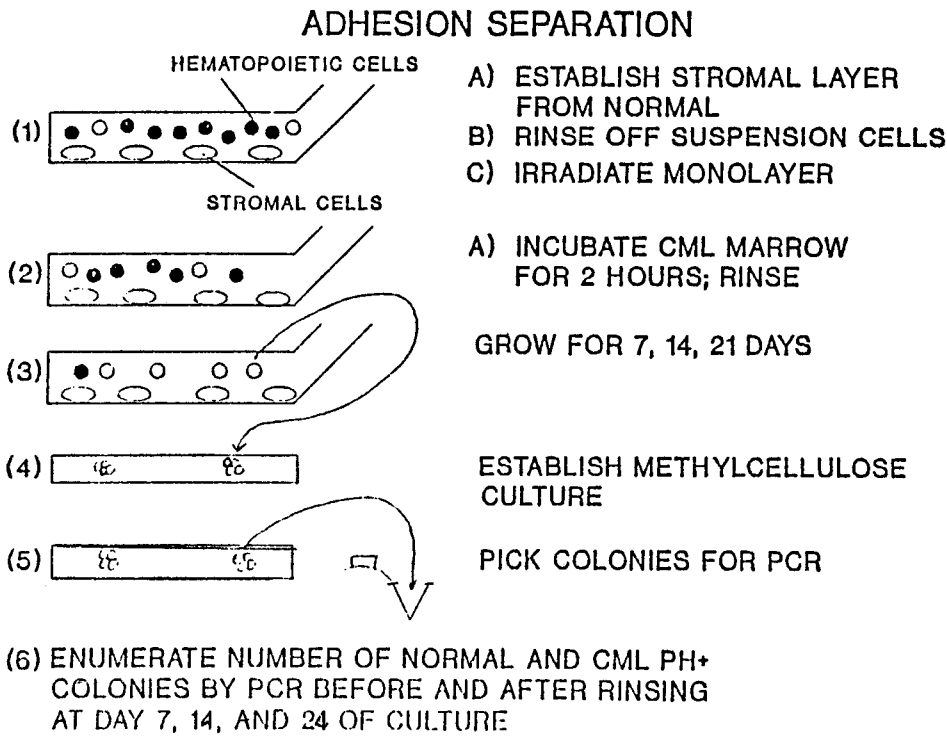

ADHESION SEPARATION
HEMATOPOIETIC CELLS
(1)
STROMAL CELLS
(2)
(3)
(4)
(5)
A) ESTABLISH STROMAL LAYER FROM NORMAL
B) RINSE OFF SUSPENSION CELLS
C) IRRADIATE MONOLAYER
A) INCUBATE CML MARROW FOR 2 HOURS; RINSE
GROW FOR 7, 14, 21 DAYS
ESTABLISH METHYLCELLULOSE CULTURE
PICK COLONIES FOR PCR
(6) ENUMERATE NUMBER OF NORMAL AND CML PH+ COLONIES BY PCR BEFORE AND AFTER RINSING AT DAY 7, 14, AND 24 OF CULTURE

FIGURE 2

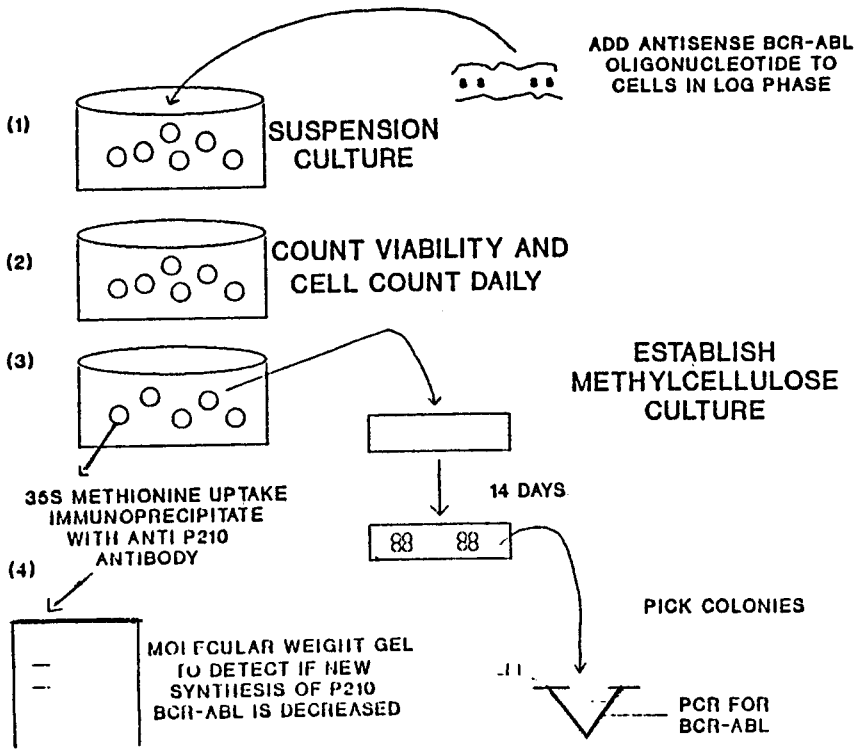
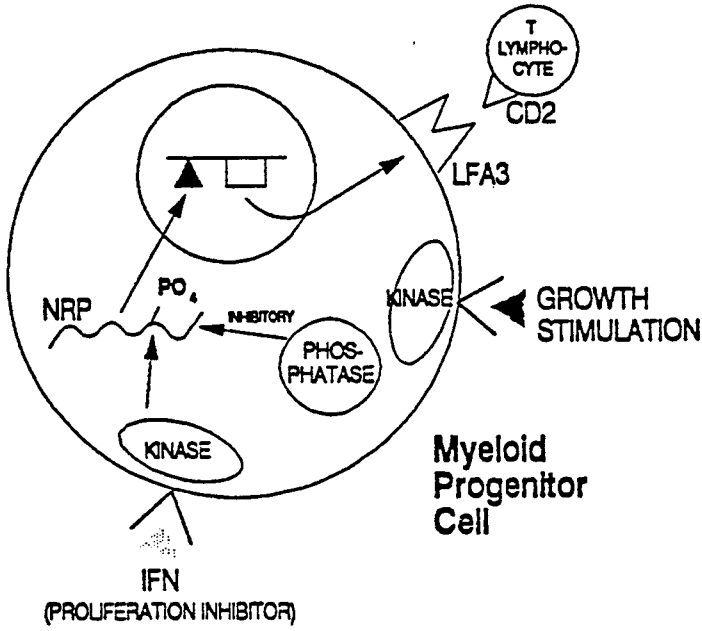

ADD ANTISENSE BCR-ABL OLIGONUCLEOTIDE TO CELLS IN LOG PHASE
(1)
SUSPENSION CULTURE
(2)
COUNT VIABILITY AND CELL COUNT DAILY
(3)
ESTABLISH METHYLCELLULOSE CULTURE
14 DAYS
35S METHIONINE UPTAKE IMMUNOPRECIPITATE WITH ANTI P210 ANTIBODY
(4)
PICK COLONIES
MOLECULAR WEIGHT GEL TO DETECT IF NEW SYNTHESIS OF P210 BCR-ABL IS DECREASED
PCR FOR BCR-ABL

FIGURE 3


T LYMPHO-CYTE
CD2
LFA3
NRP
PO4
INHIBITORY
PHOS-PHATASE
KINASE
GROWTH STIMULATION
KINASE
Myeloid Progenitor Cell
IFN
(PROLIFERATION INHIBITOR)

AUTOGRAFTING WITH CURATIVE INTENT FOR PATIENTS WITH CHRONIC MYELOID LEUKEMIA

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INTRODUCTION

Intensive therapy in marrow-ablative dose supported by transplantation of bone marrow from a suitable donor is the only established curative treatment for patients with chronic myeloid leukemia (CML) (1-3). The immunological problems associated with allogeneic bone marrow transplantation (BMT), however, remain formidable and necessitate age and histocompatibility restrictions that significantly limit the applicability of this approach (4). For BMT to have a major impact on CML, it will be essential to make the procedure safer and available to a much larger proportion of patients.

The results of syngeneic BMT (5) in patients with CML in chronic phase indicate that ablative therapy alone (i.e., without a graft-versus-leukemia effect associated with allogeneic BMT) can eradicate the leukemic clone. As residual Philadelphia chromosome (Ph1)-negative hematopoietic stem cells are known to persist in many patients (6-8), the use of autologous marrow to support intensive therapy is an attractive possibility. For this to be effective, however, a highly selective and efficient procedure for isolating Ph1-negative stem cells free of Ph1-positive stem cells is required.

We have previously shown that marrow from most patients with CML, when used to initiate long-term cultures, is spontaneously and rapidly depleted of Ph1-positive cells, whereas residual Ph1-negative hematopoietic cells are relatively well maintained and frequently become predominant within 4-6 weeks (7,9). It is presumed that this in vitro "switch" to Ph1-negative hematopoiesis, which is usually not complete at the level of the more differentiated cell compartments until after four weeks of culture, reflects a selection in favor of more primitive Ph1-negative stem cells (i.e., cells capable of reconstituting all blood cell lineages following transplantation) at some earlier time, i.e., within the first 10 days of culture. In three cases where it was possible to undertake clonality studies, it was demonstrated that at least some of the Ph1-negative

hematopoietic cells appearing in culture did not belong to the leukemic clone and hence were presumably normal (10-12).

On the basis of these observations, we initiated a study in 1987 to evaluate the use of intensive therapy supported by transplantation of cultured autologous marrow for patients with CML who were ineligible for allogeneic BMT (Figure 1). In an attempt to ensure that this treatment would be used in those likely to benefit from it, we "screened" all candidate patients by examining the behavior in culture of cells from a preliminary sample of their marrow. This report briefly reviews the results obtained to date.

PATIENTS AND METHODS

The eligibility criteria were that patients be <60 years of age, be in morphological chronic phase of CML and lack a suitable family member or unrelated volunteer to serve as a donor for allogeneic BMT. On screening of the marrow sample, the number of normal (Ph1-negative) stem cells present after an interval of 10 days was assumed to be sufficient if the number of normal clonogenic progenitors present (i.e., derived from them) after a further 3-5 weeks of incubation in long-term culture (i.e., after a total of 4-6 weeks) was $>2\%$ of the number of clonogenic progenitors found on average in analogous cultures initiated with marrow from a series of normal individuals. At the same time, the number of Ph1-positive clonogenic progenitors was required to have decreased to undetectable levels (i.e., to $<0.1\%$ of normal clonogenic progenitor levels). The time for evaluating the clonogenic progenitor content of the long-term cultures was chosen on the basis of our decision to use a 10 day culture period for the treatment of the autograft prior to BMT. This, in turn, was based on previous evidence indicating that this period might be adequate for most patients (9), as well as initial clinical experience with cultured marrow from a patient with acute myelogenous leukemia autografted in Manchester (13).

All suitable patients underwent leukapheresis on a number of occasions, and buffy coat preparations were cyropreserved to serve as an untreated reserve. The methods employed for harvesting and culturing the marrow were as described previously (14). Briefly, 2×10^{10} nucleated cells from each patient were set up in culture, and 10 days later 1.3-3.6 x 10^8/kg cells were collected and infused. In the interval, patients received etoposide 1.8 g/m^2 X 1, cyclophosphamide 2.0 g/m^2 x 3 and TBI 200 cGy x 5 or 6 (3 patients) or busulfan 1 mg/kg x 16, cyclophosphamide 60 mg/kg x 2 and melphalan 90 mg/m^2 X 1 (6 patients) or busulfan 1 mg/kg x 16 and melphalan 90 mg/m^2 (1 patient).

RESULTS

Ten patients aged 22-59 (median 43) years, 6 in 1st chronic phase (Group 1) and 4 in accelerated phase or in 2nd or 3rd chronic phase (Group 2), have been treated with intensive therapy and transplantation of cultured

marrow. One is still too early post-BMT to evaluate. In all of the other 9, satisfactory engraftment occurred (neutrophils $>1.0 \times 10^9$/L and platelets >20 10^9/L by median day 31 and 42 post-BMT, respectively,). During the initial phase of hematopoietic regeneration post-BMT, 100% Ph1-negative cells were detected in 8 patients and 94% in the ninth. In one of the female patients, clonality studies were possible using methylation analysis of a Bgl 1 restriction fragment length polymorphism (RFLP) in one of her X-linked PGK genes, as well as by analysis of the BCR locus. These studies showed that in this patient, the regenerating hematopoietic cells in vivo, like those produced after 4 weeks in vitro, showed no detectable cells with a BCR rearrangement and were polyclonal (12). Two patients (both in Group 2) died; 1 of therapy-related toxicity and 1 of recurrent blast phase disease, 1 and 4 months post-BMT respectively. The other seven patients remain well in hematological remission, with 84-100% Ph1-negative marrow cells, 5-33 months post-BMT. Four (3 in Group 1) have received no further therapy. The other 3 (2 in Group 1) are on alpha-interferon after detection of 10-16% Ph1-positive marrow cells at 12 months post-BMT.

DISCUSSION

In selected patients with CML, the consistent achievement of Ph1-negative hematopoiesis that is subsequently sustained for many months after intensive therapy and autografting with cultured marrow is gratifying. Moreover, these findings provide continuing support for the tenet that transplantable normal hematopoietic stem cells from some patients may be maintained in culture at useful levels for a period of at least 10 days. These preliminary results also suggest a potentially important role for this form of therapy and warrant its assessment in larger numbers of patients. Having established the feasibility and potential of the approach, our efforts are now also directed towards the development of modifications to the procedure that might make it easier to undertake and that could allow patients presently considered "unsuitable" to meet the current entry criteria.

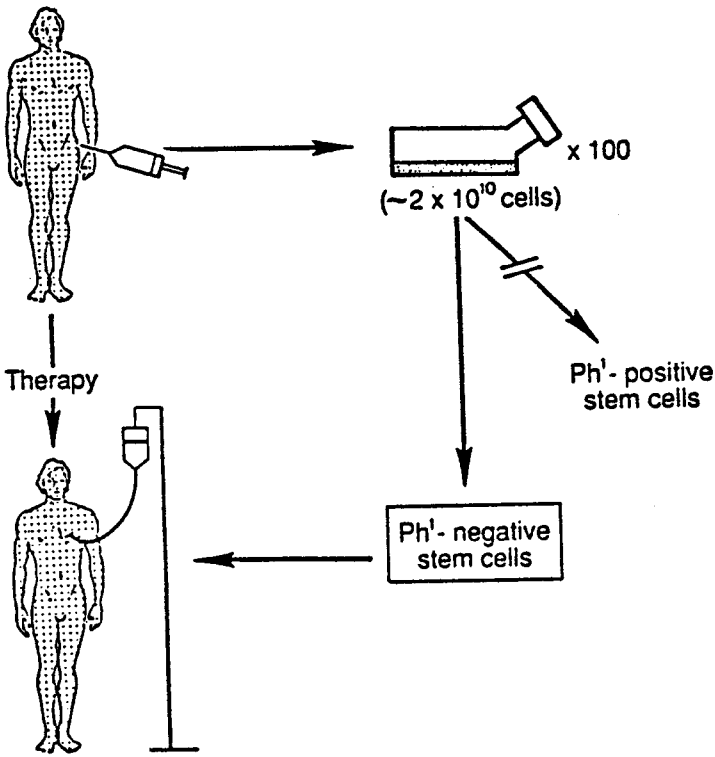
ACKNOWLEDGEMENTS

This work was supported by grants from the National Cancer Institute of Canada and the British Columbia Health Care Research Foundation. C.J. Eaves is a Terry Fox Cancer Research Scientist of the National Cancer Institute of Canada. We gratefully acknowledge the contributions of the medical, nursing, and technical staff of the Vancouver General Hospital and the British Columbia Cancer Agency, including the staff of the Cytogenetic Laboratory and Stem Cell Assay Service. We also thank Patricia Bennett for typing the manuscript. Authors' affiliations: Leukemia and Bone Marrow Transplant Program of British Columbia, Division of Hematology, British Columbia Cancer Agency, and Vancouver General Hospital; Terry Fox Laboratory of the

British Columbia Cancer Research Centre; Departments of Medicine, Pathology, and Medical Genetics, University of British Columbia, Vancouver, British Columbia.

FIGURE 1

Autografting in CML using maintenance of marrow cells in culture to select for Ph1-negative stem cells. Marrow is harvested from the patient and then incubated in tissue culture flasks for 10 days. Meanwhile, the patient receives intensive therapy at the completion of which the marrow cells are taken out of culture and infused.



CHRONIC MYELOGENOUS LEUKEMIA: IN VITRO MARROW PURGING WITH MAFOSFAMIDE AND RECOMBINANT GRANULOCYTE-MACROPHAGE COLONY-STIMULATING FACTOR

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INTRODUCTION

Chronic myelogenous leukemia (CML) is a clonal disorder arising from a neoplastic transformation at the level of the pluripotent hematopoietic stem cell (1). A characteristic marker of the disease is the presence of the Philadelphia (Ph^1) chromosome, which results from a molecular rearrangement involving the BCR and Abl genes located on chromosome 22 and 9, respectively (2).

Intensive chemoradiotherapy followed by allogeneic bone marrow transplantation has been shown to be a curative treatment modality for CML (3). However, this approach - in addition to a high rate of fatal complications mainly related to graft-versus-host disease - is restricted to $<30\%$ of CML patients, due to age limitations and availability of HLA-identical donors (3).

Experimental as well as clinical data demonstrating the persistence of functionally competent Ph^1-negative hematopoietic stem cells support the feasibility of autologous bone marrow transplantation (ABMT) in CML (4-6).

The experience of several teams performing ABMT in patients with acute leukemias using marrow purged with pharmacological agents, suggest the possibility to use such agents to decontaminate CML marrow (7).

It was the aim of the present study to evaluate the efficacy of an in vitro purging treatment of CML marrow, based on the combined effect of (a) mafosfamide and (b) a short-term (7 days) liquid culture phase allowing CML marrow cells to grow in the presence of recombinant human granulocyte-macrophage colony-stimulating factor (rGM-CSF).

Based on the observations reported herein, it can be concluded that a subgroup of CML patients exists which is responsive to chemopurging. As shown by preliminary clinical data described in the present report, this purging modality might be useful in clinical practice.

MATERIALS AND METHODS

Patients

Fifteen patients with Ph^1-positive CML were included in this study. Table I shows the main clinical and hematological data of the patients. Four patients (nos. 1, 9, 10, 11) were studied at diagnosis and prior to any treatment, the others had been diagnosed from 1 months to 3 years prior to the time of the study, and had received prior treatment with hydroxyurea and/or interferon-alpha. All patients but one were in the chronic phase of the disease at the time of the study.

Cell Separation Procedures

After informed consent, bone marrow was obtained from patients by aspiration from the posterior iliac crest at the time of examination for clinical evaluation. Mononuclear light density bone marrow cells (MNC) were separated by centrifugation on a Ficoll-Hypaque gradient (density 1.077 g/ml) at 400g for 40 min at 20C. Interface cells were washed three times and suspended in RPMI-1640 medium (GIBCO, Grand Island, NY, U.S.A.) supplemented with 10% fetal bovine serum (FBS, Hyclone, Logan, Utah, U.S.A.) and 200 mM glutamine (GIBCO, Grand Island, NY, U.S.A.).

In Vitro Purging

MNCs obtained by density gradient separation were counted and their concentration adjusted at $2x10^7$/ml. Samples were obtained for direct cytogenetic analysis while the remaining cells were divided in two aliquots to be treated with either medium or freshly diluted mafosfamide (Asta Pharma, Bielefeld, FRG). Treatment with mafosfamide (100 ug/ml) or control medium was performed by incubating the cells for 30 min at 37C in a water bath with frequent agitation. The cells were subsequently incubated for 5 min on ice to stop the reaction, washed twice, and then processed according to the experimental model depicted in Figure 1.

Suspension Culture

Untreated and mafosfamide-treated MNCs resuspended in RPMI-1640 medium supplemented with 10% FBS and 200 mM glutamine were cultured (1 x 10^6/ml, 37C, 5% CO_2) for 7 days in 75-cM2 tissue culture flasks. Both untreated and mafosfamide-treated cells were cultured with and without recombinant human granulocyte-macrophage colony-stimulating factor (rGM-CSF). At the end of the culture period the cells were harvested and progenitor cell growth as well as cytogenetic and immunological parameters were assayed.

Clonogenic Assay

The clonogenic cell content of each sample was assayed prior to and after suspension culture. The assay for pluripotent colony-forming units (CFU-GEMM), erythroid bursts (BFU-E), and granulocyte-macrophage

colony-forming units (CFUGM) has been previously described (8). Briefly, $5x10^4$ untreated or mafosfamide-treated MNCs were plated in 35mm Petri dishes in 1ml aliquots of Iscove's modified Dulbecco's medium (IMDM, Miles Laboratories, Naperville, IL, U.S.A.) containing: 30% FBS; 5×10^5 M 2-mercaptoethanol; and 0.9% (wtv) methylcellulose. Cultures were stimulated with a mixture of human recombinant colony-stimulating factors (CSF): interleukin-3 (IL-3, 10 ng/ml), granulocyte-CSF (G-CSF, 10 ng/ml), granulocyte-macrophage-CSF (GM-CSF, 10 ng/ml) and erythropoietin (1 U/ml). Recombinant IL-3, GM-CSF, G-CSF and erythropoietin were generously provided from Genetics Institute (Boston, MA, U.S.A.) and Amgen Inc. (Thousand Oaks, CA, U.S.A.). All colony-stimulating factors were used at optimal concentrations, as determined in preliminary experiments. To allow a direct comparison of the progenitor cell content of each sample prior to and after liquid culture, a fraction of each sample, corresponding to a fixed number of cells present in the liquid culture on day 0, was removed and incorporated into clonogenic assays identical to those plated prior to the suspension phase, i.e., the cells were replaced by volume. Thus, no correction was made for cell death or proliferation. After incubation for 14 days at 37C in a humidified atmosphere supplemented with 5% CO_2, the cultures were examined with an inverted microscope. Four dishes were set up for each individual data point per experiment. Mixed colonies (CFUGEMM) defined as containing at least erythroid and granulocytic cells by their in situ appearance, erythroid bursts (BFU-E) with more than 500 cells and granulocyte-macrophage colonies (CFU-GM) with more than 40 cells were all scored from the same plates.

Cytogenetic Analysis

Cytogenetic analysis and GTG-banding techniques were performed in each case prior to and after suspension culture according to standard methods. Patients showing >30% Ph^1-negative metaphases were considered responsive to the purging procedure.

Surface Marker Analysis

Phenotypic analysis was performed by indirect and direct immunofluorescence using a Coulter EPICS-Profile II flow cytometer. The following monoclonal antibodies were used: MY10 (gently provided by Prof. C.I. Civin, John Hopkins Oncology Center, Baltimore, U.S.A.), B73.1 (gently provided by Dr. B. Perussia, Wistar Institute, Philadelphia, USA) and IL2-Rl (Coulter, Hialeah, USA).

Proliferation Assay

To assay proliferative activity of untreated and mafosfamide-treated MNCS, $5x10^4$ cells in 200 ul were cultured (37C, 5% CO_2, 7 days) in 96-well, flat-bottomed microculture plates (Costar) in the same complete medium used for suspension cultures. Recombinant GM-CSF was added in the appropriate samples. Calls were pulsed with 2 uCi/well of [^{3}H]-thymidine during the last

six hours of culture and were harvested with an automated harvester onto glass fiber filters. The radioactivity was determined by liquid scintillation counting.

Statistical Analysis

Statistical analysis was performed with the statistical package Statview (BrainPower Inc., Calabasas, CA, USA) run on a Macintosh Plus personal computer. The significance of changes in the percentage of Ph-negative metaphases between samples was evaluated in each patient by the chi-square analysis.

RESULTS

Table II shows the growth of CFU-GEMM, BFU-E. and CFU-GM from untreated and mafosfamide treated CML marrow cells. Mafosfamide induced a nearly complete suppression of colony growth in all patients studied. In addition, the progenitor cell content of each sample was evaluated prior to and after suspension culture. This analysis was performed by replacing marrow by volume with no correction made for cell death or proliferation and failed to reveal any amplification of clonogenic compartments due to the presence in the liquid phase of rGM-CSF (data not shown).

Table III summarizes the results of cytogenetic analysis performed in the 15 patients studied prior to and after suspension culture of marrow cells treated with mafosfamide. Seven of fifteen cases showed 100% Ph^1-positive metaphases in the bone marrow on direct cytogenetic analysis. In the remaining cases the percentage of Ph^1-positive metaphases on direct cytogenetic analysis ranged from 47 to 90%. By definition, patients showing <30 Ph^1-negative metaphases were considered responsive to the purging treatment. Based on this criterion we could identify two groups: group I including six cases (nos. 1-6) which were responsive the purging procedure, and group II including 9 cases (nos. 7-15) which were unresponsive. Group II included 3 out of 4 patients studied at diagnosis and prior to any treatment. Among unresponsive patients were included cases nos. 7, 12, and 13 who showed 53%, 37% and 37%, respectively, of Ph^1-negative metaphases on direct cytogenetic analysis but failed to reveal any significant increase of these percentages. In the group of responsive patients the mean (+/-SD) value of Ph^1-negative metaphases on direct cytogenetic analysis was 10%($\pm$9). This value could be increased to 34% ($\pm$17) after suspension culture, 46% ($\pm$26) after suspension culture with rGM-CSF, 53% ($\pm$12) after mafosfamide treatment and 63% ($\pm$29) by combining mafosfamide treatment and suspension culture with rGM-CSF.

Table IV shows changes in proliferative activity due to mafosfamide and rGM-CSF. All patients revealed a significant increase of ^{3}H-thymidine due to rGM-CSF stimulation. Interestingly, mafosfamide induced a nearly complete suppression of proliferative activity only in cytogenetically responsive patients but usually failed to induce any suppression in cytogenetically unresponsive cases.

On the basis of these observation, four patients have been autografted with mafosfamide-purged marrow. In two cases Ph^1-positive cells reappeared 5 and 7 months after ABMT and remain as a minor population 24 months following transplantation in one patient, while the other evolved into blastic crisis 18 months following ABMT. In the third case, Ph^1-positive cells were not detected for 6 months and their reappearance 9 months after ABMT was followed by blastic transformation. The fourth case is Ph^1-negative 8 months after ABMT.

DISCUSSION

Clinical as well as experimental evidences support the existence of Ph^1-negative, polyclonal hematopoietic stem cells in the majority of patients with CML. It seems therefore attractive to consider autologous marrow transplantation as an alternative therapeutic strategy for CML patients in chronic phase. ABMT should represent a procedure able to delay the onset of blastic transformation by inducing a period of Phl-negative hematopoiesis or by reducing the size of the leukemic stem cell compartment.

The methodological approach described herein is based on the combination of a chemical purging with the cyclophosphamide derivative mafosfamide and a liquid culture purging, recently shown to be effective for isolating Ph^1-negative stem cells (9). In addition, CML marrow cells were exposed throughout the suspension culture phase to rGM-CSF, known to act at an early stem cell level (10).

The negligible number of colonies growing in semisolid culture following mafosfamide treatment made it impossible to study cytogenetic changes at the level of progenitor cell compartments. Therefore, all mafosfamide-induced cytogenetic modifications - reflecting events involving the proliferating cells - were obtained from cells growing in suspension cultures. To allow a direct comparison, all samples were analyzed by means of a similar approach.

According to data reported by the Vancouver group (11), the short-term suspension culture phase allows the emergence of Ph^1-negative cells. The use of rGM-CSF to enhance cell growth following mafosfamide treatment represents an intriguing issue. However, cytogenetic analysis performed following suspension culture as well as the evaluation of clonogenic cell content of liquid samples stimulated with rGM-CSF demonstrated that this factor is not able to selectively stimulate Phl-positive CML cells. Indeed, in some of our cases RGM-CSF "per se" was able to preferentially stimulate the proliferation Ph^1-negative cells (Table III).

Mafosfamide was effective in inducing the reappearance of Ph^1-negative cells in 6 of 15 patients studied. With the exception of case no. 1, all the responsive cases were previously treated, i.e., they had been exposed in vivo to cytoreductive therapy able to reduce the size of the leukemic compartment. A complete disappearance of Ph^1-positive cells was observed only in one case (no. 6). In addition to cytogenetic changes, mafosfamide induced immunologic modifications, such as increased expression of the natural killer cell marker

A complete disappearance of Ph^1-positive cells was observed only in one case (no. 6). In addition to cytogenetic changes, mafosfamide induced immunologic modifications, such as increased expression of the natural killer cell marker B73.1 and the IL2rec, that might play a relevant role by triggering immunological antileukemic activities.

Taken together these data allow to hypothesize that mafosfamide preferentially exerts its marked cytotoxic effect on leukemic cells, thus facilitating the reappearance of previously dormant Ph^1-negative cells that - at least in a subset of patients - might become the predominant hematopoietic population.

Based on these observations, a pilot study has been carried out in vivo to evaluate the effect of autograft CML patients in second chronic phase with mafosfamide purged marrow. All four patients transplanted so far achieved a Ph^1 chromosome negative phase ranging from 5 to 9 months. Two of these patients underwent blastic transformation 9 and 18 months following ABMT, respectively. Of the remaining, one has a minor population of Ph^1-positive cells 24 months after ABMT, while the other is still Ph^1-negative after 8 months.

The Ph^1-negative phase observed in all our patients implies that mafosfamide purging is effective in reducing the size of the malignant clone and might induce through its cytotoxic and immune actions a modification of the balance between leukemic and normal clones.

Although limited, the present results are encouraging. More extensive studies are required to optimize the ex vivo purging procedure. In order to increase the therapeutic index of mafosfamide, we are currently investigating the possibility to "prime" CML marrow cells with colony-stimulating factors and then purge them. Similarly, a larger group of patients with CML in first chronic phase is required to evaluate the relevance of a combined purging technique.

ACKNOWLEDGMENT

This work was supported in part by grants from Consiglio Nazionale delle Ricerche (nos. 88.01907.04 and 88.00847.44), and by Ministers Pubblica Istruzione (40% 50%, 1989). Authors' affiliations: (1) Department of Hematology, Bone Marrow Transplantation Unit, University of Parma; (2) Institute of Medical Genetic, University of Parma; (3) Department of Hematology, "La Sapienza" University, Rome, (4) Division of Hematology, Ospedale S. Martino, Genova, Italy.

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TABLE 1

Clinical and hematological data of the patients at the time of the study

Case	Age/Sex	Clinical Status	Peripheral Blood			Bone Marrow	Previous Therapy
			Hb g/dl	Plt $x10^9/L$	WBC $x10^9/L$	Cytogenetic (% Ph-positive)	
1.	49/F	CP	11.7	488	80	100	no
2.	48/M	CP	13.7	155	4.5	80	HU/ABMT
3.	26/M	CP	14.7	240	6.1	90	IFN
4.	37/M	2nd CP	15	180	6.8	90	IFN/CT
5.	41/M	CP	15.7	268	11	100	HU/IFN
6.	48/F	CP	12.2	156	3.9	80	IFN
7.	43/M	CP	13.4	186	6.4	47	IFN
8.	21/M	CP	15.6	348	47	100	HU
9.	35/F	CP	8.0	314	275	100	no
10.	68/M	CP	10.0	927	696	100	no
11.	58/M	CP	14.0	627	61	100	no
12.	24/M	CP	15.9	338	77	63	HU/IFN
13.	45/M	CP	14.2	298	8.7	63	IFN
14.	38/M	CP	10.8	753	19	88	HU/IFN
15.	38/M	2nd CP	9.3	97	3.0	100	HU/IFN/CT

Hb, hemoglobin; Plt, platelet counts; WBC, white blood cell counts; CP, chronic phase; ABMT, autologous bone marrow transplantation; HU, hydroxyurea; IFN, interferon-alpha; CT, chemotherapy.

TABLE 2

Progenitor cell growth from untreated and mafosfamide-treated marrow cells

Patient	Untreated Cells *			Mafosfamide-Treated Cells *		
no.	CFU-GEMM	BFU-E	CFU-GM	CFU-GEMM	BFU-E	CFU-GM
1	3±1	25±5	122±10	0	1±1	11±3
2	1±0.25	8±2	232±21	0	0	0
3	4±1	44±5	224±9	0	1±1	8±5
4	0	5±1	80±4	0	0	2±0.5
5	0	16±3	32±2	0	1±1	7±1
6	NE	NE	NE	NE	NE	NE
7	3±0.5	24±3	91±20	0	0	2±1
8	0	30±6	181±3	0	0	0
9	0	0	40±3	0	0	2±1
10	NE	NE	NE	NE	NE	NE
11	7±1	69±5	88±4	0	1±1	3±2
12	NE	NE	NE	NE	NE	NE
13	0	27±5	42±5	0	0	0
14	0	0	176±10	0	0	20±4
15	3±1	24±2	265±9	0	0	19±4

* Each value represents the mean (±SD) number of progenitors per 5×10^4 mononuclear cells plated
NE, not evaluated

TABLE 3

Percentage (and absolute number) of Ph^1-negative metaphases in bone marrow samples prior to and after suspension culture.

CASE	Direct BM	Control	GM-CSF	Mafosfamide	Mafosfamide + GM-CSF
1.	0 (10)	24 (17)	18 (22)	47* (19)	18 (17)
2.	20 (50)	30 (10)	60* (10)	60* (10)	70* (10)
3.	10 (10)	50 (10)	77* (11)	50 (8)	71* (7)
4.	10 (31)	14 (7)	18 (11)	50* (24)	75* (15)
5.	0 (10)	27 (15)	31 (13)	37 (8)	42§ (12)
6.	20 (30)	58 (19)	70 (30)	73§ (11)	100* (30)
7.	53 (15)	59 (32)	81 (21)	67 (15)	62 (24)
8.	0 (20)	9 (22)	11 (56)	ne	27* (11)
9.	0 (8)	0 (2)	0 (12)	0 (2)	ne
10.	0 (10)	0 (5)	0 (21)	0 (10)	0 (15)
11.	0 (20)	0 (25)	0 (18)	0 (10)	0 (18)
12.	37 (8)	43 (7)	31 (16)	ne	ne
13.	37 (8)	0 (13)	0 (21)	ne	ne
14.	12 (17)	11 (27)	10 (22)	6 (16)	ne
15.	0 (10)	12 (8)	ne	8 (12)	ne

ne = no analysable metaphase

§ Increases in the percentages of Ph^1-negative metaphases were statistically significant ($p < 0.05$)

• Increases in the percentages of Ph^1-negative metaphases were statistically significant ($p < 0.01$)

* Increases in the percentages of Ph^1-negative metaphases were statistically significant ($p < 0.001$)

TABLE 4

^{3}H-thymidine incorporation by CML marrow cells treated with mafosfamide and cultured for 7 days with and without rGM-CSF

Case	Control	GM-CSF	Mafosfamide	Mafosfamide + GM-CSF
1.	1,565 *	4,775	244	321
2.	1,550	7,972	160	1,660
3.	6,186	13,587	293	962
4.	2,679	8,179	2,332	3,216
5.	2,961	12,935	603	1,934
6.	ne	ne	ne	ne
7.	ne	ne	ne	ne
8.	6,383	17,236	143	219
9.	5,902	7,506	459	277
10	853	1,545	4,325	4,189
11.	3,646	9,858	3,803	2,386
12.	1,660	5,437	1,820	2,321
13.	ne	ne	ne	ne
14.	2,970	9,437	1,606	3,236
15.	2,630	13,679	3,841	10,437

* Counts per minute (mean from triplicate well); ne, not evaluated;

FIGURE 1
Schematic illustration showing the experimental design for CML marrow purging.

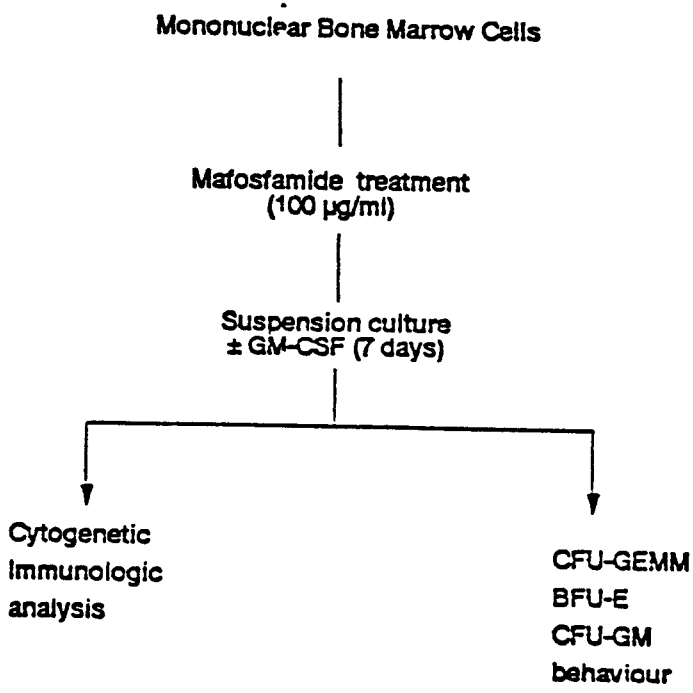
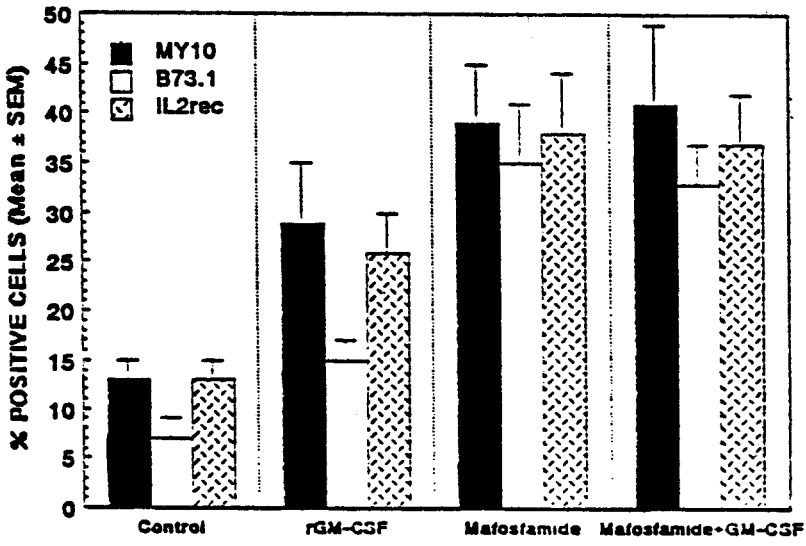


FIGURE 2

Expression of MY10,BY73.1 and IL2rec from CML marrow cells treated with mafosfamide and cultured in suspension for 7 days with and without rGM-CSF.



AUTOTRANSPLANTS IN LEUKEMIA: APPROACHES TO PREVENT LEUKEMIA RELAPSE

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Leukemia relapse is a common cause of treatment-failure following autotransplants in leukemia. Here, leukemia can recur because of persisting leukemia cells in the subject, leukemia cells reinfused with the bone marrow graft, or both. It is important to distinguish between these alternatives since their correction implies different strategies: developing more effective pretransplant therapy or developing effective means to treat the graft in vitro or post-transplant.

We analyzed leukemia relapse rates after chemotherapy, twin transplants, autotransplants and HLA-identical allogeneic transplants without graft-versus-host disease (GVHD) to determine the predominant reason(s) for relapse. We used results of twin transplants as a model of intensive therapy since the graft is free of leukemia cells and since immune antileukemia effects associated with GVHD and allogeneic graft-versus-leukemia effects do not operate.

In adults with acute lymphoblastic leukemia (ALL) in first remission, relapses occurred in 65 percent of age and time-censoring adjusted subjects receiving chemotherapy. Relapses also occurred in 35 percent of twins, in 55 percent of autotransplant recipients and in 35 percent allograft recipients without GVHD. These data suggest that more intensive therapy decreases relapse in ALL and that most relapses after autotransplants develop from the graft. They also indicate the absence of an antileukemia effect distinct from GVHD.

Comparable relapse rates in adults with acute myelogenous leukemia (AML) in first remission are 65 percent, 60 percent, 50 percent and 30 percent. These data suggest that more intensive therapy does not decrease relapses and that most relapses can be accounted for by persisting leukemia in the subject. Whether leukemia cells in the graft also contribute to relapse is not presently ascertainable. In AML there is an antileukemia effect distinct from GVHD.

Comparable relapse rates in chronic myelogenous leukemia (CML) in chronic phase are 100 percent, 95 percent, 45 percent and 15 percent. Here, recipients of T-cell depleted transplants without GVHD have a relapse rate of 60 percent. These data suggest that more intensive therapy decreases relapse

but that it is largely ineffective and that relapse can be accounted for by persisting leukemia in the subject and in the graft (or both). In CML there appear to be two distinct antileukemia effects distinct from GVHD; one appears to be mediated by T-cells.

These data regarding probable causes of leukemia relapse after autotransplants, including the efficacy of intensive therapy and possible immune antileukemia mechanisms, should be useful in analyzing results of autotransplants and in planning future trials.

FIGURE 1

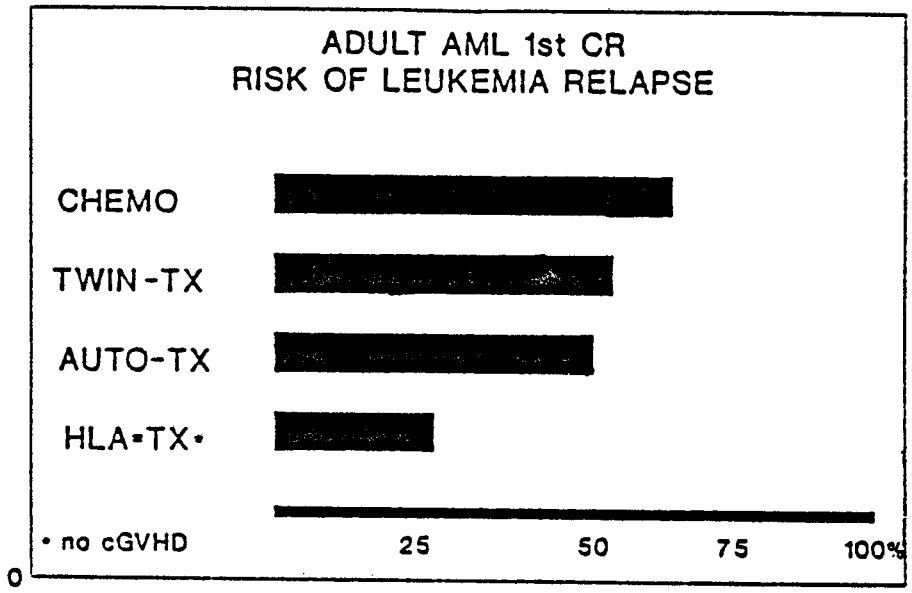


FIGURE 2

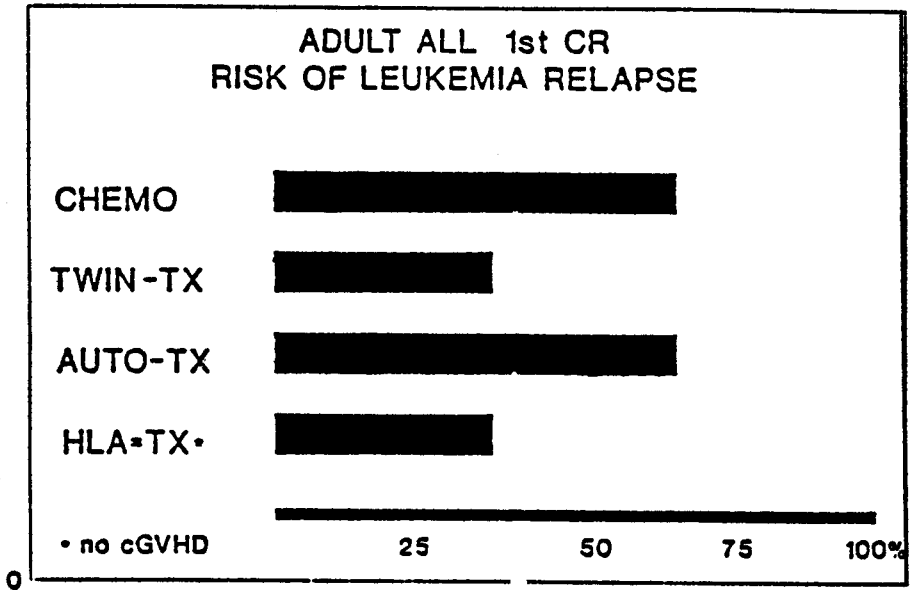
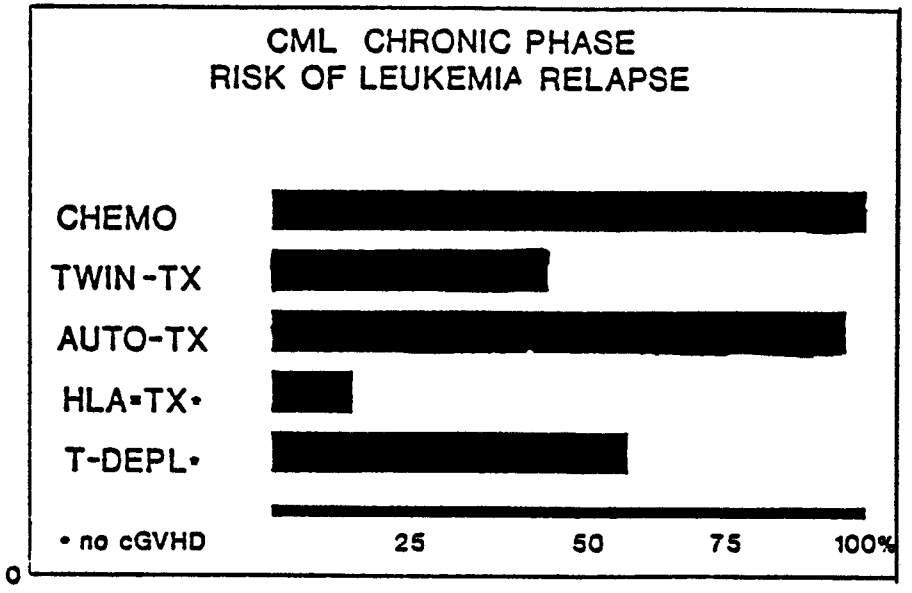


FIGURE 3



CRYOPRESERVING STEM CELLS WITHOUT CONTROLLED RATE FREEZING

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INTRODUCTION

Traditionally, bone marrow is harvested during the recovery phase after chemotherapy and is cryopreserved using a mixture of 10% DMSO with various concentrations of albumin (1-5). A few investigators utilize stem cells without freezing, usually by storing the cells at 40C in a refrigerator (6,7). This refrigerated bone marrow is in a good condition for a short duration and requires expert handling. The quality and quantity of stem cells cannot be consistently predicted so this method should be used with caution. Peripheral blood stem cells alone or in combination with marrow are also being utilized by several investigators and offer accelerated engraftment in most circumstances (8,9).

METHODS

Cryoprotectants work by decreasing the ice-crystal formation when liquid state goes through the transition phase into the solid ice state. Various agents that are able to decrease icicle formation include: Dimethyl sulfoxide (DMSO), glycerol, albumin, poly vinyl pyrolidone and hydroxyethyl-starch (HES) (6-9) Marrow is not usually cryopreserved in glycerol, because glycerol when infused into the patient may cause lactic acidosis. Washing out glycerol from the stem cells is a cumbersome process and often results in a loss of cell viability. Most institutions cryopreserve bone marrow at -196C (liquid nitrogen or vapor-phase of liquid nitrogen). Usually the source of stem cells (marrow and/or peripheral blood) are enriched for mononuclear cells. The cryoprotectant is then added and the cells are usually cryopreserved using controlled rate freezing. Lysis and clumping of mature granulocytes can occur with this method. Some investigators physically remove these cells prior to freezing, others add albumin or DNAse to minimize this problem (1-9).

Previous investigators had shown that a mixture of 6% HES, 5% DMSO and 4% albumin (HDA) was very useful in cryopreserving granulocytes. These frozen thawed granulocytes retain significant biological activity, survive

in greater numbers and cause less clumping than cells cryopreserved in 10% DMSO alone (10).

Stiff et al demonstrated a lack of clumping when unfractionated bone marrows were cryopreserved in HDA mixture. The same freezing mixture could be used to cryopreserve stem cells at -196C without controlled rate freezing (the special controlled rate freezing apparatus usually decreases temperature at 1C per minute). This control rate freezing equipment is expensive and requires trained technicians (1-11).

Our freezing method utilizing HES, DMSO, Albumin (HDA), did not cause any significant agglutination upon thawing of the stem cells and after thawing, no DNAse therapy was needed. The stem cells cryopreserved in HDA have been used in several investigations and it is clear that this freezing procedure is easy to use, less expensive and reliable (5,11,12,13).

We freeze marrow at -120C in plastic bags without controlled rate freezing. Marrow frozen at -120C has reasonable viability and growth patterns. Perhaps the marrow frozen at -196C will be viable for a longer period of time than that frozen at -120C, although the cost of freezing and handling marrow at -120C is much more practical. Table I describes the difference in viability of bone marrow cryopreserved at different temperatures. Viability results after freeze/thawing had minimal differences (6% lower viability at -196C) after 1-2 months of storage, similar viability was observed after 3 months and 7 months of freezing. In a separate study the quality of cryopreserved bone marrow remains constant over time in the first 7 months of follow up when examining viability, CFU-GM and BFU-E growth (Table II). In three bone marrows cryopreserved at -120C for 38, 40, and 80 months, we observed median of 87%, 84% and 77% viability, respectively. This suggests good long term quality of bone marrow up to 80 months of freezing.

DISCUSSION

Several authors feel that leukemic cells do not cryopreserve as well as normal hematopoietic cells. In a recent study (14), six different freezing conditions were utilized and all of the freezing mixtures tested were ineffective in preserving leukemic cells. Approximately 1% of the leukemic cells were found to be viable after cryopreservation. HSC cryopreserved in DMSO may demonstrate a decrease in the proliferation of malignant cells as DMSO can promote differentiation resulting in loss of proliferative and malignant potential. DMSO, is a well known differentiating agent and its effect on the differentiation of leukemic cells is well documented. In a recent publication the clinical toxicity of cryopreserved marrow infusion in 82 patients was reported (15). All marrows were cryopreserved in 10% DMSO and stored in liquid nitrogen. Varying symptoms of nausea, abdominal cramping and flushing were noted. Forced vital capacity decreased in most patients receiving buffy coat concentrates. A significant group of patients developed transient hypertension with 38% of these patients requiring additional medications within six hours

(15). Decreased heart rates were observed in most recipients of treated buffy-coat cells with asymptomatic bradycardia in almost half.

Eighty-eight of recipients of density-gradient separated grafts had decreased heart rates and 81% had increased blood pressure although the degrees of change were less than those experienced by the recipients of treated buffy-coat cells. Forced vital capacities were not affected by the infusion of the density-gradient separated grafts. Additional risks include the development of microemboli, hemoglobinuria, allergic reaction to BM processing reagents and infection.

In one series, the risk of bacterial contamination was reported to be 17%. Ex vivo manipulation of some of the grafts prior to infusion may have caused additional bacterial contamination. All isolated bacteria were common skin flora (16).

There are many factors which may effect the hematopoietic engraftment of cryopreserved bone marrow. We must first consider the overall quality of the HSC. The bone marrow can have long term toxicity from previous treatment. Agents like BCNU, Bleomycin are known to have chronic toxicity. The bone marrow also has to recover from the acute toxicity of the previous therapy. We like to harvest bone marrow when the blood counts are in the recovery phase after nadir. In the laboratory, room temperature during cell processing, purging agents, time spent in processing and additional reagents added may effect the hematopoietic engraftment results. At time of transplant, the duration of storage as well as the type of freezer, duration of thawing process, and quality of venous access must be considered.

Marrow engraftment also depends on the number of HSC. The viability of frozen thawed HSC, CFU-GM, BFU-E and CFU-GEMM growth of infused bone marrow is also being investigated. Table III summarizes our bone marrow engraftment data for various AuBMT protocols (5,13). The least number of cells capable of hematopoietic engraftment and variations for each protocol is also given. Several factors may be involved in hematopoietic engraftment (5,13,17). AML patients had significantly delayed engraftment. The conditioning and purging regimine for patients with AML and lymphoma was identical. The delayed engraftment in patients with AML in comparison to patients with lymphoma suggests an alteration in HSC in patients with AML.

We feel the HDA mixture described in this study is a useful method for hematopoietic reconstitution and is worthy of wider clinical use.

ACKNOWLEDGEMENTS

This paper is supported in part by the Morgan-Murray Fund and the Lisa Bilotti Foundation.

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TABLE 1

DIFFERENCE IN STORAGE at -120°C vs -196°C

	HARVEST VIABILITY	
	-120°C	-196°C
1-2 months	91%(87-94)[3]	84%(84-90)[3]
3 months	89.5%(89-90)[2]	89.5%(88-91)[2]
7 months	90%[1]	90%[1]

[# of patients]

TABLE 2

QUALITY OF BM CRYOPRESERVED AT -120°C IN 6% HES, 5% DMSO AND 4% ALBUMIN

# of Pts.	Months Cryopreserved	Percent Recovery (Range) Viability	CFU-GM	BFU-E
6	2.6 (0.3-4.6)	92(89-97)	65(37-100+)	70(29-100+)
3	7(7)	99(96-100+)	85(56-100+)	93(80-100+)

TABLE 3

Hematopoietic Engraftment of Patients Undergoing Autologous Bone Marrow Transplant

Group	Diagnosis	Protocol	No. Patients	Median No Viable Cells /Kg x10^8	Median Days (Range) for Count Recovery		
					WBC >1000	Absolute Neutrophil Count >500	Platelets >50 x 10^3
1,2A	NHL	TBI/Cyclophosphamide No purge	19	3.42 (1.62)*	12(10-19)	13(8-20)	23(13-40)
2B	NHL	TBI/Cyclophosphamide 4-HC purge	14	2.3 (0.98)	17(11-42)	17(12-42)	30(22-121)
3A	NHL	TBI/ Etoposide/ Cyclophosphamide No purge	10	2.75 (1.5)	16.5(15-23)	16(13-24)	30.5(27-40)
3B	NHL	TBI/Etoposide/ Cyclosphophamide 4-HC + Etoposide purge	13	2.0 (1.2)	14(11-13)	14(13-39)	40.5(28-67)
4	HD	Carmustine, Etoposide (250mg/m^2 x 3d) Cyclophosphamide	22	2.8 (1.5)	19.5(10-27)	19.5(10-27)	28(17-44)
5	HD	Carmustine/Etopside (350mg/m^2 x 3d) Cyclophosphamide	17	2.6 (1.3)	14.5(10-36)	14 (8-42)	28(15-41)
6	HD	TNI/Etoposide/ Cyclophosphamide	27	3.1 (1.4)	13.5(10-28)	14(10-30)	29(17-50)
7	AML	TBI/Etoposide/ Cyclophosphamide 4-HC & Etoposide purge	14	2.8 (1.4)	39(27-53)	37(16-43)	84(42-269)

*Minimum cell dose /kg x 10^8. NHL = Non-Hodgkin's Lymphoma; TBI = Total Body Irradiation; 4-HC = 4-Hydroperoxycyclophosphamide; HD = Hodgkin's Disease; TNI = Total Nodal Irradiation; AML = Acute Nonlymphoblastic Leukemia

CHARACTERISTICS OF GRAM-POSITIVE SEPTICEMIA IN PATIENTS WITH NEUTROPENIC FEVER

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INTRODUCTION

It is widely accepted that neutropenic fever should be treated with empiric antibiotics with activity against gram-negative organisms including most Pseudomonas species. During the past decade coagulase-negative staphylococci have become a frequent cause of bacteremia in neutropenic patients (1). The increased incidence of coagulase-negative staphylococcal infections has led many centers to use vancomycin in addition to antibiotics with gram-negative activity for the empiric treatment of neutropenic patients (2). However, Rubin et al. reported that witholding vancomycin until there was a proven clinical infection with coagulase-negative staphylococci did not increase morbidity and decreased cost and exposure to toxicity in those patients who did not have infections with the organism (3).

Several recent reports have described septicemia in neutropenic cancer patients caused by viridans streptococci (4,5,6). Bacteremia with viridans streptococci has caused pulmonary infiltrates, septic shock syndrome or other organ dysfunction that has resulted in the death of some patients (5,6). We are reporting a high incidence of both coagulase-negative staphylococcal and viridans streptococcal bacteremia that occurred in leukemia and marrow transplant patients enrolled in an antibiotic trial that did not include empiric vancomycin.

PATIENTS AND METHODS

Adult patients undergoing bone marrow transplant or induction or consolidation chemotherapy for the treatment of acute leukemia were randomly assigned to one of two arms of an antibiotic trial. All patients received five million units of nystatin four times a day throughout the hospitalization. Patients assigned to arm 1 received 500 mg. of ciprofloxacin twice a day from day -7 (with day 0 being the day of marrow transplant) until the patients absolute neutrophil count reached 500 cells/cu mm. At the time of first neutropenic fever (defined as a temperature of 38.5 degrees centigrade or more

when the absolute neutrophil count was less that 500 cells/cu mm) patients in arm 1 received empiric antibiotic therapy with intravenous ceftazidime, 2 grams every 8 hours. Patients assigned to arm 2 received prophylactic ciprofloxacin as in the first arm and oral ampicillin 250 mg every 6 hours. The first neutropenic fever was treated with ceftazidime and piperacillin, 3 grams every 4 hours. The ampicillin was discontinued when the piperacillin was started but ciprofloxacin was continued as in arm 1. Patients in both arms who remained febrile or had new fever after 7 days of empiric antibiotics received empiric amphotericin at the dose of 0.5 mg/kg/day until engraftment of neutrophils.

Patients with infections documented by clinical signs or microbiological tests were treated with agents appropriate for the infections. Sepsis was defined as two or more blood cultures positive for bacteria or fungus or one positive blood culture associated with fever or other clinical signs of infection.

When patients had a temperature of 38.5 degrees centigrade, 20 milliliters of blood were drawn with sterile technique from the central catheter and innoculated into an aerobic and an anaerobic blood culture bottle. This was usually not repeated more that once every 24 hours. Patients who were neutropenic and on steroids had daily blood cultures. Blood cultures were processed with a Bactec system that utilized radiometric analysis of CO_2. Bacterial sensitivity to antibiotics were measured by disk sensitivity.

RESULTS

The patients' characteristics are listed in Table 1. The distribution of patient age, sex, diagnosis, and treatment was similar between the two groups. The mean age for all 143 patients was 36 years (range 18 - 78 years).

The incidence of bacteremia was similar in both arms of the study. There were 45 separate episodes of bacteremia in 37 patients treated in Arm 1, while in Arm 2, 36 patients had 38 episodes of bacteremia. The bacterial and fungal species isolated from blood cultures are listed in table 2.

Ninety-three percent of the episodes were caused by gram-positive bacteria. The most common gram-positive bacteria was coagulase-negative staphylococci. These organisms were isolated from 34 episodes of bacteremia. Three of the episodes occurred in patients without fever or other signs of infection and the organism was grown from only one culture bottle. The remaining 31 episodes occurred in patients with fever and met our definition of septicemia. The second most common organism isolated from blood cultures was viridans streptococcus. These organisms were isolated from 31 of the 83 episodes of bacteremia and caused septicemia in 27 patients. The remaining 12 episodes of gram-positive bacteremia were caused by corynebacteria, diphtheroids, bacillus species, Enterococci and Staphylococcus aureas.

Septicemia with coagulase-negative staphylococci occurred a median of 11 days (range -7 - 67) after marrow transplant or initiation of chemotherapy in leukemia patients. Nine of the 31 (29%) coagulase-negative staphylococci septicemias were associated with the first neutropenic fever. One patient had prolonged neutropenia and many positive blood cultures despite appropriate

treatment and the removal of the central venous catheter. The patient died of hypotension and renal failure and autopsy showed a large subclavian clot proximal to where the catheter tip had been that grew coagulase-negative staphylococci.

Septicemia caused by viridans streptococci occurred significantly earlier than those caused by coagulase-negative staphylococci. Septicemia secondary to viridans streptococci occurred a median of 6 days after transplant. One patient was septicemic 45 days after transplant but the remaining septicemias occurred from the day before transplant to 12 days after transplant. Twenty-five of the 27 episodes (93%) were associated with the first neutropenic fever. Three patients developed pulmonary infiltrates and hypotension with septicemia and two of the patients went on and died of viridans streptococcal sepsis. Both patients had vancomycin and gentamicin empirically added several hours after the onset of fever when they deteriorated on ceftazidime and piperacillin.

Twenty-three of the 27 viridans streptococcal isolates that caused septicemia were available for further speciation and sensitivity testing. Seventeen of the 23 isolates were Streptococcus mitis, three were Streptococcus mutans, two were Streptococcus sanguis and one was Streptococcus anginosus. Disc sensitivity showed that only 61% of the isolates were sensitive to penicillin and only 48% of the isolates were sensitive to ciprofloxacin. Vancomycin and gentamicin were the only two drugs that the organisms were uniformly susceptible to. All of the coagulase-negative staphylococcal isolates were susceptible to vancomycin.

DISCUSSION

Many investigators have noted that coagulase-negative staphylococci are the most common cause of bacteremia in patients receiving cancer chemotherapy. Recently some centers have noted that there is an increasing incidence of viridans streptococcal bacteremia in this patient population and as in this study viridans streptococci are more frequently associated with neutropenic sepsis than coagulase-negative staphylococci or gram-negative bacteria (5,6). Othere studies have also shown that the most common viridans species causing sepsis was Streptococcus mitis (6,7).

Treatment for acute leukemia, treatment regimens containing high-dose Ara-C and a younger age were risk factors associated with viridans streptococcal sepsis in one study (6). Another study indicated that the use of quinolones for prophylaxis was associated with viridans streptococcal sepsis in leukemia patients (8). The patients in this study were adults and did not receive high-dose Ara C. However, all patients did receive oral Ciprofloxacin for bacterial prophylaxis and this may account for the marked increase in septic episodes caused by viridans streptococci that we observed.

Guiot et al. reported that intravenously administered penicillin prevented neutropenic patients from developing viridans streptococcal sepsis (9). In this study the patients receiving oral ampicillin for prophylaxis had a

similar incidence of viridans streptococcal septicemia as patients who did not receive ampicillin. This may have been because of noncompliance, poor drug absorption or penicillin resistance. We observed a number of viridans streptococcal strains resistance to penicillin similar to a previous report (7).

As reported by other authors, we observed a streptococcal sepsis syndrome that was characterized by fever, hypotension, pulmonary infiltrates and death. Some of the pulmonary complications observed with viridans streptococcal sepsis may be related to the use of high-dose Ara C (5,6,8). The incidence of pulmonary complications was not as high in our patients as reported in some studies and may be explained by the fact that our patients were not treated with high-dose Ara C.

The high incidence of viridans septicemia associated with first neutropenic fever and the early progressive sepsis despite maximum support observed in two patients has led us to add vancomycin for empiric treatment for neutropenic fever and discontinue bacterial prophylaxis with quinolones.

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TABLE 1

PATIENT CHARACTERISTICS

	Group I N = 67	Group II N = 76	Total N = 143
Median age (range)	37 years (20-75)	34 years (18-78)	36 years (18-78)
Sex M:F	33:34	35:41	68:75
Diagnosis			
HD	25	25	50
NHL	22	28	50
Leukemia	10	12	22
Solid tumor	10	11	21
Treatment			
Autologous BMT	55	62	117
Allogeneic BMT	8	9	17
Leukemia induction/ consolidation	4	5	9

Group I = decontamination - ciprofloxacin and nystatin,
empiric treatment - ceftazidime

Group II = decontamination - ciprofloxacin, ampicillin and nystatin
empiric treatment - ceftazidime and piperacillin

HD = Hodgkin's disease NHL = Non-Hodgkin's lymphoma

TABLE 2

ORGANISMS ISOLATED FROM BLOOD

	Group I N = 67	Group II N = 76	Total (% of all bacteremias)
Patients with Bacteremia	37	36	73
Episodes of Bacteremia	45	38	83
Gram-Positive Bacteria	42	35	77 (93%)
Coagulase-negative Staphylococci	17	17	34 (41%)
Viridans Streptococci	17	14	31 (37%)
Corynebacteria, diphtheroids or Bacillus sp.	5	3	8 (10%
Enterococci	2	0	2 (2%)
S. aureus	1	1	2 (2%)
Gram-negative Bacteria	2	1	3 (3.5%)
Fusobacterium	2	0	2 (2%)
K. pneumonia	0	1	1 (1.5%)
Candida	1	2	3 (3.5%)

FLUCONAZOLE FOR PROPHYLAXIS OF FUNGAL INFECTIONS IN NEUTROPENIC PATIENTS

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INTRODUCTION

Fungal sepsis and invasive fungal infections are life threatening conditions in immunocompromised neutropenic patients (1-3). The management of these infections is often disappointing. Despite the use of amphotericin-B, which remains the drug of, choice, treatment failures are not uncommon. Difficulty in diagnosis and poor outcome when therapy is delayed are the chief reasons for treatment failure. Prophylaxis of fungal infection in patients at risk has been attempted with various antifungal agents, including oral nystatin, miconazole, ketoconazole and amphotericin- B. These have all demonstrated inconsistent efficacy with the reported data still controversial (4-12).

The lack of a consistent benefit from prophylactic approaches has resulted in an increasing tendency to use amphotericin-B empirically (13-15). This approach appears to have reduced the incidence of fungal sepsis, but is associated with frequent toxic side effects especially renal dysfunction (16). This latter problem is of special concern when patients are concurrently receiving other potentially nephrotoxic drugs such as an aminoglycoside and/or cyclosporine-A.

Fluconazole is a new triazole antifungal agent available in both oral and parental forms and has a high level of penetration into most tissues and body fluids including saliva and cerebrospinal fluid (17). It has excellent in vitro activity against candida species but apparently limited activity against aspergillus species. Excretion is mainly by the renal route and side effects appear to be minor and infrequent. Fluconazole has minimal interaction with cyclosporine-A and would therefore appear to be an excellent agent to use in transplant patients (18,19). It has been shown to be effective for treatment of oropharyngeal candidiasis in patients with cancer and patients infected with the human immunodeficiency virus (20-21). Numerous studies evaluating the specific indications of fluconazole in immunocompromised patients are in progress (22-27). These reports are encouraging and have led to the evaluation of this agent as prophylaxis in neutropenic patients.

PATIENTS AND METHODS

Patients hospitalized on the Adult Leukemia and Bone Marrow Transplant Units of the UCLA Medical Center for cytotoxic therapy expected to produce neutropenia (<500 neutrophils/ul) lasting for two or more weeks were eligible. The cytotoxic treatment regimens and management have been previously described (28). Briefly, patients with leukemia received conventional doses of cytarabine and danuorubicin; patients undergoing bone marrow transplantation received combinations of total body irradiation and cyclophosphamide together with either etoposide or cytarabine in addition. Bone marrow transplant patients received cyclosporine-A for prevention of graft-versus-host disease. All patients routinely received oral antibiotic prophylaxis with norfloxacin but no antifungal prophylaxis other than the study drug. Patients were housed in individual rooms and surveillance fungal cultures were obtained from the nasopharynx, oropharynx, axilla, and inguinal skin, urine and stool or perirectal area prior to entry and weekly until end of the study. Informed consent approved by UCLA the Institutional Review Board was obtained from all patients.

At the time of initiating cytotoxic therapy, patients were randomized in a double blind fashion to receive either fluconazole 400 mg or placebo orally every day (Table 1). Once neutropenia developed patients received broad spectrum antibiotics for fever related to documented or suspected bacterial infection utilizing a double beta-lactam regimen (29). For patients continuing to be febrile while on broad spectrum antibiotics with no evidence of documented infection empiric amphotericin-B was initiated at the discretion of the attending physician. Granulocyte transfusions were not used in any patient. All patients who developed evidence of fungal infection were taken off study and treated with amphotericin-B, except if the infection was considered non-life threatening (i.e., oral candidiasis).

RESULTS

As of August 1, 1990, 80 patients were entered into the study with 75 of these completing the study (acute leukemia 36 patients, bone marrow transplant 39 patients). Eleven patients (15%) developed documented fungal infections; 7 of these had localized candidiasis (oropharyngeal); 2 had disseminated candidiasis (fungemia); 1 had pulmonary aspergillosis and 1 patient developed mucormycosis of the soft tissue in the left forearm (Table 2). A substantial number of patients 39/75 (52%) required empiric amphotercin-B for persistent fever while on broad spectrum antibiotics.

DISCUSSION

The randomization code of this double blind placebo controlled study has not been broken, so that only a preliminary analysis can be carried out at this time. The overall incidence of fungal infection (15%) is substantial but not

different to that reported in other large cancer centers (1,2). The majority of fungal infections in these immunocompromised patients is due to candida species. Fluconazole is very active against this fungal agent but appears to have limited activity against aspergillus (23). It would therefore be expected that patients receiving fluconazole prophylactically would have a reduced incidence of candida infections.

The use of empiric amphotericin-B has been considerable (52%). This probably reflects the growing tendency for this approach to be initiated for fear of not starting "established" antifungal therapy early in patients at risk for life-threatening fungal infections (13-15). This practice will most likely continue until appropriate studies firmly demonstrate that antifungal agents such as fluconazole are effective for prophylaxis of fungal infections. Indeed, a recent study evaluating the efficacy of fluconazole as prophylaxis for oropharyngeal candidiasis in susceptible cancer patients has been very encouraging (30). In this study 1/58 (2%) patients receiving fluconazole compared to 15/54 (28%) patients given placebo developed candidiasis ($p=.003$). The patients were all receiving cancer chemotherapy for metastatic malignancies, but only 14 had neutropenia (<1000 neutrophils/ul). This study demonstrated that fluconazole was effective in preventing candida infection and that the patients at greatest risk were those already colonized prior to starting therapy.

Other antifungal agents have been used for prophylaxis in susceptible patients. Nystatin has not proven to be effective and is associated with poor compliance due to its nauseating taste (3-5). Clotrimazole is well tolerated and appears to be effective in reducing oral candidiases but is not well absorbed from the gastrointestinal tract and therefore has no effect on systemic infections (11,12). Ketoconazole has been reported to be effective in reducing the risk of fungal infections but interacts adversely with other drugs, and is poorly absorbed when the acidity of the stomach is reduced thereby limiting its use (5,7,8). Fluconazole has few adverse effects and is an ideal antifungal agent for evaluating the effect of prophylaxis in patients at risk. Ongoing studies will hopefully prove its efficacy in this regard.

ACKNOWLEDGEMENT

Supported by grant CA-23175 from the National Cancer Institute and a research grant from Pfizer Pharmaceuticals.

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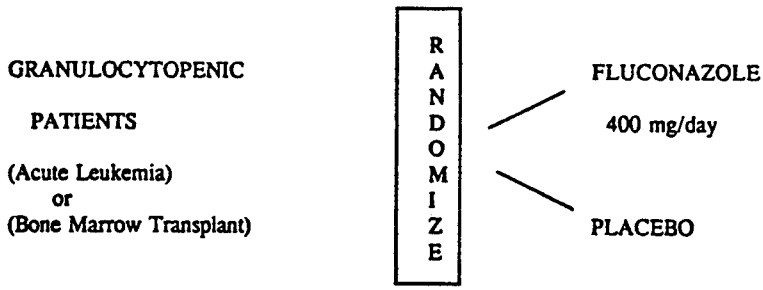
TABLE 1

STUDY DESIGN



FUNGAL SURVEILLANCE

Nasopharynx
Oropharynx
Axilla/Inguinal skin
Urine
Stool/Rectal swab

OFF STUDY CRITERIA

1. Documented fungal infection requiring therapy with Ampho-B

2. Suspected fungal infection requiring empiric Ampho-B

TABLE 2

INTERIM ANALYSIS OF STUDY

# patients completed study	75
Acute Leukemia	36
Bone Marrow Transplant	39
# patients with documented fungal infection	11/75 (15%)
Localized Candidiasis	7
Disseminated Candidiasis	2
Aspergillus	1
Mucormycosis	1
# patients requiring empiric Ampho-B	39/75 (52%)

HERPES VIRUS INFECTIONS AND ANTI-VIRAL THERAPY IN AUTOLOGOUS BONE MARROW TRANSPLANT RECIPIENTS

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INTRODUCTION

The herpes viruses are major causes of morbidity and mortality in patients undergoing allogeneic bone marrow transplantation (1). These infections have been well studied in this patient population and therapeutic strategies have been formulated to prevent or treat these infections. With the increasing application of autologous bone marrow transplantation as therapy for patients with hematologic malignancy or with selected solid tumors, it is important to understand the incidence and severity of herpes virus infections in this patient population so that therapeutic recommendations can be made to prevent or treat them. In this manuscript we will briefly review herpes simplex virus, cytomegalovirus and varicella-zoster virus infections in autologous bone marrow transplant recipients and comment on treatment and prevention of these infections.

The predictable temporal occurrence of these infections following bone marrow transplantation is the same following autologous transplantation as it is following allogeneic transplantations. Herpes simplex virus infections generally occur early following bone marrow transplantation (median 8 days), cytomegalovirus infections generally occur between months 1 and 3 following transplantation and varicella zoster virus infections generally occur late (median, 5 months) after transplantation. The viruses will be discussed sequentially based on their occurrence following transplantation.

Herpes Simplex Virus

Herpes simplex virus infections occur secondary to reactivation of latent virus and true primary infections are extremely rare. The incidence of reactivation following autologous transplantation is between 70 and 80% percent and is therefore similar to that seen following allogeneic bone marrow transplantation (2). Virtually all patients who reactivate virus develop lesions which may be quite severe. The lesions may be atypical and felt to be secondary to drug or radiation induced mucositis. Local disruption of mucosal barriers secondary to the infection may cause significant pain and provide

portals of entry for bacteria and fungi at a time when bone marrow function is markedly impaired by the preparative regimen. Herpes simplex esophagitis and rarely pneumonitis were also seen in the pre-anti-viral era. The time from onset to healing in this type of patient in the absence of antiviral therapy is a median of 21 to 28 days (3,4). Fortunately the developments in field of antiviral chemotherapy have led to the availability of compounds which demonstrate activity against the herpes viruses. Acyclovir, a deoxyguanosine analog, was developed in the 1970's (5,6). This compound has selective activity against herpes simplex and varicella zoster since these viruses encode for a virus specific thymidine kinase which activates the compound to the monophosphate. Host cellular enzymes convert the monophosphate to the triphosphate. Acyclovir-triphosphate inhibits the viral DNA polymerase and when incorporated into nascent viral DNA serves as a chain terminator of viral DNA replication. This compound is relatively non-toxic when given in doses which inhibit herpes simplex and varicella-zoster virus replication.

Several of the first clinical trials evaluating acyclovir as a therapeutic agent were performed in the bone marrow transplant population. We showed in a prospective, randomized, placebo-controlled double-blind trial that acyclovir was capable of inhibiting herpes simplex virus replication and preventing the development of culture positive lesions. In that trial bone marrow transplant recipients who had latent herpes simplex virus (defined by the presence of antibody to the virus) were randomized to receive intravenous acyclovir or placebo starting 3 days prior to transplantation and continuing for 18 days. Seven of 10 placebo recipients developed culture positive herpes simplex virus lesions while none of 10 who receive acyclovir developed culture positive lesions ($p=0.003$)(7). This clinical trial was one of the first demonstrations of the efficacy of acyclovir as an antiviral agent in humans. In a later clinical trial acyclovir was shown to be effective treatment for established herpes simplex virus infection in bone marrow transplant recipients (4). Patients were randomized to receive acyclovir or placebo. Those that received acyclovir had a dramatic antiviral effect. Lesions were free of virus a median of 3 days following initiation of therapy compared to 17 days in the placebo group ($p<0.0005$). This antiviral effect translated into a more rapid time to healing in the acyclovir group (median 14 days) compared to the placebo group (median 28 days). Based on a comparison of the results of this trial and our prophylaxis trial, we currently use acyclovir to prevent herpes simplex virus infections from occurring in our seropositive autologous bone marrow transplant patients. Our rationale is based on the fact that acyclovir can prevent reactivation of herpes simplex virus and therefore prevent lesions while treatment of active lesions will result in healing but only a median of 14 days after initiation of acyclovir therapy.

With the introduction of acyclovir into widespread clinical practice, a concern was rased about the development of resistance to the compound. To date clinically significant resistance has been reported in a small group of patients and has not emerged a major problem (8). The vast majority of

clinically significant herpes simplex virus strains which are resistant to acyclovir have been thymidine kinase mutants anecdotal experience suggests that foscarnet, a pyrophosphate inhibitor, may be effective in treating patients who develop active infection with acyclovir-resistant herpes simplex virus (9).

Cytomegalovirus

Cytomegalovirus is the major viral pathogen causing mortality following allogeneic bone marrow transplantation (10). Cytomegalovirus pneumonia following allogeneic bone marrow transplantation historically was associated with a case-fatality rate of greater than 80%. Enteritis secondary to the virus can cause significant morbidity. Infection occurs because of reactivation of latent virus or acquisition of the virus in the pre- and post- transplant time period. The vast majority of primary infections occur via transmissions of the virus through blood products. Cytomegalovirus infections in autologous bone marrow transplant patients have been less well studied than in allogenic bone marrow transplant patients. In a series of 143 patients who received autologous bone marrow transplantation at Johns Hopkins, 65 (45%) were found to have cytomegalovirus infection. Ninety-four patients had latent virus (antibody to cytomegalovirus) prior to transplantation and 44 (47%) developed infection in the post transplant period. Forty patients were seronegative prior to transplant and 20 (50%) developed infection in the post transplant period. Only 3 of 65 patients who had cytomegalovirus infection developed cytomegalovirus pneumonia. Therefore the overall incidence of cytomegalovirus pneumonia was 2% (3 of 143). This contrasts with 12% incidence (45 of 386) of cytomegalovirus pneumonia in allogeneic bone marrow transplant recipients ($p=0.0002$) in our institution. Therefore the severity of cytomegalovirus infection is much less in our autologous bone marrow transplant recipients. In fact the risk for developing cytomegalovirus pneumonia in autologous bone marrow transplant recipients is the same as for allogeneic transplants who do not develop acute graft versus host disease. This suggests the deleterious effect of graft versus host disease and/or its treatment on the immune response to the virus. We also noted in seropositive patients that cytomegalovirus infection was associated with neutrophil and platelet recovery. Patients with infection had a delay in neutrophil recovery (500 cells/ul) with a median day of recovery of 31 days compared to 24 days in patients without infection ($p=0.02$). A more dramatic finding was in platelet recovery to an untransfused count of 50,000/ul or greater. Infected patients recovered a median of 97 days after bone marrow transplantation compared to 35 days for uninfected patients ($p=0.003$) (11). A previous study demonstrated similar effects of cytomegalovirus infection on slowing the rate of platelet recovery (12). In a recent study the Seattle group has summarized their experience with cytomegalovirus in autologous bone marrow transplant recipients (13). In a series of a 159 patients, 22.5% of patients seronegative for cytomegalovirus and 61.1% of seropositive patients developed infection in the post transplant period. In contrast to our series 11 of 159 (7.0%) patients developed cytomegalovirus pneumonia which was fatal in nine cases. They were unable to detect significant effects of cytomegalovirus

infection on the rate of platelet or neutrophil recovery after transplant. Clearly more studies are necessary to define the exact role of cytomegalovirus as pathogen following autologous bone marrow transplantation.

There has been significant progress in the treatment and prevention of serious cytomegalovirus infections following allogeneic bone marrow transplantation. No controlled clinical trials employing these approaches have been performed in autologous bone marrow transplant recipients. These trials will be necessary but there are several approaches that need to be considered based on their efficacy in allogeneic bone marrow transplant recipients.

The use of ganciclovir and intravenous immunoglobulin for treatment of cytomegalovirus pneumonia has clearly reduced overall mortality from this disease in allogeneic bone marrow transplant recipients when compared to historical controls and this approach is clearly the treatment of choice in patients with the disease (14,15). The use of prophylactic immunoglobulin or the use of screened blood products has resulted in a reduction in the incidence of serious cytomegalovirus disease in allogeneic bone marrow transplant recipients who were seronegative to the virus prior to transplantation (16,17). We have not used either approach in our autologous bone marrow transplant patients since we have not encountered cytomegalovirus pneumonia in a seronegative patient following autologous transplantation. However, five of the patients in the Seattle who developed cytomegalovirus pneumonia were seronegative prior to transplantation. This should lead to consideration of the use of cytomegalovirus-seronegative blood products or leukocyte depletion of blood products. Allogeneic bone marrow transplant recipients who are seropositive for cytomegalovirus prior to transplantation may benefit from prophylaxis with acyclovir. This approach needs to be studied in autologous bone marrow transplant patients to determine its role in this patient population. The effectiveness of immunoglobulin prophylaxis of severe cytomegalovirus infection in seropositive allogeneic bone marrow transplant recipients has not been demonstrated and it has no current role in autologous bone marrow transplant recipients. Ganciclovir is currently under investigation as a prophylactic agent in allogeneic bone marrow transplant recipients. Until these trials are completed and fully analyzed, the use of this compound in autologous bone marrow transplant recipients in a similar manner is not indicated outside the context of an appropriately designed clinical trial. Finally, monoclonal antibodies against cytomegalovirus have been developed and the use of these compounds in clinical trials particularly in prophylaxis will be of importance to both allogeneic and autologous bone marrow transplantation.

Varicella-Zoster Virus

Varicella-zoster virus infections have been well studied in allogeneic bone marrow transplant recipients (1). The frequency of infection with the virus ranges from 17 to 50%. Median time to onset was 5 months following transplantation. In patients with localized zoster the rate of dissemination was high in the pre-anti-viral era (45%) and death occurred in 10% of patients. We

have reviewed the frequency, risk factors and outcome of varicella zoster virus infections in 153 patients who underwent autologous bone marrow transplantation (18). Forty three (28%) of these patients developed varicella-zoster infection after transplant. Median time of onset was five months after transplant and 90% of the cases were seen in the first year. Thirty-three of the patients (77%) presented with localized herpes zoster and ten (23%) presented with varicella. Cutaneous dissemination was observed in 15% of patients and probable visceral dissemination occurred in 5% of patients. No deaths from varicella-zoster infection were seen. However, the majority (79%) of patients received treatment with intravenous acyclovir. The major risk factor for developing varicella zoster infection was the patient's underlying disease. Patients with Hodgkin's disease and non-Hodgkin's lymphoma were at greatest risk (21 of 46; 46%) compared to leukemia patients (20 of 88; 23%) or solid tumor patients (2/25; 9%) ($p=0.002$). This study shows that varicella-zoster virus infections are common following autologous bone marrow transplantation. Because of the availability of effective anti-viral therapy for this disease, all physicians managing these patients should be aware of this complication.

The treatment of choice for varicella-zoster virus infections is intravenous acyclovir. This compound was shown to be effective in a randomized double-blind placebo controlled clinical trial evaluating the compound in the treatment of herpes zoster in immunosuppressed patients (19). In a later clinical trial acyclovir was compared to vidarabine in the treatment of varicella-zoster infections in bone marrow transplant recipients (20). Both compounds were given intravenously and acyclovir showed clear superiority as a therapeutic agent. oral acyclovir has the disadvantage of poor bioavailability. Therefore achieving levels of the compound which are capable of inhibiting varicella-zoster virus replication requires high doses of the compound (800mg) given frequently (five times a day). Use of oral acyclovir in the treatment of zoster at these doses showed superiority to placebo in non-immunocompromised patients with herpes zoster. However our preference is to use the intravenous compound in the treatment of autologous bone marrow transplant recipients with the disease.

CONCLUSION

As the clinical experience with autologous bone marrow transplantation as a therapeutic modality increases, it is clear that the herpes viruses may cause clinically significant disease in the post transplant period. It is also clear that the frequency and temporal occurrence of these infections has been well defined and most importantly that therapeutic strategies are in place to prevent or to treat these infections.

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BONE MARROW CONCENTRATION USING THE COBE SPECTRA

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ABSTRACT

Bone marrow is processed to remove red cells and debris and to concentrate hematopoietic cells. The efficacy of the COBE Spectra Cell Separator for bone marrow processing was evaluated by two methods. The COBE Spectra mononuclear cell collection program and white cell tubing set were used. In Procedure I, bone marrow was processed by a discontinuous flow method for five to six passes until 20% of the original volume was collected. The recovery of nucleated cells with Procedure I was 55 $\pm$ 11% and of mononuclear cells was 75 $\pm$ 16%. The hematocrit was decreased from 27 $\pm$ 5% to 11 $\pm$ 5%. In Procedure II, bone marrow was processed by a continuous flow method for six passes.

The recovery of nucleated cells with Procedure II was 51 $\pm$ 10% and of mononuclear cells was 84 $\pm$ 12%. The hematocrit was reduced from 24 $\pm$ 5% to 5 $\pm$ 1%. Hematopoietic precursor assays (CFU-GM) demonstrated excellent recovery of viable hematopoietic stem cells with both procedures.

INTRODUCTION

Bone marrow is processed after harvesting to remove debris and red cells and to concentrate hematopoietic precursor cells. Differential centrifugation and dextran sedimentation with collection of the buffy coat layer are the standard methods of bone marrow processing (1). The processed bone marrow contains the same proportion of mononuclear cells (MNC) and polymorphonuclear cells (PMN) as the original harvest. The MNCs contain the hematopoietic precursor cells which are needed for engraftment. Red cells and PMNs are poorly cryopreserved and may cause clumping of the marrow, hemolysis and other adverse reactions at the time of marrow infusion (2).

Density gradient centrifugation can yield a MNC-enriched product, but is a labor intensive technique (3,4). The COBE Spectra is a continuous flow cell separator which produces an enriched mononuclear cell product from blood (5). We have explored the adaptability of the COBE Spectra to bone marrow

processing. We have studied the nucleated cell (NC) and MNC recovery, MNC enrichment, RBC and PMN depletion and viability of recovered cells.

MATERIALS AND METHODS

The COBE Spectra was adapted for bone marrow processing as follows: The Spectra white cell tubing set and mononuclear collection program were utilized. Bone marrow was anticoagulated using concentrated trisodium citrate. Two procedures were utilized as outlined below. The recovery of NC, MNC and PMN and the hematocrit were determined at intervals during the processing. Viability of the recovered cells was tested by hematopoietic precursor assays (6). Eighteen autologous bone marrows were processed with Procedure I. Seven autologous bone marrows were processed with Procedure II.

Procedure I: Discontinuous Processing Method (Diagram I)

Bone marrow was processed with an inlet flow rate of 69 cc/min and collect flow rate of 3 cc/min with the interface adjusted to collect at a hematocrit of 8-10 percent. Separate bone marrow access and return bags were used. At the completion of each pass the procedure was interrupted to transfer bone marrow from the return to the access bag again. Five to six passes were carried out until 20 percent of the original volume was collected. The procedure time averaged 90 minutes.

Procedure II: Continuous Processing Method (Diagram II)

A 16 gauge, 8 inch catheter was inserted into the bone marrow reservoir bag and connected to the return line from the Spectra tubing kit. The Spectra inlet line was connected via a second port into the same bag. Bone marrow was processed continuously for 4-6 passes with an inlet flow rate of 80 cc/min. The collect flow rate was 3 cc/min for the first two passes. The remaining passes were collected at a flow rate of 1.5 cc/min. The interface was adjusted to collect at a hematocrit of 5 percent for the first four passes and a hematocrit of 10 percent for the last two passes.

RESULTS

The recovery of NC and MNC for Procedures I and II is compared in Table I. The efficiency of MNC recovery was better for Procedure II (84 $\pm$ 12%) compared to Procedure I (76 $\pm$ 16%), although total NC recovery was nearly identical for the two procedures (55 $\pm$ 11% and 51 $\pm$ 10%). Procedure II resulted in greater enrichment of MNC (84 $\pm$ 7% vs 72 $\pm$ 12%) and depletion of PMN. The recovery of MNC was proportional to the volume collected, but PMN contamination also increased proportionally to the volume collected. Procedure II resulted in collection of fewer red cells (average 9cc) compared to Procedure I (average 30 cc) in a smaller collect volume (Table II). The viability of the cells was well maintained as measured by hematopoietic

precursor assay (CFU-GM) (Table III). Ten patients have undergone autologous bone marrow transplantation using bone marrow processed by Procedure I (7 patients) or Procedure II. The time to engraftment for WBC > 500, WBC > 1000 and platelets > 25,000 was similar between the two protocols.

CONCLUSION

The COBE Spectra Cell Separator can efficiently process bone marrow in a 90-120 minute procedure. The processed bone marrow is highly enriched in mononuclear cells and depleted of polymorphonuclear cells and red cells. The overall recovery of nucleated cells is 54% $\pm$ 11% and recovery of mononuclear cells is 78 $\pm$ 15%. The continuous processing method (Procedure II) produces better recovery of mononuclear cells in a smaller volume with fewer red cells compared to the discontinuous processing method (Procedure I). Hematopoietic precursor assays (CFU-GM) demonstrate excellent recovery and viability of hematopoietic stem cells.

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TABLE 1

OVERALL CELL RECOVERY

	NC	MNC	PMN
Procedure I (n = 18)	55 ± 11%	76 ± 16%	33 ± 17%
Procedure II (n = 7)	51 ± 10%	84 ± 12%	16 ± 6%

STEPWISE CELL RECOVERY

	Collect Volume	NC	MNC	PMN
Procedure I				
	10%	43 ± 8	64 ± 10	20 ± 14
	15%	48 ± 9	71 ± 13	24 ± 15
	20%	55 ± 11	76 ± 16	33 ± 17
Procedure II				
	6%	32 ± 11	53 ± 16	8 ± 5
	10%	43 ± 12	70 ± 15	12 ± 4
	13%	51 ± 10	84 ± 12	16 ± 6

ENRICHMENT OF MONONUCLEAR CELLS

	Percentage MNC	
	Unprocessed BM	Collected BM
Procedure I	52 ± 7%	72 ± 12%
Procedure II	52 ± 11%	84 ± 7%

TABLE 2

DEPLETION OF RED CELLS

	AVERAGE VOLUME	HEMATOCRIT	RED CELL VOLUME
UNPROCESSED BM	1300 cc	26 ± 5%	350 cc
PROCEDURE I	260 cc	11 ± 5%	30 cc
PROCEDURE II	180 cc	5 ± 1%	9 cc

TABLE 3

VIABILITY OF PROCESSED CELLS

	n	% Recovery of CFU-GM
UNPROCESSED BM	13	---
PROCEDURE I	7	125 ± 30
PROCEDURE II	6	97 ± 50

TABLE 4

TIME TO ENGRAFTMENT

		Day (Range)		
	n	WBC >500	WBC >1000	Plts >25,000
PROCEDURE I	7	15 (11-33)	23 (12-33)	35 (13-250+)
PROCEDURE II	3	14 (9-17)	15 (11-17)	28 (13-60+)

FIGURE 1

PROCEDURE I: DISCONTINUOUS METHOD

INLET FLOW RATE	69 cc/min	NUMBER OF PASSES	5-6
COLLECT FLOW RATE	3 cc/min	TARGET HEMATOCRIT	8-10%
		COLLECT VOLUME	20%

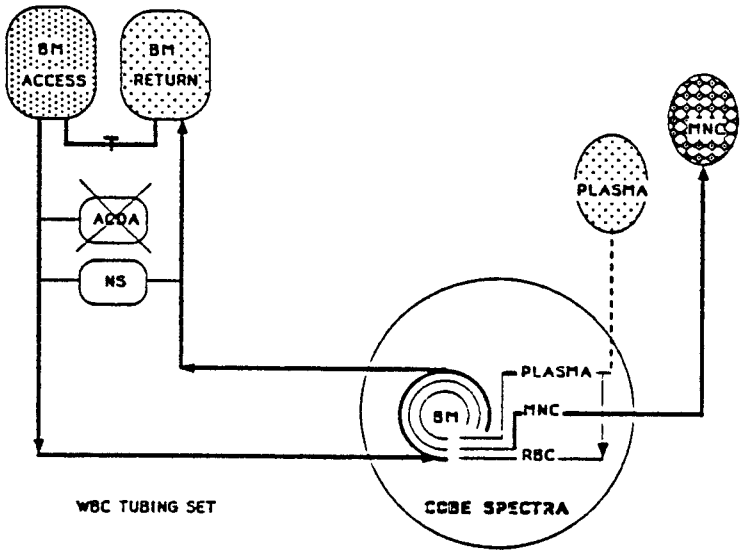
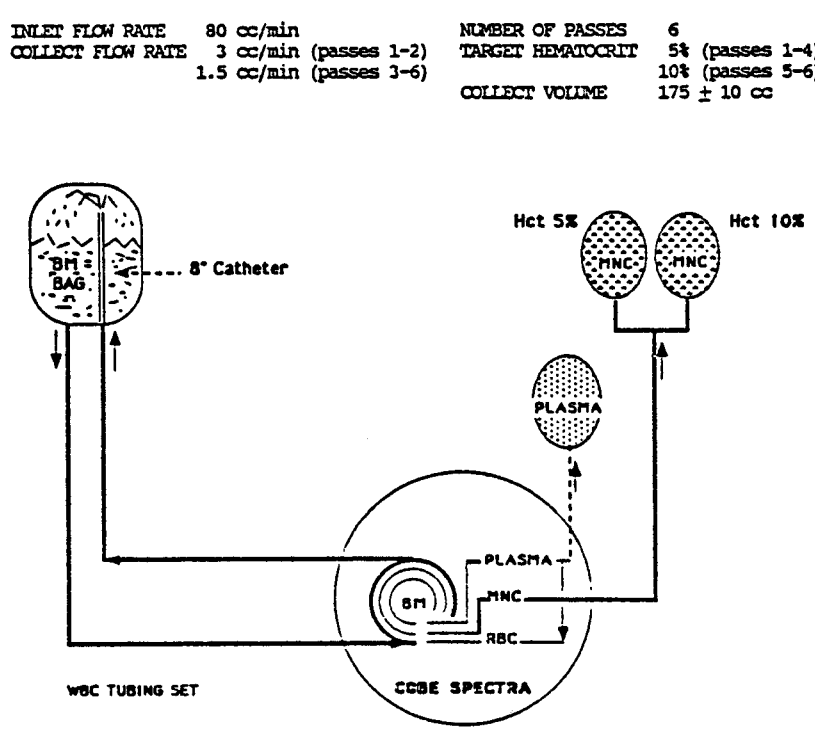


FIGURE 2


PROCEDURE II: CONTINUOUS METHOD
INLET FLOW RATE 80 cc/min
COLLECT FLOW RATE 3 cc/min (passes 1-2)
1.5 cc/min (passes 3-6)
NUMBER OF PASSES 6
TARGET HEMATOCRIT 5% (passes 1-4)
10% (passes 5-6)
COLLECT VOLUME 175 ± 10 cc
BM = BAG
8" Catheter
Hct 5%
Hct 10%
MNC
MNC
PLASMA
PLASMA
MNC
RBC
BM
WBC TUBING SET
COBE SPECTRA

BONE MARROW CELL SEPARATION WITH THE FENWAL CS3000 MACHINE

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INTRODUCTION

Several methods for hemopoietic stem cell concentration from bone marrow (BM), before its "ex vivo" treatment or cryopreservation, have been extensively investigated (1,2,3,4). The main objective of all these methods is the recovery of the majority of the stem cells in order to restore the hemopoiesis of patients when the BM is reinfused.

The extent of purification of the stem cell fraction to be reached depends on the further use of BM. Thus, "ex vivo" BM treatment procedures need processing methods yielding low final volume where the majority of red blood cells and granulocytes are removed, in order to avoid excessive reactives consumption or inactivation.

The above mentioned processing conditions are desirable as previous steps of BM cryopreservation, in order to reduce the amount of cryoprotectant and lysed cells infused into patient. With this purpose, several automated or semiautomated methods for mononuclear cell (MNC) fraction recovery using blood cell separators have been described. These methods generally involve the utilization of compounds such as ficoll (4,5) or percoll (6,7) as density gradient media, and time consuming techniques. As a consequence, the most common practice in autologous bone marrow transplantation, when "ex vivo" treatments are not needed, is the simple volume reduction and nucleated cell concentration.

In this study we present our experience in BM cell processing with the Fenwal CS3000 blood cell separator. Our experience shows that this cell separator gives a high MNC purification in an automated way and in a closed system, yielding an important reduction of the final volume.

MATERIAL AND METHODS

BM from 28 patients suffering from acute leukaemia (12), lymphomas (8) and solid tumors (8) was harvested under general anesthesia, according to standard techniques (8).

The aspirated marrow was collected and filtered through a marrow collection set (4R2104, Fenwal) and transferred into a bag containing 7% acid citrate dextrose (ACD-A) of final volume. Harvested volumes ranged from 332 to 1724 ml. BM processing was performed with a Fenwal CS-3000 blood cell separator (Baxter, Spain) using the standard "granulo" separation chamber and "A-35" collection chamber. Prior to BM processing, the separator was primed automatically.

Unprocessed marrow was transferred into a 2000 ml Fenwal bag, where we have created an internal circulation system, in order to improve cell recovery processing and its automatization, avoiding further serial connections to secondary bags, performed for separating input and output marrow fluxes (see Figs. 1 and 2). In addition, this modified bag is connected to an "H" shape tubing system for its utilization in the reinfusion phase (Figs. 1 and 2). All these steps were carried out in sterile conditions, in a laminar air flow chamber. The inlet and return line of the apheresis kit (F4R2230 Fenwal) were connected to the free arms of the "H" tubing system.

BM processing has been performed with the program not of platelet collection of the Fenwal CS3000, in an automated way, but with the following modifications : L60 (1000), L61 (0000), L71 (2000) y L78 (0950). The blood flow rate was 50 ml/min and the centrifuge speed was 1600 rpm. During all the processes ACD was not utilized.

During the first part of the procedure, plasma was collected into the transfer pack until the plasma flow rate was 10 ml/min. Then, the BM working hematocrit was adjusted with saline solution to 40% the plasma flow rate ratio (L68) was changed to 0520, according with the working hematocrit and the centrifuge speed was changed to 1400 rpm. After these modifications, the separation procedure continues automatically until the BM volume, after hematocrit adjustment, was processed five times.

When the cell recovery procedure is finished (final volume collection container was 200 ml), reinfuse procedure was performed. In five cases, the reinfuse program (platelet reinfuse procedure) was modified to get, in an automated way, the collection of the residual BM into the unprocessing BM bag and a reduction of the final volume of the collection container. In this case, once the cell recovery procedure is accomplished, halt/irrigate button was pressed.

Before reinfuse procedure was performed, tubing line fluxes were modified to allow simultaneous liquid removal from the separation cell and collection cell bag. For this purpose, the roller clamps on both the inlet and return line of H " tubing system were closed and the bypass clamp was opened. The lower part of the air trap was clamped and a tube line of the unprocesed BM was connected in the latex of the air trap (Fig 2). After then, the reinfuse process is initiated and continues automatically.

The reinfuse procedure has three steps:

A) 50 ml of plasma from the transfer pack unit and saline solution were pumped to clean the reinfuse tubing lines and to check the tubing

connections. In this step, centrifuge and blood pump are working but plasma pump doesn't work in all the reinfuse procedure.

B) Centrifugation stops and by the action of blood pump the simultaneous removal of plasma from collection and separation bags begin this plasma collection into the unprocessed BM bag.

C) Plasma collection from the two bags continues but with centrifugation (550 rpm), to avoid the loss of cells in the collection bag, until the final volume in this bag is approximately 80 ml.

Nucleated cell recovery after BM processing has been considered as the average of manual and automatic counting cells. MNC (lymphocytes and monocytes) recovery has been evaluated by cytological examination. Red blood cells and platelet reduction after BM cell separation were evaluated by automatic counting and granulocytes reduction by cytological examination.

CFU-GM and long term marrow cultures (LTBMC) using Pike Robinson (9) and Gartner & Kaplan (10) techniques, respectively, have been performed before and after BM processing.

CD34 positive cell recovery after BM cell separation has been evaluated by indirect immunofluorescence with HPCA-1 monoclonal antibody (Becton Dickinson) and flow cytometry analysis (Epics Profile, Izasa, Spain).

RESULTS

BM cell recovery results after BM processing with the Fenwal CS3000 are expressed in Table 1. A median of 74.56% (22.6-322) of MNC from the initial BM was recovered in a final product, containing just 2.7% (0-27.4) and 2.53% (0.8-18.5) of initial granulocytes and red blood cells. CFU-GM and CD34 positive cell recoveries after BM processing were 55.56% (3.6-261) and 66.53% (17.8-1446), respectively.

The modifications performed in the reinfuse program in order to accomplish a greater reduction in the final volume, after BM cell separation, have not affected final cell recovery, as it is shown in Table 2.

In the five procedures where volume reduction was performed, MNC, CFU-GM and CD34 positive cell recoveries were 90.55% (25.3109.3), 82.54% (21.4-163) and 32.89% (28.9-48.7) respectively. Moreover, granulocytes and red blood cells removal in the final product are similar to those obtained without volume reduction (table 2). This modified reinfuse program allows to an overall improvement on final volume reduction of a 10% (92.97% vs. 82.57%) compared to the standard program.

LTBMC performed after BM cell separation during 6 weeks behaved in similar way to those from Ficoll BM separation. After four weeks, all LTBMC showed normal adherent layer development and accumulative cell and CFU-GM recoveries.

DISCUSSION

"Ex vivo" BM processing is, nowadays, a widely spread practice in BM transplantation which most times requires the obtention of highly purified hemopoietic progenitors. This fact promotes the research on new automatic techniques allowing a better and more selective recovery of BM cells. These should include the development of standardized processes, in closed systems, to minimize the risk of microbial contamination.

The majority of the techniques developed until now for BM processing, are based on the obtention of the buffy coat or in the MNC fraction recovery as a final product. The first techniques mentioned have the disadvantage of the obtention of an excessive volume and high granulocytes and red blood cells contamination. The second techniques have the inconvenience of the utilization of compounds such as ficoll or percoll, being more time consuming.

In the present work, we have developed an automatic and closed system technique for BM processing with the Fenwal CS3000, obtaining an adequate MNC fraction cell recovery, with reduced red blood cells and granulocytes contamination in the final product without the utilization of density gradient agents.

In previous studies, using the IBM 2991 (5,11), Haemonetics V50 (3,12) or the Fenwal CS3000 (3,13), using Ficolldiatriazoate, Percoll or albumin, MNC recoveries from harvested marrow, were about 75%, which is similar to the results obtained in our series (74.56%) where any density gradient agent was not used.

In terms of red blood cells and granulocytes removal, our results are similar to those obtained by other author using density gradient methods (5,12). Even CFU-GM recovery, after processing, can be considered as slightly low, compared to other series (14), it could be partially explained by qualitative changes in cell fraction recovered, which condition different culture behavior. Nevertheless, CD34 positive cell recoveries, as well as, LTBMC performed after BM processing, confirms a sufficient hemopoietic progenitor recovery. Furthermore, ten patients autografted with BM processed according to this procedure showed a normal hematological reconstitution post transplantation (data not shown).

As it is well known CD34 positive cell quantification has some technical problems induced by the low number of cells bearing this antigen and its low density expression. This fact causes some aberrant results as obtained in one of our cases.

With the modifications performed in the standard reinfuse program, incorporated into the Fenwal CS3000, and in the apheresis kit, it has been possible the reduction of the final product volume from 200 ml to approximately 80 ml. As far as we know, this volume reduction is greater than those obtained with another blood cell separators performed in an automatic way, using standard separation and collection chambers, and without any collection bag manipulation. In theory, this system allows a further reduction until a final volume of approximately 50 ml without significant cell loss.

ACKNOWLEDGEMENTS

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TABLE 1

Results of 28 Bone Marrow Separation procedures using the Fenwal CS3000.

	n	Median (%)	Min-Max (%)
Nucleated cell recovery	28	20.69	3.2-45.6
MNC recovery	27	74.56	22.6-322
CFU-GM recovery	19	55.56	3.6-261
CD34 + cell recovery	12	66.53	17.8-1446
Granulocyte reduction	27	97.21	72.6-100
Platelet reduction	28	52.19	26.8-71.9
Red blood cell reduction	28	97.47	81.5-99.2
Volume reduction	28	82.57	39.7-94.6

TABLE 2

Results of 5 bone marrow separation procedures using the Fenwall CS3000 and followed by volume reduction.

	n	Median (%)	Min-Max (%)
Nucleated cell recovery	5	19.33	3.8-45.6
MNC cell recovery	5	90.55	25.3-109.3
CFU-GM recovery	4	82.54	21.4-163
CD34 + cell recovery	3	32.89	28.9-48.7
Granulocyte reduction	5	97.91	72.6-98.4
Platelet reduction	5	60.84	47.5-71.9
Red blood cell reduction	5	98.80	95.7-99
Volume reduction	5	92.97	90.8-94.6

FIGURE 1

Processing flow diagram.

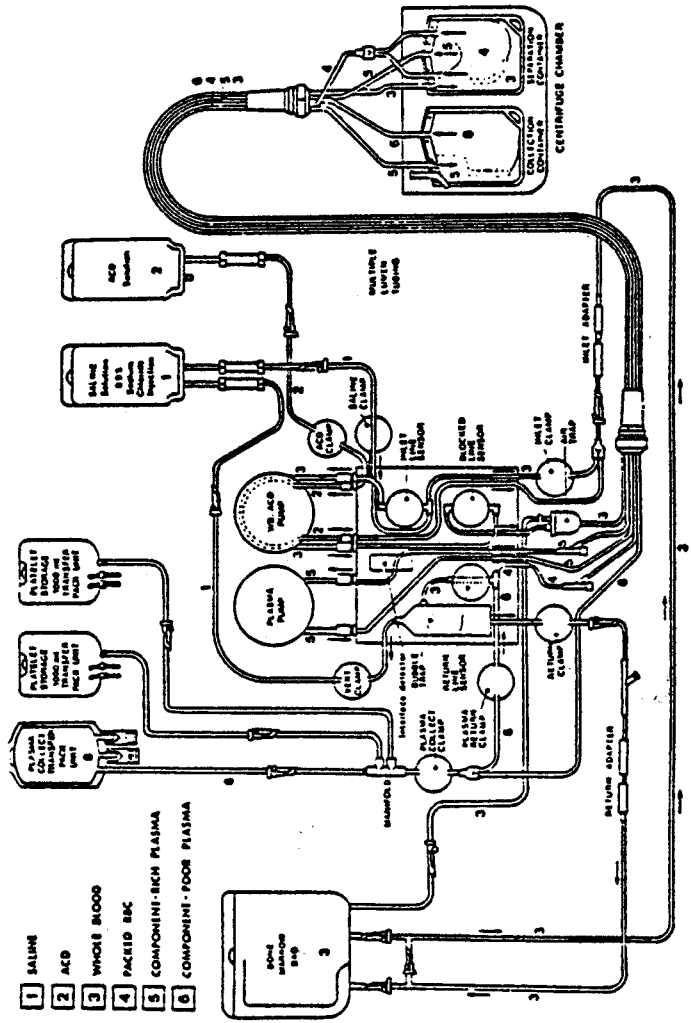
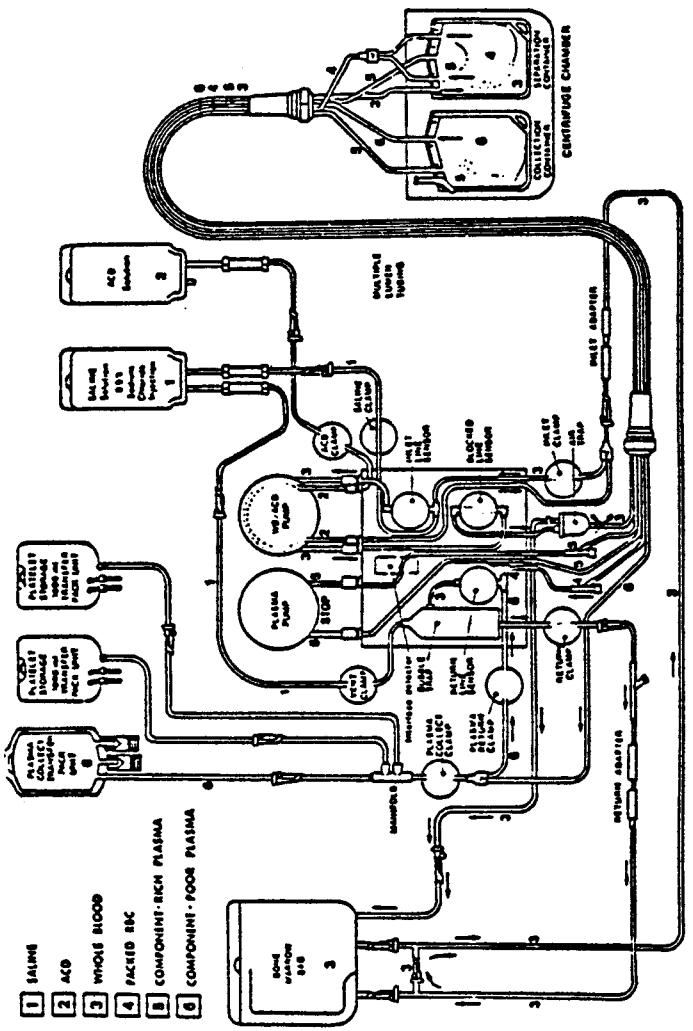


FIGURE 2

Reinfuse and volume reduction fluid flow diagram.



DOSE INTENSIVE REGIMENS IN BREAST CANCER: THE DANA FARBER CANCER INSTITUTE AND BETH ISRAEL EXPERIENCE

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INTRODUCTION

The lack of sufficiently effective cytoreductive conditioning regimens remains the major impediment to improving the disease free survival of women with metastatic breast cancer. Combinations of alkylating agents produce significant therapeutic synergy and subadditive toxicity in a variety of experimental tumor systems. Based on these observations, we have completed a series of laboratory and clinical studies focused on developing an active intensification regimen with little (or rapidly reversible) organ toxicity despite profound myelosuppression.

RESULTS

Combination phase I studies: A study of an empiric combination of cyclophosphamide, carmustine and cisplatin (STAMP 1: CBP) demonstrated that 3 alkylating agents could be combined at nearly full transplant doses. This regimen had considerable activity in metastatic breast cancer, melanoma and sarcoma, but also substantial hepatic and pulmonary toxicity (1,2).

The objective of the following sequence of studies was to develop a high dose combination regimen with a low mortality and significant activity (85% response rate) in poor risk, chemotherapy refractory patients (STAMP 3 and 5) and then to use the regimen to treat women responding to therapy at standard doses (Stamp 25). This regimen, with a 4% mortality and acceptable toxicity at the phase II dose (and an average 6 fold escalation over standard doses), has a response rate of more than 80% in previously failed breast cancer.

High dose therapy is most likely to benefit patients with minimal residual disease, those with a high risk of relapse treated in an adjuvant setting, or patients with small amounts of metastatic disease who have had very good partial or complete responses to standard dose therapy. An intensification regimen of agents known to be active at standard doses with a low mortality is a prerequisite to trials in women with a better prognosis. While profound myelosuppression is acceptable, organ toxicity, particularly if irreversible, is to be avoided.

In laboratory models, simultaneous exposure to thiotepa and 4-hydroperoxycyclophosphamide (the activated analogue of cyclophosphamide) produced striking synergy in the MCF-7 human breast carcinoma cell line, and a linear-log correlation between drug dose and cytotoxicity over 4 logs in the EMT6 murine mammary carcinoma growing in BALB/C mice (3). In addition, continuous exposure of cyclophosphamide or thiotepa was superior to bolus administration (4). These preclinical data, as well as the low single agent response rate for BCNU in breast cancer, prompted a phase I trial of high dose cyclophosphamide, thiotepa and melphalan (Stamp 3: CTL), all active agents in breast cancer at conventional doses. Dose limiting toxicity for the cyclophosphamide and thiotepa combination was oropharyngeal mucositis at thiotepa doses of more than 720 mg/m2. After reaching the dose limiting toxicity of cyclophosphamide and thiotepa, the dose of thiotepa was decreased by 25% to 680 mg/m2 and melphalan was added at a dose of 40 mg/m2 divided over 4 days in 2 patients. Mucositis precluded the use of this combination (5).

We wished to include a third active drug to decrease the development of cross-resistance. The third phase I study (Stamp 5: CTCb) added carboplatin to the cyclophosphamide/thiotepa doublet. At Indiana, 20 women with untreated breast cancer were treated with cisplatin and 9 (45%) responded (6). An EORTC study randomizing patients with measurable breast cancer between CMF and cisplatin/etoposide revealed no significant difference in response rate (7). Unlike cisplatin, carboplatin can be escalated 5 fold over standard doses, with stem cell support with dose limiting hepatotoxicity. Platinum compounds are commonly synergistic and non-cross resistant with other agents in the laboratory. Cyclophosphamide, thiotepa and carboplatin were given by continuous infusion over 96 hours followed 3 days later by autologous bone marrow reinfusion. The maximum tolerated doses of the 3 drugs are cyclophosphamide 6 gm/M2, thiotepa 500 mg/M2 and carboplatin 800 mg/M2 (8). This combination is now being utilized as an intensification regimen for women with metastatic breast cancer responding to induction therapy.

Pharmacokinetics

Interpatient variability in drug metabolism is generally substantial, and often results in considerable variation in drug bioavailability for different agents. The high coefficient of variation for BCNU (clearance of 100-324%) compared to other alkylating agents may explain its unpredictable toxicity (9). Drugs with predictable metabolism at standard doses may exhibit saturation kinetics at substantially higher doses (eg ifosfamide at doses > 5 gm/m2). At

close to lethal doses of agents with steep dose response curves, variations in bioavailability (pharmacokinetics) could profoundly affect safety and effectiveness. Pharmacokinetics were studied to determine any correlation between plasma drug concentration (area under the curve - AUC) and toxicity or response. With continuous infusion dosing schedules, serum levels and area under the curve can be determined with relatively few samples. Theoretically it should also be possible to optimize the dose in individual patients by adjustments in dose based on serum levels. Similarly if the determinants of variability were defined, more rational, safer dosing schedules could be designed.

The dose of thiotepa is linearly related to blood thiotepa levels (AUC) and the AUC, peak plasma levels, and terminal half-life of thiotepa at conventional and high doses are similar (10). Substantial interpatient variation in the systemic clearance of thiotepa resulted in widely variable drug exposures for patients treated at the same dose (coefficient of variation = 44%). Response and toxicity tended to correlate with dose ($p = .08$ and $p = .06$), and were significantly associated with area under the curve ($p = .04$ and $p < .01$) (5,8).

Combination Phase II Studies

Stamp 25 (Phase 2 Stamp 5) included four cycles of doxorubicin, fluorouracil and methotrexate (AFM) induction followed by high dose cyclophosphamide, thiotepa and carboplatin (CTCB) intensification for inoperable or metastatic breast cancer. Based on the model used in lymphoma, there are theoretical advantages to using standard dose non cross resistant induction therapy to decrease tumor bulk, diminish the likelihood of development of resistance, and use lack of response to standard dose therapy to avoid an expensive and morbid therapy in women unlikely to benefit.

Schema: AFM for 4 cycles:

	mg/M2/day	day
Doxorubicin	25	3-5
5-Fluorouracil	750	1-5
Methotrexate/LR	250	15

Restage: MR, PR or CR; on to STAMP V (Cyclophosphamide, thiotepa, carboplatin), parallel pharmacology; on to surgical resection, or radiotherapy to sites of prior bulk disease.
(See Tables 1 and 2.)

Two phase II studies have been completed, and one is currently underway using the treatment outlined above. The second differs only in that patients receive GM-CSF after cycles 3 and 4 of AMF chemotherapy. Peripheral blood progenitor cells are collected and reinfused after CTCb rather than bone marrow. Stamp 25: BM: 29 patients, May 1988 through September

1989, one toxic death. Stamp 25: PBPC, 19 patients, May 1989 through the present, one toxic death.

The first protocol has been analyzed and the follow up on all patients exceeds 1 year. Data are presented by status of patients as they entered dose intensive therapy (Table 3).

Thus, the strategy of clinical studies is rational based on laboratory observations and models. The mortality is low (4%) and does not differ significantly from the 1 to 4% for standard dose therapy (11). There is a high partial and complete response rate. While the partial responses relapse early (consistent with lack of a significant cell kill), a substantial fraction of patients who achieve a complete response continue without failure at 1-2 year follow up.

DISCUSSION

In a review of American, European and Japanese centers treating breast cancer with dose intensive therapy including 248 women responding to standard dose therapy, a total of 58% achieved complete responses and 30% were in continuous complete response at the time of data analysis (12). The complete response rate of 58% is substantially higher than the 10-20% complete response rate generally reported with standard treatment. (11,13)

The median duration of response in a large data base of over 400 women with metastatic disease under age 55 entered on two recent Cancer and Leukemia Group B studies was 8 months and the median survival was 19 months. The mortality in these standard dose studies was 3.5% (11). Women with estrogen receptor positive tumors (median survival 3 years), those who achieve a complete response with standard dose therapy (median 2.8 years) or who have only small amounts of local disease (median > 4 years) have a somewhat better prognosis (11). Only 2.5 % of these women were disease free at 36 months. The NM Anderson FAC regimen is a relatively intense treatment which does not require hematopoietic support. A total of 619 women were treated. One-hundred, sixteen (116) (19%) had a complete response. The median duration of CR was 17 months and 8 patients (1%) were in CR at 32 months (14). Sepsis and drug toxicity account for 24 of the 330 deaths (8%).

In contrast, 18 to 25% of patients on dose intensive trials are disease-free at 24 to 48 months after transplant (28 to 52 months from the start of treatment for metastatic disease).

ACKNOWLEDGEMENTS

Supported in part by U.S. Public Health Service Grant PO1CA-38493 and a grant from the Mathers' Foundation. We wish to acknowledge the excellent clinical efforts of the house staff of the Beth Israel Hospital and the Brigham and Women's Hospital and the nursing staffs of 12W at the Dana Farber Cancer Institute and the Beth Israel Hospital. We also wish to acknowledge the excellent secretarial help of Judy McBreen and Janet Ullah.

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TABLE 1

Schemata, Response and Toxicity for STAMP 1, 3, & 5

		Day from marrow reinfusion							
		-8	-7	-6	-5	-4	-3	-2	-1	0
BM Harvest and Reinfusion		X								X
STAMP 1:	Cyclophosphamide		⇑	⇑	⇑					
	Cisplatin		—	—	—	—				
	BCNU				*⇑					
	Melphalan (Dose levels 5-6 only)					⇑				
STAMP 3	Cyclophosphamide		—	—	—	—				
	Thiotepa		—	—	—	—				
	Melphalan		⇑	⇑	⇑	⇑				
STAMP 5	Cyclophosphamide		—	—	—	—				
	Thiotepa		—	—	—	—				
	Carboplatin		—	—	—	—				

*Last 12 patients given same total dose divided over four days, given BID.

TABLE 2

	STAMP 1				STAMP 3			STAMP 5		
Dose Level: dose in mg/m2										
	1-3	4(MTD)	5-6	7	1-3	4(MTD)	5-6	1-2	3(MTD)	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			
Deaths/Severe, Life Threatening & Lethal Toxicity										
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					2	0	1	2/3	0	1
Mucositis	0	1	6	0	0	0	4	6	0	3
Cardiac	0	2	0	0	0	0	1/1	1/1	0	0
Capillary-leak	0	2	0	0	3	0	1	3	0	0
Response of Breast Cancer to STAMP regimens										
		#	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
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

TABLE 3

Summary:	#	Death	Progression	No Progression	Follow up in months from: ABMT	Ind.CT
CR/CR	10	0	5	5	9-22+	17-34+
PR/CR	3	1	1	1	20+	25+
PR/PR*	4	0	1	3	13-19+	20-25+
PR/PR	12	0	10	2	18+	22+

*Bone Scan remains positive; no other disease

HIGH DOSE COMBINATION ALKYLATING AGENTS WITH AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR PRIMARY AND METASTATIC BREAST CANCER

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INTRODUCTION

Breast cancer is a common disease. There are an estimated 150,000 new breast cancer cases annually and 44,000 deaths [1]. Although adjuvant chemotherapy has modestly improved the outcome in primary breast cancer, the therapeutic results in metastatic breast cancer remain dismal and, in fact, may be worsening. The widespread adoption of the use of adjuvant chemotherapy has at the same time resulted in a high fraction of patients with metastatic disease in which the patient has received adjuvant therapy -- a feature generally associated with a poorer prognosis [2], probably due to the selection of resistant cells. More effective therapeutic approaches are clearly warranted.

The importance of dose intensification in the treatment of breast cancer has been an evolving concept in the treatment of breast cancer over the past decade. Prompted initially by the successful application of dose intensification in the acute leukemias, lymphomas, Hodgkin's disease, neuroblastoma, testicular cancer and other diseases, the use of high dose chemotherapy and ABMT represents a logical limit of the concept. In vitro and preclinical in vivo experimentation provided a sound theoretical and experimental basis for the use of high dose therapy, particularly with the use of combination alkylating agents. In extensive and elegant experiments, Skipper, Shabel, Griswold and their colleagues at the Southern Research Institute demonstrated that there was non-cross resistance among selected alkylating agents and in some settings, therapeutic synergy [3]. Clinical experience demonstrated that while the alkylating agents in general shared the common side effect of myelosuppression, they frequently differed substantially in their non-myelosuppressive side effects [4]. What was also particularly notable from these early single agent high-dose trials was that objective responses were obtainable, even in the setting of patients demonstrating clinical resistance during prior exposure to the same agents.

These observations, and others, prompted us beginning in the early 1980's to explore the role of combinations of alkylating agents in the treatment of resistant solid tumors. An initial phase I trial was undertaken in which the ability to combine several alkylating agents at full or nearly full doses was achieved, within limits (for example, more than three agents in combination were associated with excessive non-hematopoietic toxicity) [5]. Notably, however, were two features: (1) the toxicities which limited further dose escalation were novel and not predicted from the individual agents themselves [6]; and (2) that even in patients with advanced resistant disease, frequent and rapid response was noted. These objective responses were not, in general, durable. Most patients progressed within 6 months and extended remissions were, in ours and other series, very unusual. Indeed, it is now generally accepted as a treatment principle that high dose chemotherapy (except in developmental studies) is not likely to be of substantial or extended value for patients with advanced, resistant breast cancer and is not to be generally recommended. On the other hand, the ability of intensive therapy to produce objective responses, even in these advanced resistant settings, provided a superior therapeutic result to what can be obtained with non-transplant approaches. For example, the use of standard doses of known effective agents and combinations in patients with advanced breast cancer produced objective responses in only 32% of patients; few of these were complete responses. The median response duration averaged only 6.7 months [7]. For regimens of uncertain efficacy, few responses were seen and median response durations are rarely therefore reported.

The recognition of a high frequency of responses, especially complete responses, in these patients with advanced resistant cancer prompted a test of this therapeutic approach in patients who have received no prior chemotherapy for metastatic disease. We treated 22 patients with hormone receptor negative measurable metastatic disease [8]. More than half of the patients (64%) had received prior adjuvant chemotherapy and more than 40% prior radiation therapy. Only two of the 22 had solely nodal disease with lung and liver as the dominant sites of metastatic disease. Patients were treated with a single course of high dose cyclophosphamide, cisplatin and carmustine or melphalan and autologous bone marrow support and then followed without further therapy until relapse. Acute toxicity was substantial as expected from the phase I trial with therapy related mortality of 22%. The complete response rate in this trial was slightly in excess of 50% and although most patients relapsed from the unmaintained remission within 6 months, 3 of the 22 patients (14%) remain in continuous unmaintained remission now all beyond four years and with the lead patient followed in excess of 7 years [9]. The occurrence of this frequency of continuous unmaintained remission is unexpected and, particularly when coupled with similar observations from other series with induction chemotherapy, argues that there is a subset of patients with poor prognosis metastatic breast cancer that can achieve long-term disease-free remission from this therapeutic approach.

This series has several important features. First, the therapeutic outcome is clearly attributable to the high dose therapy, since no other intervention was utilized and the patients were followed without subsequent therapy until relapse or death. Given that this regimen employs drugs complementary to or at least different from standard dose chemotherapy regimens, notably cisplatin and carmustine, the potential for the use of this program in sequence with outpatient standard dose regimens is readily apparent as long as toxicities are not additive or cumulative. For example, one might reasonably be concerned that extensive adriamycin exposure might predispose patients to cyclophosphamide cardiotoxicity in the transplant setting, although this does not appear to have occurred in clinical trials.

Relapses occurred predominantly in sites of pretreatment bulk disease, or in areas of prior radiation therapy. Clearly, there is a limitation to the cytoreductive capacity of any therapeutic regimen. Bulk tumor sites appear to be resistant either for intrinsic drug resistance or due to kinetic effects. We have estimated previously from tumor volume regression rates and growth rates that a single treatment of high dose cyclophosphamide, cisplatin and carmustine when used as in this treatment schedule results in 3.5 to 5.5 logs of tumor cell kill. These data, which must be interpreted cautiously because of the assumptions made in the required extrapolations, are consistent with a prediction that eradication of disease would be expected only rarely. Tumor sensitivity may differ substantially at lower tumor burdens; clinical and laboratory experience suggest that smaller tumor burdens may be more sensitive to chemotherapy. Further, detailed analyses of clonogenic tumor numbers in experimental models indicate that tumor cell kill may be several logs greater than predicted from actual measurements of tumor volume. Optimal timing of therapy therefore may be critical to outcome.

The delineation of the therapeutic effectiveness of the high dose program as well as the pattern of failure suggested that the optimization of this approach therapeutically should be approached via two pathways: (1) in metastatic disease, tumor reduction prior to application of the high dose therapy via use of induction chemotherapy may enable more frequent and durable complete remissions; and (2) utilization of high-dose chemotherapy as consolidation in patients with high-risk primary disease may represent the optimal setting in which to utilize high dose therapy.

In the first approach, we have utilized a combination regimen of intensive adriamycin, fluorouracil and methotrexate outpatient chemotherapy as described by Jones from our group, to produce rapid cytoreduction prior to high dose consolidation [10]. The obvious limitation of this approach will be the limited capacity of outpatient regimens to produce rapid and substantial tumor regression (debulking). Although outpatient chemotherapy programs for metastatic breast cancer can produce responses in 60-80% of treated patients, the frequency of complete remissions is only in the range of 5-20%. Hence, even complete remission resulting from these techniques may mean only an approximately two log reduction in tumor volume. Thus, depending on initial tumor volume and chemosensitivity, induction therapy may be variably

effective. For this reason, we selected intensive adriamycin therapy applied as frequently as possible, in this case every 3 weeks, until maximal response. Experimental and clinical data suggest that the vast majority of the value of chemotherapy is obtained during the first few cycles and that the development of resistance is a real and frequent problem. For these reasons, we chose to limit the exposure to the induction chemotherapy to a maximum of four cycles, and followed this immediately by high dose consolidation with cyclophosphamide, cisplatin and carmustine and ABMT. Whenever possible, we utilized surgical restaging for transplant. Subsequent to the high dose therapy, we attempted to deliver radiation therapy to pretreatment sites of bulk disease. In this series, 45 patients were entered [11]. Patients were pre- or peri-menopausal and had measurable metastatic cancer which lacked hormone receptors or had failed hormone therapy. Five patients were unable to proceed to transplant because of progressive disease during AFM which precluded transplant (3), hepatitis (1) or refusal (1). Forty patients were transplanted of whom 68% were free of tumor at the end of transplant (60% of the initial 45 patients entered). The median time to progression has been 18 months and median survival is 20 months. Follow-up on this data is continuing and suggests that while the median duration of remission has been increased three-fold by the use of induction therapy, the percentage of patients remaining disease free long-term will not differ substantially from that which was achieved by the use of high dose therapy alone. Similar results have been obtained by the teams working at the M. D. Anderson Cancer Center [12] and the Dana-Farber Cancer Institute [13].

These data collectively demonstrate that high-dose consolidation results in a high frequency of complete remissions and that approximately 15-30% of patients with metastatic breast cancer, even with poor prognostic characteristics, can remain progression-free after ABMT procedures using high dose combination chemotherapy. Identification of the subpopulation responsive to high-dose therapy is not possible currently since the patients were selected for poor prognosis and are relatively uniform in their pretreatment characteristics. The data imply that further progress will be achieved in this setting only by the addition of further dose intensification, most likely by using additional dose intensification with non-cross resistant agents. The Application of this approach will likely be difficult, although advances in management of toxicity using hematopoietic colony-stimulating factors and other techniques offers the possibility to accomplish this where such approaches were not possible previously.

Currently, we are defining the appropriate timing of high dose intensification in patients who achieve optimal tumor reduction from AFM induction, i.e., achieve a complete remission, in a prospective randomized trial in which patients who achieve a complete remission following induction chemotherapy with AFM are randomized to immediate transplant, or to close follow until first sign of low volume relapse and then transplanted with the same regimen. Over ninety patients have been entered on trial and 30 patients randomized. The endpoints of the trial include disease-free and overall survival

for the two arms. Patients not achieving a complete remission to the induction therapy are immediately taken on to high-dose therapy, as we believe it is important for patients to achieve a complete remission if possible, and the CPA/CDDP/BCNU regimen is capable of converting approximately 40% of patients with a partial response to AFM into a complete remission.

As described above, the use of high-dose therapy as consolidation to standard adjuvant chemotherapy of high risk primary breast cancer represents a primary target for this therapeutic approach. The prognosis for patients in which there is extensive axillary involvement at the time of primary surgery for primary breast cancer is poor and despite adjuvant chemotherapy, relapses occur in the majority of patients. The poor prognosis of these patients is highlighted by the fact that there is little impact of adjuvant chemotherapy, particularly in the early years after primary therapy, with the majority of relapses occurring in the first 5 years. Table 1 shows the disease-free survivals for patients with extensive axillary lymph node involvement at the time of primary surgery. At five years, between 55 and 87% of patients have relapsed, depending on the standard dose series.

Because of this poor prognosis, we have undertaken a study in collaboration with the CALGB in which patients with Stage II or III breast cancer involving 10 or more axillary lymph nodes would be treated with the current intensive CAF dose arm of the CALGB adjuvant protocol with consolidation using high dose cyclophosphamide, cisplatin, and carmustine (CPA/CDDP/BCNU) and ABMT using the same doses and schedule as described previously. A preliminary analysis of this data was presented at the recent American Society of Clinical Oncology meetings [14]. The data as presented demonstrated that with a lead follow-up of 40 months and a median follow-up of 14 months, 80% of patients (53 analyzed) were, by Kaplan-Meier analysis, progression free, and 72% of patients remained event-free (relapse or death from toxicity). These data are compared to the contemporary and historical series in Table 1. These data are compelling although because of the expense, treatment related toxicity (therapy related death has been 7%), and resource requirements for this approach, confirmation in an appropriately designed randomized clinical trial is planned. The trial design is shown in figure 1 and will be conducted through the CALGB and available for intergroup participation.

The development of high-dose combination alkylating agents as a therapeutic approach to breast cancer has proceeded in a systematic fashion throughout the past 8 years. The available data has established that the therapeutic approach is effective in producing responses, progression-free and overall survival results that are at least comparable to standard dose alternatives and extended disease-free survival in a subset of patients with poor prognosis breast cancer. Ongoing randomized comparative trials should enable establishment of the magnitude of the therapeutic advantage.

ACKNOWLEDGEMENTS

We wish to recognize the important contributions of all our colleagues in the design and conduct of these studies over the years, especially RB Jones, EJ Shpall, C Gilbert, ML Affronti, B Matthias, D Coniglio, K Dukelow and all our fellows and housestaff, our nurses, and support staff. Without them these studies would not have been possible.

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20. N Robert, personal communication

FIGURE 1
Protocol design for CALGB 9082 for patients with Stage II or III breast cancer involving ten or more axillary lymph nodes.

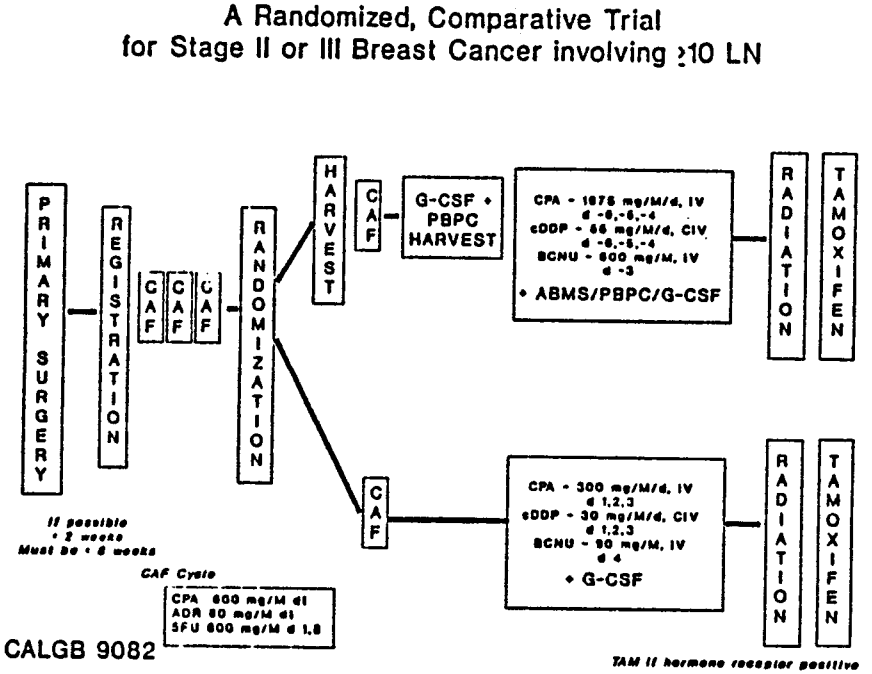

TABLE 1

Autologous Bone Marrow Transplantation in Primary Breast Cancer Involving ≥ 10 Axillary Lymph Nodes

		Relapse-Free Survival Years		
Series	n	1	3	5
Standard Dose Regimens				
Duke Standard Dose	30	58%	23%	13%
National Historical Data Base[15]	119	77%	33%	27%
Milan CMF	71	75%	52%	35%
Johns Hopkins Hospital -Abeloff 16 week regimen[16]	52	88%	54%	
MD Anderson FAC[17]	149	92%	56%	45%
CALGB 8541 CAF (3 doses rates)	63	78%	45%	
SWOG 7436 Melphalan[18]	48	72%	36%	22%
SWOG 7436 CMFVP [19]	33	92%	46%	32%
ECOG 5181 (CMFPTH),4181 (CMFPT), 6177 (CMFP(T)), 5177(CMF) [20]	319	86%	48%	28%
High Dose Therapy with AETMT(CALGB 8782 & Duke)	53	96%	80%	

TANDEM HIGH-DOSE CHEMOTHERAPY FOR METASTATIC BREAST CANCER

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INTRODUCTION

In this manuscript, we outline our approach to and past results of tandem high-dose chemotherapy for human solid tumors and future directions with this approach.

Almost a decade ago, we began developing the technique of tandem high-dose chemotherapy. This strategy was developed in part because of an early skepticism that the dose response of most human solid tumors was not steep enough to warrant expectations that a single cycle of high-dose therapy would achieve results significantly different in advanced stages from those of standard chemotherapy. Tandem high-dose therapy also offered a potential means of circumventing kinetic resistance related to the presence of a proportion of G_0 cells in a tumor population. Theoretically, the first course of therapy can initiate some of those cells into the cell cycle. Furthermore, we were concerned that drug delivery to the center of tumor masses might be problematic and that tumor reduction by the first cycle of chemotherapy might enhance the affects of subsequent cycles.

Our first efforts were focused on testing whether identical high-doses of chemotherapeutic agent could be administered in tandem within a short space of time and, on determining the maximum tolerated doses (MTDS) feasible using such an approach. Simultaneously, we anticipated modifications of our chemotherapy that would include a different, but hopefully non-cross-resistant, combination of agents in the second cycle.

PATIENTS AND METHODS

Tandem High Dose Cyclophosphamide, Etoposide and Cisplatinum: Maximum Doses of High Dose Therapy

Our approach to dose escalations in this and in our other successful high-dose therapy programs (1-3) has been more cautious and conservative than

that of other investigators in this field. We attempt to use a drug dose below the critical level where a consistent percentage of patients experience extramedullary toxic effects. We term that point the "ceiling dose". The ceiling dose is regularly associated with a clinically worrisome frequency of extramedullary toxic effects such as cardiovascular, pulmonary, hepatic, and neurological vasculitis. These effects are unacceptable in the treatment of early disease like stage II breast cancer, where the patient's median survival is lengthened significantly by even conservative therapy. Unlike in cases of acute leukemia, relapse of solid tumor such as breast cancers is not necessarily associated with early death, and median disease-free intervals are longer for the solid tumors than of acute leukemia. Furthermore, it is unlikely that we understand physiology enough to effectively modify extramedullary toxicities. The practice of defining the MTD for high-dose chemotherapy at a dose unassociated with life-threatening extramedullary toxicity is supported by the lack of clinical evidence that long-term diseasefree survival is improved by even higher doses. In leukemia, for instance, no clear evidence exists that any particular cytoreductive program is superior to the founding program of cyclophosphamide and total-body irradiation (TBI), despite obvious increases in such programs' intensity when extra drugs are added, which is exemplified by the therapies' new and increasing toxic affects (4-6). Ongoing studies are examining therapies that use higher doses of cyclophosphamide, carmustine (BCNU), and etoposide (VP-16)(CBV) in patients with relapsed Hodgkin's disease than we used in our original description of CBV (1). When we evaluate outcome in identical subgroups (equivalent number of relapses, prior therapies and tumor bulk) super CBV's is not associated with an higher proportion of long-term disease-free survivors than the low-dose therapy, but it is certainly associated with a higher rate of early mortality. Similarly, Dr. Peter Tutschka found that the combination of busulphan and cyclophosphamide employing a cyclophosphamide dose approximately 50% of that used in initial studies had an equivalent anti-leukemic effect (7). This lack of evidence that escalation to the level of serious extramedullary toxicity increases the therapeutic ratio within a single cycle further strengthened our conviction to develop and build programs based on the principle of what we now call tandem high-dose, below-ceiling-dose, low-toxicity chemotherapy.

The major combination chemotherapy that we decided to evaluate in large numbers initially incorporating cyclophosphamide and etoposide (VP-16), drugs with reversible myeloid toxic effects and, if administered below certain doses, free of serious extramedullary toxicity. Subsequently, a third drug, cisplatin was added because of its theoretical synergism with both of the first two drugs and its activity across a broad range of tumor types. We have evaluated this combination, termed CVP, in more than 150 patients at four dose levels with tumors of various types (Table 1) . Table 2 compares the doses of one course at the highest dose of CVP to of the classic one dose, STAMP therapy designed by Dr. Bill Peters (8). Our cyclophosphamide dose is slightly larger, and the cisplatinum doses are equivalent; any differences in effect, therefore, are almost totally attributed to the third drug. A carefully chosen third drug can bring the

therapeutic effects of a single cycle of our regime to an equivalent or almost of equivalent intensity to as that of other high-dose single-administration chemotherapy for human solid tumors.

A further question one could ask of our strategy is did we chose the appropriate cytoreductive therapy? Alternatives to CVP include STAMP, STAMP variants which replace the nitrosurea with thiotepa, busulphan plus cyclophosphamide, TBI in combination with many drugs, to name a few. We chose etoposide rather than a nitrosoureas like carmustine because of carmustin's life-threatening pulmonary toxic effects, particularly in patients who have undergone prior radiotherapy, and because of carmustine's propensity to produce other extramedullary toxic effects such as veno-occlusive disease and vasculitis. Therefore, because we believe that CVP is at least equivalent to alternatives, we also believe that the choice of chemotherapy comes down to determining which therapy can be administered with the least toxic effects and, therefore, can most potentially be used in the greatest variety of patients because of the lower risk of death.

Tandem CVP in Breast Cancer

We have treated approximately 150 patients with tandem CVP; the majority had breast cancer, but we also have treated patients who had extrapulmonary small cell cancer, non-small cell bronchogenic carcinoma, or adenocarcinoma of unknown origin and a small number of persons with adenocarcinoma of the bowel. Most patients have had nonprogressive disease. Chemotherapy intensification using CVP has elicited complete remission in patients whose tumors had responded partially to prior chemotherapy, and there exists a proportion of long-term survivors (no tumor progression for >2 years after therapy) with miscellaneous diagnoses (9-13). Here, we concentrate on the largest group of these patients, for whom the impact of this approach may be interpretable.

We report on the outcome of 68 of 100 patients with breast cancer whose tumors fit the clinical description of first-relapse estrogen-receptor-negative (ER-) or primary hormone-unresponsive cancer and whose tumors remained stable or responded to induction therapy. Thus, on the first challenge with hormone therapy these patients showed disease progression. We do not describe the results for patients whose tumors were resistant to induction therapy, those with ER+ positive tumors who did not receive hormone challenge, those with multiple relapses or refractory disease, and 10 patients who were treated too recently to be eligible for evaluation. During the period of study the doses have escalated progressively and a small number of patients have recently been treated with the highest CVP dose we use. The longest follow-up is, therefore, of patients who received the lowest intensity of drug therapy. The majority of these patients received their second cycle of CVP within 4 or 5 weeks of their initial therapy, and 97% of the patients received their second cycle within 3 or 8 weeks of that time, maintaining the principle of dose intensity.

Worthy of emphasis is that the median age of our patients, 43, is slightly higher than that in other studies; also, patients up to age 65 are eligible for this protocol. The median disease-free interval for our patients was just over 1 year, so we were not treating patients with indolent tumors. Also, the majority of these patients had tumor involvement at two or more sites and had predominantly visceral disease. Thus, our CVP regimen was tested on aggressive tumors in patients who had a high probability of an unfavorable outcome to conventional therapy.

The induction therapy patients received in most instances was a combination of Adriamycin and cyclophosphamide or fluorouracil, adriamycin and cyclophosphamide. We treated the patients whose tumors had relapsed within 6 months of receiving the Adriamycin combination therapy with a Velban combination. At the point of maximal response, which was a median of four cycles, patients then entered the intensification phase of therapy, which was meant to include two cycles of the CVP regime.

With induction therapy there was a 32% complete remission rate and a 50% partial remission rate; the remaining patients had stable disease. Forty-one of the 68 patients still had measurable disease when they entered the intensification phase. Two patients who originally had no evidence of disease, 5 patients who died because of toxic responses early in the therapy, and the 21 patients whose tumors had achieved complete remission before the intensification phase were not considered evaluable for intensification response. Of these 41 patients with measurable disease at the time of intensification, 46% achieved complete response through CVP therapy (Table 3). This "conversion rate" is an important indicator of the potential activity of this regimen. At the completion of intensification therapy and counting early deaths as failure, approximately 60% of the 66 evaluable patients achieved complete remission (Table 4).

Almost 20% of patients have been free of disease for more than 2 years. only one relapse has occurred more than 2 years after therapy. In a similar group of patients who had ER- or negative or hormone-refractory tumors and who were treated using two protocols by the Southwest Oncology Group progression-free survival beyond 2 years was very rare among those who were still responding or who had not progressed after 5 months of conventional therapy, progression free survivorship beyond 2 years is very rare, probably 5% at the best (14). Some insight into our patients who survived long term without tumor progression is provided in Table 5. A sizable proportion have visceral metastatic disease, particularly in the lung, and a number of these patients have exceeded their disease free interval, probably eliminating the concept that these patients have not relapsed because of the indolent nature of disease. Projected disease-free survival for patients with lesser volume of disease 1 or 2 metastatic sites is approximately 40%.

Toxicity of Tandem CVP

The mortality rate associated with the use of tandem CVP (7%) is modest given the age of the patients and the fact that they were not germ-free isolated during treatment. Two of the deaths were secondary to alpha-streptococci

sepsis, and prophylactic vancomycin administration is now routine. One death was related to a myocardial infarction and two other patients were caused by a fungal infection and a liver failure in a patient with extensive hepatic involvement by the tumor. The majority of patients experienced febrile episodes, and 50% of patients had a documented infection, predominantly gram-positive sepsis or pneumonia. The toxic effects of the first and second cycles did not differ. Severe gastrointestinal toxicity occurred only in a small proportion of patients and severe renal toxicity rarely; no other significant extramedullary toxic effects occurred (Table 6). Most importantly, we achieved our aim of avoiding serious, hepatic, vascular, cardiac, and pulmonary toxic effects.

CONCLUSIONS

Clinical Directions and Conclusions

In cases of low-volume metastatic disease (no more than two sites of metastatic disease), the two-dose double CVP regimen is a potentially effective program that has low associated mortality and morbidity. At the same time we are using tandem CVP as a model in which to evaluate new strategies of enhancing collection of progenitor cells to levels that may ameliorate absolute neutropenia. In more extensive disease or liver disease, we are uncertain whether tandem CVP therapy has caused any long-term disease-free survival, and new, more aggressive therapies must be developed. We have to accept more aggressive and newer strategies. We have conducted pilot studies of mitoxantrone/thiotepa combinations in some 80 patients who had anthracycline-refractory disease and have regularly achieved a 20-30% complete remission rate. We also have initiated a study of patients who have bulky disease or liver disease in which the first cycle of therapy is cytoxan/VP-16/cisplatinum and the second cycle is 50mg/m^2 mitoxantrone plus 900mg/m^2 thiotepa. In cases of very refractory disease, we are considering investigating the modulation of mitoxantrone resistance with cyclosporin A.

A subset of patients with stage III breast cancer do poorly and are identified by response to neoadjuvant therapy before potential mastectomy (15,16). These patients either show no response to therapy or have a significant volume of primary or lymph node disease following neoadjuvant therapy. We plan to study the effects of tandem CVP therapy, because of its acceptable rate of morbidity, in these patients and in those with stage II disease who have 10 or more nodes positive for metastatic disease. A real issue for these patients is whether Adriamycin combination therapy has changed the natural history of this disease; this is a possibility as some recent descriptions report 5- and 10-year disease-free survival rates approach 50% (17,18). Demonstrating superiority to this outcome, if real would require large numbers of patients.

Overall, we believe that such short-term high dose chemotherapies as those described here are useful for low-volume aggressive metastatic disease. Dose escalations are ongoing, and the proportion of long-term disease-free survivors continues to exceed that expected with conventional therapy. Because the best

results are achieved in patients who have only a modest amount of nodal and pulmonary metastatic disease, new strategies are needed to effectively attack more resilient bulky metastatic cancers.

ACKNOWLEDGEMENTS

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TABLE 1

TUMOR TYPE	
ER-HORMONE REFRACTORY STABLE OR RESPONSIVE BREAST CANCER	68
RECURRENT BREAST CANCER BUT PROGRESSING ON THERAPY	7
RECURRENT RESPONSIVE BREAST CANCER, NO HORMONE CHALLENGE	2
MULTIPLE RELAPSED BREAST	9
SCBC	17
OTHERS, LUNG, GL ETC	25

TABLE 2

INTENSITY OF CVP

Comparison with other therapy

	CVP	STAMP	RATIO
Cytoxan	6 g/m2	5.25 g/m2	1.14
CDDP	165 mg/m2	165 mg/m2	1.0
BCNU		600 mg/m2	
VP-16	1500 mg/m2		

TABLE 3

INTENSIFICATION RESPONSE

41 EVALUABLE - 2 NED, 20 CCR, 5 ED

RESPONSE RATE	PTS	%
COMPLETE	19	46
PARTIAL	13	32
STABLE	5	12
PROGRESSION	2	5
PR--PROGRESSION	2	5

TABLE 4

OVERALL RESPONSE

66 EVALUABLE PTS - 2 NED

RESPONSE	N PTS	%
COMPLETE	39	59
PARTIAL	15	23
STABLE	5	8
PROGRESSION	2	3
EARLY DEATH	5	8

TABLE 5

PROGRESSION-FREE SURVIVAL
GREATER THAN 2 YEARS

Disease sites	DFI	PFS
Nodes	6	139+
Lung	70	165+
Nodes	70	109+
Bone	77	145+
Lung, mediastinum	76	205+
Lung	84	129
Lung	113	157+
Nodes	128	104+
Lung, mediastinum	145	159+
Lung	245	265+

TABLE 6

TOXICITIES

Febrile Episodes	93
Sepsis: (gram-positive, mainly)	39
Pneumonia	16
GI: grades 1-2	50
Nausea: grade 3	33
Other GI toxicities	10-20
Renal: grade 3	3

No other significant extramedullary toxicity

HIGH DOSE CONSOLIDATION THERAPY WITH AUTOLOGOUS STEM CELL RESCUE IN STAGE IV BREAST CANCER

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ABSTRACT

We have treated 59 patients with metastatic breast cancer with induction (IND) chemotherapy followed by high dose intensification (ICT) and autologous stem cell rescue (ASCR). IND consisted of two treatment programs: (1) cyclophosphamide, doxorubicin, vincristine, and methotrexate with leucovorin rescue (LOMAC) in 27 patients, and (2) 5-fluorouracil, cisplatin, doxorubicin, and cyclophosphamide (FCAP) in 32 patients. ICT after LOMAC was cyclophosphamide and thiotepa (CT) with ASCR, and after FCAP was cyclophosphamide, thiotepa and BCNU (CTB) in all but 8 patients who received CT. Of the 59 patients, 37 had received adjuvant chemotherapy; none had received prior chemotherapy for metastatic disease. Responses after LOMAC IND were 4 CRs (15%) and 15 PRs (56%) and after FCAP IND 8 CRs (25%) and 11 PRs (34%). Responses after LOMAC ICT were 12 CRs and 7 PRs. Responses after FCAP ICT were 8 CRs and 5 PRs. The median time to treatment failure from reinfusion was 5 mos for LOMAC and ICT and 10 mos for FCAP and ICT. The median survival from study entry was 15 mos in LOMAC patients and 9 mos in FCAP patients. There are two LOMAC and ICT patients alive with no evidence of breast cancer at 3 years. High-dose consolidation or intensification therapy has lead to increased response rates in metastatic breast cancer requiring further investigation in randomized clinical trials.

INTRODUCTION

There is no curative therapeutic approach for patients with advanced stage IV breast cancer. Active and effective combination chemotherapy regimens for women with advanced stage IV breast cancer remain controversial. Recently, several groups have attempted to increase the complete remission rate, the initial step in rendering a small subset of advanced stage breast cancer patients disease-free, by utilizing high-dose alkylating agent chemotherapy

followed by autologous stem cell rescue (ASCR) (1). The objectives of our phase II trials in women with metastatic breast cancer, are to increase the complete response rate and survival and to investigate the toxicities of such an approach. In a prospective series in women with stage IV breast cancer who had not received prior chemotherapy for metastatic disease, we tested an initial cytoreductive program of LOMAC, then, with an intent to further increase the CR rate, we pursued FCAP. These induction regimens were followed by high-dose intensification with alkylating agents and ASCR.

METHODS

Patient Characteristics

Fifty-nine patients with stage IV breast cancer were enrolled from July 1986 to December 1989. All patients had good performance status (CALGB 2 or better) and had normal cardiac, renal and pulmonary function. Written informed consent approved by the institutional review board was obtained in all cases. Patient characteristics are described in Table 1.

Study Design and Treatment Regimens

Twenty-seven patients were treated with induction therapy of cyclophosphamide 1000 mg/m^2 days 1,22,43; Adriamycin 50 mg/m^2 days 1,22,43; vincristine 1.4 mg/m^2 (2.0 mg maximum) days 1,8,22,36,43,57, and methotrexate 200 mg/m^2 days 15,36,57 with leucovorin rescue (LOMAC). Thirty-two patients were treated with induction therapy of 5-fluorouracil 1000 mg/m^2/day for 5 days days 1-5,36-40,71-75, cisplatin 100 mg/m^2 days 1,36,71, cyclophosphamide 1000 mg/m^2 days 22, 57, and Adriamycin 50 mg/m^2 days 22, 57 (FCAP). At the conclusion of induction therapy, patients were evaluated for response. Patients achieving a CR, PR or stable disease (SD) were eligible for intensification chemotherapy. Procurement of autologous, cryopreserved stem cells was obtained in standard fashion (2,3). Eligible LOMAC patients were intensified with cyclophosphamide 7.5 gm/m^2 and thiotepa 675 mg/m^2 (CT) and eligible FCAP patients were intensified with cyclophosphamide 7.5 gm/m^2, thiotepa 675 mg/m^2, and BCNU 450 mg/m^2 (CTB) except for the last 8 patients who were intensified with CT.

RESULTS

Antitumor Response and Survival

Of the 27 eligible patients treated with LOMAC induction, 4 (15%) patients obtained a CR, 15 (56%) a PR, for a 70% overall response rate. Twenty-two patients underwent high-dose intensification therapy. At the completion of this therapy there were 12 CRs (55%) and 7 PRs (Table 2A). Of the 14 PRs intensified, 9 were converted to CRs. Two patients with stable disease were converted to partial responses after intensification. The median time to treatment failure from reinfusion was 5 months (Figure 1). At a median follow-up of 37 months, the median survival for all patients is 15 months

(Figure 2). Two patients who achieved CR after induction are currently alive and free from breast cancer beyond 36 months; however, one patient has coincidentally developed acute lymphocytic leukemia. Two patients are alive with relapsed breast cancer.

In order to improve upon overall disease free survival, we attempted to increase the number of CRs prior to intensification by modifying our induction program. This lead to the FCAP program described above. Of the 32 eligible patients treated with FCAP induction, 8 (25%) patients obtained a CR, 11 (34%) a PR, for a 59% overall response rate. Twenty-three patients underwent high dose intensification therapy. At the completion of this therapy there were 8 CRs (35%) and 5 PRs (Table 2B). Of the 9 PRs intensified, only 2 were converted to CRs. The median time to treatment failure from reinfusion was 10 months (Figure 1). At a median follow-up of 12 months, the median survival for all patients is 9 months (Figure 2).

Toxicity

The toxicities from the LOMAC & CT program have been previously described and are outlined in Table 3 (4). The median time to granulocyte recovery (PMNs > 500/ul) was 17 days and median time to platelet recovery (>50,000/ul and transfusion independent) was 53 days.

Toxicities to the FCAP program are also outlined in Table 3. Of note the FCAP induction caused more mucositis and myelosuppression necessitating therapy delays than LOMAC induction. Of the patients intensified after FCAP the number of toxic deaths was unacceptable (7/23 or 30%). Renal insufficiency and hepatotoxicity were significant. Two patients expired as a result of veno-occlusive disease (VOD). After intensifying 15 patients with CTB, we dropped the BCNU to decrease hepatic toxicity. Two patients also expired as a result of intracranial hemorrhages and three as a result of sepsis. The median time to granulocyte recovery after intensification was 19 days and median time to platelet recovery was 53 days.

DISCUSSION

In an attempt to improve upon remission rates and survival in metastatic breast cancer we explored a therapeutic approach of an initial cytoreductive induction regimen followed by "consolidation therapy". The initial induction regimen would diminish tumor burden and the intensification regimen would further decrease and eradicate tumor burden or at the very least diminish the burden in a logarithmic fashion.

Our initial results with our LOMAC induction followed by high-dose intensification with ASCR showed that we could convert a substantial number of partial responders to CRs. In order to improve upon time to treatment failure and thus disease-free survival, we attempted to increase the number of patients in CR prior to intensification and increase our intensification by adding BCNU. We successfully increased the number of induction CRs and possibly have impacted on the time to treatment failure. However, toxicity was

substantial with seven early deaths. Two patients intensified with CTB developed fatal VOD. This necessitated the deletion of BCNU from this intensification program. Two patients died of intracranial hemorrhages (one subarachnoid, one epidural); three patients died of sepsis. Nephrotoxicity was also high on the FCAP program and may be related to the use of nephrotoxic antibiotics in patients after platinum therapy.

This therapeutic approach offers a high complete response rate which is the first step in developing curative treatment strategies in metastatic cancer. Further investigation is warranted including (1) attempts to improve upon time to treatment failure with double autografts and more intensive induction therapy with growth factor support, (2) attempts to ameliorate toxicities especially related to prolonged myelosuppression, with growth factor support and stem cell mobilization techniques, and (3) once these are accomplished randomized clinical trials comparing this therapeutic approach to "standard chemotherapy".

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TABLE 1

PATIENT CHARACTERISTICS		LOMAC	FCAP
Total		27	32
Age (yr)			
	MEDIAN	44	40
	RANGE	24-62	27-56
Prior Adjuvant Therapy		17 (63%)	20 (63%)
SITES OF METASTASES*			
	Liver	11 (41%)	10 (30%)
	Lung	10 (37%)	7 (22)
	Bone	11 (41%)	16 (50%)
	Lymph nodes	3 (11%)	10 (30%)
	Chest wall	5 (19%)	4 (13%)
	Orbit	2 (7%)	
	Bone marrow	5 (19%)	10 (30%)
	Skin	1 (4%)	2 (6%)
SOURCE OF STEM CELLS			
	BONE MARROW	18	14
	PERIPHERAL BLOOD	4	9
		22	23

* Many patients had more than one site of metastatic disease

TABLE 2

INDUCTION AND INTENSIFICATION RESPONSE

A. LOMAC & ICT

		ICT RESPONSE					
LOMAC		NOT TREATED	CR	PR	SD	PD	ED
CR	4	1	3	-	-	-	-
PR	15	1	9*	5	-	-	-
SD	5	-	-	2	-	2	1
NR/PD	1	1	-	-	-	-	-
ED	1	1	-	-	-	-	-
NE	1	1	-	-	-	-	-

* 2 died after 30 days.

B. FCAP & ICT

		ICT RESPONSE					
FCAP		NOT TREATED	CR	PR	SD	PD	ED
CR	8	-	6	-	-	-	2
PR	11	2	2	5	-	-	2
SD	5	-	-	-	2	-	3
NR/PD	4	3	-	-	-	1	-
ED	2	2	-	-	-	-	-
NE	2	2	-	-	-	-	-

TABLE 3

TOXICITIES

INDUCTION	LOMAC (n=22)	FCAP (n=23)
MUCOSITIS	7	22
MYELOSUPPRESSION	4	13
TOXIC DEATH	1	1

INTENSIFICATION	LOMAC-CT (22 patients)	FCAP-CTB (15 patients)	FCAP-CT (8 patients)
MUCOSITIS	22	15	8
DIARRHEA	22	15	8
HEMORRHAGIC CYSTITIS	2	1	0
RENAL INSUFFICIENCY (DIALYSIS)	2	3	0
HEPATOTOXICITY	5	10	1
TOXIC DEATH	3	4	1

FIGURE 1
Time to disease progression from reinfusion.

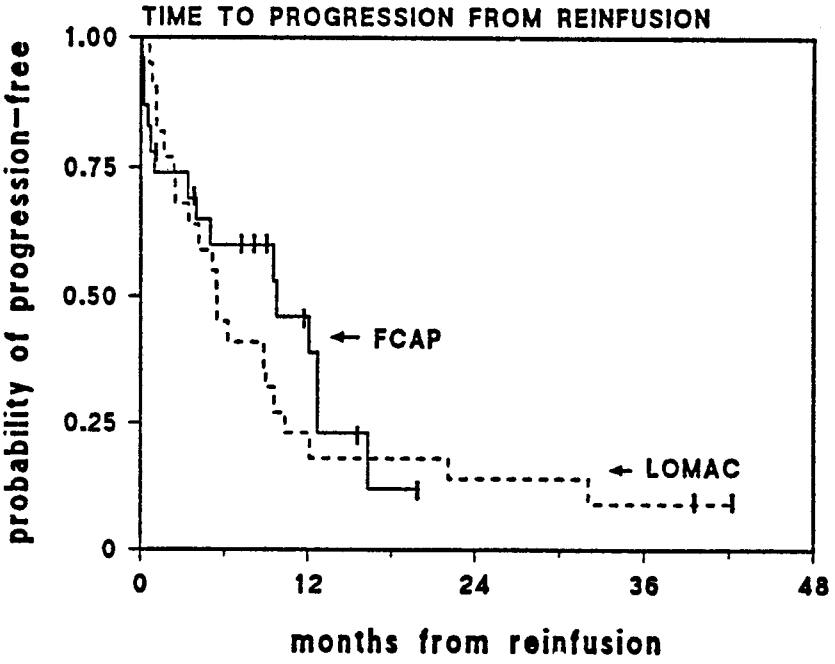
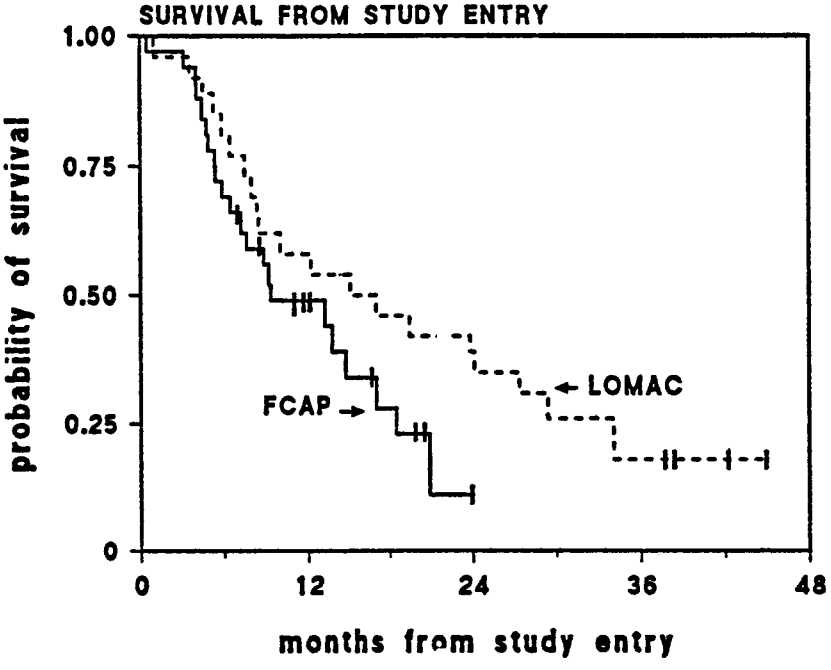


FIGURE 2
Survival curves from study entry for all patients.



HIGH-DOSE CYCLOPHOSPHAMIDE, THIOTEPA, HYDROXYUREA WITH AUTOLOGOUS HEMATOPOIETIC STEM CELL RESCUE: AN EFFECTIVE REGIMEN FOR CONSOLIDATION CHEMOTHERAPY OF EARLY METASTATIC BREAST CANCER

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INTRODUCTION

High-dose chemotherapy with autologous hematopoietic stem cell rescue has been proven effective in the treatment of breast cancer as salvage chemotherapy for patients with low dose refractory disease [1,2] as consolidation for patients with metastatic disease responding to low dose chemotherapy,[3,4,5] and more recently as part of aggressive "adjuvant" therapy for patients with low cure rate primary disease.[6] This success was predicted based upon the observation that response rate and long-term disease-free survival in breast cancer increases in proportion to dose intensity throughout the range of intensities achievable without autologous hematopoietic stem cell support [7,8,9,10] and the observation that several of the drugs effective in the treatment of breast cancer can be given in very much higher doses with autologous hematopoietic stem cell support.[11,12,13,14] However, the success of high-dose chemotherapy programs, while significant and comparable to what has been achieved in malignant lymphoma,[15] leaves significant room for improvement. Improving upon these results may not be easily achieved by using regimens developed for the treatment of other malignancies or even by extrapolating within the breast cancer setting between different disease situations. We have attempted to develop a regimen specifically for use in high-dose "consolidation" chemotherapy which recognizes these constraints.

Low dose thiotepa and cyclophosphamide are capable of curing small numbers of breast cancer patients in the surgical adjuvant setting.[16,17] In autologous hematopoietic stem cell rescue doses, thiotepa, cyclophosphamide, and hydroxyurea all have activity in low-dose refractory metastatic disease.[18] The combination of high-dose thiotepa and cyclophosphamide has been demonstrated to be effective in producing prolonged unmaintained remissions in patients with early metastatic breast cancer responding to "induction" chemotherapy.[5] Finally, hydroxyurea has been shown to potentiate the

antineoplastic affects of alkylating agents in vitro and in vivo in our laboratory.[19]

PATIENTS AND METHODS

In the first year of this study, 12 patients with metastatic breast cancer responding to outpatient chemotherapy were treated with thiotepa 150 mg/M^2 by 2 hour intravenous infusion daily for 4 days separated by 12 hours from cyclophosphamide 1500 mg/M^2 by 2 hour intravenous infusion daily for the same 4 days, followed by hydroxyurea 1500 mg/M^2 by mouth every six hours for the subsequent 3 days and then autologous hematopoietic stem cell rescue 8-12 hours after completion of the hydroxyurea (CTH-Figure 1). The patients were all pre-menopausal at diagnosis, estrogen receptor negative or borderline (<20 fmol) or had failed hormonal therapy, and no patient had intracranial metastases or significant co-morbidity, including total adriamycin dose less than 420 mg/M^2. All patients had received less than 10 months of chemotherapy for metastatic disease and less than 16 months total chemotherapy including "adjuvant." They ranged in age from 33 to 47 with a median age of 42. They had had a median of months of prior chemotherapy of 5 with a range of 3-16 and their best measurable site of metastatic disease was lung in 4 patients, liver in 4 patients, bone in 2 patients, and distant lymph node or inflammatory breast cancer 1 patient each. Seven of the patients had marrow involvement and were transplanted using autologous peripheral stem cells and 5 had no evidence of marrow involvement and were transplanted using autologous marrow (Table 1).

All patients were hospitalized in single-room isolation with HEPA filtered air and constant positive pressure to the corridor. All patients received prophylactic nystatin and those with significant antibody titer to Herpes simplex virus received prophylactic intravenous acyclovir. Irradiated packed red blood cells were given daily as needed to keep the hemoglobin at 10 gm and irradiated donor platelets were given daily if the platelet count fell below 20,000/uL. Empiric antibiotics were started at the time of first neutropenic fever. Amphotericin B (0.5 mg/kg) was started when fever refractory to antibacterial agents occurred. Patients were supported on total parenteral nutrition without heparin or lipids from the initiation of chemotherapy until at least 10 days after transplant. Patients received hydration and bladder irrigation during cyclophosphamide administration to prevent hemorrhagic cystitis.

Patients received no further chemotherapy after hospital discharge. Three patients received hormonal therapy from their referring physician despite receptor negative status or demonstrated hormonal refractoriness. Patients were evaluated for response at hospital discharge and at 100 days following transplant. These and subsequent periodic evaluations (usually every three months) included repeat of all radiographic studies demonstrating disease whether measurable or not. Patients were scored as complete or partial response according to standard criteria except that patients with only bone and bone marrow disease were considered to have a partial response if they

achieved complete symptomatic relief and were considered to remain partial responders until clinical or radiographic evidence of progressive disease. Patients achieving radiographic complete response at any time after CTH were scored as having achieved complete response at the time of CTH therapy.

Written informed consent was obtained as required and approved by the University of Nebraska Institutional Review Board. Results are calculated as of July 15, 1990. The minimum follow up was 40 weeks and the maximum follow up 72 weeks from transplant with a median of 55 weeks. The time-to-event curve was calculated using Kaplan-Meier product limit method.

RESULTS

The toxicity of the CTH regimen in this patient population was moderate (Table 2). Severe nausea and vomiting refractory to anti-emetics for several days occurred in only 1 patient. The most troublesome, acute regimen-related toxicity was moderate mucositis in 4 patients and mucositis severe enough to require parenteral narcotics in 5 patients. Life-threatening pulmonary toxicity in the form of a diffuse alveolar hemorrhage syndrome associated with unexplained fever and platelet consumption19 occurred in 4 patients, 1 of whom died. There was one other death which was due to candida sepsis and pneumonia. Time to recovery to an absolute granulocyte count of 500/uL ranged from 15 to 68 days with a median of 24 days. Time to platelet transfusion independence ranged from 16 to 100 days with a median of 24 days.

A total of 4 patients were thought to be in complete remission prior to the transplant. Of these, 1 was found to have metastatic adenocarcinoma in a bone marrow biopsy performed for evaluation of delayed hematopoietic recovery after transplant which probably reflects sampling error missing persistent disease prior to transplant. The other 3 patients remained in complete remission after transplant and are currently in complete remission at 40, 55, and 71 weeks from transplant. The remaining 8 patients were in partial remission at the time of transplant. Two of these converted to complete remission. One of these remains in complete remission at 49 weeks and the other relapsed 40 weeks after transplant. Four patients were still only in partial remission after transplant and progressed at 15, 16 and 38 weeks in three instances. One patient, however, remains progression free with persistent abnormalities on CT of the liver at 72 weeks. The two patients who died during transplant were in partial remission prior to transplant (Table 3). The Kaplan-Meier projected progression-free probability for the 11 patients who were in CR or PR after transplant is approximately 50% at one year median follow up (Figure 2).

DISCUSSION

Studies of high-dose "consolidation" chemotherapy in the leukemias and lymphomas have demonstrated that such treatments can be curative or at least produce long intervals of therapy-free, disease-free survival for selected patients.[15] A compelling body of evidence now exists that similar strategies have

produced similar results in solid tumors, especially breast cancer.[3,4,5] The development of improved "consolidation" chemotherapy for responding metastatic breast cancer will require continued investigation of new regimens in this specific setting. Clearly, regimens whose effectiveness has been established in transplant regimens for other malignancies cannot be expected to be equally effective in breast cancer. Reliance on the results of clinical trials of dose escalation in patients with advanced refractory disease may result in the exclusion of agents to which resistance would not have developed earlier, and incorrect assumptions about toxicity due to the significantly greater co-morbidity in advanced disease patients. Studies to determine the optimum "induction" and "consolidation" regimens and the determination of optimum timing of high-dose of "consolidation" are necessary to avoid drug resistance and excess toxicity. Further investigation of all these parameters is indicated because this therapeutic approach is effective in metastatic breast cancer.

The CTH combination chemotherapy program for high-dose consolidation chemotherapy in responding breast cancer was developed as a rational extension of previous clinical investigations and laboratory evidence for synergy between the drugs involved. The CTH regimen was tolerated reasonably well by this somewhat heterogeneous population of patients responding to outpatient chemotherapy for their metastatic breast cancer. The most troublesome toxicity was the diffuse alveolar hemorrhage syndrome which we have previously described.[20] This may correlate with the high frequency of moderate to severe mucositis which we observed but did not always occur in the same patients and has not been described by others. While we have seen this syndrome with other high-dose chemotherapy programs and in patients with other malignancies, the incidence in those settings is lower. Other possibilities being investigated include association with pulmonary metastases, especially "lymphangitic" and viral or other occult infections.

The CTH regimen is effective high dose "consolidation" chemotherapy for metastatic breast cancer responding to low dose "induction" chemotherapy. The 50% probability of being disease-free at one year is comparable to results achieved with similar regimens by other investigators.[3,4,5] More mature data from other centers suggest that an additional 50% of patients will relapse in the second year of follow up but that a 25% multi-year disease-free survival can be predicted.[4,5,21] As in other series, patients in complete remission prior to transplant seem to have the best probability of long-term disease-free survival.

Several of the patients in our series did not meet the usual criteria for partial remission but had symptomatic improvement of skeletal metastases and were included on that basis. Inclusion of such patients might be expected to decrease the long-term disease-free survival probability, however, one of these patients remains progression free at 49 weeks and is now considered to be in complete remission. Seven of our 12 patients were transplanted with hematopoietic stem cells collected from peripheral blood by pheresis because the marrow was histologically involved with breast cancer. No other series to date has included significant numbers of such patients. Whether marrow

involvement represents a favorable or unfavorable prognostic variable in high-dose chemotherapy has not been determined.

High-dose chemotherapy with autologous marrow transplantation as "consolidaton" chemotherapy for patients with hormone refractory, chemotherapy sensitive metastatic breast cancer is remarkably effective treatment for this disease situation. No other treatment accomplishes any significant disease-free interval off chemotherapy for any significant percentage of these patients. Further improvements in these regimens designed to improve therapeutic index will result from rational application of concepts and experience from the clinic and the laboratory.

ACKNOWLEDGEMENTS

Supported in part by NCI Grant ROl-CA45529-03 and the National and Nebraska Ladies Auxiliary of the Veterans of Foreign Wars.

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TABLE 1

HIGH-DOSE CTH "CONSOLIDATON" CHEMOTHERAPY FOR RESPONDING EARLY METASTATIC BREAST CANCER -- ELIGIBILITY

Age < 55

Pre-menopausal at diagnosis

ER negative or failed hormonal therapy

Responding to most recent chemotherapy for metastatic disease

No intracranial metastases

No significant co-morbidity

Total adriamycin less than 420 mg/M^2

Disease still chemotherapy sensitive

- less than 10 months chemotherapy for metastatic disease
- less than 16 months total chemotherapy including "adjuvant"
- in complete or partial remission

TABLE 2

HIGH-DOSE CTH "CONSOLIDATION" CHEMOTHERAPY FOR RESPONDING EARLY METASTATIC BREAST CANCER -- TOXICITY

Number	12
Nausea, vomiting	
minimal	2
moderate	9
severe	1
Mucositis	
minimal	3
moderate	4
severe	5
DAH*	4
Death	2 (1-DAH, 1-candida sepsis)
AGC < 500	15-68 days (median - 24)
Platelet supported	16->100 days (median - 24)

*DAH = Diffuse alveolar hemorrhage

TABLE 3

HIGH-DOSE CTH "CONSOLIDATION" CHEMOTHERAPY FOR EARLY METASTATIC BREAST CANCER -- OUTCOME (7/15/90)

Status before CTH	Status after CTH	No.	Duration (wks)
Complete response	Complete response	3	40+,55+,71+
Complete response	No response	1	--
Partial response	Complete response	2	40,49+
Partial response	Partial response	4	15,16,38,72+
Partial response	Died free of disease	2	--

FIGURE 1
Dose and schedule of CTH

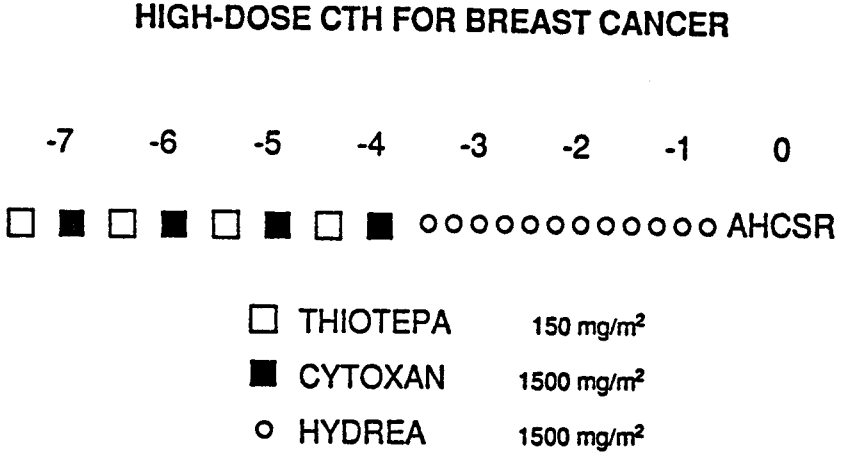
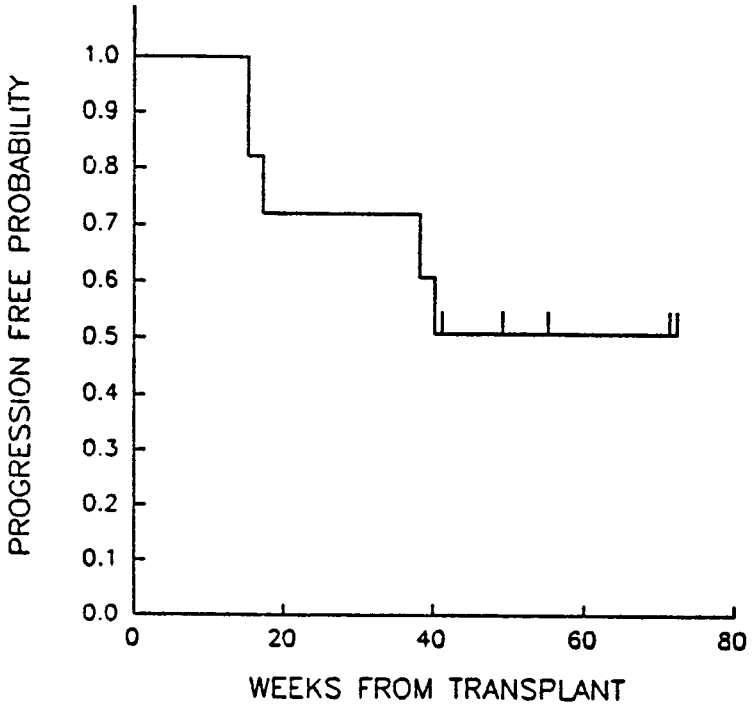


FIGURE 2
Probability of remaining progression free for 11 patients in CR or PR after CTH consolidation.



HIGH DOSE CHEMOTHERAPY (CMT) AND BONE MARROW TRANSPLANTATION (BMT) IN THE TREATMENT OF METASTATIC BREAST CANCER

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INTRODUCTION

Metastatic or recurrent breast cancer is generally a fatal disease despite the fact that significant response rates are obtained with conventional dose therapy (1). While disease that is sensitive to hormonal therapy may have a relatively protracted course (2), patients with receptor negative tumors or those who have failed hormonal therapy have a median survival of 12-18 months -- with few survivors beyond three years. The average survival duration is particularly short in patients who have received prior adjuvant chemotherapy and/or have visceral metastases (3). Over the past several years, attempts to improve the outlook in this group of patients with very intensive cytoreductive therapy and bone marrow transplantation (BMT) have shown some promise (4). This report reviews the initial results of a phase I-II study of BMT therapy for patients with metastatic or recurrent breast cancer whose tumors were either receptor negative or who had failed hormonal therapy.

PATIENTS AND METHODS

Between June 1986 and March 1990, nineteen patients with recurrent or metastatic breast cancer were entered in this study after informed consent was obtained. Patients with well circumscribed regional recurrences treatable by local therapy alone were excluded from this study. Eligibility criteria included negative hormone receptor status or failure to respond to hormonal therapy, age < 60 years, and ECOG performance status of 0 or 1. Table I details the pretransplant characteristics of the 19 patients that are the subject of this report. As can be seen from this table, patients with prior adjuvant and/or salvage therapy were not excluded from study.

The preparative regimen consisted of thiotepa, cisplatinum and cyclophosphamide. The dose and schedule of these drugs is shown in Table II along with the dose escalation scheme for the phase I portion of the study. The

last 4 patients have received doses of thiotepa and cisplatinum that were decreased to the dose set for the phase II portion of the study.

As long as there was no evidence of pelvic bone marrow involvement (by bone scan and bilateral bone marrow biopsy), autologous marrow was the preferred source for transplantation. Three patients received allogeneic BMT because of clear evidence of marrow involvement. All three were under 45 years of age and had HLA matched sibling donors. Bone marrow was collected from the posterior iliac crests by standard methods (5). Autologous marrow nucleated cells were concentrated by preparing a buffy coat on the Cobe 2991 cell washer. The volume of the cell suspension was adjusted to about 4-5 x 10^7 cells per ml in a medium containing a final concentration of 10% DMSO, 45% tissue culture medium (TC199), and 45% irradiated autologous plasma. Fifty or 100 ml aliquots were placed in polyolefin bags and cryopreserved in a Cryo-Med controlled rate freezer at a rate of 10C per minute. Reinfusion of autologous marrow was carried out as previously reported (6).

During post-transplant recovery, the patients were hospitalized in single rooms with non-laminar, HEPA filtered air. Supportive care was provided as previously described for patients with hematologic malignancies treated with BMT (7). In order to minimize the problem of the post-transplant capillary leak syndrome, total body water was maintained at the lower limits of normal in all patients by the use of diuretics and fluid restriction during the first month post-transplant. At the first sign of fever, the patients were treated with broad spectrum antibiotics. Vancomycin and/or amphotericin were added for appropriate indications or used empirically for persistent fever. Transfusions were given to maintain hemoglobin levels >10 gm per deciliter and platelets $>20{,}000$ per microliter. All patients received parenteral nutrition.

RESULTS

As can be seen from Table I, the nineteen patients reported here represent a relatively advanced group of patients. Ten of the patients were stage 3 or greater at the time of diagnosis. At the time of recurrence, 13 patients had evidence of tumor in multiple organs or tissues. The six patients with single organ involvement all exhibited multiple nodules or sites within the involved tissue. Eleven patients had received radiotherapy prior to the BMT -- 8 as adjuvant therapy after primary surgery, one as adjuvant therapy and to treat recurrent tumor, and two as salvage therapy at the time of recurrence. Among the 19 patients, 3 had received no prior chemotherapy, 5 had received prior adjuvant chemotherapy only, 5 had received both adjuvant and salvage chemotherapy, and 6 had received salvage chemotherapy at the time of recurrence. Among the 11 patients who had received salvage chemotherapy, one showed a partial response and the rest exhibited no response or progression.

Table III shows the detailed dose level, sources of marrow, engraftment, and post-transplant toxicity for patients. Sixteen of the patients whose bone marrow biopsies and bone scans were negative for tumor in the

iliac crest received autologous BMT. Three patients with marrow involvement received allogeneic transplants from HLA matched siblings. These patients received graft-vs-host disease (GVHD) prophylaxis with cyclosporine and short course methotrexate as described by the Seattle group (8). All three allogeneic transplant patients engrafted without evidence of significant GVHD. For those patients receiving autologous BMT, trilineage engraftment occurred relatively promptly. Overall, the median time to granulocytes >500 cells per microliter was 20 days and the median time to become independent of platelet transfusions was 27 days. One patient died too early to evaluate neutrophil recovery. An additional 2 patients died too early to evaluate platelet engraftment.

In addition to the predictable hematopoietic toxicity of the preparative regimen, moderate to severe gastrointestinal and hepatic toxicity was seen in most patients and significant renal toxicity was seen in a fraction of the patients. Three patients died of treatment related toxicity -- 2 with documented or presumed infection and adult respiratory distress syndrome (ARDS) and one with disseminated fungal infection. As a consequence of the GI and hepatic toxicity, the dose levels of thiotepa and cisplatinum were decreased in the last 4 patients reported here.

In analyzing the response to therapy, the patients have been divided into two groups (A and B). Group A contains the 8 patients who received no salvage chemotherapy. Group B contains the 11 patients who had received prior conventional dose chemotherapy. Table IV presents the number of complete and partial responders in each group and the number of patients who remain in continuous complete remission. One patient in Group A who had multiple bilateral pulmonary metastases prior to BMT has been counted as a complete response although a post-transplant CT scan of the chest revealed residual nodules. Since then, she has undergone bilateral thoracotomies. All suspicious nodules were resected, only one of which contained tumor. Figure 1 presents the actuarial event-free survival (EFS) for each group of patients. Although the groups are too small for much statistical precision, these curves provide a simple way to depict the duration of follow-up.

DISCUSSION

Durable remissions have been rare in late stage patients (i.e. those who fail conventional dose salvage chemotherapy) with metastatic breast cancer who receive BMT therapy. On the other hand, initial response rates to BMT therapy have been relatively high (9). This has encouraged investigators to use BMT therapy in patients at the first sign of metastatic disease. Reports from these studies indicate that complete response rates exceeding 50% are attainable. Long term follow-up of these patients suggests that an EFS plateau of 15-25% will be achieved (10-12). There are several directions which might be followed to improve these results.

First, one might utilize the preparative regimens already described after conventional dose therapy has decreased the tumor burden. Sequential studies reported by Peters (13) suggest that this approach may shift the survival curve

to the right without significantly influencing the plateau of long term EFS. Second, in the adjuvant setting, one might employ a preparative regimen which has already shown activity for recurrent or metastatic disease; selecting patients who have a high probability of relapse. Such trials are already underway and are being proposed in the cooperative group setting. The third approach to improving treatment results in metastatic breast cancer is to devise preparative regimens with enhanced antitumor activity without concomitant increases in the risk of fatal toxicity. One approach to achieving this latter aim is to fractionate therapy; i.e. administer two or more dose intensive regimens with BMT. This approach has been reported by Spitzer and his associates (12). With the preparative regimens employed, it is not clear that the plateau of EFS is significantly better than that achieved with a single transplant.

This study has focused on developing a single preparative regimen to treat patients with metastatic or recurrent breast cancer; starting with the assumption that the dose and schedule of cytotoxic agents could have a significant impact on therapeutic efficacy. The premise that the schedule of administration may critically affect the antitumor efficacy of a preparative regimen is supported by data obtained in the treatment of acute lymphocytic leukemia (ALL). Patients with ALL in second remission transplanted at Memorial Sloan-Kettering (14) after total body irradiation (TBI) followed by cyclophosphamide (CY) relapse less frequently than similar patients treated at Seattle using equivalent total doses of CY and TBI, but in the reverse order (15).

Several considerations form the basis for the preparative regimen used in this study. The pretransplant therapies associated with the highest success rates for breast cancer have consisted of combinations of alkylating agents (9). In order to maximize the dose of each drug, no more than two drugs were administered simultaneously. In order to maximize the total dose of alkylating agents, a second pair of drugs was given at the time of hematologic nadir. Finally, a dose escalation scheme was used with thiotepa and cisplatinum, since there was less experience with these agents in the BMT setting. Most of the patients studied during the phase I portion of this protocol had received prior conventional salvage chemotherapy. While a relatively high total response rate has been observed (Table IV), the durability of the responses has been equivalent to that seen in other studies with similar groups of patients. Treatment toxicities -- particularly gastrointestinal and hepatic -- have been moderate to severe. Three treatment- related deaths occurred which were due to early infection. Six of the eight patients who had not received prior salvage chemotherapy had no evidence of disease (NED) after BMT (in 1 patient the elimination of disease required post-transplant resection of a residual nodule). Four of these patients are still in remission. No fatal toxicities have been observed in this group of patients. These results may be better than those reported in other studies and merit verification in a larger phase II study.

One of the problems with the use of autologous BMT in the treatment of metastatic breast cancer is the frequency of marrow metastases. There is data to suggest that breast cancer cells can be grown in culture from marrows

of patients who have no marrow involvement detectable by standard microscopic examination (16). Several approaches may be taken to circumvent this problem. Two that have been presented at this symposium involve ex vivo marrow purging (17) or the use of peripheral blood stem cells (18). Another approach would be to use allogeneic BMT in patients under age 45 who have an HLA matched donor. Experience with allogeneic BMT in breast cancer is more limited than in the hematologic malignancies. The capacity of preparative regimens to provide sufficient immunosuppression has, therefore, rarely been tested. Thus, it is of some interest that three of the patients in this series received allogeneic BMT with clear evidence of engraftment of donor marrow.

In summary, 19 patients have been treated with a preparative regimen containing 3 alkylating agents given in a dose schedule to maximize individual and cumulative drug dose. The regimen has been sufficiently immunosuppressive to support allogeneic BMT. Response rates and durability of response in patients who have failed conventional dose chemotherapy have been similar to those reported for other regimens. In the small group of patients who have been treated with this regimen as the initial therapy for metastatic breast cancer, relatively durable remissions have been obtained in a significant fraction of patients. These results warrant a larger phase II study in patients with metastatic breast cancer who have not failed conventional dose salvage therapy.

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TABLE I

Pretransplant Patient Characteristics

	STAGE AT DX		RECEPTORS		PRIOR THERAPY ADJUVANT		SALVAGE		TUMOR SITES AT BMT						RESP TO
UTN	STAGE	TNM	ER	PR	XRT	CMT	XRT	CMT*	LG	LV	LN	CW	BN	OT	SALV RX
46	1	100	-	-	N	N	N	1	1	0	1	0	0	0	2
49	4	431	-	-	N	N	N	3	2	0	2	2	0	0	2
59	2	200	-	-	N	N	Y	1	0	2	2	2	2	1	2
62	4	331	-	-	N	N	N	0	2	0	2	0	0	1	0
63	2	200	+	-	N	N	N	1	0	0	2	0	0	0	2
66	2	210	-	-	N	Y	N	2	2	0	2	0	0	0	2
76	3	320	-	-	Y	Y	N	0	0	2	0	0	0	0	0
79	1	100	+	-	Y	N	N	0	2	0	0	0	0	0	0
110	1	100	-	+	N	N	N	2	0	0	0	0	2	2	1
111	3	220	-	-	Y	Y	N	0	0	0	0	2	0	0	0
119	3	120	+	+	N	Y	N	0	0	0	2	0	0	0	0
121	2	210	-	-	Y	Y	N	2	2	0	0	0	0	1	2
129	3	330	-	-	Y	Y	N	2	0	0	0	2	2	1	2
131	3	430	-	-	Y	Y	N	2	0	1	2	2	0	1	2
132	3	220	-	-	N	Y	Y	4	1	0	0	2	0	0	2
143	1	100	-	-	N	Y	N	0	2	0	0	0	0	0	0
148	3	320	-	-	Y	Y	N	2	0	0	1	1	2	0	2
157	3	200	-	-	Y	N	N	0	1	0	1	0	1	0	0
152	3	310	+	+	Y	Y	Y	0	0	0	0	0	2	0	2

UTN = UNIQUE TRANSPLANT NUMBER
Y = YES
N = NO
+ = POSITIVE
- = NEGATIVE
XRT = RADIOTHERAPY
CMT = CHEMOTHERAPY
** = NUMBER OF CHEMOTHERAPY REGIMENS USED*
LG = LUNG
LV = LIVER
CW = CHEST WALL
LN = LYMPH NODE
BN = BONE
OT = OTHER
0 = NONE
1 = SINGLE METASTATIC SITE
2 = MULTIPLE METASTATIC SITES
RESP TO SALV RX:
0 = UNTESTED
1 = PR
2 = PROG

TABLE II

Transplant Preparative Regimen
and Dose Escalation Scheme

DAY	TT	PT	CY	DRUG DOSES
-11	X	X		THIOTEPA (TT)
-10	X			LEVEL 1 — 225 mg/m2/d X 3
-9	X			LEVEL 2 — 300 mg/m2/d X 3
-8				LEVEL 3 — 250 mg/m2/d X 3
-7				CISPLATIN (PT)
-6				LEVEL 1 — 50 mg/m2 dose 1 & 2
-5				LEVEL 2 — 100 mg/m2 dose 1, 50 mg/m2 dose 2
-4				LEVEL 3 — 100 mg/m2 dose 1 & 2
-3		X	X	LEVEL 4 — 75 mg/m2 dose 1 & 2
-2			X	CYTOXAN (CY)
-1				FIXED DOSE — 60 mg/kg/d X 2
0	BMT			

TABLE III

BMT Dose Level, Engraftment and Toxicities

	DOSE LEVEL			DAYS TO								
UTN	TT	PT	TYPE OF BMT	POLYS >500	PLT IND	GI	CREATININE PEAK	CREATININE DAY	LIVER PEAK BILI	LIVER PEAK SGPT	LIVER PEAK ALK PH	INF
46	1	1	AUTO	14	•	13	7.7	22	6.0	136	114	0
49	1	1	AUTO	15	22	15	2.6	26	1.7	15	140	2
59	1	0	AUTO	•	•	TETE	1.5	16	11.4	TETE	TETE	1
62	1	1	AUTO	12	20	20	2.5	40	1.7	177	160	1
63	1	1	AUTO	20	26	2	4.1	7	5.3	122	196	1
66	1	1	AUTO	20	28	9	1.7	13	1.6	68	149	2
76	2	1	AUTO	22	25	18	1.2	9	1.9	196	486	2
79	2	1	AUTO	21	32	10	1.3	8	10.3	27	421	0
110	2	2	ALLO	19	26	12	2.0	13	2.8	27	431	1
111	2	2	AUTO	16	36	16	2.4	59	1.6	25	279	1
119	2	2	AUTO	12	12	9	1.2	16	0.7	31	185	1
121	2	3	AUTO	17	17	14	2.0	-2	0.9	65	114	1
129	2	3	AUTO	17	32	19	1.2	30	3.6	95	450	2
131	2	3	AUTO	11	•	14	3.2	12	24.5	110	134	2
132	2	3	AUTO	19	32	12	4.8	19	7.1	69	452	1
143	3	4	AUTO	13	19	3	0.7	15	1.1	100	262	0
148	3	4	ALLO	20	40	7	1.9	19	1.7	61	339	1
157	3	4	AUTO	22	21	5	1.2	8	0.9	474	358	2
152	3	4	ALLO	21	15	30	1.3	32	1.5	339	93	1

UTN = UNIQUE TRANSPLANT NUMBER
• = NOT REACHED BEFORE DEATH
PLT IND = PLATELET TRANSFUSION INDEPENDENT
GI = DAYS DIARRHEA
TETE = TOO EARLY TO EVALUATE
INF = INFECTION

UTN 46 & 131 DIED WITH ARDS
UTN 59 DIED OF FUNGAL SEPSIS

TABLE IV

Summary of Therapeutic Outcome

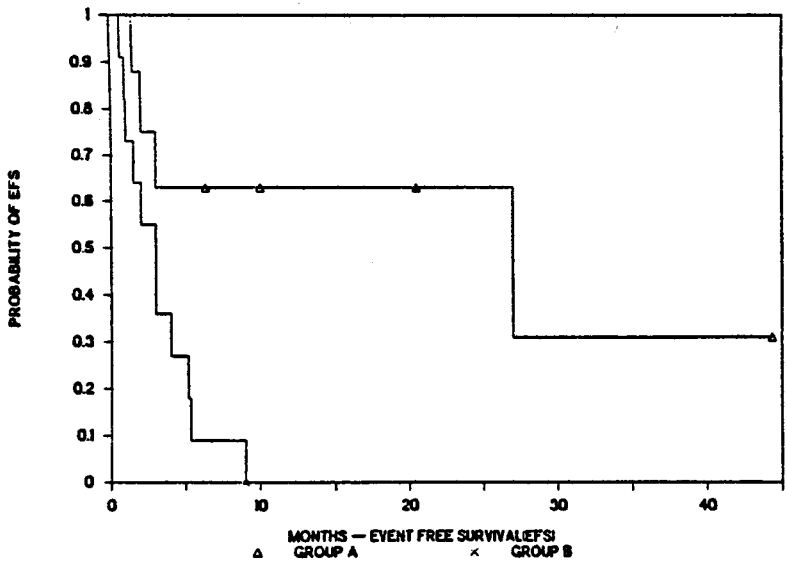
Number of Patients with	Group A*	Group B*
Therapy Related Death	0	3
Complete Responses	6**	1
Partial Response	2	7
Continuous CR	4	0

* Group A = Patients receiving no salvage CMT
* Group B = Patients receiving salvage CMT

** Includes one patient with a partial response rendered NED by thoracotomy and resection of a single residual lesion.

FIGURE 1

Actuarial event-free survival for Groups A and B. Group A = patients receiving no prior conventional salvage therapy; Group B = patients failing prior conventional dose salvage therapy.



POTENTIAL INDICATIONS FOR HIGH DOSE CHEMOTHERAPY PROGRAMS IN HIGH RISK PRIMARY BREAST CANCER

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INTRODUCTION

Experience with surgical therapy of primary breast cancer during the first half of this century demonstrated several important biological principles. First, breast cancer was curable in some instances with surgical therapy alone. Second, many patients with apparently localized breast carcinoma developed recurrent metatastic disease after "curative" local/regional therapy. Third, tumor size and regional lymph node involvement were powerful prognostic indicators, and correlated strongly with the probability of treatment failure. These principles led to the first paradigm shift some thirty years ago. The acknowledgment that the Halstedian hypothesis was not tenable led to two important directions in therapeutic research. The first one resulted in the development of breast conservation local/regional therapies. A number of prospective clinical trials have clearly demonstrated that breast conservation therapy provides cure rates equivalent to those obtained with radical mastectomy (1,2). A potentially more important line of research led to the development of combined modality therapies to improve the cure rate obtained by local/regional treatments. The last twenty years have seen the establishment of adjuvant systemic therapies as an integral part of the primary curative treatments of breast carcinoma (3). Individual clinical trials have demonstrated a decrease in the frequency of metastatic failure, as well as mortality, following adjuvant chemotherapy or adjuvant hormone treatments. Although there is ongoing controversy as to the optimal type and duration of treatment for the various prognostic subgroups, there is little argument about the fact that adjuvant systemic treatments decrease mortality (4).

The development of adjuvant chemotherapy in particular was followed with very high expectations, in part supported by the early results of adjuvant chemotherapy trials. However, more mature analysis suggested that the reduction in mortality rates by adjuvant systemic therapies was not as great as originally expected (4). This has led some investigators to suggest that completely new directions of research are needed. Others, suggest to build on

the progress demonstrated thus far. In this paper I will concentrate on the potential contributions of high dose chemotherapy regimens and the curative treatments of high risk primary breast cancer.

Current Status of Adjuvant Therapies for Primary Breast Cancer

The overview of randomized trials included about 40,000 women treated in approximately to 100 clinical studies (4). This included 28 trials of tamoxifen, 40 trials of chemotherapy, 19 trials of radiotherapy and 10 trials of ovarian ablation. The overall reductions in the odds of death during the first five years were 16% for tamoxifen versus no tamoxifen, and 11% for chemotherapy versus no chemotherapy. Both of these differences were highly statistically significant. Radiotherapy did not produce any survival benefit, and ovarian ablation appeared promising, but the differences did not reach statistical significance. The effect of tamoxifen was more pronounced in women over age 50, for whom the reduction in odds of death reached 1/5. Those trials where tamoxifen was administered for two years or longer showed a higher reduction in odds of death than those trials where tamoxifen was administered for a shorter time. The benefit from chemotherapy was most pronounced in women under the age of 50, for whom the reduction in odds of death was over 25%. The benefit was even higher for women treated with combination chemotherapy (30% reduction in mortality). The overview showed a much more modest effect for adjuvant chemotherapy in women over the age of 50.

Individual prospective trials with combination chemotherapy have shown reductions in mortality of up to 55% for women under age 50, and up to 37% for women over age 50 (5, 6). Therefore, it is clear that there are quantitative differences between the various adjuvant systemic therapies, and differences among various regimens.

Although there is much additional research to be done with standard treatment modalities, it is clear that even optimizing chemotherapy and hormone therapy within the standard dose ranges will leave much room for improvement. This is especially true for patients in the high risk categories where, despite optimal combined modality treatment, more than 50% of patients continue to relapse and die of their disease.

High Dose Chemotherapy Regimens

Numerous preclinical experiments point to the importance of a dose response correlation (7). A number of retrospective clinical reviews have also shown that there is a positive correlation between dose and response to therapy in metastatic breast cancer and dose interval in both metastatic breast cancer and primary breast cancer treated with adjuvant chemotherapy (8). A few prospective randomized trials have suggested that higher doses of currently available cytotoxic agents correlate with higher response rates, and sometimes, longer median survival times than the lower doses of the same agent or combination (Table 1) (9-14). The dose-limiting toxicity of many systemic agents is myelosuppression. With the development of autologous marrow

storage and reinfusion, higher-than-standard doses of myelosuppressive agents can be administered without the expected frequency of lethal complications. Many studies of chemotherapy with autologous marrow support have been reported by now (15). The initial small studies included patients with extensive metastatic disease who had become refractory to prior systemic therapy. While these studies demonstrated the feasibility and relative safety of this approach, and also showed that high response rates were obtainable, complete responses were few, response durations were short, and the mortality rate was high (25 to 30%) (Table 2). Subsequent studies restricted entry to patients with less extensive prior therapy, and limited metastatic disease. The complete remission rate in this group of patients was higher, but still no obvious survival benefit was detected. More recent studies limited eligibility to patients with minimal metastatic disease, and no previous exposure to chemotherapy. These patients, when treated with high dose combination chemotherapy, achieved a very high overall response rate, a high complete remission rate and response durations that parallel those achieved with standard chemotherapy (16,17). Although these studies are yet immature, there is no apparent improvement in overall survival rate. However, longer follow-up may show a subpopulation of long term survivors. Subset analysis in some of these studies has suggested that those patients treated after achieving a complete remission (either through surgery or standard chemotherapy) have a lower recurrence rate after high dose chemotherapy, confirming again the prognostic value of extent of metastatic disease (17).

These studies suggested that additional development in high dose chemotherapy programs should concentrate in two areas. The first is the development of more effective cytoreductive regimens. The second is the evaluation of existing, effective cytoreductive regimens in patients with high risk of progression or recurrence, but no detectable residual disease. An earlier session reviewed the use of high dose chemotherapy regimens in patients with metastatic disease. I will point out those subgroups of patients with primary breast cancer who might be candidates for high dose chemotherapy programs.

High Risk Primary Breast Cancer

Patients with 10 or more axillary lymph nodes involved. This subgroup of patients has a very high risk of recurrence after local/regional treatments. In various series, 80-95% of patients die of recurrent or metastatic breast cancer in the absence of systemic adjuvant treatments (18). At our own institution the 10 year disease free survival rate for this subgroup of patients is 38% after surgery and adjuvant chemotherapy (19). Therefore, every patient in this category is at high enough risk despite state-of- the-art combined modality therapy to justify the higher risk of high dose chemotherapy regimens. As a result, there are now two prospective randomized trials addressing the issue of the contribution of high dose chemotherapy to the adjuvant treatment of patients with 10 or more positive axillary lymph nodes.

Patients with less than 10 positive lymph nodes and other adverse prognostic factors. Although the number of axillary lymph nodes involved continues to be one of the strongest prognostic indicators, recent reports have suggested that other tests might contribute to the determination of prognosis (20-22). For instance, over-expression of the HER2/neu oncogene in patients with node-positive breast cancer identifies a subgroup that has a much higher risk of recurrence than a similar subgroup with identical lymph node involvement but no oncogene overexpression. Patients with estrogen receptor negative tumors also have a somewhat worse prognosis, and higher recurrence rate than their estrogen receptor positive counterparts. It is possible that S-phase fraction and ploidy also add to the identification of poor prognostic subgroups in the node positive population. At this point I would consider patients with node positive breast cancer, and overexpression of HER2/neu oncogene candidates for high dose chemotherapy studies. The role of some of the other prognostic factors in identifying poor prognostic subgroups in node positive breast cancer remains to be confirmed.

Stage III Breast Cancer. Patients in stage III breast cancer have a poorer prognosis than those in earlier stages. with the exception of patients with negative lymph nodes, these patients have a greater than 50% recurrence rate despite appropriate adjuvant systemic therapy (6). Therefore, they could be considered appropriate candidates for well designed, prospective trials to evaluate the role of high dose chemotherapy programs.

The development of combined modality regimens that include primary chemotherapy has been a major contribution to the treatment of patient with stage IIIB and inflammatory breast carcinoma. Before these strategies were developed, more than 90% of these patients developed recurrent or metastatic disease, and died of this cause (23). Several recent reports have demonstrated that 30-50% of patients with inflammatory breast cancer survived disease-free beyond 5 years, and a similar fraction of patients with stage IIIB had achieved long term disease-free survival (24-26). Analysis of prognostic factors in both of these subpopulations suggests that response to primary chemotherapy, and the extent of residual disease after primary chemotherapy, appeared to be strong prognostic indicators. Therefore, one could envision selecting patients with large residual tumor burdens, or poor response to primary chemotherapy, but who can be rendered disease-free by surgery, or radiotherapy, or both, as excellent candidates for consolidation therapy with high dose chemotherapy programs.

Node Negative Breast Cancer. Patients with node-negative breast cancer are considered to have an excellent prognosis. If one considers the outcome of an unselected group of patients with negative lymph nodes, their 5 and 10 year survival rate exceeds 80% and 70%, respectively. However, recent publications have looked at subgroups determined by various prognostic indicators. Thus, patients with poorly differentiated tumors, patients with high concentration of cathepsin D, or heat shock proteins, have a much lower expected survival rate (21,22). It is expected that multivariate analysis, taking

into consideration many of these new prognostic indicators will identify subgroups within the node-negative patient population that will have a 50% or higher recurrence rate. Should this be demonstrated and confirmed, those patients would also be candidates for investigational programs to assess the role of high dose chemotherapy regimens.

Most importantly, research of high dose chemotherapy regimens should also concentrate on improving the therapeutic index of these combinations. Thus, improvements in hematopoietic support with autologous stem cells, cytokines, and other supportive systems should be a high priority to decrease the still significant mortality rate associated with these programs. However, the first goal is to develop cytoreductive programs of the highest efficacy so that the curative potential of these strategies can be successfully established.

The dose-response concept is an attractive hypothesis. Preliminary results of high-dose chemotherapy in advanced disease have made a compelling argument to evaluate these regimens in high risk primary breast cancer. However, the role and contribution of high dose chemotherapy to the curative treatment of primary breast cancer remains to be established.

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TABLE 1

Randomized Clinical Trials to Assess the Dose - Response Correlation

Regimens (in mg/m²)	No. of Pts.	Percent Responses		P	Median Survival	P
		Complete	Overall			
Cisplatin 60[9]	18	0	0		Not stated	
Cisplatin 120	19	0	21		Not stated	
Adriamycin 35[10]	24	4	25	0.02	8	0.02
Adriamycin 70	24	17	58		20	
CMF 56-81%[11]	79	15	51	>0.05	14.5	0.03
CMFP 76-95%	86	16	63		16.4	
FEC 500/50[12]	39	2	46	0.025	Not stated	
FEC 500/100	39	13	79		Not stated	
CMF 300/20/300[13]	53	4	11	0.03	12.8	0.26
CMF 600/240/600	53	2	30		15.6	
FAC 500/50/500[14]	27	22	78	>0.05	20	>0.05
FAC 2500/100/1800	32	25	78		20	

TABLE 2

Pooled Results of High-Dose Chemotherapy Programs for Patients with Metastatic Breast Cancer

Patient Group	Induction Therapy	High-Dose Chemotherapy	No. of Patients	No. of CR (%)	No. of CR + PR (%)
Refractory	No	Single-agent	92	4 (4)	23 (25)
Refractory	No	Combination	87	38 (43)	60 (69)
Untreated	No	Combination	65	19 (29)	49 (75)
Untreated	Combination	Combination	224	117 (52)	193 (86)

Abbreviations: CR, complete remission; PR, partial remission.

NEW AGENTS IN CANCER CHEMOTHERAPY

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INTRODUCTION

Resistance to the cytoxic effects of currently available cancer chemotherapeutic agents is a major cause of clinical treatment failure. Despite the evident narrow therapeutic index of available agents, certain malignancies such as lymphomas, germ cell carcinomas and many pediatric cancers are curable with chemotherapy whereas most adult carcinomas and sarcomas are not. The fundamental differences between curable and incurable malignancies remain unclear, but understanding the relative insensitivity - or resistance - to the cytotoxic drugs which many cancers exhibit might offer prospects for improved therapeutic regimens.

Progress has been made elucidating the genetic mechanisms of drug resistance (1). Cancer cells may exhibit one or more alterations such as decreased drug uptake (2), enhanced drug afflux (the mdr phenotype) (3), increased cytoplasmic binding to glutathione or metallothionein (4) enhanced catabolism or decreased activation (5), increased or altered target proteins (6) or enhanced repair of DNA damage (7). These mechanisms may be detected in cultured cell lines or patient samples. While selection pressure (drug exposure) may be necessary to express the drug resistance phenotype, these mechanisms appear to become integral components of the cells genome.

Tumors growing "in vivo" may have additional mechanisms of resistance which are not of genetic origin, are not transmissible to later generations and are not demonstrable in cell culture monolayer. These mechanisms might be referred to as physiologic or epigenetic, a result of the unique factors which are involved in the three dimensional packing of tumor cell masses and their accessibility to blood vessels and essential nutrients. Oxygen, intracellular [H+] and the availability of energy (ATP) are only a few of the cellular environmental factors which may have a profound effect on therapeutic outcome but be totally ignored by investigations of resistance confined solely to cultured cells. These aspects of chemotherapeutic drug resistance have been the basis of recent investigation by our group.

Hypoxia results in decreased proliferation of cells with depletion of energy (ATP), elevated intra-cellular [H+] and relative resistance to many chemotherapeutic drugs and radiation therapy. In 3-dimensional tumors, cells > 140-180 U from a nutrient blood vessel are hypoxic or necrotic, since this represents the limits of 0_2 diffusion (8). Thus, these kinetically dormant, non-cycling cells are resistant to chemotherapeutic agents which are more cytotoxic to proliferating cells. In addition, 0_2 may be a necessary intermediate in the activation of many drugs for oxidation to an active compound or as a free radical intermediate (9). Many drugs cannot pass through several cell layers to reach these cells in cytotoxic concentrations.

Fluosol DA is a perfluorocarbon emulsion which noncovalently and reversibly carries 0_2. Fluosol DA and 0_2 breathing potentiate the cytotoxicity of a number of drugs including alkylating agents (except cisplatin and mitomycin C), etoposide, bleomycin and doxorubicin (10). Besides the effect Fluosol-DA may have on increasing cell proliferation by increased cellular oxygenation, the lipophilic environment provided by the fluorocarbon emulsion may sequester some drugs and either protect them from rapid inactivation by metabolism or binding in the circulation. Clinical trials of Fluosol DA and many chemotherapeutic agents are under way. Phase II doses > 400 ml/m^2 appear to exceed the 8-10 ml/kg dose needed to produce enhancement in preclinical models.

Etanidazole (SR 2508) is a 2-nitroimadazole which was developed as a radiation sensitizer. Etanidazole is a lipophilic compound which is inactive until cellular nitroreductases metabolize it under anoxic conditions whereupon it serves as an electron acceptor and a toxic bioreductive alkylating agent in its own right (11). Etanidazole also depletes cellular glutathione levels, an additional mechanism by which it may serve as a chemoradiosensitizer (12). Etanidazole has enhanced the cytotoxicity of several antineoplastic agents in preclinical studies, though clinical trials have been inconclusive regarding improved efficacy (13).

Preclinical studies of combined Fluosol-DA/0_2 breathing/etanidazole and cyclophosphamide have shown an impressive 2 log (one hundred-fold) decrease in clonogenic tumor cell survival over cyclophosphamide alone. Equally important, a normally oxygenated representative host tissue- bone marrow progenitors - showed no increase in toxicity, promising an increase in therapeutic ratio. Clinical trials are due to begin.

Cellular ATP levels must be adequate not only for cell maintenance but also must be sufficient for proliferation and repair. Depletion of ATP levels may be a proximate cause of cell death after alkylating agent exposure (14). Lonidamine (LND) is an indole carboxylic acid derivative which inhibits the rate of O_2 consumption in normal differentiated and neoplastic cells. This increases aerobic lactic acid production in normal cells but decreases lactic acid in neoplastic cells by inhibition of a mitochonchial bound hexokinase found on the outer mitochondrial surface in many transformed cells (15).

LND potentiates the cytotoxicity of a number of chemotherapeutic agents (16), radiation (17) and hyperthermia (18). It potentiates the cytotoxicity

of a number of alkylating agents, with effect being maximal at lower doses and remaining constant as the alkylating agent is escalated. In combinations with cisplatin and novobiocin or etoposide (both inhibitors of DNA topoisomerase II), LND produces substantial cell killing (about 2 logs) at doses of all 3 modulators which have minimal toxicity. In vivo trials and combinations with alkylating agents are ongoing.

The role of genetic alterations in drug resistance has also been a focus of our efforts. An enhanced sensitivity to doxorubicin is several alkylating agent resistant tumor lines prompted investigations of DNA topoisomerase II, a nuclear enzyme which plays an essential role in eukaryotic DNA replication, RNA transcription and mitosis (19). Topoisomerase II functions by transiently cleaving double stranded DNA, allowing passage of doubled stranded DNA and relegating the cleaved strands. This strand passing activity release and then restores torsional constraints in supercoiled DNA at replication points or decatenates and unknots newly replicated DNA. Several antineoplastic agents including the epipodophyllotoxins, doxorubicin, amsacrine, mitoxantrone and others form ternary complexes leading to cleavage of DNA by stabilizing the topoisomerase II molecule, which initiates a still undefined process resulting in cell death. Most cell lines which are resistant to the above agents are so because of quantitative decreases in the level of topoisomerase II or alterations in the enzyme which make it a less effective intermediate for drug toxicity.

Increases in topoisomerase II levels have been noted in several alkylating agent resistant cell lines (20, Eder unpublished observations). This observation is widespread in breast cancer cell lines and is due to overexpression of messenger RNA. In a Raji cell line resistant to nitrogen mustard, enzyme content decreased as resistance was lost suggesting a correlation (20). Novobiocin, an inhibitor of topoisomerase II may restore sensitivity when combined with the alkylating agent, by increasing DNA interstrand cross links (21). A phase I trial of novobiocin and standard dose (750 mg/m^2) cyclophosphamide has been completed and novobiocin and cyclophosphamide are being used in phase II trials of alkylating agent resistant breast cancer.

Many potential mechanisms of resistance to chemotherapeutic agents have been identified and a number of trials of modulators of resistance are in progress. Of importance it has been noted that cloned human cell lines have multiple mechanisms of resistance and that in human cancers, multiple population of cells with different or multiple mechanisms of resistance are likely. Our own data suggests that while an individual therapy targeted at a specific mechanism may enhance cytotoxicity, the effect is limited. It is likely that multiple modulators of resistance, to both genetic and physiologic factors, will be required to produce substantial tumor cytotoxicity sufficient to have clinical importance.

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IMMUNOPHARMACOLOGIC PURGING OF BREAST CANCER FROM BONE MARROW

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INTRODUCTION

High complete response rates and durable remissions have been reported for patients with advanced breast cancer who receive high dose chemotherapy and autologous bone marrow support (ABMS) [1,2,3]. A potential problem with ABMS for breast cancer is that bone marrow involvement with metastases is common. Twenty eight percent of women with newly diagnosed breast cancer and no evidence of metastases, had tumor cells detected in their bone marrow using immune-histochemical methods [4]. In a recent study of 380 patients with newly diagnosed stage IV breast cancer, radionuclide bone scans and random bilateral iliac crest bone marrow biopsies were evaluated, using routine techniques [5]. As the number of positive sites on the bone scan increased, there was an increasing frequency of marrow biopsies showing tumor. With one, two, three or more than three positive sites on the bone scan, 44%, 48%, 73%, and 94% of patients respectively, had histologic evidence of breast cancer in the marrow. The possibility of infusing clonogenic tumor into patients stimulated our efforts to develop a bone marrow purging regimen for breast cancer.

We chose to develop a combined immunomagnetic plus pharmacologic purging regimen because of our data [6] as well as other studies [7,8,9] demonstrating a superior anti-tumor effect with combined modality purging regimens, compared to the tumor cell depletion achieved with single modality regimens.

MATERIALS AND METHODS

A series of preclinical experiments were performed to optimize the ex vivo purging regimen which was used in the clinical study.

Pre-Clinical Studies

Immunomagnetic Purging (IMP). One ml mixtures of breast cancer cells plus a 10-fold excess of human marrow mononuclear cells were incubated with the a panel of anti-breast cancer monoclonal antibodies [10] for 60 minutes at 40C with frequent rotation. The cells were washed and an aliquot of immunoglobulin-coated microspheres (Dynal A.S. Corporation, Oslo, Norway and 45 North Station Plaza, Great Neck, New York) was added for 60 minutes 40C. The entire suspension was then subjected to the magnetic field generated by a small samariumcobalt magnet. The magnetospheres were rapidly attracted to the magnet, pulling the tumor cells with it[ll]. Non-adherent cells were poured off and assayed as described previously. Residual clonogenic breast cancer cells were assayed in a limiting dilution assay[6]. Bone marrow progenitor cell recovery was evaluated in tissue culture assays [6].

4-Hydroperoxycyclophosphamide (4-HC). One ml mixtures of breast cancer cells plus a 10-fold excess of human marrow mononuclear cells were incubated for 30 minutes in a 37C water bath with the appropriate concentration of 4-HC (donated by M. Colvin, JHOC, Baltimore, MD.) and TC-199 tissue culture media (Gibco Laboratories Inc, Grand Island, New York). The final incubation concentration was 2×10^7 cells/ml. Following the 4-HC incubation, the cell suspension was rapidly cooled to 40C, washed three times (centrifugation at 2900 rpm for 10 minutes) and re-suspended in TC-199 for further evaluation.

IMP plus 4-HC. The IMP and 4-HC purging procedures were performed sequentially, as described above. Both sequences of incubation (IMP first and 4-HC first) were evaluated in clonogenic tumor cell and bone marrow progenitor cell assays.

Once the small scale studies were completed, and the purging regimen optimized, a larger magnetic separation device to be used in clinical studies was constructed. The experiments were then repeated to optimize the purging regimen with large volumes of marrow that would be required for an autograft [12].

Clinical Study

The clinical marrow purging trial was designed for previously untreated stage IV breast cancer patients with significant bone or bone marrow metastases. The patients received three cycles of the Duke AFM (adriamycin, 5-fluorouracil and methotrexate) induction regimen [13]. Following hematologic recovery from the third cycle, if the tumor involvement was less than 10% of the total cells on bilateral iliac crest biopsies, the marrow was harvested. A MNC fraction of marrow was obtained using a ficolldiatrozoate density gradient (Lymphocye Separation Medium, Organon Teknika, Durham, North Carolina) on the Cobe 2991 Marrow Processor, and purged as previously described [14]. Patients then received high-dose cyclophosphamide, cisplatin, and carmustine [1] with infusion of the purged marrow.

The end-point of this phase I trial was a significant prolongation in marrow reconstitution, defined by the number of days until a peripheral

leukocyte count of 1000 cells per microliter was achieved. The first group of patients had their marrows purged with 4-HC alone, beginning with 20 ug/ml, followed by 40 ug/ml, 60 ug/ml and 80 ug/ml respectively. Once the maximally tolerated dose of 4-HC was reached, the second group had their marrows purged with the immunomagnetic technique alone. Currently accrual onto the third phase of the trial continues with combined immunomagnetic plus 4-HC marrow purging. The unpurged historical control population was a group of metastatic breast cancer patients with no bone or bone marrow involvement. This group received the same high-dose chemotherapy regimen folllowed by autologous marrow infusion with an unpurged buffy-coat fraction of marrow [1].

RESULTS

Pre-Clinical Studies

In small scale studies two incubations with antibody and magnetospheres were required for optimal removal of 3-4 logs of clonogenic breast cancer cells. An additional two logs of breast cancer could be removed by sequentially purging the marrow/tumor cell suspensions by IMP followed by 4-HC (or the reverse sequence) [6]. The concentrations of monoclonal antibodies and magnetospheres determined in the small scale studies were found to be optimal in the upscale setting [12]. One incubation with the antibodies and magnetospheres respectively removed 3.2-3.6 logs of clonogenic tumor. Increasing the number of antibody and/or magnetosphere incubations did not result in greater clonogenic CAMA cell elimination. As in the small scale studies, the addition of 4-HC to the IMP technique increased the tumor cell depletion to five logs.

Clinical Study

There was no difference in time to engraftment at the first three 4-HC dose levels of 20, 40, and 60 ug/ml respectively, compared to the controls, as shown in Figure 1.

At 80 ug/ml the engraftment period of 28 days was significantly delayed ($p=0.027$). Further escalation of 4-HC was not attempted. There was no difference in engraftment for the immunomagnetically purged group compared to the controls. Only five patients have received marrow purged with the combined IMP plus 4-HC regimen and the engraftment data is too early to discuss. To date all patients have engrafted successfully.

Unlike the days to leukocyte engraftment, there was no correlation between the days to platelet transfusion independence and 4-HC concentration.

Fifty-three percent of evaluable patients have achieved a complete remission of their breast cancer.

DISCUSSION

A series of preclinical experiments was performed to eliminate tumor from bone marrow intentionally contaminated with human breast cancer cells. In small scale IMP purging experiments, two incubations with a panel of monoclonal antibodies and magnetic microspheres respectively, produced three logs of breast cancer cell elimination. The addition of the 4-HC increased the tumor cell elimination to five logs.

To make the purging regimen relevant clinically, an upscale IMP apparatus was constructed to handle the large volumes of bone marrow required for a patient autograft. In the upscale studies, 3.0-4.0 logs of CAMA breast cancer cells were eliminated using one incubation of monoclonal antibodies and magnetospheres. The addition of the 4-HC again augmented tumor cell elimination to five logs. The major difference between the small scale and upscale experiments was that only one incubation with the antibodies and magnetospheres was required for optimal tumor cell elimination in the upscale setting.

With the preclinical data described above, a clinical phase I purging study was designed for patients with breast cancer metastatic to bone marrow. The clinical study has demonstrated that purging a ficoll-separated MNC fraction of marrow with 4-HC or the IMP method is technically feasible and produces consistent times to engraftment when infused into patients with breast cancer following high-dose chemotherapy. The maximally tolerated dose of 4-HC alone for a MNC fraction of marrow in patients with breast cancer is 80 ug/ml. IMP alone produces no delay in engraftment compared to unpurged historical controls. Evaluation of combined IMP plus 4-HC purging is progress.

The follow-up for patients on this trial is short, but to date the time to disease progression in this group is not different from our identically treated patients with advanced breast cancer that does not involve the bone marrow [15]. Obviously, longer follow up is needed to assess the therapeutic outcome in these patients.

ACKNOWLEDGEMENTS

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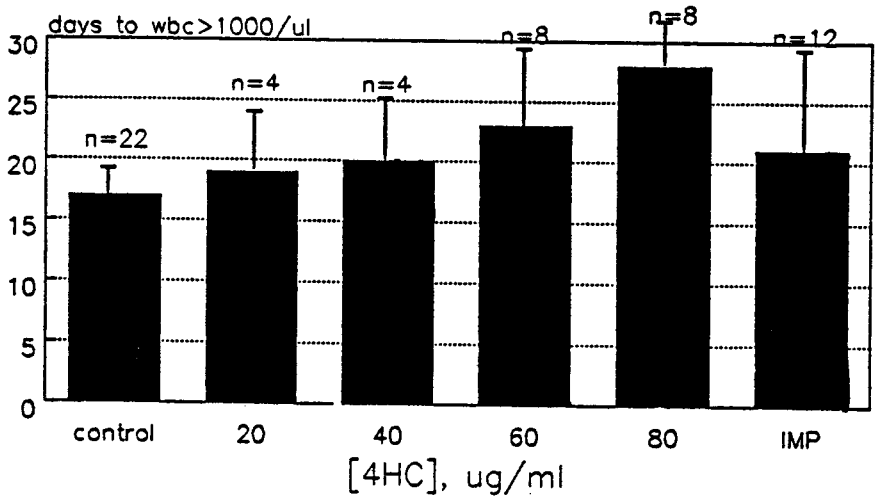
FIGURE 1



SIGNIFICANCE OF DETECTION OF TUMOR CELLS IN HEMATOPOIETIC STEM CELL HARVESTS OF PATIENTS WITH BREAST CANCER

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INTRODUCTION

Hematopoietic stem cell harvests obtained from both bone marrow or by apheresis may contain occult tumor cells. Contamination of histologically normal bone marrow aspirates [1,2] or harvests [3-5] of breast cancer patients have been reported with frequencies ranging from about 20% (Stage I patients) up to about 50% [1,4,6]. It is also clear that circulating tumor cells are present in some patients with breast cancer although the frequency of apheresis harvests containing such cells remains to be determined[4]. We describe the current status of a cohort ofpatients whose bone marrow was suspected to contain occult metastatic tumor cells as detected by culture of their marrow harvest. We also describe our initial efforts to perform similar analyses on apheresis harvests of breast cancer patients who are candidates for peripheral blood stem cell transplantation.

MATERIALS AND METHODS

Culture Techniques

Bone Marrow. Cells, fat, and particulate material collected during the filtering process were scraped from the screens and placed in HBSS. Particles were allowed to settle for 5 minutes at room temperature. Material in suspension was layered onto lymphocyte separation medium (LSMR, Litton Bionetics, Charleston SC) and centrifuged at 400 xg for 20 minutes. The cells were washed by centrifugation (400 xg for 7 minutes) and resuspended in Tris-buffered ammonium chloride for 5 minutes to lyse mature red blood cells. Complete medium was added to each tube and the suspensions washed again. Cells from patients with epithelial solid tumors were cultured using a modified method described by Coulombel et al.[7] with RPMI 1640 medium supplemented with 10% horse serum, 10% fetal bovine serum, 10^{-5}M 2-mercaptoethanol, 10^{-6}M hydrocortisone sodium succinate, 100 U/ml penicillin, 100 ug/ml streptomycin, and 2 mM L-glutamine. Flasks (25 cm^2) containing 2 x 10^7 cells

were incubated for 7 days at 37C in 5% CO2 in air, at which time the flasks were transferred to an incubator at 33C for the remainder of the study. All flasks were demidepopulated weekly. Adherent layers were trypsinized and cytospin preparations made of the resulting cell suspensions. These were blind coded and examined by the pathologist for abnormal cells.

Peripheral Stem Cells. Apheresis samples were washed once with calcium and magnesium free Hank's balanced salt solution (HBSS). The pelleted cells were resuspended in HBSS, layered onto lymphocyte separation medium, and centrifuged at 400g for 20 minutes. Cells collected from the gradient interface were washed once (400g, 7 minutes) and resuspended in Tris-buffered ammonium chloride for 5 minutes to lyse mature red blood cells. Complete medium was added to the tube and the suspension washed again. Aliquots of cells were cultured in a medium consisting of Iscovels modified Dulbecco's medium with 12.5% fetal calf serum, 12.5% horse serum, folic acid ($2x10^{-6}$M), myoinositol ($5x10^{-4}$-4M), hydrocortisone (10^{-6}M), 2-mercaptoethanol (10^{-5}M), penicillin (100 U/ml), streptomycin (100 ug/ml), and L-glutamine (2mm) as described by Douay et al.[8] Flasks (25 CM2) with 2 x 10^7 cells in 10 ml medium were cultured in a 5% CO2 in air atmosphere in a 37C incubator. 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

Biological Characteristics of Suspected Occult Tumor Cells in Marrow Harvests

It must be emphasized that there is currently no proof that the malignant-appearing epithelial-like cells amplified from marrow harvests of patients with breast cancer are, in fact, clonogenic tumor cells. Attempts to prove or disprove this proposition have been frustrating. Although such cells are immunocytochemically positive for breast tumor associated antigens, such as epithelial membrane antigen, other cells in the preparations which morphologically are almost certainly macrophages/histiocytes also show some positive staining. This may be due to phagocytosis of shed or damaged membranes or other unexplained reasons. Although fresh breast cancer cells are convincingly positive for cytokeratin expression detected by the AE-1 or AE-3 cocktail, the cells we suspect on morphological grounds to be tumor cells after 4-6 or 12-14 weeks of culture are not convincingly positive. A very limited number of samples were also negative for vimentin intermediate filaments. Consequently, we are uncertain as to the conclusions which can be drawn from such immunocytochemical studies.

Cytogenetic studies of cultured harvests from four patients have, to date, yielded only normal metaphases. However, it must be emphasized that totals of only 9, 10, 9 and 15 metaphases have been analyzed as long term cultures contain few proliferating cells. The proportion of tumor cells in such cultures by differential counting is a few percent at most. We would, therefore, predict on statistical grounds that we would need to analyze 50 to 100 cells

cytogenetically to ensure detection of abnormal cells. Therefore, cytogenetic analysis has been inconclusive. The most stringent and most convincing criterion of tumorigenicity of these cells would be to grow them in immunodeficient mice, as we have done for melanoma, lymphoma and leukemia [9,10]. Since the maximum estimated number of such cells we have obtained for transplantation into nude mice was 4.5×10^5 cells, it is likely that the mice will have to be followed for a prolonged period. The above recipients were followed for 7 months without evidence of tumor at which time they were lost due to equipment failure. Such studies are currently being emphasized. Unfortunately, results will not be available soon.

Clinical Significance of Suspected Tumor Cells Harvests

A cohort of 28 patients with initial stages I, II or III disease or with locally or regionally recurrent disease, and whose marrow harvests were studied in culture at least one year ago, have been followed to determine the clinical significance of malignant-appearing epithelial cells in their cultured marrow harvests. Of these 28 patients, 16 had positive cultures for such cells and 12 were negative. Nine of the 28 patients have had a metastatic recurrence at sites which are predominantly those expected for breast cancer (liver-2, lung-3, bone-2, lymph nodes-2). The marrow was not routinely examined. Neither age, stage or estrogen/ progesterone receptor status predicted the development of metastatic disease. However, 8/16 (50%) of marrow culture positive patients have recurred with metastatic disease compared to 1/12 (8.5%) marrow negative patients ($P < 0.05$, log rank test). The one culture negative patient who relapsed with metastatic disease had a therapy resistant chest wall tumor which ultimately disseminated to lung. Three other culture negative patients have developed regional recurrences and one of these ultimately progressed to metastatic disease.[11]

It appears, therefore, that the culture technique is a sensitive and independent predictor of metastatic disease. Clearly, it does not provide information regarding the likelihood of local or regional recurrence which are still a significant problem in breast cancer.

Detection of Abnormal Cells in Peripheral Stem Cell Harvests of Breast Cancer Patients

Morphologically abnormal cells have been detected in the apheresis harvests of 4 of 21 patients with breast cancer. These cells are not entirely identical in morphology or immunocytochemical profile to those observed in bone marrow harvests and their lineage is obscure. Three of the four patients whose harvests contained such cells have died, and one has not yet been transplanted. The remaining 17 patients without such cells are too early to evaluate.

In 1989, Kaminer et al.[12] reported two patients with breast cancer and bone marrow involvement who received high dose therapy and peripheral blood stem cell transplant (PBSCT). The patients, both of whom had brain and lung involvement, failed to respond to chemotherapy and survived no longer than

five months after transplantation. The same report described 12 additional advanced breast cancer patients who received high dose chemotherapy and bone marrow transplantation, and who also failed to achieve long term disease free survival. The authors concluded that high dose chemotherapy should be utilized earlier in the course of the disease.

In 1986, we reported a series of five patients with advanced breast cancer and bone marrow metastases who had failed conventional chemotherapy and were treated with high dose chemotherapy or chemo/radiotherapy.[13] One patient experienced an early death and the other four patients responded, but relapsed within 276 days of the transplant. These patients survived 0.5, 3.5, 5, 14 and 25 months after PBSCT. From this experience, we reached a similar conclusion to Kaminer et al.[12], i.e. that patients with metastatic breast cancer should be treated earlier in the disease course.

Between June 1988 and February 1990, we treated 12 patients with metastatic breast cancer, including bone marrow involvement, with a combination of high dose thiotepa, hydroxyurea and cyclophosphamide (THC) followed by PBSCT. They were part of a series of 17 breast cancer patients treated with THC followed either by ABMT or PBSCT [14] . The patients in this study had breast cancers that were proven to be sensitive to conventional chemotherapy and most were in partial remission at the time high dose therapy was administered. The median age of these patients was 36 years, with a range of 27-49 years. In addition to bone marrow involvement, all patients had other metastatic disease. However, patients with brain metastases were not eligible for the study. Peripheral stem cells were collected without mobilization techniques, and the collections were continued until at least 6.5×10^8 mononuclear cells per kilogram patient weight were harvested. Following transplantation, the median time to reach 0.5×10^9/L granulocytes was 24 days for 10 evaluable patients. Three patients (25%) achieved a complete response, five patients (42%) had a partial response, two patients failed to respond, and two patients had an early death and were not evaluated for response. The actuarial event free survival (survival without progression of disease) for all 12 patients at one year was 28.5%, but the length of follow-up for this series is still too short to determine if this approach has been successful.

The frequency of detection of morphologically abnormal cells in PBSC harvests 4/21 (19%) is significantly lower than in the marrow of such patients 21/46 (46%) (P=0.03). We have noted previously[6] that this might be because the PBSC cultures are less sensitive for detecting occult tumor cells than bone marrow cultures. Consequently, we performed a calibration study similar to that described for bone marrow [15], in which cells of the MCF-7 breast tumor line were added in varying proportions to a normal apheresis harvest. Cultures were sacrificed at weekly intervals and examined for the presence of positivity for MCF-7 cells. The frequency of MCF-7 cells which could be detected after one or two weeks of culture was 10^4 in 2×10^7 (1 in 2,000) apheresis-derived mononuclear cells, which is the same frequency as could be detected immediately on mixing the tumor cells with the apheresis harvest. After 3 weeks of culture, 200 in 2×10^7 (1 in 10^4) MCF-7 cells were detectable and,

after 4 weeks of culture, 70 in 2 x 10^7 (about 1 in 10^5) apheresis cells were detected. This is a similar sensitivity to that we estimated for the bone marrow cultures[15] and appears to be more sensitive than flow cytometry.[16] Therefore, we conclude that the apheresis harvests of breast cancer patients are less likely to be contaminated with tumor cells than bone marrow harvests. Apheresis harvests may be an alternative to the use of purged involved bone marrow. Alternatively, apheresis harvests may be better to purge because their lower tumor burden is a more promising starting point for purging than bone marrow. Although we have been unable to prove that the morphologically abnormal cells in these harvests are clonogenic tumor cells, their presence appears to correlate clinically with a poor outcome. This appears to be the case whether the harvest is reinfused or not, suggesting this association may be indirect as is the case in lymphoma[17]. Possibly the ability to detect suspected tumor cells in culture indicates that the patient has a reduced or absent immunological capability to eliminate a minimal tumor cell burden. If this hypothesis is correct, it would permit the culture result to predict the clinical outcome without involving the reinfusion of tumor cells as the basis of this correlation.

CONCLUSION

We conclude that the finding of malignant-appearing epithelial cells in long term cultures of marrow from breast cancer patients has both biologic and clinical significance. It has been postulated that the marrow reticuloendothelium may be the first or a transient site for metastatic disease. Our data support this hypothesis and suggest that more sensitive detection techniques, such as this, may ultimately be more reliable than predictive studies of the primary tumor in making decisions about adjuvant chemotherapy.

Additionally, PBSCT offers patients with recurrent or refractory malignancies, whose marrows are not suitable for autologous transplantation, the opportunity to receive marrow ablative therapy. While follow-up is short, especially for patients with solid tumors, there is evidence that PBSCT may be of benefit to some of these patients.

ACKNOWLEDGEMENTS

This research was supported in part by an Imogene Jacobs Memorial Grant from the American Cancer Society. We thank Don Daley and all members of the UNMC Transplant Team for valuable assistance in conducting these studies.

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AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR ADVANCED STAGE ADULT LYMPHOBLASTIC LYMPHOMA IN FIRST COMPLETE REMISSION: A PILOT STUDY OF THE NON-HODGKIN'S LYMPHOMA COOPERATIVE STUDY GROUP (NHLCSG)

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ABSTRACT

Thirty-six adult successive patients with lymphoblastic lymphoma entered a study of sequential chemotherapy consisting of an intensive LSA2-L2 - type protocol to induce first complete remission. Eighteen patients in first CR (median age 22 years, range 15-51), after receiving a conditioning regimen consisting of cyclophosphamide and total body irradiation, underwent autologous bone marrow transplantation. Of these 18 patients, at diagnosis, 2 were in stage III and 16 in stage IV, 15 showed mediastinal and 9 bone marrow involvement. The transplant procedure was well tolerated and no treatment induced deaths occurred. At this time, 14 out of 18 patients are alive and well 1 to 60 months post-transplant (median follow-up time 46 months) with an actuarial disease-free survival of 74%. This phase II study suggests that high-dose chemoradiotherapy followed by autologous bone marrow transplantation may improve long-term disease-free survival in advanced stage adult lymphoblastic lymphoma.

INTRODUCTION

Lymphoblastic lymphoma (LBL) is a distinct subgroup of high-grade malignant non-Hodgkin's lymphomas (NHL) (1), often characterized by a mediastinal mass in addition to peripheral adenopathies, rapidly involving bone marrow and peripheral blood (2).

Sequential multi-agent chemotherapy has improved the prognosis of the disease. The first encouraging results were attained in children (3-5) while only recently progress has been made in adults (6-9). However long term survival in adults with advanced stage disease, because of the high relapse rate, are still unsatisfactory (7-9).

In a small number of studies, patients with high-grade malignant NHL

in complete remission (CR) following conventional chemotherapy were treated with high-dose chemotherapy and chemoradiotherapy followed by allogeneic (BMT) or autologous (ABMT) bone marrow transplantation. When LBL were singled out of the total series, bone marrow transplant appeared to prolong survival and disease free survival (DFS) in poor prognosis patients, when performed in CR (10-15). Recently more homogeneous studies reported very encouraging results in the same group of patients (16,17). In order to confirm the real impact of ABMT in LBL, our pilot study (16) has been extended to a larger number of adult advanced stage patients in 1st CR after a sequential LSA2-L2-type regimen.

MATERIAL AND METHODS

Patients and Treatment

Thirty-six patients with lymphoblastic lymphoma classified according to the "Working Formulation" (1) were studied between January 1985 and April 1990. During this period all new patients seen at the cooperating centers were entered in the prospective study. The eligibility criteria were the following: 1) age >15 years; 2) lymph node biopsy confirming LBL diagnosis, and phenotype if possible; 3) advanced stage (II mediastinal bulky disease > 15cm., III and IV); 4) less than 25% of blasts in the bone marrow and less than 10% of circulating blasts.Immune membrane markers were determined using a panel of monoclonal antibodies (18). Informed consent was requested from every patient.

Pre-treatment tests included chest x-ray, chest computerized tomography (CT), abdominal ultrasound and/or CT scan. Two posterior iliac crest bone biopsies were carried out on all patients, as were bone marrow aspiration and a lumbar puncture for chemical and cytological examination of the liquor. Cytological or pathological tests on accessible sites were conducted in all cases.

Involvement of the pericardium, kidney, liver and spleen was based on clinical criteria alone. The stage of the disease, according to the Ann Arbor classification (19), was determined at diagnosis, after the induction/consolidation chemotherapy and just before ABMT.

In order to obtain a CR, patients were treated with a sequential regimen derived from LSA2-L2 (3), including an induction-consolidation therapy of 64 days duration, which is in line with the same phases of the original protocol. The schedule and doses of the 8 employed drugs are reported in Table I. Once a CR was confirmed after re-staging, patients were entered the second treatment step consisting of a conditioning regimen of high dose radio-chemotherapy followed by autologous bone marrow rescue.

All patients received Cyclophosphamide (CY) 60 mg/Kg on days -4 and -3, total body irradiation (TBI) 10 Gy on day -1, and bone marrow infusion on day 0. The TBI was given in a single dose, from a ^{60}Co source at a rate of approximately 4 cGy/min. The patients were adequately hydrated and received Mesna to prevent hemorrhagic cystitis, furosemide and antiemetic drugs.

Marrow Collection and Purging

Marrow was collected as described elsewhere (20). In 9 patients who presented with partially infiltrated marrow at diagnosis, the harvested marrows were purged with a dose of Maphosphamide (ASTA-Z 7557) able to spare 5% of normal CFU-GM, as reported by Gorin et al. (21). In our cases a median dose of 80 ug/2x10^7 cells was used (range 50-100) (Table III).

The marrows were placed in 5 ml plastic tubes (2.5 ml marrow and 2.5 ml dimethyl sulfoxide [DMSO] 20% in medium) and then cryopreserved and stored in the vapor phase of a liquid nitrogen freezer (13). The bone marrow aspirates contained a median of 2.0 x 10^8/Kg nucleated cells (range 1.0 to 3.5 x 10^8/Kg) and, in 2.0 x 10^8/Kg nucleated cells (range 1.0 to 3.5 x 10^8/Kg) and, in unpurged marrows, a median of 1.5 x 10^4/Kg CFU-GM (range 1.1 to 4.2 x 10^4/Kg).

Clinical Support

All patients were isolated after transplant and fitted with a Hickman catheter. The patients received intestinal tract decontamination therapy (colistin, neomycin and amphotericin B), while an empirical antibiotic therapy (an aminoglycoside and a cephalosporin) was administered intravenously if bouts of fever associated with granulocytopenia occurred. Patients showing clinical evidence of Herpes simplex were treated with acyclovir. They also received parenteral hyperalimentation, and platelet transfusions from individual donors were given for platelet counts under 20 x 10^9/l. Leukocyte-free erythrocyte concentrates were administered when hemoglobin dropped below 10 g/dl. All allogeneic cells were irradiated (20 Gy).

Statistical Methods

The DFS curve was estimated by the Kaplan and Meier method (22). The statistical analysis was performed in April 1990.

RESULTS

Patients and Tumor Responses

One patient died early of sepsis and hemorrhage; of the 35 evaluable patients, 24 achieved CR (69%), 6 attained partial remission (PR) (17%) and 5 were non-responders (NR) (14%). In spite of salvage regimen, only 2 PR patients are still alive, while all the other PR and NR patients died because of the progression disease at 7, 11, 16, 20 and 3, 3, 8, 9, 14 months respectively. Twenty-four patients in CR were registered for ABMT. One, who had persistent hypoplastic marrow four months after induction- consolidation, was registered for allotransplant and is now alive and well at 34 months from the procedure (41 from diagnosis). Three refused transplant and underwent maintenance therapy for two years. The chosen treatment consisted of weekly therapy rotating 6 different drugs (Vincristine, Adriamycin, Cyclophosphamide, Methotrexate, 6-Mercaptopurine, Prednisone). Of these three patients 2 relapsed at 10 and 22 months from CR and died at 19 and 28 months from diagnosis

respectively. The third one is alive and well at 57 months from CR and 59 from diagnosis. Two patients are now scheduled while eighteen underwent ABMT. This latter group consisted of twelve males and six females, median age 21 (range 15-43). Two were stage III and sixteen stage IV; four presented B symptoms (fever, weight loss), 15 mediastinal (83%) and 9 bone marrow involvement (50%). Six of the latter showed blasts in the peripheral blood. There was spleen involvement in seven cases, pleura and liver in four, kidney in three, gut, muscle, pericardium, bone and lung in five different ones.

Of these 18 patients, 16 were included in the study upon diagnosis, while two entered the study after receiving 6 cycles of CHOP (23) at another institution and achieving a PR (Table II). The immunological phenotype of these patients is reported on Table III.

The results of the ABMT procedure are summarized in Table IV. All patients were in first CR when transplanted.

The median time between attainment of CR and ABMT was 2 months (range 1-19 months). Only the first patient was transplanted after 19 months from CR and 18 months of maintenance chemotherapy (No.1).

Currently fourteen of the 18 patients are in continuous complete remission (CCR); the median follow-up time is 46 months (range 1-60) with an actuarial DFS probability of 74% (Figure 1). Of the nine patients who presented at diagnosis with bone marrow involvement, four died at 3, 5, 5 and 9 months post-transplant of leukemic relapse without lymph node involvement (Table IV).

Toxicity

All patients had pancytopenia (leukocyte nadir $< 0.1 \times 10^9$ /l). Marrow engraftment occurred in all. In nine patients, who had no initial bone marrow involvement, the median time to reach self-sustaining granulocyte recovery $> 0.5 \times 10^9$/l was 13 days (range 12-15), while the median time for platelet recovery $> 20 \times 10^9$/l was 22 days (range 15-53). In the other 9 patients, whose marrow had been purged, recovery was delayed, with GB $> 0.5 \times 10^9$/l, on day 35 (range on day 18 (range 12-83) and platelets $> 20 \times 10^9$/l on day 35 (range 25-105).

All patients suffered from nausea and vomiting (Grade 3), and most of them had diarrhea (Grade 2). Grade 2-3 mucositis was observed in all patients. For these reasons all patients required parenteral hyperalimentation. Abnormal liver function was observed in six patients (grade 1-2), but the elevation of liver enzymes was rapidly transient. Only one patient developed severe liver and kidney toxicity (grade 3), but again the episode was short-lived. All patients required antibiotic therapy and transfusions during the aplastic phase. Eight cases of bacterial sepsis occurred (grade 1-3), but they responded to parenteral anti-bacterial therapy. Five patients developed Herpes simplex labialis, requiring acyclovir.

DISCUSSION

The experience gained in the treatment of children shifted the therapeutic approach toward a sequential chemotherapy borrowed from protocols which proved so successful in the therapy of ALL (3-5). The employment of these treatments in adults patients yielded similar results (6-9).

However, even if overall results are very good, survival and DFS are markedly influenced by a series of prognostic factors like age (8), advanced stage of the disease and bone marrow involvement (7,9) with a 3-years DFS of approximately 20%. Most of the relapses occur while on treatment disavowing the value of a prolonged aggressive maintenance chemotherapy. The failure of conventional treatment in influencing the outcome of these high risk patients prompted the use of high dose radio- therapy and/or chemotherapy followed by bone marrow rescue. A small number of studies on the use of allogeneic BMT for the treatment of LBL in adults has shown that it is possible to improve the prognosis for patients with HLA-compatible siblings (10-12). However, this option is limited by the availability of a suitable donor.

ABMT is an increasingly safe procedure with a larger applicability, but only few data, based on very limited series, are available on ABMT in LBL in CR (13-15). Results seem encouraging; when EBMT data on patients autografted in 1st CR are scrutinized to sort out LBL cases, the 7 years DFS results of 65% (15). Recent result of the NHLCSG and the French group in advanced stages patients lend support to this approach (16,17). Our study was designed to compact the sequential induction- consolidation therapy in 64 days and to avoid any subsequent maintenance therapy after attaining a CR but to eradicate the possible minimal residual disease with megachemo-radiotherapy followed by ABMT.

This approach seems particularly suitable to high risk LBL patients, like those entered into our study, presenting one or more of those risk factors linked with poor survival such as age, advanced stage and bone marrow involvement at diagnosis. Since, in line with other investigators, the sequential treatment adopted in our protocol proved efficacious results; 24 out of 35 evaluable patients attained CR (69%) (6,8,9).

Three CR patients refused to proceed to ABMT, one underwent allogeneic BMT, two are on the waiting list and eighteen under- went ABMT in first CR. Fourteen of these eighteen are currently in CCR, off therapy, 1 to 60 months (median 46 months) after ABMT (Table IV), with a 5-years DFS probability of 74% (Figure 1). Four patients died of leukemic relapse 3, 5, 5 and 9 months after transplant. All four cases presented initial marrow involvement.

The overall toxicity of the ABMT procedure was acceptable. The mild toxicity was probably related to the good clinical conditions of the patients at the time of the procedure and probably also because none of them had received prior mediastinal radiation therapy.

This study is not able to confirm whether it is necessary to purge the bone marrow with maphosphamide (24-26), but initial bone marrow

involvement appears to be an unfavorable prognostic factor.

Because of the limited size of our study, we do not feel entitled to draw definite conclusions. However, considering the high expectancy of failure with conventional therapy, the results obtained in these high-risk patients suggest that ABMT in first CR is able to improve long-term DFS with acceptable toxicity. In addition, in comparison with the very prolonged treatment period reported in the literature, the short overall duration of treatment, comprehensive of induction-consolidation phases followed by ABMT, represents a definite improvement in terms of quality of life.

ACKNOWLEDGEMENTS

Supported by a grant from A.I.R.E.O. The Study Group: (1) Division of Hematology, Ospedale S.Martino, Genoa; (2) Division of Hematology, Ospedale Civile, Bolzano; (3) Division of Hematology, Ospedale Civile, Vicenza; (4) Division of Hematology, Ospedale Civile, Pesaro; (5) National Cancer Institute, Genoa; (6) Division of Oncology, Ospedale Civile, Sassari; (7) Division of Oncology, Ospedale Civile Noale; (8) Institute of Radiology, Genoa; (9) Institute of Hematology, Parma, ITALY.

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TABLE 1

INDUCTION/CONSOLIDATION PROTOCOL

	DOSE (m^2)		DAYS OF TREATMENT
INDUCTION			
VINCRISTINE	1.4	mg I.V.	1,8,15,22,29
ADRIAMYCIN	30	mg I.V.	8,15,22
CYCLOPHOSPHAMIDE	750	mg I.V.	15,22,29
L-ASPARAGINASE	10.000	U I.V.	8-14
PREDNISONE	40	mg P.O.	1-29
METHOTREXATE	10	mg I.T.	3,10,17,24
CONSOLIDATION			
DAUNOMYCIN	50	mg I.V.	43,46,49
CYTOSINE ARABINOSIDE	200	mg I.V.	43-49
METHOTREXATE**	400	mg I.V.	64

*continuous infusion; **I.V. MTX 1/4 dose push, 3/4 dose in 4 hours. Leucovorin rescue 15 mg/P.O. every 6 hours for 6 doses, beginning 24 hours after the end of the MTX infusion.

TABLE 2

Patients' Characteristics

Case No.	Sex/ /Age (ys)	Histology /(In.stage, Simptoms)	Med involv +/-	BM involv +/-	ExtraN involv	Previous therapy	Status at induction/ consolidation
1	F/18	LBL-NC (IV,B)	+	-	1,2	-	Diagnosis
2	F/31	LBL-C (IV,A)	+	+	-	-	Diagnosis
3	M/27	LBL-NC (IV,A)	+	+	3	-	Diagnosis
4	F/43	LBL-NC (III,A)	+	-	-	6xCHOP	PR
5	M/16	LBL-NC (IV,A)	-	+	3	6xCHOP	PR
6	F/36	LBL-C (IV-B)	+	-	1	-	Diagnosis
7	M/17	LBL-NC (IV-A)	+	-	4	-	Diagnosis
8	M/26	LBL-C (IV,A)	+	-	5	-	Diagnosis
9	M/18	LBL-NC (IV,A)	+	+	3	-	Diagnosis
10	M/15	LBL-C (IV,A)	+	+	-	-	Diagnosis
11	F/38	LBL-NC (IV,B)	+	+	1,6	-	Diagnosis
12	F/16	LBL-C (IV,A)	+	+	3	-	Diagnosis
13	M/15	LBL-C (IV,A)	+	+	3	-	Diagnosis
14	M/15	LBL-NC (IV,A)	+	+	1,2,3	-	Diagnosis
15	M/24	LBL-C (IV,A)	+	-	9	-	Diagnosis
16	M/18	LBL-NC (IV,A)	+	-	2,4,8	-	Diagnosis
17	M/30	LBL-NC (IV,B)	-	-	2,3,4,7	-	Diagnosis
18	M/27	LBL-C (III,A)	-	-	-	-	Diagnosis

In. stage: initial stage; Med involv: mediastinal involvement; BM involv: bone marrow involvement; ExtraN involv:extranodal involvement LBL:lymphoblastic lymphoma; NC: non convoluted; C: convoluted; 1: pleura; 2: liver; 3:spleen; 4: kidney; 5: muscle; 6: pericardium; 7: gut; 8: skin; 9: lung; CHOP:cyclophosphamide, adriamycin, oncovin prednisone; PR:partial response.

TABLE 3

Immunologic characterization of LBL

Cases	OKT3	OKT4	OKT6	OKT8	OKT9	OKT10	OKT11	Ia	CAL LA	B1	C-Ig	S-Ig	Immuno-type
1)	-	-	-	-	++	+	+	+	-	-	-	-	T
2)	++	++	-	++	-	++	Nd	+	+	-	-	-	T
3)	-	-	-	-	+	+	-	++	++	++	++	-	Pre-B
4)	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd	Nd
5)	-	-	-	-	-	-	-	++	++	-	-	-	NonT/B
6)	++	+	-	++	-	-	++	-	-	-	Nd	Nd	T
7)	++	-	-	-	++	-	++	-	-	-	Nd	Nd	T
8)	++	+	+	+	-	++	++	-	-	-	Nd	Nd	T
9)	++	-	-	+	+	-	++	-	-	-	Nd	Nd	T
10)	++	++	++	-	++	Nd	++	-	+	-	-	-	T
11)	-	-	-	-	-	-	-	++	++	-	-	-	NonT/B
12)	-	-	+	-	+	++	-	-	-	-	Nd	Nd	T
13)	++	-	+	-	++	++	Nd	-	-	-	Nd	Nd	T
14)	+	Nd	++	Nd	++	++	Nd	-	-	-	-	-	T
15)	++	+	Nd	+	+	Nd	Nd	-	-	Nd	Nd	Nd	T
16)	-	Nd	-	Nd	-	+	-	++	++	++	-	-	Pre-B
17)	++	++	-	++	+	-	-	-	-	-	Nd	Nd	T
18)	-	-	-	-	-	Nd	Nd	++	++	+	-	-	Pre-B

Antibody staning was scored as: -, 0 to 10%; +, 11 to 50%; ++, 51 to 100%. Nd: not determined; LBL: lymphoblastic lymphoma; Non T/B: non-T, non-B.

TABLE 4

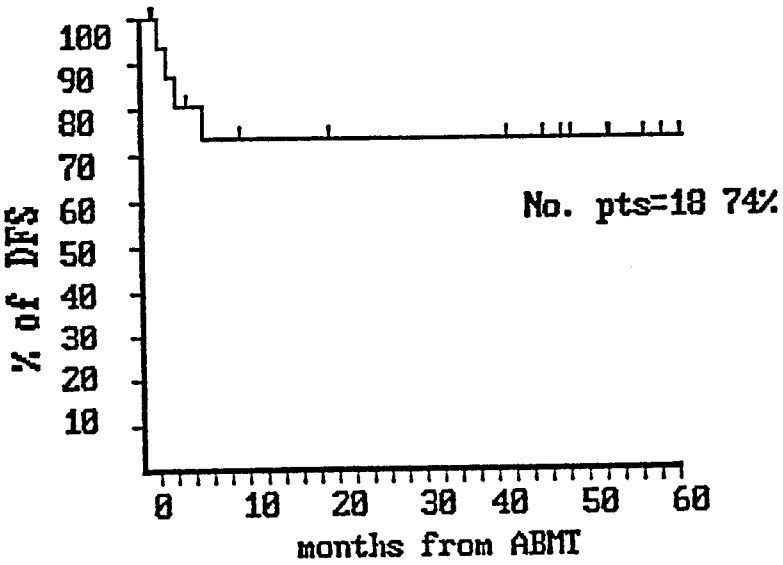
Results of cytoreductive therapy and ABMT in 1st CR LBL

Case No.	Time from CR to ABMT	Purging ($\mu g/2x10^7$ cells)	Duration of CCR(mos.) from ABMT	Cause of death (mos. from ABMT)
1	19*	-	60 +	-
2	3	70	58 +	-
3	2	100	56 +	-
4	1	-	52 +	-
5	2	50	2	leuk.rel.(3)
6	4	-	52 +	-
7	2	-	48 +	-
8	1	-	47 +	-
9	2	90	45 +	-
10	1	70	3	leuk.rel.(5)
11	3	100	41 +	-
12	2	80	7	leuk.rel.(9)
13	4	80	4	leuk.rel.(5)
14	2	60	21+	-
15	3	-	11+	-
16	1	-	5+	-
17	2	-	1+	-
18	3	-	1+	-
			med.46(1-60)	

*Transplanted after 18 months of maintenance chemotherapy.
CCR: continuous complete remission; leuk.rel.: leukaemic relapse.

FIGURE 1

ABMT in LBL. Probability of DFS for 18 LBL patients in first CR following an auotgraft.



FACTORS AFFECTING THE OUTCOME OF AUTOLOGOUS BONE MARROW TRANSPLANTATION

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INTRODUCTION

The role of intensive chemotherapy followed by autologous bone marrow transplant (AuBMT) (1-6) for patients with poor-prognosis Lymphoma, Hodgkin's disease and Acute Non-Lymphoblastic Leukemia (ANL) has been explored at Memorial Sloan-Kettering Cancer Center. 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 [Table 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 (5) for patients being treated after failing initial therapy (refractory or relapsed patients).

The data suggests that the new regimen of HF-TBI, VP-16, CTX is superior to our previous HF-TBI, CTX protocol (5). 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 (7,8,9). Results of our clinical trials for ANL using TBI, VP-16 and CTX have been previously detailed (4). The data is analyzed in this paper, to help design future protocols.

PATIENTS AND METHODS

The characteristics of all patients who received AuBMT are described in Table 1. Fourteen patients with poor-prognostic features received AuBMT while they were in CR (6 pts) or PR (8 pts) after L-17M induction (Group 1). Fifteen patients received AuBMT only at relapse/failure with HF-TBI, CTX conditioning (Group 2). The survival of the two groups is detailed in previous paper (1). Eleven of the 14 patients in Group 1 are disease-free with a median follow-up of 79+ months. The results on Group 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, 26 patients have received transplant with this new regimen, and the results are presented in Table 1. The new protocol of HF-TBI, VP-16 and CTX is slightly more toxic (predominantly ARDS in patients with residual disease and mucositis) and has a very low relapse rate with a median follow-up of 54+ months. The hematopoietic engraftment of patients in Group 3 was comparable to patients on Group 1 and Group 2. Twelve patients in the above study had low or intermediate grade lymphoma, these patients had usually failed aggressive combination chemotherapies (5). The patients characteristics are detailed in Table 2. The results of AuBMT in these patients is very encouraging and is detailed in Table 1. It is interesting to note that so far we have not seen any late relapses (after one year) in this subgroup of patients with a median follow-up of 44+ months (range 12 to 67 months).

Patients with Hodgkin's disease (see Table 2 for patient characteristics) 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 as the conditioning regimen in a manner similar to the patients with non-Hodgkin's lymphoma as described in Table 3. The results are presented in Table 1. Patients with Hodgkin's disease who have relapsed after radiotherapy and combination chemotherapy received a conditioning regimen of BCNU, VP-16 and cyclophosphamide as described in Table 3 and the survival is described in Table 1. The patients entered onto the BCNU protocol tend to be heavily pretreated and do have a higher relapse rate. Therefore, we have now increased the dose of VP-16 to 350mg/m^2 x 3 days IV and so far, only two patients out of 11 has relapsed on this higher dose combination of BCNU, VP-16, CTX.

From the data presented in Table 1, it appears that our attempts at reinduction are not successful. Only 20 to 43% of the patients achieve complete remission prior to AuBMT. The data further shows that patients who do achieve CR do exceptionally well and have a low relapse rate with less toxic complications. These findings have also been reported by other investigators (10-12). We need to develop better salvage regimens, this in turn will improve the survival of patients with lymphoma and Hodgkin's disease. In some

situations, intensive combination chemotherapy can be given with hematopoietic growth factors support (13, 14) and in these circumstances, AuBMT might not be needed. Patients with Acute Myeloblastic Leukemia received HF-TBI, VP-16 and CTX as per the protocol for patients with non-Hodgkin's lymphoma (4,5) overall 4/12 patients in second CR are alive at 57+,39+,28+ and 5+ months, one of two patients who needed high dose Ara-c plus antracycline to achieve lst CR is alive for 44+ months and five out of eight patients in first CR are doing well with median follow-up of 8+ months.

DISCUSSION

The changes implemented in the studies so far have resulted in a lower relapse rate. Unfortunately, studies carried out by other investigators and our own data (Table 1) suggests that patients who are not in remission at the time of AuBMT conditioning treatment do not do well, they either have a higher relapse rate or have toxic complications. The toxic complications vary with the conditioning regimen, but at our institution the main problem has been hemorrhage at the site of bulky disease. This problem is particularly serious in patients with mediastinal pulmonary disease and often progress to Acute Respiratory Distress Syndrome (ARDS). Other institutions are also evaluating these complications (15,16) and the results should improve further as we learn to better manage the toxic side effects.

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 decide the optimum use of AuBMT for various diseases.

ACKNOWLEDGEMENT

This paper is supported in part by the Morgan Murray Fund, United Leukemia Fund, Lisa Bilotti Fund, and the National Leukemia Association.

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TABLE 1

Summary of Autologous Bone Marrow Transplant Results for Patients with Lymphoma and Hodgkin's Disease

Group	Diagnosis No. of Patients	% CR Pre-AuBMT	Conditioning Regimen	Status After AuBMT: % Relapse CR	% Relapse IR	% Toxic Death CR	% Toxic Death IR	% Survival All Patients Median Follow-up (mo)
1	Upfront NHL/14 Poor Prognosis	43	TBI/Cyclophosphamide	20	20	0	0	79 (86)
2	Salvage NHL/15	33	TBI/Cyclophosphamide	80	50	20	30	13 (82)
3	Salvage NHL/26	20	TBI/Etoposide/ Cyclophosphamide	0	10	0	52	54 (44)
4	Salvage HD/22 Previous RT	32	Carmustine/Etoposide/ 250 mg/m^2 x 3d Cyclophosphamide	14	73	14	0	55 (25)
5	Salvage HD/17 Previous RT	41	Carmustine/Etoposide/ 350 mg/m^2 x 3d/CTX	0	20	0	20	82 (8)
6	Salvage HD/28 No previous RT	21	TNI/Etoposide/ Cyclophosphamide	17	27	0	23	75 (26)

CR = Complete remission; ABMT = Autologous bone marrow transplant; IR = Incomplete response (includes patients with partial response and disease progression) ; NHL = Non-Hodgkin's Lymphoma; TBI = Total body irradiation; HD = Hodgkin's disease; RT = Radiotherapy; TNI = Total nodal irradiation

TABLE 2

Patient Characteristics (See Tables 1, 3 for details of each group.)

	Lymphoma *Group 3	HD *Group 4	HD *Group 5	HD *Group 6
Total number of Patients	41	22	21	38
Median age at BMT	36(20-49)	28(18-37)	28(20-51)	27(15-44)
Heavily pretreated low grade lymphomas	12(29%)			
Did not achieve CR to frontline Rx	25(61%)	6(27%)	3(14%)	21(55%)
Duration of 1st CR, if attained (mos)	6(2-25)	23(3-96)	14(3-90)	10(2-55)
Number of prior treatment pre-BMN 1Rx	11(27%)	15(68%)	6(29%)	21(55%)
2Rx	23(56%)	6(27%)	15(71%)	15(39%)
3Rx	6(15%)	1(5%)	--	2(5%)
Prednisone + RT	1(2%)	--	--	--
CR pre-BMT (after BMN re-induction)	8(20%)	7(32%)	9(43%)	7(18%)

TABLE 3

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

Days	No Previous Radiotherapy Prior to ABMT (Group 6)	Previous Radiotherapy Prior to ABMT (Groups 4,5)
	Combination Chemotherapy ± Boost Radiotherapy Bulky Disease	
-10	TNI 167 rad tid	--
-9	TNI 167 rad tid	--
-8	TNI 167 rad tid	Carmustine 250mg/m^2 IV
-7	TNI 167 rad tid	Etoposide 250-350mg/m^2 IV
-6	Etoposide 250mg/m^2 IV	Etoposide 250-350mg/m^2 IV
-5	Etoposide 250mg/m^2 IV	Etoposide 250-350mg/m^2 IV
-4	Etoposide 250mg/m^2 IV	Cyclophosphamide 50mg/kg IV
-3	Cyclophosphamide 60 mg/kg IV	Cyclophosphamide 50mg/kg IV
-2	Cyclophosphamide 60 mg/kg IV	Cyclophosphamide 50mg/kg IV
-1	Rest	Rest
0	Bone marrow infusion	Bone marrow infusion

ABMT = Autologous bone marrow transplant; TNI = Total Nodal Irradiation; IV = Intravenous

RELAPSE OF MALIGNANT DIFFUSE NON-HODGKIN LYMPHOMA: THE INTERNATIONAL STUDY (PARMA)

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INTRODUCTION

Patients with advanced diffuse non-Hodgkin's lymphoma (NHL) are rarely cured after conventional chemotherapy fails. However, experience gained in Burkitt's lymphoma has shown that these lymphomas are still sensitive to intensive chemoradiotherapy after treatment failure with conventional doses [1,2,3]. With autologous bone marrow transplantation (ABMT) providing hematologic support, it has been possible to administer intensive chemoradiotherapy and to cure some children and adults with disseminated, aggressive NHL. However, 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 have led to confusion in analyzing the world experience [1,2,3]. The appropriate place for high-dose chemoradiotherapy and ABMT between front-line treatment and relapse has not yet been determined, despite encouraging results in numerous pilot studies. Several groups investigating ABMT have suggested that response to the preceding therapy may have prognostic importance in the outcome of ABMT in relapsed patients. Confirmation of these findings was the major achievement of the 100-patient retrospective analysis reported in 1987 in the New England Journal of Medicine [1].

In 1987, an international group called PARMA was organized, involving bone marrow transplant centers from around the world. Its objective was to test the value of ABMT in patients with relapsed lymphoma in a prospective, randomized trial [1]. This report will review the pilot study of the PARMA protocol, conducted in 1986 and 1987. The study objective was to confirm the response rate to the DHAP regimen [2] (table 1) (dexamethasone/high-dose cytarabine/cisplatin) in patients with relapsed lymphoma and to assess the conditioning regimen of involved-field radiotherapy and BEAC (carmustine/etoposide/ cytarabine/cyclophosphamide) [2] (table 2). Preliminary data from the ongoing randomized study will also be discussed.

PATIENTS

PARMA Pilot Study

Fifty patients aged 16 to 56 years, with first relapse of intermediate or high grade NHL (28 and 22 patients, respectively), were included in this study. The 20 worldwide centers are: Centre Leon Berard, Lyon, France; Hopital Jean Minjoz, Besancon, France; Hotel-Dieu, Paris, France; C.H.U. La Miletrie, Poitiers, France; Institut Jules Bordet, Brussels, Belgium; St. Vincent Hospital, Sydney, Australia; The Dr. Daniel Ben Hoed Cancer Center, Rotterdam, Netherlands; Hopital Jules Courmont, Pierre Benite, France; M.D. Anderson Hospital, Houston, Texas, USA; Antonie van Leeuwenhoek Hospital, Amsterdam, Netherlands; Hospital de la Princess, Madrid, Spain; Hopital des Sablons, Grenoble, France; Universita Degli Studi di Parma, Parma, Italy; Hotel-Dieu, Nantes, France, Ospedale di Pesaro, Pesaro, Italy; Ospedale S. Martino, Genova, Italy; University Hospital "Dijkzigt", Rotterdam, Netherlands; University of Nebraska, Omaha, Nebraska, USA; C.H. Paul Brousse, Villejuif, France; and Centre Claudius Regaud, Toulouse, France. The patients, 15 women and 35 men, had achieved complete response (CR) initially with a doxorubicin-containing regimen. All had normal marrow at relapse and no CNS relapses, and all had given informed consent according to the PARMA protocol and the rules of each institution. All patients but one were evaluable for response to the rescue regimen.

PARMA Randomized Study

Since July 1987, 128 consecutive patients from 43 worldwide institutions have been enrolled in this ongoing study. All patients had intermediate or high-grade NHL and had achieved a previous CR. Patients were enrolled at the time of the first or second relapse. Exclusion criteria included age 60 years or older and CNS or bone marrow relapses. Histological proof of relapse was mandatory.

PATIENTS AND METHODS

Patients in both studies received the DHAP regimen reported by Velasquez et al [2]. With the exception of patients with clearly progressive disease, bone marrow was harvested under general anesthesia following one course of DHAP. Unless the marrow had been stored previously, it was frozen according to the procedure described by Philip et al [1,3]. All patients had normal bone marrow biopsies at the time of harvest, and a second course of DHAP was initiated one day after harvest. On day 20, after the second DHAP course, patients were restaged according to the involved disease sites determined at study initiation. CR was defined as complete disease disappearance from all previously involved sites ; partial response (PR) was defined as at least a 50% tumor reduction of all previously involved sites with no new lesions; both CR and PR were defined as sensitive relapses eligible for

ABMT in the pilot study and for randomization in the ongoing PARMA protocol.

Statistical methods included comparisons of categorical data using the X2 and Fischer's exact tests. Survival curves were computed according to the Kaplan Meier method. An event was defined as a relapse, evidence of progression, or death regardless of cause. In the event-free survival curves, the date of the first event is taken into account.

PARMA Pilot Study

Among responding patients, 20 underwent involved-field radiotherapy and received the BEAC protocol. Two patients previously irradiated at the site of bulky disease did not receive radiotherapy according to the PARMA protocol. By individual center decision, two received ABMT with cyclophosphamide and total body irradiation (TBI), as reported by the Seattle Group [3]; seven were not transplanted : one toxic death and six refusals.

ABMT was performed as previously reported [1]. Pathological material was reviewed centrally in Omaha, Nebraska by D. Weisenberger, MD, using the international NHL classification [3].

PARMA Ongoing Randomized Study

Both CR and PR patients were randomly assigned to receive either four additional DHAP courses or BEAC intensification with ABMT as previously described in detail by Philip et al [1].

RESULTS

PARMA Pilot Study

Response to DHAP regimen. Of 48 evaluable patients, one was in CR before DHAP (complete surgical excision) and one was lost to follow-up. Of 28 responders (58%), seven achieved CR (15%) and 21 achieved PR (44%). No difference was observed between intermediate grade and high grade patients: 10 PR + 2 CR in the 22 responders of the high grade group, and 11 PR + 5 CR in the 26 responders + 2 non evaluable patients in the intermediate grade group.

Toxicity from DHAP Regimen. Severe neutropenia was observed in 22 of 50 DHAP courses (44%) and thrombocytopenia (platelets $<50,000$/uL) was recorded in 19 (38%). Severe renal impairment occurred in seven courses (14%), severe gastrointestinal toxicity in seven (14%), cytarabine-related neurologic disorders in two (4%), and sepsis in eight (16%). One reversible cardiomyopathy was also observed.

One toxic death from hemolytic uremic syndrome occurred in a patient who responded after the second course of DHAP.

Treatment of Sensitive Relapse Patients after DHAP Course 2. Twenty of the 29 patients (28 responding and one already in CR) received the protocol as scheduled (BEAC + ABMT). Eleven of 12 patients who still had evaluable disease (PR to DHAP) were converted to CR after ABMT (91%

response rate to BEAC). Median time to reach 1,000/uL granulocytes was 15 days ; 500/uL polynuclears, 15 days; 200/uL polynuclears, 13 days; and 50,000/uL platelets, 25 days. Two toxic deaths were recorded (hepatic and early renal failure and pulmonary bleeding) and 17 patients were in CR after ABMT. Ten patients subsequently relapsed (nine CR and one PR after ABMT); one committed suicide while in clinical CR 28 months after ABMT; and seven of 30 remain alive and disease-free at a median follow-up of 30 months (range, 24 to 32 months) from study inclusion.

Two of 29 patients were transplanted after a cyclophosphamide and TBI regimen. They are both alive and disease-free at 26 months and 28 months of follow-up. A total of nine of the 22 who received ABMT (40% overall survival) are alive and disease-free at 24 months (figure 1).

The other seven responders did not receive ABMT either because of toxic death (one) or individual decision. One refused treatment and died, and five received four to six additional DHAP courses. None of these seven responders are alive and disease-free at the present time.

PARMA Ongoing Randomized Study

After complete staging, all patients received the DHAP rescue protocol for two consecutive courses with the same intervals and schedules as the pilot study. Of 128 patients evaluable for response, 71 (55.5%) were in PR or CR (44 and 27 patients, respectively) after two courses of DHAP. Patients relapsing while receiving therapy (20%) had a lower response rate than those whose relapse occurred off treatment: 80% (e.g. 9% vs 57%, $P < .001$). Among these 71 patients, 62 were randomly assigned to receive either four additional courses of DHAP (n=34) or massive therapy with BEAC and ABMT (n=28). Involved fields were irradiated after six courses of DHAP in the first arm, and before massive BEAC therapy and ABMT in the second arm. In 66 patients, disease progression (57), patient refusal (3), technical problems (2), abnormal bone marrow cellularity (2), hepatic dysfunction (1), and renal failure (1) precluded randomization.

There was no statistical difference in terms of toxic death rate and survival at 1 year between the two arms. Further results of the first interim analysis of this ongoing international study will not be available before patient enrollment in the PARMA protocol has ended. As of June 1990, the study is still open and additional patients are required. The primary end point will be failure rate at 2 years (i.e., relapse or death regardless of cause).

ACKNOWLEDGEMENTS

Supported by a grant from Ligue Departementale de Lutte Contre le Cancer de la Haute-Savoie, de l'Ain, de l'Ardeche, du Rhone et de la Saone et Loire. Authors' affiliations:
Biostatistics Unit, Centre Leon Berard, Lyon, France; Institut Jules Bordet, Brussels, Belgium; Universita Degli Studi La Sapienza, Hematology Department, Roma, Italy; The Dr. D. Den Hoed Cancer Center, Rotterdam,

Netherlands; Hopital Jules Courmont, Pierre-Benite, France; Hopital St. Louis, Paris, France; University Hospital Leiden, Leiden, Netherlands; Antonie V. Leeuwenhoek Hospital, Amsterdam, Netherlands; C.H. Paul Brousse, Villejuif, France; Academic Medical Center, Amsterdam, Netherlands; (11) Bone Marrow Transplant Department, Centre L. Berard, 28, rue Laennec, 69373 Lyon Cedex 08, France. *The PARMA Protocol Group: J.Y. Cahn: Hopital, Besangon, France; M. Legros: Clermont-Ferrand, France; P. Fargeot: Dijon, France; C. Seban and Y. Devaux: Lyon, France; J.L. Harousseau: Nantes, France; A. Thyss: Nice, France; B. David and C. Guillard: Pessac, France; F. Guilhot: Poitiers, France; B. Pignon: Reims, France; C. Dauriac: Rennes, France; M. Janvier: St. Cloud, France; G. Laurent: Toulouse, France; P. Colombat: Tours, France; J. Biggs: Sydney, Australia; J.C. Ding: Melbourne, Australia; A. Bosly: Yvoir, Belgium; J. Fernandez-Ranada: Madrid, Spain; V. Rizzoli: Parma, Italy; A. Manna: Pesaro, Italy; G. Rosti: Ravenna, Italy; G. Van Imhoff: Groningen, Netherlands; P. Hupperets: Maastricht, Netherlands; P. Sonneveld: Rotterdam, Netherlands; W. Sieger: Berlin, Germany; B. Simmonsson: Uppsala, Sweden; S. Jagganath: Houston, Texas, USA; J. Armitage, Omaha, Nebraska, USA; R. de Bock: Edegem, Belgium; V. Liso: Bari, Italy; F. Witz: Vandoeuvre les Nancy, France; G. Tertian: Clamart, France; G. Lepeu: Avignon, France; H. Tilly: Rouen, France; H. Jirrits: The Hague, Netherlands.

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TABLE 1

DHAP Regimen

Agent	Dose	Day	Route	Time
Dexamethasone	40 mg	1-4	I.V.	15 Min.
Cisplatin	100 mg/m²	1	I.V.-C.I.	24 Hr.
Cytarabine (Ara-C)	4 g/m² (2 g/m² q12h x 2)	2	I.V.	in 200 ml normal saline 3Hrs

Legends :
I.V. :intravenous
C.I. : Continuous infusion

TABLE 2

Conditioning Regimen Schedule

	PROTOCOL REGIMEN	Mon Day 1	Tues Day 2	Wed Day 3	Thurs Day 4	Fri Day 5	Sat Day 6	Sun Day 7
Week 1*	IFXRT	X	X	X	X	X	—	—
		X	X	X	X	X	—	—
Week 2	IFXRT	X	X	X	X	X	—	—
		X	X	X	X	X	—	—
Week 3**	BCNU 300 mg/m²/24 h** (30 minutes I.V.)	X	—	—	—	—	—	—
	ETOPOSIDE 200 mg/m²/24h (100 mg/m2 I.V. twice daily)	—	X	X	X	X	X	X
	Ara-C 200 mg/m²/24h (100 mg/m² I.V. twice daily)	—	X	X	X	X	—	—
	CYCLOPHOSPHAMIDE 35 mg/kg/24h (Given as short infusion over 60 minutes)	—	X	X	X	X	—	—
	MESNA 50 mg/kg/24h (optional)	—	X	X	X	X	—	—
Week 4	ABMT (at least 48 hours after Etoposide)	—	—	—	—	—	—	X

Legends:
** : Day 20 post DHAP 2 ; ** : Day 35 post DHAP 2 ; — : no treatment ; IFXRT= involved field radiotherapy ; ABMT : Autologous Bone Marrow Transplantation.*

FIGURE 1

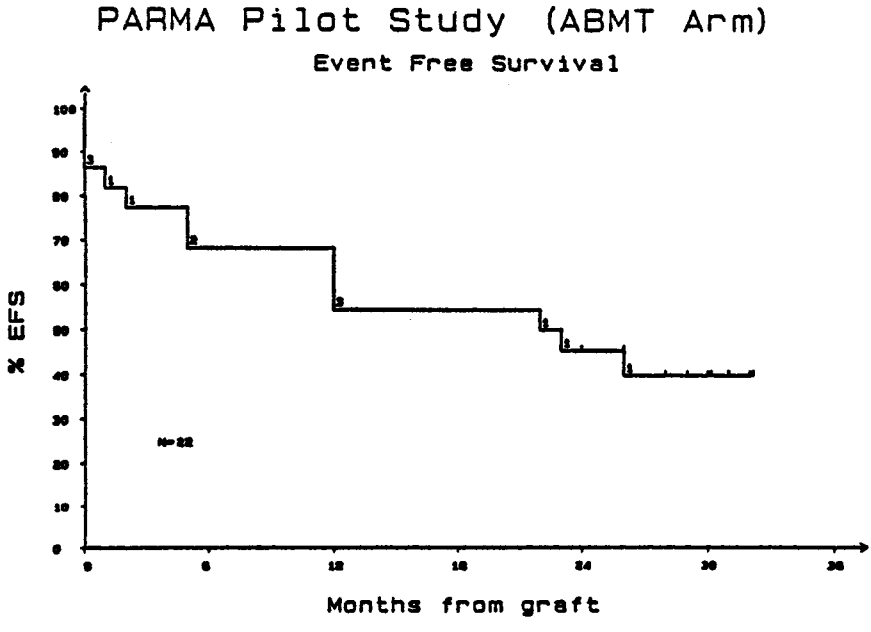

Figure 1

COOPERATIVE GROUP BONE MARROW TRANSPLANT TRIALS IN NORTH AMERICA FOR INTERMEDIATE AND HIGH GRADE NON-HODGKIN'S LYMPHOMA

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INTRODUCTION

About 50-60% of non-Hodgkin's lymphoma (NHL) patients achieve complete remission after receiving conventional chemotherapy, and relapse-free survival is attained in more than 50% of patients (1). Patients who fail to achieve complete remission, or those who relapse after therapy represent a poor-prognosis group. Re-induction with conventional doses of chemotherapy is seldom successful in producing remission or long-term disease-free survival. On the other hand, intensive chemo-radiation or chemotherapy followed by bone marrow infusion is highly successful. For example, the Nebraska Lymphoma Study Group reported that 11 of 17 (65%) relapsed NHL patients undergoing autologous bone marrow transplant (AuBMT) achieved complete remission; in contrast, only 3 of 17 (18%) patients who received conventional cisplatin-based salvage chemotherapy attained complete remission. Five-year disease-free survival differences were significant: 40% in the AuBMT group, versus none in the conventionally treated group ($p<0.001$).

While AuBMT is successful in many relapsed NHL patients, some patients are refractory to intensive therapy and others relapse after AuBMT. Some patients never achieve remission after conventional induction therapy, and the results of AuBMT in this group of "primary refractory disease" patients are poor (3). Other patients are unable to receive total body irradiation (TBI) due to lung (or other organ) damage induced by previous therapy. Moreover, many investigators believe they can identify newly-diagnosed patients at high-risk for relapse despite the fact that a complete remission has been achieved. Such a patient group might benefit from AuBMT administered as intensive consolidation therapy. Since these and other problems of AuBMT remain, several cooperative groups have undertaken trials to improve patient eligibility, increase frequency and duration of response after AuBMT, and improve

tolerance to the conditioning regimen. Some new approaches to these problems are described below.

TRIALS TO IMPROVE ANTI-LYMPHOMA RESPONSE AND DURATION

In an effort to improve antilymphoma effectiveness of high-dose chemo-radiation therapy, the North American Marrow Transplant Group (NAMTG) conducted a phase I-II trial in which high-dose VP-16 (etoposide) was added to a standard regimen of high-dose cyclophosphamide (120 mg/kg) and 1200 cGy fractionated total body irradiation (TBI) (4,5). Dose escalation was undertaken for cyclophosphamide, and in the course of this trial, patients received cyclophosphamide 150-180 mg/kg and TBI 1000-1200 cGy (6). Etoposide was given at a dose of 1800 mg/sqm over 24 hours. Seventy-seven patients ages 15-64 years (median 37 years) who had recurrent (70 patients) or primary refractory lymphoma (7 patients) underwent therapy. Forty-six of 77 patients (60%) achieved a complete remission, while 11 patients had a partial response and 3 patients did not respond. Fourteen patients died of transplant-related complications (17%), including sepsis (N=5), interstitial pneumonitis (N=5), and hepatic veno-occlusive disease (N=2). The duration of complete response ranged from 2+ to 37+ months (median 12+ months), and 24 patients (31%) remain in continuous complete remission. Thirty-three patients died of progressive NHL. The NAMTG is further refining this regimen, and plans to introduce its use earlier in the course of the patient's illness.

CALGB recently opened trial CALGB 8981 examining the use of a therapy quite similar to the NAMTG regimen. VP-16 (etoposide) 1800 mg/sqm was given iv over 26 hours on day T-7, cyclophosphamide 210 mg/kg in 3 divided doses iv over 2 hours on days T-6 through T-4, and TBI 1000 cGy given as 200 cGy in 5 fractions over days T-3 through T-1. The intent was to investigate in a multi-institutional setting the toxicity and activity of the VP-16, cyclophosphamide, TBI regimen in relapsed lymphomas patients who are given 1-2 courses of the pre-transplant cytoreduction regimen of "DHAP", e.g. dexamethasone (D), high-dose Ara-C (HA), e.g. cytosine arabinoside, and cisplatin (P) (7). The DHAP regimen has been shown to be highly effective as an interim therapy while awaiting AuBMT (8). If indicated, patients would receive 1400-2000 cGy involved-field radiation therapy (IFRT) to tumorous deposits 3-5 weeks after completion of DHAP therapy and, if feasible, a repeat course of IFRT 3 months after AuBMT.

SWOG is conducting a two-arm AuBMT phase II trial (SWOG-8942) in primary resistant disease or in relapsed patients who have achieved a complete remission in the past. Patients who are not eligible for TBI receive a new regimen containing cyclophosphamide, BCNU (carmustine), and VP-16 (etoposide) (see below), while patients who have not received prior mediastinal irradiation are eligible to receive TBI-containing therapy. All patients relapsing after a previous complete remission will receive 2 courses of DHAP therapy (or

another effective salvage regimen) to determine (for stratification purposes) if they have "sensitive" or "resistant" recurrent disease. The TBI-containing regimen is 1200 cGy (given as hyperfractionated TBI 150 cGy twice daily over 4 days, e.g. T-8 through T-5, with lung shielding after 600 cGy), VP-16 60 mg/kg iv over 4 hours on day T-4, and cyclophosphamide 100 mg/kg iv over 2 hours on day T-2.

The Children's Cancer Study Group (CCSG) will undertake a trial using AuBMT in Hodgkin,s and NHL patients who have relapsed after attaining a complete remission, or who have primary refractory lymphoma. This trial, CCG #5006, plans to employ the "BEAT" (BCNU, etoposide, Ara-C, thiotepa) regimen after successful conventional chemotherapy reinduction treatment, e.g. tumor cytoreduction after 2 cycles of therapy. Pre-AuBMT reinduction can be accomplished using either "DECAL" (dexamethasone, etoposide, cisplatin, high-dose cytarabine, e.g. high-dose Ara-C, and L-asparaginase as described in CCG #5001) or other conventional reinduction therapy. Patients may receive surgery or radiation therapy to sites of gross residual disease as prescribed in CCG #5001. The BEAT AuBMT component of the trial will consist of: BCNU (carmustine) 300 mg/sqm/day iv on day T-9, etoposide 250 mg/sqm/day iv for 3 days (T-8 through T-6), thiotepa 250 mg/sqm/day iv for 3 days (T-8 through T-6), and cytarabine (Ara-C) 3000 mg/sqm iv over 3 hours twice daily for 3 days (T-5 through T-3), and reinfusion of unpurged cryopreserved autologous marrow. After marrow infusion, patients will be randomized to receive infusions of either placebo or recombinant human granulocyte-macrophage colony stimulating factor (GM-CSF) 250 pg/sqm twice daily for 21 days (see later). If this trial is successful, this approach will be evaluated as consolidation therapy early in the course of poor-prognosis patients.

POG is undertaking a phase I-II trial in which the type of AuBMT conditioning regimen (TBI versus a non-TBI) will be based on the NHL histologic subtype. Specifically, patients less than age 21 years with either relapsed large cell lymphoma or relapsed small cell non-cleaved lymphoma (Burkitt's and non-Burkitt's NHL) will receive two courses of a new pre-transplant cytoreductive regimen 3 weeks apart. This phase I cytoreductive regimen, referred to as "ICE", will consist of ifosfamide 1.5 g/sqm twice daily on days 1-3, Mesna 400 mg/sqm four times daily on days 1-3, carboplatin 200-450 mg/sqm iv on day 3 (dose increased stepwise 50 mg/sqm from a 200 mg/sqm starting dose in patient cohorts of 3), VP-16, and GM-CSF infusions at a dose of 32-64 pg/kg from days 4-21 (or until neutrophil recovery > 1000/ul). During this trial, only Burkitt,s and non-Burkitt,s NHL patients who achieve a complete remission would receive a TBI containing regimen consisting of cyclophosphamide 1.8 g/sqm/day iv for 2 days, VP-16 400 mg/sqm/day for 3 days, and 1400 cGy TBI given as 200 cGy twice daily over 4 days. Lymphoblastic lymphoma patients are excluded from participation in the POG protocol.

The Canadian Autologous Bone marrow Transplant Group (CABMTG) recently has begun a trial using AuBMT as intensive consolidation treatment of poor prognosis lymphoblastic lymphoma patients in first complete remission.

The concept of AuBMT in first complete remission was demonstrated by Gulati et al (9) to provide for greater long-term disease-free survival in a small series of patients believed to be at high risk for relapse. The CABMTG will enroll newly diagnosed lymphoblastic lymphoma patients who are believed to be at high risk for relapse. Induction chemotherapy consists of doxorubicin 30 mg/sqm iv days 1-3, cyclophosphamide 1.0 g/sqm iv days 1-2, vincristine 1.5 mg/sqm on days 1, 8, 15, 22, L-asparaginase 10,000 units/sqm IM days 15, 17, 19, 22, 25, 28, and prednisone 40 mg/sqm days 1-28. Prophylactic intrathecal methotrexate is given. After complete remission is attained, patients 45 years or less are assigned to undergo an allogeneic BMT (providing a histocompatible family member donor is available). All other patients achieving complete remission undergo bone marrow harvest and purging with 4-hydroperoxycyclophosphamide (final concentration 100 ug/ml). For both the allogeneic and autologous groups the conditioning regimen will consist of cytarabine 2 g/sqm iv over 1 hour twice daily for 6 days (days T-9 through T-4) and 1200 cGy TBI given as 200 cGy twice daily for 3 days (days T-3 through T-1).

TRIALS TO BROADEN PATIENT ELIGIBILITY

The previously mentioned SWOG trial (SWOG-8942) involves a regimen that does not use TBI for patients who have had mediastinal radiation, or who have received significant radiation to areas outside the thorax which would make the use of TBI hazardous. Patients receive BCNU 15 mg/kg iv over 2 hours on day T-6, VP-16 60 mg/kg IV over 4 hours on day T-4, and cyclophosphamide 100 mg/kg IV on day T-2. The hope is that this regimen will provide effective therapy in patients unable to receive TBI.

As part of the POG NHL trial, large cell NHL patients who achieve complete or partial remission after "ICE" chemotherapy would receive a non-TBI regimen. This therapy will consist of BCNU 112.5 mg/sqm/day iv for 4 days, VP-16 200 mg/sqm twice daily for 4 days, and cyclophosphamide 0.9 g/sqm twice daily for 4 days.

TRIALS TO REDUCE MORBIDITY AND MORTALITY

Hematopoietic growth factors can dramatically shorten chemotherapy-induced neutropenia and have been a major advance in the treatment of cancer (10). Several colony-stimulating factors have been used in high-dose chemotherapy trials to improve patient tolerance. Brandt and co-workers (11) conducted a phase I trial in metastatic (but previously untreated) malignant melanoma and breast cancer patients using high dose BCNU, cisplatin, and cyclophosphamide followed by increasing doses of the mammalian, glycosylated recombinant GM-CSF (prepared by Sandoz Research Institute). Not only was recovery faster in patients who received GM-CSF infusions compared to historic controls, but infections were reduced and considerably less hepatic, pulmonary, and renal dysfunction were observed.

These effects were attributed to treatment of occult infection and augmentation of neutrophil and monocyte killing of microorganisms.

The ECOG recently completed a phase II trial (EST P-Z488) using GM-CSF (Sandoz Research Institute) in 16 relapsed NHL patients (12). No attempt was made to identify patients who were responding to conventional doses of chemotherapy. Patients received 1200-2000 cGy radiation to all sites of active or previous bulk disease, cyclophosphamide 60 mg/kg/day iv (days T-6, T-S), fractionated TBI 1200 cGy over 3 days (days T-3 through T-1), and re-infusion of unpurged autologous bone marrow (5). Infusions of GM-CSF 11 pg/kg/day over 4 hours were used beginning 3 hours after marrow re-infusion and continued until both neutrophil count exceeded 1500/ul and platelet count exceeded 50,000/ul for 3 consecutive days, or for up to a total of 30 days. No evidence of capillary-leak syndrome occurred, and toxicities included moderate to severe gastrointestinal dysfunction and transient hypertension and myalgias. Recovery of neutrophils in this heavily pretreated group of patients occurred a median of 13 days after AuBMT (range: 9-30 days), while platelet transfusion independence occurred at a median of 23 days (range: 12-100 days). Three infections occurred: one central venous catheter-related Staphylococcus epidermidis bacteremia, one Giardia lamblia stool infection, and one case of fatal interstitial pneumonitis.

Since this approach appeared promising, a successor trial has been initiated in diffuse large cell lymphoma patients. The same preparative regimen will be used, followed by unpurged autologous marrow, since the effects of growth factor infusions are clouded when purged marrow is used (13). Three hours after marrow re-infusion, patients will receive either placebo or recombinant human GM-CSF (derived in E. coli) at one of two doses (5 and 10 ug/kg/day) iv over 4-6 hours. Unlike the earlier ECOG trial, patients who are in second complete remission are eligible to participate. Study endpoints include time to neutrophil recovery, number of infections, hospital days, transfusions, and duration of antibiotic usage. This trial will help develop a database for future combination recombinant hematopoietic growth factor trials.

A similar design has been devised by CCSG, in which patients are given the "BEAT" conditioning regimen. During CCG #5006, patients will receive, in randomized fashion, infusions of either placebo or GM-CSF 250 ug/m^2 twice daily for 21 days after marrow infusion.

SUMMARY

AuBMT is an extremely successful therapeutic modality. Several of the major cooperative groups in North America have either initiated, or plan to undertake investigations to improve the tolerance and outcome of AuBMT. Successful completion of such studies will allow this treatment approach to be offered to more NHL patients.

ACKNOWLEDGEMENTS

Supported, in part, by grants from the Sandoz Research Institutes, and grants P30CA43703, and CA21115 and CA14548 from the National Institutes of Health, National Cancer Institute, and the United States Public Health Service. Authors' affiliations: From the (1) Eastern Cooperative Oncology Group (ECOG), (2) Southwestern Oncology Group (SWOG), (3) North American Marrow Transplant Group (NAMTG), (4) Pediatric Oncology Group (POG), (5) Cancer and Leukemia Group B (CALGB), and the (6) Canadian Autologous Bone Marrow Transplant Group (CABMTG), and the (7) Children's Cancer Study Group (CCSG), and (8) Ireland Cancer Center, Cleveland, Ohio, (9) Vancouver General Hospital, Vancouver, British Columbia, (10) Stanford University Medical Center, Stanford, California, (11) Baylor University, Dallas, Texas, (12) University of Florida, Gainesville, Florida, (13) University of Louisville, Louisville, Kentucky, (14) Wake Forest University, Winston-Salem, North Carolina, (15) Loyola University, Maywood, Illinois, (16) University of Cincinnati, Cincinnati, Ohio, and (17) Fred Hutchinson Cancer Center, Seattle, Washington, (18) Washington University, St. Louis, Missouri, (19) University of Iowa, Iowa City, Iowa.

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AUTOLOGOUS MARROW TRANSPLANTATION FOR NON-HODGKIN'S LYMPHOMA: HIGH DOSE ETOPOSIDE AND MELPHALAN WITH OR WITHOUT TOTAL BODY IRRADIATION

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INTRODUCTION

We have employed a new intensive therapy regimen injunction with autologous bone marrow transplantation (ABMT) for patients with selected hematologic malignancies (1). The treatment consists of the administration of high doses of etoposide and melphalan with or without total body irradiation (TBI). In order to determine the efficacy of this regimen and document its side-effects, we evaluated patients with relapsed intermediate or high grade non-hodgkin's lymphoma (NHL) deemed suitable candidates for ABMT. To further assess the role and impact of abmt in this patient population, we analyzed outcome in terms of the patient group actually referred for transplant.

PATIENTS AND METHODS

Patients

Entry into the transplant program was restricted to patients aged 16 to 60 years with intermediate or high grade NHL who had previously achieved a complete response (CR) with an anthracycline-containing regimen and who relapsed but had chemotherapy-sensitive disease. The latter was defined as a partial response of at least 50% reduction in tumor bulk in all evaluable lesions after salvage chemotherapy. In addition, patients with poor prognosis high grade nhl in CR-1 were also eligible. Thirty consecutive patients with NHL were evaluated. Twenty-five had intermediate grade or immunoblastic nhl, while five had high-grade disease (lymphoblastic lymphoma and burkitt's lymphoma). Two patients underwent transplant for poor prognosis Burkitt's lymphoma during CR1. There were 17 males and 13 females. The median age was 41 years.

Pre-Transplant Salvage Chemotherapy Regimens

In order to establish eligibility for ABMT, patients received DHAP (2) as first line salvage chemotherapy, primarily to determine if the relapsed disease

was sensitive to chemotherapy but also in an attempt to reduce tumor bulk before transplant (3). In patients otherwise suitable transplant candidates who failed to respond to DHAP, second and third line salvage chemotherapy regimens were offered in order to assess chemosensitivity to other antineoplastic drugs. Second line salvage consisted of augmented CVP, based on the standard CVP protocol but with the cyclophosphamide dose increased to 4 gm/m^2. Third-line salvage consisted of mini-BEAM, a protocol for relapsed/refractory Hodgkin's disease devised by A. Goldstone and D.C. Linch (University College Hospital, London). Depending on tumor bulk at relapse (> 5 cm), patients underwent local radiotherapy prior to transplant. Those who failed all the salvage chemotherapy regimens received palliative care. A schema of the approach to treatment before transplant is shown.

Intensive Therapy Regimen: Etoposide, Melphakan + TBI

The intensive regimen is administered as follows:

Etoposide: 60 MG/KG IV x 1	Day -4
Melphalan: 140-160 MG/M^2 IV x 1	Day -3
+ TBI: 500 cGY Midplane Dose at 50 cGY/MIN	Day 0
Thawed cryopreserved autograft	Day 0

The etoposide is administered as a 5 hour infusion in 5 litres of normal saline. The dose of melphalan is increased to 160 mg/m^2 in patients not receiving TBI. Patients who had not previously received dose limiting radiotherapy are given TBI. Unpurged cryopreserved autografts are employed in all cases. Marrow harvesting, performed on an out-patient basis, has been previously described (4).

Patients are managed in single bed transplant-designated rooms until granulocytes of 0.5 X 10^9/l are reached and receive empiric broad-spectrum antibiotics for fever, as well as prophylactic red blood cell transfusions to maintain the hemoglobin, 90 g/l and platelet transfusions to achieve platelet counts of, 20 x 10^9/l.

RESULTS

The intensive therapy regimen was very well tolerated with little Grade 3 toxicity. Few patients required intravenous morphine to control pain associated with oral mucositis. The non-hematologic toxicity in 21 evaluable patients is described below:

Percentage with Toxicity	Grade 1-2	Grade 3-4
n/v	81	5
diarrhea	62	14
mucositis	57	19
rash	24	0
pulmonary	19	0
renal	24	0
hepatic	5	0

One treatment-related death, due to sepsis, was observed (n = 28, incidence 3.6%).

Hematologic recovery in this patient group was acceptable as indicated below:

	Median (days)	Range
anc > $0.5 \times 10^9/l$	25	15-61
platelets > $20 \times 10^9/l$	29	15-150

Outcome post-transplant was as follows. Of the 30 patients, 2 were too early to evaluate, 3 patients did not respond to the intensive therapy regimen and 10 patients relapsed at a median of 7 months post-transplant with a range of 3-10 months. Fourteen patients remain in continuous complete remission at a median of 18 months post-transplant with a range of 4 to 36 months. Projected disease-free survival at 3 years is 45%.

We also examined the impact of ABMT in NHL by determining the outcome of patients referred for transplant. Of 45 patients referred for ABMT, 22 proceeded to transplant. Fourteen of the 22 transplanted patients (64%) underwent intensive therapy and autotransplant while in CR. Complete responses were achieved in the relapsed patients by administration of the salvage chemotherapy regimens described earlier. Of the 22 patients who underwent ABMT, 10 remain in continuous CR at a median of 18 months after transplant. All referred patients ineligible for transplant died within one year of commencing salvage chemotherapy.

DISCUSSION

We have demonstrated that the intensive therapy regimen of high dose etoposide, melphalan with or without TBI is effective and very well tolerated in patients with relapsed NHL. Extramedullary toxicity is mild and long-term disease free survival is 45%. However, in order to determine the true impact of ABMT in patients with NHL, we analyzed our results in terms of the number of patients referred for transplant. We found that approximately one-half of the patients referred for ABMT underwent transplant and, of those, one-half remain disease-free at a median of 18 months post-transplant. Therefore, we can

expect approximately 20-25% of patients referred for ABMT to be long-term disease-free survivors post-transplant. No long-term disease-free survivors were documented in the group referred for transplant who were ineligible for, and did not receive, ABMT.

Our approach provides a means to evaluate the contribution of conventional dose salvage chemotherapy to long-term disease-free survival post-transplant, if the transplant-associated intensive therapy regimen remains the same.

ACKNOWLEDGEMENTS

This study was supported by a grant from the National Cancer Institute of Canada. Dr. Keating is a Senior Research Scientist of the Institute.

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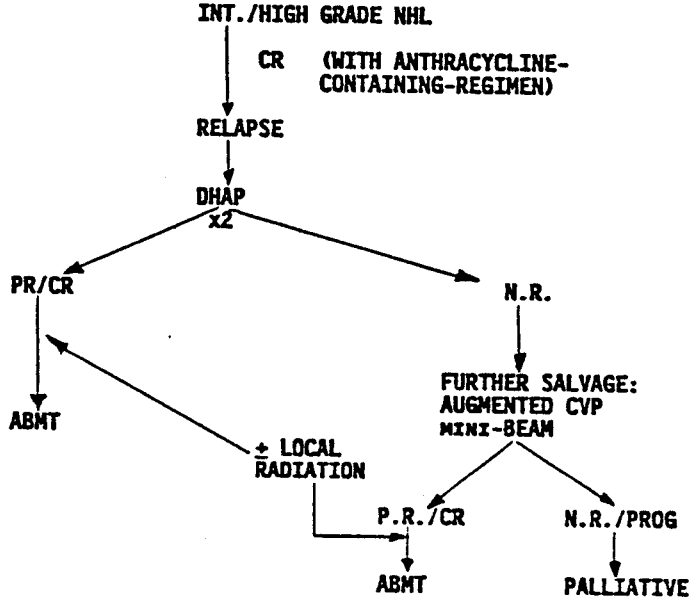
FIGURE 1
Schema of the approach to treatment before transplant.



PROG = PROGRESSION OF DISEASE
N.R. = NO RESPONSE
P.R. = PARTIAL RESPONSE
C.R. = COMPLETE RESPONSE

RESULTS OF THE BEAM PROTOCOL IN AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR INTERMEDIATE OR HIGH GRADE NON-HODGKIN'S LYMPHOMA IN FIRST SENSITIVE RELAPSE: A STUDY OF THE FRENCH AUTOLOGOUS BONE MARROW TRANSPLANTATION GROUP

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SUMMARY

Nineteen patients treated in first sensitive relapse of intermediate or high grade Non-Hodgkin's Lymphoma with BEAM (BCNU, VP16, Etoposide, Cytosine Arabinoside [Ara C], Melphalan) conditioning regimen and autologous bone marrow transplantation (ABMT) are reported. Fifteen patients were in second complete remission and four in second partial remission. Six patients relapsed and one toxic death was observed. With a median follow up of 42 months, the actuarial disease free survival is 62% and the actuarial survival is 61%. No prognostic factor could be found in this small number of patients. So we confirm the good efficacy and small toxicity of BEAM protocol and that ABMT can cure a high percentage of relapsed NHL still responsive to conventional chemotherapy.

INTRODUCTION

Patients with intermediate or high grade Non-Hodgkin's Lymphomas (NHL) who fail conventional chemotherapy, either with primary resistant disease or after relapse, are rarely cured by conventional salvage chemotherapy. Since 1978 (1), many reports (2-27) have been published about the efficacy of high dose chemotherapy with or without total body irradiation and autologous bone marrow rescue in the treatment of bad prognosis NHL, but there is also a wide range in the results with a disease free survival between 15% and 69% according to some studies. Many prognostic factors can explain the discrepancy between these results: persistence of chemosensitivity to conventional salvage chemotherapy (19), completion of response to the salvage therapy (8-37), tumor burden at ABMT (28),histology (9,25), age (25), performance status (28), but also conditioning regimen, ex vivo purging and previous therapy can play a role.

A new conditioning regimen, the BEAM protocol (BCNU, Etoposide, Cytosine-Arabinoside, Melphalan) was started in 1983 and the first results were presented in 1986 (29) and in 1988 (30). We present here the long term results of patients treated in France in first chemosensitive relapse of intermediate or high grade NHL.

PATIENTS AND METHODS

Nineteen patients in first chemosensitive relapse of intermediate or high grade diffuse NHL are included in this retrospective study.

Informed consent was obtained for each patient according to the requirements of institutional review board for human investigations of each institution. Details of the patients are summarized in table I. The age range of the patients was 7 to 61 years (median = 33 years) with three patients below 20 years. There were 15 men and 4 women. According to the National Cancer Institute Working Formulation (31), there were five mixed diffuse, six diffuse large cell, three immunoblastic, three lymphoblastic and two Burkitt's lymphomas.

At diagnosis ten patients presented constitutional symptoms and nine patients had extranodal disease, six with a single localisation and three with two or more localizations bone, bowel (3 patients each); skin, waldeyer's ring (2 patients each); lung, liver, chest wall. Only one patient had bone marrow involvement. Five patients had a bulky disease (defined as a node larger than 10 centimeters in its greatest diameter or extensive abdominal disease) and five patients had more than three nodal sites of involvement. Initial treatment included one chemotherapy regimen in 13 patients and two regimens in 6 patients. All patients received Doxorubicin before relapse. Eight patients received localized radiation therapy and 2 patients had CNS irradiation as meningeal prophylaxis.

The median duration of the first complete remission was ten months (5 to 41 months) with eight relapses on therapy and eleven relapses off therapy. Eight relapses were localized in nodes, two in nodes and bone marrow, three were nodal and extranodal (bone (2), testes (2), lung (1), chest wall (1) and six pure extranodal diseases (pure CNS (3); breast (1); bowel (1); lung (1)).

The median length between relapse and ABMT was 4 months (2 to 9 months). A median of two courses (range, one to six) and a median of six drugs (range two to ten) had been given for the treatment of relapses. Three patients had also CNS irradiation because of CNS relapse.

At time of ABMT fifteen patients were in second complete remission (CR2) and four patients in second partial remission (PR2) with a minimal residual disease in nodes (two patients), bone marrow (one patient with small nodular involvement) and bowel (one patient).

Preparative Regimens

All patients received the BEAM protocol (29) with BCNU 300 mg/m^2 on day 1 given over 30 minutes IV, VP16 100 mg/m^2 every 12 hours (200

mg/m^2/day) over 30 minutes IV on days 2 to 5, Cytosine-Arabinoside (ARA C) 100 mg/m^2 every 12 hours (200 mg/m^2/day) over 30 minutes IV on days 2 to 5 and Melphalan 140 mg/m^2 IV in five minutes on day 6. Four patients also received a boost of radiotherapy at 20 Gy in involved fields after ABMT.

Bone Marrow Procedures

Marrow was histologically evaluated by bone marrow biopsy within 3 weeks of harvest. Only one patient with Burkitt's lymphoma had a minimal residual disease in the bone marrow when harvested.

The technique of bone marrow harvest, cryopreservation and reinfusion has been previously described (32). The median dose of infused nucleated cells was 1.6 x 10^8/kg of body weight (range 0.3 to 3.3 x 10^8/kg) and the median dose of CFU-GM was 1.8 X 10^4/kg (range 0.1 to 5.7 X 10^4/kg).

Supportive Care

In all patients, a central catheter was implanted. Some patients were treated in single rooms with reversed isolation, some in laminar air flow rooms. All patients received oral antibiotics, Amphotericin B suspension for selective decontamination of the gut and sterile food was given until the granulocyte counts were above 0.5 x 10^9/1.

Empiric broad spectrum antibiotics and in some cases antifungal therapy, were given intravenously for febrile episodes associated with granulocytopenia. Platelets from single donors and leukocyte-free red cell concentrates were administered routinely in order to maintain platelets above 30 x 10^9/1 and hemoglobin above 100 g/l. All allogenic blood cells were irradiated.

Evaluation and Statistical Analysis

At relapse, and before ABMT, all patients were evaluated by means of physical examination, blood chemistry profile, peripheral blood count, chest and abdominal CT scanning, bone marrow biopsy. Staging studies were repeated at day + 30 and 6 month intervals.

Complete response was defined as the disappearance of tumor according to all indexes. Partial responses were defined as >50% reduction in cross-sectional tumor dimensions without the appearance of new lesions.

Disease free survival and total survival were calculated from the day of marrow transplantation according to the method of Kaplan and Meier (35). For prognostic factors, disease free survival curves were compared by the log rank test (36).

RESULTS

Survival and Disease-Free Survival

At present twelve patients are alive, and eleven are disease-free, with an actuarial disease free survival of 62 and a median follow up of 42 months (12-60). Nine of them have a follow up longer than 2 years. Six patients

relapsed 4, 4, 4, 8, 9 and 28 months after ABMT and five died of disease. Two patients died, one of transplant related toxicity and one at home of sudden unexplained death, 35 days after graft. The associated Kaplan Meier plot is shown in figure 1.

Prognostic Factors

Despite the small number of patients, we tried to find some correlation with prognostic factors in the seventeen evaluable patients. Regarding status at initial diagnosis, we were unable to find any correlation between prognosis and age, stage, number of involved node areas, bulky disease, number of extranodal involvements ($p > 0.1$). According to the histology, two out of the four diffuse mixed, one out of the five diffuse large cell, one out of the three immunoblastic, two of the three lymphoblastic and zero of the two Burkitt's lymphomas have relapsed. Relapses were also observed in three out of the eight B and two out of the three T phenotype lymphomas and in five out of the ten patients with constitutional symptoms. But the numbers are too low to permit to draw any statistical conclusion.

According to the relapses before ABMT, the fact that the relapses occurred on or off therapy did not appear of prognostic value ($p > 0.5$). Two of the seven evaluable patients with pure nodal relapse and four out of the ten patients with extranodal involvement failed after ABMT. However, if CNS and bone marrow involvement did not seem of prognostic value, three out of the five patients with atypical extranodal involvement died of disease.

According to the status at ABMT and about the four patients in second partial remissions, one patient with nodal medullar residual disease relapsed eight months after ABMT, one patient died of toxicity and two patients (with gut and bone marrow residual diseases) are disease free 40 and 42 months after ABMT. The patient who relapsed was the patient who had the most important tumor burden at time of transplantation. According to the ex vivo treatment, two of ten patients without purging and four out of the seven patients with immunological or chemical purging had subsequent relapse.

Engraftment

One patient died of toxicity before day 30 and was not evaluable for hematologic recovery. The median day of recovering sustained leukocytes $> 1.0 \times 10^9/1$ was day + 19 (range 10 to + 29) and for neutrophils $> 0.5 \times 10^9/1$ day + 20 (range 10 to + 30). Platelets recovery to $50 \times 10^9/1$ was 26 days (range 14 to > 150). Only one patient grafted after immunologic purging of bone marrow, had no recovery of platelets at day +60 and relapsed at day +140. Globally we observed no significant increase in the delay of recovery in patients receiving purged bone marrows.

Acute and Chronic Toxicities

Two patients died during therapy, one of infection (aspergillus pneumonia) at day 15 and one of sudden death at day 35. Six bacterial septicemias (three gram positive cocci and three gram negative) were observed

in five patients. Pneumonitis occurred in five patients: two fungal (aspergillus), one due to mycoplasma and two of unknown origin, one patient developed also a transient veno-occlusive disease of the liver.

If nausea and vomiting were mild, mucositis of grade 2 or 3 according the W.H.O. criteria (37) was observed in most of the patients but was always resolved by day 20.

DISCUSSION

We present here the updated results of BEAM protocol in first sensitive relapse of intermediate or high grade NHL. We confirm the good efficacy of the preliminary reports (29-30) with an actuarial disease free survival of 62% and the low toxicity whilst only one toxic death has been observed among the nineteen patient.

These results appear to be as good as or better than those already published in the treatment of NHL in relapse: 36% of DFS in the study of Philip et al (19) including 44 patients in sensitive relapse, 69% of DFS in the study of Takvorian with 49 patients grafted with minimal residual disease (but some patients with first partial remission or with low grade NHLs were included in this trial). Our good results can have two explanations: 1) the very good efficacy of the conditioning regimen; 2) the selection of the included patients.

According to the efficacy of the conditioning regimens in ABMT, two factors seem to be important: the intensity of the drug schedules and the use of drugs which have not been already included in the previous standard chemotherapies (38). According to the first point, we cannot consider that Carmustine, VP16 and Cytosine Arabinoside are used at a very high level in the BEAM regimen. According to the second point, Carmustine, Melphalan and less usually cytosine arabinoside, are drugs which are not commonly utilized in the first line or second line conventional chemotherapies of NHL: this fact may play a role in our good results.

Some authors have tried to define some prognostic factors about patients who received ABMT for NHL: age over 50 years (25), a Karnofsky performance score of at least 80 (28), high grade histology (25), lymphoblastic histology (8), no bulky tumor (any tumor mass > 7 cm) (28), lack of previous CR before ABMT (19) responsiveness to the last conventional chemotherapy regimen (19,28) and the completion of this response (8,38).

About the selection of patients, all the French patients grafted for sensitive relapse of intermediate or high grade non Hodgkin's lymphoma appear to have been included in this study except those of one center. However, the fact that fifteen out of nineteen patients of our study were in CR and that three of the four patients in second PR had minimal residual disease, could play a role in our good results.

In this small study of selected patients in sensitive relapse, we tried to find some prognostic factors which could be different from those of larger unselected studies. In fact, we were unable, because of the small number or of

the selection of patients, to find any significant prognostic factor. We can only observe that two out of the three patients with lymphoblastic lymphoma, five out of the ten patients with initial constitutional symptoms and three out of the five patients with atypical extranodal involvement at relapse (as described for low grade NHL (39)) failed after graft. Nevertheless, two out of the three patients with pure CNS relapse seem to be cured as previously described (6,40). As Philip et al (19), we also did not find the prognostic value of relapses on or off therapy. But the schedules of the drugs used in initial therapy and the completion of response to salvage conventional chemotherapy may be two factors influencing these conclusions.

Most of the failures after ABMT are focal. So the place of radiotherapy in preparative regimens for ABMT remain to be determined. TBI does not seem to increase significantly the cure rate, but is associated with a higher early death rate (19,28). Localized radiotherapy just before ABMT or later after recovery is also used by many investigators but no conclusion can be drawn now about the effectiveness of this approach (38). In our study four patients received localized radiotherapy post-ABMT: three remain disease free, the last one who was a Burkitt lymphoma relapsed as leukemia four months after ABMT.

CONCLUSION

In conclusion, this report indicates that ABMT can induce a long term disease free survival of more than 60% in sensitive relapses of NHL. BEAM seem to be a very efficacious conditioning regimen with light toxicity in this type of patients.

ACKNOWLEDGEMENTS

This work was supported by grants of "Association contre le Cancer", Villejuif, France. Authors' affiliations:(1) Marrow Transplant Unit, Department of Hematology, CHU Bretonneau, 37044 Tours Cedex; France; (2) Marrow Transplant Unit, Centre Leon Berard, Lyon; (3) Marrow Transplant Unit, Hopital Saint Antoine, Paris; (4) Marrow Transplant Unit, Hopital Jean Minjoz, Besancon

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TABLE 1

PATIENT'S CHARACTERISTICS AND RESULTS

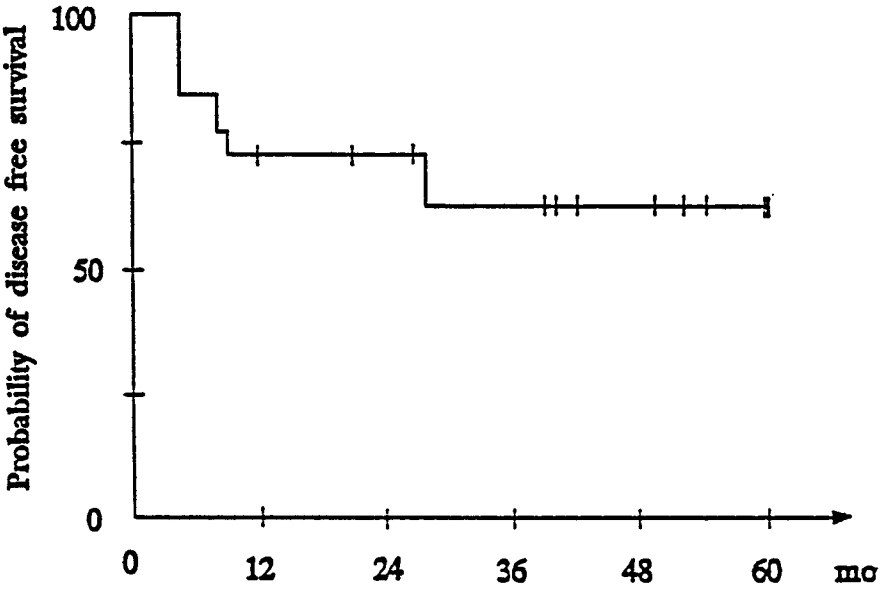
	n	RESULTS Early Death	Relapses	Alive and Well
- Total	19	2	6	11
- Histology				
- mixed diffuse	5	1	2	2
- diffuse large cell	6	1	1	4
- immunoblastic	3	0	1	2
- lymphoblastic	3	0	2	1
- Burkitt	2	0	0	2
- Immunology				
B	8	0	3	5
T	3	0	2	1
Unknown	8	2	1	5
- Prognostic Factors at Diagnosis				
- Numbers of nodal sites involved				
≤ 3	14	1	5	8
> 3	5	1	1	3
- Tumor burden				
< 10 cm	14	2	5	7
> 10 cm	5	0	1	4
- Numbers of extranodal involved sites				
none	10	0	5	5
1	6	2	1	3
≥ 2	3	0	0	3

TABLE 1 Cont.

- Constitutional symptoms				
A	9	2	1	6
B	10	0	5	5
- Relapses				
- on therapy	8	0	2	6
- off therapy	11	2	4	5
- Localizations of relapses				
- pure nodal	8	1	2	5
- nodes + bone marrow	2		0	0
- pure extranodal	6	0	2	4
- CNS	3	0	1	2
- breast	1	0	1	0
- bowel	1	0	0	1
- lung	1	0	0	
- Status at ABMT				
- CR2	15	1	5	9
- PR2	4	1	1	2
- In vitro purging				
- Yes	7	0	4	3
- No	12	2	2	8
- Involved fields radiotherapy				
- Yes	4	0	1	3
- No	15	2	5	8

FIGURE 1

Kaplan-Meier estimate of the probability of disease free survival (DFS) in 19 patients undergoing Autologous Bone Marrow Transplantation for poor prognosis Non-Hodgkin's Lymphoma. Each tick mark represents a surviving patient in complete remission



PRELIMINARY ANALYSIS OF HIGH DOSE ETOPOSIDE CYTOREDUCTIVE REGIMENS AND AUTOLOGOUS BONE MARROW TRANSPLANTATION IN INTERMEDIATE AND HIGH GRADE NON-HODGKIN'S LYMPHOMA

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INTRODUCTION

Strategies for improving the results of intensive therapy and autologous transplantation in high risk lymphoma patients include increasing the efficacy of the ablative regimen, reducing the morbidity and mortality in the immediate post-transplant period and incorporating this modality in the primary treatment of selected patients. The single agent activity of etoposide (VP16) in refractory lymphoma and its spectrum of toxicity make it an attractive candidate drug for combined use with marrow grafting. Blume et al. have defined the maximum tolerated dose of an escalating dosage of VP16 in combination with fractionated total body irradiation in advanced hematologic malignancies (1). This was followed by a phase I-II study designed to define the maximum tolerated dose of total body irradiation (TBI) in combination with both high dose VP16 and high dose cyclophosphamide (CY) in refractory lymphomas (2). It is well recognized that patients previously exposed to critical doses and volumes of radiotherapy are not candidates for TBI. Therefore, a phase I-II study was conducted at Stanford University and The City of Hope Medical Center to determine the MTD of escalating doses of carmustine (BCNU) in combination with fixed doses of VP16 and CY. This report represents a preliminary analysis of these high dose VP16 regimens in selected patients with intermediate and high grade lymphoma treated at Stanford.

PATIENTS AND METHODS

Patients were eligible for the study if they were less than 55 years of age with recurrent diffuse large cell, diffuse mixed or diffuse small non-cleaved cell (DSNC) lymphoma. In addition, patients with DSNC (Burkitt's or

non-Burkitt's lymphoma) histologic subtype were eligible in first remission if they presented with stage IV disease and an elevated serum lactic dehydrogenase. Patients with sensitive disease who had failed to achieve a complete remission were also allowed on this study. Also eligible were patients with intermediate or high grade lymphoma which represented a conversion from an initial low grade histology.

A minimal disease state obtained with conventional chemotherapy, irradiation or both was required for study entry. Minimal disease was defined as a complete remission or a >75% reduction in an initially bulky mass, a single node < 2 cm in maximum horizontal diameter, or 10% or fewer residual tumor cells in the bone marrow. Active central nervous system (CNS) involvement was not an exclusion criteria. The choice of conventional chemotherapy was based upon prior treatment, with an attempt to use alternative drugs which were non-cross resistant with previous therapy or the ablative regimen. Radiotherapy was used to boost treatment to bulky sites in selected cases.

Patients were assigned to one of two high dose VP-16 regimens, based upon prior therapy (Figure 1). Those with a history of prior chest irradiation in excess of 20 Gy, age > 50 years or prior pelvic irradiation and an inadequate bone marrow harvest received a combination of BCNU 15 mg/kg on day -6, VP-16 60 mg/kg as a single injection day -4, and cyclophosphamide (CY) 100 mg/kg given as a single injection day -2. All other patients received fractionated total body irradiation (FTBI) 12 Gy, given in ten treatments day -8 through day -5, VP-16 60 mg/kg day -4, and CY 100 mg/kg day -2. Cryopreserved bone marrow that had been previously treated with a panel of monoclonal antibodies (Mab) and complement was rapidly thawed and reinfused on day 0.

Bone marrow was harvested and treated *in vitro* with a panel of B- or T-cell monocional antibodies and baby rabbit complement. Patients with B-lineage lymphomas had their marrows treated with a mixture of anti-CD9, -CD10, -CD19, -CD20 antibodies while Tlineage marrows were treated with a mixture of anti-CD2, -CD3, CD4, -CD6, -CD6, -CD8, -CD28 antibodies. Lymphomas of uncertain lineage received both antibody panels. Peripheral stem cells were routinely collected as a backup to ensure engraftment. In selected cases, peripheral stem cells were administered on day 0 to supplement an inadequate harvest or exclusively in patients with a history of prior pelvic irradiation. After November 1988 patients were eligible to participate in a double-blind placebo trial of rh-GM-CSF (granulocyte-macrophage colony stimulating factor, Schering/Sandoz Study Group, New Jersey) administered posttransplantation.

All patients were evaluated at study entry and pretransplant with physical examination, chest X-ray, computed tomographic scans, bone marrow biopsies, blood chemistry profile and complete blood counts. Post-transplant restaging was every other month for the first year and every three monthly for the subsequent year. Freedom from progression and survival were calculated

from the date of transplantation, expressed as Kaplan-Meier plots (3) and compared according to the method of Gehan (4).

RESULTS

Patient Characteristics

Twenty-six patients with high risk intermediate and high grade lymphoma who achieved a minimal disease state were transplanted between April 1988 and June 1990 (Table 1). Fifteen patients were prepared with FTBI/VP16/CY and 11 with BCNU/VP16/CY. This included fifteen males and eleven females. The median age was 41 years with a range from 24 to 53 years. Eighteen patients were in their first relapse and one each was in second or third relapse from prior remission. Three patients were primary induction failures. One patient with marrow and CNS positive DSNC lymphoma was in first remission. Two patients had lymphomas which had transformed from an initial low grade histology. The median duration of first complete remission for the twenty patients with recurrent disease was 5 months.

The histologic subtypes included 21 cases of diffuse large cell lymphoma (cleaved/noncleaved or immunoblastic), three cases of diffuse mixed small cleaved and large cell and two cases of diffuse small non-cleaved cell (Burkitt's/non-Burkitt's) lymphoma. All patients had received prior chemotherapy alone or together with irradiation. Cytoreductive therapy prior to transplant was highly variable. A single patient with minimal disease received no therapy, two patients with CNS disease received intrathecal methotrexate, and five patients were treated with irradiation. Multiagent chemotherapy incorporating doxorubicin and cyclophosphamide was used in 7 cases, CEPP (cyclophosphamide, etoposide, procarbazine and prednisone) in 7 cases and DHAP (cisplatin, cytosine arabinoside, dexamethasone) in 10 cases. In seven patients more than one cytoreductive maneuver was required to achieve a minimal residual disease state.

Transplant and Engraftment

The median bone marrow cell dose was 0.40 and 0.67 x 10^8/kg, respectively. Two patients were rescued with peripheral mononuclear cells only at doses of 10 and 6 x 10^8/kg. Two patients treated with FTBI and one patient treated with the BCNU-based regimen received combined marrow and peripheral mononuclear cells. Twenty-two of the 26 patients were enrolled in the GM-CSF study. In vitro Mab and complement treatment was performed on the marrows from 13 of 15 FTBI/VP16/CY patients and 8 of 11 BNCU/VP16/CY patients.

All patients achieved granulocyte engraftment with the exception of one early toxic death (see below). The median time to absolute neutrophil count (ANC) greater than 500/MM^3 was 22 days for patients on the FTBI regimen and 20 days for those only the BCNU arm. Time to ANC >500 for the two patients rescued with peripheral mononuclear cells was 28 days and 11 days. Platelet engraftment defined as $>25{,}000/MM^3$ was achieved in all but four

patients at a median time of 38 days and 29 days for patients receiving FTBI/VP16/CY and BCNU/VP16/CY regimens, respectively. Three patients failed to platelet engraft prior to death at 15, 22 and 149 days. One additional patient has been followed less than one month post-transplant. Three patients received GM-CSF on a compassionate basis when engraftment had not occurred by day 28, and four patients received their peripheral mononuclear cells as a boost due to sluggish engraftment.

Acute toxicity according to preparative regimen is noted in Table 2. Two acute in-hospital deaths were observed in patients receiving BCNU/VP16/CY: one due to veno-occlusive disease on day 15 and a second due to a combination of fungal sepsis and cardiomyopathy on day 22. Two cases of diffuse alveolar hemorrhage were encountered, both in patients prepared with FTBI/VP16/CY. Both were managed with high dose steroids, which were then rapidly tapered with clinical improvement. Six patients met the criteria for interstitial pneumonitis; four had received FTBI and two had received BCNU. All have subsequently improved. Other acute toxicities were similar to those observed with high dose chemoradiotherapy regimens. Although fever in the setting of neutropenia was nearly universal, only nine bacterial infections (8 in the blood) were documented. Viral infection, mainly due to Herpes simplex or zester, was diagnosed in 15 patients. Three patients had documented infection with Aspergillus, two were recognized post-mortem. Mucositis was commonly observed, but no patient had severe mucositis, defined as grade 3 according to the Southwest Oncology Group autologous bone marrow transplantation toxicity criteria. Erythema, desquamation and vesiculation of the skin was observed with both regimens; no patient had severe skin (grade 3) toxicity. Other toxicities observed included one case each of inappropriate ADH (antidiuretic hormone) and CNS (subarachnoid) hemorrhage. The latter was small and the patient has fully recovered.

Preliminary Therapeutica Results

With a range of followup from one to 26 months, median eight months, 17 of the 26 patients are currently alive and free of disease (Table 3). Seven patients have developed recurrent lymphoma of whom three are living and four are dead. Two additional, transplantrelated deaths have occurred as outlined above. There are no significant differences in overall survival or freedom from disease progression when the two preparative regimens are compared. The actuarial one year survivals are 74% and 59% for the FTBI/VP16/CY and BCNU/VP16/CY regimens, respectively ($p = 0.18$). Actuarial one year freedom from progression probabilities are 63% and 64% for FTBI- and BCNU-based regimens, respectively.

Relapses occurred in 4 patients in first relapse, one patient in third relapse, one induction failure and the single patient with Burkitt's lymphoma in first remission. Three patients had a history of CNS disease: two at relapse and the patient transplanted in first CR. All three patients developed recurrent disease in the bone marrow. One patient with peripheral T-cell lymphoma/leukemia developed recurrent disease in marrow and liver, again

with a leukemic picture. The remaining three patients developed disease recurrence in areas of preexisting adenopathy. The initial remission durations in these patients were 0, 1, 2, 4, 5 and 6 months plus one patient transplanted in first CR.

DISCUSSION

In this preliminary analysis, we report the data on 26 patients who received one of two high dose etoposide regimens for high risk intermediate and high grade lymphoma The most significant observation is the low regimen-related mortality. Two acute, inhospital deaths were seen, both in patients prepared with BCNU/VP16/CY. No acute deaths were noted among 15 patients receiving an ablative regimen consisting of FTBI/VP16/CY. This treatment related mortality is comparable to that reported for primary chemotherapy of intermediate and high grade lymphomas. These data compare favorably with data from the European Bone Marrow Transplant Group and the Fred Hutchinson Cancer Research Center (5,6). Very low regimen-related mortality has been reported by Freedman et al. in selected B-cell lymphoma patients prepared with FTBI/CY at the Dana-Farber Cancer Institute (7) A previous report combining data from our institution and The City of Hope indicated a regimen-related mortality of 4.7% in a group of 105 lymphoma patients receiving these ablative regimens (8).

Our results support earlier observations regarding the ability to rescue patients with *in vitro* manipulated bone marrow and peripheral mononuclear cells. Of note, four of seven relapses occurred in bone marrow which had received ex vivo purging. It is not possible with current technology to determine whether relapse occurred from residual tumor in the patient or tumor cells which were reinfused.

The curative potential of either of our high dose etoposide regimens awaits longer followup and adequate patient numbers to properly address the clinical parameters of potential prognostic significance. In addition to the ablative regimens, patient selection and histologic variation increase the difficulty of comparing outcomes in different studies. Even among the intermediate grade lymphomas, there is a marked difference in the natural history of follicular large cell and diffuse small cleaved cell lymphoma when compared with diffuse large cell and mixed subtypes. Furthermore, discordant lymphomas are being recognized with increased frequency, making it essential that the subtype of marrow involvement is also reported. With the early use of bone marrow transplantation as a consolidative treatment for first remission, it is important to address these variables with valid concurrent or historical control groups.

ACKNOWLEDGEMENTS

Supported in part by NIH Grant PO 1 CA 49605.

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TABLE 1

Characteristics of 26 Patients

FTBI/VP16/CY	15
BCNU/VP16/CY	11
Median age	41 yr
(Range)	(24-53)
Male:Female	15:11
Category	
First complete remission	1
First relapse	18
Second/third relapse	2
Induction failure	3
Histologic transformation	2
Median duration of first remission	5 mo

TABLE 2

Toxicity

CATEGORY	FTBI/VP16/CY n = 15	BCNU/VP16/CY n = 11
Veno-occlusive disease		1
Diffuse alveolar hemorrhage	2	
Interstitial pneumonitis	4	2
Documented infection		
Bacterial	6	3
Viral	8	7
Fungal	1	2
Cardiomyopathy		1
Inappropriate ADH		1
CNS hemorrhage		1

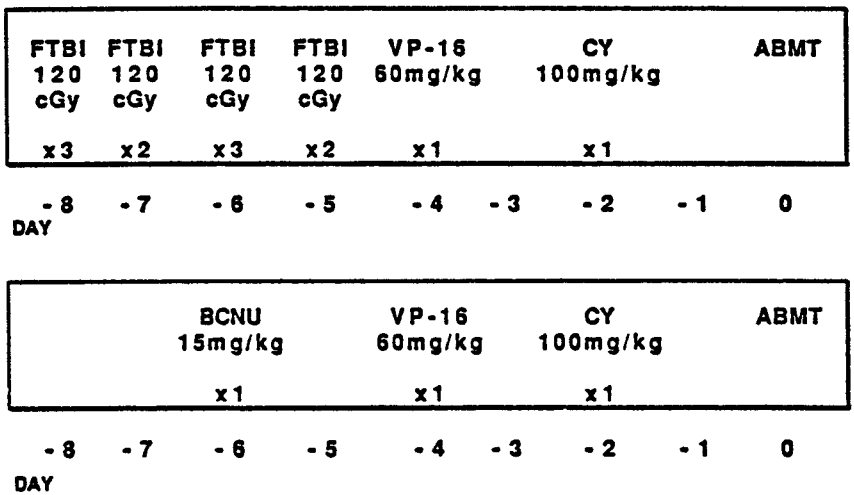
TABLE 3

Therapeutic Results

REGIMEN	FREEDOM FROM PROGRESSION	SURVIVAL
	Actuarial probability at one year	
ALL	62%	68%
FTBI/VP16/CY	59%	74%
BCNU/VP16/CY	64%	63%

FIGURE 1

High dose VP-16 ablative regimens.



AUTOLOGOUS BONE MARROW TRANSPLANTATION IN LOW GRADE B CELL NON-HODGKIN'S LYMPHOMA

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INTRODUCTION

High dose therapy and autologous bone marrow transplantation (ABMT) has been shown to be a potentially curative modality for patients with relapsed intermediate/high grade NHL (1-11). Considering the large numbers of patients who have undergone ABMT for NHL, relatively few with relapsed low grade histologies have been treated with this approach (3,4,6,7,9,12-14). The lack of interest for ABMT in low grade lymphomas has largely been based upon the evidence that this is a disease with a very long natural history and therefore excessive treatment related toxicities associated with aggressive therapy would not be acceptable. Moreover, the high frequency of overt bone marrow infiltration, even following aggressive chemotherapeutic regimens, has been a major obstacle (15). To date, less than 100 patients with low grade NHL have undergone ABMT worldwide. Many of these patients had disease resistant to conventional therapy prior to ABMT and the vast majority were selected for lack of bone marrow infiltration.

In this study, we report the results of 69 patients with low grade NHL who had sensitive disease and good performance status. At the time of ABMT, fifty-one patients retained a low grade histology, while 18 had tumors which had undergone histologic transformation to a higher grade NHL. All patients were uniformly treated with high dose chemoradiotherapy and the bone marrows were purged with anti-B cell monoclonal antibody (mAb) and complement. As expected with these histologies, 51 patients had a history of bone marrow infiltration and 30 had bone marrow involvement at the time of ABMT. The treatment associated mortality was very low. Kaplan-Meier actuarial analysis indicated a 50% probability that patients will remain disease free for 43.6 months.

METHODS

Patients were eligible for this study if they were less than 65 years of age; had relapsed low grade NHL as defined by the International Working Formulation, after standard chemotherapeutic regimens; and had lymphoma cells that expressed the CD20 (B1) antigen as previously described (4). In addition, patients with sensitive low grade NHL but who had failed to enter complete remission after one or more standard chemotherapeutic regimens were eligible. All patients had to achieve CR or minimal disease state as described. Preparative therapy consisted of cyclophosphamide, 60 mg/kg of body weight, infused on each of two consecutive days before radiotherapy. TBI, was administered in fractionated doses (200 cGy) twice daily on three consecutive days (total of 1200 cGy) in all patients. Patients were cared for as previously described.

Bone marrow was obtained, treated in vitro as previously described (4), and stored within four weeks of its use in transplantation in all patients except one. The bone marrow cells were treated with combinations of anti-B1, -B5, and J5 (anti-CD10) and rabbit complement as described. The median number of infused cells was 3.73 X 10^7/kg (range 1.47-12.35 x 10^7/kg), with 85-95% viability as measured by trypan blue dye exclusion.

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

Complete remission (CR) was defined as the disappearance of all measurable and evaluable disease. Disease-free survival (DFS) was calculated from the day of marrow transplantation (day 0). Disease-free survival curves were estimated by the method of Kaplan and Meier (16,17) and compared by the log rank test.

RESULTS

Patient Characteristics

Sixty-nine patients with B cell NHL in sensitive relapse or incomplete first remission who attained a minimal disease state underwent ABMT (between December, 1982 and November, 1989). Fifty-one of these patients (median age 42) retained a low grade histology at ABMT (49 follicular and 2 diffuse) whereas 18 patients (median age 43) had a history of low grade NHL which had undergone histologic transformation to an intermediate grade NHL. At diagnosis, 48 of the 69 patients had FSC histology. Patients who underwent histologic transformation generally had diffuse histology at ABMT, the majority were either DLCL (n=7) or DM (n=5). The majority of patients with both

low grade or transformed histology had a history of bone marrow infiltration at some time during the course of their disease.

Prior Therapy

All patients were previously treated with combination chemotherapy. The mean number of regimens with which patients were treated was 3 for both low grade and transformed histology patients (range 2-8 for transformed, 2-7 for low grade). Approximately one-third received prior involved field radiotherapy and two patients had been previously treated with TBI (150 cGy). A prior CR was documented in only 36 (71%) of the low grade patients (median prior longest disease free interval 8 months, range 2-66 months) while 14 (78%) of the transformed patients had previously achieved a CR (median prior longest disease free interval 7 m, range 3-87 months). Prior to ABMT, only 20 of the 51 low grade patients were in CR whereas 31 had only attained a minimal disease state. At the time of bone marrow harvest, 24 of these patients had residual BM involvement, the majority with 5% or less of the intratrabecular space, however, 5 patients had between 6 and 20% (significantly higher numbers of patients had between 5-20% involvement when the overall marrow cellularity was examined). In contrast, 10 of the 18 patients with transformed histologies were in CR prior to ABMT, with 6 having minimal residual lymphomatous marrow infiltration at harvest.

Treatment Outcome

One patient with FSC NHL died an acute in-hospital treatment-related death due to thrombocytopenic cerebral hemorrhage which occurred at day 36. Two late deaths from non-lymphomatous causes were observed. One of the patients with transformed histology who had received low dose TBI 10 years prior to ABMT, multiple chemotherapeutic regimens, and involved field radiotherapy to the pelvis developed acute non-lymphocytic leukemia at 47 months, and died without evidence of lymphoma following an HLA-matched allogeneic bone marrow transplant (at 49 months). An additional patient with FM histology treated with multiple chemotherapeutic regimens and involved field radiotherapy died of a myelodysplastic syndrome at 14 months, two months after bone marrow relapse with lymphoma.

Of the 69 patients, 44 remain in complete unmaintained remission with a median follow-up of 20 months. There is no significant difference in the DFS for patients with either low grade or transformed NHL (Figure 1) ($p=0.201$). Following relapse, 14 of 24 patients are alive (median follow-up of 17 months). This is in contrast to patients who relapse following ABMT for intermediate/high grade NHL, where the overall survival is essentially identical to the DFS. Kaplan-Meier actuarial analysis indicated a 50% probability that patients with low grade and transformed NHL will remain disease free for 25 and 44 months respectively.

Of the 23 patients who relapsed, the overwhelming majority of patients with both low grade ($n=14$) and transformed histologies ($n=4$) relapsed in sites of prior disease (Table 3). Entirely new sites of disease were observed in only

5 patients, none of whom had bone marrow involvement at harvest. Eight of the 23 relapses involved the marrow, 6 of whom had bone marrow infiltration at the time of harvest.

There was no significant difference in the DFS for patients with a history of CR or lymphomatous marrow infiltration at any time during their disease course for low grade patients and when all patients are considered together (data not shown). However, when the 51 patients who retained a low grade histology were examined separately, patients in CR at ABMT demonstrated significantly better DFS than patients in a minimal disease state (PR) (Figure 1) ($p=0.010$). Moreover, at the time of marrow harvest, the presence of overt bone marrow involvement adversely affected the DFS when the entire patient population was examined ($p=0.042$). Although there is a similar trend for patients who had low grade histology at ABMT, it is not statistical significant ($p=0.30?$)(data not shown).

DISCUSSION

It is now generally accepted that high dose ablative therapy and ABMT is a potentially curative modality for patients with relapsed intermediate/high grade NHL (18). However, the use of this approach for patients with low grade histology has been limited. Two reports (13,14) have recently examined the treatment results of ABMT in patients with a history of follicular lymphoma. In these studies virtually all patients were selected for uninvolved bone marrow at harvest. Considering the high frequency of bone marrow involvement in low grade NHL, 43% of patients in the present study had bone marrow infiltration with lymphoma at harvest. In contrast to patients with intermediate/high grade NHL who have undergone ABMT, patients with a history of low grade histology who had marrow infiltration at harvest had a significantly worse DFS than patients with uninvolved marrow. Several series have demonstrated that patients with intermediate/high grade NHL who undergo high dose therapy with residual but sensitive disease and enter CR following ablative therapy, have a similar DFS to patients in CR at ABMT (1,3,7,10,19,20). The present study suggests that patients with low grade NHL who could attain a CR prior to ABMT have an improved DFS over patients with residual disease at ABMT. Our patients were treated with multiple aggressive salvage regimens in an attempt to attain a minimal disease state or CR prior to ABMT. Therefore, many of those who were transplanted with residual disease, either in BM or nodal sites most likely harbored residual resistant lymphoma.

The treatment outcome for patients who have undergone histologic transformation employing conventional therapy has been reported to be poor (21,22). The limited number of studies which have included patients with transformed NHL who have undergone ABMT, report a high treatment related mortality and low DFS (11,13). Our patients with transformed histologies had disease which was still sensitive to conventional therapy, which is contrast to other reports of ABMT in histologic transformation and is likely to account for the observed differences in treatment associated mortality and DFS. An

additional finding from this study is that following ABMT, the DFS for patients with transformed NHL is essentially identical to the overall survival. This is analogous to that seen in patients with relapsed intermediate/high grade NHL who undergo ABMT. In contrast, patients with low grade NHL can survive for prolonged periods following relapse, in the present series from 1+ to 38+ months, with a median of 17 months.

The majority of studies employing high dose therapy and ABMT in NHL have included patients with no evidence of marrow involvement with lymphoma at harvest. Since it is clear that patients with histologically uninvolved bone marrow and peripheral blood can have residual lymphoma cells as detected by sensitive techniques molecular techniques, the reinfusion of tumor involved autologous marrow or peripheral blood it at least a theoretical concern (23-26). Four reports have attempted to deal with this problem in patients with low grade NHL. Three studies have reported the use of anti-B cell MAbs and complement in the treatment of patients with both histologically involved and uninvolved BM (4,6,14). Another approach has been to utilize autologous peripheral blood stem cells as an alternative to autologous marrow (27). These studies have not examined the influence of marrow involvement upon treatment outcome. The present study suggests that the DFS for patients with marrow involvement at harvest is significantly less than for patients with uninvolved BM. We are presently unable to determine whether relapse results from endogenous residual tumor cells or from reinfused tumor cells contaminating the bone marrow. However, since the majority of relapses occur in sites of prior bulk disease, including the BM, tumor cells reinfused with the bone marrow may not contribute to relapse. Specifically, the observation that 6 of the 8 BM relapses occurred in patients with marrow involvement at harvest might suggest that reinfused tumor cells preferentially "home" to the marrow. Alternatively the BM is a site of bulk disease where disease is not completely eradicated by the conditioning regimen.

Although longer follow-up will be necessary, the present study and others suggest that some patients with relapsed low grade NHL experience prolonged DFS following high dose therapy and ABMT. Since the long term DFS following ABMT for relapsed patients with intermediate/high grade NHL is predicted to be approximately 40%, perhaps high dose therapy may improve the DFS for patients with incurable low grade NHL. A report from Young et al. employing aggressive combination chemotherapy and total nodal radiotherapy, suggests delayed intensive treatment will lead to a lower probability of achieving CR and therefore the chance of long term cure (28).

Another approach to previously untreated patients with low grade NHL is the use of high dose therapy and ABMT in first remission. At Dana-Farber, to date, 16 patients with previously untreated stage III/IV low grade NHL who have attained a minimal disease state following 6 cycles of CHOP have undergone ABMT. To date, only one acute in-hospital toxic deaths has occurred with earlier hematologic engraftment than observed in relapsed patients undergoing ABMT. Although follow-up is limited, the remaining 15 patients remain in CCR from 1+ to 27+ months. The effectiveness of high

dose therapy in low grade lymphoma remains controversial. However, considering the very low treatment associated mortality for relapsed patients with sensitive NHL undergoing ABMT, earlier treatment may be justified for patients with incurable lymphoma. Low grade NHL may be analogous to chronic myelogenous leukemia (CML) which was deemed incurable until bone marrow transplantation was evaluated (29,30). It is generally accepted that allogeneic BMT for CML in accelerated phase or blast crisis is inferior to that seen in chronic phase. Moreover, earlier BMT for patients in chronic phase CML appears to lead to an even better DFS (31). The investigation of the role of more intensive treatment strategies for low grade NHL may demonstrate a similar finding.

ACKNOWLEDGEMENT

Supported by NIH grant CA34183, supported by PHS grant 5KO8 CA01 105 (ASF) and the Leukemia Society of America (SNR, Fellow).

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TABLE 1

Patient Characteristics

	Low Grade	Transformed
Total	51	18
Age at ABMT		
< 35	4	4
35 - 50	42	11
> 50	5	3
Sex		
male	35	11
female	16	7
Histology at Diagnosis[a]		
FSC	35	13
FM	11	5
other	5[b]	0
Histology at ABMT		
FSC	35	0
FM	14	0
FLC	0	2
DLC	0	7
DM	0	5
DSC	0	4
other	2[c]	0
Hx of BM Involvement	39	12

a FSC - follicular small cleaved cell WF-A
FM - follicular mixed small cleaved and large cell WF-B
DIL - diffuse intermediate lymphocytic lymphoma
DLC - diffuse large cell WF-G
DM - diffuse mixed small-large cell WF-F
DSC - diffuse small cleaved cell WF-E
FLC - follicular predominant large cell WF-D
SL - small lymphocytic WF-C

b DLC-2; DSC-1; DIL-1; SL-1.

c SL-1; DIL-1.

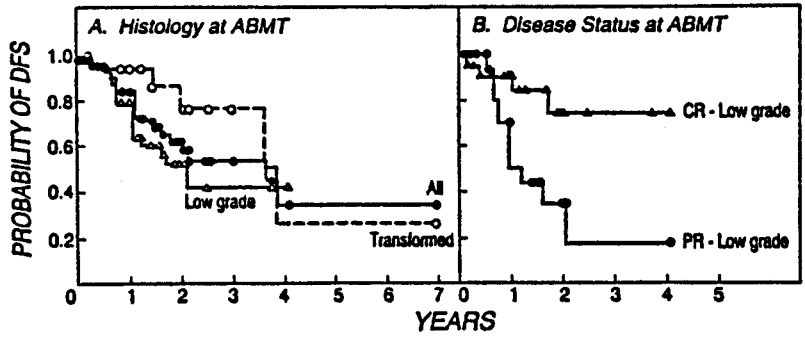
TABLE 2

	Prior Therapy	
	Low	Transformed
Total	51	18
Prior Therapy		
Chemotherapy	51	18
Local Radiotherapy	16	4
TBI	1	1
Prior Response		
PR	15	4
CR	36	14
Status at ABMT		
PR	31	8
CR	20	10
BM involvement at ABMT		
undetected	27	12
$\leq 5\%$	19	5
6 to $\leq 20\%$	5	1

TABLE 3

	Clinical Outcome	
	Low	Transformed
Total	51	18
Treatment associated deaths	2	1
CCR	32	12
Relapse	18	5
Relapse (alive)	13	1
Sites of relapse		
Prev. Site	14	4
Prev. + New	3	0
New	1	1
BM Relapse (total)	7	1
BM + at Harvest	5	1
BM - at Harvest	2	0

FIGURE 1



ABLATIVE THERAPY WITH AUTOLOGOUS BONE MARROW TRANSPLANTATION AS CONSOLIDATION OF REMISSION IN PATIENTS WITH FOLLICULAR LYMPHOMA

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SUMMARY

Since June 1985, 38 patients (age range 29-61, median 43 years) with follicular lymphoma and no evidence of transformation to high grade histology have received myeloablative therapy (Cyclophosphamide 60mg/kg x 2 and total body irradiation 2OOcGy x 6) with autologous bone marrow transplantation (CY + TBI + ABMT). The marrow mononuclear cell fraction has been treated in vitro with 3 cycles of the monoclonal antibody anti-CD20 and baby rabbit complement. At the time of treatment, 22 patients were in complete remission, residual disease was present in 16 (lymph nodes < 2cm diameter: 8; < 10% bone marrow infiltration: 4; lymph nodes and bone marrow: 1; other sites: 3. Twenty-nine patients were in second remission, 6 in third, and 3 in > third, respectively.

Thirty-two patients are alive, six have died (2 treatment related deaths, 2 following recurrence, 1 from secondary acute myelogenous leukaemia, 1 from an unrelated cause). Twenty five patients continue in remission between 3 months and 5 years (median follow up 2 years). Recurrence has occurred in 9, with transformation to high grade histology in 3. It remains to be established whether such intensive consolidation therapy prolongs survival.

INTRODUCTION

Despite sensitivity to both chemotherapy and radiotherapy, follicular lymphoma remains almost universally fatal with conventional treatment (1-4). In view of the encouraging results with very intensive therapy supported by ABMT in a proportion of patients with high grade lymphoma in whom conventional therapy has failed (5-11), this approach is currently being evaluated as consolidation of second or subsequent remission in patients with follicular lymphoma in the hope of prolonging remission duration and hence survival. In view of the frequency of bone marrow involvement in follicular

lymphoma, the marrow is being treated in vitro with a monoclonal antibody directed against the CD20 (B1) antigen and rabbit complement.

PATIENTS AND METHODS

Patients

The patient's clinical characteristics at the time of receiving CY + TBI + ABMT are shown in Table 1. All had received several different previous treatments (median 3, range 2-7). In 13/38 patients, more than 1 treatment was required to achieve complete remission or a "minimal disease state" prior to administering CY + TBI + ABMT, 15 patients required an Adriamycin containing regimen.

Reactivity with anti-CD20 was demonstrated by indirect immunofluorescence and flow cytometric analysis of bone marrow, or by immunoperoxidase staining of lymph nodes or other tissue in all but 2 cases where fresh tissue biopsy material was not available.

In Vitro Treatment of Marrow

Bone marrow was aspirated under general anaesthetic and the mononuclear cell fraction treated in vitro with 3 cycles of anti-CD20 (Coulter Clone anti-B1, Coulter Immunology, Hialeah, Florida) and baby rabbit complement (Pel-Freez, Wisconsin) as described by Nadler et al (12) and cryopreserved. The bone marrow was thawed and re-infused within 24 hours of the last dose of TBI.

Treatment

Cyclophosphamide 60mg/kg (with mesna) was administered as a one hour intravenous infusion on 2 consecutive days. TBI was given as 6 fractions of 200cGy each, over 3 days. Three patients who had clinical evidence of residual lymph node enlargement and one with residual skin involvement received additional radiotherapy concurrent with the TBI.

Patients remained in hospital until the absolute neutrophil count exceeded 0.5 x 10^9/l. Oral non-absorbable antibiotics (Framycetin, Nystatin and Colistin) (13) were prescribed although not always consistently taken, prophylactic Acyclovir was continued for one year. Intravenous antibiotics were commenced empirically in patients who became pyrexial (38C), the combination was altered on the basis of bacteriological isolates when appropriate. Single donor platelets were administered when the platelet count fell below 20 x 10^9/l or when clinically indicated. HLA matched donors were used if alloimmunisation was suspected or demonstrated. Cytomegalovirus (CMV) negative blood products were used in patients who were demonstrated to be CMV-ve. All blood products were irradiated with 5000cGy.

RESULTS

Clinical Toxicity

The early toxicity and late complications are shown in Table 2.

Haematological Recovery

The mean number of mononuclear cells reinfused was 2.6 x 10^7/kg (range 1.1 - 6.9 x 10^7/kg). The mean time to engraftment was 27 days (median 25 days, range 14-90 days) and 31 days (median 25 days, range 13 - 150 days) for neutrophils (> 0.5 x 10^9/l) and platelets (> 20 x 10^9/l) respectively. The majority of patients left hospital after 4 weeks (range 3 - 8.5 weeks). The median number of red cell and platelet transfusions required was 8 and 7 units respectively (range 2-48 and 2-54). Five patients required HLA identical platelets.

Immunological Recovery

Immunoglobulin recovery. A reduction in the level of IgG, IgM and IgA was seen in the majority of patients as shown in Table 3.

T cell recovery. A relative excess of circulating CD8+ (T suppressor) cells resulting in a decrease in the ratio of CD4+ (T helper) cells to CD8+ cells was observed for approximately one year (Table 4).

Survival

Thirty-two patients are alive, six have died; two as a consequence of the transplant procedure (1 cerebral haemorrhage at 29 days, 1 systemic fungal infection at 3 months), and two of progressive lymphoma following relapse. One patient died from secondary acute myelogenous leukaemia at 4 years, having presented 8 years prior to receiving CY + TBI + ABMT and another died from an unrelated cause.

Duration of Remission

Twenty-five patients remain in remission between 3 months and 5 years with a median follow up of 2 years. Nine have relapsed, 6 with follicular lymphoma between 3 and 24 months, 3 with transformation to high grade histology at 10, 22, and 46 months. None of the five patients who were treated at a time when the bone marrow showed "minimal residual disease" have relapsed (maximum follow-up 2 years).

DISCUSSION

At St. Bartholomew's Hospital, a conservative approach comprising observation if appropriate and short courses of Chlorambucil given to achieve clinical remission, with repeat biopsy at relapse results in a median survival of 9 years (4). In patients presenting with advanced disease, the median survival from the time of second remission is six years but decreases with each

subsequent remission. The incurability of follicular lymphoma thus justifies an experimental approach.

Recent studies suggest that the use of more intensive therapy, namely proMACE MOPP, followed by total nodal irradiation (14) and, in patients with stage III disease, sequential intensive chemotherapy and radiotherapy (15) may prolong disease free survival and overall survival respectively. In general, patients with follicular lymphoma have not been treated with myeloablative therapy requiring autologous bone marrow support because, on the one hand, of a reluctance to use treatment which has a potential mortality in patients whose survival is not necessarily immediately threatened by the disease and on the other, because of its propensity for bone marrow involvement.

The early treatment related mortality in the study to date (2/39) is lower than that reported in most series (5, 6, 8-10) and reflects the fact that patients were in remission at the time of treatment and therefore had a very good performance status. The development of myelodysplasia and secondary AML in 2 patients is of great concern and is presumably a consequence of the total amount of alkylating agent administered over a prolonged period rather than the use of CY + TBI + ABMT per se.

With a few exceptions, haematological recovery was reasonably prompt and similar to that reported for patients whose marrow had not been treated in vitro (5-10). The delayed recovery of immunoglobulins consequent upon B cell depletion of the marrow and the preponderance of CD8+ cells for a prolonged period confirms the experience of Anderson et al (16). The contribution of the in vitro treatment remains unproven though studies from St. Bartholomew's Hospital using a semi-quantitative adaptation of the polymerase chain reaction have demonstrated a 5 to 50 fold reduction in the number of t(14;18) translocation containing cells in 10/10 patients in this study (17).

In the context of the natural history of follicular lymphoma these results are obviously preliminary but the freedom from recurrence pattern seems superficially better than that seen previously at St. Bartholomew's Hospital with conventional therapy (4). However, the number of patients is relatively small and the follow up short. Moreover the patients in this study represent a selected group who received this treatment as consolidation of complete or "near complete" remission. Longer follow up will determine whether such therapy can be curative.

ACKNOWLEDGEMENTS

We are indebted to the medical and nursing staff of Annie Zunz and Dalziel wards at St. Bartholomew's Hospital for their excellent care and to the staff of the Radiotherapy Department. Anti-CD20 and complement were generously provided by Coulter Immunology, Hialeah, Florida as was an EPICS C flow cytometer. We also thank Claire Parfitt for preparing the manuscript.

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TABLE 1

Clinical Characteristics at the time of receiving Cy + TBI + ABMT

Median Age:		43 (range 28-61 years)
Remission:	2ND:	29
	3RD:	6
	>3RD:	3
Karnofsky performance status		90%-100%

TABLE 2

Toxicity and Late Complications

Mortality		Early Toxicity		Late Complications	
Cerebral Haemorrhage	1	Fever	All	Cataracts	2
Systemic Fungal Infection	1	Septicaemia	22	H. Zoster	1
		Pneumonia	3	AML	1
		Mucositis	7	RAEB	1

TABLE 3

Immunoglobulin Levels

	Time From CY + TBI + ABMT in months			
	3m	6m	9m	12m
IgA (N.R. = 0.8 - 4.0 g/l)				
Mean	1.3	1.1	1.0	1.2
Median	1.1	1.0	0.9	1.1
Range	0.3 - 3.6	0.4 - 2.9	0.3 - 2.2	0.4 - 2.5
IgG (N.R. = 7.0 - 18.0g/l)				
Mean	8.6	8.4	8.5	9.3
Median	8.8	8.8	9.2	9.3
Range	4.8 - 12.5	4.2 - 11.9	5.4 - 11.9	4.8 - 13.8
IgM (N.R. = 0.4 - 2.5g/l)				
Mean	1.3	1.2	1.0	1.1
Median	0.9	0.8	0.9	1.1
Range	0.2 - 4.4	0.2 - 5.2	0.4 - 2.1	0.4 - 1.9

TABLE 4

T Cell Recovery

Mean % Reactivity with monoclonal antibodies directed against T cell antigens

Months	1	3	6	9	12	18	24
CD3	45	27	27	30	32	24	32
CD4	17	17	16	15	18	21	26
CD8	29	27	29	29	27	23	22
CD4/ CD8 Ratio	0.6	0.5	0.7	0.8	0.8	0.9	0.9

A POSSIBLE NEW APPROACH TO THE MANAGEMENT OF FOLLICULAR NON-HODGKIN'S LYMPHOMA : EARLY AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR CONSOLIDATION IN FIRST REMISSION

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INTRODUCTION

In the past decade, several studies, including ours, have clearly established in the role of autologous bone marrow transplantation (ABMT) for consolidation of remission in patients with intermediate or high grade non Hodgkin's lymphomas (NHL) in chemosensitive relapse (1-4). For patients in first remission (CR1) some studies, including a report from the European group on a large series of 698 patients of whom 193 were autografted in CR1, have indicated long-term disease free survivals (DFS) as high as 70% at 8 years. However, the inclusion of ABMT very early in the management of the disease still remains a matter of debate in the absence of randomized studies comparing this approach to several modern front line chemotherapy regimens which currently achieve a cure rate of over 50%.

In contrast, patients with follicular lymphomas (so called low grade malignancy), although they may run an indolent course for many years in some cases, still retain a poor prognosis in the long run: 40 to 70 % of the tumors eventually transform either from a follicular to a diffuse type or from the small cleaved cell to the large cell proliferation category, both modifications resulting in a more aggressive course of the disease (5,6). Recent series indicate a median survival from 4 to 8 years, but, with a few possible exceptions for patients treated in stage I/II at presentation, the cure rate has remained below 10%. Indeed, several reports have suggested that follicular lymphomas are incurable by conventional chemotherapy.

There is very little experience with ABMT in follicular lymphomas at the present time (1,4,7-9). The possible impact of high dose consolidation therapy + ABMT applied in early CR1 is unknown.

In this paper we report on our preliminary experience in 9 patients with follicular lymphomas who were consolidated in remission by high dose chemotherapy (BEAM regimen) followed by ABMT with marrow purged by

mafosfamide, as part of a prospective trial. We also include an update on one of our earlier patients treated 11 years ago.

PATIENTS AND METHODS (See Table 1)

This report concerns 10 patients with follicular NHL autografted in remission. One patient was treated in April 1979: he received the TACC regimen and unpurged marrow. The other 9 patients were treated between July 1987 and January 1990. For these patients the median follow-up from diagnosis and from ABMT are respectively 33 (range 13 - 42) and 11.5 (range 2 - 31) months.

The median age of the population was 40 years (range 31 - 48). The sex distribution male/female was 3/7. Originally four patients had a follicular small cell (FSC) lymphoma, and 6 had a follicular mixed (FM) lymphoma.

At initial staging, only 4 patients had no detectable extranodal disease. Among the other 6, bone marrow was infiltrated in 4, liver in 2, digestive tract in 2 and skin in 1 patient. All ten patients had been previously treated by various multi-drug therapies, and 3 had surgery for tumor resection. At the time of ABMT, seven patients were in CR1, 2 in first partial remission and 1 in CR2.

Patient 1 received the TACC regimen as pre-transplant modality. All other 9 patients received the BEAM. Except for the first patient autografted in 1979, all other patients had their marrow treated in vitro with mafosfamide (CFUGM LD 95). Three patients received purged marrow with non-detectable CFUGM. Four patients received GMCSF (250 ug/m^2/day) as part of another parallel randomized study.

Following ABMT, 3 patients (1, 6, 8) received additional localized radiotherapy on previously involved abdominal fields. No other antitumor therapy was administered post ABMT. CR duration was evaluated after autografting, and total survival from initial diagnosis. The probability of remaining in remission was calculated using the Kaplan and Meier's method (10).

RESULTS

Engraftment was rapidly achieved in all patients. The median time to reach 0.5 10^9 PMN/l, 0.1 % reticulocytes and 50 10^9 platelets/l after ABMT was respectively 18 days (range 12 - 29), 17 days (range 12 - 24) and 39 days (range 10 - 90). For the 3 patients whose CFUGM were not detectable, the median time was respectively 19 days (range 12 - 29), 18 days (range 16 - 24), 23 days (range 12 - 41).

No death related toxicity was observed. Sepsis occurred in 4 patients during aplasia: bacterial gram positive septicemia bacterial gram negative septicemia. Severe fungal esophagitis. All these infections were resolutive under appropriate therapy. There was no CMV infection. Interstitial pneumonitis of undetermined origin occurred in patients 2, 4, and 5, and was

resolutive in all 3. Mucositis was observed in 2 patients. Finally, 1 patient developed a moderate spontaneously resolutive liver veno-occlusive disease. At the present time all patients are alive: of the 2 patients autografted in partial remission, both went into CR after the procedure. This includes patient 1 who was our first patient with follicular lymphoma to be autografted 11 years ago for consolidation of abdominal NHL. This patient has remained in unmaintained CR for 9 years when he relapsed with superficial nodes above the diaphragm. The other patient autografted in PR (no. 6) remains in CR1 10 months post ABMT. Of the other 8 patients autografted in CR, 1 has relapsed 2 months later (no. 9) and the other 7 have remained in persisting unmaintained remission for a median of 8 months (range 3 - 31) ; 2 patients are beyond 2 years after ABMT. Of interest is that the 2 relapses (patients 1 and 9) correspond to the only 2 patients of this series with a tumor mass greater than 10 cm in diameter at time of initial diagnosis.

DISCUSSION

In this report we present our preliminary observations of follicular lymphomas in remission treated by high dose chemotherapy consolidation followed by infusion of marrow purged in vitro by mafosfamide (CFUGM LD95). In the 10 autografted patients, none experienced severe toxicity and there was no toxic death. Of the 9 patients included since July 1987 in a prospective trial of early consolidation of remission (7 CR1, 1 PR1, 1 CR2) by the BEAM regimen, 8 remain in unmaintained CR, but the follow-up is short and only 2 patients are beyond 2 years post ABMT. That the follow-up is too short to get any reliable information in terms of antitumor efficacy, is further documented in our own institution by the unfortunate evolution of patient 1 who happened to be one of the first follicular lymphoma autografted 11 years ago. This patient was considered as cured by current standards when he presented a late relapse 9 years post- transplant. In contrast to intermediate and high grade NHL, the best therapeutic modality for low grade NHL remains to be established. With a few exceptions, patients with stage III/IV low grade follicular lymphomas, have frequent relapses, histologic conversion into a diffuse form, and eventually die from tumor. Divergent treatment approaches have been proposed varying from as little as no initial therapy and a watch and wait attitude to more aggressive strategies up to the inclusion of ABMT at some stage of the evolution, usually late in the course of the disease. It seems that initial aggressive therapy is indeed beneficial: for instance, in a randomized study comparing watchful waiting versus aggressive modality treatment in indolent lymphomas at the National Cancer Institute (11), 50% of patients randomly assigned to "watch and wait" have required introduction of therapy at 34 months and the CR rate in patients crossed over has only reached 43%. In contrast, in the group randomly assigned to receive front line chemotherapy, 78% have achieved CR and the median duration of initial remission will be longer than 4 years.

The experience of high dose consolidation with ABMT in follicular lymphomas is limited and presently concerns almost exclusively advanced disease: in a study of a series of 49 NHL treated after relapse, with cyclophosphamide and total body irradiation followed by ABMT with marrow purged in vitro with anti-B1 monoclonal antibody and complement, Takvorian et al (4) report on 6 patients with low grade disease, who behaved similarly to those with intermediate grade with an estimated 84% probability of disease-free survival at 4 years. In a series of 18 patients autografted in Omaha (26) with cyclophosphamide and TBI for failure to previous 1-4 chemotherapy regimens, 8 (42%) remain alive in continuous CR from 3 + to 18 months + post transplant. Interestingly, these 8 patients include 6/8 patients with no histologic transformation at transplant. Similarly, at Saint Bartholomew's Hospital in London, 24 of 35 patients with follicular lymphomas transplanted in or beyond CR2 remain in remission although the longest follow-up is of 4 1/2 years (12). Finally, in a series of 16 patients consisting of 9 FM and 7 FSC autografted in France with the BEAM (n = 12) or cyclophosphamide + TBI (n = 4), in or beyond CR2, 8 are alive and well 2 to 40 months after BMT and there was no toxic death (13). Our decision to establish a protocol of autograft in patients with low grade NHL in CR1 whenever possible or otherwise in PR1 arose from our wish to use maximum consolidation at a time of early chemosensitive minimal residual disease. We also chose to systematically purge the marrow in vitro in view of the reported high frequency of marrow and/or blood involvement in this particular variety of NHL (14). Indeed with no significant morbidity and no toxic death until now, we believe that our first goal to provide aggressive therapy early and nevertheless avoid unacceptable toxicity, has been achieved. With all 10 patients of this series alive at the time of writing and 8 in continuous CR 3 to 31 months post ABMT, we propose autografting with BEAM and marrow purged with mafosfamide as a feasible alternative aggressive therapy for low grade NHL as early as in CR1. The assessment of the anti-tumor efficacy of this procedure needs further studies and a longer follow up.

ACKNOWLEDGEMENTS

Authors' affiliations: (1) Service des maladies du sang Hopital Saint Antoine, Paris, France (Pr. A. Najman); (2) Formation associee Claude Bernard, Assistance Publique - Paris : Unite de Recherche sur les greffes de cellules souches hematopoietiques (Pr. N.C. Gorin); (3) Unite de cryobiologie de moelle osseuse: Fondation Centre National de Transfusion Sanguine (Dr. M. Garetta); (4) Service des maladies du sang : CHU de Lille (Pr. F. Bauters).

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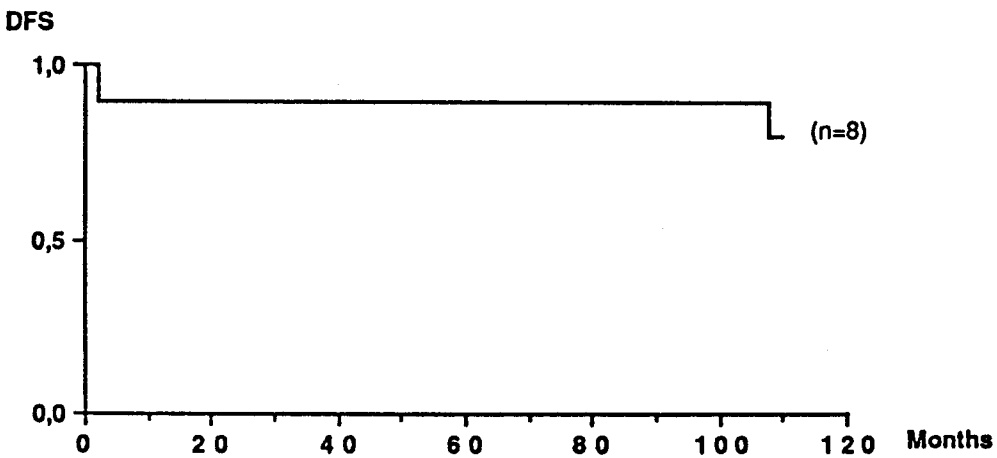
FIGURE 1
Disease free survival in follicular NHL autografted in first remission.



HIGH DOSE CHEMOTHERAPY WITH STEM CELL RESCUE FOR THE TREATMENT OF FOLLICULAR LOW GRADE NON-HODGKIN'S LYMPHOMA

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INTRODUCTION

Patients with disseminated follicular low grade non-Hodgkin's lymphoma (NHL) are very rarely cured with standard chemotherapy regimens (1-3). Although most of these tumors are initially responsive to chemotherapy, the majority will relapse and eventually result in the death of the patient. In addition, 40%-70% of the cases of low grade follicular NHL will transform to a higher grade malignancy with a much poorer prognosis (4-6). There is accumulating evidence that high dose chemotherapy with autologous bone marrow transplantation (ABMT) or peripheral stem cell transplantation (PSCT) can result in some cures for relapsed diffuse aggressive NHL (7,8). In an attempt to see if this modality of treatment could also produce long term disease free survival in patients with low grade follicular NHL, we have now utilized this treatment in 33 patients with low grade follicular NHL.

MATERIALS AND METHODS

Thirty-three patients with an original diagnosis of follicular low grade NHL have undergone high dose chemo/radiotherapy with ABMT or PSCT at the University of Nebraska Medical Center between 4/1983 and 1/1990. The characteristics of this patient population are outlined in table 1. The patients ranged in age from 27 - 53 years with a median of 40. Nineteen of the patients still had a histology that was in the low grade category at the time of transplantation (follicular mixed = 10, and follicular small cleaved = 9). Fourteen of the patients had some element of a diffuse histology at the time of transplantation. All of these patients had been treated with at least one prior doxorubicin - containing regimen, and six had also received involved field radiation therapy.

Thirty of the 33 patients had some evidence of residual disease at the time of transplantation, while 3 were in a second or subsequent complete remission (CR). In previous years, autologous bone marrow was used if it was found to be histologically negative for lymphoma. However, most recently,

autologous peripheral stem cells have been used as the rescue source in all of the patients with follicular low grade NHL due to the fear of occult lymphomatous contamination in the bone marrow.

The patients were transplanted with cyclophosphamide 60 mg/kg x 2 days and total body irradiation (TBI) 2OOcGy twice daily for 3 days if their prior radiotherapy did not preclude this treatment approach. If they were unable to receive TBI, they were prepared for transplant utilizing one of two chemotherapy only regimens: BEAC (carmustine 300 mg/m^2 x 1, etoposide 100 mg/m^2 x 8, cytarabine 100 mg/ml 8, and cyclophosphamide 35 mg/kg x 4) or BECH (carmustine 300 mg/m^2 x 1, cyclophosphamide 2.5 gm/m^2 x 2, hydroxyurea 2.0 gm/m^2 continuous infusion, and etoposide 150 mg/m^2 x 6). Following the preparative regimen and the appropriate rest period, the previously collected cryopreserved bone marrow or peripheral stem cells were thawed and reinfused per protocol.

RESULTS

Of the 33 patients transplanted for follicular low grade NHL under these protocols, 22 are progression free following the transplant with a median follow-up of 14 months. Seven of the 33 patients have relapsed from 1 - 13 months post transplant. There were four early transplant related deaths in this patient cohort. All of these deaths were secondary to overwhelming fungal sepsis or bacterial sepsis with adult respiratory distress syndrome (ARDS).

The overall survival of this patient group is 66%, with the longest patient follow-up at 83 months (Figure 1). The progression free survival is 52% in the same group of patients. When the patients are divided into those who are still in follicular phase at the time of the transplant compared to those with some diffuse component, there is a striking difference in outcome. The patients with a diffuse component have a much higher early death rate, perhaps due to the longer presence of disease with more previous chemotherapy (Figure 2). There were a few patients with a diffuse component that did have a longer progression free survival; however, none of these had a diffuse large cell component - these patients all had a diffuse mixed or diffuse small cleaved component. All of the patients with a diffuse large cell component are either on the survival curve soon after transplantation, or have died either of toxicity or recurrent disease.

DISCUSSION

Despite initial complete remissions of follicular low grade NHL, most all patients with this histology will eventually relapse and succumb to their disease. Many different treatment approaches have been used for this disease, ranging from an initial watch and wait strategy to aggressive combination chemotherapy. Initial results from a randomized trial utilizing these two approaches by Young et al (9), has shown some advantage in the disease-free survival of patients treated with the aggressive approach. In this study, patients

were randomized to an initial "watch and wait" strategy versus PROMACE-MOPP (Prednisone, Methotrexate, Doxorubicin, Cyclophosphamide, Etoposide, Mechlorethamine, vincristine, Procarbazine, and Prednisone) plus total nodal irradiation in advanced indolent lymphomas. The disease-free survival rates were significantly different between the two arms, 51% for the PROMACE-MOPP group vs. 12% for the watch and wait group at 4 years. However, this had not translated into an overall survival advantage at the time of publication. This data along with the success of high dose therapy and transplantation in diffuse aggressive lymphomas, have been the basis for the trial of high dose therapy with ABMT or PSCT in the follicular low grade lymphomas.

Our data demonstrates that patients with this type of lymphoma can successfully undergo transplantation. Although the progression free survival is of interest, much longer follow-up will be necessary to identify patients who may have been cured by this technique. This treatment is very costly - not only in terms of money, but also toxicity. For example, our series had a 12% toxic death rate in our initial 33 cases. Because patients with relapsed follicular low grade NHL may live for a number of years, these facts must be weighed prior to proceeding with this treatment. This treatment should perhaps best be utilized for patients who are initial induction failures, have relapsed disease, or are fairly young patients. It would seem that these subgroups would be best served by this treatment approach. Patients with transformed follicular lymphomas are a special group of patients. While our analysis shows that patients with transformed lymphomas may have a higher early death rate, there were some long term disease free survivors with a diffuse component other than diffuse large cell. Furthermore, other centers have not found these same results in transformed lymphoma patients.

Finally, the optimal conditioning regimen and source of stem cells have yet to be identified. Due to the radiosensitivity of the follicular low grade lymphomas, TBI has often been utilized in the conditioning regimens. However, it is unknown if TBI is a necessary component as there have been no randomized trials. Furthermore, it is unclear if the use of purged bone marrow or peripheral stem cells as the rescue product will decrease the relapse rate in these patients. since most relapses occur in sites of previous bulky disease, the rescue product contamination may not play a large role in the overall outcome. This study is very preliminary and further patient enrollment with much longer follow-up will be necessary to determine if the long term disease-free survival of patients with follicular low grade lymphoma has been altered by this treatment.

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TABLE 1

Patient Characteristics

Patients	33
Age (median)	27-53 (40)
Histology at transplant	
FSC	9
FM	10
Diffuse/ Follicular	14
Prior Therapy	
Chemo	33
Radio	6

FIGURE 1

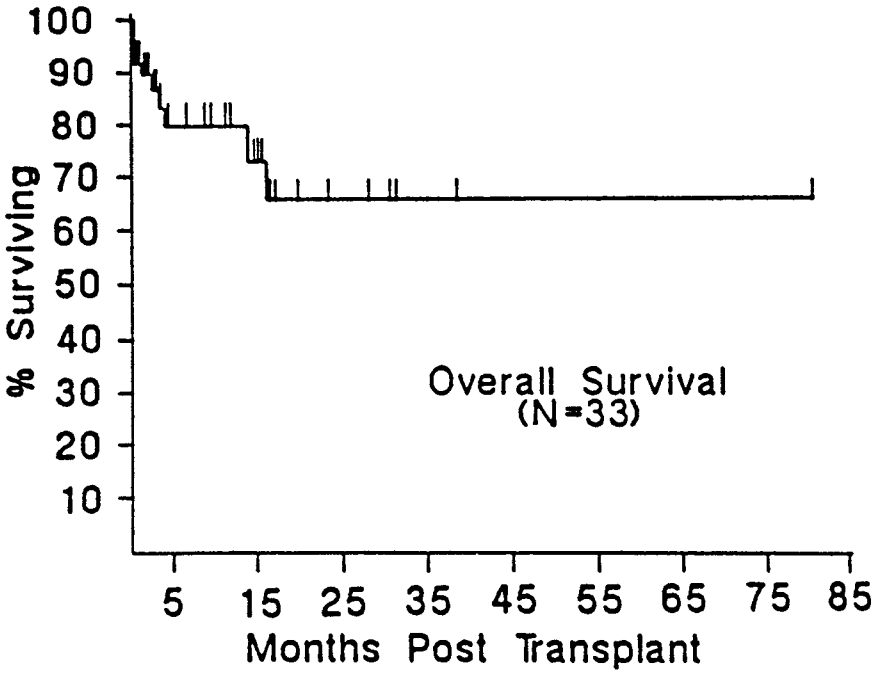
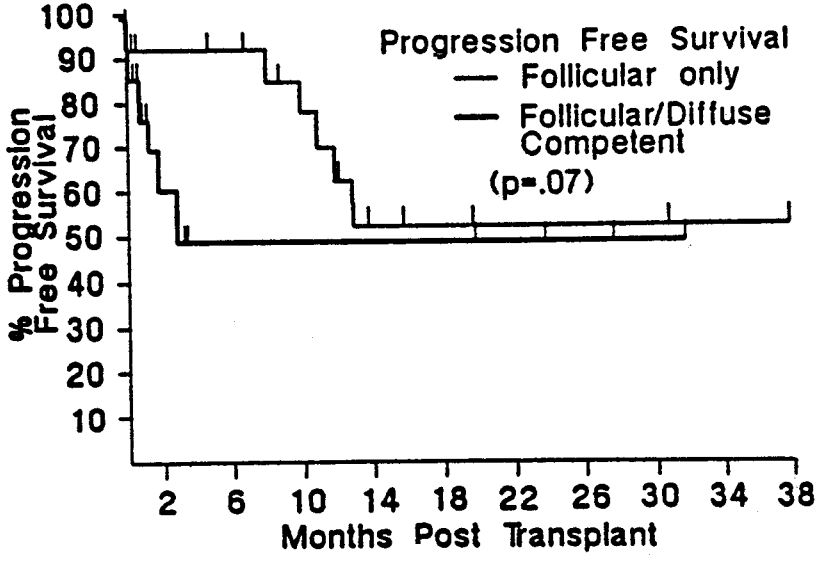

100
90
80
70
60
50
40
30
20
10
% Surviving
Overall Survival
(N=33)
5
15
25
35
45
55
65
75
85
Months Post Transplant

FIGURE 2


100
90
80
70
60
50
40
30
20
10
% Progression Free Survival
Progression Free Survival
— Follicular only
— Follicular/Diffuse Competent
(p=.07)
2
6
10
14
18
22
26
30
34
38
Months Post Transplant

MARROW TRANSPLANTATION FOR HODGKIN'S DISEASE: STUDIES WITH SEQUENTIAL TRANSPLANTATION

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INTRODUCTION

High dose chemotherapy with autologous bone marrow transplantation is now recognized as standard therapy for patients with Hodgkin's disease that has failed to respond to, or relapsed after standard chemotherapy (1). A variety of different schedules and doses have been used as the preparatory regimen for cytoreduction prior to marrow transplantation (2). A common denominator in several trials has been the use of carmustine, etoposide and cyclophosphamide (CBV) in high doses, that may or may not be myeloablative but are certainly extremely myelosuppressive (3-6). Autologous stem cells, whether derived from the bone marrow or the peripheral blood are used in conjunction with high dose therapy in order to shorten the period of myelosuppression (7). Certain patients with these high dose therapies do extremely well, and as many as 70% of patients with good prognostic variables such as minimal prior therapy, good performance status survive free of disease long term with CBV type regimens (8). Patients who have had multiple prior regimens with poor performance status, with a relapse that is resistant to subsequent standard dose therapy also do poorly with aggressive high dose therapy.

Goldie and Coldman have hypothesized that tumors that are marginally responsive to one regimen may respond better to alternating non cross resistant regimens (9). This hypothesis has been tried out in many different trials and current data favor the use of potentially non cross resistant regimens in patients with Hodgkin's disease and non-Hodgkin's lymphoma (10, 11).

In 1987 the bone marrow transplant service at New York Medical College began to evaluate the use of sequential potentially myeloablative chemotherapeutic regimens in patients with advanced, refractory or relapsed Hodgkin's disease who had a poor prognosis and compared them with concurrent patients with Hodgkin's disease with good prognostic features who were treated with a single cycle of high dose chemotherapy. The disease free survival of the 67 patients entered in these trials were compared with 24

patients who underwent a single marrow transplant for advanced, poor prognosis Hodgkin's disease and have been previously reported elsewhere (4).

PATIENTS AND METHODS

Patients with advanced Hodgkin's disease that had failed to respond to or relapsed after multiagent chemotherapy were eligible to enter these trials provided they had an adequate cardiopulmonary reserve, i.e. DLCO > 40%, EF > 40, cellular marrow and adequate hematologic parameters. Patients with marrow involvement were eligible if > 4.0×10^8 mononuclear cells/kg could be pheresed and cryopreserved. Patients with disease responsive to subsequent therapy who also exhibited prognostic features such as a minimal residual disease, good performance status and minimal prior therapy were given BCNU 400 mg/m^2, etoposide 1800 mg/m^2 and cyclophosphamide 6 gm/m^2 (BEC-2) (Figure 1) and then transplanted with autologous marrow and/or peripherally harvested, blood derived stem cells. Patients with poor prognostic features including Hodgkin's disease that did not respond to initial or subsequent standard dose chemotherapy, patients who had failed > 2 prior regimes or had bone marrow involvement were eligible to participate in sequential marrow transplant trials. Patients with poor prognostic feature were first transplanted with the BEC-2 regimen and upon blood count recovery and return of performance status and cardiopulmonary reserve to near normal, were retransplanted with one of two potentially non-cross resistant regimens which included ThioTEPA, cytosine arabinoside and vinblastine (TAVe) or ThioTEPA, mitoxantrone and carboplatinum (JM8) (TMJ).

Prior to administration of myeloablative chemotherapy, cytoreduction was attempted. Selected patients were radiated to sites of bulk disease. The exact chemotherapy used was usually decided in consultation with the referring physician.

Patients with progressive disease on the last chemotherapy regimen used were offered the sequential approach. Patients with minimal disease after an attempt at cytoreduction were treated with one cycle of myeloablative therapy. Patients with persistent disease after one transplant were offered a second bone marrow transplant. Patients without response to the first transplant were no offered retransplantation. All patients who signed a consent for transplantation were included in the analysis.

Marrow was cryopreserved at -80C using the method of Stiff et al (12). Alternatively, marrow was stored at 40C and this was the preferred method of storage for patients undergoing a marrow transplant in a short while. In patients with marrow involvement and in patients who had previous radiation therapy to the pelvis stem cells were pheresed using a Haemonetics V-50 (Haemonetics Labs, Braintree, MA) or a Cobe spectra 500 (Cobe Labs, Lakewood, Co).

Survival was calculated using the method of Kaplan and Meier (13). All data reported are for progression free survival. Survival was counted from the date of the first transplant to the date of last follow-up, death or progression.

Response criteria were used and included: Complete remission (CR): complete resolution of all physical, biochemical and roentgenographic evidence of disease for at least 100 days. Patients with roentgenographic changes suggestive of residual disease underwent biopsy in order to establish a complete remission. Partial remission: > 50% reduction in the summed products of the perpendicular diameters of the bi-dimensionally measurable lesions for > 1 month. Minor remission: a 25-49% reduction. Stable disease: < 25% change for > 3 months. Progression: increase in tumor size over previous.

Patients were given allopurinol and cotrimoxazole during chemotherapy and up to 1 day prior to marrow transplant. Cotrimoxazole was resumed once count recovery occurred. Acyclovir was given prior to and during the period of cytopenia.

Patients seronegative for cytomegalovirus (CMV) were given blood products from donors who were seronegative for CMV. All blood products were irradiated.

All patients signed informed consent approved by New York Medical College Institutional review board.

Patient Characteristics

Sixty-seven patients entered these trials. Patient characteristics are outlined in Table 1. There were 34 males and 33 females. The median Karnofsky performance status was 80 (range 30-100). The initial stage was as follows: Stage I = 1, stage II = 20, stage III = 27 and stage IV = 19. Histologically nodular sclerosis Hodgkin's disease was diagnosed in 58 patients while there were 8 patients with mixed cellularity and 1 with lymphocyte predominant Hodgkin's disease The median number of previous chemotherapy regimens was 2 (range 1-5). The median time from diagnosis to protocol entry was 12 months (range 4 months to 10 years). Twenty-one patients had good prognostic features and were offered 1 BMT only after BEC-2 therapy. Forty-six patients had poor prognostic features and were offered sequential bone marrow transplants. Twelve patients had disease which was refractory to initial chemotherapy and 34 patients had experienced a relapse refractory to subsequent chemotherapy.

RESULTS

Of the twenty-one patients with sensitive relapse of Hodgkin's disease treated with one course of high dose BEC-2, sixteen are alive and free of disease (71%) (figure 2) with follow up to 32 months. There were 2 therapy related deaths. Of the 46 patients with high risk Hodgkin's disease, 16 achieved complete remission and 12 had partial remission (figure 3). Five patients had therapy related mortality. Three patients, all responders, refused a second bone marrow transplant and 3 patients were felt to have excessive toxicity after BEC-2. Four patients are too early to have a second bone marrow transplant. Twenty-five patients underwent a second transplant: 12 using the TATe regimen and 13 with the TMJ regimen. Twelve of these 25 patients are

alive in continuous complete remission with follow up to 40 + months (figure 4). Compared with a historical group of 24 consecutive patients with Hodgkin's disease primarily in the "high risk" category (figure 5), these patients appear to be doing significantly better. Relapses among patients with high risk Hodgkin's disease treated with sequential transplants and sensitive relapse treated with BEC- 2 only were equal: 6 of 25 patients with high risk disease relapsed as opposed to 4 of 21 patients with sensitive relapse (p=ns). Of note patients with refractory relapse of Hodgkin's disease undergoing sequential transplants have significantly fewer relapses compared to sensitive relapse of Hodgkin's disease: 1 of 11 vs. 4 of 21 ($p < .05$). The overall progression free Kaplan-Meier survival estimate for patients undergoing sequential transplantation was similar to the low risk sensitive relapse category.

Seven patients had overt evidence of bone marrow involvement and 2 patient had been radiated to the pelvis. These 9 patients had peripheral stem cells as the sole source for hematopoietic reconstitution. Forty-three patients had cryopreserved marrow and 15 patients had marrow stored in the liquid phase (40C). Five of these patients had both marrow and peripheral stem cells. The time to white blood count recovery was significantly faster in patients receiving peripheral stem cells alone or in combination with bone marrow compared to patients receiving bone marrow alone: 12 days VS. 17 days ($p < .05$). The median number of red cell transfusions used following BEC-2 therapy was 8 (1-56) and a median of 41 (0-202) units of platelets were transfused.

The major toxicities encountered with BEC-2 chemotherapy are listed in Table 2. The mucositis noted with BEC-2 therapy was severe enough to require morphine sulfate injections in 20 of 67 patients. Sixteen patients required > 1 day of morphine sulfate. Probanthine appeared to reduce the severity of mucositis significantly. Thirty-six patients had a doubling in serum bilirubin or other hepatic function tests. Only 2 patients had a bilirubin > 8 mg/dl, and 1 expired due to hepatic toxicity. Twenty- one patients experienced interstitial pneumonitis or an asymptomatic decrease in diffusion capacity. This was generally steroid responsive but caused mortality in 2 patients. Eight patients experienced hemorrhagic cystitis. This was generally managed with hydration but occasional patients required invasive procedures including instillation of dilute formalin. Two patients both with poor performance status died prior to bone marrow transplantation but were included in the overall survival calculations since they had signed consent.

Twelve patients were treated with TAVe. All experienced grade IV mucositis. Severe myalgias occurred in 9 and were accompanied with ileus in 7 and colitis in 5 patients. Two patients required surgery for signs of peritonitis. Eight patients had a chemical hepatitis. Four patients receiving 3 to 6 gm/m^2 of Ara-C in combination with ThioTEPA developed signs of cerebellar dysfunction including dysdiodokinesis, past pointing and intention tremors. Ara-C was not given to any other patients. Hemorrhagic cystitis occurred in 4 patients and required invasive procedures in 1. Of note none of

these 4 patients had experienced hemorrhagic cystitis with cyclophosphamide in the BEC-2 regimen. Five patients died following TAVe therapy.

Thirteen patients were treated with TMJ. This combination was much better tolerated. six experienced grade IV mucositis, 3 had severe hepatitis, and 2 experienced colitis secondary to clostridium difficile. There were no instances of myalgias, ileus or CNS dysfunction. There were 2 early deaths: 1 due to hepatic failure and 1 secondary to an intracranial bleed.

Patients receiving BEC-2 only had a mortality rate similar to those receiving sequential BMT with BEC-2 and TMJ: 7/67 vs. 2/13 ($p=ns$). Patients receiving TAVe after BEC-2 did worse in this regard: There were 5 episodes of toxic death ($p<.007$).

DISCUSSION

While Hodgkin's disease is a chemotherapeutically responsive illness, at least 15% of patients never enter a complete remission with initial therapy and another 15-20% relapse after primary therapy (14, 15). While some subgroups can be salvaged successfully with standard doses of chemotherapy, high dose chemotherapy is often necessary to help achieve long term survival. Even in patients treated with high dose chemotherapy, however, success is not guaranteed. Fifty percent of patients relapse and die as a result of their disease following this type of therapy. Also, the various high dose chemotherapeutic regimens that have been employed thus far have side effects and innate toxic mortality. At New York Medical College we have attempted to identify risk groups in patients who have failed standard dose chemotherapy and/or radiotherapy. Patients with standard risk of relapse after high dose chemotherapy with carmustine, cyclophosphamide, and etoposide. Patients at high risk of relapse, i.e., those with primary refractory Hodgkin's disease or with a refractory relapse of Hodgkin's disease, are treated with alternating potentially non-cross resistant high dose chemotherapeutic regimens in conjunction with bone marrow transplantation in an attempt to reduce relapse and improve disease-free survival. Since standard regimens used in bone marrow transplantation are not effective for the majority of patients with refractory relapse or primary refractory Hodgkin's disease, sequential transplantation is worthy of study. Our data would indicate that regimens such as TMJ are relatively well tolerated and in fact reduce the relapse rate in such patients.

In patients with overt marrow involvement at the time of the first transplant, sequential transplants can be performed using autologous peripheral stem cells. Patients receiving peripheral stem cells alone or in combination with bone marrow had a significantly faster count recovery, requiring fewer transfusions and had a shorter length of hospital stay. These stem cells were collected in the unperturbed state. Whether this is superior to stem cells harvested after stimulation by growth factors (16) or when counts are recovering following chemotherapy (17) is as yet unclear and will require a randomized prospective study. While we did not use growth factors in our

study, others have shown that GM-CSF or G-CSF can reduce the time to count recovery. Using unperturbed peripheral stem cells we have seen count recovery within 10 days. since the Reed-Sternberg cell expresses GM-CSF receptor, one would intuitively be concerned about potentially stimulating residual disease.

Hemorrhagic cystitis can generally be prevented with hydration and mesna (18). A surprising finding was one of hemorrhagic cystitis after ThioTEPA. Whether this was related to viral infection or a second insult with an alkylator is unclear. Nevertheless it was successfully prevented by using mesna with ThioTEPA.

Ara-C has been associated with CNS dysfunction especially when used at doses of 36 gm/m^2 over 6 days (19). In combination with high dose ThioTEPA one or two doses of high dose Ara-C 3 gm/m^2 resulted in significant neurologic toxicity.

BCNU has been associated with pneumonitis in several studies. This phenomenon is dose related with significantly greater pulmonary toxicity at doses > 450 mg/m^2 (6, 20). With the recent report of pulmonary toxicity due to BCNU becoming apparent 17 years after exposure (21), future programs should seek to limit the use of this agent. Regimens such as TMJ may be explored as front line therapy for the future.

While ideally one should only transplant patients with good performance status early in the course of their disease, in reality this is not always feasible. For patients who are at high risk of relapse following high dose chemotherapy and ABMT, sequential marrow transplantation presents a viable alternative.

ACKNOWLEDGEMENTS

The authors wish to thank the nursing and medical house staff of 7 South, Westchester County Medical Center and Ms. Ann Fulgum for secretarial assistance.

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TABLE 1

PATIENT CHARACTERISTICS

# ENTERED	67
# MALES	34
KPS	80 (30-100)
PRIOR DRUGS	8 (4-15)
PRIOR REGIMENS	2 (1-5)
SENSITIVE RELAPSE	21
PRIMARY REFRACTORY	12
REFRACTORY RELAPSE	34
INITIAL STAGE:	
STAGE I	1
STAGE II	20
STAGE III	27
STAGE IV	19
HISTOLOGY:	
LP	1
NS	58
MC	8
PRIOR DISEASE INVOLVEMENT:	
NODES	52
LUNG	18
MARROW/BONE	12
SPLEEN	7
LIVER	6
CNS/CORD	4
CHEST WALL	1

TABLE 2

	TOXICITY	
BEC-2		
	MUCOSITIS	58
	HEMORRHAGIC CYSTITIS	8
	COLITIS	6
	HEPATITIS	36
	PNEUMONITIS	21
	POSITIVE BLOOD CULTURE	18
	TOXIC DEATH	5
	PRE BMT DEATH	2
TAVe		
	GR IV MUCOSITIS	12
	MYALGIAS	9
	HEPATITIS	8
	CYSTITIS	4
	CNS DYSFUNCTION	4
	PNEUMONITIS	4
	ILEUS	7
	COLITIS	5
	TOXIC DEATH	5
TMJ		
	GR IV MUCOSITIS	6
	HEPATITIS	3
	COLITIS	2
	CYSTITIS	1
	CNS DYSFUNCTION	0
	ILEUS	0
	TOXIC DEATH	2

FIGURE 1
Not submitted.

FIGURE 2

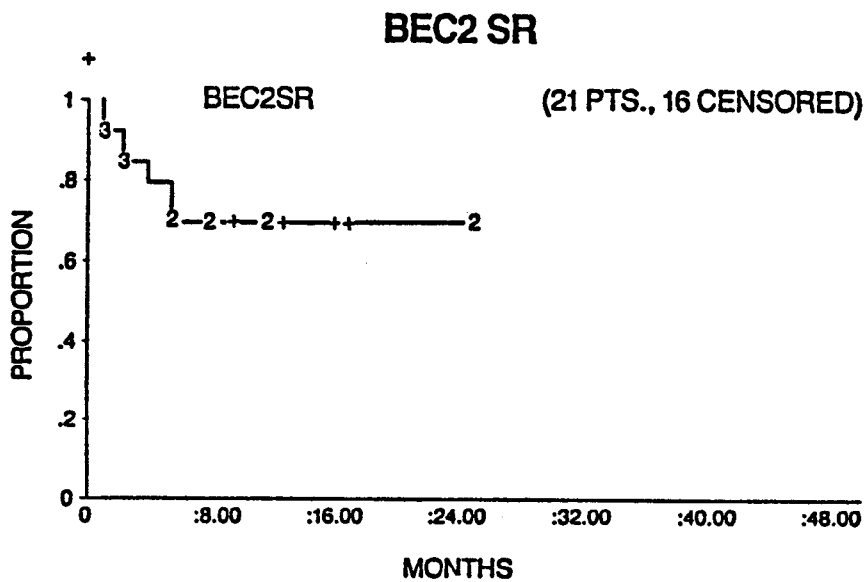


FIGURE 3

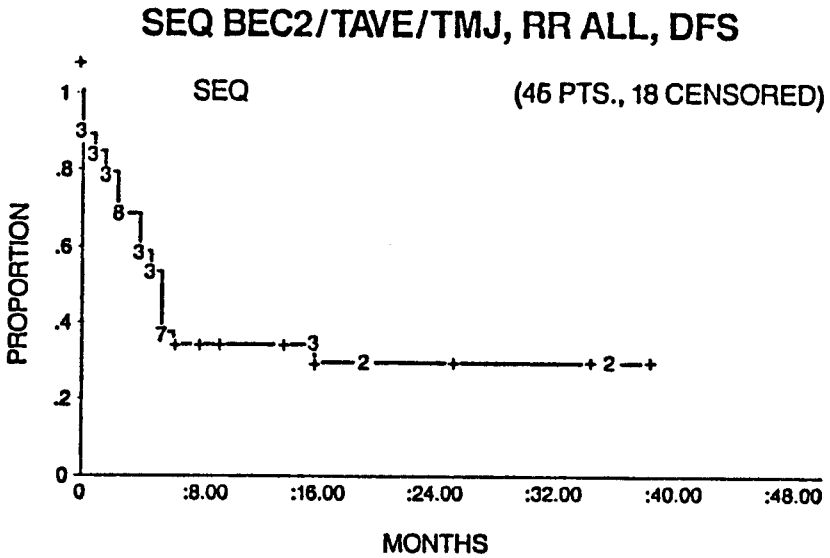


FIGURE 4

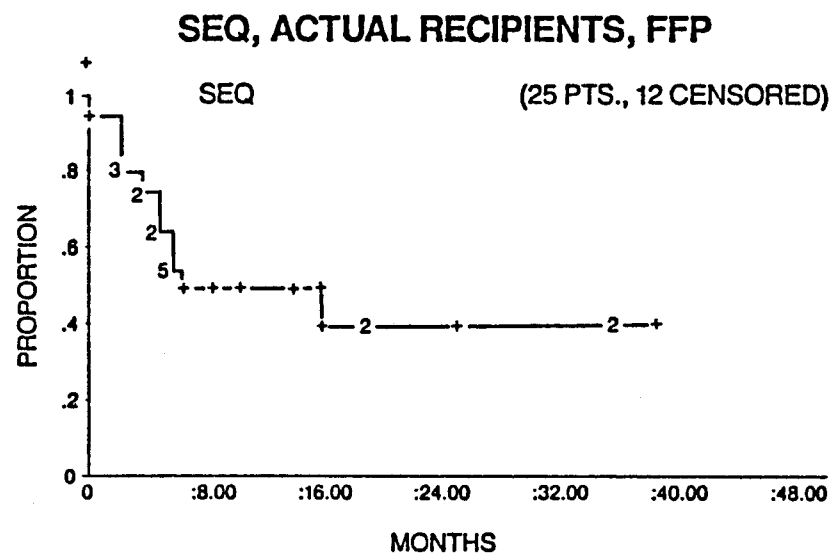
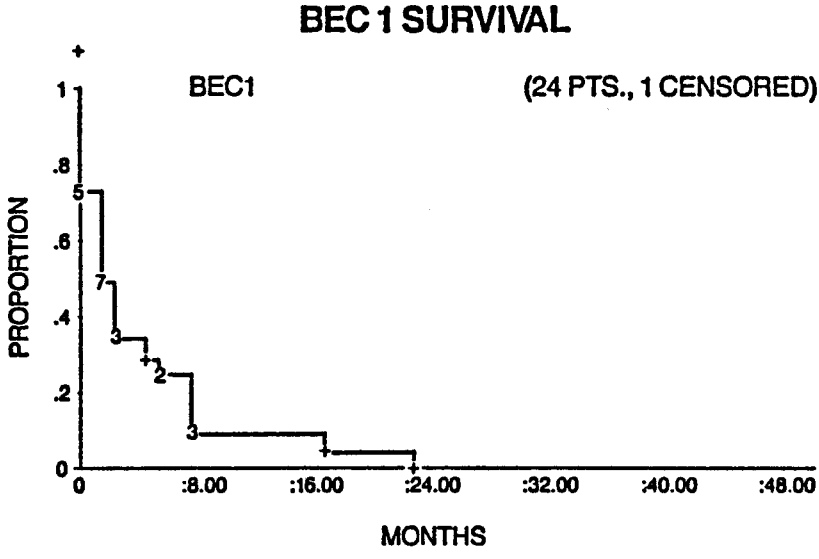


FIGURE 5


BEC 1 SURVIVAL
BEC1
(24 PTS., 1 CENSORED)
PROPORTION
1
.8
.6
.4
.2
0
5
7
3
2
3
0
:8.00
:16.00
:24.00
:32.00
:40.00
:48.00
MONTHS

AUGMENTED CBV REGIMENS AND AUTOLOGOUS BONE MARROW TRANSPLANTATION IN HODGKIN'S DISEASE

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SUMMARY

A total of 162 Hodgkin's disease patients, incurable with conventional therapy, were treated on one of three sequential protocols using the "backbone" of augmented doses of "CBV" (i.e., cyclophosphamide [C] 7.2 g/m^2, BCNU [B] 0.5-0.6 g/m^2 and VP16-213 [V] 2.4 g/m^2) before reinfusion of previously-harvested and cryopreserved but otherwise unmanipulated autologous bone marrow (or in 2 cases, peripheral blood) reinfusions. Some patients received conventional chemotherapy and/or local radiation (L-RT) before augmented CBV for cytoreduction, but not as a test for chemosensitivity; patients so treated were entered on study at the time of such therapy and all were subsequently transplanted.

In all three studies, CR/PR rates of 80-90% were observed; the toxic death rate was -20% in the first study but 6% and 3%, respectively, in the subsequent two. The progression-free survival for patients in these studies was 48% (median follow-up 3.0 years), 61% (median follow-up is -2 years), and 76% (median follow-up 1.0 years), respectively.

We conclude that the original augmented CBV regimen and the modifications we explored are active in progressive HD compared with the parent regimen; the modifications appear to have decreased toxicity, with no obvious loss of antitumor activity.

BACKGROUND AND RATIONALE

Previous studies using chemoradiotherapeutic conditioning regimens (usually cyclophosphamide and total body irradiation [CY+TBI]) before

autologous bone marrow transplants (AUBMT) for patients with advanced Hodgkin's disease (HD) have produced a finite cure rate, even in some patients with "refractory" disease. However, the use of conditioning regimens including TBI at doses -1000 cGy is limited by an excessive rate of pulmonary regimen-related toxicity (RRT) -- especially in patients previously given thoracic radiotherapy (1). Since a substantial fraction of patients with Hodgkin's disease receive mediastinal irradiation as part of primary (or occasionally secondary) therapy, this is a major limitation to the use of CY+TBI conditioning. Accordingly, investigators from Houston (2) had previously reported results using conditioning without TBI, namely "CBV" (total cumulative doses: C = 6.0 mg/m^2, B = 300 mg/m^2, V = 600-750 mg/m^2). Most failures were due to progression and not toxicity; since each of the components of CBV could be given in higher doses as single agents (3) than were given in this report, an augmented regimen was chosen for evaluation for patients with progressive Hodgkin's disease. Although a formal dose escalation phase of this combination was not undertaken, there was previous information regarding the use of cyclophosphamide and VP16-213 (4) and we have had prior experience using BCNU at escalated doses, both alone (5) and in combination (6).

Also, it should be emphasized that the use of augmented CBV was closely correlated with efforts to treat patients who had not been extensively exposed to standard chemo- or radiotherapy. However, no patient was excluded from any of the studies noted below for this reason.

MATERIALS AND METHODS

During the years 1985-1990, three protocols have been used; they differed only in the conditioning regimen used. The first contained augmented CBV as illustrated in Figure 1A; the second used infusional VP16-213 (CBVI) at an identical dose given at a rate of 70 mg/m^2 per hour (Figure IB), and the final used CBVI plus a decreased dose of BCNU (to 500 mg/m^2) and the addition of cisplatin (50 mg/m^2 daily x 3) (CBVIP) (Figure 1C).

For patients anticipated to have residual chemosensitivity and/or relatively localized disease amenable to radiation therapy, such therapy was given with "British MOPP" (MVPP) in conventional doses (7) for a median of two cycles. Also, patients were eligible to receive L-RT (total cumulative doses of 1500-3000 cGy) to areas of bulk disease. It is important to re-emphasize that neither the MVPP nor the radiation therapy were used to "test" patients for sensitivity to either of these agents; patients were not routinely restaged after these therapies, and all went on to AUBMT.

Bone marrow was harvested from the iliac crests in most cases, although 2 patients had peripheral blood stem cells collected (and eventually reinfused) when the iliac crest sites were not suitable for harvest. Marrow was routinely cryopreserved but otherwise not purified (i.e., "purged") of potentially-contaminating malignant HD stem cells.

Certain patient characteristics are listed in Table 1; of particular emphasis is the fact that all patients were deemed incurable with conventional therapies for HD. All patients were re-staged, underwent routine organ toxicity screens, and gave informed consent as per each center's institutional review board.

RESULTS

Augmented CBV

Between March 1985 and December 1987, 56 patients with advanced Hodgkin's disease received augmented CBV as detailed in Figure 1A (8). Results are as indicated in Table 2; the complete remission (CR) rate was 80%, with an additional 4% partial remissions (PR); the PR patients had ongoing regression when they died of toxicity. These facts, plus the occurrence of 9% early (i.e., pancytopenic) deaths before day +30, revealed that only 7% of patients were true non-responders. Actuarial freedom from progression was 48% at a median follow-up of 3 years (range, 2.0 to 4.5). The cumulative progression rate was 40%.

The toxic death rate was 21%, due to bacteremia (presumably related to the severe mucositis universally observed in these patients) as well as pulmonary regimen-related toxicity (RRT) presumed due to the BCNU; unlike previous interim analyses, our final analysis suggests that pulmonary RRT appeared more commonly in patients previously given mediastinal radiotherapy. There were no deaths due to prolonged myelosuppression (i.e., absolute neutrophil count [ANC] $< 0.5 \times 10^9/L >$ day +30). In our evaluation of prognostic factors associated with progression-free survival, only the presence of "B" symptoms at study entry was found to be significant.

We concluded that the augmented CBV regimen was very active, but also toxic. Therefore, both of the subsequent studies noted below have emphasized toxicity reduction.

Augmented CBV with Infusional VP16-213 (CBvi)

Between January 1987 and December 1989, a total of 72 patients were transplanted using this protocol, which altered the method of VP16-213 administration; VP16-213 was given as a -34-hour infusion at a dose rate of 70 mg/m^2 instead of the twice-daily x 6 schedule used in the parent regimen. As previously reported (9), results in this study were similar; a CR/PR rate of 72% and 21% was noted. Eighteen patients progressed; 11 died of toxicity, including 4 due to bacteremia and 1 due to pulmonary RRT. Of interest was the striking reduction of the severe mucositis noted from the parent regimen and the possible improvement in progression-free survival compared to the original study.

Augmented CBV with Infusional VP16-213, Decreased BCNU Dose and the Addition of Cisplatin (CBViP)

The above study was initiated in an attempt to further reduce the toxicity of the primary regimen, both by using the continuous infusion

VP16-213 of CBVI and by a 100 mg (16%) reduction in BCNU dose. However, because we were concerned about the possibility of compromising antitumor results by these modifications, we added conventional doses of cisplatin, an appealing agent in this setting due to both putative synergy with VP16-213 and activity as a single agent in advanced Hodgkin's disease. As of 01 August 1990, we have entered a total of 34 patients on this study; results are preliminary (10). However, 81% of patients attained CR and 6% PR, with 9% non-responders. To date, the cumulative incidence of progression is 24% at a median follow-up of 1 year; this compares favorably with the relapse rate in the initial study, although again it must be emphasized that it is possible that the more recent group was more favorable (as defined by disease status) and that the follow-up is shorter. There have been only 2 treatment-related deaths, although non-fatal pulmonary RRT is still seen with roughly the same frequency. Therefore, this regimen may be producing less fatal toxicity, although relapse continues to be a problem.

Hematologic Recovery

Assessment of hematopoietic recovery was not a major goal of these studies; however, only 11 of 162 patients (7%) died of pancytopenic complications, and most patients had ANC recovery prior to day +21.

CONCLUSIONS

We have now explored two modifications of augmented doses of CBV. CBvi clearly reduces morbidity of the parent regimen (mainly mucositis), and perhaps mortality. This modification, plus the BCNU dose decrement in CBVIP, appears to have reduced fatal RRT -- despite the addition of cisplatin. The effect of these measures on progression-free survival is uncertain and further follow-up will be required.

ACKNOWLEDGEMENTS

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TABLE 1

PATIENT, DISEASE AND TREATMENT VARIABLES

	CBV	CBVi	CBViP
N	56	72	34
Age (years)			
Median	27	28	29
Range	13-56	14-27	13-51
Disease Status (%)			
Induction Failure	27	21	21
Untreated Relapse	57	22	79
Sensitive Relapse	2	43	-
Resistant Relapse	16	14	-
Histology (%)			
Nodular Sclerosing	82	90	82
Mixed Cellularity	18	8	18
Other	-	1	-
Prior Therapy (%)			
Exposure to < 7-8 drugs	7	3	12
L-RT	62	63	32

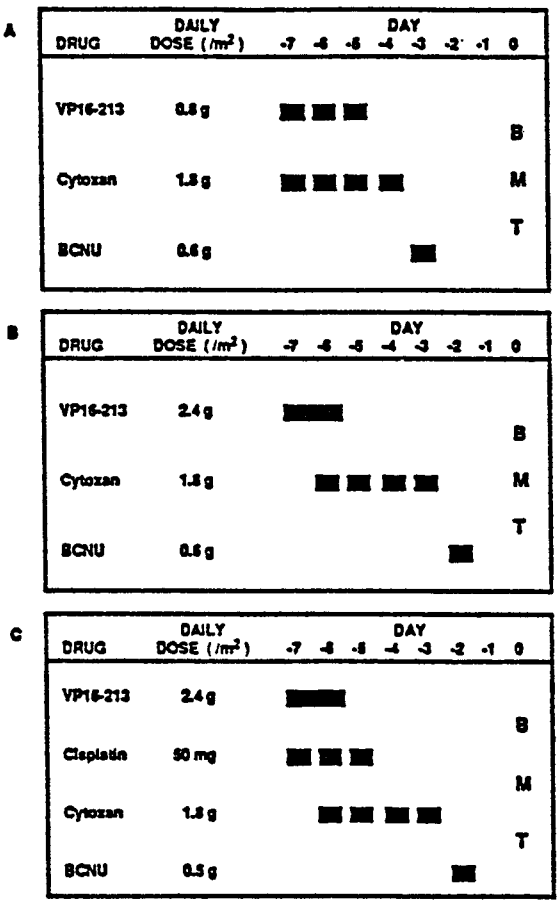
TABLE 2

RESULTS

	CBV	CBVi	CBViP
ANTITUMOR RESULTS (%)			
Complete Remission	80	72	81
Partial Remission	4	21	6
Progression-free Survival	48	61	77
Treatment-related Deaths	21	8	6
TREATMENT-RELATED DEATHS (%)			
Sepsis	13	6	-
Idiopathic IP	9	1	-
Cardiac Necrosis	-	6	3
VOD	-	3	-
Other	-	-	3
Total	22	15	6
PROGNOSTIC FACTORS FOR PROGRESSION-FREE SURVIVAL	"B" Symptoms	Disease Status	N/A

FIGURE 1

(A) Augmented CBV conditioning regimen;
(B) Augmented CBVI conditioning regimen;
(C) Augmented CBVIP conditioning regimen.



NINE YEARS' EXPERIENCE WITH ABMT IN 128 PATIENTS WITH HODGKIN'S DISEASE: AN ITALIAN STUDY GROUP REPORT

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ABSTRACT

One-hundred, twenty-eight patients with Hodgkin's disease in remission or who had failed a mechlorethamine, vincristine, procarbazine and prednisone (MOPP), a doxorubicin, bleomycin, vinblastine and dacarbazine (ABVD) and/or lomustine, etoposide and prednimustine (CEP) regimens have been treated with a high-dose therapy (HDT) containing cyclophosphamide, etoposide, carmustine (CVB) and autologous bone marrow transplantation (ABMT).

Forty patients were treated while they were in resistant or progressive disease states using alternating MOPP/ABVD protocol; 15 patients received ABMT in first relapse; 51 patients had a complete remission (CR) with first-line therapy but later relapsed and then received conventional salvage therapy; 16 achieved no response or progression ("resistant relapse" patients) and 35 responded partially or completely ("sensitive-relapse" patients). The other 22 patients received ABMT in remission.

Following HDT, 56 patients (52.8%) achieved CR and 23 patients (21.6%) achieved a partial remission for an overall response rate of 74.4%. Sixteen patients failed to respond and died in progressive disease 1 to 10 months (median 6 months) after ABMT. High-dose therapy produced severe toxicity including vomiting (100%), mucositis (75%) and liver enzymes and alkaline phosphatase elevations (51%). There were 10 treatment-related deaths. A multivariate analysis identified poor performance status and resistant-relapse patients as very important adverse risk factors for survival immediately after ABMT. These results, while validating this procedure for inducing remissions in advanced highly-treated patients, at the same time confirm the need of employing this approach in first relapse or in second complete remission after standard therapy and before ABMT or, in first complete remission in very high

risk Hodgkin's disease patients. Our experience in 15 very poor prognosis Hodgkin's disease patients transplanted in first CR is very interesting.

INTRODUCTION

There has been a radical change in the outcome of patients with untreated, advanced stage Hodgkin's disease. However, patients who fail a first-line chemotherapy regimen do not have a favorable outlook, particularly for patients resistant to MOPP/ABVD protocol. Because of the poor results with conventional salvage therapies, clinical trials were begun to evaluate the appropriate place for HDT and ABMT. This approach offers one way to circumvent treatment resistance by increasing the dose of available cytotoxic agents and radiotherapy while ameliorating myelo-toxicity by infusion of hematopoietic stem cells. In this report several Italian investigators have pooled their data on the use of HDT and ABMT in the treatment of refractory or relapsed Hodgkin's disease.

MATERIAL AND METHODS

Patient Characteristics

Between July 1981 and July 1990, 128 patients with Hodgkin's disease were treated with HDT and ABMT. The patients' clinical characteristics are shown in Table 1. There were 86 males and 42 females with a median age of 25 years (range, 11 to 51 years). Nodular sclerosis histology was the most prevalent subtype (78 patients). Eight-two patients had stage IV and the majority of patients had B symptoms (89 patients). Fifteen patients received ABMT in first relapse after receiving the alternating MOPP/ABVD protocol. Forty patients had progressive disease while receiving either the alternating MOPP/ABVD plus radiation protocol or MOPP followed by ABVD and CEP protocols. Fifty-one patients had recurrent disease after complete remission (16 patients began "resistant relapse" after salvage chemotherapy and 35 patients were "sensitive relapse").

Twenty-two patients received ABMT in remission: seven patients in 2nd or 3rd, and fifteen patients with very poor prognosis in first complete remission.

Supportive Care and Treatment Protocols

In all patients, a central venous catheter was inserted 12 hours before HDT. The patients were maintained in single rooms, received prophylactic allopurinol, antiemetics, and oral non-adsorbable antibiotics and antimycotics, and were administered intravenous (IV) hydration until day 5 post-transplantation. Urine output was maintained at 150 mL/h. In sixty patients, the HDC consisted of a modified regimen of cyclophosphamide (1.5 g/m^2/d for four days), etoposide (150 mg/m^2/d for four days), and carmustine (BCNU) (150 mg/m^2/d for four days) (1). The marrow was reinfused 48 hours after this therapy. Subsequently, we decided to increase the doses of BCNU (200

mg/m^2/d) and etoposide (250 mg/m^2/d for four days) in the other fifty-three patients (CVB-2 Protocols). For patients in first complete remission (15 patients), the doses of cyclophosphamide and etoposide were the same of CVB-2 protocols but BCNU was reduced to 150 mg/m^2/d for three days.

Evaluation and Statistical Analysis

Patients who did not achieve 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 then classified according to their responsiveness to this protocol as having had either "resistant-relapse" when no response or disease progression were observed immediately before HDT/ABMT, or "sensitive-relapse" when at least a partial response was observed immediately before transplantation (2). Before HDT, all patients were evaluated by means of physical examination, blood-chemistry profile, peripheral blood count, chest radiography, chest and/or abdominal computed tomographic (CT) scanning and bilateral bone marrow biopsies. Follow-up restaging was carried out every 2 to 4 months after transplantation or as clinically indicated. Complete remission was defined as the disappearance of clinical and radiological evidence of Hodgkin's disease. Partial remission was defined as a reduction of 50% or more in measurable disease for at least 1 month. Complete remission patients did not receive 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 July 1990.

RESULTS

Tumor Response and Survival

The outcome of HDT in relation to the patients' characteristics before ABMT is summarized in Table 2. Fifty-six patients achieved CR (52.8%) with a median duration of 32 months and 23 patients achieved PR (21.6%) with a median duration of 9 months, for an overall response rate of 74.4%. Twenty-eight of 56 complete responders subsequently relapsed 5 to 34 months (median, 10 months) post therapy. Sixteen patients failed to respond and died in progressive disease 1 to 10 months (median, 6 months) post ABMT.

Table 2 outlines the results obtained with HDT in patients of different categories. Nine of 15 patients received transplantation during a first relapse and achieved complete remission; two patients achieved a partial remission for an overall response rate of 73.3%. Five of nine complete remission patients are disease-free at a median of 36 months post-transplantation. Forty patients, who never achieved a prior complete remission in the course of their disease, underwent transplantation while their disease was progressive during alternating MOPP/ABVD protocol alone or salvage therapy. Eighteen patients (45%) obtained a CR and nine achieving a partial remission: Eight of 18 complete responders are alive and well 19 to 82 months (median, 46 months) post-

transplantation. Four of 16 (25%) "resistant-relapse" patients and twenty-five of 35 (69.4%) "sensitive-relapse" patients achieved complete remission after transplantation.

The analysis of the progression sites showed that 82% of relapses occurred primarily at the initial site of disease, whereas only 18% occurred in other sites (none in the marrow).

Thirteen of 15 (86.6%) patients transplanted in first CR remain alive in unmaintained CR at a median time of 28 months (range, 12 to 64 months). In the other two patients, reasons for failure included relapse of Hodgkin's Disease (one patient) or death due to interstitial pneumonitis.

Toxicity

High-dose therapy produced significant neutropenia and thrombocytopenia in these heavily pretreated patients. All patients had a WBC count $< 0.5 \times 10^9$/L for a median of 16 days (range, 9 to 33 days) and platelet count $< 10 \times 10^9$/L for a median of 18 days (range, 13 to 57 days). Fever during neutropenia, which warranted IV antibiotic and antimycotic therapy, was present in all patients.

There were ten treatment-related deaths: five patients died of interstitial pneumonitis, four patients of cardiac failure and one patient of viral hepatitis and candida pneumonitis (Table 3).

DISCUSSION

According to the United States and the European Bone Marrow Transplantation Group (EBMTG) results, approximately two-thirds of endstage patients can expect to achieve a complete remission after HDT and many remain in such state for prolonged periods.

The aim of our study, which was started more than 9 years ago, was to improve, by using HDT, salvage results in the advanced heavily pretreated patients. In this study we showed that it is possible to achieve a high response rate (74.4%) with a low proportion of early deaths in 106 resistant Hodgkin's disease patients with very bad prognoses. Superimposable results have been obtained by others in similar patients.

Recently, Goldstone presented the EBMTG experience on 778 patients receiving ABMT for Hodgkin's Disease: 43% of patients achieved complete remission and 26% achieved partial remission, for a total response rate of 69%. Despite the advanced nature of most patients grafted, the results are good in terms of remission rate, although relapses remain a major problem (3). All these results, as well as those concerning smaller groups of patients, are similar to ours and prove the validity of HDT/ABMT for 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 Hodgkin's disease patients. Our preliminary results seem very interesting (4) and therefore we feel that ABMT when employed in first CR should probably

be the treatment of choice for patients with systemic symptoms and more then one extranodal site of Hodgkin's Disease who achieve CR after MOPP/ABVD protocol; the reduction of the BCNU dose should avoid the risk of interstitial pneumonitis. Clearly more patients and more time are necessary to assess final conclusions.

ACKNOWLEDGEMENT

Supported in part by Associazione Italiana Ricerca Emato-Oncologica (A.I.R.E.O.)

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TABLE 1

Clinical Characteristics	
Age	25 years
(Median)	(Range: 11-51)
Sex	M: 86; F: 42
Histology	NS: 78 (60.9%)
Systemic Sympt.	89 (69.5%)
Prior Chemoth. Regimens (=>2)	95 (74.2%)
Prior Radiotherapy	79 (61.2%)
Staging at ABMT	IV: 82 (63.5%)
Status at ABMT	Patients
First Relapse	15
Prog. Disease	40
Resist. Relapse	16
Sensitive Relapse	35
2^{nd} - 3^{nd} CR	7
1^{st} CR	15

NS: Nodular sclerosis;

TABLE 2

RESULTS ACHIEVED WITH CVB PROTOCOLS IN 106 PATIENTS WITH ADVANCED HODGKIN'S DISEASE

DISEASE STATUS	PATIENTS	TOXIC DEATHS	CR	PR	NR	RLPs	CCR
FIRST RELAPSE	15	2	9 (60%)	2	1	4	5
PROGRESSIVE DISEASE	40	3	18 (45%)	9	10	10	8
RESISTANT RELAPSE	16	3	4 (25%)	6	3	2	2
SENSITIVE RELAPSE	35	2	25 (69.4%)	6	2	12	13

TABLE 3

CVB PROTOCOLS

TOXICITY

VOMITING	100%
MUCOSITIS	75%
LIVER TOXICITY	51%
HERPES VIRUS GENERALIZATION	1.6%
INTERSTITIAL PNEUMONITIS	1.6%

CAUSE OF DEATH (N=86)

HODGKIN'S DISEASE	75	(62.5%)
INTERSTITIAL PNEUMONITIS	5	(4.1%)
CARDIAC FAILURE	4	(3.3%)
VIRAL HEPATITIS AND CANDIDA PNEUMONITIS	1	(1.6%)

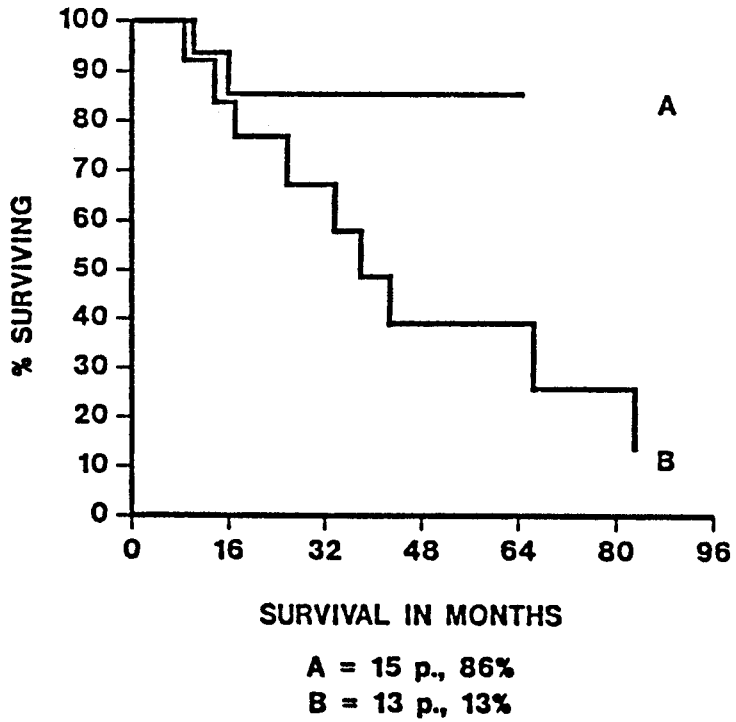
TABLE 4

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.
CVB-3 (REGIMENS USED FOR PATIENTS IN CR-1)				
Cy	6000	IN	I.V.	30 MIN.
VP-16	1000	FOUR	I.V.	120 MIN.
BCNU	450	DAYS	I.V.	PUSH

FIGURE 1

Disease free survival time according to treatment group for 15 patients with poor risk Hodgkin's disease. Outcome is superior for the intensified arm (MOPP/ABVD/ABTM) (A) compared with historical group (MOPP/ABVD) (B).



HIGH DOSE CYCLOPHOSPHAMIDE, CARMUSTINE, AND ETOPOSIDE IN HODGKIN DISEASE: FOLLOW-UP OF 128 PATIENTS

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INTRODUCTION

There have been remarkable improvements in the results of treatment for Hodgkin Disease [HD] over the last twenty-five years. More than 50% of patients with advanced disease can expect to be cured with modern front-line chemotherapy regimens.[1-3] Nonetheless, a substantial proportion of patients remain who do not attain an initial remission, or who relapse after achieving a remission. Despite the large number of salvage regimens which have been developed for these situations it seems unlikely that more than 10% of such patients can expect prolonged disease-free survival [DFS].[4] The poor results of conventional chemotherapy salvage regimens for HD have led to wider use of strategies that employ high-dose chemo-radiotherapy followed by autologous bone marrow or peripheral stem cell transplantation.[5-9] We report here transplantation results on a group of 128 HD patients most recently reported when the minimum follow-up period was six months.[10] All patients have now been observed for at least 2.5 years from the time of transplant.

PATIENTS AND METHODS

All patients were treated at the University of Nebraska Medical Center or M.D. Anderson Cancer Center after informed consent was obtained. Patients were treated with the CBV regimen consisting of cyclophosphamide 6 gm/m^2 from day -6 to -3, carmustine 300 mg/m^2 on day -6, and etoposide 600 mg/m^2 (n=24), 750 mg/m^2 (n=36), or 900 mg/m^2 (n=68) from day -6 to -4. Additional details of chemotherapy administration, supportive care, and bone marrow or peripheral stem cell processing have been reported previously.[11] Survival curves were calculated from the day of transplant (day 0) according to the method of Kaplan and Meier.[12] Comparison of survival curves used the log-rank test. Complete remission [CR] was defined as disappearance of all clinical and radiographic evidence of disease for at least one month. Residual

lymph nodes measuring <1 cm were not considered abnormal. Partial remission [PR] was defined as greater than 50% reduction of measurable disease for at least one month. Patients with stable small residual masses for at least six months were classified as achieving CR. Gallium scans were utilized for staging at the end of the study period and were required to be negative for a patient to be coded a CR. Early death [ED] was defined as failure to survive the transplant hospitalization.

PATIENT CHARACTERISTICS

Patient characteristics are shown in table 1. All patients were transplanted prior to January 1988. The median age of patients in this series was 28. There were 78 males. The Zubrod performance status was ≤ 1 in 93% of patients. The median time interval between HD diagnosis and transplant was 40 months. Patients had failed a median of two chemotherapy regimens prior to transplantation. (A patient who responded to ABVD following relapse from MOPP was considered to have failed only one regimen. MOPP-ABVD and similar therapies were counted as only one regimen.) Ninety-one patients had had prior radiotherapy and 70 had extranodal disease at the time of transplant. Autologous bone marrow was used to rescue 109 patients. The remainder received autologous peripheral stem cells (primarily because of prior pelvic radiation) or both marrow and stem cells.

RESULTS

The response to transplantation is shown in Table 2. Fifty-six patients (44%) attained a CR following transplantation. Ten patients were in a second or subsequent CR at the time they were transplanted and continued in CR. An additional seven patients had an apparent PR following their transplant and were converted into CR by means of involved-field radiotherapy to small residual extranodal masses. Overall, 73 patients (57%) were in remission following transplantation. Forty-four patients had a PR or failed to respond to high-dose therapy. There were 11 early deaths in this series (7 diffuse alveolar hemorrhage/adult respiratory distress syndrome; 1 cardiac tamponade; 2 candida sepsis; 1 gram negative sepsis). Seven of the patients experiencing early death had evidence of persistent HD.

The overall survival [OS] and DFS for the entire group of 128 patients are shown in figures 1 and 2. Fifty-eight patients remain alive (median follow-up 44.3 months) with a projected OS of 24%. Thirty-four patients remain alive and continuously free of disease with projected DFS of 23%. The DFS is 40% (figure 3) for those patients transplanted following failure of only one chemotherapy regimen vs. 19% for those transplanted after failing more than one prior regimen ($p=0.04$). Similarly the DFS for those failing either one or two prior chemotherapy regimens is 28% (figure 4) vs. 15% for those patients transplanted after failing more than two prior regimens ($p=0.02$).

With prolonged follow-up we have now identified six patients experiencing relapse at intervals exceeding 24 months from transplantation (26-33 months). Four of these patients with "late" relapses remain alive from 6+ to 25+ months following their relapse.

DISCUSSION

With a minimum follow-up interval of 2.5 years, 34 patients are alive in continuous remission. The 57% CR rate is similar to other reported series.[5-9] Only prospective trials will demonstrate the superiority of any particular regimen. The projected DFS of 23% contrasts with a 31% DFS projected when these same patients were previously reported after a shorter follow-up interval.[10] The difference is primarily related to the presence of six relapses beyond 24 months, and underscores a need for long term follow-up in order to critically evaluate the true impact of transplantation for HD.

Examination of figures 3 and 4 emphasizes the superior results attained when transplants are carried out early prior to multiple chemotherapy salvage regimens. Although prospective randomized studies have not been performed we feel that our results of transplantation after failure of just one chemotherapy regimen compare favorably with the majority of reports examining the use of ABVD-type regimens following relapse from MOPP-type regimens.[4] Our results in more heavily pretreated patients also compare favorably with other conventional salvage regimens for HD.[4,13-15] We feel that autologous transplantation for HD may be indicated following failure of any single front-line chemotherapy regimen and certainly after failure of both MOPP- and ABVD-type regimens or their use in combination.

Figures 3 and 4 also indicate that differences in DFS between groups can be explained by higher CR rates in less heavily pretreated patients rather than by a lower relapse rate in these patients. A similar situation may be observed in the small group of patients who were converted to CR with localized radiotherapy following transplant. Four of 7 patients in this group remain continuously free of disease from 41+ to 60+ mo. following transplantation. Although some of these patients may have actually been in remission prior to receiving post-transplant radiation they illustrate the principle that rare patients can experience long-term DFS with radiotherapy after a relapse from chemotherapy.[16]

CONCLUSION

Results of transplantation in this large series of patients demonstrate that CBV can produce long term DFS in a significant proportion of patients with relapsed HD. Our results also show that some patients achieving a PR after transplantation can experience long term DFS with localized radiotherapy following their transplant. Results are clearly superior when transplantation is performed early and we believe that this form of therapy should be considered after failure of a single front-line HD chemotherapy regimen. Relapses as late

as 33 months following transplant have now been observed and emphasize a need for continued follow-up and reporting of these patients.

ACKNOWLEDGEMENTS

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TABLE 1

Patient Characteristics

Median Age (range)		28 (11-57)
Sex M:F		78:50
Performance Status:	0	54 (42%)
	1	65 (51%)
	≥2	9 (7%)
Diagnosis to Transplant (mo.)		40 (60-226)
Chemo Regimens Failed		2 (1-6)
Prior Radiotherapy		91 (71%)
Extranodal Disease at Transplant		70 (55%)
Rescue Source:	Autologous Marrow	109 (85%)
	Autologous Stem Cells	15 (12%)
	Both	4 (3%)

TABLE 2

Transplantation Results

Complete Remission	56 (44%)
Continued Complete Remission	10 (8%)
Complete Remission after XRT	7 (5%)
Partial Remission	30 (23%)
No Response/Progression	14 (11%)
Early Death	11 (9%)

FIGURE 1

Overall survival for all patients treated with CBV.

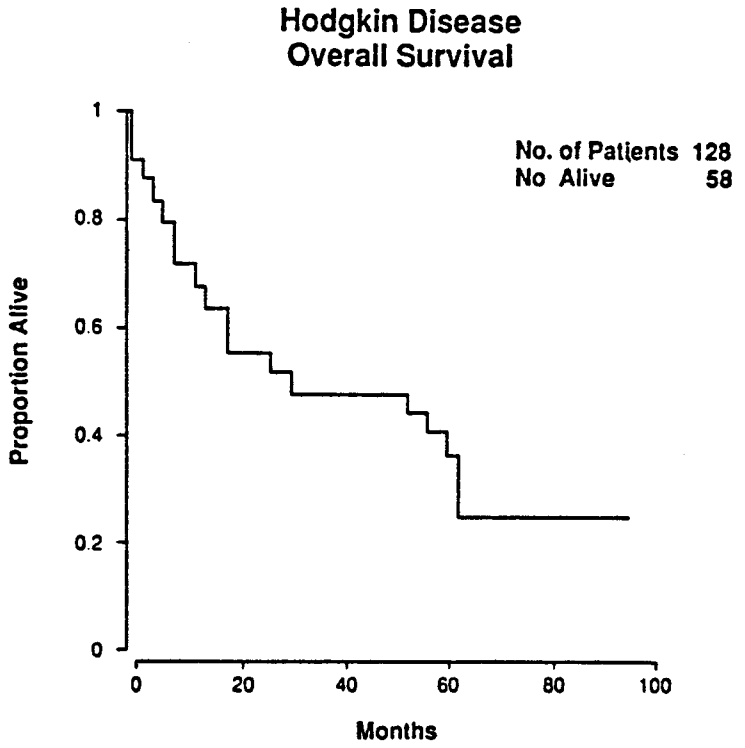


FIGURE 2

Disease-free survival for all patients treated with CBV.

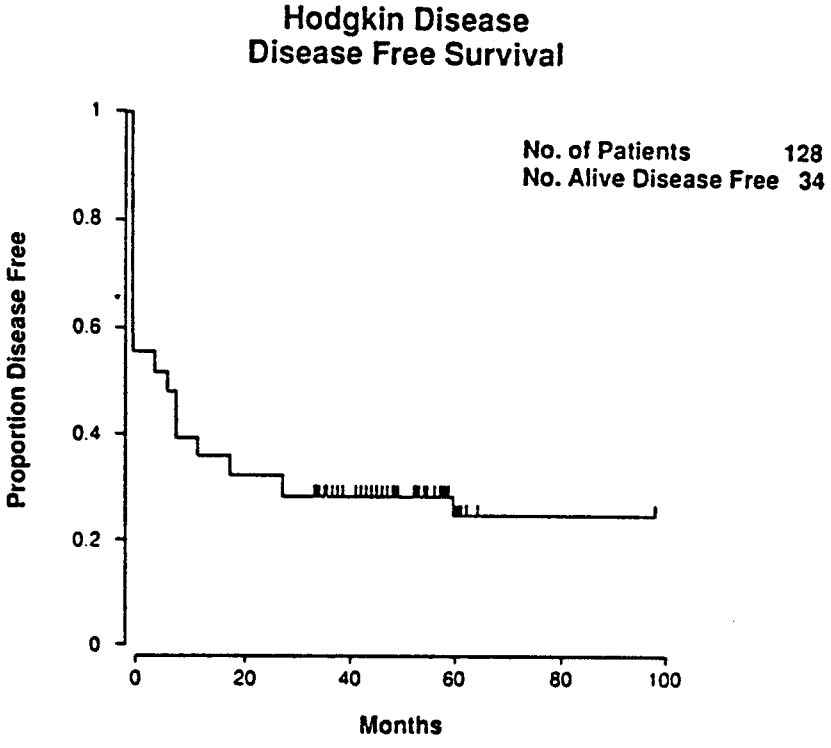


FIGURE 3

Comparative disease-free survival for patients transplanted after failure of 1 prior chemotherapy regimen vs. failure of > 1 prior regimen.

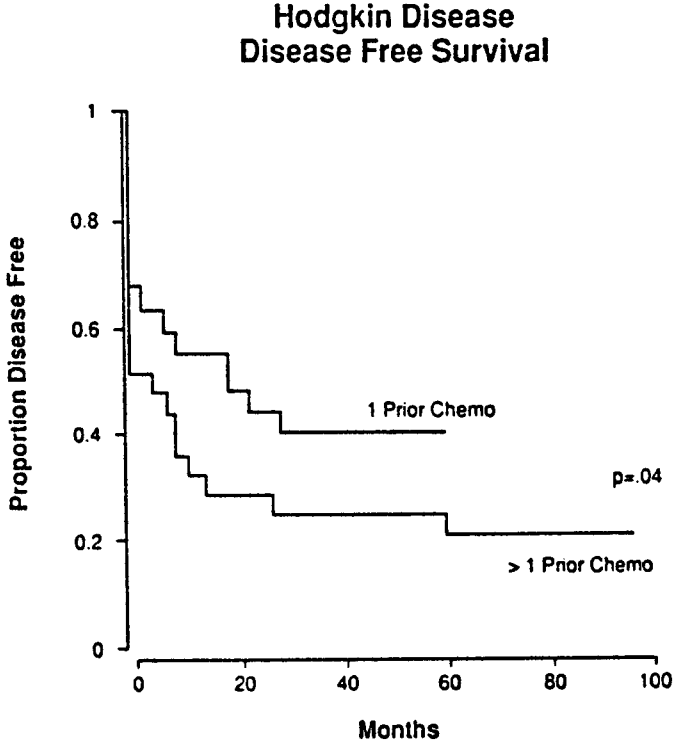
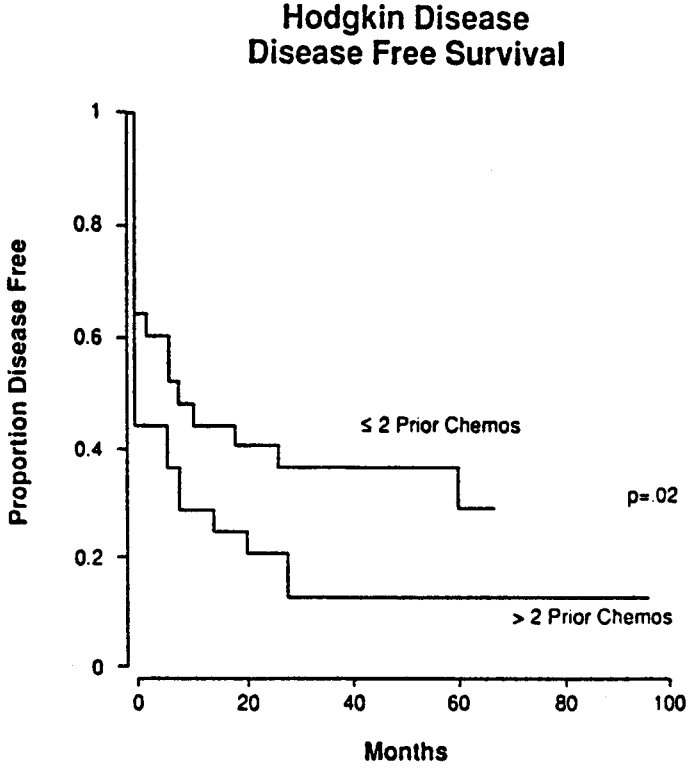


FIGURE 4

Comparative disease-free survival for patients transplanted after failure of 1 or 2 prior chemotherapy regimens vs. failure of >2 prior regimens.



AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR HODGKIN'S DISEASE: COOPERATIVE GROUP TRIALS IN THE UNITED STATES

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INTRODUCTION

Hodgkin's disease is a relatively uncommon malignancy with an annual incidence of approximately 3/100,000 in the United States (1). Prognosis of newly diagnosed patients is dependent on several factors including stage, age, presence or absence of constitutional symptoms, histologic subtype, and response to initial therapy (2). Patients with limited stage disease are usually cured with the proper use of radiation therapy; and, during the past 20 years, the prognosis of patients with advanced stage disease has improved dramatically due to the development of effective combination chemotherapy regimens (3-7). However, patients who either do not achieve a remission with their initial chemotherapy, have a remission that lasts less than one year, or who have experienced multiple relapses have a poor prognosis (2,8). Except with the use of intensive therapy and autologous or allogeneic transplantation, these patients are unlikely to be cured with any currently available treatment program although they may still have responsive disease (8).

Over the past ten years there has been an marked increase in use of intensive chemotherapy with or without total body irradiation (TBI), in conjunction with hematopoietic support, for patients with relapsed and refractory Hodgkin's disease (9-23). The vast majority of these patients have undergone autografting since the use of allogeneic bone marrow transplantation (BMT) is limited primarily by the availability of suitably matched donors, and may be further restricted to patients who have not received prior radiation, if TBI is to be part of the preparative regimen for transplant (11,12,21). Additionally, it would appear from some studies that the overall results with autologous BMT may be comparable to allogeneic transplantation for Hodgkin's disease (12,21), although there has never been a prospectively controlled trial to test this assumption.

With the increasing number of reports in the literature of the potential benefit of autografting in relapsed and refractory Hodgkin's, some of the U.S. Cooperative Groups have initiated Phase II trials to further evaluate this treatment approach. The purpose of this paper is to summarize the objectives, eligibility criteria, and treatment design of those studies.

PATIENTS AND METHODS

Cooperative Group Trials

A summary obtained from the Cancer Treatment Evaluation Program listed only three Cooperative Group trials (CALGB, ECOG, RTOG) utilizing autologous BMT for Hodgkin's disease that were active in the United States. By contacting other Cooperative Groups, one additional trial (SWOG) was identified. In each of these studies, bone marrow, rather than peripheral blood precursors, are being used to support the patient after intensive therapy. None of the Studies use hematopoietic growth factors or in vitro marrow purging; only patients with marrows morphologically free of disease at the time of harvest are eligible.

Cancer and Leukemia Group B

CALGB protocol 8783 was open to accrual in December, 1987 with the primary objective to further determine the activity of augmented CBV (cyclophosphamide, carmustine, etoposide). This study represents the first multi-institutional autografting study to be undertaken in CALGB. Eligibility requirements attempt to capture patients relatively early in their disease course, but who, by the nature of their treatment failure, are not expected to be cured without intensive therapy. These criteria include failure to achieve a complete remission (CR) after $>$ 6 cycles of primary treatment including both MOPP and ABVD (or equivalent regimens) ; progressive disease after $>$ 2 cycles of treatment with 2 separate regimens of primary treatment including both MOPP and ABVD (or equivalent regimens); or patients who have relapsed $<$ 12 months following their initial chemotherapy induced CR. Patients who have been in remission for more than one year and patients who are originally treated with radiation therapy only are not eligible until they subsequently relapse.

In an attempt to achieve minimal disease status prior to transplant, patients are first eligible to receive cytoreductive therapy based on their treatment history. Prior MOPP failures can receive up to three cycles of ABVD with or without involved field irradiation (IFR) and, alternatively, prior ABVD failures can receive up to three cycles of MOPP + IFR. Patients who have failed both MOPP and ABVD are not eligible for further chemotherapy, but may receive IFR. IFR (20 cGy in 10 fractions) is given if there is residual nodal disease after chemotherapy that measures $>$ 2 cm, and these nodal areas have not received prior irradiation, and provided that 90% or more of all disease sites (nodal and contiguous extranodal disease) can be included by IFR. Parenchymal metastases (liver, lung, etc) are not to be treated by IFR, but these do not exclude patients from entry on study.

All patients, regardless of response to the pretransplant chemotherapy + IFR, are then eligible for intensification, if their underlying health status has not changed during cytoreductive therapy. The preparative regimen for transplant is outlined in Table 1 and includes etoposide 2400 mg/m^2 by continuous intravenous infusion over 34 hours on days -8 and -7, cyclophosphamide 1800 mg/m^2 over 2 hours beginning 2 hours after the etoposide is completed and given daily on days -7, -6, -5, and -4, and carmustine (BCNU) 600 mg/m^2 as a two-hour infusion on day -3. Marrow is reinfused on day 0, 48-72 hours after the BCNU.

Eastern Cooperative Oncology Group (ECOG)

ECOG protocol P-A488 opened in June, 1989 with the objectives to determine the response rate, duration of response, and survival of patients with relapsed Hodgkin's disease treated with high-dose chemotherapy and autologous bone marrow rescue, and to determine the toxicity of a sequential combination of high-dose chemotherapy agents. Patients > 15 and < 60 years old who have relapsed > 6 months after completing chemotherapy, with or without prior radiation therapy, (1st or 2nd relapse) that had led to a CR or stable partial remission (PR) are eligible if they have objectively measurable disease, an adequate bone marrow harvest, and no intercurrent illness or organ dysfunction that would preclude intensive chemotherapy. Patients are first given pretransplant cytoreductive therapy with one of a variety chemotherapy regimens, or with local field radiation therapy, and when maximum response is obtained (CR, PR with no further improvement on 2 consecutive cycles, or stable non-responding disease after completion of 2 full cycles), they are eligible for intensification course 1. Patients with progressive disease on pretransplant cytoreductive therapy are ineligible for transplant. Adequate bone marrow to support both intensification courses is obtained in a single harvest prior to beginning any cytoreductive therapy.

Course 1 is outlined in Table 1 and consists of carmustine 400 mg/m^2 as a 6 hour infusion on day -8, cyclophosphamide 60 mg/kg as an 8 hour infusion on days -7 and -6, and etoposide 400 mg/m^2 as 4 hours every 12 hours on days -5, -4, and -3. Autologous marrow is infused on day 0. Patients who are in CR or PR after recovering from course 1 are eligible for further therapy provided they have a performance status of 0 or 1, are free of active infections, and have been off parenteral antimicrobials for 2 weeks. Course 2 (Table 1) includes vinblastine 4mg/m^2 by continuous infusion on days -6 and -5, followed by thiotepa 450 mg/m^2/d over 4 hours on days -4 and -3. The previously harvested bone marrow is infused on day 0 of course 2.

Southwest Oncology Group (SWOG)

The recently activated (April, 1990) SWOG protocol 9011 will evaluate the response rate and survival of patients with either "sensitive" or "resistant" relapsed or refractory Hodgkin's disease treated with either a chemotherapy only regimen or a chemotherapy plus TBI regimen. In addition, the

non-hematopoietic toxicities of these regimens will also be assessed in this patient population.

All patients who relapse after achieving a CR are required to have a minimum of 2 courses of salvage chemotherapy, or radiation therapy, to first determine if they have "sensitive" or "resistant" disease. Patients who have failed both MOPP-ABVD regimens, DHAP (24) is the recommended salvage therapy. Patients failing in the mediastinum, who have not had prior radiation, salvage chemotherapy is recommended if they are otherwise eligible for the TBI-based preparative regimen.

Patients with prior thoracic irradiation will be prepared for transplant using a chemotherapy only regimen (Table 1) consisting of carmustine 15 mg/kg over 2 hours on day -6, etoposide 60 mg/kg over 4 hours on day -4, and cyclophosphamide 100 mg/kg over 1 hour on day -2. Patients without prior thoracic irradiation will receive fractionated TBI 150 cGy twice daily on days -8, -7, -6 and 5, followed by etoposide 60 mg/kg on day -4, and cyclophosphamide 100 mg/kg on day -2 as noted above. Marrow infusion is on day 0.

Radiation Therapy Oncology Group (RTOG)

The RTOG protocol 87-01 was activated in September, 1987 and was recently closed. This was a phase I-II dose escalation study of ^{90}Yttrium antiferritin for the treatment of advanced Hodgkin's disease in patients who had failed MOPP-ABVD or similar treatments. The objectives of protocol 87-01 were to determine the maximum tolerated dose of ^{90}Yttrium-labelled antiferritin that could be delivered in conjunction with autologous BMT, to determine the response rate of chemotherapy-resistant Hodgkin's disease when the ideal dose of ^{90}Yttrium antiferritin was administered in two or three cycles, to determine the influence of autologous BMT on the interval between treatment cycles, and to determine the non-hematological toxicity of high dose radiolabeled antiferritin.

Patient eligibility included age < 55 years, chemotherapy resistant measurable disease at diagnosis or relapse, prior radiation < 30 Gy to the mediastinum and < 20 Gy to both iliac crests, an adequate bone marrow harvest no HLA/MLC compatible sibling donor, and tumor targeting with ^{111}Indium on scan.

The treatment schema included external beam irradiation (150 cGy x 2 days) to areas of known disease > 5 cm^2 prior to the first injection of antibody to increase the amount of antibody deposited in the tumor. Patients whose disease could be targeted with ^{111}Indium labelled antiferritin antibody were then eligible to receive up to three cycles of ^{90}Yttrium antiferritin using a different antibody producing species (rabbit, pig, baboon) each cycle. Autologous marrow was infused three weeks after the antibody. Dose escalations in cohorts of three patients were undertaken until a maximum dose of 50 mci was given on each cycle. At the maximal tolerated dose (MTD) further patients were entered to determine response.

DISCUSSION

Each of the Cooperative Groups Trials is attempting to further define the role of intensified therapy in patients with refractory and relapsed Hodgkin's disease. In the CALGB, ECOG and SWOG studies, disease "sensitivity" is being tested by pretransplant cytoreductive therapy with either chemotherapy, radiotherapy or both. In the CALGB and SWOG studies, patients, are eligible for transplant regardless of response; while in the ECOG study, only patients achieving a CR or PR to proceed on to transplant. The SWOG and CALGB studies will attempt to assess the relative effectiveness of the Subsequent transplant regimens in relative effectiveness of the subsequent transplant regimens in both "sensitive" and "resistant" disease, whereas the ECOG protocol, will focus on that subset of patients with "sensitive" Hodgkin's disease who might benefit the most and would be able to tolerate the double autologous transplant therapy (14).

Although radiation therapy is a highly effective modality for the treatment of Hodgkin's disease, the majority of autografting studies have used chemotherapy only as the preparative regimen for transplant. This is based on the experience that the majority of patients presenting for transplant will have had prior irradiation, especially to the mediastinum, that would exclude the safe delivery of TBI containing regimens (25). In the studies that have been able to safely use chemoradiotherapy preparative regimens, the outcome appears comparable to chemotherapy only programs (18). The SWOG study will further explore the use of TBI in selected patients who have not had extensive prior radiation.

The RTOG is exploring the role of targeted radiation therapy through the use of radiolabeled antibodies. Ferritin is a tumor associated protein found in Hodgkin's disease and other malignancies. In RTOG protocol 83-09, there was demonstrated activity of ^{131}I-antiferritin with the major toxic ^{90}Yttrium restricted to bone marrow depression. Subsequently, ^{90}Yttrium labelled antiferritin was used since it is a more powerful beta emitter and has the theoretical advantage of determining whether a dose response relationship may be achieved with radiolabeled antibody. pilot data demonstrating activity of ^{90}Yttrium served as the basis for the dose escalation schema for RTOG 87-01 (26). Preliminary analysis of this recently closed study indicates between a 30 and 50 percent CR with ^{90}Yttrium-antiferritin compared to a 40 percent PR with ^{131}I-antiferritin. The CR rate appears to be related to tumor targeting with Yttrium-antiferritin rather than dose escalation. Currently, Hodgkin's disease that targets with Yttrium-antiferritin is now being investigated with protocols involving both ^{90}Yttrium-antiferritin and chemotherapy with autologous BMT at the University of Nebraska and the Johns Hopkins University. Further analysis of the data from RTOG 87-01 is pending.

SUMMARY

Autologous BMT appears to improve disease free survival and potential for cure in patients with relapsed and refractory Hodgkin's disease. with the potential for large numbers of patients to be uniformly treated on studies through the Cooperative Group Clinical Trials mechanism, response rates, toxicities, and therapeutic efficacy of the different preparative regimens will hopefully be better defined.

Many areas for ongoing investigation of the role of intensification therapy remain open in Hodgkin's disease including 1) defining the optimal preparative regimen for autologous (and allogeneic) transplantation; 2) defining the role of hematopoietic growth factors; 3) defining the role of autologous BMT compared to aggressive chemotherapy programs which utilize growth factor support; 4) defining the role, if any, of intensification therapy in first or second CR; 6) defining the role, if any, of marrow purging; 7) further investigating the role of progenitors collected from the peripheral blood compared to bone marrow in the various intensification programs; and 8) further defining the relative roles of autologous and allogeneic bone marrow transplantation in Hodgkin's disease.

ACKNOWLEDGEMENTS

Supported in part by grants CA31946, CA14548, CA21115, P20CA43703 from the National Institutes of Health, the National Cancer Institute, and the United States Public Health Service. Authors' affiliations: 1. Cancer and Leukemia Group B (CALGB); 2. Eastern Cooperative Oncology Group (ECOG); 3. Southwest Oncology Group (SWOG); 4. Radiation Therapy Oncology Group (RTOG); 5. Cancer Center at Wake Forest University, Bowman Gray School of Medicine, Winston-Salem, North Carolina; 6. Washington University School of Medicine, St. Louis, Missouri; 7. University of Iowa, Iowa City, Iowa; 8. Ireland Cancer Center, Case Western Reserve University, Cleveland, Ohio; 9. University of Connecticut Health Center, Farmington, Connecticut; 10. Stanford University Medical Center, Stanford, California; 11. Loyola University, Maywood, Illinois; and 12. The Johns Hopkins Oncology Center, Baltimore, Maryland

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TABLE 1

Hodgkin's Disease
Preparative Regimens for Autologous Bone Marrow Transplantation

DRUG	DOSE	DAY
Cancer and Leukemia Group B		
Etoposide	2400 mg/m^2 by CIVI* over 34 hours	-8, -7
Cyclophosphamide	1800 mg/m^2 over 2 hours	-7, -6, -5, -4
Carmustine	600 mg/m^2 over 2 hours	-3
Eastern Cooperative Oncology Group		
Course 1		
Carmustine	400 mg/m^2 over 6 hours	-8
Cyclophosphamide	60 mg/kg over 8 hours	-7, -6
Etoposide	400 mg/m^2 over 4 hours every 12 hours	-5, -4, -3
Course 2		
Vinblastine	4 mg/m^2/d by CIVI*	-6, -5
Thiotepa	450 mg/m^2 over 4 hours	-4, -3
Southwestern Oncology Group (SWOG)		
Prior thoracic irradiation		
Carmustine	15 mg/kg over 2 hours	-6
Etoposide	60 mg/kg over 4 hours	-4
Cyclophosphamide	100 mg/kg over 1 hour	-2
No prior thoracic irradiation		
Fractionated TBI	150 cGy twice daily @ 5-20 cGy/min	-8, -7, -6, -5
Etoposide	60 mg/kg over 4 hours	-4
Cyclophosphamide	100 mg/kg over 1 hour	-2

*CIVI = continuous intravenous infusion
All patients supported with marrow infused on day 0

DOUBLE AUTOLOGOUS BONE MARROW TRANSPLANTATION OF ACUTE LEUKAEMIA AND LYMPHOMA-40 CASES

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INTRODUCTION

The Bloomsbury Transplant Group has carried out double autologous bone marrow transplantation (ABMT) for malignant lymphomas and acute leukaemia. We present the experience of double transplants in 40 patients with Non-Hodgkin's Lymphoma (NHL) (3), Hodgkin's Disease (HD) (2), acute lymphoblastic leukaemia (ALL) (12), and acute myeloid leukaemia (AML) (23).

In leukaemia high dose chemo-radiotherapy followed by ABMT affords the opportunity to give very intensive therapy to a wider range of patients than is possible with allogeneic transplantation. A major disadvantage might be the possible contamination of harvested marrow with minimal residual disease. Some centres have carried out "in-vitro" purging in an attempt to remove any residual disease (1). There are however technical and logistical problems with this approach. We have therefore argued that remission induction arises through non specific marrow ablation with subsequent faster regeneration of normal haemopoiesis compared to the leukaemic clone. If therefore an initial autograft is performed and then in the early regeneration phase, when it is argued that the leukaemia content of the marrow will be at its nadir, the marrow is reharvested and the ABMT procedure repeated, it in effect leads to an "in -vivo" purging procedure.

In lymphoma, high dose combination chemotherapy may lead to meaningful disease free survival in patients with relapsed disease (2). There is however often relapse in previous sites of disease, suggesting the failure to eradicate host disease. The double autograft procedure affords a method of giving dose intensification. The second cycle of intensive chemotherapy with ABMT rescue being given as soon as possible on recovery from the first cycle.

PATIENTS AND METHODS

Lymphoma

Our first 12 transplant patients with lymphoma, referred many years ago, were considered for the UCH double transplant option (3). Out of these patients with lymphoma, 5 patients eventually received a double autograft. The reasons for the other patients not receiving the second graft are listed in table 2.

Three patients had NHL and 2 patients had HD. Four patients had relapsed after one or more lines of chemotherapy. One patient had shown a partial response (PR) to conventional first line chemotherapy. All patients had received a chemotherapy regimen containing adriamycin and 1 patient received localised radiotherapy. Four patients had chemosensitive disease and 1 patient had chemoresistant disease at the time of ABMT. The median age of patients was 39 years and all patients were males.

The chemotherapy regimen for the first autograft was UCH I and the second chemotherapy regimen was UCH II. UCH I and II was a 2 cycle regimen designed to introduce as many new potentially non-crossresistant drugs as possible. UCH I was based on the UCH double protocol for patients with acute leukaemia (Table 1), but adriamycin was dropped as it was argued that all patients would have received it anyway. In UCH II, methotrexate replaced the cyclophosphamide in UCH I.

If the UCH double protocol was employed one marrow harvest was performed and if more than $1.2x10^8$/l nucleated cells/Kg body weight were obtained post-processing, the marrow was divided and half given after each course. UCH II was given as soon as there was haematological recovery after UCH I. The median time between ABMT 1 and 2 was 2 months (Range:1-7).

Acute Leukaemia

In the patients with acute leukaemia, remission was established by morphological and karyotypic analysis of bone marrow immediately prior to each harvest. Bone marrow was harvested and cryopreserved as described previously (4). No ex vivo manipulation of the marrow was carried out. The conditioning regimen used for transplant was UCH chemotherapy (Table 1). One patient, UPN 199, received his first graft with the UCH chemotherapy protocol and the second with cyclophosphamide and TBI. After the first part, as soon as satisfactory regeneration was achieved (neuts $> 1.5x10^9$/l; platelets $> 100 \times 10^9$/l), a second harvest was carried out and the patients offered the choice of a second autologous transplant, providing there were no contraindications to this (see Table 2).

Acute Lymphoblastic Leukaemia

Nineteen patients were intended to receive the double graft procedure and of these, 12 received both parts of the procedure (Table 2). There were 5 females and 7 males and the median age of these patients was 26 years (range 12-47 years). Six patients were transplanted in first complete remission (CR)

and 6 were transplanted beyond first CR. All patients received one of the Medical Research Council (MRC) United Kingdom Acute Lymphoblastic Leukaemia (UKALL) protocols according to their date of presentation for induction and consolidation (UKALL VI, IX or X). All patients received intrathecal methotrexate as CNS directed therapy and, in addition, patients received cranial irradiation (18 or 24 Gy) after the early intensification block.

For ALL like AML, the marrow was not split but harvested a second time following recovery from the first graft. The mean marrow dose collected for ABMT 1 was 1.8 x 10^8/l and ABMT 2 was 1.1 x 10^8/l. The median time gap between ABMT 1 and ABMT 2 was 2 months (range: 1-3).

For the patients in 1st CR, the median time between remission and ABMT 1 was 4.5 months (Range: 1-6 months). Of the patients transplanted beyond 1st CR, 2 were in first relapse and 4 were in 2nd CR (Table 5). The median duration of 1st CR in these patients was 32 months. All the patients in first relapse had bone marrow stored during the first remission. Two of the patients in 2nd CR also had their marrow stored in first remission, whereas the remaining 2 patients had reinduction chemotherapy followed by bone marrow harvest. The patients in 2nd CR underwent ABMT 1 within a median time of 2 months (range:1-10), after achieving 2nd CR.

Acute Myeloid Leukaemia

Patients with acute myeloid leukaemia (AML) M1-M6 were eligible to be entered on the Bloomsbury double autograft programme.

Initially all patients received induction therapy with Daunorubicin, Cytarabine and 6-Thiguanine on a DAT 1+5 or 3+10 protocol. They received one or two consolidation courses with the same agent. The UCH conditioning regimen was used for both grafts in all patients. Bone marrow was reharvested once again on satisfactory regeneration of counts (neuts. > 1.5 X 10^9/l; platelets > 100 x 10^9/l). The second ABMT was then performed shortly afterwards, using the same regimen.

Fifty-one patients were eligible for the double autograft programme but only 23 eventually received it (Table 2). The median age of these patients was 37 years and there were 10 females and 13 males. Twenty-one patients were in 1st CR and 2 patients in 2nd CR.

The mean dose of marrow collected for ABMT 1 was 1.5x10^8/l and for ABMT 2 was 1.7x10^8/l. The median time between ABMT 1 and ABMT 2 was 2.5 months (Range 1 - 7).

In the patients transplanted in 1st CR the median time between remission and ABMT 1 was 3.5 months (range 2-7). The 2 patients transplanted in 2nd CR had their bone marrow harvested and stored in first remission and underwent ABMT within 1-2 months of achieving second remission.

RESULTS

Lymphoma

Of the 5 patients that received the double autograft, 1 had chemoresistant disease and 4 had chemosensitive disease at ABMT. Two of these patients, both NHL, are alive with median survival of 87 months post ABMT II. One patient, UPN 101, is deemed to be in partial remission (PR) due to persistent autoimmune haemolytic anaemia. The other surviving patient, UPN 65, received post-autograft radiotherapy to a residual abdominal mass. Two patients have died with relapsed disease. Both patients achieved a complete remission to ABMT but one patient, UPN 34, died within 7 months of the procedure. The other patient relapsed within 5 months of ABMT for HD and died approximately 5 years later. One patient, UPN 61, died in remission from cardiac failure 4 months post ABMT. Of the 5 patients who received the double autograft, there were no procedure related deaths (Table 4). Procedure related morbidity is summarised in Table 4. Haematological reconstitution is summarised in Table 3.

Acute Lymphoblastic Leukaemia

Of the 6 patients in 1st CR receiving the double autograft, 2 patients had procedure related deaths. One death was due to cardiac arrest and the other one due to pneumonitis. Only one patient is alive with leukaemia free survival of 47 months. The rest of these patients are now dead with a median leukaemia free survival (LFS) of 20 months.

Of the 6 patients who were beyond 1st CR at ABMT, only one patient, UPN 51, is alive approximately 8 years post transplant. There were 2 procedure related deaths, one from pneumonitis and the other from aspergillosis. The other 3 patients died with LFS less than 10 months. The procedure related morbidity is summarised in Table 3.

Acute Myeloid Leukaemia

Of the 21 patients who received the double autograft in 1st CR, the median time of remission to transplant was 4 months. Sixteen patients (74%) are alive in CR (median LFS 39.5 months) (Fig 1). One patient, UPN 218, relapsed 21 months post double autograft. He was reinduced to 2nd CR and received a 3rd ABMT using BU/CY regimen. Post 3rd ABMT he developed drug induced pneumonitis which settled on steroids. He is now 17 months into 2nd CR with a normal Karnovsky score. One patient was lost to follow-up and has been censored.

Two patients who received the double autograft were in 2nd CR and their duration of 1st CR was 2 and 34 months respectively. Both patients are dead with relapsed disease. The duration of 2nd CR was 5 and 9 months respectively.

DISCUSSION

The patients who received the double autologous transplant procedure for lymphoma had all relapsed after salvage therapy or only shown a partial response to first-line therapy. This group of patients with NHL and Hodgkin's disease has a very poor prognosis (5). Although the numbers treated with the double ABMT protocol are too small to draw conclusions with regard to long term survival, it is interesting to note that 2 patients out of 5 (40%) are alive with survival of 83 and 91 months post ABMT. However, the procedure related mortality was 25% during ABMT 1 and, although a selected group proceeded onto the second procedure with no further mortality, the incidence of infection and other side effects was high (Table 4). We have now abandoned the UCH I + II autograft procedure in these patients in favour of the BEAM regimen (6,7). The mortality rate with BEAM chemotherapy is in the order of 10-20% for patients with relapsed or refractory NHL or Hodgkin's disease (6,7). The toxicity rate increases in patients with chemoresistant disease. It is interesting to note that the 2 survivors with NHL had chemosensitive disease and thus in such cases double transplantation may be a valid approach. Indeed, most patients relapse at sites of existing disease, suggesting a failure of disease eradication. We cannot say the results with one BEAM ABMT are better or worse than the double graft approach but certainly the logistics of one graft as opposed to two are simpler.

Some centres have embarked on pilot studies in ABMT using maximum tolerated doses of combinations of drugs in patients with refractory lymphoma (8). At the higher doses, the incidence of pneumonitis increases. Indeed, BCNU increased from 300mg/m^2 to 600mg/m^2 results in a rise of treatment related mortality from 5% to 22% (8). In our small series of patients, BCNU was delivered in divided doses to 600mg/m^2 and in these selected patients there was no procedure related mortality. There is clearly a need for the investigation of the means of delivering high dose combination chemotherapy by the use of double autografts.

It is also worthy of note that patients with lymphoma where the bone marrow harvest was divided in two and half given after each course of chemotherapy, the neutrophil and platelet recovery after ABMTs 1 and 2 are similar. In contrast, in acute leukaemis, the neutrophil and platelet recovery is longer in ABMT 2 (Table 3).

In ALL the results obtained with the double autograft procedure in patients in first CR or beyond first CR are poor and there are only two long term survivors. One of these survivors, UPN 51, has already had a very long first CR and the prolonged survival post ABMT may be a reflection of the slow tempo of the disease. Due to the poor results achieved using UCH chemotherapy as conditioning, we are now using melphalan-TBI and the results may be more promising (9). The fact that these ALL results are so poor with two grafts could have a variety of explanations and these include the following possibilities: induction therapy was too gentle; the grafts should have been done

with TBI; the grafts should have been purged; and the grafts were done too early in the disease. We do not know the answer to any of this.

In AML beyond first CR, we have only transplanted two patients with the double autograft procedure. Both patients died, with LFS of 5 and 9 months. In consideration of the small numbers involved, it would be difficult to draw any conclusions, but it should be pointed out that meaningful long term survival can be achieved in AML beyond first CR using Bu/Cy chemotherapy (10).

For patients with AML who receive the double autograft procedure in first CR, there is a projected LFS of 74%. This is significantly better than that in patients receiving only a single autograft. There was a 6% mortality rate for the first procedure and a 0% rate for the second procedure. The mortality during the second graft procedure is also low in AML with only 2 out of 28 patients developing pneumonitis, this is less than with TBI conditioning regimens. The patients who undergo the second graft are obviously selected and this must contribute to the low procedure related mortality rates observed with the second procedure. However, despite this selection, the patients would still be in a high risk category for allogeneic transplant because of their age. These results compare favourably, both in terms of LFS and morbidity, with results of allogeneic transplant for AML in first remission (11). The relative decreased morbidity is further stressed by the fact that one patient, UPN 218, received a third ABMT with BU/CY conditioning approximately 22 months post ABMT 2.

It is interesting to note that the procedure related mortality and morbidity is greater in patients with lymphoma or ALL as compared to AML in this series. This may be because conventional dose chemotherapy can achieve cure rates in a higher percentage of patients with lymphoma or ALL as compared to AML. The patients considered for the double autograft programme with lymphoma or ALL were in the poor risk group whereas this selection may not have taken place for AML.

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TABLE 1

CONDITIONING REGIMENS

	DAY 1	2	3	4	5	6
UCHI-LYMPHOMA						
Cyclophosphamide (1.5g/m)	•	•	•			
BCNU (300mg/m)	•					
Cytosine Arabinoside (100mg/m)	• •	• •	• •	• •		
ABMT						•
UCHII- LYMPHOMA						
Methotrexate (1.0g/m)	•					
BCNU (300mg/m)	•					
Cytosine Arabinoside (100mg/m)	• •	• •	• •	• •		
ABMT						•
UCH- AML and ALL						
Cyclophosphamide (1.5g/m)	•	•	•			
BCNU (300mg/m)	•					
Cytosine Arabinoside (100mg/m)	• •	• •	• •	• •		
Thioguanine (100mg/m)	• •	• •	• •	• •		
Adriamycin (50mg/m)	•					
ABMT						•

TABLE 2

REASONS FOR FAILURE TO PROCEED TO SECOND TRANSPLANT

	LYMPHOMA	ALL	AML
Delayed Haemopoietic Recovery	-	1	7
Inadequate second BM Harvest	-	-	1
Cardiac Toxicity	-	1	3
Hepatitis	-	-	3
Infection During ABMT	1	-	1
Psychiatric Problems	-	-	1
Relapse Before Second ABMT	2	-	4
Refusal	1	1	5
Toxic Death	3	1	3

TABLE 3

HAEMATOLOGICAL RECONSTITUTION

TIME TO:(MEDIAN)	LYMPHOMA (N=12) ABMT1	(N=5) ABMT2	ALL (N=19) ABMT1	(N=7) ABMT2	AML (N= 51) ABMT1	(N=28) ABMT2
WCC $1.0X10^9/l$	17	17	15	22	19	19
NEUTROPHILS $0.5x10^9/l$	24	22	18	26	21	22
PLATELETS $50X10^9/l$	25	26	23	35	31	53

TABLE 4

PROCEDURE RELATED MORBIDITY AND MORTALITY

MORBIDITY	LYMPHOMA	ALL	AML
Pneumonitis	-	3	3
Cardiac Toxicity	-	1	3
Infection: Viral	-	2	2
Fungal	-	1	-
Bacterial	5	8	20
Haemorrhage	-	-	-
Haemorrhagic Cystitis	1	-	-
Subclavian Vein thrombosis	1	-	-
MORTALITY			
ABMT1	3/12 (25%)	1/19 (6%)	3/51 (6%)
ABMT2	0/5 (0%)	4/12 (33%)	0/23 (0%)

TABLE 5

PATIENT DETAILS-LEUKAEMIA

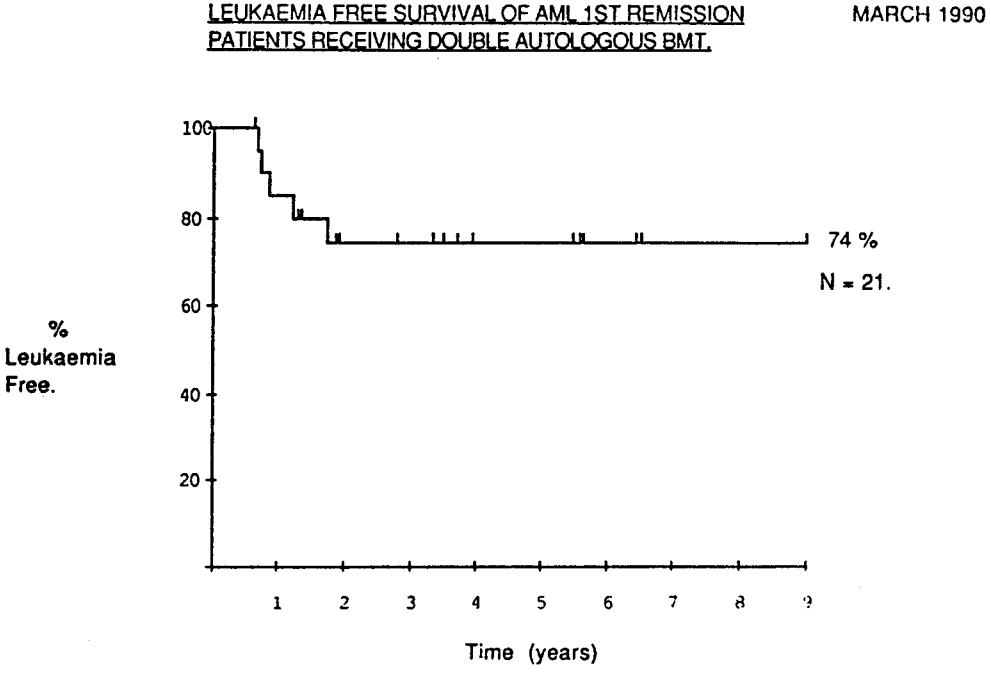
UPN	AGE	SEX	FAB/IMMUNO -PHENOTYPE	STATUS AT TRANSPLANT	DURATION OF 1st CR (MTHS)	CR TO ABMT (MTHS)	ABMT1 TO ABMT2(MTHS)	LFS	STATUS
ALL BEYOND 1st CR									
51	35	F	C-ALLA	1 REL	70	-	3	95+	ALIVE CR
39	20	M	C-ALLA	1 REL	32	-	3	6	DEAD RELAPSE
107	47	M	C-ALLA	2nd CR	36	2	2	10	DEAD RELAPSE
121	38	M	T	2nd CR	30	1	3	4	TOXIC DEATH
116	22	F	NULL	2nd CR	28	10	3	10	DEAD RELAPSE
199	29	M	C-ALLA	2nd CR	36	3	2	3	TOXIC DEATH
AML BEYOND 1stCR									
98	44	M	M1	2nd CR	2	1	2	9	DEAD RELAPSE
125	21	M	M1	2nd CR	34	2	3	5	DEAD RELAPSE
ALL 1st CR									
69	24	F	C-ALLA	1st CR		5	3	30	DEAD RELAPSE
103	16	M	C-ALLA	1st CR		6	2	42	DEAD RELAPSE
150	47	M	C-ALLA	1st CR		1	2	2	TOXIC DEATH
189	39	F	PRE-B	1st CR		6	1	7	TOXIC DEATH
201	22	F	T	1st CR		2	2	20	DEAD RELAPSE
212	14	M	C-ALLA	1st CR		4	2	47	ALIVE CR
AML 1st CR									
19	24	M	M1	1st CR		2	2	104	ALIVE CR
105	44	F	M4	1st CR		6	3	74	ALIVE CR
108	39	F	M1	1st CR		8	2	73	ALIVE CR
143	48	M	M1	1st CR		3	3	63	ALIVE CR
147	35	M	M5	1st CR		4	2	63	ALIVE CR
148	26	M	M1	1st CR		5	3	62	ALIVE CR
209	17	M	M2	1st CR		4	3	43	ALIVE CR
218	26	M	M4	1st CR		7	2	21	ALIVE CR
223	43	F	M4	1st CR		2	2	41	ALIVE CR
237	16	M	M1	1st CR		3	3	38	ALIVE CR
241	55	F	M5	1st CR		3	4	15	DEAD RELAPSE
245	46	M	M4	1st CR		5	7	36	ALIVE CR
259	16	M	M2	1st CR		4	1	18	LOST TO FOLLOW-UP
272	32	F	M2	1st CR		3	3	30	ALIVE CR
307	21	F	M5	1st CR		5	4	9	DEAD RELAPSE
320	41	F	M1	1st CR		3	1	19	ALIVE CR
323	39	F	M2	1st CR		3	3	7	DEAD RELAPSE
324	26	M	M4	1st CR		5	3	19	ALIVE CR
331	48	F	M2	1st CR		3	2	10	DEAD RELAPSE
361	45	M	M4	1st CR		6	2	9	ALIVE CR
426	33	F	M2	1st CR		3	3	6	ALIVE CR

TABLE 6

PATIENT DETAILS-LYMPHOMA

UPN	AGE	SEX	STAGE	HISTOLOGY	PREVIOUS TREATMENT	Δ TO ABMT (MTHS)	STATUS AT ABMT1	ABMT1 TO ABMT2 (MTHS)	STATUS POST ABMT2	DFS	OS	STATUS
34	38	M	III	DLCL	CHOP/MBCOD	25	RESIST REL.	1	CR	6	7	DEAD REL
65	39	M	IV	DMCL	CHOP/MBACOD	10	RESP.REL.	2	PR (DXT->CR)	85	91+	ALIVE CR
101	47	M	IV	DLCL	CHOP/DXT	46	PRI.	2	PR (AIHA)	-	83+	ALIVE NP
61	55	M	III	HD	MOPP/BACOP	52	RESP.REL.	4	CR	4	4	DEAD IN CR
88	28	M	IIB	HD	MOPP/CCNU/BCNU/VB/P	47	RESP.REL.	2	CR	5	72	DEAD REL

FIGURE 1


LEUKAEMIA FREE SURVIVAL OF AML 1ST REMISSION PATIENTS RECEIVING DOUBLE AUTOLOGOUS BMT.
MARCH 1990
100
80
60
40
20
% Leukaemia Free.
74 %
N = 21.
1
2
3
4
5
6
7
8
9
Time (years)

AUTOGRAFTING IN LYMPHOMA: PROSPECTS FOR THE FUTURE

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INTRODUCTION

The lymphomas have been considered prime candidates for dose intensification and marrow rescue at relapse. With longer follow-up of patients treated with high-dose chemo/radiotherapy regimens, it is now becoming more apparent where the future indications lie. In this article we would like to consider the prospects for the future in lymphoma transplantation.

HODGKIN'S DISEASE

Case Selection

The selection of patients and exact timing of ABMT in patients with advanced Hodgkin's disease (HD) remains controversial. It seems reasonable at the present time that patients with HD should only be considered for ABMT following relapse of the disease, although there is now data from Italy (1), considered elsewhere in this volume, which may indicate that there are indeed some patients with HD who have such a poor prognosis that they are candidates for ABMT in first remission. There is broad agreement on the current indications for ABMT in HD among the centres reporting substantial series of patients (2-5), and these can be summarised as follows: (i) patients who fail to achieve first complete remission with induction therapy; (ii) patients who relapse within six months of achieving complete remission with conventional first line therapy; (iii) patients who fail to maintain remission with two different modalities of therapy, i.e. either relapsed from second remission or failed to achieve a second remission after relapse from first remission; (iv) patients who fail two chemotherapy regimens either sequentially, or in an alternating regimen, or as a hybrid; or (v) patients who initially fail radiotherapy and subsequent chemotherapy with one regimen if this is within one year of diagnosis, or if the failure is within the site of the original radiotherapy field. This range of indications takes into account the fact that early failure from conventional combination chemotherapy is a factor indicating poor prognosis in HD (6-8). Patients who have achieved complete remission after first line MOPP or MOPP-like treatment and have maintained this for a period of at least a year before relapsing can yield a second complete response rate of between

50 and 70% after treatment with non-cross resistant regimens like ABVD (9). The failure from second complete remission in this group of patients is slow, with approximately 20% alive at 5 years from second complete remission (10). Thus, in this group of patients in second complete remission, one cannot justify the use of ABMT which has a procedure-related mortality of approximately 10% (11). Nonetheless, it should be remembered that such patients grafted early in relapse are also earlier in the natural history of their disease then those patients with HD who are exposed to a third or fourth consecutive chemotherapy regimen before consideration for BMT. When these latter patients are transplanted, it is better to compare their survival from time of failure to two regimens with that of transplanted patients grafted immediately after failure of two regimens in order to avoid a significant selection bias. In other words, like must be compared with like.

Further Dose Intensification

In terms of HD at least, TBI has not proven to be a popular ablative regimen (12) and the most popular ablative chemotherapy regimens have continued to be the BEAM regimen and CBV. We do not have much doubt that the high doses of CBV, in comparison with the BEAM regimen, do indeed increase the complete remission rates for Hodgkin's patients at transplantation, but we also have no doubt that the morbidity/mortality of such regimens is much higher than that associated with the BEAM regimen in the pre-transplant situation, particularly in relation to BCNU lung toxicity. Whether growth factors can make much difference to the morbidity of such procedures is discussed in the next section.

How else might one proceed in relation to further dose intensification? In HD in particular it makes sense that dose intensification might be better performed by a combination of a chemotherapy autograft associated with local radiation to sites with a potential high risk of relapse. Whether this radiation is carried out before or after the transplant remains to be determined. We feel that irradiation before bone transplant significantly increases the morbidity of the transplant and, in the case of the lung, puts the patient at very high risk of pneumonitis (12). Whereas, post-graft irradiation, although difficult to do in the presence of a "fragile" blood count, can at least be controlled by interruption, if necessary, thus prolonging the time for the radiation dose to be given in order that it can be given safely. We are unable yet to be certain of a long term benefit for irradiation post-ABMT (Figure 1), although there is a suggestion that relapse may be delayed in the radiotherapy group, as compared to the non-irradiated group. However, because these two groups were determined by whether or not the patients received radiotherapy earlier in the disease history, this retrospective comparison may not be entirely valid. The patients who are eligible for irradiation may be those who were initially treated with combination chemotherapy without previous irradiation, i.e., those who presented with advanced disease. These patients might be being compared with ineligible patients already irradiated in a particular site, who therefore may have presented as stage I or II disease and not been given chemotherapy until

relapse. The only true comparison would be to select patients who are eligible post transplant, in remission, for "spot weld" radiotherapy to a particular site, and then randomise these patients in properly controlled trials.

Another consideration in the context of dose intensification in the question of elective early double graft procedure. We did a small number of these with variable results some years ago (13) but did not proceed for the following reasons. We argued at the time that if a patient remitted with the first graft, there would probably be no additional benefit to a second autograft, unlike the case of AML in which second autograft may be of benefit in eliminating minimum residual disease from the marrow itself. We also argued that if the converse was true, i.e., that the patient did not remit with the first autograft, then a repeat of the same regimen would in this case not be helpful. However, perhaps this argument is not a useful one in patients who have a very good partial remission from the first autograft. The second problem relating to the selection of the ablative regimens for the first and second autografts and the question of selecting two regimens, which when given close together with a small number of weeks intervening, would be the least toxic, particularly to the lungs. In consideration of a second autograft for lymphoma, the question now arises as to whether late relapse HD patients after transplant, i.e. 3 to 5 years out, might be usefully given a second transplant at a time at which the toxicity of the second graft might be not too great.

Another way to consider dose intensification might be to give further chemotherapy before transplant but without marrow rescue, in an attempt to minimise tumour bulk. This might facilitate a better ultimate response from the high dose therapy and transplant itself. We have attempted this in our own group with the mini-BEAM regimen, given as one or two doses before transplant with BEAM (Table 1). The rationale behind this is to determine which apparently poor prognosis patients are indeed suitable for the transplant procedure. We have used the mini-BEAM therapy both to produce cytoreduction and of course to determine sensitivity to the individual drugs used in the BEAM regimen. Although there is the theoretical risk of inducing drug resistance and selection of clones in the context of BEAM transplant, we feel that in this poor risk group, it is more important to determine drug sensitivity and facilitate cytoreduction. We have altogether treated 16 HD patients in this fashion. In one group of ten patients with relapsed bulky disease (i.e. large mass of greater than 10 cm) who received mini-BEAM before proceeding to BEAM ABMT, the median number of lines of conventional dose chemotherapy was 3 (range:2-4). Most patients received two courses of mini-BEAM (1 patient received 1 course of mini-BEAM and another patient received 3 courses). None of these patients went on to complete remission after mini-BEAM. Seven out of 10 patients showed no partial response (i.e. >50% reduction in tumour bulk) and 3 patients showed no response to mini-BEAM. All of these 10 patients proceeded to BEAM ABMT and 5 patients are alive with progression free disease at a median term of 10 months post ABMT (range 6-26 months). Two patients are alive with relapsed disease at 6 months and 12 months post ABMT receiving palliative therapy. Three patients died with

relapsed disease within 3 months of the ABMT procedure. Although longer follow-up is needed for this group of patients, a 50% progression free survival in a very poor risk group suggests that the approach outlined above is worth further exploration, Five further patients had mini-BEAM in an attempt to clear bone marrow involvement so that they could then proceed to the BEAM ABMT.

This to us seemed a more sensible approach than using a peripheral stem cell transplant with all its demanding requirements of resources. Furthermore, in light of the data presented elsewhere in this volume by Sharp and co-workers, there is the worry that peripheral stem cell collection may be contaminated with disease (14). Each of the 5 patients received 2 courses of mini-BEAM and at the end of this all 5 had no evidence of Hodgkin's disease in the marrow as assessed by bilateral iliac crest trephine biopsies. Three of these patients are alive and progression free with a median follow-up of 12 months post- ABMT (range: 12-14). One patient is alive with progressive disease 11 months post-ABMT and 1 patient died with progressive disease 8 months post-ABMT. What we have already learned is that there is a considerable price to pay for this mini- BEAM/BEAM approach in that (i) there is increased lung toxicity from the additional BCNU and melphalan, and (ii) there is significant prolongation of the platelet recovery in those BEAM transplants that have been previously exposed to one or two courses of mini-BEAM. The median time for achieving 50 x 10^9/l was 61 days (range: 17-130), approximately twice the time to platelet recovery for an ordinary BEAM transplant.

The Role of Growth Factors

There is much controversy as to the role of these factors in ABMT and G-CSF (15), GM-CSF (16) and MCSF (17) have all now been used in lymphoma transplants to try and ameliorate morbidity and mortality. These agents clearly shorten neutrophil recovery after ABMT, but what this actually achieves in terms of morbidity and mortality, i.e. toxic deaths, febrile days, length of stay in hospital and ultimate cost of transplant, is by no means clear. Not only that, it is doubtful as to whether there is significant shortening of platelet recovery time in ABMT and, though this may indeed be forthcoming from growth factor combinations in the future, it is not clearly established at the present time. How can one, therefore, account for the vastly different conclusions which various groups have come to following the use of these factors in their programmes? In our view, those groups, such as our own, who aim for toxic death ratio of no more than 10% in their programmes will not see a considerable improvement in their morbidity and mortality by the introduction of growth factors into their transplant regimens. The price that these groups pay for their low toxicity is a lower complete remission rate than with the use of more toxic regimens and growth factors bring little increased benefit to them. On the other hand, for the groups who have a 30-40% Day 100 mortality rate as a result of much more aggressive regimens, it would be more reasonable to expect that the introduction of growth factors into such programmes may

ameliorate morbidity, in a variety of ways, and possibly decrease toxic death. So far, none of the studies utilising growth factors in ABMT for lymphoma have shown a significant change in toxic death rates. If, however, such evidence were forthcoming, it may persuade groups using less aggressive regimens to increase the regimen dosage and introduce growth factors.

The other potential use of growth factors may of course be in the situation where one or more courses of mini-BEAM can be much more easily given before a transplant.

Allogeneic Versus Autologous BMT in Hodgkin's Disease

It is possible that the end result of these two types of transplant may in the end be similar, the increased morbidity of the allogeneic transplant being compensated by the graft versus lymphoma effect that such a transplant may produce. There has been little evidence for this since the EBMT data shows that patients allografted in the presence of active HD have a very high mortality from the transplant itself. If one considers the use of allogeneic transplant as being relevant to those patients with marrow involvement at the time of transplant (i.e. those who could not have an autograft) then the situation is clearer, since allograft patients are likely to undergo great toxicity from the transplant itself and are unlikely to survive it. Also, there is no data to suggest, either in NHL or HD, that patients who have had marrow involvement earlier in the disease but not at the time of transplant do any worse with an autograft than patients who have never had marrow involvement (EBMT registry data, unpublished). Such previous marrow involvement is often quoted as an indication for allogeneic transplant but on the basis of the available data cannot be considered as such. The question of graft versus lymphoma effect in allogeneic transplantation remains controversial and there had been no evidence of such an effect until the most recent presentation of the Seattle group (18). If indeed there is some graft versus lymphoma effect in allogeneic transplantation, then allograft must now be considered more seriously and the question of whether the indications for allogeneic transplant should be age related becomes important. It would seem reasonable that patients under the age of 20-25 years with HD might be considered for an allograft, but this approach may be too toxic for patients with HD over the age of 30 years. One may speculate that morbidity from allogeneic transplant might be more considerable for a comparable regimen in HD than in leukaemia and NHL. The general toxicity of the procedure would be added to the underlying immuno-paresis of the HD patients not generally seen in leukaemia or NHL. Nonetheless, with these recent assertions from Seattle, the whole question of allogeneic transplantation in HD may need significant reappraisal. Our own views have hitherto been reflected in our patient activity, whereby in 250 lymphoma grafts, we have only elected to carry out 3 allogeneic transplants.

Purging

In HD the question of purging seems particularly irrelevant since the exact nature of the Hodgkin's cell remains to be elucidated. If one looks at the

more general problem of purging in lymphoma transplants, it is in B-cell lymphomas in particular that monoclonal antibodies have been most widely used (19), and in which bone marrow purging may seem the most useful. Having always been unattracted to the concept of purging, we have been particularly dubious about its use in lymphoma ABMT since lymphomas can be seen to relapse at the sites of previous disease and not in the autologous marrow. Even if there was contamination of the autologous marrow, its relevance within that relapse is impossible to disentangle because of the predilection to relapse elsewhere. However, this strongly held belief may be challenged by the hypothesis that relapse at previous sites of disease could be due to reinfusion of lymphoma cell in contaminated marrow then making their way to specific sites at which they may have specific homing receptors and appropriate microenvironments allowing them to proliferate. In other words, relapse at sites of previous bulk disease may be coming from infused contaminated marrow. This is a difficult concept to grasp and one which should not lead us to assume that purging the marrow is automatically worthwhile. The only studies that lead one to accept the concept of useful purging in lymphoma come from the purging of overtly contaminated B-cell lymphoma marrows by the Boston group (19) where it has been shown that some of these patients have long term disease free survival. However, large numbers of patients will be needed to evaluate the efficacy of purging and to disentangle the relative contributions of the efficacy of ablative regimens and patient selection from the issue of contaminated marrow. In patients who relapse post ABMT, the use of techniques like clonal immunoglobulin gene rearrangements or polymerase chain reaction may be of benefit in allowing the detection of minimal residual disease in reinfused marrow and its role in patients who relapse post-ABMT.

It is certainly possible that in clinical situations such as B-cell lymphoma and ALL (where purging is most commonly used) we are indeed purging the autograft of residual disease while still failing to cure most patients. The evidence thus may be that it is failure of host ablation rather than failure of purging which results in relapse. An approach to this problem might be to consider the role of adjuvant antibody given to the host as the only means to remove residual disease not sensitive to chemotherapy or radiation.

NON-HODGKIN'S LYMPHOMA

Many of the issues discussed above are as relevant in NHL as in HD. We will discuss here the question of randomised trials, the issue of first remission transplantation and the future for studies in low grade lymphoma.

Randomized Trials of Bone Marrow Transplantation in Non-Hodgkin's Lymphoma and Hodgkin's Disease

Whilst any randomised trial involving allogeneic transplantation in lymphoma is clearly impossible for the moment, there are several which are going ahead in NHL, randomising patients with relapsed disease to conventional salvage chemotherapy versus ABMT. Amongst these are the Parma study

which randomises patients still responsive to the DHAP chemotherapy to either continue with DHAP or undergo ABMT with BEAC (20). This study has taken some years to accrue adequate numbers on a multinational basis. Amongst the problems associated with this study are the heterogeneity of induction therapy rendered unavoidable by the multinational nature of the study. In the British National Lymphoma Investigation (BNLI) relapsed high and intermediate oracle NHL is randomised to either high dose BEAM chemotherapy with a transplant or low dose mini-BEAM repeated on two or three occasions and salvage chemotherapy. The strengths of this latter study are that all the patients come from the BNLI study and have the same induction chemotherapy. Furthermore, the mini-BEAM versus BEAM study gives an opportunity to review dose escalation using the same drugs as a basis for the chemotherapy arm versus the transplantation arm. In this study, patients are also randomised in both arms, to either recover GM-CSF or placebo to study the effects of growth factors in this model situation. However, the BNLI study has certain disadvantages in that it is also recruiting slowly because patients are being selected from one country only, and secondly mini-BEAM can hardly yet be regarded as conventional chemotherapy although there is data emerging that suggests that it is a useful salvage chemotherapy regimen in lymphoma (21). The third disadvantage of this study is that three courses of mini-BEAM do not equate to the dose of BCNU and melphalan that is in the high dose BEAM chemotherapy (Table 1). In HD, the only randomised study is the British National Lymphoma Investigation (BNLI) study, again of BEAM versus mini-BEAM in patients who fail two regimens on the LOPP/EVAP study.

First Remission Bone Marrow Transplantation in Non-Hodgkin's Lymphoma

It is very difficult to define those patients who will have a high probability of relapse from remission once they have entered it. It is much easier to define those who have a high probability of not reaching first remission. Candidates for BMT in first remission would include: (i) patients with massive bulk, diffuse large cell lymphoma and presenting with a high LDH (22); (ii) patients presenting with stage IV peripheral T-cell lymphoma, as such patients always ultimately fail conventional chemotherapy (23); and (iii) adult patients with lymphoblastic lymphoma presenting with CNS and/or bone marrow involvement or a raised LDH who appear most frequently to do badly on conventional chemotherapy even on reaching first remission (24). The structure of these studies will have to be formulated and could take two general forms. Either, patients would complete 6 courses of conventional chemotherapy, or the equivalent, and then be randomised to first remission intensification with ABMT versus no further treatment, or alternatively, patients would be restaged after 3 or 4 conventional courses of chemotherapy, or equivalent, and those in first complete remission or very good partial remission randomised either to intensification with ABMT or to 3 further courses of conventional chemotherapy or equivalent. Both these structures for a

randomised study are reasonable and it is likely that different groups will use different formats.

The special problems associated with attempted first remission transplantation in NHL, in terms of a prospective study, have already been demonstrated by the relatively unsuccessful attempts to get underway an international study by the EBMT into first remission transplantation of T-cell lymphoblastic lymphoma. The data in the literature for the conventional therapy of the poor prognosis patients presenting with CNS and/or bone marrow disease at diagnosis shows that for adults at least, no more than 20% have long term survival (24). In the EBMT registry, if one selects such patients who have then gone on to have a transplant in first remission, the long term disease free survival from first remission looks to be about 60% in this heterogenous group of patients, induced and consolidated in various ways and reported to the registry after transplantation. An attractive proposition has therefore been to try and begin a randomised study of such poor prognosis patients with common induction and consolidation and randomising at the time of remission to an early first remission transplant with CY/TBI versus the harvest of bone marrow and delaying the transplant until relapse or second remission. Whilst thought by many members of the EBMT to be an excellent study, this study has failed to attract significant recruitment for a variety of reasons. These reasons include the fact that lymphoblastic lymphoma is an uncommon disease and the fact that many of these patients are not induced and consolidated at the transplant centres but referred in from elsewhere. Their induction and consolidation therapies may therefore be beyond the control of the transplant centres. The study may therefore have to be restructured and accept a variety of induction and consolidation therapies before entering the patient to the study at the time of transplant.

Special Problems Associated with Transplantation in Low Grade Lymphoma

A variety of single centre one arm pilot studies (25-26) have suggested that patients with low grade lymphoma can do well with ABMT. These studies have used protocols in which patients with minimal residual disease are selected for elective chemotherapy/TBI autograft after "in vitro" bone marrow purging with monoclonal antibodies. Such studies are in danger of becoming anecdotal folklore unless they enter patients with a prognosis on conventional therapy comparable to that of the transplant group. For such studies, one must consider patients in second or third remission who might have a 3-5 year survival on conventional therapy and prospectively randomise them in a study versus a transplantation arm in which purging is itself randomised. Low grade lymphoma is a common enough disease for this to be contemplated on a multinational basis and the time is ripe for such a study to begin.

OVERVIEW

ABMT in lymphoma is an exciting clinical area and holds real promise in these conditions for which sensitivity to drug and radiation therapy remains, even in relapse. However, we must not forget that the long term toxicity of the high dose regimen is at present unknown and we must remember that with agents eg., high dose BCNU hitherto used some years ago for CNS tumours in childhood, there is now evidence emerging of long term toxicity, such as serious lung pathology in many recipients (27). The concept of greater and greater dose escalation of such agents, perhaps made possible by including growth factors in clinical studies in the short term, must be tempered by consideration of the consequences of such regimens ten years on, a not unrealistic prospect for some lymphoma patients.

Finally, it is a source of no little amazement to us that on the North American continent, bone marrow transplantation has become an accepted form of treatment in many lymphomas with no clear cut evidence whatsoever from prospective studies of benefit over conventional salvage chemotherapy. Although in a sense this is gratifying to those of us who have promoted lymphoma transplant for a number of years, it cannot be the correct way to proceed when we are beholden to maintain clinical ethics, scientific standards and the conservation of resources. We should not repeat the follies of fifteen years during which time the issues in relation to bone marrow transplantation in acute leukaemia have never been resolved. Only now are appropriate large scale randomised studies of transplantation versus conventional chemotherapy in acute leukaemia being carried out. The same must not be allowed to happen in the lymphoma field.

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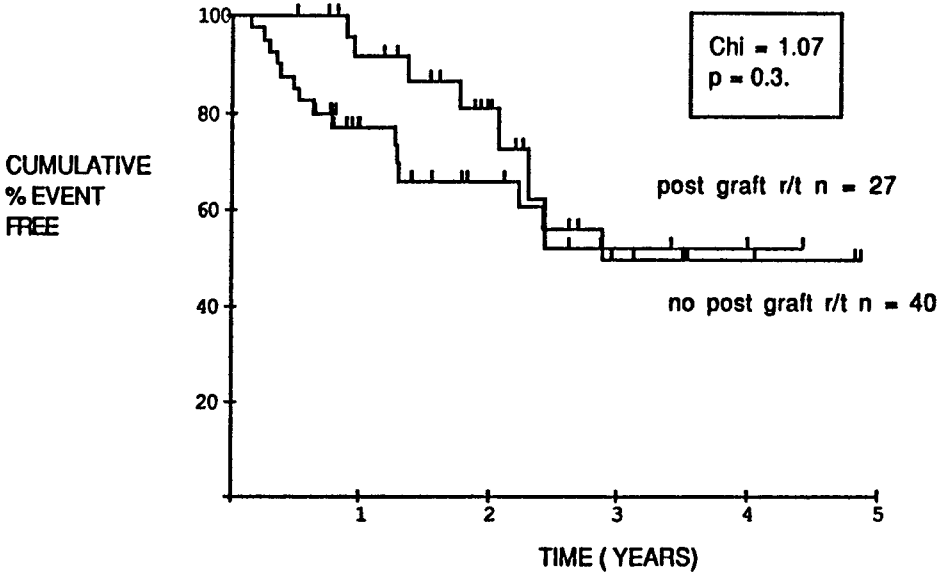
TABLE 1

BEAM / Mini-BEAM Relative Doses

	BEAM	Mini-BEAM
BCNU	300mg/m^2	60mg/m^2
ETOPOSIDE	800mg/m^2	300mg/m^2
ARA-C	1600mg/m^2	800mg/m^2
MELPHALAN	140mg/m^2	30mg/m^2

FIGURE 1

Relapse or Progression Free Survival After BEAM and ABMT in Hodgkin's Disease: The Effect on Outcome of Early Post-Graft Radiotherapy in Patients in PR or NR After ABMT.



A COCKTAIL OF MONOCLONAL ANTIBODIES AGAINST HLA-Dp/Dr AND ADHESION MOLECULES (CD56 AND CD54) AS A TOOL FOR MAGNETIC PURGING OF BONE MARROW IN MULTIPLE MYELOMA

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INTRODUCTION

Multiple Myeloma (MM) is a malignant disease of Plasma cells, primarily residing in the bone marrow. Conventional therapy consists out of regular courses of chemotherapy containing alkylating agents and localised radiotherapy. Although good responses are initially obtained, the mean survival is estimated to be 3.5 years. Since more than 30% of newly diagnosed patients are under the age of 60 years, more intensive treatment strategies, aiming to cure the disease, have been worked out. High dose chemoradiotherapy with autologous bone marrow transplantation (ABMT) has given promising results (1) in otherwise end stage patients. Since ABMT is performed with cell suspensions containing large quantities of malignant cells, purging of the NM marrow, might enhance the outcome. Considering the possibility of immunological purging, the immunobiology of multiple myeloma has to be taken into consideration. Starting as the level of pre B cells and leading to the plasma cell stage, intraclonal maturation in MM occurs. Using different approaches, several authors (2,3) have shown that precursor plasma cells with immature B cell characteristics and surface antigens, are part of the malignant clone. B cell antigens disappear at the final plasma cell stage, while a gain of few lineage non-specific antigens is seen. They are scarcely and heterogeneously distributed. Recently we have reported the strong presence of CD56 (Leu19; HNK-1) on malignant plasma cells (4). Since CD56 is accepted to be the Neural-cell adhesion molecule (N-CAM) (5), we undertook a search for other adhesion molecules in MM. In this study, we report on the in vitro use for immunological purging of antibodies against adhesion molecules CD56 and CD54 and against a monomorphic epitope shared by HLA-DR and HLA-DP antigens present on all precursor plasma cells.

MATERIAL AND METHODS

Patients

Bone marrow samples of twenty randomly accrued MM patients and five patients with monoclonal gammopathy of undetermined significance (MGUS) were analysed for cytoadhesion molecules. NM patients were clinically staged according to Durie and Salmon. Six patients were in stage I, two in stage II and twelve in stage III. Thirteen MM patients were previously untreated, three were relapsing and four had refractory disease at the moment of analysis. Bone marrow aspirates of 5 hematological normal individuals served as controls.

Myeloma Cell Lines

The origin and main features of the ten myeloma cell lines used in this study have been published. The cell lines 8226/Dox and L363 were provided by Dr. B. Durie (Arizona Cancer Centre, AZ). The LP-1 cell line was a gift of Dr. L. Pegoraro (Institute de Medicina Interna, Torino, Italy). The ARH-77 and RaJI (Burkitt lymphoma) cell line was provided by Dr. R. Levy (Stanford University, CA). Six other lines (Fravel, Karpas 707, OPM-1, JJN3, EJM and U266) were provided by Dr. Nilson (University of Uppsala, Sweden). Cell lines were cultured in RPMI 1640 medium supplemented with 5% fetal calf serum, antibiotics and L-glutamine (Gibco Europe, Belgium) at 37C in a humidified 5% $C0_2$ incubator. Before labeling cells were washed in phosphate buffered saline (PBS) containing 1 % bovine serum albumin (BSA).

Monoclonal Antibodies

CD56 (N-CAM) expression was characterised by Leu19 (Becton Dickinson, Mountain View, CA) or NKH-LA (Coulter, Immunology Hialeah, FL). CD56 (ICAM-1) was detected by RR 1/1 produced and kindly provided by Dr. Rothlein (Dana-Farber Cancer Institute, Boston, MA). Leu-CAM's were identified by a monoclonal antibody with specificity for the common beta chain (CD18) (Janssen Biotech, Olm, Belgium). The monoclonal antibody DH12 directed against the common beta-1 subunit of the VLA antigens, was a generous gift of Dr. J. Cassiman (University of Leuven, Belgium). The monoclonal antibody 2c9 recognized a frame work antigen of HLA Dp/Dr as defined by the IVth leukocyte typing conference (8).

Phenotypic Analysis

For the detection of antigen expression on plasma cells of patients, use was made of cytocentrifuge preparations of mononuclear cell suspensions isolated from bone marrow, and peripheral blood samples by Ficoll-Hypaque centrifugation. These preparations were made immediately after sample collection and stored at -20C until analysis. After thawing, the glass slides were labeled using an immunogold-silver staining method described elsewhere (6). Surface antigen expression of myeloma cell lines was performed by immunogold silver staining of cells in suspension (7). All slides were examined in bright-field microscopy.

Immunomagnetic Purging Elements

The efficacy of the purging procedure was examined using several cell lines, marked in advance with the DNA stain H342 (Calbiochem, La Jolla, CA). Purging conditions were simulated by mixing labeled tumor cells with nominal mononuclear bone marrow cells. After incubation with each monoclonal antibody an antibody combination, cells were incubated with goat anti mouse IgG coated Dynabeads M450 and separated using a magnetic particle concentrator (MPC- 1) (Dynal, Oslo, Norway). After treatment remaining tumor cells were counted using a Leitz fluorescence microscope. The clonogenic capacity of the bone marrow before and after immunomagnetic purging was assessed by the CFU-GM assay.

Myeloid colonies were cultured in a double layer system using human placentas conditioned medium as source of colony stimulating activity. After 12 days of culturing at 37C and 5% $C0_2$, colonies consisting of at least 40 cells were counted using an inverted microscope.

RESULTS

Expression of Adhesion Molecules

Plasma cells. The expression of adhesion molecules on plasma cells was examined by immunogold-silver staining of fixed cytospin preparations of mononuclear cell suspensions of bone marrow. An individual was accepted as being positive, if more than 50% of his plasma cells were expressing the antigen. In 80% of the MM cases N-CAM was expressed. ICAM-1 was positive in 95% of the patients while 55% of the patients expressed the integrin beta-2 subunit (table 1). The expression of the latter molecule was very weak in all positive cases. The mean number of N-CAM positive plasma cells was 78% with a range of 4 to 100%. ICAM-1 and LEU-CAM showed a mean expression of 94% and 54% with a range of respectively 30 and 1 to 100%. Among 5 hematological normal individuals no significant N-CAM positivity of the plasma cells was observed. In all cases normal plasma cells expressed ICAM-1 very strongly while CD18 expression was very weak or absent. Circulating plasma cells of one PCL patient and two out of four end stage NM patients were N-CAM negative. In all cases circulating plasma cells expressed ICAM-1 while Leu-CAM was negative for the PCL patient and one end stage MM patients.

Myeloma Cell Lines. The results of the phenotypic analysis are summarized in Table 2. The N-CAM antigen was expressed in three cell lines (LP-1, Karpas 707 and OPM-1) which all lacked a co-expression of B cell antigens. The seven N-CAM negative cell lines included are those that were derived from PCL patients and the one derived from an extramedullary myeloma patient. ICAM-1 was strongly expressed in all cell lines with the exception of OPM-1. The beta-1 integrin antigens showed also a broad distribution and were only lacking in one cell line (U266). The beta-2 integrins were variably present without defining a clear picture.

Reactivity with Progenitor Cells

The results of CFU-GM recoveries are presented in Figures 1a and 1b. The use of the HLA class H reacting antibody 2c9 in combination with complement resulted in a nearly complete inhibition of progenitor cell growth. When 2c9 was used, combined with magnetic beads, a CFU-GM recovery up to 80% could be observed. After complement lysis of normal marrow cells with monoclonal antibodies against adhesion molecules CD54 and CD56, no significant inhibition of CFU-GM growth was observed.

Immunomagnetic Purging of Tumor Cells

With a tumor cell to bead ratio of 1:100 and two consecutive treatments the use of 2c9 resulted in a depletion efficiency of 4 logs in a mixture containing 1% tumor cells. In the same conditions, combination of monoclonal antibodies against the CD54 and CD56 antigens removed from a mixture containing 10% Karpas 707 or OPM1 myeloma cells respectively 4 and 2.9 logs of tumor cells.

DISCUSSION

Following a previous study (4), in which we demonstrated that most malignant plasma cells strongly express the CD56 (N-CAM) antigen, we analysed the expression of other adhesion molecules on myeloma cells. The integrin beta-1 subunit, associated with the VLA antigens, was widely expressed in plasma cells of all patients and on myeloma cell lines.

The pattern of expression of the beta-2 integrins was found to be more heterogenous. Only 55% of MM patients had a detectable CD 18 expression, while the CD 11 subunits were not always clearly co-expressed, CD54 (I-CAM) was found to be strongly present on normal and malignant plasma cells, as well as in most myeloma cell lines. The expression profile of CD56 (N-CAM) was substantially different compared to the other adhesion molecules. CD56 is present on plasma cells of 80% of MM patients, but absent on normal, MGUS or leukaemic plasma cells. Only 3 out of 10 MM cell lines were N-CAM positive. Cell lines derived from plasma cell leukaemias, extramedullary plasmacytoma or refractory myeloma were N-CAM negative. These findings suggest that adhesion molecules, especially N-CAM, play a critical role in the interaction of plasma cells with the bone marrow environment. Since N-CAM and I-CAM are strongly expressed by malignant plasmacells, and nearly absent on other haemopoietic cells, we analysed their possible use for immunological purging. In order to ascertain the depletion of the malignant B cell clone - including the precursor plasma cells - we added the monoclonal antibody 2c9 directed against the HLA Dr/Dp framework antigen (8). Using Ig gene rearrangement analysis we proved in a previous study that lymphoid cells enriched by this antibody were part of the malignant clone (3). Our in vitro studies provide evidence that simultaneous use of CD56 and CD54 as well as HLA-Class II antibody in an immunomagnetic purging setting, is not toxic for the haemopoietic stem cell. More than 4 logs depletion was obtained when myeloma cell lines were tested as target cells. Up to now, only the HLA class

II antibody 2c9 was used in a clinical ABMT setting and gave a fast haemopoietic recovery and stable engraftment (data not shown). Other workers have used monoclonal antibodies such as PCA-1, BL-3 and J5 in order to remove myeloma cells (9). However, the heterogeneity and often weak expression of these plasma cell antigens do not permit a generalised use. Recently, an immunotoxin was described claimed to recognize the whole B cell maturation cycle (including plasma cells) and offering a good purging efficiency (10). The nature of this antigen is not well defined and might not cover all malignant cells. We therefore conclude that the use of a cocktail of monoclonal antibodies directed against the cytoadhesion molecules CD56 and CD54, as well as an HLA class II antigen structure is an optimal candidate in immunological purging of MM marrow.

ACKNOWLEDGEMENTS

This study was supported by grants no. 3.0033.88 from the National Foundation of Scientific Research (NFWO) Belgium and the "Kom op tegen Kanker" Foundation, Belgium.

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TABLE 1

Expression of Adhesion Molecules in Plasma Cells

	N-CAM (CD56)	ICAM-1 (CD54)	β1 integrins (CD29)	β2 integrins (CD18)
Bone marrow PC				
MM	16/20	19/20	15/15	11/20
MGUS	1/5	5/5	2/2	5/5
normal	0/5	5/5	5/5	4/5
Circulating PC				
PCL	0/1	1/1	1/1	0/1
MM(a)	2/4	4/4	3/3	1/4

TABLE 2

Expression of Adhesion Molecules in Myeloma Cell Lines

Cell line	CD56	CD54	CD18	CD11a	CD11b	CD11c	CD29
L363	0	100	0	0	0	0	100
JJN3	0	100	0	0	0	0	100
U266	0	100	0	0	0	0	0
LP-1	97	100	0	0	0	0	94*
FRAVEL	0	100	100	100	47	0	100
KARPAS 707	100	100	19	22	0	0	100
OPM-1	100	0	76	81	0	0	100
ARH-77	0	100	100	100	0	0	100
EJM	0	100	0	0	0	0	100
8226/Dox	0	100	100	100	0	0	100

Results are presented as percentage of positive cells.
* very weak expression.

Figures 1A and 1B

Recovery of precursor cells was determined after immunomagnetic treatment and complement lysis of normal bone marrow samples. Results are presented as percentage recovery of normal CFU-GM growth.

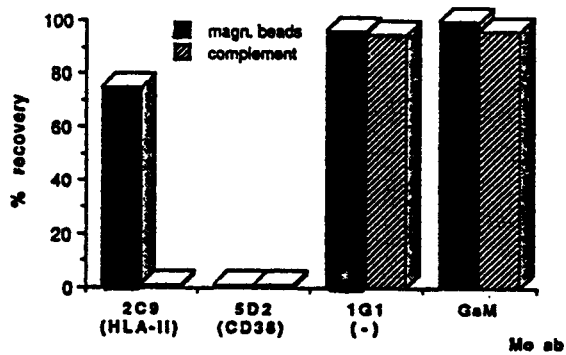


Fig 1a

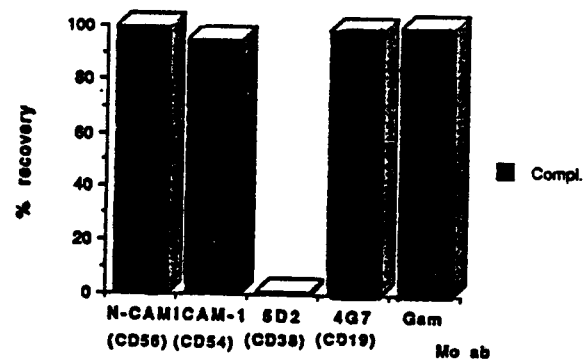


Fig 1b

Figure 2A

Mononuclear bone marrow cells were mixed with H342 stained RAJI cells (Burkitt Lymphoma line) in a ratio of 99:1. After incubation with the HLA class II antibody 2c9 and goat anti- mouse coated Dynabeads M450, cells were separated by magnet. Effects of one and two cycles of treatment were compared.

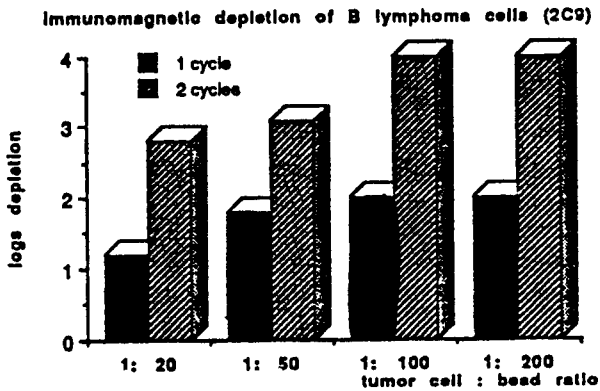
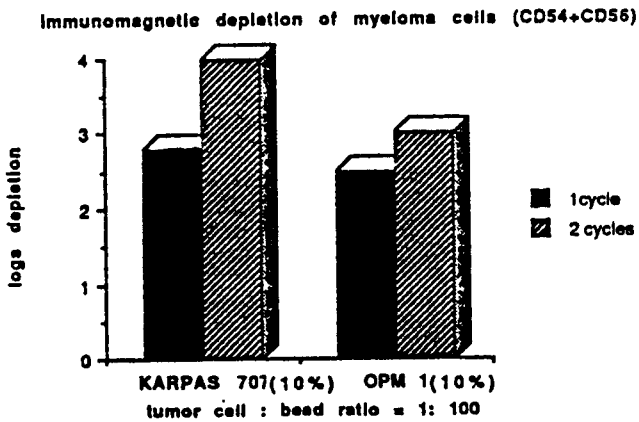


Figure 2B

Mononuclear bone marrow cells were mixed with H342 stained Karpas 707 and OPM-1 cells in a ratio 9:1. The mixtures were immunomagnetically treated with the combination of antibodies against the CD54 and CD56 antigens.



REPEATED HIGH DOSE THERAPY IN REFRACTORY MYELOMATOSIS

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SUMMARY

A second cycle of high dose therapy was administered to 20 patients with advanced and refractory myelomatosis upon disease progression from a preceding course after a median of 10 months. All 20 patients received at least one cycle that was supported by hemopoietic stem cells including 8 patients receiving transplants with both courses, 3 only with the first and 9 with the second cycle. of a total of 28 cycles with transplantation, 15 included total body irradiation (TBI, 850 cGy) with either melphalan or thiotepa; 18 cycles were supported with autologous marrow, 6 with blood stem cells, 2 with both autologous marrow and blood stem cells, and 2 with allografts. Of the 15 patients receiving autotransplants with a second cycle of high dose therapy, stem cell collection was performed after the first cycle in 12 including 5 patients following prior transplantation (3 with TBI). Eighty percent achieved > 75% tumor cytoreduction with the first cycle (CR, 15%), with a median progression-free survival duration of 6 months; 75% responded to the second cycle (CR, 0%) with a median progression-free survival of 3.5 months; the overall median survival was 22 months. Median progression-free survival was longer after TBI-containing regimens, especially when administered with the first cycle of high dose therapy. In the absence of cumulative extramedullary toxicity, morbidity was related mainly to the duration of marrow aplasia which was about 2 weeks shorter after the first than after the second cycle of high dose therapy. These results demonstrate that even older patients (median age, 50 years) can tolerate repeated including marrow-ablative chemo(radio)therapy, justifying our expectation that current trials with double transplants within 6 months and with autografts obtained entirely prior to the first cycle of high dose therapy may be less toxic and more effective, especially when applied during remission.

INTRODUCTION

There has been an increasing interest in applying intensive therapy for patients with symptomatic myelomatosis. Thus, worldwide, almost 500 patients have been reported to have undergone autologous (about 350) or allogeneic transplantation (about 150 patients) (1). We have recently reported our results with TBI and melphalan or thiotepa in 55 patients receiving marrow autografts following induction or salvage therapy with the VAD regimen (2). Over 80% of the 41 patients treated in remission or for primary drug resistance were projected to be alive at 4 years compared to a median survival of only 7 months among the 14 patients presenting with resistant relapse. In the absence of an adverse impact of tumor cell contamination of marrow autografts on relapse-free or overall survival in that study, current clinical trials employ more intensive remission induction with mutually non-cross resistant combinations such as VAD (3), intermediate doses of melphalan (4), EDAP (5), to be followed by 2 cycles of melphalan at 200 mg/M^2 3 to 6 months apart, supported by marrow and blood stem cells harvested prior to marrow-ablative therapy.

We report here our experience with 2 successive cycles of high dose therapy in 20 patients with advanced myeloma refractory to alkylating agent therapy and VAD, who received at least one of the 2 cycles in conjunction with hemopoietic stem cell support. This pilot study was conducted in selected patients with refractory myeloma who, in anticipation of relapse even despite high dose therapy, had sufficient quantities of stem cells collected either prior to any high dose therapy or who underwent additional stem cell collection upon recovery from a preceding high dose therapy regimen.

PATIENTS AND METHODS

Patient Characteristics

Demographic characteristics and details of therapy and stem cell support are summarized in Tables 1 to 4. Three patients underwent transplantation with their first treatment, 9 with their second and 8 with both cycles of therapy (Table 2). Of the 28 courses with transplantation, 18 were supported with 6 autologous bone marrow, 6 with peripheral blood stem cells (6), 2 with allografts, and 2 patients received both autologous bone marrow and blood stem cells. The marrow-ablative regimen consisted of TBI (850 cGy) with either melphalan (140 Mg/M^2; 12 patients) or thiotepa (750 mg/M^2; 3 patients); 13 patients received other regimens such as melphalan 140 mg/M^2 without TBI (one patient), melphalan 200 mg/M2 (4 patients), busulfan and cyclophosphamide (2 patients) or a combination of cyclophosphamide, BCNU and etoposide (CBV; 6 patients) (Table 3). Five patients received TBI with the first and 10 with the second course of treatment. The time interval between the 2 courses of therapy ranged from 3 to 51 months with a median of 10 months (Table 3).

Stem Cell Collection

Of the 15 patients receiving autotransplantation with the second cycle of therapy, 3 had stem cells collected before and 12 after the initial high dose therapy regimen, including 5 after transplantation 3 of whom had received TBI (Tables 3 and 4). The time interval to stem cell collection after the initial cycle of high dose therapy ranged from 3 to 18 months (median, 9 months); all 3 patients with prior TBI had collections performed after a minimum of 5 months (Table 4). The median yield from all autologous bone marrow collections was 2.2×10^8 mononuclear cells per kilogram (range 1.1 to 4.3), and the yield from blood collection 3.8×10^8 cells per kilogram (range .7 to 7.5). one marrow collection procedure was sufficient to procure enough stem cells in all patients not yet exposed to high dose therapy, while a median of 2 procedures were required for patients who had received prior intensive therapy ($p=.3$).

Antitumor Effect

The initial cycle of intensive therapy effected objective responses (>75% tumor cytoreduction) in 80% of patients with a complete remission rate of 15% (Table 5). The median progression-free survival was 6 months (range, 1 to 25 months) and significantly longer among the 5 patients who had received TBI as part of their initial regimen (8 vs 3 months, $p=.03$) (Figure 1). The second cycle of high dose therapy included TBI in 10 patients, none of whom died from toxicity and 9 patients achieved remission; this compares with one early death and 6 responses among 10 patients not receiving TBI. Unlike the observation with the first treatment cycle, inclusion of TBI with the second course did not extend progression-free survival beyond results achieved without TBI. Although progression-free survival curves after the first and second cycle of therapy were superimposable (Figure 2), added TBI extended progression-free survival durations beyond those observed after the corresponding 15 courses without TBI (medians of 7 and 3 months; $p=.09$) (Figure 3). Total survival following the initial cycle of high dose therapy ranged from 5 to 64 months with a median of 22 months (Figure 4), which compared to a median of 15 months for 21 other patients receiving only one cycle of TBI-containing high dose therapy for VAD-refractory myeloma ($p=.2$).

Hematologic Recovery

Hematologic recovery was significantly faster after the first than after the second cycle of high dose therapy; thus, levels exceeding 500 granulocytes/ul and 50,000 platelets/ul were reached after 3 weeks following the first cycle compared to 5 and almost 7 weeks, respectively, after the second cycle of treatment ($p=.02$, granulocytes; $p=.002$, platelets) (Table 6). Shorter time intervals from first therapy (< one year) and younger age (< 50 years) were also associated with a prompter recovery, but only for the first cycle of high dose therapy administered. Thus, the above blood counts were reached

within 3 weeks in younger patients with shorter treatment as compared to almost 4 weeks for those with one or both adverse features ($p < .1$).

CONCLUSION

In the absence of cumulative extramedullary toxicity, bone marrow-ablative treatment could be administered relatively safely even when hemopoietic stem cells had been collected after prior high dose therapy. Longer progression-free survival after TBI attests to the dose-intensity concept also pertaining to myelomatosis, as had been evident from comparisons (7) of groups of patients receiving melphalan alone or with added TBI. These data provide the background rationale for current investigations that explore the value of repeated high doses of melphalan (200 mg/M^2) (8) supported with hemopoietic stem cells obtained exclusively prior to the first cycle of such high dose therapy. It is anticipated that hematologic recovery will be faster than when stem cells were collected after high dose treatment. Based on the extension of remission and survival from maintenance therapy with interferon--alpha (9), our current "Total Therapy" program for newly diagnosed patients with symptomatic myeloma calls for post-transplantation maintenance therapy with this biologic agent until relapse.

ACKNOWLEDGEMENT

Supported in part by CA 37161, CA 23077 and CA 28771-11 from the National Institutes of Health, Bethesda, MD.

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TABLE 1

PATIENT CHARACTERISTICS

N	20
Age > 50 years	8
Disease Status	
Primary Unresponsive	10
Resistant Relapse	10
Tumor Mass	
Intermediate/High	9
Immunoglobulin Isotype	
IgG	12
Other	8
VAD-Refractory	20

TABLE 2
Details of High Dose Therapy

Regimen	N	Median Months Between Treatments (Range)
no Transplant - Transplant	9	8 (3 - 39)
ABMT	7	
ABMT + APBT[a]	1	
APBT	1	
Transplant - no Transplant	3	13 (8 - 35)
ABMT	1	
APBT	2	
Transplant - Transplant	8	15 (6 - 51)
ABMT - Allo BMT	1	
ABMT - ABMT	3	
ABMT - ABMT + APBT	1	
APBT - ABMT	2	
APBT - Allo BMT	1	

[a]ABMT, Autologous Bone Marrow Transplantation
APBT, Autologous Peripheral Blood Transplantation

TABLE 3
Details of Therapy

Parameter	N
Total High-Dose Therapy (HDT) courses	40
HDT courses with TBI	15
HDT courses with Transplant	28
Patients receiving TBI	15
Patients receiving Transplant	20
Time Interval HDT-1 to HDT-2	
$\leq$ 12 months	12
$\geq$ 12 months	8
Stem Cells Collected for HDT-2	
Prior to HDT-1	3
Post HDT-1	
$\leq$ 12 months	8
$\geq$ 12 months	4

TABLE 4
Stem Cell Collection

Parameter	N
Collected prior to HDT-1	14
for HDT-1	10
for HDT-2	3
for HDT-1 and HDT-2	1
Collected after HDT-1	5
after Transplant without TBI	2
after Transplant with TBI	3
after HDT-1 without Transplant	7
Time Interval after HDT-1	
> 12 months (TBI)	4 (1)
6-12 months (TBI)	3 (1)
< 6 months (TBI)	5 (1)

TABLE 5
Response Analysis

Regimen	N	%R	%CR	%ED	Median Months PFS*
HDT-1	20	80	15	0	6
+ TBI	5	100	0	0	8
- TBI	15	73	20	0	3
HDT-2	20	75	0	15	3.5
+ TBI	10	90	0	0	3.5
- TBI	10	60	0	20	3

*progression-free survival

TABLE 6
Hematologic Recovery

Parameter	N	Median Days to Granulocytes > 500/μL	p	Median Days to Platelets > 50,000/μL	p
HDT-1	20	23	.02	22	.002
HDT-2	20	34		47	

FIGURE 1

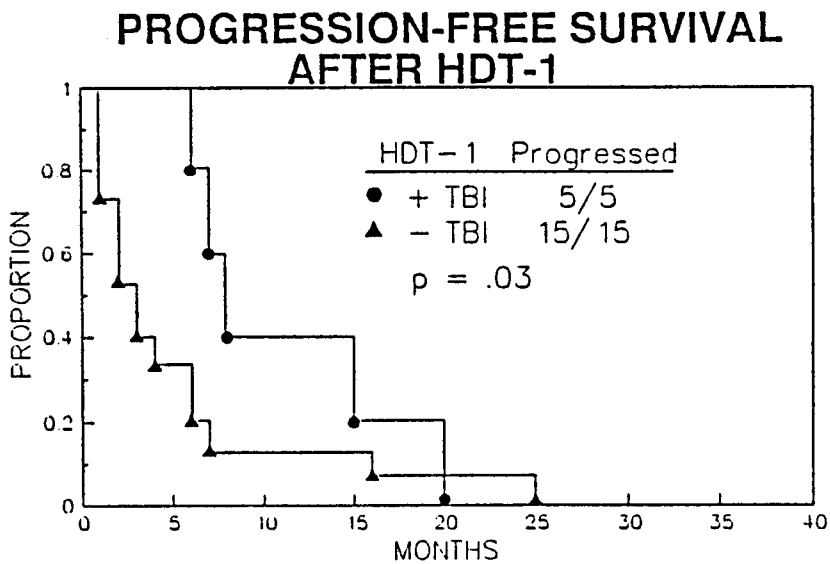


FIGURE 2

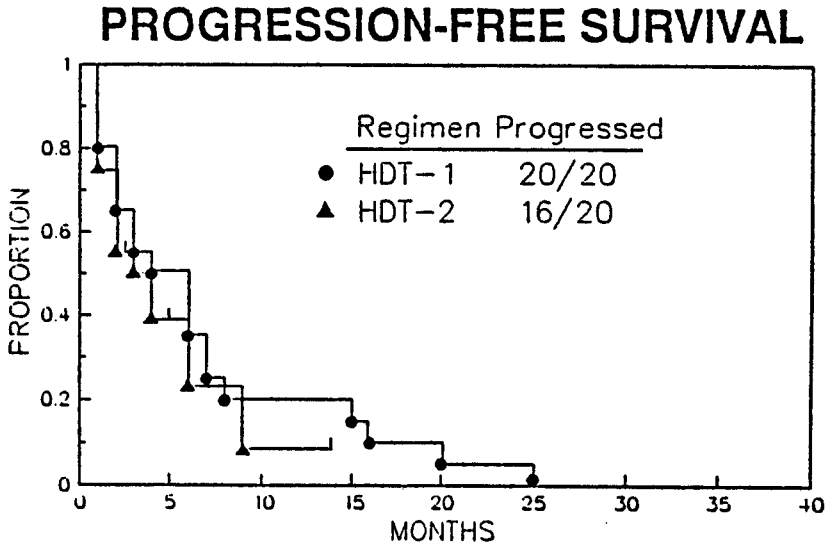


FIGURE 3

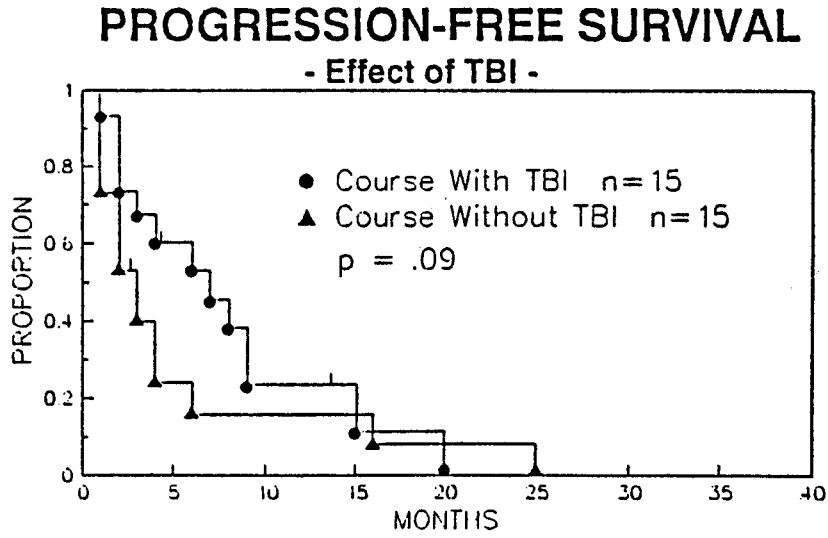
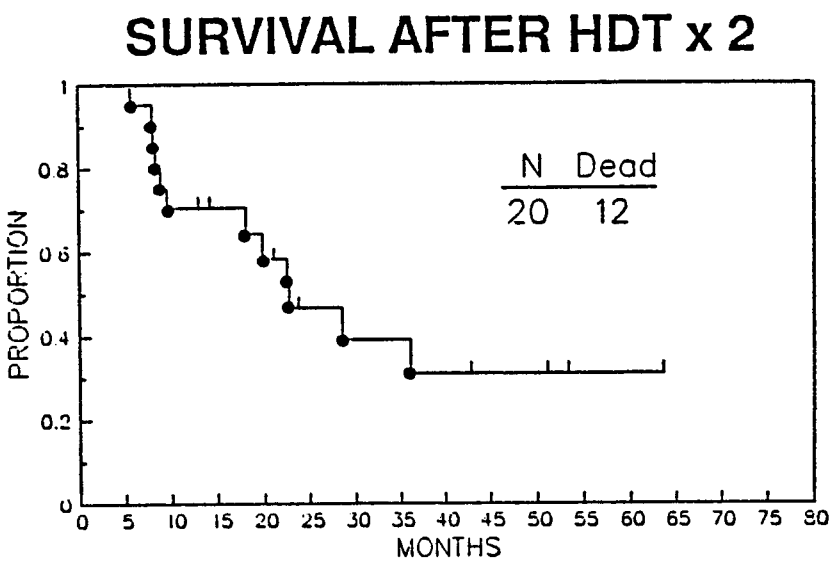


FIGURE 4


SURVIVAL AFTER HDT x 2
N Dead
20 12
PROPORTION
0
0.2
0.4
0.6
0.8
1
0 5 10 15 20 25 30 35 40 45 50 55 60 65 70 75 80
MONTHS

AUTOLOGOUS BLOOD STEM CELL TRANSPLANTATION IN HIGH RISK MYELOMA

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INTRODUCTION

Autologous blood stem cell transplantation (ABSCT) has been proven to be feasible for the treatment of patients with various hematological malignancies, in particular chronic myelogenous leukemia (1) and acute leukemia (2). Recently, high-dose chemotherapy or chemoradiotherapy followed by autologous (3,4,5) or allogeneic (6) bone marrow transplantation (BMT) has been proposed as treatment for patients with high-risk multiple myeloma (MM). The use of allogeneic BMT is limited by the low number of MM patients under the age of 45-50 years with an HLA identical donor. The feasibility of autologous BMT (ABMT) has previously been demonstrated in MM patients (3,4,5). Even if CR can be achieved when a significant number of "mature" malignant cells are still present in the transplant products (3,5), ABSCT may be an interesting alternative to ABMT, as peripheral blood stem cells (PBSC) may be less contaminated with malignant cells than bone marrow (7). Moreover hemopoietic reconstitution following ABSCT may be quicker than that following ABMT (2) and thus may make this treatment safer for older patients.

This report describes our experience in 18 patients with high-risk MM who received high-dose cyclophosphamide (HD CYC) followed by PBSC collection and subsequently underwent ABSCT.

PATIENTS AND METHODS

Eighteen patients (14 males 4 females) with a median age of 48 years (range : 33 - 57) underwent ABSCT for high-risk MM. At diagnosis, 15 patients had stage III MM according to the Durie-Salmon staging (8) while one patient had a stage I MM and two others, plasma cell leukemia. The monoclonal component was IgG in 12 cases, IgA in three cases and pure Bence-Jones in the three other cases. Prior to transplantation all patients received HD-CYC (7 g/m^2) in order to collect PBSC (9). Ten patients (Group

A) received HD-CYC as early therapy after diagnosis (median time between diagnosis and HD-CYC: 4 months, range 3-11) and had received before only one type of chemotherapy: melphalan - prednisone (MP) regimen (10) in four cases, VAD regimen (11) in five cases, M2 protocol (12) in one case. Eight others patients (Group B) received HD-CYC as third or subsequent therapy (median time between diagnosis and HD-CYC : 31.5 months, range : 23 - 72). All these patients had received at least two different types of chemotherapy regimens prior to HD-CYC : VAD and MP regimens in four cases, VAD and VMCP or VBAP (13) regimens or M2 protocol in four cases. Two Group A patients and six Group B patients were considered non-responsive to chemotherapy at the time of starting HD-CYC, as the decrease in the tumor burden was less than 50 % (14). After HD-CYC induced aplasia, at least six leukaphereses per patient were performed to collect PBSC. Thereafter the bone marrow was harvested as a precautionary measure.

Before transplantation, the conditioning regimen consisted of a combination of high-dose melphalan (140 mg/m2) (HDM) and fractionated total body irradiation (TBI) (8 - 15 Gy) for 17 patients. Before HDM - TBI, three of the 17 patients received cyclophosphamide (60 mg/kg/day on two consecutive days). One patient in whom the CFU-GM content of the leukaphereses and bone marrow products were low received HDM alone. Twelve patients received PBSC alone, while six other patients in whom the CFU-GM content of leukaphereses products was below 2 x 10^4 kg received both bone marrow and PBSC. After transplantation, complete remission (CR) was defined by the disappearance of marrow plasma cells and the monoclonal component (the latter evaluated by immunoelectrophoresis and immunofixation) and partial remission (PR) by a decrease of at least 50% in measurable paraprotein and bone marrow infiltration (4).

RESULTS

No toxic death was observed and all the patients were evaluable for hematological recovery and response after transplantation. The median number of CFU-GM cells infused was 5.3 x 10^4 kg (range 2.3 - 69.4) in the 12 patients (Group A: 9, Group B: 3) who received only PBSC and 2 x 10^4 kg (range 0.4 - 7) in the six patients (Group A: 1, Group B: 5) who received both bone marrow and PBSC. Engraftment was observed in every case, but one of the three patients who received less than 2 x 10^4 CFU-GM /kg required subsequent GM-CSF treatment. The median time to reach 500 granulocytes/mm3 and 50,000 platelets/mm3 was 13 days (10 - 93) and 45 days (4 - 200) respectively.

After transplantation, seven patients achieved PR and 11 patients CR. Four patients (PR : 2 ; CR : 2) relapsed 5, 5, 10 and 18 months after ABSCT while 14 patients are still in CR (9 cases) or PR (5 cases) with a median follow-up of 16 months (3 - 25) after ABSCT. It is interesting to note that only one of the ten Group A patients relapsed as compared with three of the eight Group B patients.

DISCUSSION

This study reports the preliminary results of autologous transplantation in high-risk MM using PBSC collected after HD-CYC treatment. After ABSCT, the rate of hematopoietic reconstitution was not significantly different from that observed in other studies of ABSCT in MM patients (7, 15, 16).

Considering the high levels of CFU-GM in the blood after HD-CYC induced aplasia in man (17) we have applied this strategy to MM. The median number of CFU-GM collected in Group A patients was higher than that collected in Group B patients (5.3 vs 0.65 x 10^4/kg). This may be explained by the shorter time interval between diagnosis and PBSC collection which is probably related to the number of courses of chemotherapy previously received. In another report, low levels of blood CFU-GM were observed in heavily pretreated MM patients (18). Reducing the time between the diagnosis and the PBSC collection may be effective in increasing the number of hemopoietic stem cells harvested. Hemopoietic growth factors such as GM-CSF. may help to achieve this goal (19).

The time of transplantation may be important to obtain the best results with either auto or allografting in MM. Two groups suggested transplantation as first-line therapy for patients with high-risk MM and recommend BMT in patients who have failed to respond to second-line treatment (3,20). Our results would not recommend ABSCT for chemoresistant patients. Even if some response can be achieved, a high relapse rate is usually observed (3 relapses in 8 patients). However we observed a high CR rate in our study even though it may be explained by the fact that ten of the 18 patients were transplanted early after diagnosis.

The absence of malignant mature plasmocytic cells in the transplant product does not seem to be an absolute criteria for CR after ABMT for MM (5). However, a decrease in the number of tumor cells may be an important feature to increase the CR rate and/or the duration of response after transplantation. Some in vitro studies of peripheral blood collected from NM patients suggest that the malignant clone is present amongst circulating cells (21). However, a recent study using similar techniques did not confirm these results (22). Previous studies reported that malignant cells were not detectable in leukaphereses products collected after high-dose chemotherapy (7, 15, 23).

A comparison between PBSC and bone marrow cells using highly sensitive methods to detect residual disease and between the results of ABMT and ABSCT are needed to assess the importance of PBSC collection if transplantation is to become an efficient means of treatment for high-risk MM.

ACKNOWLEDGEMENTS

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OUTCOME FOR CHILDREN WITH NEUROBLASTOMA RECEIVING MARROW-ABLATIVE TREATMENTS SUPPORTED BY AUTOLOGOUS MARROW INFUSIONS

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INTRODUCTION

Children with neuroblastoma (NBL) mostly present with metastases and their cancer usually becomes chemoresistant after first responding to treatment (1). Patient age and disease stage are both risk factors, children without metastases and infants having a better outcome. Failure of standard treatment in most patients has led us to try stronger measures, and during the last 14 years about 600 children have undergone myeloablative treatment and allogeneic or autologous infusions. About half these children have relapsed since but a third are alive and well up to 10 years later. Between 5% and 15% have died of complications, mostly infections but a few of hepatic veno-occlusive disease.

The first protocol to test this most intensive of all treatments (2) used melphalan alone in children with already resistant disease, and produced only temporary responses. Melphalan was chosen because of its efficacy in adult cancers,its steep dose-response curve, and our hope that it would block tyrosine metabolism in the neuroblast. Since then cyclophosphamide, cis-platinum, etoposide, vincristine and doxorubicin have all been added or substituted in different studies, often together with systemic and local-site irradiation (3-6). There is still no consensus about which is the best regimen.

The three largest clinical trials are those of the European Bone Marrow Transplant Group, the Children's Study Group, and the Pediatric Oncology Group (POG) (4-6). Though precise risk factors have not been defined, patients given myeloablative treatment in first remission have done significantly better than those treated in relapse or later remissions. Adding more drugs seems to have added more toxicity than efficacy; more children have died of toxicity without reducing relapses. A difference between these series is that relapses have continued to be seen for up to ten years in the French trials but very rarely after two years in North America. We don't know why this is, because the myeloablative treatment regimens have been similar. In this paper I will use data from 94 children treated on POG 8340 protocol (6) to relate patient,

disease and treatment factors to outcome in terms of frequency and sites of relapse. This will illustrate what we have achieved so far, and what questions and difficulties remain.

METHODS

The POG conducted protocol 8340 between March 1984 and December 1988. All patients had metastatic NBL and received myeloablative treatment in either first or second complete (CR) or partial (PR) remission. We based the diagnosis on either histopathology or marrow cytopathology plus urinary catecholamine levels, and defined remission extent clinically (though this was confirmed pathologically after further surgery in many cases). During this pilot protocol we changed the regimen in several ways:

(a) The first 74 children received melphalan 60mg/m^2 intravenously (IV) daily for three days.
(b) The last 20 received etoposide (VP16) 300mg/m^2 daily for six days infused IV + cyclophosphamide (CY) 300mg/m^2 IV twice a day for six days during the VP16 infusion.
(c) The first 27 received total body irradiation (TBI) 1.5Gy at 10Gy/minute twice daily for three days.
(d) The last 67 received 2.0Gy TBI twice daily for three days.
(e) Thirty-one children received local irradiation (LRT) 1.2Gy twice a day for five days to residual disease sites just before TBI.
(f) Eighty-two marrows were purged immunomagnetically using five monoclonal antibodies and paramagnetic beads (7).

Marrows from patients at other centers were transported for purging (8) at the University of Florida and were returned in liquid nitrogen when needed. Marrows were reinfused the day after finishing TBI.

The protocol was approved by the NCI and all institutional review boards, and parents and older children signed consent.

I have used the log rank test to compare event-free survival (EFS) probabilities from the end of treatment to relapse or death, and constructed EFS curves using the product limit estimate. Because this was a pilot study I have used univariate methods of analysis only, and have not tried to compare results with those of children given less potent treatment on concurrent POG protocols.

PATIENTS

The 94 children treated on this protocol represent about 15% of all POG patients with metastatic NBL diagnosed during the time of the study. The 41 girls and 53 boys were aged from one to 14 years. Sixty-one had both skeletal and marrow metastases, the rest having disease in only one of these sites and/or liver, lymph node, lung, brain metastases. All had received at least six courses of combination chemotherapy before myeloablation. Of the 62 patients in first remission 28 were clinically in CR and 34 in PR. Only four of

the 32 treated after relapse were clinically in CR, reflecting how hard it is to achieve second CRs in patients with this disease. We treated the last child 20 months ago and the longest follow-up is 71 months.

RESULTS

Figure 1 shows what has happened to the 94 patients. Fifty-four (57%) have relapsed since treatment ended and 11 (12%) have died of complications, 10 from infections and one from a brain hemorrhage 4 months after treatment with a normal blood count. Twenty-nine are alive and well, 28 more than two years and 13 more than three years since treatment finished. Eighty percent of the relapses were in the first year; the latest relapse we have seen so far was at 23 months. The median follow-up of the well children is 34 months.

Table 1 shows relapse patterns. Multi-site relapses have been the rule, and less than 25% have involved one site only. Relapse in the retroperitoneum, marrow and bone has been equally frequent, and has mirrored the disease pattern at diagnosis. There have been only four isolated marrow relapses, and only five relapses in sites treated with LRT.

The state of the disease at the time of treatment has had an important bearing on outcome, shown by the difference according to the patients' remission number. Significantly more children in first remission (39%) than in second remission (16%) remain well and free of relapse (Figure 2). And nine of the 11 fatal complications have also been in children in second remissions.

Demographic variables (age, sex, race) have not affected outcome significantly in univariate analyses.

There are, however, differences in the effect of treatment variables, particularly the radiation components. Children given 12Gy TBI have had fewer relapses than those given 9 Gy; and those receiving LRT have relapsed less often than those not so treated. These differences are significant after correcting for remission number (Figures 3 and 4). The change in the ablative chemotherapy during the study has had no apparent effect either way. There is no difference in EFS probability between those given melphalan and those given VP16 + CY (Figure 5).

I have summarized these associations with treatment variables in Table 2.

DISCUSSION

These results suggest that a proportion of children with metastatic NBL will have long remissions after myeloablative chemoradiotherapy and autologous marrow infusions. These findings are like those of other series (4,5), in which the myeloablative regimen has been strengthened by adding more drugs without however lessening relapse frequency. We did not find switching from melphalan to VP+CY reduced relapses, but these drugs seem to have been equally effective when used with the 12 Gy TBI dose. The difficulty is that

escalating the chemotherapy with even more drugs may add more toxicity than efficacy.

Modestly augmenting the radiation component may however reduce relapses without adding toxicity, because NBL is very radiosensitive (9). A dose response to TBI has been shown in the rat BNML leukemia model, where a 33% TBI dose increment kills another log of cancer cells (10). This approximates the difference between 9 and 12 Gy, suggesting this increment in our TBI regimen may have had significant therapeutic value.

Giving low-dose LRT to residual disease also seems to have been of significant benefit to our patients, particularly as we restricted it almost completely to those in PR. We expected them to have more relapses than those treated in CR, but the reverse has been true (68% CR 1 compared with 56% PR 1). The rationale for low-dose LRT is that relapses are often in sites involved at diagnosis, suggesting that occult disease persists even in those clinically in CR. Perhaps it should be included in every child's treatment.

Though we have seen fewer relapses after one year and none more than two years after treatment, we don't know if these children are cured because the disease has recurred up to 10 years later in other series (4). A difficulty in comparing results between series has been a lack of uniform staging and response criteria. NBL is rarely confined to single organs, surgical staging and restaging are used variably, complete response to induction is unusual, and terms such as "very good partial response" confound comparison. The recently developed consensus staging system will help resolve this difficulty (11).

As well as a low incidence of relapse in irradiated sites, we have seen few relapses confined to the marrow. This could be due to in vitro marrow purging, which has been included in most trials. There is no proof that it is needed, though, and because it is labor-intensive and quite expensive its value should be established. The high relapse rate in spite of myeloablation is probably due to persisting cancer in the patient, and makes better in vivo treatment a priority. A phase three trial of purging vs. non-purging therefore seems premature, because it would be inevaluable until or unless we can reduce the relapse rate from cancer persisting in vivo.

The value of myeloablation relative to non-ablative treatment is unresolved, nor is the best marrow source known. Theoretical advantages of using allogeneic marrow are that it comes from a normal donor, so that it is undamaged by previous chemotherapy, has no cancer in it, and it may indeed exert an anti-cancer effect. But using autologous marrow has the advantage that it is available to all potentially, and is associated with less toxicity. We compared the outcome recently of 125 children receiving allogeneic marrow and 350 receiving autologous marrow at centers all over the world (12). Relapses were equally frequent but there were more early deaths after allogeneic marrow infusions, suggesting if anything an advantage for using autologous marrow.

Despite these uncertainties, we do have data about the identity of the best candidates for myeloablative treatment. Children under one year should be excluded because of their better outlook and the risk of greater toxicity.

Remission number at the time of treatment has been the dominant factor predicting outcome in other series as well as ours (4,5), leading some to restrict the use of myeloablation to children in first remission. Though patients who have suffered relapses probably have more resistant disease, they may still be candidates for new myeloablative regimens. Molecular biology, for example N-myc oncogene amplification, is proving useful for diagnosis and staging (13) and it is likely also to refine prognostic factors and help us choose suitable patients for this treatment.

Two North American phase three protocols currently in progress comparing myeloablation and autologous marrow infusions to continuing chemotherapy in children with metastatic NBL should settle which is the better treatment. If the former is better the biggest task will still be to lessen relapses. The POG plans to compare in its next group-wide randomized study a regimen of intensified chemotherapy consolidation after induction to the currently best induction/consolidation treatment known to date. Everyone will then receive myeloablative chemotherapy, 12 Gy TBI, low-dose LRT to persisting local disease sites, and autologous marrow infusions. A further randomization may be included to examine the effect of a biological response modifier after recovery from myeloablation (14).

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TABLE 1

SITES OF RELAPSE IN 54 PATIENTS

MULTIPLE SITES	42	SINGLE SITES	12
RETROPERITONEUM	52%	CORTICAL BONE	54%
BONE MARROW	61%	ALL OTHER	57%
RELAPSES NOT INVOLVING BONE MARROW			43%
RELAPSES LIMITED TO BONE MARROW			7%
RELAPSES IN IRRADIATED SITES			16%

TABLE 2

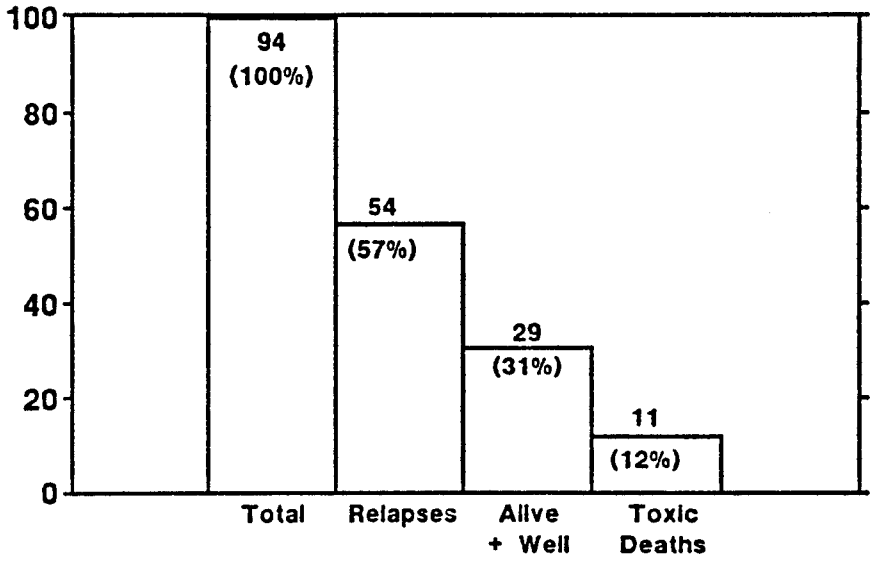
EVENT-FREE SURVIVAL OF THE 94 CHILDREN ACCORDING TO DISEASE AND TREATMENT VARIABLES

	N	FAILED*	EXPECTED	P VALUE	TOXIC DEATHS
REMISSION 1	61	37	49.7	.001	2
REMISSION 2	33	28	15.0		9
REM 1,TBI 12Gy	50	29	41.8		1
REM 1,TBI 9Gy	11	8	8.4	.001	1
REM 2,TBI 12Gy	20	16	11.2		6
REM 2,TBI 9Gy	13	12	4.6		3
REM 1, LRT YES	16	9	11.5		0
REM 1, LRT NO	46	29	38.2	.01	2
REM 2, LRT YES	16	13	7.0		5
REM 2, LRT NO	16	14	7.9		4
MELPHALAN TBI**	50	33	31.1	NS	4
VP16 CY TBI	20	12	13.9		3

* RELAPSES OR TOXIC DEATHS
** ONLY PATIENTS RECEIVING 12Gy TBI ANALYZED

FIGURE 1

Overall Outcome of 94 Children Treated With Myeloablation and Marrow Infusions (Log Rank)



28 of the 29 patients in remission finished treatment more than two years ago. The latest relapse was 23 months after completing.

FIGURE 2
Probability of Remaining Alive and Well According to Remission Status at the Time of Myeloablatioin

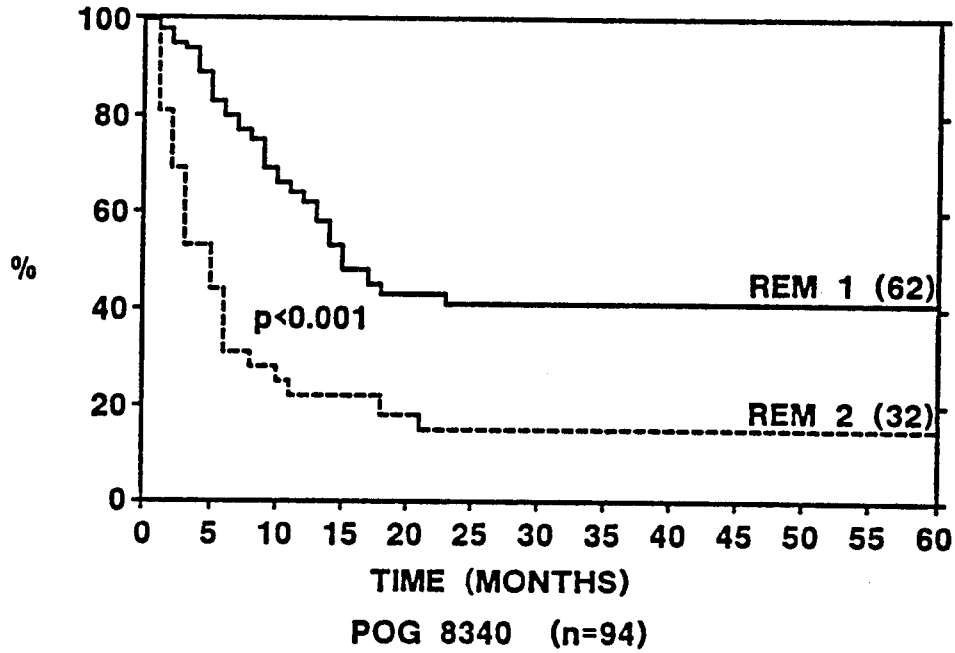


FIGURE 3
Probability of Remaining Alive and Well According to TBI Dose and Use or Not of Local Irradiation

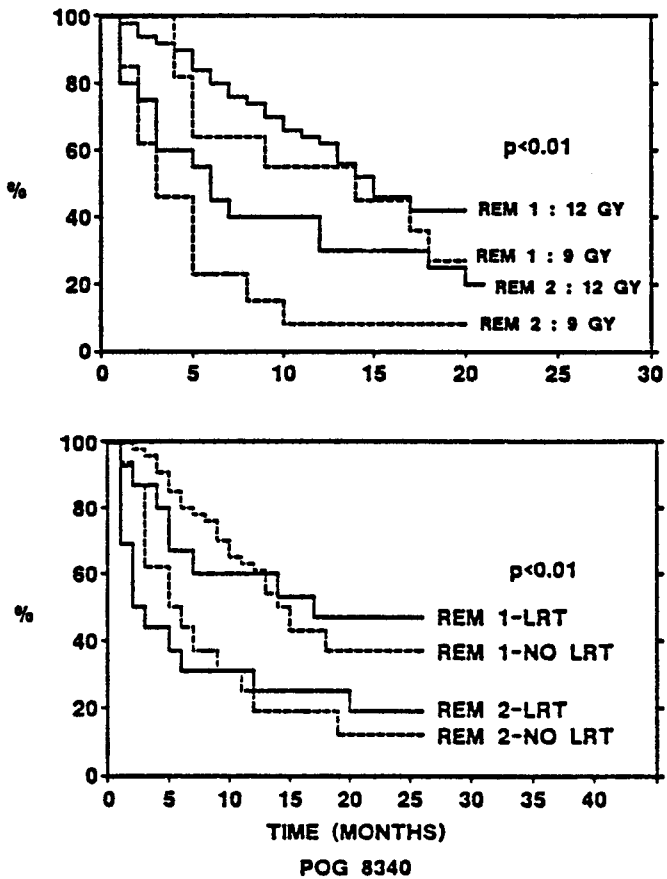
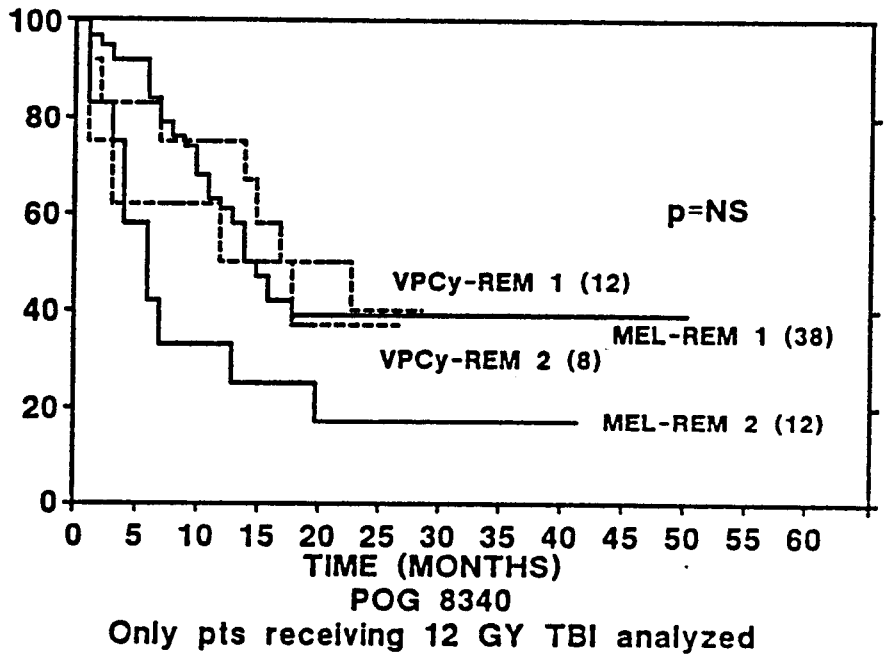


FIGURE 4
Probability of Remaining Alive and Well According to Use of Melphalan or VP16-Cytoxan (Only Patients Receiving 12GY TBI Included)



HIGH-DOSE CHEMOTHERAPY WITH BONE MARROW RESCUE IN CHILDREN AND YOUNG ADULTS WITH RECURRENT HIGH-GRADE BRAIN TUMORS

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INTRODUCTION

Brain tumors represent approximately 20% of childhood cancer, superceded only by the acute leukemias in frequency. Although adjuvant chemotherapy appears to have improved the progression- free and overall survival for newly-diagnosed children and adolescents with medulloblastoma (1) and high-grade astrocytoma (2) in conjunction with surgery and irradiation, nevertheless, the majority of children with high-grade astrocytomas still succumb to their disease. Furthermore, the outcome for newly-diagnosed children with brain stem tumors (BST) and ependymomas remains poor (3,4), with less than 25% of such children being long-term survivors, and with little or no proven benefit from adjuvant chemotherapy. Once children develop recurrence of these high-grade brain tumors, then the likelihood of even meaningful palliation, let alone cure, is extremely small with conventional modalities of therapy, especially in the current era when the majority of such children will have already been exposed to multi-drug chemotherapy regimens as part of their initial therapy.

Since March of 1986, a total of 13 largely pediatric oncology centers in the United States and Canada have participated in a series of phase I/II studies of high-dose (presumed marrow- ablative) chemotherapy followed by bone marrow rescue, in children and young adults with recurrent high-grade brain tumors. We specifically chose not to evaluate a BCNU-containing regimen, at least initially, since a body of literature already demonstrated significant pulmonary and neurologic toxicity in adult patients treated for high-grade astrocytomas with a high-dose single-agent BCNU regimen (5-7). We specifically chose to evaluate initially a two-drug combination of Thiotepa

and Etoposide because of some data suggesting the efficacy of these drugs against brain tumors and phase I data on their use in high-dose with marrow rescue in other solid tumor systems in adults (8,9). Further, we rationalized that synergy could be anticipated between an alkylating agent and a topoisomerase-II inhibitor, via inhibition of DNA repair mechanisms, and that two drug regimens would be more efficacious than one in tumors of such proven individual heterogeneity pathologically, cytogenetically and in their response to drugs in vitro in tumor stem cell clonogenic assays. More recently, in a pilot study conducted at four of the participating institutions, we have added either Carboplatin or BCNU to the two-drug regimen in appropriate patients without significant prior exposure to either of these drugs. This short manuscript summarizes our experience with the first 49 consecutive patients with recurrent brain tumors treated in these studies.

METHODS

Patient Characteristics

The characteristics of the patients are listed in Tables 1a and 1b. Only one patient was over 23 years of age at time of study entry. All patients were treated at the time of tumor recurrence, except for three patients with glioblastoma multiforme (GBM) arising as second malignancies. All patients had received maximal surgical resection when feasible prior to study entry, with the exception of 6 children with diffuse, intrinsic, hypodense pontine lesions on CT scan who were not biopsied. Most patients had received both irradiation and chemotherapy prior to study entry. (See Table 1b).

Bone Marrow Harvesting and Reinfusion

In only four patients was marrow harvested just prior to study chemotherapy without cryopreservation, and reinfused 48 hours after completion of the Etoposide infusion. In all other patients, marrow was harvested at varying periods prior to study chemotherapy and cryopreserved, and reinfused 48-72 hours after completion of the Etoposide.

A minimum of 1×10^8 nucleated marrow cells per kg body weight were reinfused in all except one case. All patients were reinfused with autologous marrow, except for one patient who received marrow from his HLA-matched sibling who had previously served as his bone marrow donor for a prior transplant for acute lymphoblastic leukemia in second remission.

Chemotherapy Regimens

Study chemotherapy regimens include: Regimen A, Thiotepa 300mg/M^2/day and Etoposide 500mg/M^2/day on days -5, -4 and -3, administered in 28 patients. Regimen B, Carboplatin 500mg/M^2/day on days -8, -7 and -6, prior to Thiotepa and Etoposide as in Regimen A; this has been administered in 9 patients. Regimen C, BCNU 100mg/M^2 twice daily on days -8, -7 and -6, prior to Thiotepa and Etoposide as in Regimens A and B; this has been administered in 12 patients. Thiotepa is administered as a continuous

infusion over 3 hours, Etoposide as a continuous infusion over at least 5 hours, Carboplatin as a continuous infusion over 4 hours and BCNU as a continuous infusion over one hour.

Evaluation of Response

Patients are evaluated at day +28 following marrow reinfusion for disease response, by either CT or MRI imaging with and without contrast enhancing agents. A complete response implies disappearance of all tumor on imaging. A partial response implies greater than 50% shrinkage of all tumor on imaging. Imaging studies, laboratory studies and physical examinations are conducted in follow-up at monthly intervals, to assess tumor response, clinical status and toxicities of therapy. The results have been analyzed as of August 20th, 1990.

RESULTS

Toxicity of Therapy (See Table 2)

While all three regimens are characterized by the usual toxicities seen in patients undergoing autologous marrow reconstitution following marrow-ablative chemotherapy, namely, thrombocytopenia with bleeding tendency, neutropenia with susceptibility to bacterial and fungal sepsis, and anemia with requirement for packed cell transfusions, all three regimens are also characterized by a very significant degree of oro-pharyngeal mucositis, such that about 16% of patients have required elective intubation for impending or actual upper airway obstruction secondary to mucositis. Despite this, the duration of the required intubation has usually been no more than 48-96 hours, with dramatic resolution of mucositis. At least one case of aspiration pneumonia appears related to aspiration of oro-pharyngeal debris in a somnolent patient. Surprisingly, a small number of patients have had such mild mucositis as to permit continued oral intake throughout the post-marrow reinfusion period.

More specific toxicities appear related to the addition of either Carboplatin (Regimen B) or BCNU (Regimen C). These have resulted in an unacceptable mortality with these two regimens, at least at the doses of drugs employed currently. The mortality rate in Regimen B (4 of 9 patients = 44%) appears largely due to the development of a syndrome of "distributive shock", characterized by dramatically decreased peripheral vascular resistance ("warm shock without sepsis") and associated with hepato-renal failure; this has been reported now in 3 patients, all treated with the addition of Carboplatin, and sharing prior cranio-spinal irradiation and multiple courses of platinum-containing chemotherapy regimens.

The three toxic deaths with Regimen C, the BCNU-containing regimen, were all characterized by the development of acute renal failure and, in the case of two patients with significant prior exposure to CCNU, acute encephalopathy. All three patients had received prior cranio-spinal irradiation and the third

patient, although not exposed previously to a nitrosourea, had significant prior exposure to Ifosphamide and Carboplatinum.

One patient treated on Regimen A died of veno-occlusive disease, having been entered on study with a significantly elevated SGPT (ALT).

Neurologic toxicities have been common with all regimens, of varying character and etiology. In general, one commonly sees a transient deterioration of neurologic function, both motor and cognitive functions, during the chemotherapy. Many patients experience somnolence, confusion, short-term memory loss and less commonly combativeness in the early days following administration of the chemotherapy. These have tended to recover within days to weeks. Such neurologic dysfunction is conspicuously lessened in patients who have had only involved-field irradiation or no irradiation at all, and patients who have good renal function and no prior exposure to either nitrosourea or Ifosphamide.

Cases of interstitial pneumonitis have not developed among the 12 patients treated with Regimen C, employing 600mg/M^2 of BCNU. However, once such death has occurred in a newly-diagnosed patient treated with the same dose of BCNU on a Regimen C protocol for newly-diagnosed GBM (not discussed in this manuscript).

Poor or failed marrow engraftment has occurred in 4 patients. One patient with poor platelet engraftment was harvested with a low platelet count considered secondary to Ranitidine. One patient with delayed engraftment had a poor harvest following intensive chemotherapy and cranio-spinal irradiation; this patient also had anti-platelet and anti-white cell auto-antibodies and subsequently proved to be HIV-infected. One patient was harvested from the posterior iliac crest within a few months of completing cranio- spinal irradiation; this patient failed to engraft and received an autologous marrow boost. A second patient with a GBM developing 8 years after cranio-spinal irradiation for medulloblastoma (without adjuvant chemotherapy) had poor engraftment of both white cells and platelets following reconstitution with marrow harvested from the posterior iliac crests. It has since been recommended to investigators participating in this study that patients with a history of prior spinal irradiation be harvested from the anterior iliac crests rather than the posterior iliac crests, since sufficient scatter (from a spinal dose of 3600cGy) could lead to permanent damage to the marrow stroma both in the vertebrae and posterior iliac crests.

Responses (See Tables 3 and 4)

The best responses have occurred in patients with high-grade astrocytomas, with a complete response (CR) rate of 24% (5/21) and a partial response (PR) rate of 24% (5/21). Eight of these patients received the two-drug chemotherapy Regimen A, producing 2 CR, 3 PR, 2 SD and 1 PD. Nine patients received the BCNU-containing 3-drug Regimen C, producing 2 CR, 1 CCR, 2 PR, 1 SD, 1 PD and 1 early toxic death. Three patients received the Carboplatin-containing 3-drug Regimen B, producing 1 SD, 1 early toxic death, and 1 SD at day 28 subsequently becoming a good PR after 4 months (patient

with AA). Two of the four CRs, all in patients with GBM, have been maintained now for 23 months without evidence of tumor recurrence; both of these two patients received the 3-drug BCNU-containing Regimen C. The two remaining CRs, both treated with the two-drug Regimen A, developed tumor recurrence at 9 and 10 months post-marrow reinfusion. The single GBM patient treated with Regimen C in CCR following surgical resection, developed tumor recurrence after 7 months.

Partial and/or complete responses have been observed in patients with recurrent medulloblastoma, pineoblastoma, ependymoma, choroid plexus carcinoma, choriocarcinoma and unbiopsied brain stem tumors. However, these responses have usually been of short duration. Two patients with unbiopsied brain stem tumors remain stable at 84+ and 140+ days post-marrow reinfusion. One ependymoma patient treated in CCR with the two-drug regimen, continues without disease recurrence 22 months post-marrow reinfusion.

DISCUSSION

The responses observed in patients with recurrent high-grade astrocytoma, particularly in those patients with GBM, are encouraging, with a 48% CR+PR rate overall. One cannot begin to consider curing patients, or even extending the time to disease progression, without first identifying strategies that are capable of producing responses in a phase II setting. The Thiotepa-Etoposide +/- BCNU-containing regimens used in these studies would appear to have produced higher response rates than any previously published phase II trials in adults or children with high-grade astrocytomas, either at recurrence or at initial diagnosis. These data particularly contrast with the recent Children's Cancer Study Group experience with the "8-in-1" regimen in newly-diagnosed children with high-grade astrocytomas, administered as two cycles two weeks apart prior to irradiation; of almost 100 sets of CT and/or MRI scans evaluated, not a single CR was observed, and the PR rate was less than 20%. The duration of responses following ABMT in these studies has been unimpressive, except for the patients with recurrent GBM who achieved CR; 2 of a total of 15 GBM patients (13%) remain free of recurrence almost 2 years following treatment.

These regimens have considerable morbidity and mortality, and it is possible now to identify those risk factors which are predictive of significant toxicity. Patients who have been exposed to "significant" doses of nitrosourea (i.e. above 400mg/M^2) are at risk for neuro- and nephro- toxicities if treated with the BCNU- containing regimen. Patients who have received cranio-spinal irradiation and intensive chemotherapy regimens, incorporating nephrotoxic agents (e.g. cisplatin, Ifosphamide) are at significant risk if treated with the Carboplatin-containing regimen, for a newly-described syndrome which our Intensivists have termed the "distributive shock" syndrome, characterized by decreased peripheral vascular resistance with hypotension and a "warm shock"

state accompanied by hepato-renal failure and significant erythrodermia and desquamation.

Having identified which patients are at risk for significant toxicities from these regimens, one may also now identify patients who are unlikely to gain benefit in terms of durable response from these regimens. Patients with bulky residual tumor at the time of study entry, particularly those who have failed prior irradiation and conventional multi-agent chemotherapy regimens, are unlikely to achieve significant and durable responses to marrow-ablative therapy. The obvious conclusions are that marrow-ablative regimens should be evaluated earlier in the natural history of malignant childhood brain tumors. We have embarked upon a study of the three-drug BCNU-containing regimen in newly-diagnosed patients with high-grade astrocytomas and diffuse intrinsic brain stem tumors, prior to irradiation. We would also strongly advocate using these marrow-ablative regimens in patients with recurrent malignant brain tumors who can be rendered surgically grossly free of residual tumor prior to study entry, or can be placed at least into a good PR, with minimal residual tumor, by the judicious use of conventional chemotherapy regimens of proven activity (e.g. Ifosphamide, Etoposide and/or Carboplatin, in patients with ependymoma or PNET/MB).

Finally, significant morbidity can be reduced by rescue with marrow that has not previously been exposed to considerable stem cell toxic chemotherapeutic agents. Accordingly, harvesting and cryopreservation of bone marrow prior to initiation of conventional multi-agent chemotherapy and/or spinal irradiation should be considered in patients at high risk for relapse despite conventional therapeutic strategies.

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TABLE 1a

PATIENT CHARACTERISTICS AT STUDY ENTRY

Characteristics		Patient Numbers	Percentage of Total
Patient Number		=49	
Age in Years:	median age = 9.6 yrs range = 8 months - 32 years		
Sex:	females	=18	37%
	males	=31	63%
Pathology:	Glioblastoma multiforme	=15	
	Anaplastic astrocytoma	= 6	
	Medulloblastoma/PNET	= 8	
	Ependymoma/Malignant ependymoma	= 8	
	Pineoblastoma	= 3	
	Anaplastic oligodendroglima	= 1	
	Choroid plexus carcinoma	= 1	
	Primary intracranial choriocarcinoma	= 1	
	Intracranial malignant melanoma	= 1	
	Unbiopsied brain stem tumor	= 6	
Treatment Regimen:			
	Regimen A: Thiotepa-Etoposide	=28	
	Regimen B: Thiotepa-Etoposide-BCNU	=12	
	Regimen C: Thiotepa-Etoposide-Carboplatin	= 9	

TABLE 1b

PATIENT CHARACTERISTICS AT STUDY ENTRY

Characteristics		Patient Numbers		Percentage of Total
Previous Surgery:			=43	88%
Previous Radiation Therapy:			=46	94%
CNS irradiation for current malignancy		=42		
CNS irradiation for previous malignancy		= 4		
External beam irradiation		=45		
Craniospinal irradiation		=17		
Whole brain irradiation		= 4		
Involved-field irradiation		=23		
Conventional irradiation	=16			
Hyperfractionated irradiation	= 7			
Total body + whole brain irradiation		= 1		
Stereotactic irradiation		= 2		
No previous irradiation:			= 3	6%
Children less than 1 year of age	= 2			
CNS melanoma	= 1			
Previous chemotherapy:			=40	82%
Single drug regimens only:		= 2		
Thiotepa	= 1			
Beta-interferon	= 1			
Multi-drug regimens:		=38		
"Eight-drugs-in-one" regimen	=20			
Regimens with DDP/CBDCA	=32			
Regimens with CCNU/BCNU	=28			
Regimens with Ifosphamide	= 7			
Regimens with high-dose cytoxan	= 5			
Regimens with Etoposide	= 8			
No previous chemotherapy for current tumor:			= 9	18%
Second primary brain tumor	= 2			
Second malignancy following A.L.L.	= 1			
Unbiopsied brain stem tumor	= 3			
GBM	= 1			
CNS melanoma	= 1			
Pineoblastoma	= 1			

TABLE 2

TOXICITIES OF HIGH-DOSE CHEMOTHERAPY REGIMENS

THROMBOCYTOPENIA:	Intra-tumoral hemorrhage, fatal	(1)
	Subdural hemorrhages, minimal	(3)
	Intraventricular hemorrhage	(1)
	Pulmonary parenchymal hemorrhage	(1)
	Gastro-intestinal hemorrhage	(4)
	Hemorrhagic cystitis (1 severe)	(2)
LEUKOPENIA:	Bacterial Sepsis	(4+)
	Candidal Sepsis	(2)
	Aspergillosis, disseminated, fatal	(1)
	Reactivated HSV infection	(1)
	Interstitial Pneumonitis, non-fatal	(1)
FAILED/DELAYED ENGRAFTMENT:	Failed engraftment-marrow boost	(1)
	Delayed engraftment	(1)
	Poor platelet engraftment	(2)
PLATELET REACTION:	Salmonella-infected transfusion - fatal	(1)
OROPHARYNGEAL MUCOSITIS:	Pain, anorexia, superinfection	
	Elective intubation for obstruction	(8)
	Aspiration pneumonia	(1)
DIARRHEA:	Minimal to moderate, transient	
ERYTHEMA, DESQUAMATION, HYPERPIGMENTATION:	Universal, minimal to severe, transient	
	Avoid pressure points, adhesive dressings	
HEPATOTOXICITY:	Veno-occlusive disease (1 case, fatal)	(1?2)
PULMONARY TOXICITY:	Pulmonary hemorrhage, transient, severe	(1)
	Interstitial pneumonitis (1 CBDCA - PCP?)	(1)
NEPHROTOXICITY:	Acute renal failure (3 BCNU - fatal)	(4)
	Hemorrhagic cystitis (1 severe)	(2)
OTOTOXICITY:	Acute hearing loss, reversible (CBDCA)	(1)
NEUROTOXICITY:	Irreversible fatal brain stem dysfunction - two cases with BCNU regimen	(1?2)
	Transient memory loss, disorientation, somnolence	

TABLE 3

DISEASE RESPONSE AT DAY +28 POST-MARROW REINFUSION

TUMOR TYPE	TOTAL	CR	PR	SD	CCR	EARLY TOXIC DEATH	PD
GLIOBLASTOMA MULTIFORME	15	4	4	2	1	2	2
ANAPLASTIC ASTROCYTOMA	6	1	1	3	0	1	0
BRAIN STEM TUMORS (No Bx)	6	0	1	4	0	1	0
EPENDYMOMA	8	1	1	3	1	2	0
MEDULLOBLASTOMA	8	2	2	2	1	1	0
PINEOBLASTOMA	3	1	1	0	0	0	1
CHOROID PLEXUS CARCINOMA	1	0	1	0	0	0	0
CHORIOCARCINOMA	1	0	1	0	0	0	0
CNS MELANOMA	1	0	0	0	1	0	0
TOTAL EVALUABLE FOR RESPONSE =	49	9	12	14	4	7	3
		18%	25%	29%	8%	14%	6%

TABLE 4

DURATION OF RESPONSE: TIME TO DISEASE PROGRESSION OR DEATH

TUMOR TYPE	DAYS - TIME TO PROGRESSION/DEATH	MEAN
GLIOBLASTOMA MULTIFORME	10, 14, 16, 28, 42, 53, 61, 94, 125, 205, 272, 300, 314, 636+, 654+	188+ days
ANAPLASTIC ASTROCYTOMA	24, 29, 115, 168, 338+, 360	181+ days
BRAIN STEM TUMORS (No Bx)	24, 28, 60, 84+, 105, 140+	74+ days
EPENDYMOMA	14, 24, 35+, 55, 100, 115, 115, 608+	133+ days
MEDULLOBLASTOMA/PNET	17, 38+, 55, 56, 99, 121, 131, 155	84+ days
PINEOBLASTOMA	28, 76, 140	81 days
CHOROID PLEXUS CARCINOMA	125	125 days
CHORIOCARCINOMA	81	81 days
CNS MELANOMA	83	83 days

PATTERN OF FAILURES IN PATIENTS RECEIVING UNPURGED AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR NEUROBLASTOMA

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INTRODUCTION

A marrow ablative therapy (MAT) followed by autologous bone marrow transplantation (ABMT) is presently considered part of the modern treatment for disseminated neuroblastoma responding to first line therapy (1-8). After the initial enthusiasm following the pioneering experiences of the early eighties, it has become evident that nearly 50% of patients undergoing MAT and ABMT relapse within 12 months (1-8) and that relapse is the main cause of failure in patients grafted. The outcome of patients receiving an allogeneic bone marrow is no better, since approximately one third experience a relapse and another third dies from complications (9).

Despite a lack of evidence that the presence of residual tumor cells in the infused bone marrow can result in the return of overt disease, most investigators have adopted the practice of purging electively the harvested bone marrow either pharmacologically, or immunologically (3-10). However, the role of purging in reducing the risk of tumor regrowth remains unproven. I n this study we report our experience on the use of MAT followed by unpurged ABMT in children with poor prognosis neuroblastoma and we focus on the pattern of failures developed after the ABMT.

MATERIAL AND METHODS

Eligibility requirements were that patients had either resistant or relapsed (later defined as "after disease progression") or previously untreated disseminated neuroblastoma (later defined as "before disease progression"). Thirty-four children bad fulfilled these criteria; 29 had bone marrow infiltration at some time in their disease.

Eligible patients were treated according to the Protocol AIEOP NB-85 previously described (Fig. 1) (7,10,11). The response to the induction therapy

was assessed by a change in the size of the tumor, urinary catecholamine excretion, bone lesions and bone marrow infiltration, according to the following criteria complete remission (CR) required complete disappearance of all measurable tumor; partial remission (PR) required a 50% or more reduction of all tumor lesions; stable disease (SD) required no significant change of any tumor lesion; progressive disease (PD) required either an increase of any tumor lesion, or the appearance of new lesions.

Bone marrow was harvested after two evaluations performed at 4-week intervals. Each evaluation included aspirates obtained from four different sites as well as trephine biopsy specimens from two sites. Aspirates were studied using traditional cytomorphology and indirect immunofluorescence using monoclonal antibody UJ 13 a (kindly provided by J.T. Kemshead, London, UK) (12). The marrow was considered free of neuroblastoma when cytomorphology and trephine biopsies were negative with less than 2% fluorescent cells. Both evaluations showed a normal bone marrow in 25 cases. In the remaining 9 cases the marrow was harvested with "minimal" residual disease, consisting of few neoplastic cells (<5/slide) with displaying ganglionic differentiation at histology in 8 cases and a clump of neuroblastoma cells with regressive phenomena at cytology in 1 case. However the harvested bone marrow was negative at both cytology and immunofluorescence staining in all cases. The MAT protocol consisted of vincristine, fractionated TBI and melphalan (Fig. 1) as previously described (7).

RESULTS

Patients Grafted After Disease Progression

Of the 10 children in this group 7 received MAT while in second or later CR, 1 in VGPR and 2 in early PD after good response. Four of the 7 children grafted in CR relapsed at 7-17 months (median 8 months) and subsequently died. The three remaining children are in continuous CR at 47, 56 and 59 months respectively. The child grafted in VGPR developed local PD and is alive with stable disease (SD) at 54 months after being treated with 3 courses of 131-Metaiodobenzylguanidine therapy. Two children grafted in PD achieved CR but relapsed after 2 and 3 months respectively and died.

Patients Grafted Before Disease Progression

Of the 24 children in this group 10 underwent ABMT while in CR, 5 in VGPR and 9 in PR. Of the 10 children grafted in CR, 6 relapsed at 7-23 months (median 15 months) and died while 4 are in continuous CR at 31, 38, 50 and 58 months respectively. Three of the 5 children grafted in VGPR had SD for 5, 6 and 15 months respectively, then developed PD and died, another child achieved CR, relapsed after 5 months and died; the last child grafted in VGPR achieved CR, which has persisted for 31 months. Of the 9 children grafted in PR, 2 had SD for 2 and 4 months respectively and died with PD, 1 died from multiple organ failure 5 days after ABMT; six other children achieved CR: 4 of them relapsed after 11, 12, 15 and 19 months respectively

and died; the a remaining children are in continuous CR after 33 and 51 months.

Progression-Free Survival

The 5-year progression-free survival is 29% (SE=0.07) for all the 34 grafted children, 30% (SE=0.14) for the 10 children grafted after disease progression and 2$% (SE=0.09) for the 24 children grafted before disease progression. In detail, progression free survival is 41% (SE=0.11) for the 17 children grafted in CR and 20% for the 15 grafted in VGPR or in PR. There is no significant difference between these figures.

Failures

Failures (relapse or disease progression) were documented in about of 34 children (67%) and occurred at a median time of 9 months (2-23) after ABMT (Table 1). The bone marrow was the first site of relapse in 15 cases, as an isolated site in 5 children, in combination with other sites in 10 children (primary 3, cortical bone 4, nodes 1, bone and nodes 1, nodes and lung 1) (Table 2). Seven of the nine children with "minimal" bone marrow infiltration before harvesting relapsed in the bone marrow (as an isolated site in a cases, with primary, or cortical bone, or nodes and lung in 5 cases). Of the 29 children with bone marrow infiltration at some time in their disease, 19 relapsed: 15 in the bone marrow, (10 of them with relapse also in other sites), 4 in sites other than bone marrow (Table 3).

DISCUSSION

Over the past few years more than 300 neuroblastoma patients have been grafted in Europe (13) and more than 100 in the United States (2,5,6). Most authors have electively purged the bone marrow utilizing, among the others, murine monoclonal antibodies adsorbed by anti-mouse immunoglobulin-coated magnetic beads (immunomagnetic purging) or cytotoxic compounds such as maphosphamide (4) a cyclophosphamide derivative.

Two critical problems responsible of the failure of the purging procedures in clinical trials are the heterogeneity of malignant cells and the variations in the tumor cell characteristics. For these reasons, several groups have proposed a combination of purging methods such as 6 hydroxydopamine and ascorbic acid (15) or the combination of sedimentation, filtration and incubation with monoclonal antibodies (5) or differential agglutination with lectin (16) and dye-mediated photolysis (17).

Few authors have recently proposed to overcome the problem of purging a minimally infiltrated bone marrow by utilizing peripheral blood stem cells presuming that neuroblastoma cells spread in the blood only in patients with overt disseminated disease (18,19).

Despite the evidence of the efficacy of most of these purging procedures in experimental models, the real significance of minimal bone marrow disease has not been defined yet. In particular, the detection of

micrometastases in the bone marrow biopsies does not imply that harvested bone marrow cells are contaminated with malignant cells (20). In our experience, one out of 9 patients, who had at harvesting trephine biopsy positive for a "minimal" tumor infiltration, is surviving disease-free 51 months after the ABMT.

Moreover the comparison of the progression-free survival of 88 patients treated in LMCE mainly with a MAT including vincristine, melphalan and fractionated TBI, and bone marrow purged with immunomagnetic depletion, with that of 53 patients treated in Genova with the same MRT and unpurged bone marrow (13) suggests that bone marrow purging may not produce any real advantage (Fig. 2). To the same conclusions drives the information from Lyon that the chance of relapse is twice as high in patients with bone marrow micrometastases at the time of ABMT than in patients without micrometastases (20) even after purging.

In our patients, bone marrow was extensively evaluated before harvesting and harvested only if free of neuroblastoma or if infiltration was "minimal".

Bone marrow has been the main site of failure after ABMT, mostly in association with other sites. This has also been the experience reported in most of the series where purged bone marrow had been given (3-5). Although in those series different induction and ablative regimens had been used, the similar timing and pattern of relapses observed suggests that recurrence results more from the failure of MAT in eradicating minimal residual disease, rather than from the reinfusion of neuroblastoma cells by ABMT. The pattern of failures seen after allogeneic bone marrow transplantation further supports this opinion (9).

CONCLUSION

The majority of failures occurring post-ABMT for neuroblastoma have involved the bone marrow mostly in association with primary or distant sites; however the pattern of failures did not show a clear correlation with the pattern of residual tumor prior to ABMT. Only one patient developed lung metastases and node dissemination as a possible consequence of the reinfusion of malignant cells together with the bone marrow (21). Despite that, careful evaluation of residual disease prior to ABMT remains crucial to clarify the pathogenesis of failures after ABMT and the role of purging in neuroblastoma.

ACKNOWLEDGEMENTS

We thank Monica Robiglio for her help in preparing the manuscript and Paola Mini for typing the manuscript.

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TABLE 1

Outcome of patients according to status prior to ABMT

Site of Recurrence	Extent of Residual disease prior to ABMT								Total
	None	BM	BM+ Primary	BM+ LN	Bone+ Primary	Liver	Primary	VMA	
No recurrence	7	1			1	1			10
BM	3	1	1						5
BM + Bone	2	2							4
BM + Primary		1					2		3
BM + LN							1		1
BM + LN + Lung		1							1
BM + LN + Bone									1
Bone	1								1
Primary	4						2	1	7
Toxic death			1						1
Total	17	6	2	1	1	1	5	1	34

TABLE 2

Pattern of failures after ABMT

Site/s	Cases	Months from ABMT ranges (median)
BM	5	4-20 (9)
BM, primary	3	2-15 (6)
BM, bone	4	7-19 (12)
BM, nodes	1	5
BM, bone, nodes	1	15
BM, nodes, lung	1	4
Primary	7	1-23 (8)
Bone	1	10
Total	23	2-23 (9)

Abbreviations: ABMT, autologous bone marrow transplantation; BM, bone marrow.

TABLE 3

Pattern of failures of 29 children with bone marrow infiltration at some time in their disease, according to the bone marrow status before harvesting.

Outcome	Bone marrow at harvesting	
	Neg.	Pos.
Number of cases	20	9
Relapsed	12	7
Bone marrow (BM)	3	2
BM + other sites	5	5
Other sites	4	-
Alive disease free	8	1
Toxic death	-	1

FIGURE 1

Protocol AIEOP NB-85. Abbreviations: PTC, peptichemio; VCR, vincristine; c.i., continuous infusion; CY, cyclophosphamide; CDDP, cisplatin; VM26, teniposide; Dox, doxorubicin; TBI, total body irradiation; L-PAM, melphalan.

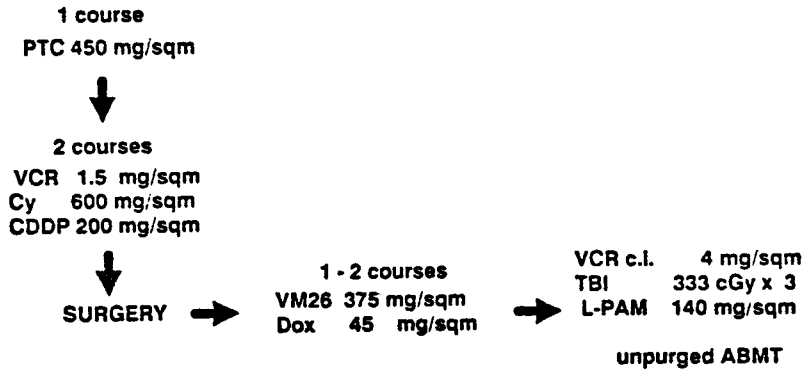
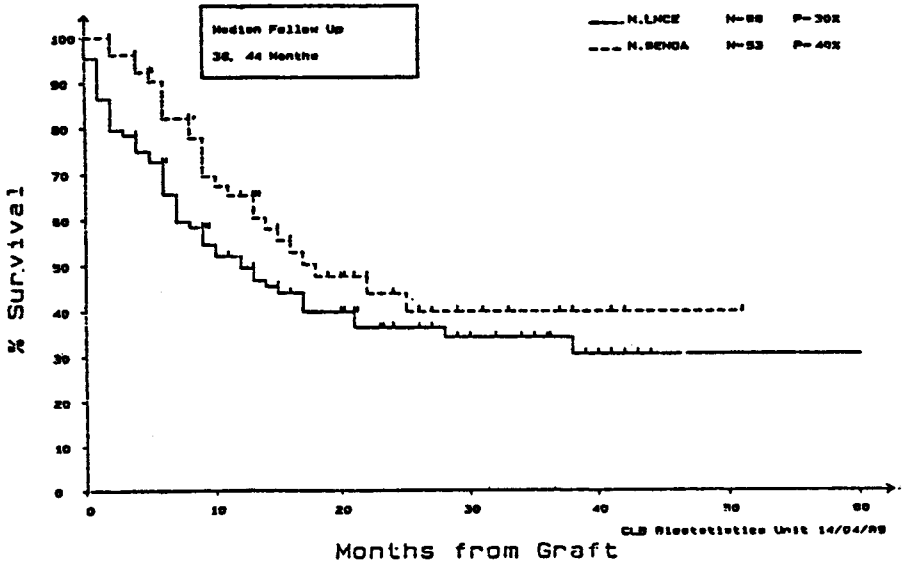


FIGURE 2

Survival of patients grafted in LMCE with purged bone marrow and in Genova with unpurged bone marrow. EBMT 89 Neuroblastoma; Genoa vs. LMCE.



IMMUNOMAGNETIC PURGING PROCEDURE IN ABMT: CLINICAL RESULTS IN A GROUP OF UNSELECTED CHILDREN WITH STAGE IV NEUROBLASTOMA

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INTRODUCTION

The principle of immunomagnetic depletion (IMD) is to target small magnetic beads on tumor cells by means of specific monoclonal antibodies (MoAbs) and then to remove the coated tumor cells from the BM through a flow system using permanent samarium cobalt magnets (1-5).

This method was first used for ABMT in patients with neuroblastoma and further extended to common acute lymphoblastic leukemia or lymphoma (6). Among the different physical methods for purging ex vivo malignant cells from the BM graft, the IMD separation is now the most widely used in clinical trials because of its simplicity, its reproducibility, its lack of toxicity, and the possibility of using this technique to remove any tumor cell for which specific MoAbs are available.

In an initial publication, the development of effective immunomagnetic purging methods in the neuroblastoma (NB) model had been rendered difficult by the lack of rapid and sensitive method to detect viable neuroblastoma cells remaining after in vitro treatment of the marrow (1). The IMD was then subsequently modified using various experimental models with a good agreement between investigators in the definition of the technical parameters (2-5).

On normal BM samples artificially contaminated with cells from an NB cell line prestained with the vital DNA dye Hoechst 3342, the IMD enabled the elimination of more that 3 log neuroblastoma cells from contaminated samples. The use of a cocktail of MoAbs rather than a single agent did not improve malignant cell depletion (4,6). However, the applicability of experimental results to clinical trials is questionable, since the antigen expression may be more heterogenous in tumors and the selection of appropriate antibodies would require the determination of their binding efficiency on each patient's tumor cells. Thus, a cocktail of antibodies is still used in clinical purging procedures. One-hundred, fourteen (114) children with stage IV neuroblastoma were treated

at diagnosis in one of the centers of the French cooperating group LMCE and entered in the same protocol of induction chemotherapy followed by consolidation treatment with megatherapy and BMT (protocols LMCE1 and LMCE3 reviewed in 7 and 8). They received a single course or a double course of megatherapy (the second graft was harvested and purged between the 2 courses of megatherapy). The IMD of the graft was performed in our center for 71 children (54 single grafts and 17 double grafts).

We will summarize here the clinical results obtained with IMD in this group of 71 children.

Since the efficiency and the toxicity of the procedure, the hematological or the immunological recovery after the graft, may largely depend on the clinical status of the patients at time of BM harvest and previous chemotherapy, the analysis of this group of unselected and homogeneous patients must enable a better evaluation of the IMD procedure in clinics.

MATERIALS AND METHODS

Therapeutic Protocols

All children were included in the LMCE1 or LMCE3 protocols. They received induction therapy with PE-CADO, VP16-CDDP-CADO or IVAD, followed by a consolidation treatment with megatherapy (VCR, high-dose Melphalan, TBI) and ABMT, or double megatherapy (first course with BCNU, VM26 and CBDCA, second course as above) (7,8).

Magnetic Depletion Procedure

The magnetic depletion procedure was performed as previously described (3,4,6). Briefly, after harvesting BM cells were separated on a ficoll, washed, resuspended in PBS at $20x10^6$ cells/ml, incubated at 4C for 30 minutes with 5 anti-NB MoAbs kindly provided by J. Kemshead (UJ13A, UJ127.11, H-11, aThy-1, UJ181.4, at appropriate dilution), washed twice and incubated with magnetic immunodisperse 4-5 um polystyrene microspheres (Dynal ME450) at the concentration of 2 mg/ml and for $10x10^6$ BM cells and pushed through a magnetic system. The last two steps (bead incubation and magnetic separation) were repeated twice. Samples were then resuspended in appropriate medium for the freezing procedure.

Detection of Minimal Residual Disease in Bone Marrow

Bone marrow micrometastases were detected by the analysis of 4 trephine biopsies and 4 aspirates. In parallel, residual NB cells were detected on cell suspension by a dual-immunofluorescence analysis with UJ13A or anti-GD2 (Hoescht) MoABs in combination with an anti-panleukocyte MoAB of IgM isotype (G. Janossy) (9110).

Immunological Follow-Up

Analysis of lymphocyte subpopulations in the peripheral blood was performed as previously described (11) by dual-immunofluorescence and cyto-

fluorometric analysis (FACScan, Becton-Dickinson) with the following monoclonal antibodies (Becton Dickinson): CD45, leuM3, anti-leu4-FITC (CD3), anti-leu3a-FITC (CD4), anti-leu2b-FITC or anti-leu2b-PE (CD8), anti-leul9-PE (CDs6); 2H4-PE, (CD4sRA), S6Fi-PE from Coulter, UCHLI-FITC (CO45RO) from Dako-France.

RESULTS

Toxicity of the Procedure on Progenitor Cells (Table I)

The IMD was very weakly toxic to BM precursors as evaluated by their in vitro clonogenic efficiency (CFU.GM/$2x10^5$ MN cells), but the loss of BM cells was substantial; recovery of MN cells after the purging procedure ranged from 37 to 42%, without difference between first and second harvests (single graft, and first or second grafts in the double procedure).

Hematological Recovery

The median time to recover granulocytes ($0.4x10^9$/l) was 24.5 days (8-100) after a single graft, 24 days (12-43) after the 1st graft and 36 days (18-100) after the 2nd graft in the double-graft regimen. The median time to recover platelets ($50x10^9$/1) was respectively 40 days (22-100), 22 days (12-54) and 80 days (30-NR) in the single and double-graft programs (1st and 2nd grafts). Delay to engraftment (defined as more than 45 days to reach $0.5x10^9$ PN and/or more than 60 days to reach $50x10^9$ platelets) was observed in 24 patients.

As shown in table II, this complication was more frequent after the second graft. The analysis of risk factors (table II) showed that the incidence of viral infections and BM involvement before the autograft were similar to those observed in other children in this study, but the 6 patients presenting veno-occlusive disease in the LMCE1 protocol were delayed in recovering both platelets and granulocytes. The number of CFU.GM and mononuclear cells reinjected in to these patients was in the normal range.

As previously described (12,13), those patients presented with an excess of CD8+ leu7+ lymphocytes in the blood and the BM. Four patients received therapy with anti-CD8 monoclonal antibody for 6 days, with successful engraftment in 2.

Detection of Minimal Disease in Bone Marrow

In 64 cases of autografting, the detection of minimal residual disease on the day of BM harvest was performed both by the histological analysis of the trephine biopsies and the immunological analysis of the harvested cell suspension before and after the purging.

In 37 cases, the BM was pathological; malignant cells were detectable by the morphological and/or the immunological analysis. The detection of malignant cells in the autograft before the purging was positive in 23 cases, with contamination ranging from 10^{-2} to 10^{-5} malignant cells; after purging, none of the autograft samples contained any residual NB cell detectable by

immunological analysis; less than 10^{-5} residual NB cells might thus be left after purging.

Fifteen (15) of the 20 patients included in a double-autograft program and analyzed at time of the first BM harvest had residual BM metastases. A second question thus arose, to define whether or not the first course of megatherapy (BCNU-VM26-CBDCA) enabled purging of the BM in vivo. In vivo cleansing of the BM after the first course of megatherapy was observed in only 3 of the 15 patients; one patient with normal BM before megatherapy progressed with the appearance of a BM metastasis after the first course of megatherapy. Thus, in vivo purging of the BM by the first course of megatherapy was not efficient and did not permit avoidance of a purging procedure for the second BM harvest. One must note that in 42 patients having received megatherapy including TBI (VCR, high-dose Melphalan, TBI) (either in the single-graft protocol or for the second graft of the double-graft program), 16 had BM micrometastases before megatherapy; these were detectable in only 5 patients after megatherapy; 2 patients with negative BM before megatherapy later progressed with the appearance of malignant cells in the BM. Finally, when we analyzed the disease-free survival from graft of patients treated with megatherapy and ABMT (first or second grafts in the doublegraft program), we found no significant difference in relation with the presence or absence of BM micrometastases at time of harvest, or with the presence or absence of malignant cells in the autograft.

Immunological Reconstitution and Rationale for Immunotherapy by Interleukin-2 in the Months Following ABMT

We previously showed that in the months following the ABMT, patients had abnormal T cell subset distribution in peripheral blood, with an inverse CD4/CD8 ratio, whereas functional circulating NK cells recovered ever since the first months post-graft and exceeded normal values up to 6 months postgraft (6). We analyzed here the immunophenotypic characteristics of circulating T cells during the first months post-graft. As shown on table III, patients presented with a profound defect (both in percentage and absolute numbers) of the suppressor-inducer T cell subset (CD3+ CD4+ 2H4+ T cells); the percentage of helper-inducer T cells (CD4+, UCHL1+ T cells) was normal (14,15). Moreover, these patients presented a large excess (both in percentage and absolute number) of memory cytotoxic T cells (CD3+, CD8+, S6F1+ or CD3+, CD8+, UCHLi+); the percentage of CD8+ T suppressors is normal, but the absolute number is lower than in normal children (16).

Six of the patients had systemic administration of IL2 between day 60 and day 90 post-graft (see legends table III). IL2 induced minor modifications of the T cell subsets; in particular, these children still presented a defect of the suppressor-inducer T cell subset and a large increase of the memory cytotoxic T cell subset. Furthermore, as expected from experimental data, we observed a major increase of the circulating NK cell population (CD56+ CD3-) which coexpressed CD8, a marker known to reflect the activation of NK cells.

DISCUSSION AND CONCLUSION

The immunomagnetic procedure initially described by Treleaven (1) has been modified and allows 3 log elimination of malignant cells in experimental models (3,4,6). The clinical questions were then to prove that it was nontoxic, and clinically useful. In this series of 71 homogeneous patients, received in our institute since diagnosis and included in the same protocols of chemotherapy, 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. The only risk factor clearly associated with hematological abnormalities was the presence of veno-occlusive disease and the presence in the peripheral blood of an excess of circulating CD8+ leu7+ cells (12,13). It was recently reported that TNFA is increased in patients presenting toxic complications after AMBT (17). The role of various cytokines in the delay of engraftment in our group of patients is under investigation.

The detection of minimal residual disease both in the BM and the autograft proved that the IMD was useful and efficient in more than 50% of the patients. Moreover, "in vivo purging" of neuroblasts from the BM by the first course of megatherapy was inefficient and did not permit to avoid the use of the IMD for the second autograft.

Finally, it has been demonstrated in animal models that chemotherapy and/or irradiation may favor the antitumoral effect of IL2 by inhibiting suppressor T cells (18). Our analysis of the T cell subset in the months following ABMT confirms this hypothesis, as it enables us to confirm the expansion of NK cells by systemic IL2-administration in the months following the graft.

ACKNOWLEDGEMENTS

This work was supported by the Association pour la Recherche sur le Cancer (ARC, grant no.6519) and by the Comite de la Savoie of the Ligue Nationale contre le Cancer (grant 1988).

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TABLE I

TOTAL BM MONONUCLEAR CELLS AND CFU.GM HARVESTED AND REINJECTED IN IMMUNOMAGNETIC DEPLETED ABMT
Analysis of 71 patients

	HARVESTED BM						REINJECTED BM		
	Before Purging (median range)			After Purging (median range)			after Thawing (median range)		
	CFU.GM /2x10^5 MN cells	CFU.GM/kg x10^4	MN cells/kg x10^8	CFU.GM /2x10^5 MN cells	CFU.GM/kg x10^4	MN cells/kg x10^8	CFU.GM /2x10^5 MN cells	CFU.GM/kg x10^4	MN cells/kg x10^8
LMCR Single graft									
n=54 Pts	220	25.0	2.45	171	6.7	0.78	140	3.2	0.58
	(23-800)	(2.1-87.8)	(0.65-6.65)	(11-600)	(0.3-42)	(0.20-2.4)	(11-420)	(0.3-16.5)	(0.15-2.34)
RECOVERY				93 %	27 %	41 %			
LMCR - Double graft									
n=17 Pts									
1st graft	190	18.4	2.10	127	5.5	0.81	110	2.6	0.64
	(30-560)	(6.2-86.0)	(1.68-4.27)	(40-500)	(1.8-10.0)	(0.36-1.38)	(40-500)	(1.3-11.7)	(0.22-1.05)
RECOVERY				71 %	33 %	37 %			
2nd graft	150	19.0	3.46	130	5.0	1.05	60	2.8	0.75
	(75-400)	(7.1-73.4)	(1.65-5.30)	(2-400)	(0.7-15.0)	(0.29-3.54)	(30-220)	(0.7-8.6)	(0.21-2.2)
RECOVERY				64 %	32 %	42 %			

TABLE II

DELAY TO HEMATOLOGICAL RECOVERY AFTER ABMT

Clinical characteristics of the 24 patients

	Single graft		Double graft *
DELAY TO ENGRAFTMENT	14 ptts (25%)		10 ptts (58%)
PN $<0.5 \times 10^9$ day 45	7 ptts (13%)		6 ptts (35%)
PLTS $<50 \times 10^9$ day 60	10 ptts (19%)		8 ptts (47%)
GRAFT FAILURE	4 ptts (7%)		2 ptts (11%)
CFU.GM/kg **		3.2×10^4	
MN cells/kg **		0.52×10^8	
BM INVOLVEMENT before autograft ***	6 ptts (11%)		6 ptts (35%)
VEINO-OCCLUSIVE DISEASE ***	5 ptts (9%)		1 ptt (6%)
VIRAL INFECTION ***	3 ptts (6%)		1 ptt (6%)
PROGRESSION ***	1 ptt (2%)		

* Delays to engraftment were always observed after the second graft.

** median (range) for the 24 patients; there was no difference between single and double graft. These values are similar to those observed in the control group (see table I).

*** Incidence of the complication in the 24 patients; this incidence is similar to that observed in the control group with the exception of the VOD, as described in the text.

TABLE III

IMMUNOPHENOTYPIC CHARACTERIZATION OF THE CIRCULATING LYMPHOCYTES IN CHILDREN AFTER ABMT *

	CD_3+	CD_4+ $2H_4+$	CD_4+ $UCHL_1+$	CD_8+	CD_8+ $2H_4+$	CD_8+ $UCHL_1+$	CD_8+ S_6F_1+	$CD_{56}+$	$CD_{56}+$ CD_8+
NORMAL CHILDREN	39	17	9	20	12	2	4	6	1
absolute number of lymphocytes $x10^9/l$ 3.3 (1.1-5.9)	35-43	5-32	3-15	7-30	7-18	1-7	1.5-5	2-15	0.5-4
day 60 post ABMT	12	3	8	38	13	19	27	18	5
absolute number of lymphocytes $x10^9/l$ 1.2 (0.2-2.2)	8-15	0.4-5	7-11	12-61	12-36	4-31	17-38	10-28	1-4
After IL2 therapy **	14	3	14	25	10	14	14	42	18
absolute number of lymphocytes $x10^9/l$ 5.5 (3.6-6.5)	7-20	0-5	7-22	12-35	4-11	8-35	10-22	30-62	7-27

* The NK cell population was defined as $CD_{56}+$ CD_3-; CD_8 coexpression on NK cells was analyzed with the combination $CD_{56}-CD_8$ and CD_8-CD_3.
Within the T cell population, helper-inducer T cells were defined as CD_3+ CD_4+ $UCHL_1+$ and helper-suppressor T cells as CD_3+ CD_4+ $2H_4+$; memory cytotoxic T cells were defined as CD_3+ CD_8+ $UCHL_1+$ (memory) and CD_3+ CD_8+ S_6F_1+ (cytotoxic).
In an attempt to avoid surestimation of the CD_8+ S_6F_1+ T cell subset, we only considered the bright CD_8+ population; S_6F_1+ cells with a dim expression of CD_8 correspond to NK cells.

** In the $LMCE_3$ protocol, patients in partial remission before megatherapy received IL2 (Eurocetus) ($3x10^6$ U/m^2/day during 5 days in 2 cycles, with 1 week rest between the 2; the course of IL2 therapy was repeated twice) between day 60 and day 90.

HIGH DOSE CHEMOTHERAPY WITH AUTOLOGOUS BONE MARROW TRANSPLANTATION IN WILMS' TUMORS: DATA OF EBMT REGISTRY

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INTRODUCTION

Wilms' tumor represents approximately 6% of childhood malignancies. The modern combined treatment leads to at least 80% long term disease-free survival; complete remission is achievable even after relapse in 6-40% of the cases (1-2).

A number of factors able to influence the clinical course and the eventual outcome, when present at diagnosis or at time of relapse, have been identified (3-4-5). In the selected group of patients with poor prognosis after relapse new strategies are justified, but a large number of patients is necessary to assess their efficacy (6-7-8).

In this search the European Bone Marrow Solid Tumor Registry (EBMT STR) has been examined to obtain data on the use of high dose chemotherapy with autologous bone marrow transplantation (ABMT) in children with Wilms' tumor.

PATIENTS AND METHODS

In the period February 1985-February 1990, 23 children from 6 European Centers (table 1) with histologically documented Wilms' tumor were registered in the EBMT STR following treatment with high dose chemotherapy and ABMT.

Four patients from a single center have been excluded from this analysis because they had received high dose chemotherapy and ABMT in first CR as a part of front line treatment (all these patients are alive, disease free more than 3 years after ABMT). Thus, 19 children who received high dose chemotherapy in salvage strategies were available for this analysis.

There were 10 males and 9 females. At diagnosis, median age was 5 years (range 10 months-15 years), 4 patients had stage I Wilms' tumor, 2 stage II, 4 stage III and 9 stage IV. Three stage IV patients had progressed during first line treatment: 2 achieved CR and 1 PR with a salvage therapy. Eleven

children (4 stage I, 1 stage II, 3 stage III and 3 stage IV) had obtained a 2nd CR after relapse; all but one had poor prognostic factors (5) at relapse. One stage II patient relapsed twice and achieved a 3rd CR. One stage III and 1 stage IV patient obtained PR after a relapse and 2 stage Ill patients were in PD after 2nd relapse.

Most of the various salvage therapy employed included Cisplatin together with VP16 (6), or Ifosfamide with Adriamycin (7). Median interval between diagnosis and ABMT was 21 months (ranges 5-61 months). At ABMT there were 14 patients in CR and 5 with residual or progressive disease after second line treatment. Seven different regimens of high dose chemotherapy were administered (details in Table 2); 7 patients received also involved field radiotherapy. All patients, except one who had had multiple bone metastases, received cryopreserved unpurged autologous bone marrow.

RESULTS

Toxicity

Three patients died of pneumonitis 29-30-45 days after ABMT, in one case a fungal infection was documented at autopsy. Two of them had received bilateral lung irradiation 15 days and 6 months, respectively, before conditioning with Vincristine and Melphalan, the third patient had received BCNU therapy with Carboplatin, VP16 and Cyclophosphamide as high dose. Two patients developed a transient acute renal failure, one a severe hemorrhagic cystitis followed by chronic renal insufficiency. All patients experienced profound myelosuppression and all but one had fever. All patients achieved complete bone marrow recovery except three patients who died of pneumonia before platelet recovery.

Clinical Course

Out of the 5 patients who had measurable disease at transplant, 2 died of toxicity, 3 achieved CR, 2 relapsed at 4-9 months and one is alive disease-free at 17 months. Of the 14 patients grafted in CR, 1 died of pneumonia, 8 relapsed at 3-23 months (median, 6), and 5 are alive disease free at 3-42 months (median, 6). Relapses occurred in the previous sites of disease in 7 patients, in different sites in 3. Time to relapse ranged between 3-23 months (median, 6).

The overall and progression-free survival for the 19 patients are 23% and 18%, respectively, with a follow up ranging from 1-46 mos (median 11 mos) (fig. 1).

DISCUSSION

The actual multidisciplinary treatment of children with Wilms' tumor is highly successful and great efforts are now directed to reduce the side effects of treatment. Large multicenter studies have identified patients at risk of relapse at diagnosis (3) and prognostic factors for children who relapse. At

relapse, unfavorable histology, short duration of initial remission, initial therapy with three drugs, and relapse at a site other than the lungs, are adverse prognostic features (4-5), and survival of these patients with conventional therapies is poor.

We have reviewed data collected in the EBMT STR on the use of high dose chemotherapy in this group of patients.

Although the number of patients is small and various high dose regimens were employed, some considerations can be made:

a) High dose chemotherapy regimens may be given to selected children with relapsed Wilms' tumor although risks of pulmonary and renal toxicities seem to be high (9-10).

b) Children with relapsed Wilms' tumor remain sensitive to high dose chemotherapy even after more than one attempt of retrieval (in fact, 3 out of 5 evaluable patients achieved CR and 1 is alive almost 2 years after ABMT).

c) Five out of the 14 patients who received high-dose chemotherapy in CR as consolidation remain in CR at 3-42 months.

d) Relapses tended to occur early (median 6 months).

In conclusion, a salvage attempt with high dose chemotherapy in children with resistant or poor prognosis relapsed Wilms' tumor appears justified and multicenter trials with common protocols are warranted.

ACKNOWLEDGEMENTS

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TABLE 1

EBMT Solid Tumor Registry:

Centers registering Wilm's Tumor cases

Partecipating Centers	Cases	Investigator
Genova, Istituto G. Gaslini	10	G. Dini
Villejuif, Institut G. Roussy	4	O. Hartmann
Marseille, Hopital de la Timone	2	J.L. Bernard
Barcelona, Hospital St. Creu	1(5)	N. Pardo
Lyon, Centre L. Bérard	1	T. Philip
Valencia, Hôspital La Fe	1	V. Castel

TABLE 2

High Dose Chemotherapy Regimens

Regimen		Number of Patients
Vincristine	4 mg/sqm in 5 days	10
Melphalan	180 mg/sqm 6th day	
(involved field radiotherapy		7)
Cisplatin	200 mg/sqm in 5 days	2
VP16	1 g/sqm in 5 days	
Melphalan	140 g/sqm 6th day	
VP16	1 g/sqm in 5 days	2
Ifosfamide	4 g/sqm in 2 days	
Melphalan	140 mg/sqm 6th day	
BCNU	300 mg/sqm 5th day	1
Vincristine	3 mg/sqm in 4 days	
Melphalan	180 mg/sqm 5th day	
Busulfan	20 mg/Kg in 4 days	3
Cyclophosphamide	120 mg/kg in 4 days	
BCNU	200 mg/sqm 1st day	1
VP16	1 g/sqm in 5 days	
Carboplatin	800 mg/sqm in 5 days	
Cyclophosphamide	100 mg/Kg in 2 days	

FIGURE 1

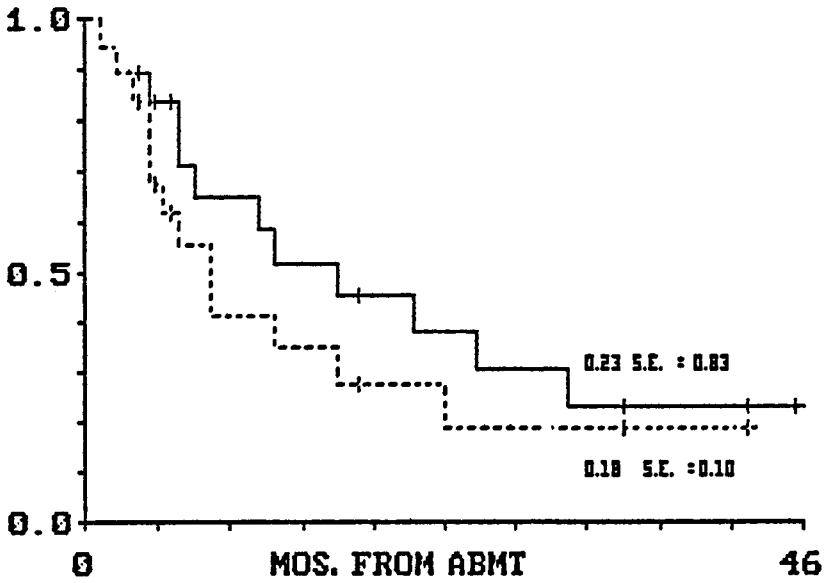

Fig. 1 Overall (____) and event free (-----) survival (projected at 46 mos.) of the 19 Wilms' tumor children who received high dose chemotherapy and ABMT.

STRATEGY INCLUDING SURGERY, HIGH DOSE BCNU FOLLOWED BY ABMT AND RADIOTHERAPY IN SUPRATENTORIAL HIGH GRADE ASTROCYTOMAS: A REPORT OF 98 PATIENTS

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INTRODUCTION

We present here the results of 98 patients treated between March 1986 and June 1989. Our preliminary results were presented at the 1988 Houston symposium. At that meeting, we presented the background of this trial and the therapy which would be used due to patients' poor prognosis despite conventional treatment. At that point we estimated that the treatment program was feasible with a low toxic cost and required a short hospitalization time.

PATIENTS AND METHODS

Ninety-eight patients entered the program. Eighty-five were treated in Centre Leon Berard in Lyon, 10 in the regional hospital of TOURS and 3 in Centre Rene Huguenin in St Cloud.

Patient age ranged from 16 to 65 years with a median of 48 (47 for men and 49 for females). Fifty-three were males and 45 females (sex ratio: 1.28).

At induction of treatment, 70% had a good performance status (PS 0 or 1) 84% were ambulatory or semi-ambulatory (26 patients with a PS grade 0; 42 patients with a PS grade 1; and 14 patients with a PS grade 2). Sixteen had a bad performance status (13 grade 3, 3 grade 4).

Among the 98 patients, three could not have surgery and diagnosis was established on arteriography and CT scan-criteria. Following the Kernohan staging we treated : 20 grade III astrocytomas and 75 grade IV astrocytomas or glioblastomas. Median age is lower for grade III than grade IV : 43 years (Range 16 to 62) and 49 years (Range 17 to 65), respectively.

Nine patients were treated at time of relapse and 89 as a first line treatment. For the latter patients, according to the possibility of surgery based on the size of the tumor and its location in the brain, 2 Strategies have been used: Arm 1 when surgery was done first (72 patients), and Arm 2 when

BCNU was used pre-operatively (17 patients) (Table 1). The treatment plan was the following:

Arm 1

-Cyto-reductive surgery as wide as possible on day 0.
-Early post-operative chemotherapy on day 3 after histology confirmation, combining. VM 26, 150 mg/m^2
-BCNU, 100 mg/m^2 in a 5 mn IV short infusion, through a peripheral venous access.
-PROCARBAZINE (day 3 to 10), 100 mg/m^2/day, per os.

The bone marrow harvesting was scheduled between day 21 to 24, as previously reported; 1x10^8 mononuclear cells/kg were harvested.

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 60C. On the afternoon of marrow harvest, the high dose chemotherapy (BCNU: 800mg/m^2) was administered diluted in 100 ml of 5% dextrose over a 2-hour infusion, using a peripheral venous access. BCNU was preceded 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 subtentorial brain, followed by a localized boost of 21 Gy in 7 fractions to the tumor or tumor bed. Every radiotherapy field was designed using the dosimetry CT scan planning system.

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 scheduled to receive early post-operative chemotherapy (VM 26, BCNU, PROCARBAZINE) and finally were irradiated in the same delay as in Arm 1.

Patients were treated in single rooms, without isolation procedures. Except for the 3 initial patients, monitoring was organized on an out-patient basis with 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 evaluated by the evolution of the PS during the follow up period.

Only 16 patients received the early post operative chemotherapy because of the difficulty to manage it due to the number of surgery departments involved. Planned doses were administered in 11 courses and the mean delay after surgery was 6 days (Range 1 to 15) and it was finally decided to stop this chemotherapy in March 1987.

RESULTS

The toxicity observed in the 98 patients is similar to the first observations we presented and much lower in the most recently treated patients. Marrow harvesting was possible any time; mean of 565 ml of marrow blood could be collected. It contained 1.30 10^8/kg mononucleated cells and 1.6 10^4/kg GMCFUC. The marrow infusion contained a mean of 0.98 10^8/kg MNC (Range 0.33 to 6.43) and 3.36 10^4/kg GMCFUC (Range 0.00 to 27.4). Using furosemide at the end of anesthesia, we did not observe decrease of consciousness and enhancement of neurologic impairment or cranial hyperpressure which was experienced in 5 of the first patients and suspected to be related to hydration administered during general anesthesia for marrow harvesting.

Hematologic toxicity remained very mild: 12% of the patients had severe aplasia grade V with neutrophil less than 500/mm^3 and 6% with WBC less than 1000/mm^3. Grade III is observed in 15% for neutrophils and in 18% for WBC. Mean time of neutropenia for patients with grade 3 and 4 toxicity is 10 days. Mild or no toxicity (grade 0-1-2) is observed in 73% for neutrophils and in 76% for WBC. Concerning platelets thrombopenia grade IV (less than 25,000/mm^3) was observed in 12% of patients, grade III in 19% and grade 0, I, or II in 69%. For patients with grade III or IV mean duration of thrombopenia is 8 days. Nadir for WBC, neutrophils and platelets is on day 21. Immediate tolerance of BCNU was good. We observed 1 sinusal tachycardia, 5 transient febrile episodes and 2 cutaneous rashes.

Nausea and vomiting were mild: 31 grade 0.58 grade I and II and 9 grade III. Visceral complications were also manageable. Of great concern, lung complications were observed 24 times in 20 patients: 5 embolisms of which 2 were fatal and 19 pneumonitis (20 %). Six occurred in the first month after BCNU and 9 in the first two months. Among the 9 occurring in the post ABMT period, 6 were partial and 3 diffuse with 2 life-threatening requiring assisted ventilation. Ten pneumonitis occurred later and often intricated with neurologic degradation and corticosteroid treatment. The bronchoalveolar lavage when realised showed: 5 Candida Albicans, 1 streptococcus pneumonia, 1 pneumocystis carinii. Finally, 4 interstitial pneumonitis were observed: 2 were regressive and 2 were fatal (1 superinfection with pneumocystis carinii, 1 intricated with progression of the disease).

Concerning hepatic complications, most of them were biological changes only: 8% of patients had a minor cytolysis with grade I and II

enhancement of AST - ALT; 58% of patients had GGT increased before BCNU probably due to the anticomitial treatment. No changes were observed during the post ABMT period. Two patients presented a jaundice: 1 HBV hepatitis and 1 CMV fatal infection combining hepatitis (perhaps veno-occlusive disease) pneumonitis and fever.

Forty-seven infectious episodes including pneumonitis and hepatitis already described were observed: 19 (40%) in the first month after BCNU. The others occurred later and are not related with the treatment program, intricated with the disease progression. Nine patients presented a phlebitis.

We observed 5 toxidermia, of which 2 were severe. The most serious presented extensive cutaneous edema, erythema and desquamation associated with a 26,000/mm^3 eosinophilia. In these patients, symptoms progressively disappeared when phenytoin anticomitial treatment was discontinued.

Renal function remained normal except for 3 patients: (2 patients with a grade I toxicity and one patient with a grade II).

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 86 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 bled 3 days after the end of the radiotherapy course in a sudden coma; the necropsy showed a cerebral hemorrhage where 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, 30 critical events are recorded in the first month after ABMT and the total toxic death rate is 6.1% (6 patients): (1 septic shock, 2 hemorrhages, 1 pneumonitis, 1 CMV infection, 1 brain toxoplasmosis infection). Two patients died from embolism and are not considered as a complication of treatment but of the disease itself.

Survival

Survival is the major evaluation criteria in this study as tumor response evaluation is difficult due to the surgical reduction associated with BCNU. Even for the whole program evaluation, CT scan seems to be better for evaluating relapse (preceding clinical progression of 2 to 3 months) rather than tumor response itself.

Median follow up in this study is now 28 months. Overall survival is 10% at 36 months with a median survival at 11 months after ABMT and 12 months after surgery for the whole group (98 patients). The survival of the 89 patients treated in the initial phase of their disease is the same: Median survival 11 months after ABMT (there is no difference if surgery is performed before or after BCNU): patients treated in Arm one: 12 months median survival and

in Arm two: 9 months median survival (curve no. 1). Regarding the quality of surgical resection, a macroscopically complete resection was possible in 36% of patients treated by surgery first compared to 70% for patients treated first by high dose BCNU. For those patients it was noted during surgery a nodular aspect of the tumor and a more marked limit with the normal brain. Furthermore, tumors seemed to be less hemorrhagic. Concerning histopathological observations, all tumors were widely necrotic with a very thick capillary endothelium, but in all cases viable astrocytoma tumor cells were remaining: 12 grade IV and 2 grade III. For the 6 patients having a biopsy before BCNU we observed no change in the histological staging. All had residual viable tumor cells and the major modifications due to the high dose BCNU seem to concern first capillary endothelium becoming thicker with sclerohyalin aspect; necrosis also seems to be wider.

Therefore, for our patients a macroscopically complete surgical resection does not allow a better survival between patients of Arms 1 and 2. However, for patients treated in Arm 1 only, the difference of survival between macroscopically complete resection and other operative procedures is significant with a 4 month greater survival for patients with a complete resection ($p < 0.05$). Thus patients of Arm 2, despite an easier operative procedure after BCNU, have probably a poorer prognosis due to the initial surgical contraindication. On the other hand, we found two differences in the survival of the whole group according first with age and performance status, secondly with histological grading.

We observed a difference in survival for patients under or over 30 years old which is significant: 17 months versus 10 months, respectively ($p < 0.05$). In the same way survival is better for patients with a good performance status: 13 months for PS = 0.1 or 2 versus 3 months for PS = 3 and 4, p (0.001). The best survival is observed for patients under 50 years with a good performance status (0, 1 or 2). Their median survival is 17 months versus 10 months for older patients or those with a poor PS, and versus 2 months for older patients with a bad PS (curve no. 2).

The histological grading seems to determine the greatest difference in survival. In fact, among the 86 patients with a known histology, the median survival of 17 grade III is 14 months compared to 11 months for the 69 grade IV. At 3 years 33 % grade III are surviving. If we consider only the 72 patients treated in the Arm 1, the difference is greater with a 31 month median survival for grade III (15 patients) and an 11 month survival for grade IV (57 patients), and with 38% grade III versus 5% grade IV survivors at 3 years. Thus, histological grading seems to remain the most acute prognostic factor at this time (curve no. 3).

In glioblastomas, quality of life is of concern for those patients, due to the poor prognosis and their neurologic impairment. In an attempt to evaluate the quality of life we analyzed the evolution of the PS during the follow up of patients: 64% of patients surviving 12 months are still ambulatory or semi-ambulatory (PS = 0, 1 or 2) and 46% are strictly ambulatory (PS = 0,1). In contrast, after the first year PS decreases rapidly and patients often require

constant help (table 2). We then observe that patients seem to be stabilized from a neurological point of view, keeping the same PS they had after surgery during the first year (which corresponds to the median survival of the patients) and then patients rapidly get during relapse.

CONCLUSIONS

In conclusion we think that such a strategy can be ethically considered in those patients as initially accepted by the Lyon's University committee as the 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 remain poor for the whole group but quality of life is acceptable during the first year and the results are better for younger patients with a good performance status. Furthermore, the results are encouraging for patients with grade III astrocytomas. At this point we decided to pursue this procedure for young patients with a macroscopically complete resection of grade III astrocytomas. For other patients with residual tumor after surgery (grade III and IV), we designed a new phase I-II high dose nitrosourea compound Fotemustine trial to potentially increase the efficacy. Fotemustine plasma kinetics is investigated for each patient. Fourteen patients have been treated with 600 to 800 mg/m^2 Fotemustine in 2 days for one hour infusions. Myelotoxicity is controlled by ABMT but seems to be more important than BCNU at the 800 mg/m^2 level. Toxic death has not been experienced until now. Efficacy is not yet evaluable but progression has not been observed at the first evaluation after completion of the treatment program. Nevertheless, 3 patients died from relapse, and 11 are still alive 1 to 10 months after Fotemustine.

TABLE 1

GLIOMAS = ARM 1

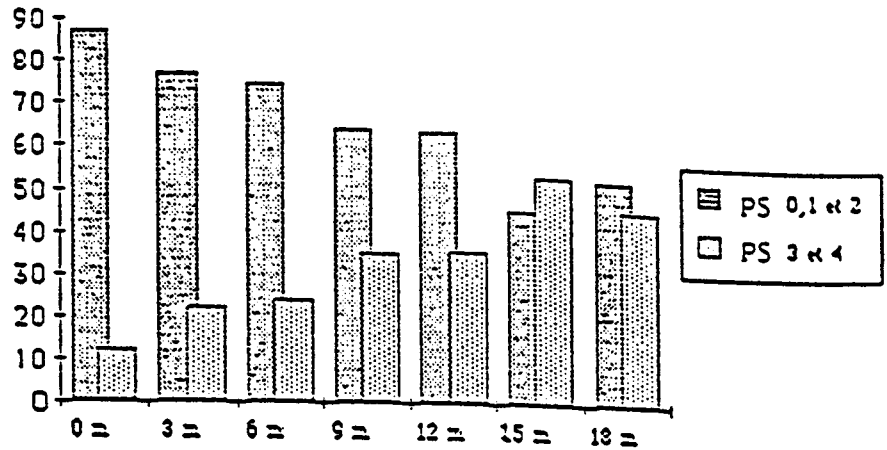
D 0	Surgery	
D 3	VM 26: BCNU:	150 mg/m^2 100 mg/m^2
D 3 to 10	Procarbazine:	100 mg/m^2
D 21	Marrow Harvesting High Dose BCNU:	800 mg/m^2
D 24	Marrow Infusion	
D 45 to 63	Radiotherapy	45 Gy

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: BCNU: Procarbazine	150 mg/m^2 100 mg/m^2 100 mg/m^2
D 45 to 63	Radiotherapy	45 Gy

GLIOMAS STRATEGY

TABLE 2



Evolution in percent of performance status.

FIGURE 1

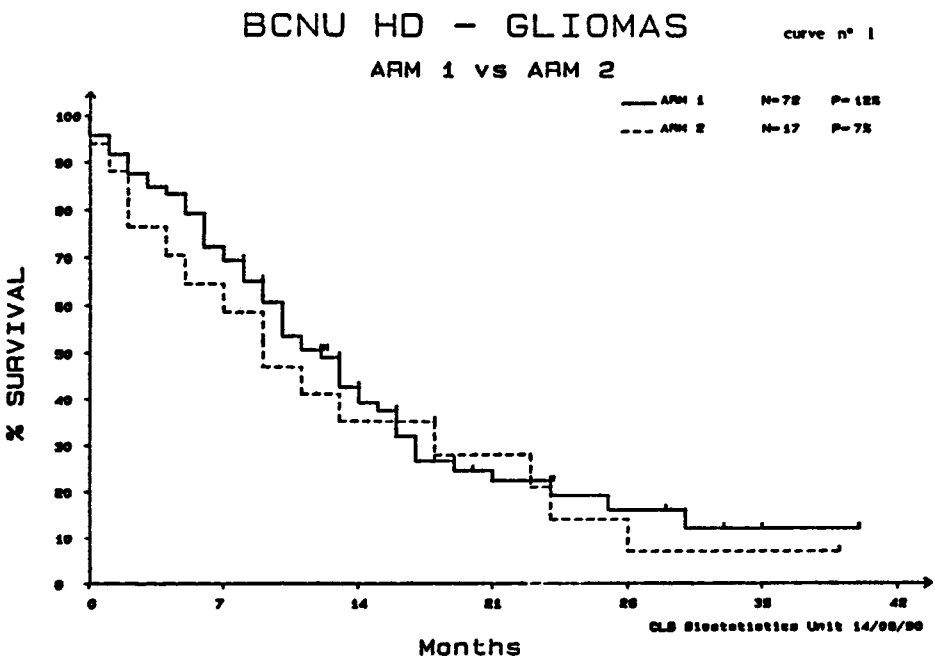


FIGURE 2

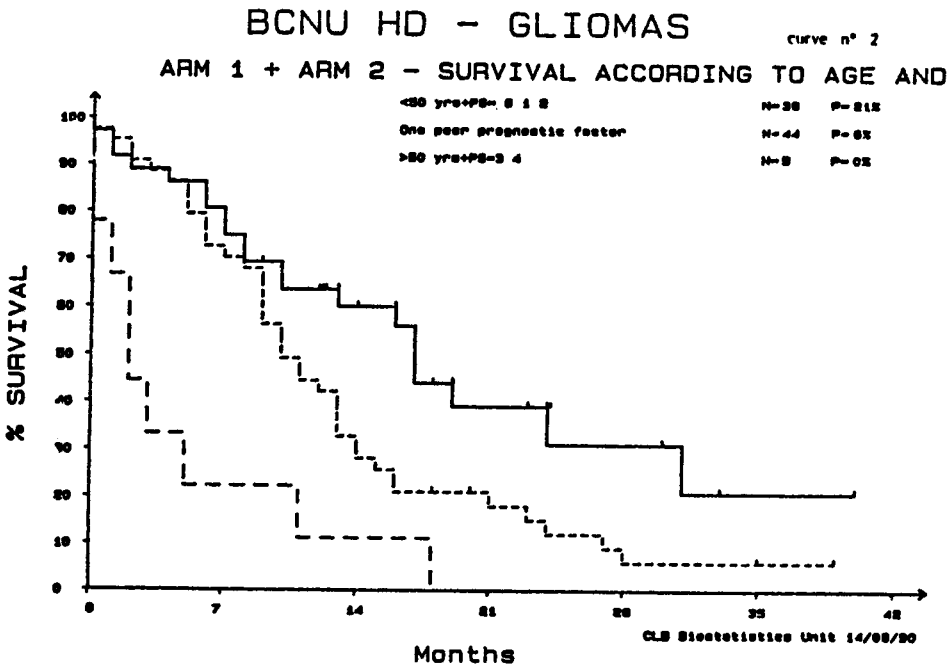
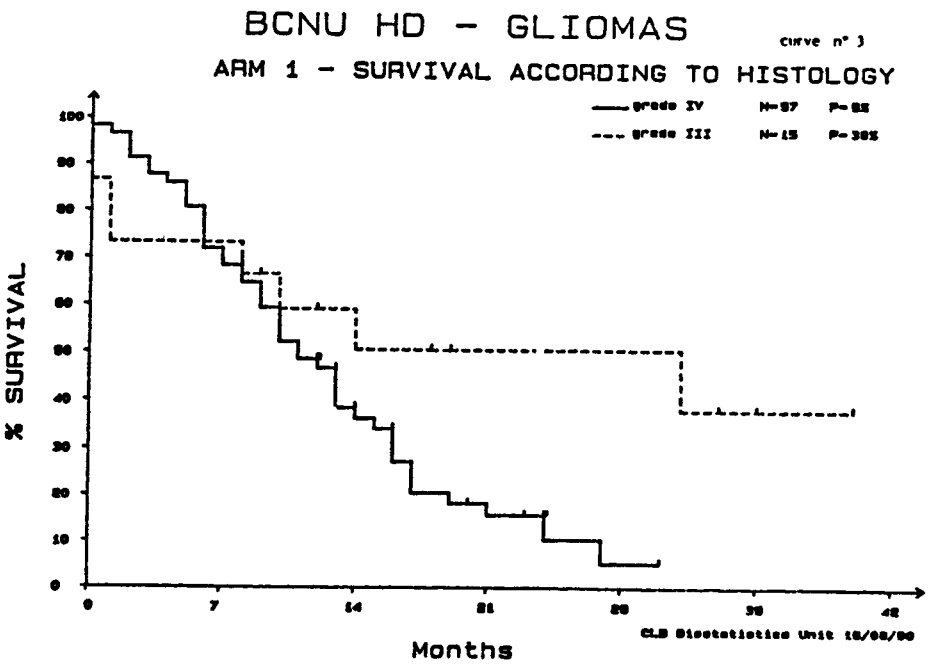


FIGURE 3



DOSE INTENSIVE THERAPY FOR GERM CELL NEOPLASMS

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ABSTRACT

Dose intensive chemotherapy for recurrent or refractory germ cell cancer has evolved over the last decade. Initial experience using high dose regimens including single agents such as cyclophosphamide, thiotepa and VP-16 demonstrated that responses could be obtained in the majority of patients but these responses tended to be of short duration. Second generation studies focusing on this disease site incorporated drugs and principles more specific to germ cell cancer. Large studies performed at Indiana University and in several European centers have demonstrated that the addition of high dose carboplatin or cisplatin to other regimens can result in long term survival in otherwise incurable patients. with the results of initial pilot studies now confirmed, high dose carboplatin-based chemotherapy is moving to be come a portion of first salvage therapy and in some centers entering protocols of initial therapy for poor risk patients. In addition, based on the success of some of the early studies in germ cell cancer, similar protocols are being investigated in other disease sites such as ovarian cancer, neuroblastoma, lymphoma, and breast cancer.

INTRODUCTION

Clinical trials in the treatment of germ cell cancer have been particularly fruitful. Development of clinical trials and careful interpretation of results of these investigations have led to chemotherapy treatments that cure 75%-80% of patients with disseminated disease.[1] The use of new compounds within the context of these clinical trials has led to marketing of drugs such as cisplatin, etoposide and ifosfamide with proven clinical activity in a variety of other neoplastic diseases. Perhaps of more importance, clinical investigations in germ cell cancer have helped shape and validate general chemotherapy principles such as the importance of intensive induction therapy, the futility of low dose maintenance treatment and minimizing treatment for patients with a well-defined good prognosis.[2,3,4]

An alternative to the addition of new drugs to chemotherapy combinations has been exploring the effect of intensifying the doses of conventional drugs in order to improve therapeutic outcomes. Preclinical investigation of the effect of dose of certain drugs on cell kill has generated favorable dose- response curves for many common anticancer drugs. Hryniuk and others have reviewed clinical studies in breast cancer, ovarian cancer and other disease sites and correlated projected dose- intensity measured in mg of drug administered per square meter of body surface area per week ($mg/m^2/wk$) with clinical outcome.[5] Clinical investigation of high dose therapy has shown benefit for patients with recurrent hematologic malignancies, but this approach has not yet been found to be generally applicable for patients with solid tumors.

From many aspects, germ cell cancer is an ideal disease site to test the concept of dose-intensity. Laboratory evidence suggests a steep dose-response curve for cisplatin, the most active single agent in this disease. Several clinical trials have suggested improved outcome for patients with germ cell cancer who receive more cisplatin-intensive regimens.[6,7] With the availability of carboplatin, true dose-intensity can be achieved in the four to five fold range when coupled with autologous bone marrow support and preliminary trials suggest that such dose escalation can overcome some degree of cisplatin resistance.

Given the evidence of the effect of dose on outcome and the utility of prior clinical investigations in this disease, it seems most reasonable to use this disease site as a template to develop newer approaches to treatment of other solid tumors. In particular, patients with recurrent germ cell cancer are suited to investigation of high chemotherapy regimens requiring autologous bone marrow rescue. These patients are typically young, have few concomitant health problems and only rarely have bone marrow involvement with tumor. The results of previous investigations using high dose therapy in germ cell cancer and an overview of current and future directions of high-dose chemotherapy trials will be summarized herein.

PATIENTS AND METHODS

Early Trials of High Dose Chemotherapy in Germ Cell Cancer

Early clinical trials using marrow ablative chemotherapy in testis cancer enrolled highly refractory patients in broad dose-seeking investigations of high dose therapy. These trials were not disease-site specific and accrued patients with a variety of different malignancies. Regimens included high dose cyclophosphamide, etoposide and thiotepa either alone or in combination. Results from these broad studies are difficult to interpret, but several conclusions can be drawn. First, response to therapy was common. About 80% of patients in these early trials achieved an objective response with about 25% obtaining a complete remission.[8] Second, response to therapy was brief.

Remission duration was usually measured in weeks and only very rarely did patients have enduring benefit. Nonetheless, these high response rates prompted a second generation of studies of high dose therapy incorporating agents and principles more specific to germ cell cancer.

Indiana University Studies

Between September 1986 and July 1988, 29 patients with recurrent germ cell cancer at Indiana University were entered on a larger phase I/II trial in conjunction with Vanderbilt University. [Nine] Entry criteria included either failure of an initial cisplatin containing regimen and a salvage regimen with cisplatin and ifosfamide or cisplatin-refractory disease as defined by progression on cisplatin or within four weeks of the last dose.

The treatment protocol was designed to deliver two consecutive courses of high dose carboplatin and etoposide with autologous bone marrow rescue. The rationale for this protocol lies in the clinical and preclinical evidence of a steep dose-response curve for cisplatin in a variety of human cancers. Cisplatin dose escalation is limited to about twice the conventional dose by neurotoxicity, renal impairment and ototoxicity. Carboplatin is a second generation platinum coordination complex with an activity profile similar to cisplatin. However, the toxicity profile is substantially different with the dominant toxicity being myelosuppression.

Eligible patients underwent bone marrow harvest with a target yield of 2×10^8 mononuclear cells/kg. Carboplatin and etoposide were then administered on day -7, -5, and -3 followed by infusion of half of the cryopreserved bone marrow on day 0. After hematopoietic recovery, patients were assessed for toxicity and response. Patients experiencing unacceptable non- hematological toxicity or failing to respond were excluded from a second course of therapy. Patients were given a fixed dose of etoposide (400 mg/m^2 QOD x 3) and varying carboplatin doses - (300 mg/m^2 - 600 mg/m^2 QOD x 3). After determination of the maximum tolerated combination of carboplatin and etoposide, patients were treated with carboplatin 500 mg/m^2 and etoposide 400 mg/m^2 QOD x 3.

Twenty-nine patients were given 49 courses of therapy. Nine patients obtained complete remission and six obtained a partial remission for an overall response of 528. Four of the patients remain in CR at 30+, 28+, 20+, and 18+ months. In addition, one patient obtained a complete serologic response and partial radiographic remission. At 24 months, the patient remains with normal tumor markers and continued slow resolution of his multiple small pulmonary nodules. These abnormalities are thought to represent involuting necrotic fibrous tissue. Two of these long term survivors had exhibited progressive disease while receiving cisplatin-based regimens. An additional patient developed a therapy-related myelodysplastic syndrome and died 28 months after high dose treatment free of germ cell cancer.

The primary toxicity of this regimen was myelosuppression. Four patients died during the granulocytopenic nadir. Death was attributed to sepsis in three patients and veno-occlusive in the fourth. Clinically significant enterocolitis was seen as the primary non-hematologic toxicity. Ototoxicity, renal impairment, and neurotoxicity were not clinically significant complications.

This study demonstrated that very highly refractory germ cell cancer could be cured with high dose therapy as third-line treatment. An important

corollary was that overt cisplatin resistance could be overcome in some cases by massive doses of carboplatin and etoposide. A confirmatory trial of this important study has recently completed accrual goals through the Eastern Cooperative Oncology Group.

European Studies

Serial studies of high dose chemotherapy at the Institut Gustave-Roussy have demonstrated activity in recurrent germ cell cancer.[10] These investigators developed a high dose regimen with cisplatin 40 mg/m^2/day 1-5, etoposide 350 mg/m^2/day 1-5 and cyclophosphamide 1600 mg/m^2/day 2-5. Autologous bone marrow was reinfused 72 hours after completing Chemotherapy. Mesna was given at a dose of 1 g/m^2/day 2-5 as a urothelial protectant. This regimen (PEC) was given to 19 patients with recurrent germ cell tumor. All patients had received previous cisplatin based chemotherapy. Myelosuppression was the only significant toxicity and extramedullary complications were mild. Two of the 19 patients died from toxic consequences of treatment. Fifteen patients were evaluable for response. Among the 12 patients with chemotherapy resistant disease, there were two early deaths and the remaining ten patients experienced tumor progression with a median survival of seven months. Of seven patients with non- resistant relapse, there were two early deaths and five patients remain in continuous complete response at 3+, 45+, 48+, 49+, and 51+ months.

The succeeding study at the same institution enrolled patients felt to be at high risk of failure with conventional therapy based on massively elevated tumor markers.[11] Therapy consisted of two courses of cisplatin, vinblastine, etoposide and bleomycin in conventional doses followed by a single course of high dose PEC. Thirty-two poor risk patients were entered. Of these, 21 patients (65%) achieved disease-free status. Five died of rapidly progressive disease early in the course of treatment. Three patients did not respond to initial therapy and did not go on to high dose treatment. Of the patients obtaining disease-free status, six have relapsed. The remaining 15 patients remain free of disease with a median follow-up of 18 months.

A preliminary report from Linkesch and colleagues in Austria combines features of the PEC protocol and the protocols from Indiana University along with a hematopoietic growth factor.[12] In this study, high-dose chemotherapy with carboplatin (2000 mg/m^2), etoposide (1500 mg/m^2), and cyclophosphamide (60 mg/kg x 2) was given to patients with recurrent and refractory germ cell cancer. Twelve patients received the high dose chemotherapy with autologous bone marrow rescue and an additional four patients received the same treatment with GM- CSF along with autologous bone marrow rescue. The hematologic toxicity appeared to successfully modulated by the use of GM- =CSF. Of the 12 patients receiving therapy without the hematopoietic growth factor, the median time to an absolute granulocyte count > 500 was 20 days compared to 12 days for the four patients receiving identical therapy plus GM-CSF. The median time to hospital discharge was 24 days in the group of patients not receiving GM-CSF compared to 15 days in the group receiving GM-CSF.

Non-hematologic toxicity included grade 3 and 4 diarrhea in four patients, renal toxicity in two patients and ototoxicity in two patients. There was no significant neurotoxicity, hepatotoxicity or cardiac toxicity and there were no therapy related deaths. Overall, eight patients achieved a partial response and three achieved durable complete responses lasting 7+, 9+, and 13+ months. A larger study by Rosti and colleagues in Raveena, Italy used the basic carboplatin and etoposide protocol skeleton developed at Indiana University and has begun to expand it with the addition of ifosfamide.[13] In this study, 21 patients were entered after failing primary and usually secondary therapy for germ cell tumors. Therapy consisted of a similar dose and schedule of carboplatin and etoposide as used at Indiana University, but, recently, have added ifosfamide at a dose of 12 gm/m^2 over 3 days. Again, the primary toxicity was myelosuppression and no significant renal toxicity, ototoxicity or neurotoxicity was encountered. Overall, 13 patients received one course, seven received two courses and one patient received three courses. There was one therapy related death due to veno-occlusive disease. There were eight complete remissions including three patients who had unresectable partial remissions with prior therapy. The duration of complete remissions were 2, 4, 4, 1+, 2+, 4+, 5+, 5+, and 33+ months. This study confirms that high dose carboplatin/ etoposide-based chemotherapy can result in cure in patients with recurrent or resistant germ cell cancer.

Current Studies

With the promising results of these early trials of high dose chemotherapy in patients with highly refractory germ cell cancer, investigators have begun to use similar regimens earlier in the course of treatment. Indiana University and the Eastern Cooperative Oncology Group have begun a trial of high dose chemotherapy as a part of initial salvage treatment. Patients with recurrent germ cell cancer are given two courses of conventional therapy with vinblastine, ifosfamide and cisplatin followed by a single round of high-dose carboplatin and etoposide.

Other investigators have begun to use high dose chemotherapy as a portion of primary treatment for patients with poor prognostic features. A multicenter French study randomizes poor risk patients to receive either cisplatin, vinblastine, etoposide, and bleomycin alone or the same combination followed by high dose chemotherapy with cisplatin, etoposide, and cyclophosphamide. Memorial Sloan Kettering Cancer Center has begun a trial of high dose carboplatin and etoposide for patients who have a suboptimal serologic response to conventional initial therapy.

CONCLUSION

Future Directions

Germ cell cancer has served as a model for development of new drug combinations and validation of chemotherapy principles. This uniquely chemotherapy sensitive tumor should be used as a guide to developing new

strategies of high dose chemotherapy. As such, this disease site should be selected to investigate new drugs or new combinations. Plans for high dose trials using newer platinum coordination complexes are being formulated.

Applying the lessons learned from investigations in leukemia and lymphoma, high dose therapy in germ cell cancer may be more effective when used as part of initial salvage therapy when the patient has not become resistant to treatment and has undergone cytoreduction with conventional treatments. Several current trials reflect this approach and investigations in this clinical setting are warranted. In addition, the use of hematopoietic growth factors may extend the dose range of currently available agents, allow for multiagent high dose combinations and improve tolerance of high dose therapies.

Our current ability to predict outcome of therapy in patients with poor risk features is not sufficiently precise to identify a risk group that is incurable. In fact, it is difficult to define a risk group with a less than 50% cure rate with standard chemotherapy. As such, it is difficult to recommend that high dose therapy be used as part of initial treatment. With the success of standard therapy, comparative trials with and without high dose chemotherapy would be very difficult to accomplish in this rare tumor. Newer technologies such as flow cytometry and cytogenetics may help define a risk group in whom the rigors of high dose therapy could be justified, but currently, investigative efforts are more properly confined to the setting of recurrent disease after primary standard dose therapy.

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HIGH DOSE CHEMOTHERAPY AND AUTOLOGOUS MARROW RESCUE IN POOR PROGNOSIS OVARIAN CARCINOMAS

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INTRODUCTION

Ovarian carcinoma is frequently a fatal malignancy in women, partly because it is usually diagnosed at an advanced stage (1-3). FIGO stages III and IV have a poor prognosis, with a survival rate $<20\%$ at 5 years. Alkylating agents have some benefit in treating these patients, and Melphalan at a standard dosage produces a 47% response rate (6). The introduction of Cis-Diamine-Dichloro-Platinium (CDDP) improved the response rate and the disease-free survival of advanced stages (7,8,9). However, after the first line therapy, treatment is not yet well established, particularly for patients with residual tumor at the second look surgery.

In this study, we analyze the impact of high dose chemotherapy (HDC) in 48 patients with poor prognosis ovarian carcinomas.

PATIENTS AND METHODS

Forty-eight patients between February 1981 and March 1990 were studied. Initial FIGO stages were III (38 patients) or IV (10 patients); histologic types were serous tumors (42 patients) mucinous tumors (3 patients) clear cell tumors (2 patients) and granulosa cell-tumor (1 patient). At time of HDC, their median age was 46 years (range 22 to 57). All patients underwent an initial debulking surgery, then a median of 6 cycles (range 4 to 12) of chemotherapy containing CDDP. Second look surgery was performed in 43 patients (5 have evidence of refractory disease).

Results of initial therapy were: refractory disease: 12; partial response ($T>2$ cm): 11; partial response ($T<2$ cm): 11; complete response: 14. Out of the 48 patients, 44 received HDC after the first line therapy. So, at time of HDC, status of patients were: refractory disease: 15; partial response ($T>2$ cm): 10; partial response ($T<2$ cm): 12; complete response: 11. Thirty four patients received Melphalan alone, IV bolus, at a dosage ranging from 140 mg/m^2 to 240 mg/m^2. Marrow was infused 24 hours after Melphalan.

Twelve patients received cytoxan 60 mg/kg two consecutive days and Melphalan 140 mg/m^2 the third day; one patient received Busulfan 4 mg/kg each day, during four days before Melphalan 140 mg/m^2. In these cases, marrow was also infused 24 hours after Melphalan. One patient received CARBO.PEC : VP16 350 mg/m^2/day, day 1 to day 5, Cytoxan 1600 mg/m^2/day, day 2 to day 5, Carboplatin 300 mg/m^2/day, day 2 to day 5. Marrow was infused on day 8. All patients were hydrated with 3 l/m^2 and received Mesna for uroprotection with Cytoxan. In all cases, marrow was cytologically normal.

RESULTS

Response

Thirty patients were not evaluated for response (CR or PR without third look surgery). Four patients, with refractory disease, failed to response, 8 achieved a partial response defined as a diminution of tumor size > = 50% for at least 4 weeks (RD : 7, T>2 cm : 1) and 6 patients achieved a complete response (RD : 2, T>2 cm t 3, T<2 cm : 1). The overall response rate was 78 % among evaluated patients.

Toxicity

Haematological toxicity (Table 1) and extra-haematological toxicity (table 2) were no different between the different conditioning regimen. Granulocytes were inferior to 0.5x10^9, 1 during 13 days (range 6 to 45) and platelets inferior to 50x10^9/1 during 26 days (range 13 to 300). Severe mucositis, diarrhea and bacteremia occurred in 35%, 30%, 32% of patients. However, 4 patients died from the procedure: 2 with extensive aspergillosis, one with viral hepatitis and one from a secondary acute myeloid leukaemia 10 months after HDC.

Duration of Response

Fifteen patients had a refractory disease at time of HDC. Four failed to respond, 11 progressed in a median of 3 months. However, one progressed 50 months after HDC and three are alive 11, 36, 69 months after HDC with disease.

Out of the 10 patients with tumor >2 cm, 6 progressed in a median of 7 months (range 4 to 33) and 3 are alive with non evolutive disease (10+, 69+, 72+). Two patients with tumor <2 cm are alive with non evolutive disease (3+, 50+), 9 relapsed in a median of 14 months (range 3 to 52). Eleven patients were in complete response at time of HDC : 5 relapsed (17 months) (7,24), 6 are alive NED (4+, 10+, 21+, 26+, 46+, 47+). Overall 11 patients are alive NED in a median of 26 months (range 3 to 12).

DISCUSSION

Among evaluable patients, 78% responded to HDC. This is a very high response rate, particularly for patients with a refractory disease after a first line therapy with CDDP. Few other data are available on the response rate to HDC of ovarian carcinomas, especially after initial therapy by CDDP (12, 13, 14).

Haematological and extra-haematological toxicity were acceptable, and not different between the conditioning regimen. However, 4 patients died from the procedure. Two had a refractory disease, with a Karnofsky $< 50\%$ at time of HDC, but one who died from AML had a small residual tumor at time of HDC.

Duration of response was short, but was longer in patient with small residual disease than in patient with refractory disease. This study seems to show an effect of the status at time of HDC. However, it's difficult to define the impact of HDC in patients with complete remission after a first line therapy. Further studies are needed, to compare it to radiotherapy or intra peritoneal therapy.

We are now trying to increase the duration of response in patient with refractory disease or minimal response after initial therapy in which HDC and ABMT is probably the only possible salvage. Two ways are used : the increase of conditioning regimen (pre-intensification with Ifosfamide-CDDP 200 mg/m^2, then association of carboplatin 1600 mg/m^2-Cytoxan-VP16 for RD, association of carboplatin 1200 mg/m^2, VP16, Melphalan in small responders) and utilization of immunotherapy post ABMT.

ACKNOWLEDGEMENTS

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TABLE 1

HEMATOLOGICAL TOXICITY

Infused nucleated cells/kg x 10^8:	2 (0.2; 4.2)
Days with granulocytes $<0.5x10^9/1$:	13 (6; 45)
Days with platelets $<50x10^9/1$:	26 (13; 300)

No difference between : Melphalan > or < 180 mg/m^2

Melphalan and Melphalan-Cytoxan

TABLE 2

NON-HAEMATOLOGICAL TOXICITY

Mucositis moderate or severe:	35 %
Diarrhea moderate or severe:	30 %
Bacteremia:	32 %

Median of hospitalization (days) : 35 (21-70)

DOSE INTENSIVE THERAPY FOR ADVANCED MELANOMA

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INTRODUCTION

The results of standard treatment for metastatic malignant melanoma have been disappointing. A wide variety of agents, alone and in combination, have been tried, with DTIC (dacarbazine) most extensively evaluated. The response rate (complete and partial) in patients receiving DTIC, alone or in combination, is about 20% (1,2). We, and other investigators, have explored dose intensive therapy with alkylating agents with the hope of improving the response in patients with metastatic malignant melanoma. Previous reviews have analyzed these studies (3,4); this manuscript will summarize our phase I - II trials with single and double alkylating agents and preliminary results from a trial combining the biologic response modifier interleukin-2 (IL-2) with a dose intensive regimen.

MATERIALS AND METHODS

Patients

Patients eligible for treatment had metastatic melanoma and met the general requirements for participation in our phase I and II trials of dose intensive therapy and autologous marrow rescue. Informed consent, approved at each institution, was obtained before beginning treatment.

Marrow Processing

For protocols requiring autologous marrow, the marrow was collected and cryopreserved by standard methods (5) before the high dose therapy was administered. Histology was normal and the marrow was collected a minimum of 4 weeks after previous chemotherapy. The marrow was cryopreserved with dimethyl sulfoxide and was kept at -196C in the liquid phase of liquid nitrogen. Three to four days after completing chemotherapy, the marrow was thawed and rapidly infused intravenously.

Chemotherapy Regimens

We have conducted five phase I-II trials: three with single agents (BCNU, melphalan, thioTEPA) and two with two agents (BCNU/melphalan, melphalan/thioTEPA). We currently have three phase II trials using thioTEPA/cyclophosphamide, etoposide/cyclophosphamide, and IL2/thioTEPA. During the phase I dose-escalation studies, a modified Fibonacci scheme was used. For the single agent trials, the total dose was administered over 3 days, given intravenously over 2 hours (BCNU, thioTEPA) or by rapid bolus (melphalan). When combinations were used, the starting dose for each agent was 50-80% of the maximally tolerated dose achieved during the phase I single agent study. Thus, for BCNU and melphalan, the starting doses were 50% of each (i.e., BCNU 600 mg/m^2 and melphalan 90 mg/m^2) and were escalated individually to 100% of each. When the melphalan-thioTEPA trial was started, the melphalan was started at the 50% level and the thioTEPA was given at 80% of the single-agent maximum tolerated dose (900 mg/m^2). The third, and current, trial uses thioTEPA (900 mg/m^2) and cyclophosphamide (150 mg/kg) without dose escalation. For patients not eligible for this trial, a dose-intensive regimen, based on our acute leukemia study (6), of etoposide (4200 mg/m^2, 70 mg/m^2/hr) followed by cyclophosphamide (200 mg/kg, 50 mg/kg/day) without marrow support is given. At one of our institutions (Washington University), an NCI supported trial of IL-2 and thioTEPA (with marrow support) has been in progress since December, 1988. Patients were to receive 2 cycles of IL-2 (100,000 u/kg IV bolus q8 hr to 15 doses) followed by thioTEPA (900 mg/m^2) and after recovery another two cycles of IL-2.

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). The duration of response was calculated from the day of marrow infusion. The results of our phase I and II studies have been reported and are updated for this report (7-15). For statistical comparisons, the confidence interval method described by Simon, Fisher's exact test, or chi-square method were used (16).

RESULTS

The responses of patients treated for advanced melanoma with maximally tolerated doses of BCNU, melphalan, or thioTEPA are found in Table 1. The doses were determined to be the maximally tolerated doses in the phase I trials. The dose-limiting toxicities from these trials were hepatic (acute yellow atrophy) and pulmonary (interstitial fibrosis) with BCNU, mucositis with melphalan, and CNS (organic brain syndrome) with thioTEPA. Only in the phase I study using thioTEPA was a dose-response effect noted at 900 mg/m^2, with significantly higher response rate in patients receiving > 900 mg/m^2

compared with patients who received lower doses. For each of the drugs, however, a comparison (Table 2) with the response rates reported for conventional doses demonstrates a significantly higher response rate with the high dose used with marrow support ($p<0.05$ by confidence interval). The overall response rates for melphalan and thioTEPA were higher than for BCNU. The difference can be accounted for by a significantly poorer response of patients who had received prior therapy before high-dose BCNU (Table 3). There were also better responses in patients who had more limited disease (soft tissue involvement, i.e., confined to skin and/or lymph node) compared to patients with visceral metastatic disease. Overall, 10/15 (67%) responded with skin and/or lymph node disease, while 51/97 (53%) patients with visceral metastases responded. The median duration of unmaintained response was similar for all three agents: BCNU 6 months (range: 2 - 46+ mo.); melphalan 4 months (range: 2 - 14 mo.); thioTEPA 4 months (range: 2 - 31+ mo.). Of note, 10-15% of the patients had responses that lasted more than 1 year.

Since there were no overlapping non-myeloid toxicities, we embarked on a series of phase I-II combination alkylator studies. The first, CARMEL, involved combining melphalan and BCNU (4,12). We began at 50% of the maximally tolerated doses from the phase I single agent studies (melphalan 90 mg/m^2, BCNU 600 mg/m^2) and escalated each drug individually. While we had hoped to escalate each to the full single dose level, this expectation was unrealistic if the phase I trial had truly identified the single agent maximum tolerated dose. The pattern of marrow recovery was similar to that observed with each agent alone. The pulmonary (noninfectious diffuse interstitial pneumonitis and respiratory distress syndrome) and gastrointestinal (diarrhea) toxicities were increased in frequency at the highest level (100% melphalan and BCNU) compared to the lower combination levels or the single agent frequencies. Overall, there were 58 patients entered, with 6 CR and 28 PR, for a response rate of 59%. The response rate showed a trend for improved responses with increasing doses (44% at the lowest, 59% at the highest levels), but too few patients were entered at each level to be statistically significant. The response rate resembled melphalan alone and was marginally better than BCNU alone, but the duration of response resembled BCNU with a median duration of unmaintained response of 5 months (range: 2 - 30+ mo.), with 15% of patients responding for more than 1 year.

The significant dose escalation possible and the dose response seen with thioTEPA prompted the second study with melphalan and thioTEPA, MELT (13). With the recognition of an inability to reach full maximally tolerated doses when used in combination, we started at 50% melphalan (90 mg/m^2) and 80% thioTEPA (900 mg/m^2). Seven patients were treated, but no escalations were accomplished because of significant liver and lung toxicity not previously encountered with these drugs.

When the availability of intravenous melphalan occurred because of formulation problems, we substituted cyclophosphamide (150 mg/kg) in combination with thioTEPA. The regimen consists of thioTEPA 300 mg/m^2 and cyclophosphamide 50 mg/kg daily for three days with marrow rescue after

3 days rest. As part of a phase II study, 6 patients with melanoma have been treated. Marrow recovery has been within 4 weeks and none of these patients had severe grade 3 or 4 toxicity. Five of the six (83%) responded, with 3 CR and 2 PR. Three patients had skin and/or lymph node involvement. All three responded (2 CR), and the responses were of 8+, 15, and 24+ months. The patients with visceral metastases responses were all less than six months.

We have recently employed the use of a dose intensive regimen which does not require marrow support for patients with advanced hematologic malignancies. Etoposide (4200 mg/m^2) and cyclophosphamide (200 mg/kg) were given without the need for marrow (6). We have applied this treatment for patients with involved marrow or for patients not eligible for a transplant. We have treated only one patient with melanoma. Stabilization of disease for 4 months was achieved. Further accrual will be needed.

A summary of the clinical trials using high-dose combinations can be found in Table 4. The results are not significantly better than those obtained with single agents, except there seems to be a trend for improved duration of response. In an effort to improve the results, the investigators at Washington University have added interleukin-2 (IL-2) to the approach (15). The scheme included the administration of IL-2 for 2 cycles, followed by high-dose thioTEPA (900 mg/m^2) with marrow support, and then two more cycle of IL-2. Eight patients have been entered since December, 1988. Six have had visceral disease, 2 had prior chemotherapy, and one had previously received a biologic response modifier. All eight patients received the first two cycles of IL-2. None of the 8 responded. Two patients did not go on to receive the high-dose thioTEPA: one because of severe toxicity from IL-2 and one because of progressive disease on IL-2. Six patients underwent the high-dose portion of the protocol; 5 are evaluable, one had a partial response. Only one patient has received the last two cycles of IL-2, and has had stable disease. Four patients did not get the second IL-2 due to toxicity from the high-dose chemotherapy, previous IL-2 toxicity, progressive disease, or patient refusal. Thus, while it is an interesting concept to combine different therapeutic approaches, there are many problems in completing this approach.

SUMMARY

Dose escalation is possible with marrow support. There is a dose response effect observed for patients with advanced melanoma treated with high doses of alkylating agents, singly or in combination. Unfortunately, the duration of unmaintained response was short, regardless of chemotherapy regimen, with about 10% of patients have responses of more than 1 year. Efforts to improve the results with the addition of the biologic response modifier IL-2 have not yet been successful, but the trial is still on-going. Another approach, multiple courses of dose intensive therapy, have been tried in a few patients. No conclusions can be drawn from these preliminary studies.

Since patients with limited disease appear to have the best results with the dose-intensive approach, it would seem more practical to extend this type

of therapy to patients with less extensive disease, but with a poor prognosis for cure. One such group would include patients who achieve a complete response to initial therapy (surgery, radiotherapy, and/or chemotherapy) for recurrent disease. Another suitable situation might be the use of high-dose therapy in the adjuvant setting for high risk stage 11 patients. Such a trial has been recently closed at Duke University. The results are now being analyzed (21).

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TABLE 1

Advanced Melanoma: Response to High-Dose Therapy and Autologous Marrow Transplantation

Drug b	Number of Patients	Response a			
		CR	PR	RR (%)	95% C.I.
BCNU	31	4	10	45	24-56
Melphalan	26	6	12	69	50-83
ThioTEPA	55	4	25	53	40-65

a See text for definition: RR, response rate, determined by dividing the total number of patients responding by the total number of patients and multiplying the fraction by 100 to yield per cent. 95% C.I. is the 95% confidence interval.

b See text for doses administered.

TABLE 2

Advanced Melanoma: Comparison of Responses to Conventional Dose and High Dose Therapy with Autologous Marrow Transplantation

Drug b	Number of Patients	Response a	
		Rate (%)	95% C.I.
BCNU	110	15	10-23
BCNU/ABMT	31	45	29-62
Melphalan	24	17	7-36
Melphalan/ABMT	26	69	56-90
ThioTEPA	55	16	9-28
ThioTEPA/ABMT	55	53	40-65

a Response rate determined by dividing the number of patients responding by the total number and multiplying the fraction by 100 to yield per cent. 95% C.I. is the 95% confidence interval.

b Conventional dose is presented on the top line, the high dose with autologous bone marrow transplantation (ABMT) is presented on the line below.

TABLE 3

Advanced Melanoma: Effect of Prior Therapy and Extent of Disease on the Response to High Dose Therapy with Autologous Marrow Transplantation

Treatment Group b	Response Rate (%) a			
	BCNU	Melphalan	ThioTEPA	Total
Prior Therapy	2/15 (13)	10/16 (63)	16/31 (52)	28/62 (45)
No Prior Therapy	12/16 (75)	8/10 (80)	13/24 (54)	33/50 (66)
Skin +/- Lymph	3/5 (60)	1/2 (50)	6/8 (75)	10/15 (67)
Visceral Disease	11/26 (42)	17/24 (71)	23/47 (49)	51/97 (53)

a Number of patients responding / Number patients treated: per cent in brackets.

b Prior therapy refers to patients who received previous chemotherapy. Skin +/- lymph is skin and/or lymph node involvement only: visceral disease is visceral organ metastases.

TABLE 4

Advanced Melanoma: High Dose Combination Regimens with Autologous Marrow Transplantation

Reference	Drug Regimen b	Number of Patients	Response a		
			CR	PR	RR (%)
17	CY, CDDP, BCNU +/- L-PAM	19	1	10	65
18	BCNU, L-PAM	17	2	5	41
12	BCNU, L-PAM	58	8	28	59
19	CY, ThioTEPA	2	1	0	50
14	CY, ThioTEPA	6	3	2	83
20	CY, ThioTEPA, L-PAM	1	1	0	100
21	CY, CDDP, L-PAM	10	1	5	60
21	CY, CDDP, ThioTEPA	4	0	2	50
	TOTAL	117	15	52	57

a See text for definition: RR, response rate, determined by dividing the total number of patients responding by the total number of patients and multiplying the fraction by 100 to yield per cent.

b CY=cyclophosphamide: CDDP=cis-platinum: BCNU=carmustine: L-PAM=melphalan: thioTEPA=triethylenethiophosphoramide.

A PHASE I/II STUDY OF HIGH-DOSE CYTOXAN/VP-16/CARBOPLATIN WITH AUTOLOGOUS BONE MARROW RESCUE

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ABSTRACT

Seventeen patients have received 18 courses of high-dose cytoxan (6 g/m^2 over four days), VP-16 (1800 mg/m^2 over three days), and carboplatin (800-1600 mg/m^2 over four days by continuous infusion) and autologous marrow or peripheral stem cell rescue. The MTD of this regimen included these doses of cytoxan and VP-16 with a total of 1600 mg/m^2 of carbOplatin. Acute renal failure was the dose limiting toxicity and was observed in two patients out of 11 evaluable courses at the MTD. Non-hematologic toxicity was otherwise modest with an overall response rate of 67% in a wide variety of solid and hematologic neoplasms.

INTRODUCTION

High-dose chemotherapy regimens have been utilized with and without autologous bone marrow support for several years (1,2). These treatments have been most effective in the therapy of Hodgkin's and non-Hodgkin's lymphoma and acute leukemia (3,4). More recently, this approach has also been applied to more common solid tumors such as ovarian and breast carcinoma with encouraging results (1,5). The major obstacles facing such therapies remain the prolonged duration of neutropenia and thrombocytopenia following marrow reinfusion, its associated infectious and non-infectious organ toxicity, and tumor recurrence following initial responses. While the utilization of recombinant human growth factors and techniques such as peripheral blood stem cell harvest and reinfusion promise to decrease both the hematopoietic and non-myeloid toxicity associated with these regimens (6,7), it is clear that new combinations of chemotherapy drugs will be needed to improve the duration of response and frequency of long term survival.

Cyclophosphamide and VP-16 have been used in a number of transplant regimens for both hematologic and non-hematologic tumors (1,2). Additional

drugs commonly employed in the transplant arena include BCNU, cisplatin, and thiotepa (8,9,10). Recently, an analog of cisplatin, carboplatin, has been made available for clinical use in the United States and has shown itself to be a useful drug in the high-dose setting (11,12). Its major side effect at standard doses is myelosuppression, thus making it an attractive alternative to cisplatin as the renal, hepatic, and neurologic dose limiting toxicities of carboplatin are not seen until the drug dose is escalated 4-5 fold over that used in standard clinical practice. The current report describes a phase I dose escalation study which commenced with a fixed dose of cyclophosphamide and VP-16 given as divided doses over 4 and 3 days respectively combined with escalating doses of carboplatin administered as a continuous infusion over 96 hours.

PATIENTS AND METHODS

Eligibility

Patients with histologically documented non-leukemic malignancy that was not curable by standard means were eligible for enrollment. All patients were >16 years of age, performance status of 0-2 (CALGB), creatinine clearance >60 cc/min, transaminases and bilirubin <3 x normal, and a pretreatment platelet count >100,000 and neutrophil count >1,000/ul. Patients with marrow hypoplasia or previous tumor involvement were reinfused with peripheral blood stem cells.

Treatment Schema

All patients were treated with prophylactic antibiotics in rooms equipped with unidirectional air flow and HEPA filtration. Reverse isolation procedures including gowns and gloves were required for all patient contact. Irradiated, CMV specific blood products were administered through leukocyte-depleting filters.

Cyclophosphamide was administered as a fixed dose of 750 mg/m^2 q 12 hours x 8 doses beginning on day -7. VP-16 was administered as 300 mg/m^2 q 12 hours for 6 doses. Carboplatin was initially begun at a dose of 800 mg/m^2/day over 4 days by continuous infusion (Table 1).

Three patients were treated with this first dose level, 4 patients were treated with 1200 mg/m^2 of carboplatin, and 11 courses were administered to 11 patients at a dose of 1600 mg/m^2 of carboplatin. Dose escalations were based on a modified Fibonacci schema that required at least three evaluable patients to complete treatment prior to dose escalation. Autologous bone marrow (14 courses) or peripheral blood stem cells (4 courses) were administered 72 hours following completion of the carboplatin infusion.

RESULTS

The pretreatment characteristics for the 17 patients enrolled on the study are presented in Table 2.

Hematologic Toxicity

Hematologic toxicity with this regimen was expectedly severe. All patients required intravenous antibiotics with a median time to neutrophil recovery >500/ul of 24 days and for platelets >20,000 of 23 days post marrow reinfusion. No difference was observed in the median time to count recovery between patients who received autologous marrow and those who were reinfused with peripheral blood stem cells. The median number of red blood cell transfusions was 10 (range 4-14) units and the median number of platelet transfusions was 14 (range 4-55) separate infusions. The median hospital stay was 34 days with a range from 24-51 days. Engraftment was significantly delayed in three patients, two of whom had their marrow harvested after receiving two and three cycles, respectively, of high-dose carboplatin (1200 mg/m^2) during the several months prior to transplantation. Each patient exhibited prolonged thrombocytopenia which lasted 75 and 90 days respectively. The third patient with delayed engraftment had an initially good recovery following autologous marrow reinfusion, but developed CMV viremia at day +26 with subsequent prolonged graft failure and requirements for platelet transfusions of approximately 100 days. This patient's neutrophil count remained between 500 and 1,000/ul during this period during while she received DHPG.

Non-Hematologic Toxicity

There were four cases of candida sepsis. One of these patients also had renal failure and died eleven days following marrow reinfusion. There were fatal cases of P. aeruginosa (day +10) and E. coli (day +3) sepsis and three additional cases of non-fatal bacteremia. The one case of severe CMV viremia occurred in a patient who was sero-positive for CMV prior to initiation of the treatment and received CMV-positive blood products during her hospital stay.

Acute renal failure was the dose limiting toxicity of this regimen and was observed in two of eleven patients treated with 1600 mg/M^2 of carbOplatin. One patient had underlying diabetes and had received three cycles of cisplatin and cytosine arabinoside for her large cell lymphoma prior to the transplant. The second patient had already been treated with 800 mg/M^2 of carboplatin on this transplant regimen six months earlier and achieved a partial remission of her ovarian cancer for six months duration. During her second cycle she received 1600 mg/m^2 and promptly developed renal failure that required dialysis prior to marrow reinfusion. She went on to develop candidal sepsis and died 14 days following marrow reinfusion. Both of these patients were very obese and were dosed on total body weight when, in retrospect, lean body mass would have been more appropriate.

Other organ toxicities have included two cases of grade 3 mucositis and modest elevation of serum transaminases and bilirubin. No treatment courses resulted in clinical veno-occlusive disease, transaminase elevations above 300 or bilirubin levels >5.0. Several patients have also noted mild tinnitus and paresthesias with documented decreases in high frequency hearing but clinically significant deficits have only occurred in patients previously heavily treated with

cisplatin. One patient developed congestive heart failure that responded well to digoxin and diuretics.

Responses

Fifteen of the 18 treatment courses are evaluable for response table 3). The duration of response in the two patients who achieved CR following transplantation were 2.5 and 7 months. Of the 8 patients who achieved a PR, 1 remains progression free at 3+ months while the other patients have relapsed at a median of 3. 5 months (range 2-6). The two patients with large cell lymphoma had disease progression at 2 and 3 months respectively.

DISCUSSION

The substitution of carboplatin for cisplatin in high-dose chemotherapy regimens can result in significant dose escalation of platinum-based chemotherapy with acceptable additional toxicity. Assuming a 4:1 (w/w) ratio of equivalently effective doses of carboplatin to cisplatin, the MTD of this regimen (cyclophosphamide, 6 gm/M^2; VP-16, 1800 mg/M^2; carboplatin, 1600 mg/M^2) represents a 2 to 3 fold increase over current transplant doses of cisplatin. While the response rate in the current trial was quite high (67%) in this group of heavily pre-treated patients, the median duration of response was not significantly different from that obtained with several other regimens in comparable patient populations. Whether or not this increased platinum dose will result in a significant improvement in the duration of response in either solid or hematologic neoplasms remains to be determined in appropriate Phase II studies of less heavily pre-treated patients.

It is interesting to note that while non-myeloid toxicity was no greater than usual, delayed marrow engraftment was observed in two patients who underwent bone marrow harvesting following therapy with high-dose carboplatin (1200 mg/m^2), administered in conjunction with GM-CSF (Sandoz-Schering) on an N.C.I. sponsored protocol. Despite GM-CSF use during the induction therapy, normal peripheral blood counts at the time of marrow harvest, and reinfusion of adequate numbers of nucleated marrow cells (2.1 and 2. 3 X 10^8 nucleated cells/kg respectively), the time to engraftment in these two patients was very long. This was particularly apparent in their prolonged thrombocytopenia (>75 days) and reflects both the marrow damaging properties of high- dose carboplatin and the variable effects of GM-CSF given post transplant on platelet recovery. Studies are currently underway to evaluate the feasibility of combining sequential courses of high-dose carboplatin followed by this three drug transplant regimen and reinfusion of marrow that was harvested prior to the carboplatin induction treatment.

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TABLE 1

HIGH-DOSE TREATMENT SCHEMA

Drug	Total Dose mg/m2	Schedule	DAYS -7	-6	-5	-4	-3	-2	-1	0
CPA	6000	q 120 X 8	X	X	X	X				
VP-16	1800	q 120 X 6	X	X	X					
CBDCA	800-1600*	C.I. X 960	X	X	X	X	X			
I.V. hydration and foley			X	X	X	X	X	X	X	
Stem cell reinfusion										X

*MTD dose = 1600 mg/m^2

TABLE 2

PATIENT CHARACTERISTICS

Enrolled	17 (18 courses)
Median Age	41
Sex	Women - 14
	Men - 3
No. Prior Regimens	2 (Range 1-4)
Histologic Types :	
Ovarian	4
Breast	8
NHL	2
Squamous Cell	1
SCLC	1
Peritoneal Ca	1

TABLE 3

		RESPONSES			
Tumor	Evaluable	C.R.	P.R.	C.R.+P.R.	E.D.
Ovarian[a]	4		3	3	1
Breasts[b]	6	2	1	3	1
Lymphoma	2		2	2	
Squamous CA	1				1
SCLC	1		1	1	
Peritoneal	1		1	1	
TOTALS	15	2	8	10 (2 -7 mos)	

a) One patient achieved a P.R. for 6 months and died of renal failure and candida sepsis following a second transplant procedure; 1 patient was in C.R. at the time of BMT and remains progression free at 9 months.

b) Two patients are not evaluable for response. One patient had bone only disease and remains progression free at 3 months; 1 patient was in C.R. at the time of BMT and remains progression free at 3.5 mos.

BUSULFAN, CYCLOPHOSPHAMIDE, VP-16 (BUCYVP) CONDITIONING FOR BONE MARROW TRANSPLANTATION (BMT)

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ABSTRACT

More intensive conditioning regimens may decrease relapse and result in improved survival after bone marrow transplantation (BMT), if the regimen-related toxicity is not excessive. We have utilized Busulfan (4 mg/kg/d x 4), Cyclophosphamide (60 mg/kg/d x 2) and escalating doses of VP-16 (15 mg/kg to 60 mg/kg) to treat 20 patients with advanced malignancies. The patients included 13 with non-Hodgkin's lymphoma (NHL), 3 acute myelogenous leukemia (AML), 1 acute lymphocytic leukemia (ALL), 1 refractory anemia with excess blasts in transition (RAEB-T), and 2 solid tumors. Five patients received allogeneic BMT, 12 autologous BMT, 2 autologous peripheral blood stem cell transplants (PBSC) and one syngeneic BMT. Median time to engraftment with WBC > 500 was 14 days (range 9-66 days), WBC > 1000 was 15 days (11-72 days) and with platelet count > 50,000 was 26 days (12-105 days). Stomatitis requiring a morphine drip was noted in all patients treated with > 45 mg/kg VP. Skin toxicity ranged from hyperpigmentation to superficial desquamation. Dose limiting toxicity was hepatic, with fatal veno-occlusive disease (VOD) in two patients who received 45 mg/kg VP. Three patients died early (less than three months), two with VOD, one with aspergillus sepsis. Fourteen patients attained complete remission (CR), 2 partial remission (PR). Fourteen patients remain alive, 10 in continuous complete remission (CCR) 3+ - 40+ months (median 20 months) after transplant. Actuarial survival and disease-free survival at two years are 69% and 43%, respectively. Of the 13 patients with NHL, nine attained CR, and seven remain in CCR. Actuarial survival and disease-free survival at two years for NHL patients are 85% and 38%.

INTRODUCTION

Dose intensive therapy with bone marrow transplantation can be effective treatment for patients with malignant diseases. More effective conditioning regimens are needed to improve the efficacy and reduce the relapse

rate after transplantation. The combination of high-dose busulfan and cyclophosphamide (BuCy) with allogeneic or autologous bone marrow transplantation has been successfully utilized in patients with hematologic malignancies such as acute and chronic myelogenous leukemia (1,2,3). The regimen is well-tolerated with a low rate of non-hematologic life-threatening toxicities. Patients with high-risk features, such as relapsed acute leukemia, chronic myelogenous leukemia in accelerated or blast phase or relapsed lymphoma, have a high relapse rate after bone marrow transplantation.

VP-16 (etoposide) has a broad range of antitumor activity against leukemias, lymphomas and many solid tumors. The limiting toxicity of VP-16 is myelosuppression, with minimal other organ toxicities (4). We have explored the addition of VP-16 to the BuCy conditioning regimen for bone marrow transplantation for patients with hematologic malignancies and selected solid tumors. VP-16 has been added in a dose escalation scheme from 15 mg/kg to 60 mg/kg of body weight in order to assess for dose-related toxicity.

MATERIALS AND METHODS

All patients gave informed consent to participate in the protocol as approved by the Institutional Review Board of the treating institution. Patients were treated at Pacific Presbyterian Medical Center, San Francisco, CA or Alta Bates Hospital, Berkeley, CA.

Twenty patients with hematologic malignancies or selected solid tumors were treated between August 1986 and April 1990. Patient characteristics are shown in Table I. The patients included 13 with NHL, 3 AML, 1 RAEB-T, 1 ALL and 2 solid tumors. The median age of the patients was 35 years with a range of 19-48 years. All patients were treated with combination chemotherapy with or without irradiation (median 3, range 1-6 previous therapies) given to maximum response prior to initiation of this regimen. At the time of treatment nine patients were in CR, eight in PR and three bulky disease (refractory).

The treatment regimen consisted of busulfan 4 mg/kg/day orally for 4 days followed by cyclophosphamide 60 mg/kg/day intravenously for 2 days. VP-16 15-60 mg/kg was given intravenously over 6-10 hours with continuous electrocardiographic and blood pressure monitoring. Seventy-two hours later patients received HLA-matched sibling donor allogeneic BMT (5 patients), cryopreserved autologous BMT (12 patients), autologous PBSC transplant (PBSC) (2 patients) or syngeneic BMT (1 patient). Patients receiving allogeneic BMT received graft-vs-host disease prophylaxis with Cyclosporin and prednisone.

RESULTS

The median time to engraftment (Table II) with WBC count > 500 was 14 days (range 9-66 days), WBC > 1000 was 15 days (11-72 days) and Platelets > 50,000 was 26 days (12-105 days).

Toxicity (Table II) was graded according to the scale of Bearman (5). Dose-related toxicity included stomatitis, with all patients requiring a morphine drip at a dose of VP > 45 mg/kg and skin toxicity ranging from hyperpigmentation to superficial desquamation. Hepatic toxicity was dose-limiting, with fatal VOD in two patients who received 45 mg/kg VP. There were three early deaths (two VOD, one sepsis).

Response and outcome are shown in Table III. Survival and disease-free survival for all patients and the subset with NHL are shown in Diagrams I and II. Fourteen patients attained CR, two PR. Fourteen patients remain alive, ten in continuous CR 3+ - 40+ (median 20 months) after transplant. Actuarial two-year overall survival and disease-free survival are 69% and 43%, respectively. Of the 13 patients with NHL, nine attained CR and seven remain in CCR. Actuarial two-year survival and disease-free survival for NHL patients are 85% and 38%, respectively. See patient summary (Table IV).

DISCUSSION

The BuCy conditioning regimen has shown efficacy in the treatment of acute and chronic leukemias. The BuCy regimen is reported to be well tolerated, with less pulmonary toxicity than TBI regimens. The relapse rate for patients with advanced disease after BMT is high with most regimens and more efficacious conditioning regimens are needed. VP-16 has little reported non-hematologic toxicity and a broad spectrum of activity against leukemias, lymphomas and solid tumors.

We have found that the BuCyVP regimen is efficacious is the treatment of patients with advanced non-Hodgkin's lymphomas and leukemias. Most patients had received several previous chemotherapy regimens and radiation prior to transplantation. We have found dose-related skin (hyperpigmentation, desquamation) and mucous membrane (stomatitis) toxicities. Two cases of fatal VOD were noted at a dose of 45 mg/kg of VP, both cases in heavily pretreated patients. A recent report of BuCyVP, with 60 mg/kg VP, for allogeneic transplantation for acute leukemia did not find hepatic toxicity (6), whereas other workers have reported à 9.1% incidence of reversible VOD with BuCy (7). Our group of heavily pretreated patients may have an increased risk for hepatic toxicity, limiting the dose of VP-16.

CONCLUSIONS

BuCyVp is an effective conditioning regimen for patients with non-Hodgkin's lymphomas and other hematologic malignancies. Dose-related toxicities are hepatic (VOD), dermatologic (hyperpigmentation, desquamation) and stomatitis. Hepatic toxicity is dose-limiting in this group of heavily pretreated patients.

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TABLE 1

PATIENT CHARACTERISTICS

	Median	(Range)
Age	35	(19 - 48)
Previous Therapies	3	(1 - 6)
Diagnoses	NHL	13
	AML	3
	RAEB-T	1
	ALL	1
	Other	2
Status at BMT	CR	9
	PR	8
	Refractory	3
Type of BMT	Allo	5
	Auto	12
	PBSC	2
	Syn	1

TABLE 2

TIME TO ENGRAFTMENT

	Days	Range
WBC > 500	14	9-66
WBC > 1000	15	11-72
Platelets > 50,000	26	12-105

TOXICITY

	Median	Range
cardiac	0	0-2
Renal	0	0-2
Pulmonary	0	0-1
Hepatic	1	0-4
CNS	0	0-2
Stomatitis	2	1-2
GI	1	0-2
Skin	1	0-3

TABLE 3

	RESPONSE
Complete Response	14
Partial Response	2
No Response	1
Unevaluable	3

	OUTCOME
Continuous CR	10
Alive in Relapse	4
Dead	6

	CAUSE OF DEATH
Progressive Disease	3
Sepsis	1
VOD	2

TABLE 4

PATIENT SUMMARY

PT NO.	AGE	DIAGNOSIS	NO. PREV. THERAPIES	STATUS AT BMT	DOSE VP-16	TYPE BMT	RESP	OUTCOME	SURVIVAL (MONTHS)
1	31	RHABDO	4	REFRACT	15	AUTO	NR	D (PD)	4
2	46	NHL-CML	3	PR	15	AUTO, PBSC	CR	CCR	40+
3	20	ALL-L2	3	PR	30	ALLO	CR	REL 3 MO D (PD)	4
4	35	RAEB-T	1	PR	30	ALLO	CR	CCR	28+
5	26	AML	3	CR	30	ALLO	CR	REL 4 MO D (PD)	4
6	32	NHL-NLC	5	PR	30	AUTO	CR	REL 9 MO	24+
7	33	NHL-NPDL	5	PR	30	AUTO, PBSC	PR	REL 19 MO	22+
8	19	AML	1	CR	30	AUTO, PURGE	CR	CCR	21+
9	45	NHL-NSCC	5	PR	30	AUTO	PR	REL 6 MO	11+
10	39	AML-POST-BREAST CA	1	CR	45	SYN	CR	CCR	22+
11	29	NHL-T-CELL IMMUNOBLAST	1	CR	45	AUTO	CR	CCR	22+
12	41	NHL-CM	1	PR	45	AUTO	CR	CCR	20+
13	43	NHL-T-CELL	3	REFRACT	45	AUTO	CR	REL 7 MO	19+
14	38	NHL-NM	3	CR	45	AUTO	CR	CCR	15+
15	46	NHL-DLC	3	CR	45	AUTO	UNEV	D (VOD)	2
16	38	NHL-NSC TRANSF TO NLC	4	CR	45	AUTO	CR	CCR	10+
17	48	NHL- LO TRANSF TO HI GRADE	4	CR	45	ALLO	UNEV	D (VOD)	1
18	38	NHL-LG CELL IMMUNOBLASTIC	2	CR	45	AUTO	CR	CCR	7+
19	33	NHL-NSCC	4	PR	45	ALLO	CR	CCR	3+
20	27	NEUROBLASTOMA	6	REFRACT	60	AUTO	UNEV	D (ASPER)	1

FIGURE 1

Diagram I: Actuarial survival and disease-free survival for all patients treated with BuCyVP.

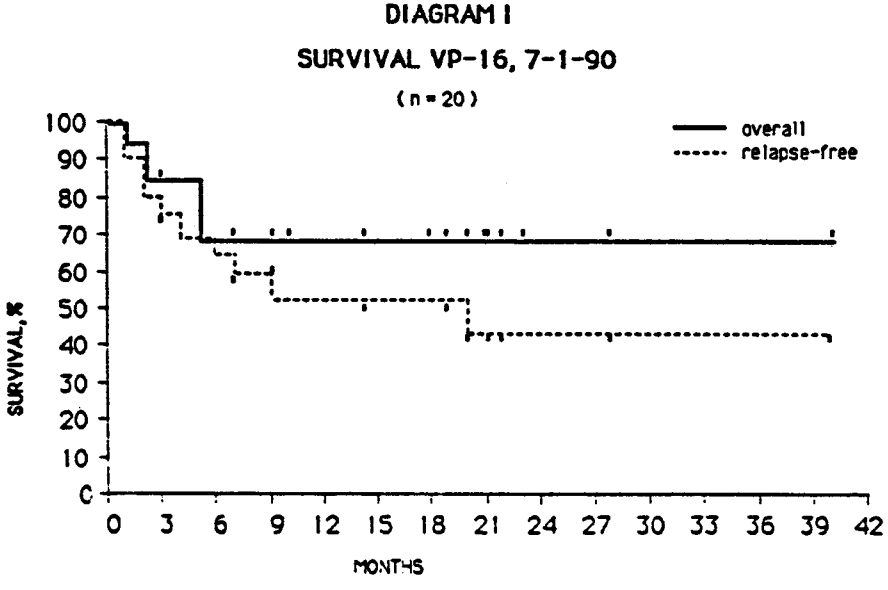
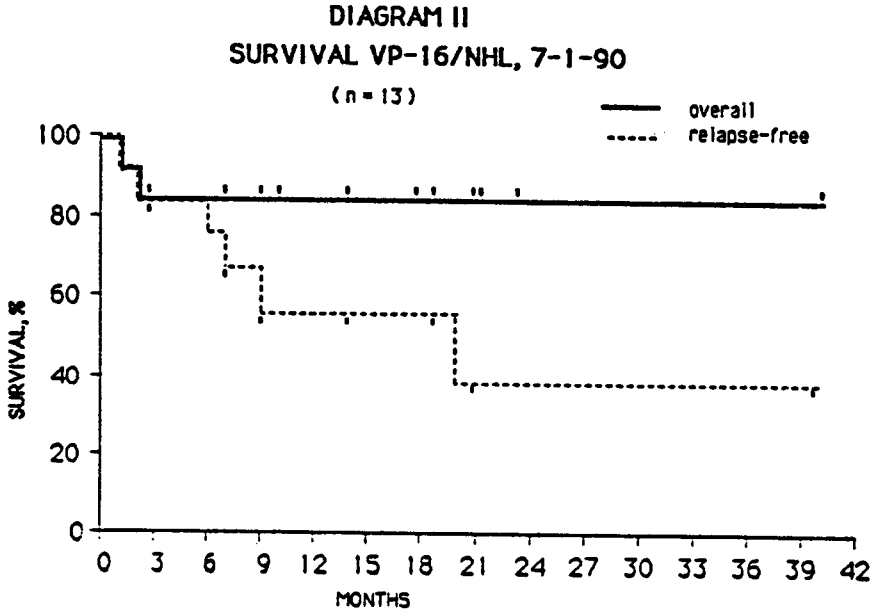


FIGURE 2

Diagram II: Actuarial survival and disease-free survival for patients with non-Hodgkin's Lymphoma.



HIGH-DOSE CHEMOTHERAPY WITH CARBOPLATIN AND VP16 ± IFOSFAMIDE IN GERM CELL TUMORS: THE ITALIAN EXPERIENCE

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INTRODUCTION

Germ cell tumors are considered as potentially curable diseases even when they present with advanced widespread stages. In fact with the effective upfront regimens such as PVB or PEB up to 80% of the patients achieve complete remission (CR), and usually these cases are cured (1) and this is of course something unusual in Medical Oncology. But for refractory to first line, Cisplatin containing regimen patients as well as for relapsing (20 to 25%) patients, the prognosis is still dismal. In the last five years some trials investigated the possibility of employing high dose chemotherapy in germ cell tumors with discording results (2,3,4). Carboplatin, due to the toxicity profile of this compound, has been hypothesized to be a suitable agent for high dose programs. Even at standard doses, it has been employed with success mostly for seminoma (5). Nichols recently reported that using high doses they had 44% objective responses in 32 previously treated patients with 4 cases being relapse-free in excess of 1 year (4). Motzer and colleagues recently reported that Carboplatin can be considered as active as Cisplatin when combined with Etoposide and Bleomycin in poor risk patients (6).

This paper will report the first Italian experience in Germ Cell high-dose program using Carboplatin, VP 16 and Ifosfamide.

PATIENTS AND METHODS

From July 1986 until May 1990, 23 patients (21 male and 2 female) with germ cell tumors underwent ABMT at Ravenna Hospital. Mean age was 25 years with a range of 18-50. All had been previously treated with Cisplatin containing regimens. The employed schedule was Carboplatin 1,350 mg/sqm in 3 days, VP16 1,800 mg/sqm in 3 days while the last six cases received also Ifosfamide at the dosage of 12 gr/sqm in 3 days (VP16 reduced to 1,200 mg/sqm). At the time of PMN <500 ul, patients were located in reverse room isolation until PMN >500ul. Platelets were infused if bleeding or if PLT $<20{,}000$ ul; RBC were infused to maintain hematocrit $>30\%$. Prophylaxis

consisted of ketokonazole, paromomycin and Vitamin A. Amikacine and ceftazidime were administered in case of temperature >38C, waiting for culture tests.

The week before ABMT and the week after discharge, patients were asked to undergo neurophysiologic investigations to detect Carboplatin neurotoxicity. Fifteen of twenty-three (15/23) patients were transplanted while in progression of their disease; 6/23 received ABMT in non-resectable partial remission, one with stable disease and one as intensification for high risk second CR (case #17). (Table 1).

This study was a double graft program, and patients were offered second ABMT if progression was not evident after the first course.

RESULTS AND TOXICITY

Table 1 shows the results obtained in this population of pretreated germ cell tumors. The response is specified as on tumor markers (all cases had at least one elevated level at ABMT) and on lesions. Most of the CRs were pathologically confirmed; a complete remission on markers was considered as fall to normal values for at least one month, while partial remission was identified when the tumor marker levels had a log decrease. Two patients died of transplanted related procedures, so 15/21 patients (71%) had PR or CR on seric markers and 11/21 patients (52%) achieved objective responses: 8 CR and 3 PR. Table 2 shows the details and the outcome of the 8 CR cases.

As clearly indicated in the Table the major results (5/23 CR - 22%) where registered in "sensitive relapsing" patients. These data are superimposable in terms of responses to Nichols' responses (4). This regimen was well tolerated, and the toxic death rate was among the lowest in this high dose studies (9%). One patient died of veno occlusive disease and one for rapidly growing tumor both at day 15 post-transplant.

Details on toxicity are given in Table 3. Stomatitis was the major toxic effect being severe (grade 4 WHO) in 5/32 courses (15%). One case of viral hepatitis was detected 3 months after ABMT.

DISCUSSION AND CONCLUSIONS

This is the first Italian Cooperative non-randomized study on high dose chemotherapy in Germ Cell tumors. Five of twenty-three (5/23) patients, not curable with Cisplatin combinations, obtained durable CR. As for studies in other solid tumors, one of the best prognostic factors seems to be the time of transplantation (7). In fact, all continuous complete remissions (CCR) were achieved in previously responding patients, where they were offered ABMT. This regimen was well tolerated (with or without Ifosfamide) and maybe higher doses of one or more of the drugs can be tested. One important point is that a minority (10%) of the patients achieved CR (even if of short duration) which they never obtained before with standard Cisplatin regimens. This had already pointed out in the Indiana trial (4). The multiple neurophysiologic investigation

performed by one of us (F.V.) using neurologic symptom and disability scores, sympathetic skin reflex, computed stabilography electroneurography on peroneal, sural and median nerves did not show any difference between the pre- and post- ABMT evaluation, suggesting no peripheral neurotoxicity due to high dose Carboplatin. Moreover the number of reinfused cells in our trial was a median of 0.57 10E8/kg/b.w. This number is nearly half than that used by the Indiana group (4,8) with a similar schedule, suggesting that at these doses a small amount of cells is able to restore in two weeks normal hematopoiesis.

ACKNOWLEDGEMENT

Istituto Oncologico Romagnolo (Italy) Grant 90262.1.

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TABLE 1

PATIENTS CHARACTERISTICS AND RESULTS

	HISTOL.	PREV. THERAPY	PREV. CR	STATUS AT ABMT	RESPONSE MARK.	RESPONSE LESIONS	ABMT #	DURATION (weeks) RESP	DURATION (weeks) SURV
1	MIX	PEB/PEI/S	N	PD	PD	PD	2		8
2	MIX	PEB/PEI/S	Y	PD	PD	PD	1		8
3	CHOR	PEB/MIX	N	PR	CR	CR	1	144+	144+
4	MIX	PEB/PE	Y	PD	CR	PR	1	13	16
5	EXT/EC	PEB/PEI/S	N	PD	PD	PD	1		13
6	EC	PEB/VIP	N	PD	CR	CR	3	12	16
7	MIX	PVB/PEI	N	PD	PR	SD	2	12	34
8	YST	PVB/VAC/S	Y	PD	PR	//*	1	8	44
9	EC	PEB/PEI	Y	PD	TOXIC DEATH V O D				
10	EC	CARIOUS	Y	PD	RAPIDLY GROWING TUMOR DEATH				
11	YST	PEB/OTHERS	Y	SD	CR	CR	2	12	15
12	CHOR	PEB/XR	N	PD	PR	SD	1	8	22
13	EXT/TC	VARIOUS	Y	PR	SD	PR	2	4	20
14	MIX	PEB/PEI/XR	N	SD	SD	SD	1	9	30
15	MIX	PEB/PEI/S	Y	PD	SD	SD	1	6	10
16	MIX	PEB/PEI/OTH	Y	PD	CR	CR	1	6	32
17	EC	PEB/S/PEI	Y	CR2	CR	CR	1	36+	36+
18	MIX	PEB	N	PR	CR	CR	2	40+	40+
19	MIX	VARIOUS	Y	PD	CR	SD	2	28	32+
20	MIX	PEB/VIP	Y	PD	PR	SD	2	9+	9+
21	CHOR	PEB/PEI	N	PR	CR	CR	2	24+	24+
22	MIX	PEB	N	PD	CR	PR	1	8	12+
23	MIX	PEB PEI	Y	PR	CR	CR	2	9+	9+

MIX = Mixed Histology, CHOR = choriocarcinoma, EXT = Extragonadal; EC = Embryonal carcinoma, YST = Yolk Sac Tumor, TC = teratocarcinoma, S = Surgery, XR = radiation
* seric only disease

TABLE 2

* 7 cases achieved Complete Remission

* 1 case maintained previous Complete Remission

* 5 cases are still in Complete Remission at 2.5 up to 38+ months from ABMT with no further therapy.

* Among the 5 durable CR, 4 were achieved in non-resectable partial responders and one in the patient transplanted in second CR.

TABLE 3

TOXICITY

DATA REGARDING 32 COURSES IN 21 PATIENTS

(2 deaths within 30 days excluded)

	DAYS	RANGE
POLYS < 500 ul	16.2	7 - 22
PLT < 50,000 ul	15.4	6 - 26

DAYS WITH FEVER (>38°C) MEAN 4.9 RANGE 0 - 10

SEVEN DOCUMENTED BACTERIEMIAS: St. Haemoliticus, St. Epidermidis (3), Pseudomonas, St. Aureus (2).

STOMATITIS (WHO grade 4) in 5/32 courses (15%)

GASTROINTESTINAL BLEEDING: 2 courses (resolved)

#PLT bags infused each course	mean 25	(range 6 - 66)
#RBC bags infused each course	mean 8	(range 2 - 14)

REMISSION INDUCTION OF ADJUVANT ARTHRITIS IN RATS BY AUTOLOGOUS BONE MARROW TRANSPLANTATION

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INTRODUCTION

In a previous publication we reported that total body irradiation with a lethal dose followed by rescue with bone marrow transplantation is a highly effective treatment of adjuvant arthritis in rats (Van Bekkum et al. 1989). The treatment is most effective when given within 2-5 weeks after the beginning of the clinical manifestations of the disease. Transplantation of bone marrow at a later stage causes regression of the inflammation, but the bony deformations are not repaired. Local irradiation of the affected joints did not influence the course of the disease. It was also demonstrated that the curative effect of bone marrow grafting is unrelated to the presence of Mycobacterium tuberculosis antigen. Surprisingly, transplantation of syngeneic bone marrow was as effective as grafting bone marrow from rats of a strain that is not susceptible to the induction of arthritis by adjuvant. Apparently, the success of the treatment is not dependent on the genotype of the bone marrow graft. Clinically, autologous bone marrow transplantation is much less risky than allogeneic transplantation; another advantage of autologous bone marrow is that it is virtually available to every patient.

Therefore, it seemed of interest to investigate the effect of autologous bone marrow grafting in our arthritis model.

MATERIALS AND METHODS

The induction of arthritis in the susceptible Buffalo rat was similar as reported before, using one intradermal injection of complete Freund adjuvant at the base of the tail. This resulted in severe progressive arthritis becoming clinically apparent at 2 weeks after sensitization in about 70% of the animals. At 3 - 4 weeks after sensitization the swellings of the ankle and wrist joints of the diseased rats were measured with calipers. The arthritic score was calculated for each individual animal as the sum of the thicknesses of the 4 paws following substraction of the measures taken before sensitization. The

progression of the disease was recorded by similar measurements taken at weekly intervals.

Autologous marrow was collected from arthritic rats at the same time after sensitization as the treatment of other groups with syngeneic bone marrow took place. For this purpose the femur was surgically exposed, two holes were drilled at some distance through the bone and the marrow was flushed out with Hank's solution. The yield was between 6.10^6 and 8.10^7 cells per rat and this was returned intravenously after the animal had been irradiated. The dose of total body irradiation was 9 Gy. Pseudo-autologous bone marrow was collected from sacrificed arthritic rats with the same severity and stage as the recipients. It was employed prior to the experiments with real autologous transplants in order to limit the surgical interventions on animals suffering from arthritis. For the pseudo-autologous and the syngeneic grafts, 5×10^7 bone marrow cells per recipient were used.

The results summarized in Figure 1 show that essentially similar results were obtained with autologous and pseudo-autologous bone marrow grafts as with grafts from healthy syngeneic rats, that is complete and lasting remissions. The bone marrow from arthritic rats (pseudo-autologous) was equally effective as the marrow from rats that had not responded to sensitization with adjuvant. We investigated whether this remarkable result could be produced by subjecting rats to a sublethal dose of total body irradiation, as such treatment is expected to result in a comparable reduction of the immune system and subsequent repopulation from a few surviving stem cells, as occurs in the case of supralethal irradiation and rescue with syngeneic bone marrow or autologous bone marrow.

Figure 2 shows that only the highest doses of total body irradiation caused remissions and these were somewhat less pronounced than those observed after lethal irradiation and marrow grafts. Since the bone marrow of rodents contains far less T lymphocytes than that of humans, it could be questioned as to whether the results with autologous bone marrow grafts may be applied to the clinical situation.

Therefore, another group of rats was given total body irradiation and normal syngeneic bone marrow to which 5×10^7 spleen cells from arthritic rats were added. This treatment resulted in similar lasting remission as following grafting of syngeneic bone marrow only.

Finally, we studied the effects of local irradiation of the arthritic limbs versus irradiation of the trunk and head with the limbs shielded, using doses of 8 and 9 Gy. Both these treatments were ineffective, suggesting that near complete eradication of both the local and the systemic populations of auto-reactive lymphocytes is required simultaneously for remission induction. These observations are in accordance with the clinical experience that severe arthritis cannot be permanently controlled by a course of fractionated total lymphoid irradiation (Strober et al. 1985 and Trentham et al. 1987). Experiments are in progress to establish whether cures can be obtained in our model by using high dose chemotherapy instead of whole body irradiation with or without bone marrow transplantation.

It is of interest that a remission of rheumatoid arthritis has been described in two leukemic patients following successful treatment of their leukemia with high dose cytosine arabinonucleoside, daunorubicin and m-ASA (Roubenoff et al. 1987).

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FIGURE 1

Effects of TBI and Pseudoautologous or Autologous Bone Marrow on Arthritis in Rats

Results of two different experiments are depicted. Relative response was obtained by normalizing arthritic scores for each group of rats at 100 at 4 weeks after sensitization, i.e. just before treatment. All subsequent scores are expressed as percent of this value. Solid lines represent the experiment with pseudoautologous bone marrow. Broken lines refer to the experiment with real autologous marrow. Controls were given no treatment.
syn b.m. = syngeneic bone marrow from naive rats;
a.b.m. = bone marrow from syngeneic donors suffering from acute arthritis.

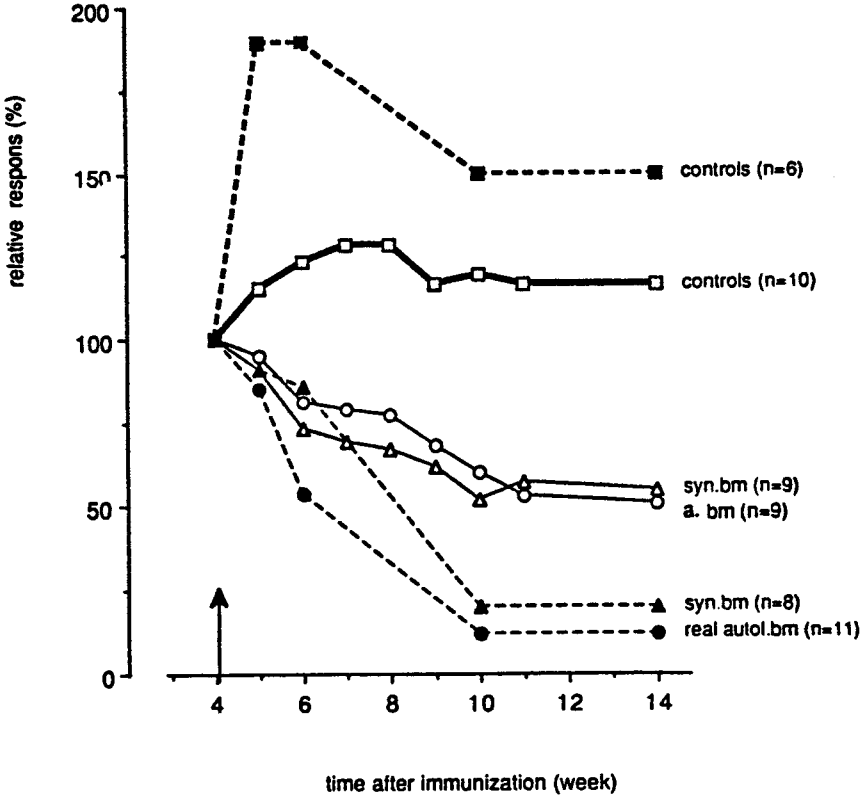
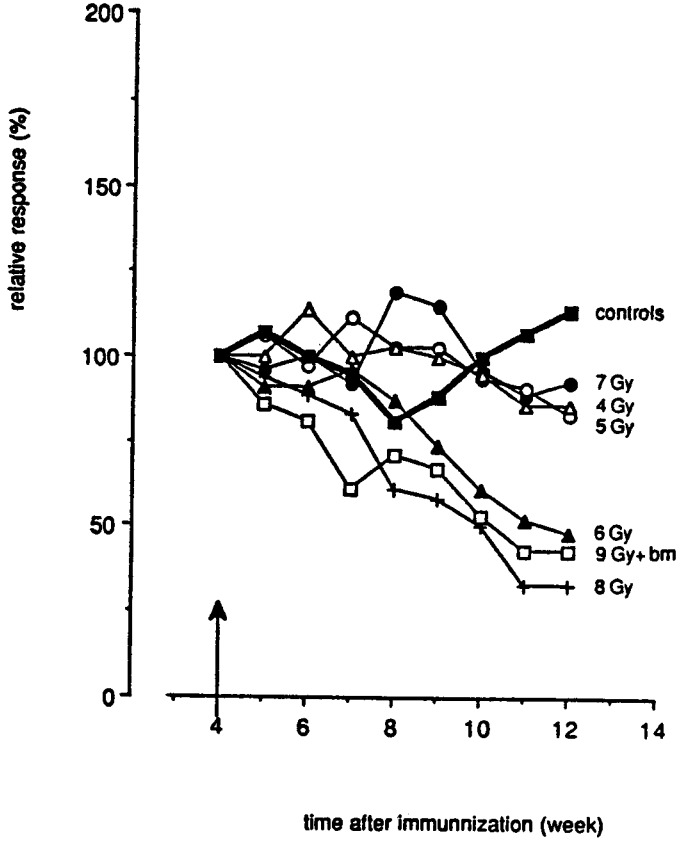


FIGURE 2

Effect of Graded Doses of TBI on Arthritis in Rats

Relative response is explained in legend of Figure 1. Controls were untreated arthritic rats. The group indicated with 9 Gy+bm received 9 Gy TBI and 5 x 10^7 bone marrow cells from syngeneic naive donors. All groups consist of 5 rats.



MAGNETIC ROSETTING : IMMUNOSELECTION FOR LIGHT AND ELECTRON MICROSCOPY

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ABSTRACT

Dynabeads M-450 are magnetizable polystyrene microspheres 4.5 micro meter in diameter to which antibodies can either be physically adsorbed or covalently attached. Antibody coated Dynabeads can be used to label cell surfaces and to separate the rosetted cells by application of a external magnetic field. This technique is being widely used for purging in bone marrow transplantation. We have developed a novel technique, using cell lines K-562 and U-937 and the previously undescribed monoclonal antibodies CH-F42 and CH-E25, where Dynabeads can also be used to label cells at the ultrastructural level. This technique can thus provide a useful bridge between light and electron microscopy. The preservation of specific rosettes at the ultrastructural level without the formation of artifactual aggregates requires rapid but gentle fixation followed by neutralization of excess glutaraldehyde with ethanolamine.

INTRODUCTION

Magnetic microspheres appear to offer a useful addition to the range of immunolabelling and separation methods since they combine both high efficiency and specificity. However, experimental conditions such as the time required for bead to cell binding to occur, the ratio of beads to target cells and the use of centrifugation or mechanical shaking to improve rosette formation differ considerably between studies (1,2). In the current study experimental conditions are optimized for the use of Dynabeads.

From the earliest reports it was realized that magnetizable microparticles, by virtue of the electron density conferred by their iron content, might make useful markers for transmission electron microscopy (3,4). However, this application has not been pursued, although other heavy metals have been incorporated into microparticles, partly with the purpose of transmission electron microscopy in view but principally for scanning electron microscopy (5). It would be very useful to investigate their applicability in electron microscopy.

MATERIALS AND METHODS

Magnetic Monosized Polymer Particles

Dynabeads M-450 (Dynal, Oslo) were used. For direct rosetting, CH-F42 and CH-E25 (control) were physically adsorbed on to uncoated Dynabeads. For indirect rosetting Dynabeads pre-coated with sheep anti-mouse IgG were used. Cell lines used in this study were K562 and U937. Materials and methods in detail have been described elsewhere (6).

RESULTS

Optimal Conditions for Binding of Beads to Cells

Optimum conditions for both direct and indirect techniques were determined. The use of centrifugation to bring the beads and the cells in close contact followed by an incubation on ice for various periods, proved to be a good method. It was found that the longer the incubation period, the higher was the proportion of cells binding beads, and also the number of beads bound per cell. However, even with extended incubation periods, bead to-cell ratios of 20:1 or 40:1 were required for maximal binding.

It was found, for instance, that in tests with peripheral blood mononuclear cells (PBMNC) treated with anti-CD3 and rosetted with anti-mouse IgG coated beads, bead-to-cell ratios of 40:1 resulted in a marked degree of non-specific binding after only 30 minutes of incubation (Figure 1). In contrast, even after 90 minutes of incubation minimal non-specific binding was observed with ratios up to 20:1. By 120 minutes some non-specific binding was observed at all the bead-to-cell ratios used (Figure 1).

These experiments indicate that the optimum conditions for specific binding of Dynabeads to labelled cells are 90 minutes incubation with a 20:1 bead:cell ratio.

Specific Binding of Beads to Target Cells for Magnetic Separation

Since a proportion of cells bound only very low number of beads (1 or 2), it was necessary to determine whether this binding was specific and if these cells could be magnetically attracted. Normal mononuclear cells were treated with anti-CD3 antibody and were allowed to rosette with anti-mouse IgG coated Dynabeads for 30 minutes, in a deliberate attempt to form a significant proportion of low bead number rosettes, at a bead-to-cell ratio of 20:1. The cells and rosettes were then incubated with FITC anti- mouse Ig for a further 30 minutes. Antigen positivity by fluorescence and Dynabead binding, and the number of beads bound per fluorescent cell were determined both before and after magnetic attraction. It was observed that cells binding 1 or 2 beads were indeed antigen positive by fluorescence, and further- more, the presence of similar proportions of cells with 1 or 2 beads in the population concentrated by magnetic attraction indicates that attachment of such low numbers of beads is sufficient for magnetic separation. In contrast to the original bead/cell suspension, very few cells had no beads bound after having been magnetically

separated, However, this can not totally explain the overall increase in the number of beads per fluorescent positive cell observed following magnetic attraction. It is possible that mechanical and spatial factors during this procedure increase the rosetting efficiency. Such factors have previously been suggested to have this result during centrifugation in conventional red cell rosetting.

Efficiency and Specificity of Negative Selection Using Dynabeads

The experiments described above determined the binding parameters of beads to the target cells and defined a range of optimal conditions for specific bead/cell rosetting. Further experiments were included to test whether these conditions were also optimal for specific depletion of antibody-labelled cells. The indirect method was tested with PBMNC treated with anti-CD3 antibody and anti-mouse IgG coated beads, and the direct method was tested with K562 and CH-F42 coated beads. Target cell ratios of 5:1, 20:1, and 40:1 were used with 30, 90, and 120 min incubation periods. Following magnetic separation of CD3 positive cells, unbound fractions were stained with FITC anti-mouse Ig. The CH-F42 depleted fraction was first incubated with CH-F42 antibody and then stained with FITC anti-rat Ig. The presence of contaminating positive cells in the depleted fractions was determined by examination with fluorescence microscope (Table 1). A bead to target cell ratio of 40:1 at 90 minutes resulted in a negative fraction completely devoid of CD3 positive cells, when more than 500 cells were counted. Bead-to-target cell ratios of 40:1 at 30 min, or 20:1 at 90 min, were slightly less effective, resulting in negative fractions containing less than 1% CD3 positive T cells. The ratio of 5:1 at 120 min and 20:1 at 30 min were effective at removing approximately 90% of CD3 positive cells. Similar results were observed in the direct method using antibody CH-F42 and cell line K562.

Following these experiments, a bead:cell ratio of 40:1 for 90 min was used, with a range of monoclonal antibodies recognizing different cell types. These conditions were highly specific and effective for all the monoclonal antibodies used.

Recovery of Positively Selected Cells

A number of attempts were made at using different monoclonal antibodies in immunomagnetic rosetting to recover positively selected cells, by incubating bead/cell rosettes at 37C in tissue culture medium as described previously (2). However, even after 48 hours incubation cells could not be recovered free from the attached beads.

Depletion Efficiency Using More Than One Monoclonal Antibody

When two monoclonal antibodies recognizing completely separate antigens were used together (anti-CD4 and anti-CD8), the depletion efficiency was comparable to the removal of the corresponding cell types individually. Negatively selected fractions were free of contaminating cells. Cells in the

positive fractions bound a similar number of bead-to-cells in experiments using single antibodies.

Optimal Conditions for Processing of Dynabeads for Electron Microscope

The fixation protocol, comprising a brief primary fixation with glutaraldehyde in very dilute suspension followed by quenching with ethanolamine and postfixation with osmium tetroxide was essential to frequent the formation of non-specific aggregates while still preserving good ultrastructural morphology. Fixation also prevented the spontaneous disassembly of rosettes which may occur if the samples are stored for some time in a refrigerator. If quenching with ethanolamine was omitted, large non specific mixed aggregates of cells and beads were observed by both light and electron microscopy. This may be due to the formation of increasing number of crosslinks between free amino groups, leading to aggregates. Ethanolamine provides free amine groups to react with the excess glutaraldehyde and stops formation of such aggregates.

In the electron microscope (Figures 3-4) the beads were revealed to contain a uniformly distributed but heterogeneous population of electron-dense particles which usually fell in the range of 10-55nm and which permitted the beads to be easily visualized even in unstained sections (Figure 3). The rippled appearance of the beads in the electron microscope was a sectioning artifact and was reduced in harder blocks. The proportion of K562 cells forming rosettes with CH-F42 appeared consistent in both light and electron microscopy. CH-E25 did not form any rosettes with K562. Neither CH-F42 nor CH-E25 formed rosettes with U937.

A gap was sometimes evident between the Dynabeads and the surface of the cells to which they were rosetted (Figures 3 and 4). Tight contacts between cells and Dynabeads could also be found (Figure 4). Results from serial sectioning suggested that tight contacts exist between the cell surfaces and most if not all of the Dynabeads.

DISCUSSION

In this study conditions were optimized for the application of commercially available magnetizable microspheres in immunolabelling and immunomagnetic separation. Furthermore, a novel technique has been developed for the application of Dynabeads for immunolabelling in electron microscopy.

It was found that optimal conditions for specific binding of Dynabeads with the target cells requires an incubation time on ice of between 60 and 90 minutes, with a bead:target cell ratio of approximately 20:1. These conditions were also found to result in highly specific and effective depletion of particular lymphocyte sub-populations from PBMNC. Although the use of higher bead-to-target cell ratios induced an unacceptable level of nonspecific binding in the original detection system, these non-specifically bound cells could not be separated using the magnet. This would suggest that non specific interactions

are of low affinity and do not result in magnetic attraction of antigen negative cells which are in contact with beads. The experiments indicate that the desired purity of the negative fraction determines the requisite bead-to-target cell ratio and incubation time. When fractions depleted to below detectable levels of positive cells (e.g > 1 in 500) are required a 40:1 ratio for 90 minutes is appropriate. However, sufficiently pure cell populations can be obtained using longer incubation periods with low bead:cell ratios, or short incubation periods with high bead:cell ratios. The precise conditions selected therefore depend on application, cost and time considerations.

A large proportion of antibody positive cells only had 1 or 2 beads attached to their surfaces. In fact, using generally accepted criteria for rosetting with, for instance, red cells (3 or more particles/cell) (7) a considerable proportion of positive cells would not be considered to form true rosettes. However, for separation by magnetic attraction, only one bead was required to be specifically bound per antigen positive cell.

Dynabeads have proved to be entirely suitable as immunological labels in both light and electron microscopy, although it must be emphasized that the fixation protocol for electron microscopy is crucial in preventing the formation of non-specific aggregates.

As a consequence of the relative sizes of the particles, 4.5 micrometer in the case of Dynabeads as compared to 5-40nm for colloidal gold, Dynabeads are unable to locate surface antigens as precisely as colloidal gold. Like colloidal gold, Dynabeads are electron dense and so visible by transmission electron microscopy even in unstained sections. However, unlike colloidal gold, Dynabeads are large enough to be also easily visible in the light microscope without the necessity of contrast enhancement by silver staining or the requirement of expensive facilities for video image processing (8,9).

In many though not all cases, electron microscopy revealed a gap larger than the expected span of an IgM molecule between the Dynabeads and the cells to which they were rosetted. The most likely explanation to account for this observation is that in those cases where gaps appeared, tight contacts were out of the plane of the section. It was demonstrated by serial sectioning that such contacts did exist and they were therefore probably present in most if not all cases. It is possible that the size of the gap might be increased by cell shrinkage during processing for electron microscopy, especially if the Dynabeads were already "locked in" to the polymerizing resin matrix. It is also possible that redistribution of cell membrane associated molecules during fixation and subsequent processing for electron microscopy might allow the distance between the Dynabeads and the cells to increase while covalent attachments were still maintained. This is perhaps unlikely as there is little evidence of stained material spanning the gap between the cells and the beads, gaps between the components of rosettes are not unprecedented. For example, they have been observed in rosettes formed between erythrocytes and lymphocytes during an investigation of IgM receptors on the surface of hairy cells in leukaemic reticuloendotheliosis (10).

Previous studies have reported considerable difficulties when removing Dynabeads from positively selected cells (11,12). This study confirmed this point. It may limit the value of Dynabeads for positive selection in those cases where removal of the beads is subsequently necessary. However, as a negative selection matrix, Dynabeads provide an attractive alternative to other methods of depletion described, since it is possible to obtain high yields of very pure cells in a relatively short time and at low cost.

Dynabeads used as immunological markers are able to form a useful bridge between light and electron microscopy. Additionally, the Dynabeads have the intrinsic advantage for which they were primarily conceived that, by the application of an external magnetic field, they can be used as a mean of separating the cells of interest for further study.

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TABLE 1

Percentage of OKT3+ fluorescent cells in OKT3 depleted fractions (PBMNC)

Time	Bead: Target Cell Ratio		
	5:1	20:1	40:1
30 Min	21.4±5.8	10.3±2.6	9.2±3 5
90 Min	18.6±3.4	0.7±0.35	0.0
120 Min	15.0±4.5	0.0	0.0

Results are mean ± SD of three experiments.

FIGURE 1

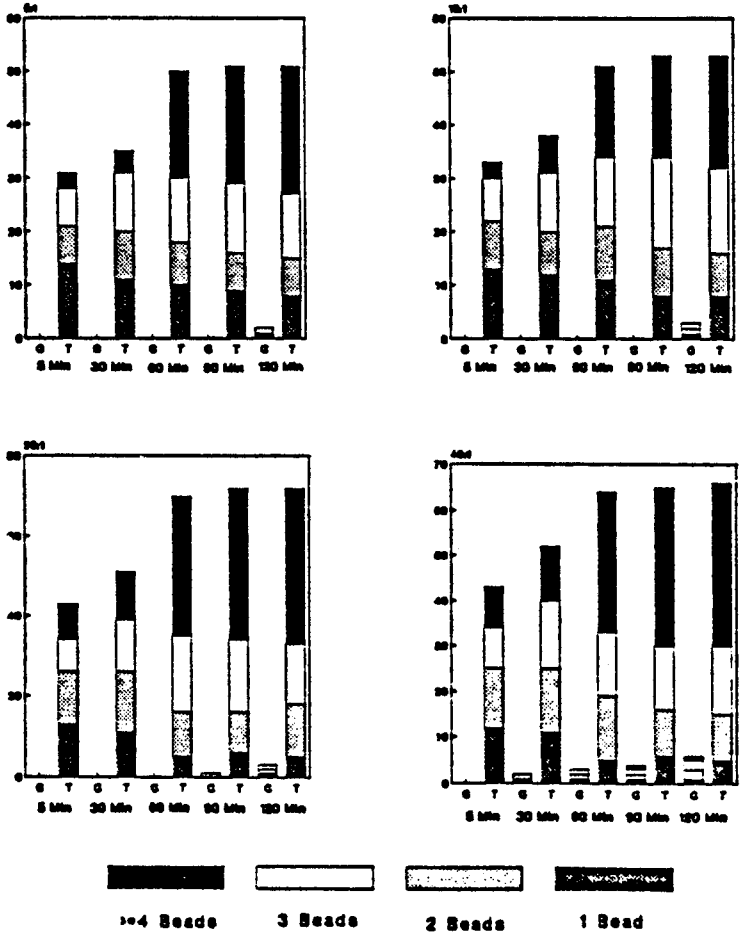

Fig 1: Shows specific and non-specific binding of beads to cells at different bead:target cell ratios and incubation periods. PBMNC were incubated with anti- CD3 antibody on ice. After two washings in H/HBSS, the appropriate number of anti-mouse IgG coated Dynabeads were added to the cells. The cells were then centrifuged and kept on ice for different lengths of time.

FIGURE 2

K562 cells rosetted with CH-F42 coated Dynabeads.

Fig 2: Phase contrast micrograph of K562 cells. Both cells are surrounded by CH-F42 coated Dynabeads. (original magnification: x 400)

FIGURE 3

K562 cells rosetted with CH-F42 coated Dynabeads

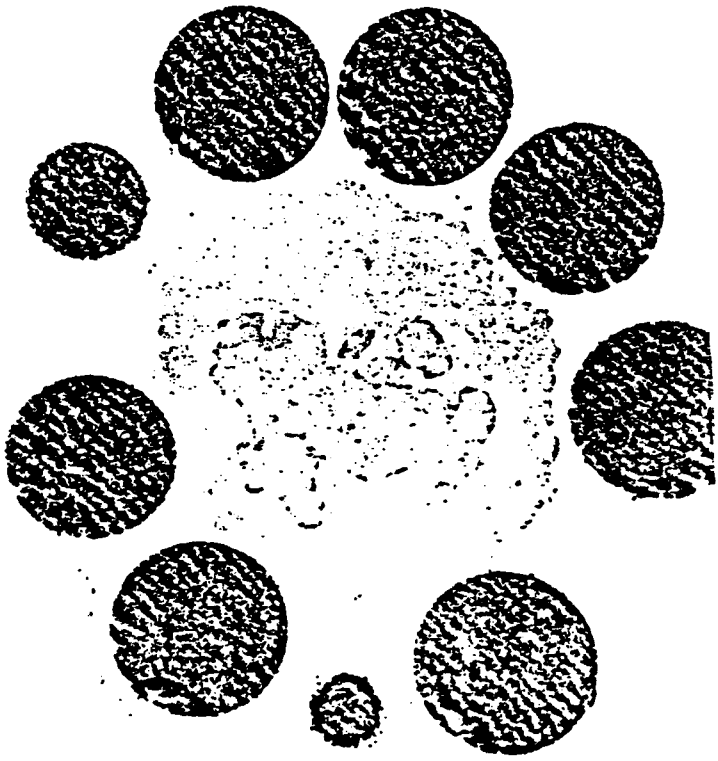

Fig 3: Electron micrograph of an unstained section through a rosette between a K562 cell and several CH-F42 coated Dynabeads. LR white resin (original magnification: x 9000)

FIGURE 4

K562 cells rosetted with CH-F42 coated Dynabeads

Fig 4 : Higher power electron micrograph of a close contact between a CH-F42 coated Dynabead and the surface of a K562 cell. Spurr's resin section stained with uranyl acetate and lead citrate (original magnification: x 20000)

FIGURE 5

K562 cells rosetted with CH-F42 coated Dynabeads.

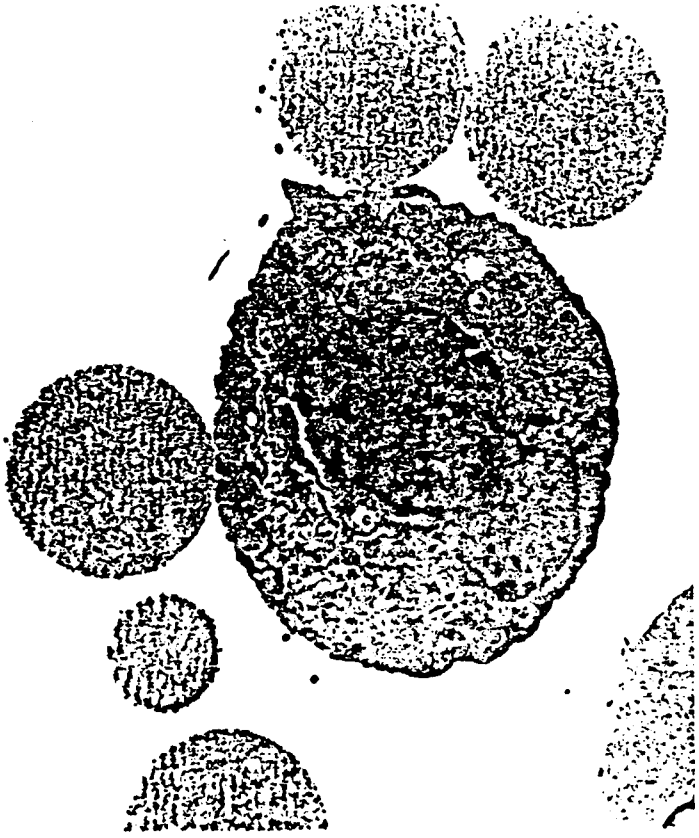

Fig 5 : Electron micrograph of a rosette between a K562 cell and three CH-F42 coated Dynabeads. Spurr's resin section stained with uranyl acetate and lead citrate. In this plane of sectioning two of the Dynabeads were in close contact with the cell surface while the third was not (original magnification: x 9000)

LINKER-MODULATED BIODISTRIBUTION OF IN-111 AND Y-90 LABELED MOAB ANTIFERRITIN IMMUNOCONJUGATES IN NUDE MICE AND DOGS

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ABSTRACT

High tumor uptake and rapid clearance from normal organs are essential for any radioimmunoconjugate to be considered as a clinically useful diagnostic and therapeutic agent. The usually slow transit of radioactivity through the liver and blood is detrimental since it lowers the target to non-target ratio and delivers undesirable radiation to normal organs. To mitigate this problem, two labile chemical linkages (EGS and DST) were introduced between monoclonal antiferritin antibody (QCI) and a chelating agent (DTPA) to modify the biodistribution. The biodistribution of these metabolizable linkers was evaluated in nude mice implanted with human hepatoma xenografts (HepG2). Biodistribution was compared to a stable non-cleavable DSS linkage and a thiourea linkage which was prepared by direct conjugation of isothiocyanatobenzyl-diethylenetriamine-pentaacetic acid (ITCB-DTPA) to the antibody. These immunoconjugates, containing two DTPA molecules per antibody, were radiolabeled with In-111 or Y-90, purified by Sephadex G50 chromatography, and tested for immunoreactivity. The labeled immunoconjugates were injected into the tail vein of nude mice bearing a subcutaneous, ferritin positive, human hepatoma (HepG2) graft. The tumor targeting was similar for the four immunoconjugates studied. Tumor to normal organ ratios were enhanced for EGS linkage in comparison to the other two stable linkages. EGS linkage showed low retention of radioactivity in the liver, blood, and spleen, although the kidney evidenced the clearance of activity at early time points. Serial immunoscintography data confirmed the biodistribution study. As the mouse model does not represent the high liver uptake of monoclonal antibodies (MoAb) in the human liver, beagle dogs were used to explore the retention of radiolabel in the normal liver. Following injections of In-111 labeled radioimmunoconjugates, serial SPECT scans were performed. Autopsies were conducted 7 days after injection. Quantification obtained by SPECT scans was confirmed by direct counting of tissue samples obtained at autopsy. The EGS linked immunoconjugate significantly reduced the dog liver activity when compared to the thiourea linked immunoconjugate. Rabbit polyclonal anti-dog ferritin showed a 4x lower uptake when compared

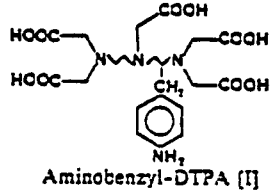
to thiourea linked QCI. The chosen animal models (mice and dog) appear to allow for optimal preclinical analysis of chelated radiolabeled monoclonal antibodies for diagnosis or treatment.

INTRODUCTION

The clinical usefulness of radioimmunoconjugates might be improved by interposing chemical linkages between the chelate-isotope complex and the immunoglobulin. In an attempt to reduce normal organ activity for In-111 labeled immunoconjugates, a readily metabolized chemical linkage can be placed between the antibody and chelator. Ideally, the radiolabeled chelate will be cleaved from the antibody by normal tissue metabolism and the chelated radiometal can be eliminated rapidly through the kidneys, thereby reducing the background activity. Quadri *et al.* (1); Haseman *el al.* (2); and Paik *et al.* (3) have reported that the introduction of a diester linkage between the antibody and a radiometal chelate increases the clearance rate of the activity from blood and substantially reduces the activity in normal organs such as bone marrow, kidney, and liver. The target-to-normal organ activity ratio can be amplified if the labeled immunoconjugate remain bound to the tumor target. In this communication we report on a comparison between two stable linkages and two chemically labile linkages in experimental animals. Using thiourea (ITCB) and hydrocarbon linkages (DSS) as relatively stable controls, diester (EGS) and tartaramide (DST), two chemically labile linkages, were introduced between the MoAb and aminobenzyl-DTPA derivative in order to evaluate pharmacokinetics in a tumor model system.

MATERIALS AND METHODS

Synthesis of 1-(p-aminobenzyl) derivative of DTPA (I)



Aminobenzyl-DTPA [I]

This compound was synthesized with an aminobenzyl group on the backbone of the chelate as previously described (5,6) and has a primary amino group available for conjugation with a bifunctional cross-linking agent. This diethylenetriamine-pentaacetic acid (DTPA) derivative was selected as a chelate over conventional cyclic DTPA, as it secures better stability of the In-111 or Y-90 complexes in vivo due to availability of an additional carboxyl ligand.

Placement of Chemical Linkages

The primary amino group of compound [1] was reacted with a carbonyl group of N-hydroxysuccinimide ester of disuccinimide tartarate (DST),

disuccinimidyl suberate (DSS), ethylene glycol bis(succinimidylsuccinate) (EGS); the remaining carbonyl group of the diester was then reacted with the amino group of the antibody to introduce the linkage between the chelator and the antibody.

Introduction of Labile Linkages

The method of Abdella et al. (4) was adapted to conjugate monoclonal antibody (IgG) with the labile linkers when DST and EGS were used as cross-linking agents. For the introduction of a linker, the cross-linking agent dissolved in dimethylsulfoxide was mixed with aminobenzyl-DTPA dissolved in phosphate buffer at pH 7.0. The solution was stirred gently at room temperature. Antibody in phosphate buffer was rapidly added to this solution while stirring. The conjugation reaction was continued for 2 hours at room temperature.

Introduction of Hydrocarbon Linkages

The reaction procedure for the introduction of the hydrocarbon chain was the same as that for the introduction of the labile linkage, except that disuccinimidyl suberate (DSS) was used as a cross-linking agent. Immunoconjugates were purified from unconjugated chelate moieties before radiolabeling with $^{111}InCl_3$ for biodistribution studies. The linker placement and chemical structure of immunoconjugates are described in Figure 1.

Introduction of Thiourea Linkages

This compound was synthesized with an isothiocyanatobenzyl group on the backbone of the chelate as previously described (5,6). 1-(p-Isothiocyanatobenzyl)-DTPA was reacted with antibody amino groups to conjugate through a thiourea bond. Monoclonal antibody was reacted with ITCB-DTPA at a molar ratio of 4 in 0.2M bicarbonate buffer at pH 8.4 at room temperature for 2 hours. For the biodistribution, MoAb-thiourea-benzyl-DTPA was purified from unconjugated DTPA by centricon filtration.

Radiolabeling and Determination of Chelates per IgG

An assay for the determination of the average number of chelates per IgG was performed on small aliquots of the coupling reaction mixture before separation of the unreacted chelating agent. This aliquot was diluted to 0.5 ml with 0.1M sodium acetate, pH 5.5, and 0.1 ml of $InCl_3$ solution of a known concentration spiked with a trace amount of ^{111}In. The amount of indium metal added must be twice the amount of the ligand (coupled and uncoupled) in the aliquot. After one hour the labeled antibody was separated from other ^{111}In complexes by thin layer chromatography (TLC) analysis and the number of chelate moles/mole antibody was calculated. Radiolabeled IgG was determined by instant thin layer chromatography (ITLC-SG) using a 2:2:1 solution of methanol:10% NH_4OAc: 0.5M citric acid. Indium-111-chelated IgG appeared at R_f-0, ^{111}In-DTPA derivative at R_f-0.55, and ^{111}In-citrate at R_f-1.0 in TLC

analysis. An average number of 2 DTPA chelates were conjugated to antibody molecules in these antibody-linker chelates.

An aliquot of pure $^{111}InCl_3$ in 0.1M HCl was equilibrated with acetate/citrate buffer at pH 5.5. 100ul of an antibody-linker-chelate conjugate was added, mixed well, and incubated at room temperature for 30 minutes. The labeled protein was separated from low molecular weight compounds by the Sephadex G50 gel column chromatography using O.1M PBS. The labeled IgG was collected and assayed in a dose calibrator, and the labeling efficiency was determined. Yttrium-90 radiolabeling was achieved by using ^{90}Y acetate solution of pH 6.0 at room temperature in a manner similar to the ^{111}In chelation.

Biodistribution Studies

A human tumor (HepG2) was xenografted into the left hind leg of nude mice by a subcutaneous injection of human tumor cells ($1x10^7$ cells). When the size of the tumor was approximately 0.5 to 1cm in diameter, the mice were injected with purified ^{111}In or ^{90}Y-labeled immunoconjugates (40 uCi, specific activity 2 mCi/mg) via the tail vein. The animals were sacrificed at 1, 2, 4 and 6 days after the injection of the radioimmunoconjugates. Organs were excised, weighed promptly and counted in a gamma counter.

Imaging Studies

Athymic nude mice bearing xenografted HepG2 tumor were injected with 65 uCi of QCIEGS-aminobenzyl-DTPA-In-111 (EGS) and QCI-isothiocyanatobenzyl-DTPA-In-111 (ITCB) for immunoscintographic analysis. A GE 400 AT gamma camera with pulse height analyzer was set at 10% to accept 172 and 274 kev energy peaks of In-111. Sequential images at 12, 24 and 48 hours post-injection were taken with parallel hole collimator 4 cm from the dorsal surface of the animals. Static images were acquired in a 128x128 pixel matrix using a dedicated computer.

Dog Studies

Female beagle dogs were used to analyze the retention of radiolabel in the normal liver. After In-111 labeled immunoconjugate injections, serial planar gamma camera images and SPECT scans were performed followed by autopsies 7 days postinjection. Quantification obtained by SPECT scans was confirmed by direct counting of tissue samples excised at autopsy in a gamma counter.

RESULTS

Quality Control

Stability studies, characterization of immunoconjugates, and quality control analyses were performed (Table 1). HPLC analysis of these immunoconjugates showed one major peak with a retention time identical to that of the native antibody, and a minor peak (5%) representing the dimer.

^{111}In-labeled immunoconjugates were purified by DEAE affinity HPLC and affinity chromatography to ensure that the labeled antibody preparations were all immunoreactive and radiochemically pure, so that any differences in their biodistributions would result primarily from the differences in chemical linkage.

Biodistribution

MoAb-EGS-DTPA and MoAb-DST-DTPA conjugates labeled with ^{111}In were injected intravenously into animals and the pharmacokinetics were compared to those of the stable DSS and thiourea linked conjugates labeled with the same radioisotope as shown in Figure 2 (A-D). Excellent tumor localization was achieved on day 2 following injection. The DST linker immunoconjugate did not target as well as the ones containing EGS or the stable linkers. There was a rapid clearance of blood activity with DST and EGS containing immunoconjugates compared both to DSS and thiourea containing immunoconjugates. For the labile linkers the liver uptake was three to four-fold lower when compared to the stable thiourea and DSS linker conjugate. Localization of DSS linked conjugates in normal organs such as blood, liver, kidney and spleen was very similar to the biodistribution patterns for the thiourea linked conjugate (Figure 2). Bone marrow uptake was not observed in any of the mice the study. The biodistribution results for EGS and DST linkers did not demonstrate any appreciable uptake of activity in normal tissues when compared to that of the control conjugates. Similar biodistribution patterns were obtained when ^{90}Y radiolabeled MoAb-EGS-DTPA and thiourea linked immunoconjugates were compared in the nude mice tumor model as illustrated in Figure 3 (A-D).

Tumor Targeting

Tumor to target ratios and image quality improved over time as demonstrated in Figure 4. A significant amplification of the target to non-target ratios was achieved by EGS linker conjugates which was confirmed by imaging studies. Radioimmunoconjugate injections resulted in a preferential localization of the radiolabel in the tumor and excellent visualization within 24 hours of injection. The EGS radioactivity localized in the bladder/kidney region of the mouse at 24 hours, indicating clearance of EGS chelate-In-111 through the urine. The isotope uptake by the abdominal organs, primarily the liver, is less intense with the EGS linker chelate than with the ITCB-DTPA chelate. In the case of the EGS linker, the liver activity was apparent in early scans, but declined rapidly, indicating clearance of the isotope, probably due to hydrolysis in the liver (Figure 4).

IN-111 Labeled Immunoconjugates in Dog Model

The EGS linked immunoconjugate significantly reduced (-50%) the dog liver activity when compared with thiourea linked immunoconjugate. Rabbit polyclonal anti-dog ferritin showed a 4x lower liver uptake when compared with relatively stable non-cleavable linked QCI. The results are summarized in the Table 2.

Liver volume and activity predictions based on SPECT scans were in accordance (+/- 5%) with autopsy data. Less than 15% of injected In-111 activity was excreted in the dog's urine. The liver half-life was monophasic (~3 days). The half-life in the blood was similar for all immunoconjugates (biphasic with t1/2 of 30 minutes and 2.5 days respectively).

DISCUSSION

The labile linker-chelates clear the blood more rapidly than the stable ITCB-DTPA or DSS chelate. Virtually all circulating activity is eliminated for the EGS-chelate within 48-96 hours. Radioactivity in the normal tissue is lower with the labile linker-chelate than with either the non-cleavable DSS linker or ITCB-DTPA. The effective half-life in the normal tissue is also shorter for the labile linkers. The conjugate concentration and effective half-life in the tumor are comparable for the ITCB-DTPA chelate and the labile linker-chelate. The pharmacokinetic patterns are similar for ^{111}In-labeled and ^{90}Y-labeled immunoconjugates in the nude mouse tumor model indicating that under these circumstances the indium isotope can be used as a predictor (dosimetry test) for subsequently injected yttrium labeled antibodies.

Immunoscintography confirmed all three significant observations gained from the biodistribution studies: 1) good tumor targeting by both labels; 2) clearance of EGS activity from the liver; and 3) rapid excretion of the EGS chelate through the kidney in the urine.

These data demonstrate that in mice when a labile linker is placed between the antibody molecule and the chelator, minimal non-specific uptake in non-target tissues and improved localization of the isotope to the tumor target result.

Radiolabeled monoclonal antibodies (In-111, Y-90) have targeted subcutaneously implanted human tumors well in murine models *in vivo* and have not shown enhanced uptake in normal liver of mice or rats. However, in man unsatisfactory results have been obtained with radiolabeled monoclonal antibodies, i.e. short dwell times in tumor, rapid and irreversible normal liver uptake and short blood half lives. Short tumor half-lives have frequently been long enough for diagnostic purposes yet too brief for delivery of substantial radiation doses to the tumor. In the absence of a predictive animal model, there is no alternative to direct testing of monoclonals for RIT in human patients. Further exploration of other experimental animal species (such as the dog) with monoclonal antibodies might provide a better prediction for the presence or absence of human normal liver uptake than the uninformative small rodent models. A predictive animal model would obviously facilitate and accelerate the analysis of the linkerchelate modification of monoclonals in human radiolabeled immunoglobulin therapy.

Our preliminary results in beagle dogs indicate that the high uptake by the normal human liver is reproduced in this model, and that liver uptake is dependent on the species in which the antibody is produced as well as on the applied linker-chelate chemistry (Table 2). The combination of the chosen

animal models (mouse and dog) appears to allow for optimal preclinical analysis of radiolabeled monoclonal antibodies for diagnosis or treatment. Tumor targeting can be evaluated in the mouse model. Normal tissue toxicity (bone marrow, liver) can be evaluated in the dog. Radiolabeled (^{90}Y) antiferritin appears to cause dose limiting liver damage, if bone marrow toxicity is reversed by a bone marrow transplant (7). Optimal clinical application of monoclonal antibodies will require a further decrease in uptake by the normal human liver. Labile linker-chelates might provide an important improvement in the therapeutic ratio of monoclonal antibodies in human cancer patients.

ACKNOWLEDGEMENTS

This work supported by NIH grant CA43791, and Hybritech, Inc.

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TABLE 1

Quality Control Analysis of Radioimmunoconjugates

Analysis	Ester Linkage (EGS)	Tartaramide Linkage (DST)	Thiourea Linkage (SCNBZ)	Hydrocarbon Linkage (DSS)
DTPA/IgG	1.8	1.8	2	1.5
Specific Activity	2 mCi/mg	2 mCi/mg	2.5 mCi/mg	2 mCi/mg
Colloid Formation	none	none	none	none
Serum Stability*	80-85%	92-97%	99%	99%
Cross Linking	<5%	<5%	none	<5%
Immunoreactivity	85%	86%	90%	90%
Dose Injected	30-40 μCi/Animal	30-40 μCi/Animal	30-40 μCi/Animal	30-40 μCi/Animal

*Protein bound fraction after 24 hours incubation at 37°C in serum

TABLE 2

Liver Uptake of In-111 Labeled Mouse MoAb in dogs (N=2)

Antibody	Linker-Chelate	Specificity	Dose Injected	% in Liver per mCi
Poly (Rabbit (Anti-dog-ferritin)	Thiourea (ITCB-DTPA)	Ferritin dog	2.0mCi	16
MoAb QCI (Mouse) (Antihumanferritin)	Thiourea (ITCB-DTPA)	Ferritin man	1.0mCi	64
MoAb QCI (Mouse) (Antihumanferritin)	Diester (EGS-ABDTPA)	Ferritin man	1.35mCi	33

FIGURE 1
Chemical structures of antibody-linker-DTPA conjugates.

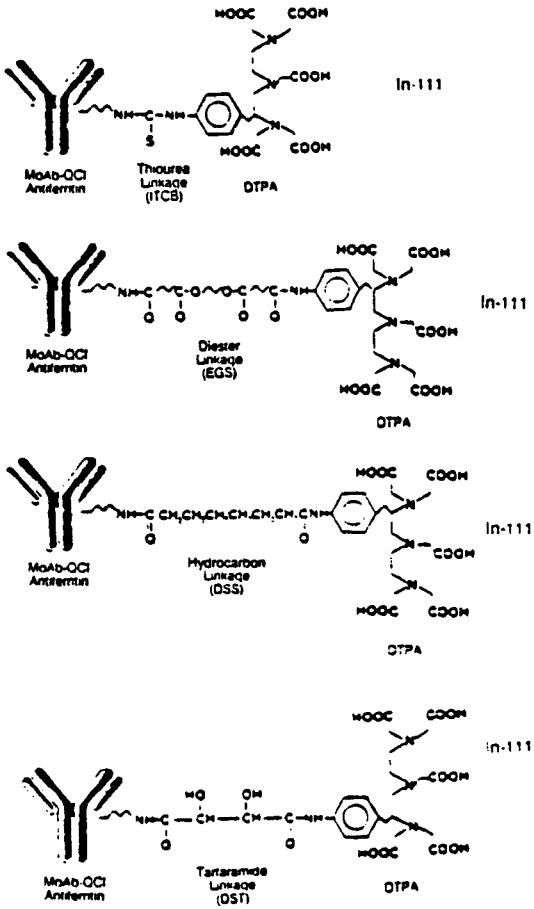


FIGURE 2
Biodistribution of In-111 Labeled QCI-Linker-Chelate Conjugates in Nude Mice (n=6) Xenografted with Hepatoma at Different Time Intervals: (A) Day 1; (B) Day 2; (C) Day 4; (D) Day 6.

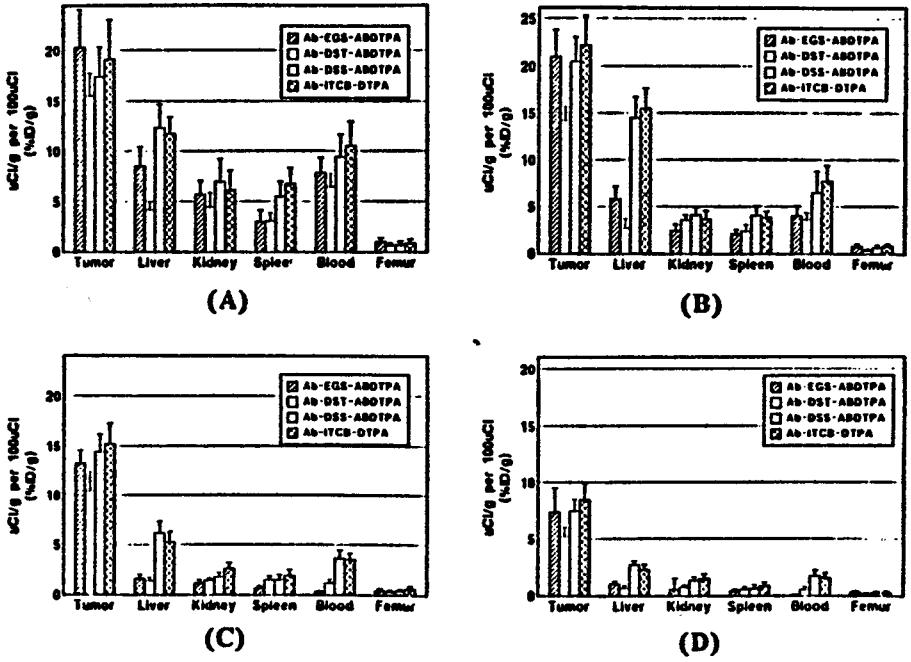


FIGURE 3
Biodistribution of Y-90 Labeled QCI-Linker-Chelate Conjugates in Nude Mice (n=6) Xenografted with Hepatoma at Diffeent Time Intervals: (A) Day 1; (B) Day 2; (C) Day 4; (D) Day 6.

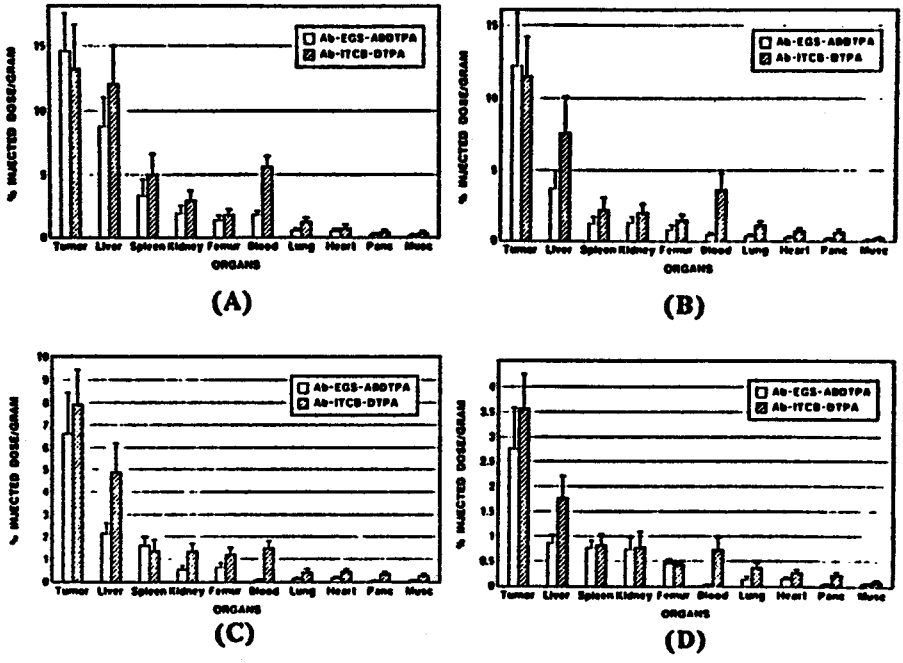
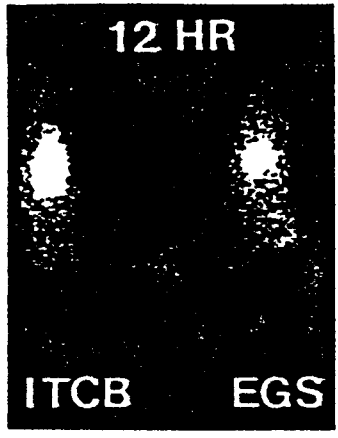
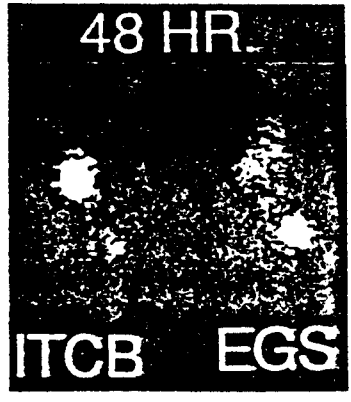


FIGURE 4
Immunoscintograph of QCI-EGS-Chelate (EGS) and QCI-Isothiocyanato-Chelate (ITCB) After Injection of Radiolabeled Immunoconjugates into Nude Mice Bearing Human Xenografted (HepG2. Hepatoblastoma) in Left Rear Flank of Leg. Radioisotope Concentration Shown in Upper Light Area is the Abdominal Region: Tumor is Located Lower Right Section of Light Area. A: 12 Hours Images, B: 24 Hours Images, C: 48 Hours Images.





INCORPORATION OF RADIOLABELED IMMUNOGLOBULIN ADMINISTRATION IN CONDITIONING REGIMENS FOR BONE MARROW TRANSPLANTATION

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INTRODUCTION

External beam radiation therapy provides local cancer treatment. Intravenous Radiolabeled Immunoglobulin Therapy (RIT) is a new modality with the potential to provide systemic radiation to cancer patients. The source and specificity of the inmunoglobulin, the physical characteristics of the isotope and the chemistry employed in the linking of the isotope to the immunoglobulin (1) determine dose deposition in many different parts of the body. Phase I-II studies of RIT in man have demonstrated anti-tumor activity and indicated bone marrow as the dose limiting tissue (2-8). Toxicities in other organ systems have not been encountered so far. RIT might provide an effective new element in the conditioning of cancer patients for bone marrow transplantation. RIT is not expected to add to the morbidity and mortality, if an effective bone marrow transplant is performed, and RIT might have a better therapeutic ratio than total body irradiation with external beam. In this communication we review recent information obtained in various studies in our laboratories pertaining to the inclusion of RIT in conditioning for bone marrow transplantation.

MATERIAL AND METHODS

Animals

Nude mice (male, 8 weeks old), ACI rats (male, 12 weeks old), and beagle dogs (male or female, 6-18 months old) were purchased and utilized in an AAALAC approved facility.

Human Patients

Individuals with recurrent Hodgkin's disease were treated with 90-Yttrium labeled antiferritin on one of the three possible protocols: Protocol

1, dose-escalation study including an autologous bone marrow transplant (RTO6 87-01); Protocol 2, 20 mCi of Yttrium labeled antiferritin without bone marrow support; Protocol 3, 20 mCi or 30 mCi of Yttrium labeled antiferritin followed by cyclophosphamide, VP-16 and BCNU chemotherapy and autologous bone marrow transplantation, the latter at the University of Nebraska Medical Center (9). Approved institutional or RTOG informed consent forms were signed by all patients. All patients received prior injection with In-111 labeled antibodies. Treatment with Yttrium-90 labeled antibodies was only given if serial scans following Indium injection showed good tumor targeting and no evidence of dechelation.

Antibodies

Immunoglobulins with specificity for human ferritin were obtained from immunized rabbits, pigs or baboons for human patients. QCI054 (mouse IgG_1, monoclonal antibody) against human antiferritin was obtained courtesy of Hybritech, Inc., San Diego, CA, and utilized in mice, dogs and human patients. Rabbit anti-rat and rabbit anti-dog ferritin was made at Hopkins and utilized in rats and dogs, respectively. B72.3 (mouse IgG1, monoclonal antibody against extract of human mammary carcinoma cell line) was obtained courtesy of Cytogen, Princeton, NJ, and utilized in dogs (10).

Radionuclides

Yttrium-90 was applied in dose escalation studies and studies of normal tissue toxicity and cancer treatment. Indium-111 was used in pharmacokinetic studies, imaging and tumor dosimetry.

Chemical Linkages

Unless otherwise indicated, all immunoglobulins were linked with diethylenetriamine pentaacetic acid (DTPA) through a stable thiourea bond, courtesy of Hybritech, Inc., San Diego, CA. Two dogs were injected with Indium labeled QCI containing a labile ester ethylene glycol bis (succinimidyl succinate) (EGS) between the antibody and DTPA (1). A larger group of dogs was injected with Indium or Yttrium labeled site specific chelate conjugate of B72.3 (11).

Imaging Studies

Serial planar view gamma camera images and SPECT scans were performed in dogs and human patients injected with Indium labeled antibodies for pharmacokinetic data, determination of dechelation, for tumor targeting and for tumor dosimetry (12,13).

RESULTS AND DISCUSSION

Antibody

Monoclonal anti-ferritin QCI is taken up by the normal liver of dogs and human patients (approximately 50% of injected activity). The EGS linker

immunoconjugate decreases liver uptake in dogs (1). Only approximately 10% of the injected dose is taken up by the liver of mice Or rats. Monoclonal QCI (i.v.) did target 1 out of 4 patients with Hodgkin's disease, while polyclonal antiferritin targeted 45 out 50 patients, including the 3 patients who did not target with QCI. This difference is significant at $p<0.01$ in a Fisher exact test. An example of tumor targeting with polyclonal Indium labeled antiferritin is provided in figure 1. Anti-antibodies were not induced in any Hodgkin's patients. QCI did target subcutaneously implanted human hepatoma cells in nude mice (14).

Radionuclides: Indium-111

Volumes and radioactivity in dog livers were predicted with serial planar scans and SPECT scans, and were found to be accurate $\pm$ 5% when compared to autopsy data (15). Dechelation was observed in a single patient out of 50 patients. Subsequent infusions of Indium labeled antibodies in the same patient did not dechelate. Tumor dosimetry was possible in approximately two-thirds of the patients. It is difficult to assign doses to small tumor volumes (<2cm diameter) and volumes contiguous with high activity normal tissues such as heart, major vessels, liver. Under the assumption that the Indium predicted for the subsequent Yttrium labeled antibody intravenous administration, tumor doses between 10 and 20 Gy in 1 week were calculated for patients receiving 30 mCi of Yttrium labeled antiferritin.

Radionuclides: Yttrium-90

Hematological side effects were dose limiting. Twenty mCi of Yttrium appeared to induce similar hematologic toxicity as 50 mCi Iodine-131 labeled antiferritin. Radioimmunoconjugates were stable. Urine activity was less than 10% of injected dose, while the serum half-lives were approximately 2 days. Physical half life of Yttrium-90 is 2.7 days. Tumor half lives were frequently longer than the physical half life.

Prescription

Equitoxic doses were determined in rats, dogs and human patients (Table 1). The results reflect the similarities between systemic chemotherapy and RIT, and indicate that radiolabeled antibodies are currently best prescribed in activity per kilogram recipient, as this correlates best with the concentration of the dose limiting normal tissue target, the hemopoietic stem cell.

Timing of RIT Injection

Bone marrow cells injected too quickly after the radionuclide administration are ineffective due to lack of "space" in the bone marrow (18) or residual radioactivity in blood or bone marrow (19). For human patients 20 mCi of Yttrium labeled antiferritin didn't create sufficient space to make a bone marrow transplant necessary or effective (14). Bone marrow cells ($1x10^8$ cells/kg i.v.) injected 10 days after 4 mCi Yttrium labeled antiferritin per kilogram, effectively restored hemopoiesis in dogs (figure 3).

Bone marrow transplantation 10 days after 5.5 mCi of Yttrium labeled antiferritin per kilogram was no longer effective. Dosimetric calculations based on serial blood and bone marrow samples indicated that bone marrow dose rates at day 1 0 after injection were approximately 0.5 cGy/hour and 1.25 cGy/hour for dogs receiving 4 mCi and 5.5 mCi/kg, respectively. These calculations are in accordance with earlier reports of LD_{50} studies in dogs in constant low dose rate radiation fields (20). LD_{50} doses appear to be independent of dose rates until approximately 1 cGy/hour. Dose rates lower than 1 cGy/hour apparently allowed for cellular proliferation in the bone marrow and led to higher LD_{50} doses. The radiation sensitivity of in situ or transplanted bone marrow cells is expected to be the same (21). The bone marrow radiation dose activity is determined by the injected activity, the amount of bone marrow targeting of the radiolabeled antibody, and the kinetics of the disappearance and decay of the radionuclide in the bone marrow. In dogs antiferritin and B72.3 antibodies did not target bone marrow and showed a biphasic disappearance curve in blood and bone marrow. The rapid component probably reflected liver uptake of conjugate and elimination in urine of chelated isotopes; the slow component (T 1/2 of 2.5 days) reflected mainly the physical decay of the intact radioimmunoconjugate in the bone marrow compartment. Bone uptake, indicative of unbound radioactive Yttrium, was not observed. Radiolabeled antibodies with specificities directed against membrane antigens of cells in the hemopoietic system do target in the bone marrow (22), and will be more difficult to incorporate in conditioning schedules for bone marrow transplantation due to the prolonged high bone marrow radioactivity. For the same reason, it would be difficult to use repeated RIT infusions ("fractionated" RIT), although the initial experience with B72.3 in dogs indicated that two fractions (day 0-4 or day 0-8) of RIT caused less hematologic toxicity than a single fraction of RIT (Vriesendorp et al., in preparation).

Normal Tissue Damage

Radioactivity in the circulatory system caused lymphopenia within 24 hours after injection in rats, dogs and human patients. There was a positive dose effect correlation. Granulopenia was more pronounced in larger species (table 1) and correlated positively with activity injected/mg and negatively with hemopoietic stem cell concentration. Thrombopenia was also controlled by bone marrow damage, but in addition by the amount of radiation received by the liver. Endothelial (hepatic vein) radiation damage probably trapped platelets in the liver and, after high injected activities, caused veno-occlusive disease, identical to the histological picture seen in human radiation hepatitis (23). In figure 3 alkaline phosphatase levels are summarized for dogs receiving escalating doses of polyclonal Yttrium-90 labeled antiferritin. The highest activity caused lethal radiation hepatitis. Kidney toxicity was not observed. Other normal organ toxicity was seen in rats, where dose escalation led to rapid mortality from intestinal toxicity, but not in dogs or human patients.

Tumor Responses

Radiolabeled anti-ferritin was used as a single agent in refractory Hodgkin's disease. Summarized information for protocols 1 and 2 is contained in table 2, updated to August 1, 1990. The patients treated with Iodine-131 were reported earlier (3). The Yttrium-90 patients in protocols 1 and 2 were done later (18 and Vriesendorp et al., in preparation). Response rates were higher for the Yttrium treated patients (61% vs 40%). Complete responses were significantly more frequent after Yttrium ($p<0.005$). Responses lasted a median 6 months with a range from 2 to 26+ months. Five patients continue in complete response beyond 8 months. Approximately one-third of the recurrences were in previously uninvolved areas. Responses were more common in patients in the small volume disease (<30 cm) and long (> 3 years) histories of Hodgkin's disease. Response rates in small numbers of patients were not improved by escalation of injected activity. No correlation was found so far between responses and calculated tumor doses. Yttrium labeled antiferritin given to 11 poor risk Hodgkin's patients prior to high dose cyclophosphamide, VP-16 and BCNU followed by bone marrow transplantation (protocol 3) caused no extra toxicity. Response rates remain to be determined by longer follow-up.

CONCLUSION

RIT appears to offer the right modality for inclusion into conditioning regimens for bone marrow transplantation. It is an active, but presently not curative, single agent. Bone marrow toxicity is dose limiting, but can be corrected by a well time transplant. The therapeutic ratio of RIT appears to be excellent in comparison to other cancer chemotherapeutic agents. Yttrium-90 appears to be a better isotope than Iodine-131 for treatment. The optimal chemistry for linking Yttrium to the immunoglobulin remains to be determined. Current preparations (in particular monoclonal antibodies) have unacceptably high liver uptake in larger animals (dogs) or human patients (1,8). Hodgkin's disease provides the best disease model for further attempts at improvements of RIT in man. Both normal tissue toxicity and tumor responses of RIT can be evaluated in patients with end stage Hodgkin's disease. Cancer patients with other histologies are often candidates for other experimental protocols, or do not respond sufficiently to the currently available RIT protocols. Further dose escalation by a factor of 5, leading to tumor doses greater than 50 cGy in one week, appear to be achievable with RIT by further exploration of radioimmunoconjungate chemistry and interactions between RIT and other modalities. In the laboratory, the nude mouse and the beagle dog appear to offer the best models at present for tumor targeting and normal tissue toxicity, respectively. After optimization of RIT in animal models and in patients with Hodgkin's disease, other cancer patients with more common tumors might also become candidates for experimental RIT. The prime concern remains to exploit the excellent therapeutic ratio of RIT.

ACKNOWLEDGEMENT

This work was supported by NIH grant CA-43791, Bethesda MD; Hybritech, Inc., San Diego CA; and Cytogen, Inc., Princeton NJ.

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TABLE 1

TABLE 1 GRANULOPENIA IN RAT, DOG AND MAN AFTER RABBIT ANTI-SPECIES SPECIFIC FERRITIN LABELED WITH YTTRIUM-90			
Species	Relative Hemopoietic Stem Cell Concentration*	Equivalent RIT Toxicity per mCi/kg	Equivalent Toxicity per Chemotherapeutic Agent mg/kg**
Rat	6.7	6	7
Dog	1.1	1	2
Man	1	0.5	1

*Vriesendorp and van Bekkum, 1984 (16)

**Freireich, et al., 1966 (17)

TABLE 2

TABLE 2 RADIOLABELED ANTIBODY IN END STAGE HODGKIN'S DISEASE				
Response rate	Iodine-131	Yttrium-90	X_2	P
RR	15/37	22/36	3.09	<0.1
CR	1/37	10/36	--	<0.005 (Fisher exact)
PR	14/37	12/36	0.16	>0.5

FIGURE 1

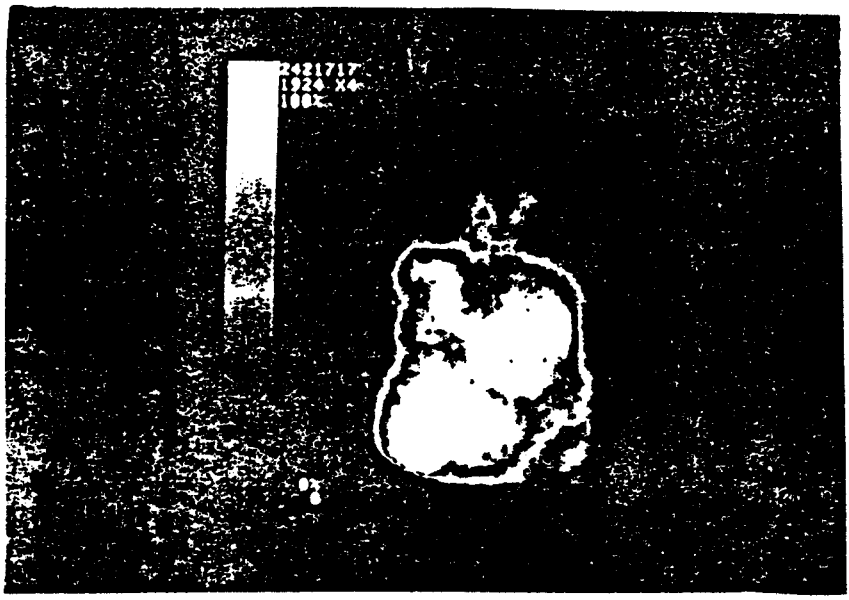


FIGURE 2

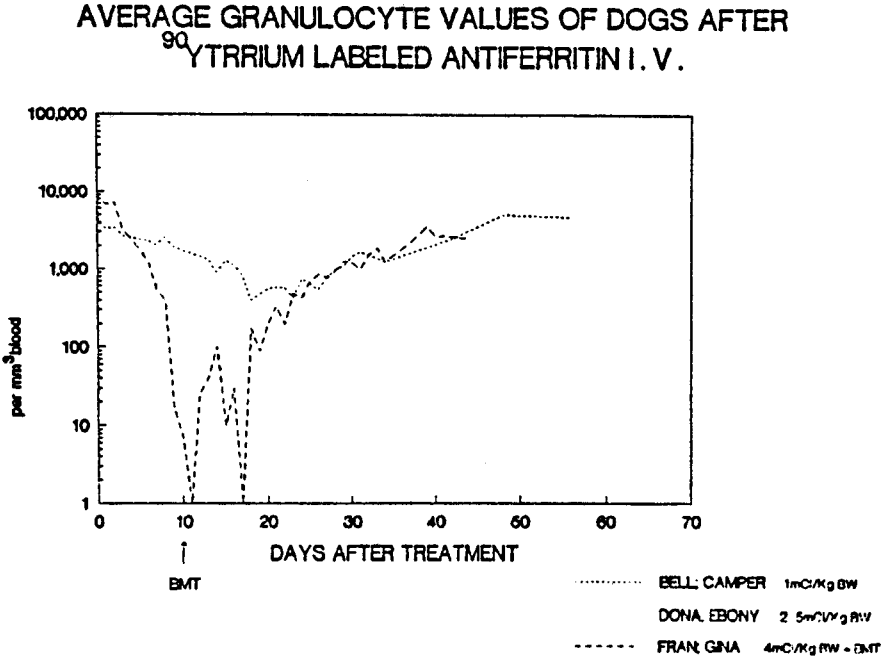
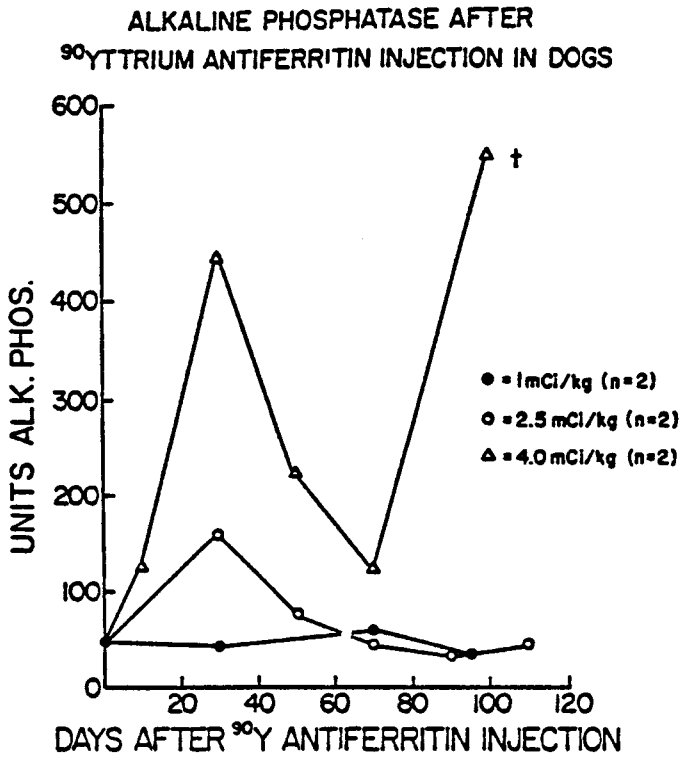


FIGURE 3


ALKALINE PHOSPHATASE AFTER
90YTTRIUM ANTIFERRITIN INJECTION IN DOGS
UNITS ALK. PHOS.
600
500
400
300
200
100
0
20
40
60
80
100
120
DAYS AFTER 90Y ANTIFERRITIN INJECTION
†
● = 1mCi/kg (n=2)
○ = 2.5 mCi/kg (n=2)
△ = 4.0 mCi/kg (n=2)

GENETIC ENGINEERING IN MARROW CULTURES

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INTRODUCTION

(In this paper, B= beta; Y= gamma.) Autologous transplantation of genetically-altered bone marrow for the potential treatment of many gene-based disorders has been a promising concept which is coming closer to realization (1). Autologous marrow comprised of cells with abnormal genes would be harvested, and a cloned normal gene would be transferred and inserted into the genome of these cells. The deleterious effect of the abnormal genes would thus be overcome so that the altered cells which are then transplanted, will function normally. Another application of this technology would be transfer of potentially useful genes to normal marrow being used for bone marrow transplantation. For example, transfer of a drug resistance gene would protect the transplanted marrow from the effects of anti-tumor agents in cases of post-transplant relapse.

The methods of delivery of genetic material into cells are numerous, and may be divided into chemical, such as calcium phosphate precipitation, physical, such as electroporation or lipofection, or viral, using viruses which have been engineered to contain the cloned gene that is being transferred. The most promising group of viruses are the retroviruses which have been found to transfer genetic material into intact mammalian cells with the greatest efficiency. Briefly, retroviruses infect the cell and shed their viral coat, which enables their RNA to be converted by reverse transcriptase within the cell cytoplasm to double-stranded DNA. The DNA then enters the nucleus and integrates into the genome of the cell. The integrated proviral DNA is capable of producing further virions which exit the cell and infect other cells. A number of investigators have devised ways to cripple these viruses such that it would be possible to insert a gene of choice into the viral genome, so the virus would be capable of infecting and integrating this gene into the cell, but would not have the ability to reconstitute itself and, therefore, could not reinfect (2). This was done by replacing essential elements of the viral genome with the gene of choice, the essential components then being produced by a helper-cell containing the missing sequences.

In view of the promising results noted using these retroviral vectors, particularly in murine experiments, our group has studied their use in

transferring genes to normal human hematopoietic progenitors in culture. We used two vector retroviruses, N2, and pZIP NEO, both containing a selectable, marker gene, neomycin resistance, which when transferred to a cell, confers resistance to the neomycin analog, G418. In long-term bone marrow cultures, uninfected cells which were cultured without G418, initially decreased in number, but then remained constant beyond 120 days (3). Culture of uninfected cells in the presence of G418, in contrast, resulted in cell death within 15 days. Cells infected with either N2 or pZIP NEO and cultured in the presence of G418, however, grew with similar characteristics to the previously described uninfected cells grown in the absence of G418 indicating:

a. that the cell growth was not affected in long-term cultures by infection with these viruses, and
b. infection does indeed confer upon these cells resistance to the neomycin analog.

In addition, non-adherent cells were removed from the cultures in serial fashion, and by semi-solid cloning assays, were shown to be capable of differentiating along at least two of the hematopoietic cell lines, myeloid and erythroid, for the duration of the culture.

To confirm the presence of the transferred neomycin resistance gene in the G418-resistant cells, we performed a series of DNA analyses by the Southern blot technique. When the human myelomonocytic cell-line, U937, was infected with N2, and selected with G418, the transferred neomycin resistance gene was found to be present at about the same level as endogenous controls. However, when human bone marrow cells which were surviving appropriately in the presence of toxic doses of G418 were assayed, the transferred neomycin resistance gene was not detected in conditions where at least one copy in ten was able to be recognized. This was all the more puzzling, since RNA from the neomycin resistance gene was found in these cells. Further studies have elucidated an explanation for these findings. A few cells, that is less than 10% of the entire marrow population, did indeed contain the transferred gene, and were too few in number to be detected by Southern blot. However, the cells containing the transferred gene were able to confer resistance to the uninfected cells surviving in the presence of G418 due to leakage of the gene product, neomycin phosphotransferase into the culture medium. Although this was a satisfactory explanation, it was disappointing in that it demonstrated that While these murine retroviruses were able to transfer genes with high efficiency to homogenous human hemopoietic cell-lines, gene transfer efficiency to normal human bone marrow was much lower. Therefore, to adequately use this technology in the context of autologous transplantation of genetically altered bone marrow, gene transfer to normal human bone marrow, with particular emphasis on the primitive, self-replicating, multipotential stem cell, had to be improved.

Consequently, we initiated a series of experiments with the following rationale to improve retroviral gene transfer efficiency. First, the stem, or

other progenitor cells, should be enriched, providing more numerous targets. Then the enriched cells should be cycled in a synchronized way by exposure to hemopoietic growth factors, since cell-cycling is necessary for integration of the retroviral DNA (4). Induction of cell-cycling was felt to be particularly important with the primitive stem cells, since they are believed to be largely quiescent, which may contribute to low gene transfer efficiency. Cycling, which reflects integration, since one depends on the other, would be measured by DNA and RNA analyses, in addition to G418 resistance of cultured cells.

METHODS

Protocol

Mononuclear cells from peripheral blood or bone marrow were first cultured in liquid medium for varying periods prior to infection with the retroviruses. Three different media were examined: medium with 10% (v/v) fetal calf serum (FCS) only, medium with FCS plus erythropoietin (EPO) and burst promoting activity (BPA), and medium with FCS plus 10% (v/v) CA5637-CM. After retroviral infection, cells were then plated in semisolid medium containing EPO and BPA. Duplicate plating was performed with G418 present. Erythroid bursts were counted after a 2 week incubation, and cells harvested for DNA and RNA analysis. Transfer efficiencies were determined by the percent G418-resistant bursts.

Peripheral Blood Mononuclear Cells and Bone Marrow Cells

Peripheral blood was obtained by venesection and bone marrow by aspiration of normal, healthy adult volunteers after signing informed consent approved by the Committee for Protection of Human Subjects of The Children's Hospital of Philadelphia. Heparinized blood or bone marrow was diluted with HBSS [Gibco], layered over Ficoll-Hypaque [Pharmacia], and centrifuged at 600g for 40 minutes at 20C. The interfaced cells were washed twice with HCSS, and resuspended. Cells ascertained to be viable by Trypan blue staining were counted.

5FU Exposure

Cells were exposed to 5FU (5-fluorouracil) (Adria) at 50ug/ml for peripheral blood and 200ug/ml for bone marrow for 2 hours at 37C, after which the cells were washed to remove the 5FU.

Hematopoietic Hormones

Erythropoietin and burst-promoting activity (EPO/BPA) for semi-solid BFU-E cloning assays consisted of a combination of recombinant TCerythropoietin [Amgen] 1U/ml, and either 5% (v/v) Mo-T cell-line conditioned-medium for the experiments using pN2, or IL-3 [Amgen] 1U/ml for the experiments using beta(Y)-SVX. CA5637-CM 10% (v/v) was generated in 7 days from cultures in which cells had just reached confluency. EPO/BPA or CA5637-CM used for preincubation purposes were added to interface cells at

a concentration of 1-3x10^6/ml inIscove's [Gibcol, and 10% (v/v) FCS (Hyclone lot nos. 1111883 in N2 studies, 1111831 in D(l)-svx studies). The cells were incubated from 2 hours to 5 days, concentrated by centrifugation, and then placed in the semi-solid cloning assay described below.

Retroviral Constructs

All experiments using these constructs were performed according to the NIH Guidelines for Research Involving Recombinant DNA Molecules (May 7, 1986). The recombinant retrovirus pN2 was obtained from a high-titer,6 virus-producing helper PA12 cell-line, V6 (greater than 10^6 colony forming units CFU/ml as titered on NIH 3T3 cells) (3). The Beta (Y)-SVX retroviral construct was prepared by insertion of the 3.55 kb Sna BI-Xba I fragment containing the B-globin gene into the vector, pzipNeopSV(x) (3) in reverse transcriptional orientation. A Sac II-Not I-Sfi I-Spe I polylinker with Bam HI ends (Boehringer-Mannheim) was cloned into the unique Bam HI site of pzipNeoSV(x), and the Not I site used for cloning the globin insert after blunt-ending the insert using the Klenow fragment of DNA polymerase. The Beta-globin gene was modified [Beta(Y)] by replacing the Acc I-Bam HI fragment containing most of the second exon with the equivalent fragment from a human Y-globin gene. The construct was transfected into the psi-AM amphotropic packaging line (3), and a clonal producer line isolated and titered as 5 x 10^4 CFU/ml on 3T3 cells.

Retroviral Infection of Cells

Infection protocols were as previously described for MEL cells and peripheral blood mononuclear cells (5). Briefly, for peripheral blood or bone marrow mononuclear cells, N2 virus stock at a concentration adjusted to 2x10^6 CFU/ml and 0.8ug/ml of polybrene was added to cells at a concentration of 1-3x10^6/m1, and incubated at 37C for 2 hours. B(Y)-SVX virus stock at a concentration of 5x10^4 CFU/ml with 0.8ug/ml of polybrene were added to cells at a concentration of 0.5-1x10^5/ml, and incubated as above.

Semi-Solid BFU-E Cloning Assays

These were performed as previously described (5). Briefly, after preincubation, cells were resuspended to 1-3x10^5/m1 in medium containing Iscove's, 0.9% (h/V) methylcellulose [Dow], 30% (w/v) FCS, 9mg/ml BSA-fraction V [Sigma], 1.4x10^{-4}ME [Sigma], and 1U/ml of Epo/BPA. The cells were plated at 1ml/dish in 35mm tissue culture plates [Nunc/Corning], and incubated in 5% CO_2 at 37C in humidified air for 14 days. Colonies (greater than 100 hemoglobinized cells) were then counted. G418 concentration curves were performed on these cells indicating no growth in uninfected controls at a dose of 400ug/ml. For the experiments using N2, G418 was added at 400ug/ml, and for the experiments using D(l)-SVX, G418 was added at 500ug/ml except where noted. Semi-solid CFU-E cloning assays. These were performed according to Leary and Ogawa (6), as modified by Brandt et al (7).

Semi-Solid CFU-GM Cloning Assays

These were performed as previously described (3).

DNA Analysis

DNA was extracted from the BFU-E cultures (5), and PCR (polymerase chain reaction) was performed. The endogenous B-globin gene oligonucleotide primers (1. and 3.) produced a 400 bp PCR fragment, while primers used for detection of B(Y) sequences (1. and 2.) gave a 500 bp PCR fragment. Primers (4. and 5.) were also used for monitoring integration of the NEO^r gene yielding a characteristic 300 bp PCR fragment.

1. Beta-globin antisense (-55 to -36 relative to CAP site):
   5' AGCCCTGGCTCCTGCCCTCC 3'
2. 3' splice acceptor region from MoLv:
   5' AGGTCCTTTCCAGCGAGGTT 3'
3. Beta-globin sense (-455 to -437 relative to CAP site):
   5' CATCCATTCTGTCCTGAAG 3'
4. NEO^r sense (5 to 24 relative to 5' end of NEO^r in N2):
   5' CAAGAGACAGGATGAGG 3'
5. NEO^r antisense (286 to 305 relative to 5' end of NEO^r in N2):
   5' GTCCCTTCCCGCTTCAGTGA 3'

Depending on the sample, 10 to 100 ngs of template DNA was used, while for primers, 100 pgs was used. Samples were run on a Perkin-Elmer thermocycler for 30 cycles of 1 to 2 minutes at 94C, 1 to 2 minutes at 56C, and 1.5 to 2 minutes at 72C, or on a Coy tempcycler for 30 cycles of 1 minute at 95C, 1 minute at 55C, and 2 minutes at 72C. After a single chloroform extraction, a sample containing 15ul of a 100ul reaction was electrophoresed on a neutral 2% (w/v) agarose gel. NEO^r analysis was performed by DNA blotting (5) and probing with a radiolabelled 2.3 kb Bam HI/Hind III fragment of pBR Neo. B(Y) analysis was performed by blotting DNA onto BioRad zeta probe membrane according to the manufacturer's instructions and hybridizing with a radiolabelled 0.8 kb 5' Sna BI-Bam HI B-globin gene probe.

RNA Analysis

Total cytoplasmic RNA was extracted (5), reverse transcribed and the globin-specific cDNA was amplified by PCR using primers specific for the 5'-untranslated region and third exon of the B-globin gene (see Map of B(l) - SVX and A-globin gene in Figure 4). Approximately 0.1 to 0.2 ugs of the amplified products were electrophoresed, and blotted as described above. An end-labelled oligonucleotide specific for the second exon of the Y-globin gene was used for hybridization to detect the retrovirally transferred B(Y) transcript. A 500 bp plasmid fragment (10 and 100pgs) containing the Y-globin second exon was used as a positive control. Oligonucleotide primers were as follows:

Beta 5'UT sense (14 to 33 relative to CAP site):
5' ACACAACTGTGTTCACTAGC 3'
Beta 3rd exon antisense (1365 to 1384 relative to CAP site):
5' AGTGATGGGCCAGCACACAG 3'
g 2nd exon antisense (388 to 407 relative to CAP site):
5' GCTTTATGGCATCTCCCAAG 3'

RESULTS

Initially, to test our hypothesis that enriched and cycling stem cells would result in higher gene transfer following retroviral vector infection, committed progenitor cells, specifically the BFU-E, were used (5). In addition, improved gene transfer to BFU-E would be useful for the study of transferred globin genes, necessary for therapy of hemoglobinopathies, in a primary cell which was both normal and differentiating, rather than the cell-lines that are more frequently employed. For the BFU-E assays, peripheral blood which contains a high proportion of BFU-E's, was used. In some of our experiments the mononuclear cells from peripheral blood were first exposed to 5FU (5-fluorouracil). The rationale for use of 5FU was the same as that for its intravenous administration to mice a few days prior to bone marrow harvesting, which has been found to substantially increase the number of primitive stem cells in the harvest (8).

The effects of preincubating the cells with growth factors prior to infecting with the N2 retrovirus at different times were examined. Growth factor Combinations were either erythropoietin and burst-promoting activity, both of which are necessary for the growth of BFU-E's, or conditioned-medium from the human bladder carcinoma cell-line, CA5637, which includes a mixture of growth factors (IL-1, IL-3, IL-6, GM-CSF, G-CSF, M-CSF) (9). The best results as measured by G418 resistance, were following preincubation with CA5637 conditioned-medium for 18 to 24 hours (Figure 1). In these experiments, the presence of the neomycin resistance gene has been confirmed by DNA analysis (Figure 2).

On repeating these experiments, but first exposing the cells to 5FU, the combination of 5FU exposure followed by preincubation with CA5637 conditioned-medium for 18 to 24 hours, resulted in an even greater efficiency of transfer as measured by G418 survival (Figure 3). This needs further study.

A different retroviral construct, pZIP NEO, containing a B-globin gene was also used in similar experiments. The second exon of the B-globin gene had been replaced by that of the Y-globin gene which would allow the transferred globin to be distinguished from endogenous globin. These experiments confirmed that preincubation with CA5637 conditioned-medium resulted in a higher gene transfer efficiency in the same way when using a different virus which also carried more than one gene of choice. The presence of the second transferred gene, B(@)-globin, was shown by DNA analysis (Figure 4), with evidence of transcription of this gene (Figure 5). We believe this model will be useful for further studies of globin genes where enhancer

sequences shown to be important for improved expression could be studied in a more normal environment than human cell-lines. The model may also be useful for the study of other lineage-specific genes using different committed progenitors.

Similarly, we have initiated a number of experiments applying the same rationale to the primitive, multipotential, self-replicating stem cell from normal human bone marrow. Figure 6 shows preliminary results of the use of 5FU exposure followed by incubation with CA5637 conditioned-medium prior to infection, and the measurement of the presence of G418-resistant CFU-GM's and CFU-blasts (CFU-GM measuring the more committed myeloid progenitors, and CFU-blasts measuring the more primitive progenitors). Preincubation for two hours with the CA5637 conditioned-medium resulted in a higher efficiency gene transfer to the CFU-blasts as measured by G418 resistance, but this was not true at 24 hours. After 24 hours of preincubation, in contrast, the number of resistant CFU-GM's was increased. This indicates that the timing of exposure to growth factors is probably critical for cells at different stages of development.

CONCLUSION

Other techniques to enrich and cycle cells, particularly to synchronize the cell-cycling, need to be developed before use of marrow cultures to improve gene transfer efficiency is optimized. Recent reports on both micro-culture of highly enriched stem cells (10, 11), and growth factors thought to be active on very early hematopoietic cells (12) are encouraging and may lead to improved gene transfer efficiencies. Therefore, cultures of marrow cells are not only useful for studying the effects of gene transfer to hematopoietic cells, but may also provide a model of cell culture manipulation which would improve gene transfer efficiency to a level useful for clinical gene therapy.

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FIGURE 1
Percent G418-resistant BFU-E's from normal human peripheral blood divided into three culture plates following preincubation with either erythropoietin (EPO) and burst-promoting activity (BPA), or CA5637 conditioned-medium for 2-4, 18-24, and 48 hours before infection with N2.

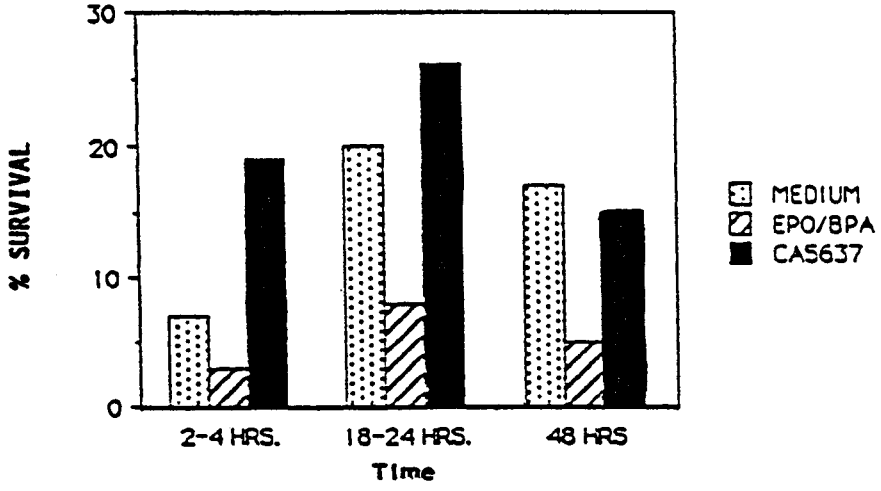


FIGURE 2
Detection of the viral NEO^r gene in PCR products amplified from N2-infected erythroid burst DNA with NEO^r specific primers. 1. Size marker (BRL 123 ladder); 2. N2-infected BFU-E after 24-h liquid culture in FCS-medium alone; 3. N2-infected BFU-E after 24-h liquid culture in FCS-medium plus CA5637-CM; 4. uninfected U937 cell-line (negative control); 5. N2-infected U937 cell-line (positive control). 6. Uninfected mononuclear cells from normal peripheral blood.

FIGURE 2

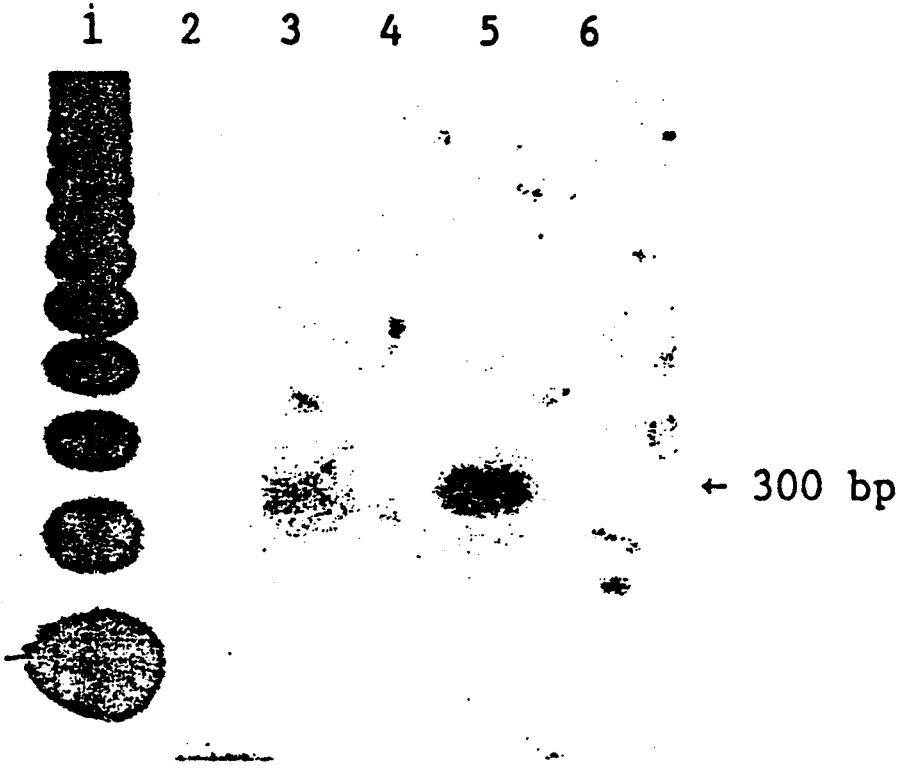


FIGURE 3
Percent G418-resistant BFU-E's from normal human peripheral blood divided into three culture plates following exposure to 5FU and preincubation with either erythropoietin (EPO) and burst- promoting activity (BPA), or CA5637 conditioned-medium for 2-4, 18-24, and 48 hours before infection with N2. 5FU/EPO/BPA for 2- 4 hours, and medium and 5FU at 48 hours resulted in no G418- resistant BFU-E's.

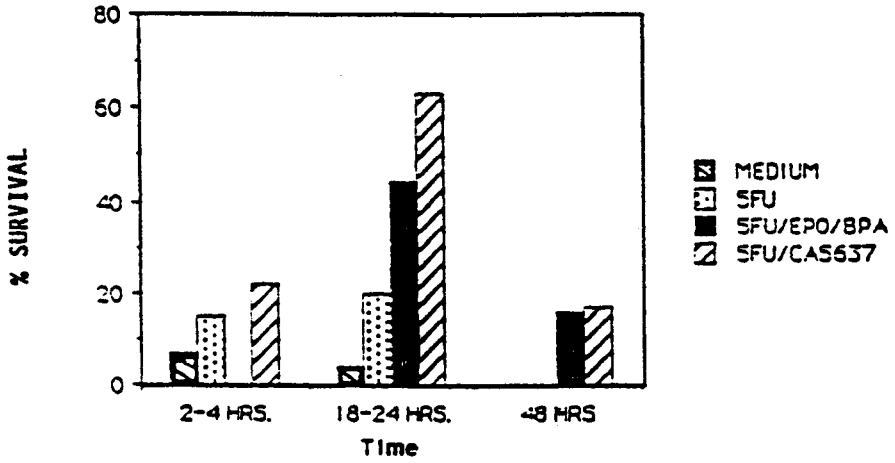
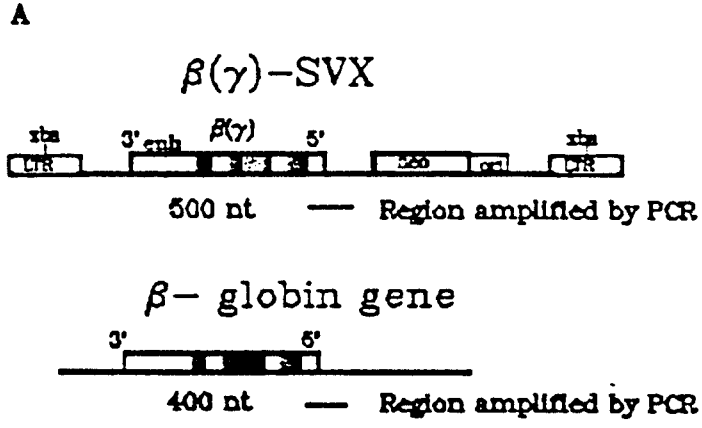


FIGURE 4
Detection of the viral B(Y)-globin gene in PCR products amplified from B(Y)-SVX-infected erythroid burst DNA. (A) Maps of the B(Y)-SVX retroviral construct and the A-globin gene. Indicated are regions amplified in the B(Y)-SVX and endogenous B-globin genes. (B) PCR products using B-globin primers: 1. uninfected BFU-E. 2. B(Y)-SVX-infected BFU-E. PCR products using B(Y) -SVX primers: 3. B(Y)-SVX-infected MEL cell-line (positive control). 4. Uninfected BFU-E. 5. B(Y)-SVX-infected BFU-E.

FIGURE 4


A
β(γ)–SVX
xba
3'
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xba
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LTR
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Region amplified by PCR
β– globin gene
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5'
400 nt
Region amplified by PCR

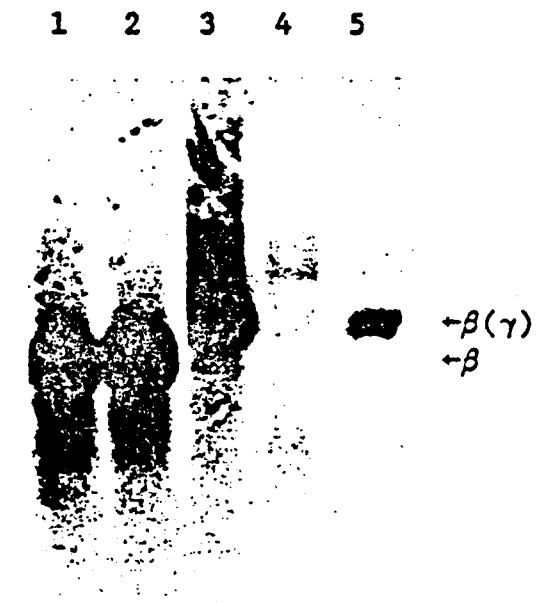
B
1
2
3
4
5
←β(γ)
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FIGURE 5
Detection of the viral B(Y)-globin transcripts in PCR products amplified from B(Y)-SVX-infected erythroid burst RNA. (A) Maps of B(Y)-globin and endogenous B-globin mRNA's. Indicated are regions amplified in cDNA's prepared by reverse transcription of the B(Y)-globin and endogenous B-globin mRNA's. (B) Agarose gel (1.5% wt/vol) of PCR products using B-globin primers: 1. Uninfected BFU-E. 2. B(Y)-SVX-infected BFU-E after 3-day liquid culture in FCS-medium plus CA5637-CM. 3. Reticulocyte RNA (negative control). 4. Ten picograms of B(Y)-globin 500-bp plasmid fragment (positive control). 5. 100pg of B(Y)-globin 500bp plasmid fragment (positive control). (c) Autoradiogram of a Southern blot of the gel shown in panel B. hybridized with a probe specific to the Y-portion of B(Y). Lanes 1 through 5 are the same as in panel B.

FIGURE 5

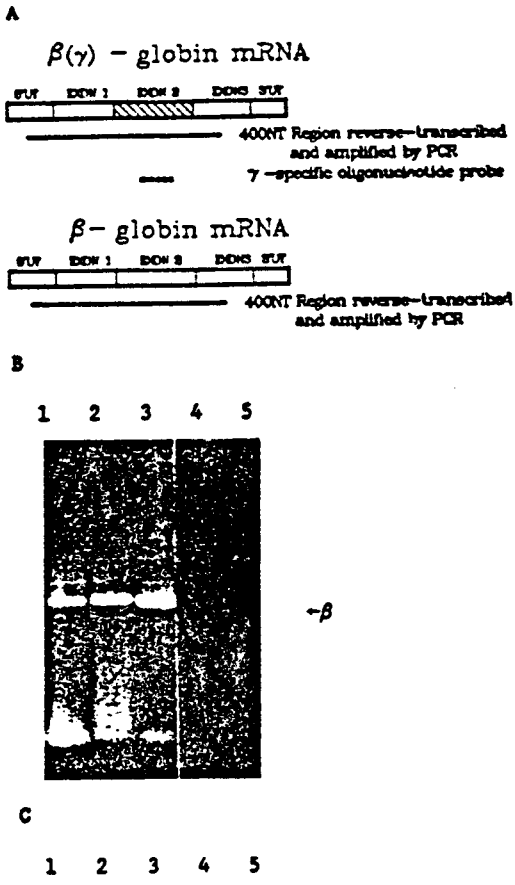
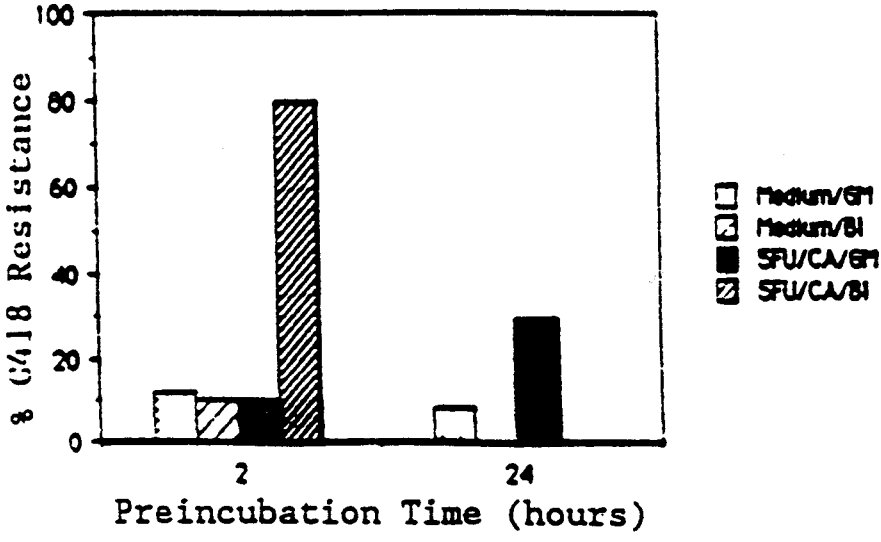

A
β(γ) – globin mRNA
5'UT
EXON 1
EXON 2
EXON3
3'UT
400NT Region reverse-transcribed and amplified by PCR
γ -specific oligonucleotide probe
β– globin mRNA
5'UT
EXON 1
EXON 2
EXON3
3'UT
400NT Region reverse-transcribed and amplified by PCR
B
1 2 3 4 5
←β
C
1 2 3 4 5
←β(γ)-plasmid
←β(γ)

FIGURE 6
Percent G418-resistant CFU-Bl's (Bl) and CFU-GM's (GM) from normal human bone marrow divided into three culture plates following exposure to 5FU and preincubation with CA5637 conditioned-medium for 2 and 24 hours before infection with N2. Medium/Bl and 5FU/CA/Bl at 24 hours resulted in no G418-resistant colonies.



IMMUNOCYTOCHEMICAL DETECTION OF P-GLYCOPROTEIN ON NORMAL MARROW MYELOID CELLS

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INTRODUCTION

A characteristic feature of multidrug resistant (MDR) cell lines developed *in vitro* is decreased accumulation of intracellular drug associated with overexpression of a membrane glycoprotein termed P170 or P-glycoprotein (Pgp) (1). The Pgp is the product of MDR1 gene (2) located on human chromosome 7 (3). A positive correlation is found to exist between the amount of this protein on the cell surface and the degree of drug resistance (4-10). A variety of human tissues including the liver, colon, small intestine, kidney, adrenal cortex, adrenal medulla and the lung have been demonstrated to express the MDR1-mRNA at intermediate to high levels. Cancers originating from these tissues are intrinsically resistant to natural product drugs such as the anthracyclines, vinca alkaloids and epipodophyllotoxins. On the other hand, the bone marrow (BM) and some other tissues are reported to express negligible amounts of MDR1-mRNA and the cancers derived from these tissues may initially respond to the natural product drugs but may eventually develop resistance (11).

The availability of monoclonal anti-Pgp antibody MRK-16 (MRK-16 MoAb) with specificity to the external epitope of Pgp on the cell membrane (12) enabled us to test the possibility of selective *ex vivo*, pretransplant elimination of multidrug-resistant residual tumor cells from remission marrows of cancer patients by treatment with this antibody. Using a simulated remission BM created by mixing drug-resistant tumor cells derived from a doxorubicin-resistant Pgp positive myeloma cell line (10,13) with irradiated BM mononuclear cells, we have already shown that several logs of tumor cells can be eliminated by three cycles of treatment with MRK-16 MoAb and rabbit complement. Similar treatment did not adversely affect BM granulocyte-macrophage colony forming cells (GM-CFCs) (14). Ex vivo purging of drug-resistant tumor cells from the bone marrow could be a valuable supplementation to current methods of purging, such as chemopurging and

immunopurging in a number of tumor types, such as acute leukemia, myeloma, and breast carcinoma.

As a sequelae, we explored the possibility of detecting multidrug-resistant tumor cells in postchemotherapy remission marrows from cancer patients using immunocytochemical techniques. The novel findings which have resulted from this work are reported here.

MATERIALS AND METHODS

Cell Line

The Pgp positive, doxorubicin-resistant 8226/DOX40 cell line was obtained from Dr. W. Dalton, The University of Arizona, Tucson, AZ, and maintained as described earlier (14).

Antibodies and Immunoglobulin Reagents

Anti-Pgp antibody MRK-16, a murine monoclonal antibody of IgG2a subclass was prepared in the laboratory of Drs. H. Hamada and T. Tsuruo as described earlier (12). MRK-16 MoAb was stored at -20C in lyophilized form. When required for use, the lyophilized antibody was reconstituted with distilled water and stored in 5 uL aliquots (25mg/mL) at -70C. Further dilutions were made in phosphate-buffered salt (PBS) solution, pH 7.5 (GIBCO, Grand Island, NY), supplemented with 1% bovine serum albumin (BSA) (Sigma Chemical Company, St. Louis, MO). Murine monoclonal antibodies MY7 (anti-CD13, subclass IgG1), MY9 (anti-CD33, subclass IgG2b), and PCA-1 (plasma cell-specific, subclass IgG2a) were purchased from Coulter Immunology, Hialeah, FL). Normal mouse IgG1 (MsIgG1), IgG2a (MsIgG2a) and IgG2b (MsIgG2b) derived from NS-1 hybridoma clone #2T8-2F5, #7T4-IF5 and mouse myeloma MPC- 11 (Coulter Immunology, Hialeah, FL) were used as negative controls.

Bone Marrows

Seven normal human BM specimens were obtained as aliquots of marrows aspirated from normal individuals for transplantation into allogeneic recipients. Twenty-one multiple myeloma, 11 breast carcinoma and 7 chronic myelogenous leukemia patient marrow specimens were aliquots of marrows aspirated for storage for future autologous transplantations.

Prior to BM aspiration, all the 21 myeloma patients had received treatment with corticosteroids. In addition, 15 of these patients had also received anthracyclines, vinca alkaloids, alkylating agents and interferon, and 3 patients had received alkylating agents. All 11 breast carcinoma patients had received prior chemotherapy with anthracyclines, alkylating agents and 5FU. In addition 6 of these patients had received vinca alkaloids. And one of these 6 patients had also received corticosteroids. Among CML patients, 2 patients received hydroxyurea and alpha 2B interferon. In addition, 1 patient also received alkylating agents and 2 others received alkylating agents,

epipodophyllotoxins and corticosteroids. One patient received only alkylating agents.

Preparation of BM Specimens for Immunoenzyme Staining Analysis

The light density mononuclear cells (MNCS) were separated by centrifugation over Ficoll-hypaque (density 1.077 g/ml; Ficoll from Pharmacia Fine Chemicals, Piscataway, NJ; Hypaque from Winthrop Laboratories, New York). The slides were prepared for staining by spinning 100 ml aliquots of a 1 X 10^6 ml suspension of MNCs in RPMI 1640 medium supplemented with 10% fetal bovine serum in a cytospin 2 centrifuge (Shandon Inc., Pittsburgh, PA). The slides were fixed with cold acetone (4C) for 5 min, air dried and stored at 4-10C until used.

Single and Double Marker Analysis using Immunoenzyme Assays

Single marker analysis was carried out using immunoalkaline phosphatase method on acetone-fixed cytospin preparations of cells using a Vectastain Avidin-Biotin-Conjugated alkaline phosphatase (ABC-AP) kit (Vector Laboratories, Burlingame, CA). The slides were first washed with 0.5% BSA in PBS for 5 min and then incubated with diluted normal horse serum (blocking reagent) for 20 min. This was followed by successive incubations with (1) 50 ul of MRK-16 MoAb (primary Ab) in 1% BSA in PBS, pH 7.5, at a concentration of 20 ug/ml for 30 min, (2) diluted biotinylated horse antimouse IgG for 20 min, (3) diluted ABC-AP for 15 min, (4) substrate solution (phosphatase substrate kit 1, Vector Red) for 15 min. The slides were washed with 0.5% BSA in PBS between successive incubations except after blocking reagent wherein the excess serum was blotted. The slides were counterstained with hematoxylin for 1 min, washed in tap water for 5 min, and dehydrated in 70, 90 and 100% alcohol each for 1 min. The slides were then cleared in xylene and mounted in permount. In negative control slides, the primary antibody MRK-16 was excluded and replaced by MsIgG2a. In each experiment 8226/DOX_{40} cell line was used as a positive standard. The number of positive cells in stained BM slides was counted microscopically and the staining intensity of positive cells was graded in decreasing order from high 4^+(++++) equivalent to 8226/DOX_{40} to low 1^+ which was just a visible reaction.

The double marker analysis was done using the single enzyme, alkaline phosphatase used sequentially for both markers (AP/AP method) or by sequential use of two enzymes, horse radish peroxidase and alkaline phosphatase (HPO/AP method). AP/AP method was carried out as described by Schmetzer and Gerhartz (15) with some modifications. The fixed slides were washed with PBS, blocked with normal goat serum and then successively incubated with the first primary antibodies (My7 + My9 or PCA- 1), and goat anti-mouse IgG conjugated chromogenic AP Substrate (Vector Kit III - blue). The slides were washed with PBS between successive incubations and after development of staining. Then they were immersed in 2N HCI at 37C for 15 min to inactivate AP. The staining procedure was then repeated on the same slides using successively MRK-16 (as second primary antibody), AP-conjugate

and Vector AP Substrate Kit I - Red). The slides were washed with PBS, immersed in 100% alcohol, air-dried and mounted in glycergel mounting medium (DAKO Corporation, Santa Barbara, CA).

HPO/AP method was carried out as described by Mullink et al (16) with minor modifications. HPO-immunoassay was done using Vector ABC-Peroxidase staining kit. Chromogenic peroxidase substrate AEC (reddish brown) was purchased from Cambridge Research Laboratory, Cambridge, MA. The staining procedure is similar to that described earlier for ABC-AP except that the slides were incubated with 3% H_2O_2 for 5 min before staining. AP immunoassay was performed after development of color with HPO assay, as described earlier in AP/AP method. The sequence of primary antibodies was same as before.

RESULTS

Immunoenzyme Staining Analysis of Normal and Patient Bone Marrow with MRK-16 MoAb

A significantly large proportion of BM specimens from normal individuals (4/7), and patients with either multiple myeloma (16/22), breast carcinoma (6/11) or leukemia (5/7), stained positive with MRK- 16 MoAb (Table 1). The control slides stained with MsIgG2a were negative. The number of positive cells ranged between 2% and 90% and the degree of staining intensity between $1^+(+)$ and $4^+(++++)$. A greater proportion of BM specimens from all sources contained positive cells in the range of 1-25% than in 26-50% and above. No specimen among normal donors contained >50% positive cells.

In most BM specimens, the positive cells were myeloid in appearance. Pgp positive cells from a I + normal marrow and a 4+ myeloma marrow (++++) are shown in Figure 1. In two myeloma specimens, one with high (54%) and the other with low (4%) plasma cell content, positive plasma cells were also observed.

Phenotypic Identification of Pgp Positive Cells by Sequential Double Staining Analysis using Single and Double Immunoenzyme Assays

The morphological identification of positive cells as predominantly myeloid cells was further substantiated by sequential double staining of BM specimens with MRK - 16 MoAb, and a cocktail of anti-myeloid monoclonal antibodies My7 (anti-CD13) and My9 (anti-CD33). Two marrows (code numbers M39 and M40) derived from two multiple myeloma patients, one with high percentage of plasma cell number (15%) and the other with low number (1%), respectively were analyzed.

Both the double enzyme method (ABC-HPO/AP) and the single enzyme method (AP/AP) were attempted in double staining analysis. These methods have the propensity for cross-reactions between various reagents used in the assay, and therefore, the specificity of reactions was ensured by using appropriate controls.

The Pgp positive MDR cell line 8226/DOX_{40} used as a positive control showed dual staining with PCA-1 and MRK-16, single staining with PCA-1 and PBS, or PBS and MRK-16, and no staining with MsIgG and MsIgG (Figures 2 & 3). The subclass of mouse IgG corresponded with subclass of the primary Ab used.

Both myeloma marrows showed dual staining with My7 + My9 MoAbs and MRK-16 MoAb (Figures 2E & 3E). There were no detectable plasma cells present in M40 marrow (Fig 2I) and the slides stained with PCA-1 and MRK-16 showed single blue staining with MRK-16 of cells with myeloid morphology (Figure 2G, brown staining of myeloid cells due to incomplete inhibition of endogenous peroxidase activity with H202). Despite the presence of plasma cells in M39 marrow (Figure 3H), only single staining of plasma cells with PCA-1 was seen in slides stained with PCA-1 and MRK-16 (Figure 3F).

The various control slides stained sequentially with My7 + My9 or PCA-1 and PBS or PBS and MRK-16 showed single staining whereas the control slide stained with MsIgG and MsIgG showed no staining and excluded the occurrence of non-specific reactions between reagents.

DISCUSSION

Contrary to the reports from other laboratories (11), we have demonstrated that Pgp is expressed at detectable levels on normal BM cells. Pgp expression was also detected on marrow cells from cancer patients in remission after chemotherapy. These cells were identified morphologically and phenotypically as myeloid cells. Pgp expression in marrow myeloid cells of cancer patients may be related in fact to their exposure to cytotoxic chemotherapeutic drugs. This likelihood is supported by the findings of Carmichael et al (17) who showed that treatment of mice with a low cytotoxic dose of cyclophosphamide resulted in a significant increase in glutathione (GSH) and GSH transferase content in the marrow and peripheral blood. These increases were accompanied by an increased resistance to a high dose of the cytotoxic compounds. GSH transferase levels were restricted to the granulocyte fraction. The authors concluded from these data that the enhanced levels of glutathione in cells resulting from cytotoxic insult could be a general response of cells to cytotoxins and may be important in both antitumor therapy as well as the initiation of chemical toxicity and carcinogenicity (elevation of GSH and GSH transferase levels have been related to the initiation of chemically induced neoplastic growth) (18, 19), since bone marrow cells in addition to being sensitive targets for chemotherapeutic agents are also the sites of malignant transformation. The occurrence of myelodysplastic syndrome (MDS) and acute myelogenous leukemia (AML) following cytotoxic therapy or carcinogen exposure is well known (20). These results concur with our results which showed that in some postchemotherapy remission patients, detectable levels of Pgp expression were found on myeloid cells; in others with lack of detectable Pgp levels, elevated levels of GSH and GSH transferase might be present as alternative mechanism of defence. There was no correlation between presence

or absence of Pgp on cells and the type of drugs used for treatment. Further phenotypic analysis of normal marrows using other antibodies such as anti-lymphocyte antibodies directed against mature T and B cells and their precursors should reveal whether Pgp expression is restricted to myeloid cells or not.

Since the normal donors have had no prior exposure to MDR-related drugs, the Pgp expression in these individuals must be related to exposure to some toxic agents other than these drugs. Considerable variability in Pgp expression (range 0% to 36%) observed among normal marrows (without known prior exposure to MDR-related drugs) suggests that Pgp expression in normal marrow is subject to regulation by some internal and/or external factors Since Pgp is known to function as a drug-efflux pump (21, 22), Pgp expression by normal BM cells indicates prior exposure to toxic agents. Thorgeirsson et al (23) have demonstrated that MDRI gene expression may be part of the many changes associated with malignancy. The authors showed that in chemically-induced neoplasia (as well as following regeneration) of rat liver, levels of messenger RNA for MDRI gene were elevated in both preneoplastic and neoplastic lesions. Based on these findings, we hypothesize that Pgp expression in the normal marrow may be reflective of previous exposure to carcinogenic agents and may or may not be part of multiple changes associated with malignancy. To assess the potential role of environmental carcinogens in Pgp expression, we are now examining marrows from myelodysplastic patients; MDS is considered to represent a preleukemic condition resulting from known or unknown exposure to carcinogens. Experiments are also in progress to induce Pgp expression *in vitro* in Pgp negative cells to elucidate the mechanism of Pgp expression in myeloid cells.

Our findings suggest several possibilities for clinical application of these data. For example, if our hypothesis about Pgp expression in BM cells is proven right, it could form the basis for early diagnosis of preleukemia. Furthermore, if Pgp is found to be expressed clonally, it might be possible to intervene the process of leukemogenesis in preleukemia cases, and treat overt leukemia by selective removal of Pgp positive clones from the bone marrow by *in vivo or in vitro* treatment with MRK- 16 MoAb conjugated to toxins. And given the frequent detection of MDRI MRNA on myeloid leukemic relapse, leukemic cell removal would be possible by taking advantage of these surface expressions. The present status of AML marrow purging with MoAb is less than satisfactory because of the lack of specificity of these MoAbs for leukemia antigens with the problems of sparing of tumor cells and compromising the survival of pluripotent stem cells and engraftment.

Our earlier studies showed that GM-CFUC survival was not affected adversely by three-cycle treatment with MRK-16 MoAb and rabbit complement (14). Of the three myeloma marrows that were so treated, two contained 21% and 14% Pgp positive cells and the third showed less than 1% cells. Of the three breast carcinoma marrows treated, two were negative and the third showed less than 1% cells. Although no definite conclusions can be drawn from these small numbers of positive samples, the data suggest that either Pgp

is not present on progenitors of GM-CFCs or that the density of antigen was too low for removal with the antibody and complement.

Immunoenzyme staining method was used in this study because in addition to its sensitivity for detection of an antigen, it also offers the advantage of morphological identification of stained cells.

In conclusion, our results suggest that further information on cell lineage and cell differentiation stage of the Pgp positive cell in the marrow must be procured before *ex vivo* manipulation of drug resistant tumor cells in the patient BM is attempted.

ACKNOWLEDGMENTS

The authors gratefully acknowledge the technical help of Karen Dubowy, Eva Menefee and Mira Shah for their assistance in the preparation of the manuscript, and Dr. William S. Dalton of the University of Arizona, Tucson, AZ, for kindly providing us with the drug-resistant cell lines established in his laboratory. This work was supported by Grant No CA23077 from the National Cancer Institute.

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TABLE 1

IMMUNOCYTOCHEMICAL DETECTION OF P-GLYCOPROTEIN EXPRESSION IN THE BONE MARROW (BM) OF CANCER PATIENTS AND NORMAL INDIVIDUALS

Source of BM	No. of MRK-16$^+$ Samples/Total No. (%)	No. of MRK-16$^+$ Samples % Positive Cells 1-25	26-50	>50
Normal Donors	4/7 (57)	3	1	-
Multiple Myeloma	16/22 (73)	10	4	2
Breast Cancer	6/11 (54)	4	1	1
Leukemia (CML)	5/7 (71)	3	1	1

FIGURE 1

Detection of Pgp positive cells in a normal marrow and a myeloma patient marrow by immunoalkaline phosphatase (IAP) assay. 8226/DOX_{40} cell line: Giemsa staining (A), IAP staining with MRK-16 (B), and MsIgG2a (C). IAP staining of normal marrow with MRK-16 (D), MsIgG2a (G), and of myeloma marrow with MRK-16 (E), and MsIgG2a (F).

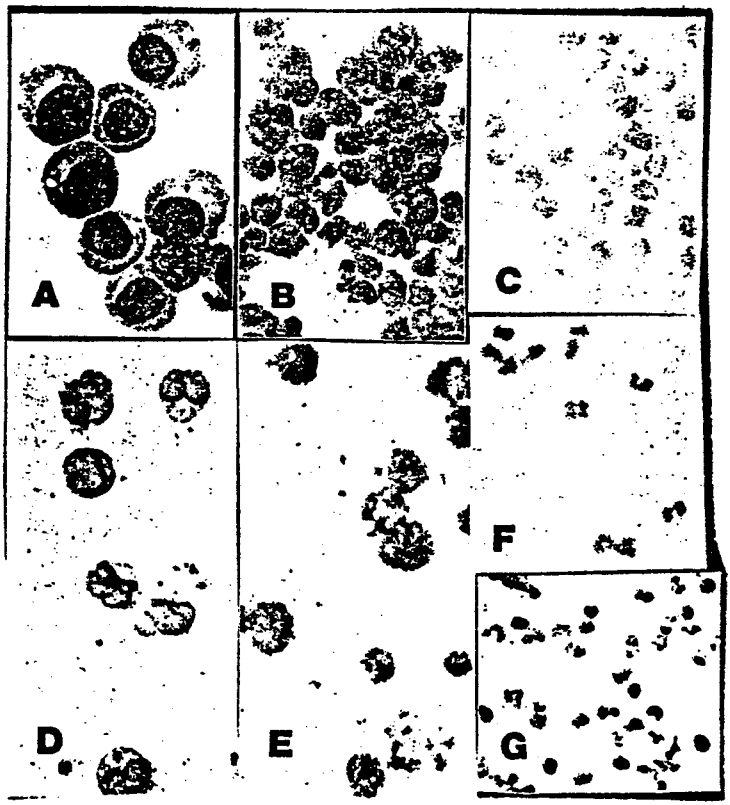


FIGURE 2

Phenotypic identification of Pgp positive cells by double staining analysis using double enzyme ABC-HPO/AP method. 8226/DOX_{40} cell line stained sequentially with PCA-1 & MRK-16 (A), PCA-1 & PBS (B), PBS & MRK-16 (C) and MsIgG & MsIgG (D). Myeloma marrow M40 stained with My7 + My9 & MRK- 16 (E), My7 + My9 & MsIgG (F), PCA-1 & MRK-16 (G), My7 + My9 & PBS (H), PCA - I & PBS (I), PBS & MRK- 16 (J) and MsIgG & MsIgG (K).

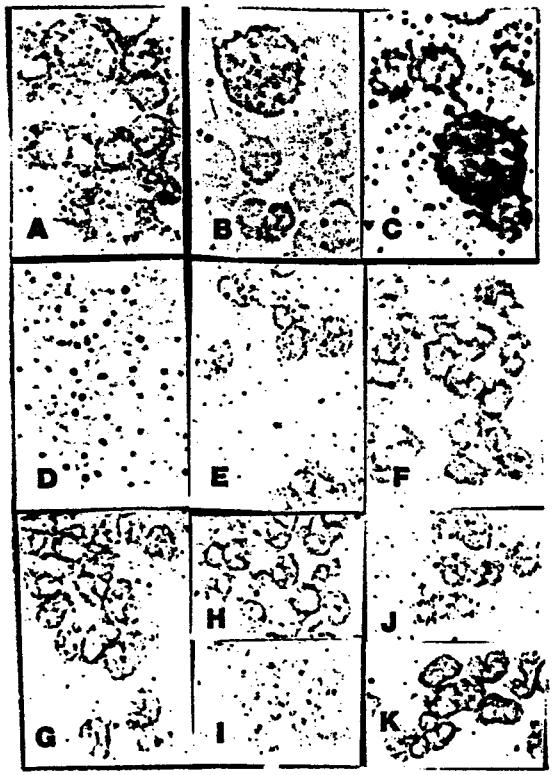
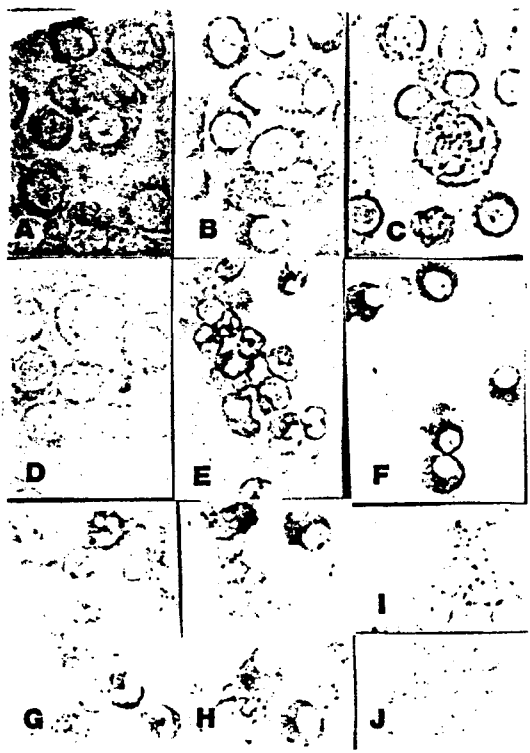


FIGURE 3

Phenotypic identification of Pgp positive cells by double staining analysis using single enzyme AP/AP method. 8226/DOX_{40} cell line stained sequentially with PCA-1 & MRK-16 (A), PCA-I & PBS (B), PBS & MRK- 16 (C), and MsIgG & MsIgG (D). Myeloma marrow M39 stained with My7 + My9 & MRK- 16 (E), PCA-1 & MRK-16 (F), My7 + My9 & PBS (G), PCA-1 & PBS (H), PBS & MRK-16 (I), MsIgG & MsIgG (J).



IMMUNOTHERAPY WITH ANTI-B4-BLOCKED RICIN FOR B-CELL LEUKEMIAS AND LYMPHOMAS: IMPLICATIONS FOR THE POST-ABMT PATIENT

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INTRODUCTION

Despite recent advances in conventional combination chemotherapy for the treatment of patients with B-cell non-Hodgkin's lymphomas (NHL), the majority of patients ultimately die of their disease. The effectiveness of high dose combination chemotherapy with bone marrow reinfusion for patients with responsive relapsed NHL is a widely accepted salvage regimen. Although initial high rates of complete remission are obtained, only 25-50% of these patients enjoy long term disease free survival following autologous bone marrow transplantation (ABMT) (1,2).

Several reasons exist to explain why patients relapse after ABMT. Dose escalation of the chemotherapy to maximal cytotoxic doses is limited by non-specific end organ toxicity. Attempts to further dose escalate have been associated with increased morbidity and mortality. Furthermore, there are clones of lymphoma cells which are resistant to both standard and high-dose chemoradiotherapy. The challenge remains to identify a therapeutic modality that will enhance the durability of a complete remission in patients who initially achieve a clinical complete remission or minimal residual disease state following ABMT. The cytotoxic mechanism of this modality must be able to circumvent tumor cell resistance.

Immunotherapy with monoclonal antibodies directed against differentiation antigens on the cell surface of malignant lymphocytes potentially affords such a modality. Potent toxins may be conjugated to the monoclonal antibodies, with the monoclonal antibody used as a vehicle to deliver the cytotoxic agent directly to tumor cells. Such antibody-toxin conjugates are termed immunotoxins. By selecting a toxin which has a cytotoxic mechanism different from that of standard chemotherapeutic agents, one may potentially kill cells which are resistant to standard therapy.

This paper describes the results of two Phase I clinical trials using Anti-B4-blocked ricin (Anti-B4-bR), a novel immunotoxin directed against the

B4 antigen on malignant B cells. The potential role of this immunotoxin in patients with relapsed leukemias and lymphomas as well as in patients who have undergone ABMT is considered.

PATIENTS AND METHODS

Patients eligible for entry in both trials had B-cell NHL, B-cell CLL, or B-cell ALL which had relapsed from conventional primary and salvage regimens. All patients were required to have tumor cells which demonstrated reactivity with the anti-B4 or anti-B1 monoclonal antibody by either flow cytometric analysis or immunoperoxidase staining. (B1 positive tumors were accepted inasmuch as all tumcrs that are B1 positive are also B4 positive) (3).

Anti-B4-bR (supplied by Immunogen, Inc., Cambridge, MA) is an immunoconjugate linking the anti-B4 monoclonal antibody to a derivatized whole ricin molecule. The anti-B4 monoclonal antibody identifies a B lineage restricted glycoprotein which is expressed throughout normal B cell ontogeny and on all B cell leukemias and lymphomas (4). Ricin is a toxic lectin which consists of two subunits, A and B, linked by a single disulfide bond. The A-chain is an enzyme that inactivates the 60S subunit of eucaryotic ribosomes, and the B-chain binds to galactose terminated oligosaccharides that are ubiquitous on eukaryotic cell surfaces (5). The non-specific toxicity of the immunoconjugate has been limited by blocking the two galactose binding sites on the ricin B chain by linking natural ligands on to the binding sites in a chemically stable way (6). The in vitro cytotoxicity of Anti-B4-bR is approximately 10 times lower than that of whole ricin for B4 positive cell lines. Nanomolar concentrations of Anti-B4-bR are able to kill four to five logs of 84 positive tumor cells in in vitro systems.

In an initial Phase I clinical trial, Anti-B4-bR was administered by daily bolus injection for five days, with patients eligible for retreatment at 4 week intervals subject to the absence of production of human anti-mouse antibody (HAMA) or human anti-ricin antibody (HARA). Patients were treated in groups of three, and, if no significant toxicity was observed, the dose was to be escalated in the following scheme: 1, 5, 20, 40, 60, 80 ug/kg of Anti-B4-bR per day for five consecutive days. Doses were escalated until NCI CTC Grade 3 toxicity was reached. For the purposes of this study, Grade 3 hepatotoxicity in terms of transaminase elevations was defined as a 5-20X increase in SGOT and SGPT. A total of 25 patients were treated. Twenty-three patients had NHL, one had CLL, and 1 had ALL. Ages of these patients ranged from 20 to 64. Eight patients had relapsed after prior ABMT, and one after allogeneic bone marrow transplantation.

In a second Phase I trial, which is currently in progress, Anti-B4-bR is being administered by continuous infusion over seven days. Patients have been treated in groups of three, and, if no significant toxicity was observed, the dose was escalated from 10ug/kg/day to 70ug/kg/day in increments of 10ug/kg/d. The first 15 patients received a 20ug/kg bolus of drug prior to beginning the constant infusion. After these patients were treated, the bolus

was eliminated because the bolus may have contributed to toxicity without improving drug delivery. As in the previous trial, patients were eligible for retreatment at 28 days subject to the absence of development of HAMA/HARA. To date, 27 patients have been treated. Nineteen patients had NHL, 4 had CLL, and 4 had ALL Eleven of the patients had relapsed after ABMT and 16 had relapsed after conventional combination chemotherapy. Patients ranged in age from 26 to 65 years old. The majority of the patients with lymphoma had bulky lymphadenopathy.

All tumors were restaged for response measurement at 28 days using CT scans, gallium scans and bone marrow biopsies to re-examine sites of disease involvement. Physical examinations were performed weekly after therapy to examine for more transient responses.

RESULTS

The initial Phase I trial was undertaken to determine the quantitative and qualitative toxicities of Anti-B4-bR administered by daily bolus injections for five days. The primary objective of this study was to define a maximal tolerated dose (MTD) and the dose limiting toxicity (DLT). Table I summarizes the observed toxicity. Despite transient elevations of transaminases seen in the majority of patients and transient hypoalbuminemia, patients failed to manifest any clinical symptomatology.

At the lowest daily dosages, transient elevations of SGOT and SGPT occurred, but resolved within 5 days of completing therapy. Two of three patients treated at 60 ug/kg/day demonstrated Grade Ill hepatotoxicity with elevations of SGOT and SGPT between 10 and 13 times the upper limit of normal that persisted for approximately one week. Because of this toxicity, the dosage was decreased to 50 ug/kg/day and nine additional patients were treated at that dose level. As was true for the patients treated at lower doses, all nine of these patients developed transient elevation of transaminases without impairment of hepatic synthetic function. Moreover, although all nine patients demonstrated approximately a 25% reduction in serum albumin from baseline, none developed capillary leak syndrome.

Blood levels of the immunoconjugate were measured at regular intervals on each day of therapy. Although high peak blood levels could be achieved immediately after the bolus injection, these levels were maintained only transiently. Thus, therapeutic blood levels (100-200ng/ml predicted from in vitro cytotoxicity assays) were rarely achieved for longer than six hours. HAMA and HARA were produced in 50% of the patients, limiting subsequent courses of therapy.

In the 25 patients treated, there was one complete response, two partial responses and 10 responses that were transient or mixed. In the case of transient responses, patients had a significant reduction in adenopathy which was not maintained over a period of four weeks. In mixed responses, some lymph node groups in a patient regressed while others progressed.

Following the completion of the bolus injection Phase I trial, a second Phase I study was undertaken to determine the toxicities of Anti-B4-bR when it is administered by continuous infusion over seven days. This study is in progress. As with the bolus injection trial, the primary objective of this study is to define the MTD and DLT. Preclinical studies in mice and monkeys revealed that higher doses of Anti-B4-bR could be administered by continuous infusion than by bolus injection with decreased toxicity. Furthermore, prior in vitro studies had demonstrated that maximal cytotoxicity developed when Anti-B4-bR was in contact with cells for prolonged periods of time. Thus, it is hoped that the higher blood levels maintained for increased periods of time in patients when the immunotoxin is administered by continuous infusion might prove clinically advantageous.

Again, in the second Phase I trial, all patients demonstrated transient elevations of SGOT and SGPT (Table 1). At 50 ug/kg/d, the peak ratio of SGPT/upper limit of normal was 9.4 and the peak level of SGOT/upper limit of normal was 12.9. At 60 ug/kg/d, the peak SGPT was 9.0 times the upper limit of normal and the peak SGOT was 9.1 times the upperlimit of normal. Finally, at 70 ug/kg/d, the peak was 12.5 times upper limit of normal for SGPT and 9.1 for SGOT. All patients for whom follow-up data are available have had a return of their transaminases toward normal following therapy. Thirteen patients in this trial demonstrated transient hypoalbuminemia as defined by a 20% reduction in serum albumin during the infusion. Twenty patients developed low grade fever during the infusion without evidence of active infection. Two patients have had transient thrombocytopenia without bleeding. No patients have experienced central nervous system, cardiac, renal, or pulmonary toxicity to date. There has been no leukopenia or anemia resulting from therapy. Blood levels without bolus injection were maintained for extended periods of time in all patients receiving more than 40 ug/kg/day by continuous infusion. It remains too early to correlate blood levels with either response or toxicity.

HAMA was detected in 13 of 27 patients for whom data are available to date. HAMA developed between 10 and 38 days of completing the infusion. While repeat courses of Anti-B4-bR have been administered to five of the 27 patients treated, multiple cycles of drug administration in many patients have been precluded by the development of HAMA.

In 27 patients evaluated to date, there has been one complete response, four partial responses, and seven transient or mixed responses. The MTD has not yet been reached and we continue to enroll patients in this Phase I trial.

DISCUSSION

Anti-B4-bR has been administered in two Phase I trials, both by bolus injection and by continuous infusion. Of the 52 patients treated, 25 patients have shown a response to therapy with at least a transient reduction in the bulk of their disease. Virtually all patients had bulky disease, and all had been refractory to prior standard therapies. The only significant toxicity observed

thus far has been transient elevation of SGOT and SGPT which return toward normal within one week of completing therapy. Thus, Anti-B4-bR appears to have significant biological activity for patients with B cell malignancies. The optimal mode of administration and frequency of repeat courses remains to be determined in future Phase I and II trials.

Moreover, questions remain as to the optimal timing of the delivery of this agent in relation to standard chemotherapy. Although potentially therapeutic blood levels of Anti-B4-bR have been achieved for extended periods of time by administering the drug by continuous infusion, it is possible that the drug may not be able to penetrate deep inside bulky lymph node masses to expose all cells to its cytotoxic action. One way to surmount this difficulty would be to administer Anti-B4-bR concomitantly with or following standard chemotherapy.

A second issue in administering anti-B4-bR concerns the development of HAMA. Although the vast majority of the patients treated to date were seriously ill, secondary to long-standing leukemias and lymphomas, the overwhelming number of these patients were immunologically intact and capable of producing HAMA/HARA. The occurrence of HAMA/HARA limits the delivery of repeat courses of the immunotoxin because subsequent administration of Anti-B4-bR results in binding of the drug by antibody and, as a result, lower blood levels of the agent. Moreover, it is possible that in the presence of elevated titers of HAMA antigen-antibody complexes could form and result in immune complex mediated reactions.

Surmounting the problem of HAMA production may be possible by treating patients with immunosuppressives such as cyclosporine or azathioprine. However, the use of such immunosuppressive agents in patients with leukemia and lymphoma may lead to disease progression or markedly increased risk of infection. The HAMA response conceivably could be blunted by altering the schedule of delivery of Anti-B4-bR. For example, administering the immunotoxin by prolonged continuous infusion may decrease HAMA formation. Alternatively, one could select a sub-population of patients for treatment who have a decreased ability to mount an immune response. Patients who have undergone ABMT meet this requirement.

At the Dana-Farber Cancer Institute, patients with responsive relapsed NHL have been treated with high-dose cyclophosphamide (120mg/kg as 60mg/kg/day for two days) and total body irradiation (1200cGy as 200cGy bid for three days) followed by reinfusion of autologous bone marrow which has been purged with anti-B cell monoclonal antibodies (2). With a median follow-up of 40 months, the disease free survival appears to be just under 40%. Furthermore, B cell reconstitution has been extensively studied in relapsed NHL patients who have undergone ABMT (7). By two to three months following ABMT, most patients have normal numbers of T cells, NK cells and monocytes. However, only 40% of patients have normal numbers of B1 positive cells at three months after ABMT. Consistent with these findings are the low serum immunoglobulins which are at their nadir between three and six

months after ABMT. Although these patients are transiently deficient in B cell function, there are few infections related to this immunodeficiency.

Thus, patients who have completed ABMT for responsive relapsed lymphoma represent a patient population with both a significant risk of recurrent disease and a potentially limited ability to develop HAMA. We are currently undertaking a study to treat patients with Anti-B4-bR beginning 60 to 100 days following ABMT (Figure 1). Since the bone marrow of these patients has been purged to deplete normal and malignant B cells and the patients have been treated with marrow ablative doses of chemoradiotherapy, the immunotoxin may be administered during a window of time when these patients have significantly impaired B cell function. Furthermore, by delivering therapy to patients with no clinical evidence of residual disease, we may be able to deliver Anti-B4-bR more efficiently to the tumor cells and permit an increased number of cycles to be delivered. Unlike the patients treated in our previous Phase I trials, these patients have very few B4 antigen positive normal and neoplastic B cells. Therefore, Anti-B4-bR will not be cleared from the blood rapidly by binding to the massive antigen load expressed on normal and neoplastic B cells. This may yield higher blood levels at lower administered doses of Anti-B4-bR.

The role of Anti-B4-bR in the treatment of B cell malignancies remains to be determined. Since Anti-B4-bR kills malignant B cells by inhibiting protein synthesis, it is theoretically capable of killing residual lymphoma and leukemia cells which are resistant to high dose therapy. The encouraging preliminary results with this agent in the two Phase I trials that have been described suggest a potential clinical role for this drug in both patients with relapsed lymphoma and in patients who have completed ABMT.

ACKNOWLEDGEMENTS

Supported by NIH grant CA34183.

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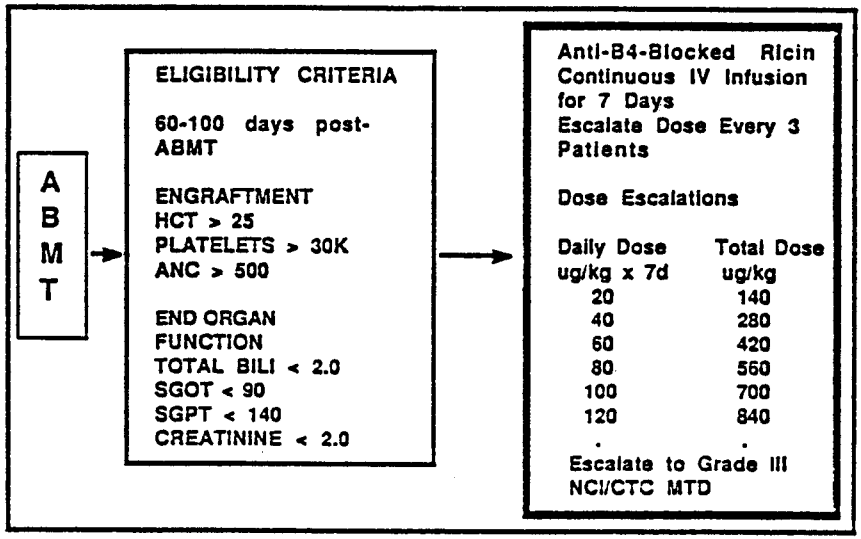
TABLE 1

Toxicity of Anti-B4-bR Administered by Bolus Injection and Constant Infusion

	BOLUS INJECTION	CONSTANT INFUSION
Total Patients	25	27
Elevation SGPT & SGOT	23	27
Thrombocytopenia	3	2
Hypoalbuminemia	18	13
Fever	0	20
Leukopenia/Anemia	0	0
CNS Toxicity	0	0
Renal Toxicity	0	0
Pulmonary Toxicity	0	0

FIGURE 1
Schema for administration of Anti-B4-bR after ABMT



IMMUNOSTIMULATION POST AUTOLOGOUS BONE MARROW TRANSPLANTATION WITH THE NOVEL DRUG LINOMIDE: AUGMENTATION OF T- AND NK- CELL FUNCTIONS

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ABSTRACT

Immunostimulatory therapy is now considered as adjuvant therapy to ABMT in order to improve remission duration and reduce relapses of leukemia. Five adults with acute myeloid leukemia (AML) received the new immunomodulator Linomide post-ABMT. Patients were conditioned with Busulphan plus Cyclophoshamide and received Linomide 0.3 mg/kg/week p.o. in cycles of 3 weeks followed by 3 weeks of rest, up to 6 months. Four patients are evaluable. FACS analysis shows cyclic increases in CD16+ and CD56+CD3-NK cells. Increased killing of the NK sensitive K562 and the NK resistant Daudi cell line cells followed the same cyclic pattern. T cell responses to PHA and MLC reactivity also increased during Linomide therapy. In vitro production of TNFA and IFNY followed a similar cyclic pattern. Side effects were, in general, mild and consisted most frequently of nausea and muscle stiffness. We show here that Linomide post ABMT induces broad immunostimulatory effects and offers potential therapeutic benefit with regard to leukemia-free survival.

INTRODUCTION

Over the last ten years, a considerable bulk of evidence has emerged supporting the role of the immune system in tumor surveillance. Especially the importance of natural killer (NK) cells (1) and lymphokine activated killer (LAK) cells (2) is suggested. That cellular defense mechanisms contribute to leukemia-free survival after bone marrow transplantation (BMT) is implied by the finding that relapse rates differ between patients receiving non-T-cell depleted, T-cell depleted and identical twin marrows (3). Low numbers of NK cells were reported in patients who subsequently relapsed after autologous BMT (ABMT) (4) and loss of NK cell activity preceded relapse of leukemia (5). Apart from the effects of high dose chemo-radiotherapy, the bone marrow transplant procedure in itself seems to generate an antileukemic effect. Both

allogeneic and autologous BMT, but not chemotherapy, induced elevated numbers of NK and activated "LAK"-like cells (6,7). Evidence that immunotherapy can improve survival after BMT was reported from rodent models, were the antileukemic effect of marrow transplantation was further enhanced by interleukin-2 (IL-2) therapy (8). An increased in vitro cellular production of tumor necrosis factor (TNF-alpha, TNFA) and y-interferon (IFN-gamma, IFNy) was also documented in patients receiving IL-2 infusions after BMT (9,10). This cytokine release was also shown to inhibit the clonogeneic growth of myeloid blasts in vitro. IL-2 therapy is, however, hampered by major toxicity (11) and is presently administered on an in-patient basis.

In this paper we describe the immune-stimulatory effects of a new oral drug, Linomide (quinoline-3-carboxamide, LS2616), in patients after ABMT. Linomide has mild side effects and can be used on an out-patient basis. The drug was found during screening of anti-inflammatory agents, and was shown to have potent immune-stimulatory properties. Linomide strongly stimulated NK cell function in mice, and increased the numbers of bone marrow NK cell precursors (12). In addition, Linomide enhanced delayed-type hypersensitivity in rats (13), mitogen and alloantigen responses in mice (14). Linomide also improved survival of virus infected animals (15) and accelerated the rejection of cardiac allografts (16). Taken together, these findings indicate that Linomide increases NK cell activity and interacts with macrophage T-cell interactions. The immune-stimulatory effects of Linomide are also documented in rodent tumor models, were Linomide reduced both tumor cell growth and metastatic processes by NK dependent and NK independent mechanisms (17,18). In a dose finding study in patients with advanced renal cell carcinoma, it was found that the immunostimulatory effects of Linomide followed a bell-shaped curve with optimal effects at 0.2-0.4 mg/kg once weekly (19). Encouraged by the profound immune-stimulatory effects, mild side effects and measurable tumor regression in two out of three long-term treated renal cancer patients, we decided to use Linomide as adjuvant therapy after ABMT. In order to establish the immunological effects, Linomide was administered in repeated cycles of three weeks followed by three weeks of rest, thereby permitting each patient to be its own control.

MATERIAL AND METHODS

Patients and Treatment Protocol

Five consecutive patients with AML undergoing ABMT were studied. Informed consent was obtained from all patients, and the study was approved by the Institutional Review Board. Their age ranged from 48 to 57 years. All recipients were conditioned with Busulphan (16 mg/kg) and cyclophosphamide (120 mg/kg). Hematological engraftment was successful in every case. All patients received Linomide 0.3 mg/kg orally once a week, starting the day of marrow infusion. Treatment was continued for three weeks followed by rest for three weeks. The cycle was then repeated up to 6 months. Four patients

are alive, although one patient has relapsed in his leukemia. One patient died 13 weeks after ABMT in an intracerebral hemorrhage.

Peripheral blood samples (40-60 ml) were obtained before start of the conditioning therapy and from once a week to every third week for up to 6 months. Samples during treatment periods were always taken 2 days after the dose.

Preparation of Mononuclear Cells and Immunofluorescence Staining

Mononuclear cells (MNC) were isolated from heparinized blood samples by standard Ficoll-Paque (Pharmacia Uppsala Sweden) separation. Cells were divided into two parts, one used for immunofluorescence staining, and one frozen in liquid nitrogen for functional experiments. In the latter experiments, all samples from a given patient were thawed at the same time and used for in vitro studies. Cell surface markers were determined by direct staining of cell suspensions as described previously (7), using monoclonal antibodies (Becton Dickinson, Mountain View, CA) directed against CD3, CD16, CD56 and CD14. Absolute lymphocyte counts were obtained with an automatic blood cell counter (Technicon H1, Tarry, NY)

Flow Cytometry

Stained cells were analyzed on the FACStar (Becton Dickinson) flow cytometer equipped with a 5 W Argon ion laser run at 0.5 W. At least 10,000 viable lymphocytes were gated and analyzed by one- or two-color fluorescence. For enumeration of CD14+ cells scatter gates were set on lymphocytes plus monocytes.

Lymphocyte Proliferative Responses

Proliferative responses of patient lymphocytes to alloantigens and PHA at various time points were performed in triplicate microtiter wells. Cells (10^6/ml) were cultured in the presence of either media alone (RPMI 1640 with 10% human AB serum), with PHA 1 ug/ml for three days, or with a pool of irradiated allogeneic stimulator lymphocytes for six days. At the end of culture, cells were pulsed with 1 uCi/well of tritiated thymidine, incubated for six hours before harvesting and counting in a liquid scintillation counter.

Cytotoxicity Assays

The standard four-hour ^{51}Cr release assay was used. NK cell sensitive K562 and NK cell resistant Daudi cell line cells were labelled with 200 uCi sodium chromate for one hour. Effector to target (E:T) cell ratios of 60:1, 30:1, 15:1 and 7.5:1 was used. The percentage specific lysis for each target was determined.

Peripheral Blood Mononuclear Cell Culture for Cytokine Production

Cells were cultured with or without PHA for three days as described above. Supernatants were harvested and stored at -70C.

Determination of Cytokines Cell Supernatants

IFNy, and TNFA, were measured using commercially available immunoradiometric assays (Medgenix, Fleurus, Belgium).

RESULTS

After ABMT, patients were treated with Linomide in repeated cycles of three weeks on the drug followed by three weeks off the drug. In four patients a total of 13 evaluable cycles of Linomide were analyzed. One patient, who developed a Quincke oedema received only one incomplete cycle and was therefore excluded. The remaining four patients received two to four treatment cycles and were evaluable. Side effects were generally mild and consisted mainly of nausea/vomiting and muscle stiffness.

Changes in Cell Phenotype

An increase in NK cell numbers was observed during the majority of treatment cycles. An increase on Linomide was defined as a value > 50% (mostly >100%) higher than the value preceding Linomide. The proportion of both CD16+ and CD56+CD3-NK cells was increased during 10/13 cycles. The absolute cell counts were elevated during 9/12 end 8/12 cycles, respectively. A typical patient is shown in Figure 1. A relative increase in CD14+ cells was observed during 7/11 cycles and an absolute increase during 7/10 cycles.

Changes in Killer Cell Activity

Prospective changes in the cytotoxic capacity of patients peripheral blood lymphocytes were analyzed against two different target cells. Because of the cytopenia during the first weeks after ABMT, cells were seldom available for studies in the early period. During 10/10 treatment cycles an enhanced killing of the NK sensitive cell line K562 was noticed with subsequent decline off treatment. Killing of the NK-resistant Daudi cell line increased during 7/10 therapy periods. A typical patient is shown in Figure 2.

PHA and MLC Responses

Changes in T-cell responses were measured by proliferative responses to PHA and alloantigens. A pool of irradiated lymphocytes were used as stimulator cells in the MLC reaction. A similar response pattern was seen in both assays. An increase in PHA response was observed during 5/9 treatment cycles and in MLC reaction during 6/7 cycles. A typical pattern is shown in Figure 3.

Cytokine Production by MNCs

Increased spontaneous in vitro secretion of TNFA was observed during 5/6 Linomide treatment periods. Spontaneous IFNY production in vitro was generally low with values around the detection limit of the assay (0.2 IU/ml), thus making clear distinctions difficult. However, in PHA stimulated cell

cultures an increase in IFNy production were noticed during 6/7 treatment cycles. Typical results are shown in Figure 4.

DISCUSSION

Immunotherapy of human cancer is an area in rapid progress. However, the conclusion that immunological mechanisms cure tumor patients is at the present time not justified. It could be argued that the mere appearance of a tumor is evidence that the immunosurveillance has failed. Immunotherapy is however an attempt to boost the efficacy of the immune system to a level which will eradicate cancer. That tumor regression can occur after immunostimulation is evident from numerous experimental and clinical trials with LAK/IL-2 therapy (2). The success of immunotherapy is probably more likely if: 1) the tumor cells are sensitive to the killer mechanisms of the immune system, 2) the tumor burden is as small as possible and 3) immunocompetent cells and/or their precursors are available in the patient. Therefore, leukemic patients undergoing bone marrow transplantation in remission should be ideal subjects for this type of therapy. Several reports have shown that leukemic blast cells are susceptible to NK and LAK cell killing (6,20,21) and patients transplanted in complete remission of their leukemia have a low tumor load. Reversible severe combined immunodeficiency always occurs after both allogeneic and autologous marrow transplantation (22). This immunodeficiency is aggravated by the presence of graft-versus-host disease (GVHD). Despite this, there is an inverse relationship between the degree of GVHD and leukemia-free survival (3). It is plausible that alloreactive donor T cells, responsible for the GVHD, also take part in elimination of leukemic cells. Further, activated NK cells constitute a considerable proportion of early post-transplant lymphoid cells (7) and possess antileukemic activity (6,23,24). Therefore, it is clear that host immunocompetent cells are present.

In this paper we describe the immunostimulatory properties of the novel drug Linomide post ABMT. The relative and absolute numbers of CD16+ and CD56+CD3NK cells increased in a cyclic fashion in four patients repeatedly treated with the drug. Both phenotypes are characteristic for the cell type responsible for the oncolytic activity obtained at LAK therapy (25). The fact that CD56+CD3- cell numbers increased during Linomide therapy is of great interest and contrasts with published data on IL-2 therapy after ABMT. In the latter context, no increase was recorded for this population, whereas CD16+ cells were elevated (26). Cytotoxic NK-like cells represent a heterogenous population with different targets and functions (25). The ability of Linomide to generate both CD16+cells, which are important effectors in antibody-dependent cell-mediated cytotoxicity, and CD56+ cells could be of therapeutic advantage. The numbers of CD14+ cells, another cell type certainly involved in tumor defense (27), also increased cyclically during Linomide therapy. Significant increases in NK and LAK cell activity were also generated. The elevation of cytotoxic activity was of the same magnitude as that reported after IL-2 infusion (26). The proliferative T cell responses to

PHA and MLC (allogeneic cells) were also increased by the drug. Both mitogen responses and MLC reactivity are typically depressed for months after marrow transplantation (22) which may be due to a reduced T-helper cell activity. The enhanced responses noted during Linomide therapy may therefore reflect a stimulated T-helper cell function, which could be beneficial with regard to viral and bacterial infections. T-helper cells are also important in tumor resistance (27). Linomide therapy was also associated with increased cytokine production. The spontaneous in vitro production of TNFA followed a cyclic pattern with enhanced production during treatment periods. Unstimulated cells secreted low levels of IFNY, whereas PHA driven cells secreted high levels upon in vivo "priming" with Linomide. These results are different from IL-2 therapy, where spontaneous IFNy production increased in vitro. If this represents a true phenomenon or merely a difference in assay precision remains to be established. The biological implications of an increased capacity for cytokine production are several. TNFA and IFNy both possess direct tumouricidal activity against myeloid blasts (28) in addition to their ability to induce other cytokines. Both cytokines are also known to enhance granulocyte, monocyte and B cell functions, with potential benefits in resistance against infections.

We have shown that Linomide therapy after ABMT results in increased numbers of phenotypic NK/LAK cells exhibiting enhanced cytotoxic function together with increased cytokine production. Together with the finding of elevated CD14+ cells and improved T cell proliferative responses, this broad immunological stimulation could be of potential therapeutic benefit with regard to leukemia-free survival. The mild side effects of Linomide with the convenience of an oral drug administered on an out-patient basis make this drug an interesting alternative to IL-2 therapy post ABMT. A multicenter randomized placebo controlled clinical trial with Linomide post ABMT is necessary to determine whether the observed immunostimulation will improve remission duration. Such a clinical trial will be initiated in the near future.

ACKNOWLEDGEMENTS

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FIGURE 1
Prospective changes in the relative numbers of CD16+ and CD56+CD3- cells in peripheral blood in one patient after ABMT.

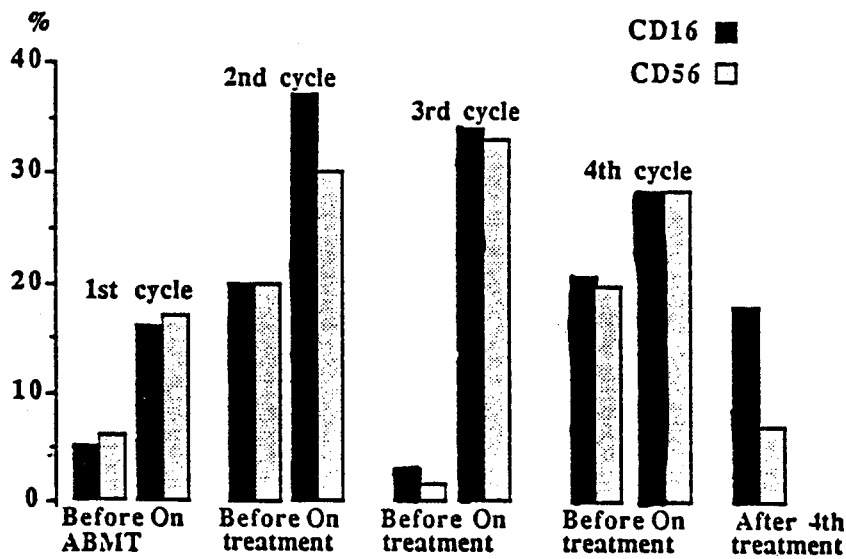


FIGURE 2

Prospective changes in the cytotoxic capacity of peripheral blood lymphocytes against the NK sensitive K562 cell line and the NK resistant Daudi cell line. The effector:target ratio was 30:1.

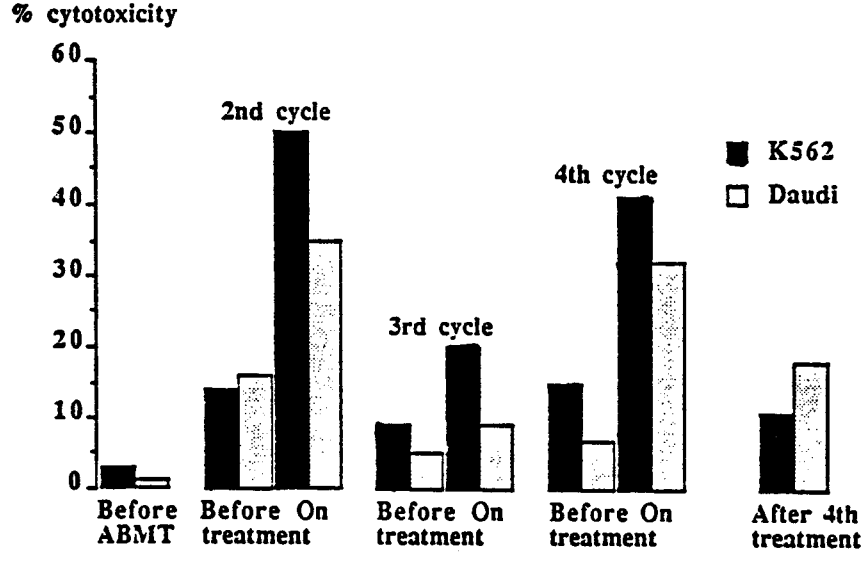


FIGURE 3
Changes in T cell proliferative responses before, on and after Linomode therapy.

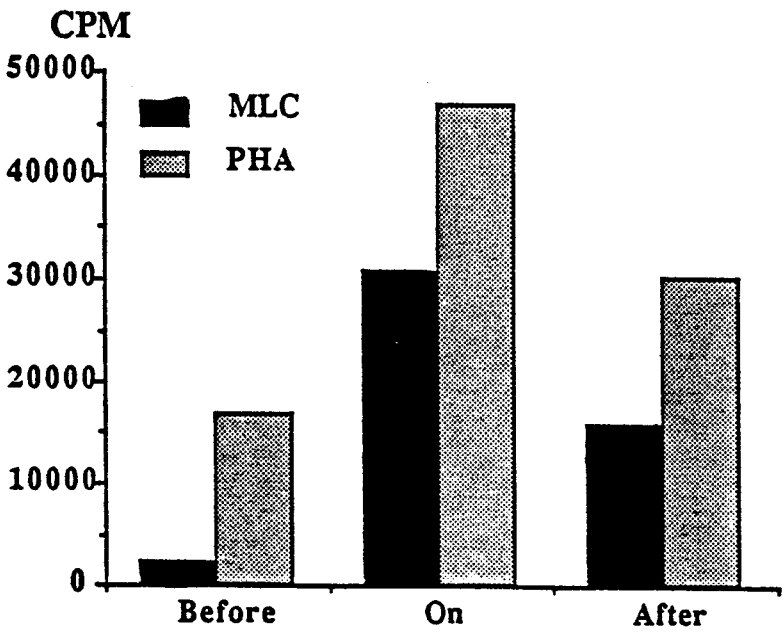
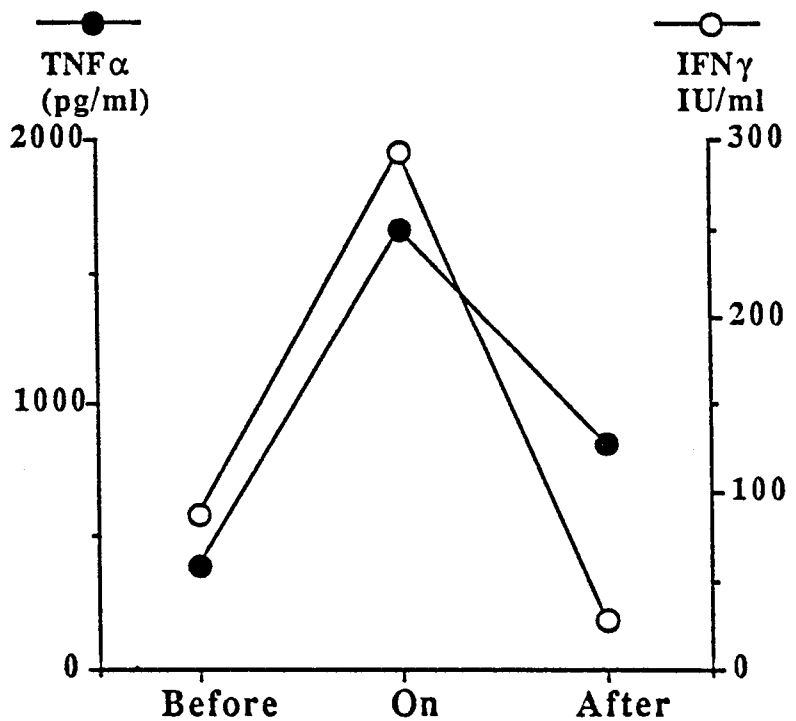


FIGURE 4
Spontaneous production of TNFA and PHA stimulated IFNY production in vitro, before, on and after Linomide treatment.



PERIPHERAL STEM CELLS : MOBILIZATION BY MYELOSUPPRESSIVE CHEMOTHERAPY

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INTRODUCTION

Recovery Phase Peripheral Blood Stem Cells for Autotransplantation

Increased peripheral blood levels of granulocyte-macrophage colony-forming-units (CFU-GM) during haematological recovery from chemotherapy induced myelosuppression were first described in 1976 and it was suggested that autologous haemopoietic reconstitution (HR) could be produced by peripheral blood stem cells (PBSC) harvested at this time [1]. Further investigation was limited because some animal [2] and human [3,4] studies suggested that PBSC had inferior HR capacity compared with bone marrow (BM), although others disagreed [5-8]. Subsequent reports of more successful mobilization, collection and autotransplantation in man employed recovery phase PBSC and produced rapid HR after transplantation, with safe neutrophil and platelet counts by the beginning of the third week [9-12].

METHODS

The Importance of Collecting a Large Number of CFU-GM

Two studies of HR after recovery phase PB autotransplantation have found a significant correlation between CFU-GM dose and short and long term HR but not between mononuclear cell dose and HR [13,14]. Adequate numbers of recovery phase PBSC produce trilineage bone marrow engraftment by day 7 post graft and recovery to safe levels of neutrophils and platelets by 9 to 14 days. The sustained HR suggests that the high levels of CFU-GM in recovery phase PBSC are associated with high levels of pluripotent stem cells. Thus the initial engraftment probably results from relatively late stem cells, adequately measured by the CFU-GM assay, whereas the later phases of engraftment result from pluripotent stem cells. Various minimum safe CFU-GM doses have been proposed [14,15]. The collection of PB containing a large number of CFU-GM is thus important for achieving both rapid and sustained HR following transplant.

Proposed Mechanism of the Haemopoietic Rebound During the Recovery Phase Following Myelosuppressive Chemotherapy

The most primitive pluripotent stem cells are metabolically and mitotically quiescent and less sensitive to most cytotoxic agents than the more mature progenitor cells. Depopulation of the mature cells by chemotherapy presumably sends signals which stimulate the pluripotent stem cells to repopulate the committed and maturing compartments. Many myelosuppressive chemotherapy regimes produce severe but reversible bone marrow hypoplasia followed by vigorous haematological recovery which usually starts 10-14 days after the chemotherapy [8-12]. The platelet and leukocyte counts rise rapidly and monocytosis, reticulocytosis and large mononuclear cells which resemble atypical lymphocytes occur in the peripheral blood. During the rapid rise in blood counts the levels of single and multiple lineage progenitor cells rise up to one-hundred-fold above the mean in normal subjects for periods between 4 and 7 days [9,16].

The release of normal haemopoietic cells from leukaemia associated inhibition in remitting patients may also contribute to the vigorous recovery and overshoot. This may explain why higher levels of PBSC are seen in patients entering remission from AML [9,17] than after cytotoxic chemotherapy in non-AML patients [16], and why successful collection in AML requires the achievement of remission [18]. Remission is not mandatory in other diseases where high dose cyclophosphamide (Cy) is particularly effective [16]. The drug is active as a single agent against most lymphomas and solid tumours and is followed by brief cytopenia and vigorous recovery, suggesting selective killing of more mature cells and stimulation of haemopoiesis. Patients receiving less myelosuppressive consolidation chemotherapy for AML [9,17] or CHOP, MACOP-B and conventional dose cisplatin/Cy [unpublished data] often have absent or smaller increases in CFU-GM levels.

PBSC Mobilization in Acute Myeloid Leukemia

We have performed successful PBSC mobilization in 65 patients with AML, early in first remission in 49 and after less intense consolidation chemotherapy in 16 who had either unsuccessful first remission collections or a slow or delayed recovery in which it was difficult to recognize a period of rapidly rising blood counts suggesting high PBSC levels. Twenty-two of these cases have been reported previously [18]. The remission induction chemotherapy was either DAT, 737 or HIDAC-37. The consolidation chemotherapy was modified DAT or 525. Table I shows the chemotherapy regimen, the number of patients, and the numbers of mononuclear cells (MNC) and CFU-GM collected.

The differences between the groups are not significant. The important conclusions are (1) all of these remission induction regimens can produce high CFU-GM levels, (2) either consolidation regimen can achieve high levels, which suggests a more satisfactory protocol may be the collection of PBSC after 3rd, 4th or later consolidation at a time when potential leukaemic contamination may be further reduced.

PBSC Mobilization in Lymphoma, Myeloma and Solid Tumours

We have performed 37 cyclophosphamide mobilizations using a single dose of 4gm/m^2 in 34 patients. Twenty of these cases have previously been reported [16]. We have preliminary information on 8 mobilizations in 7 patients using Cy 7gm/m^2, given as 4 hourly infusions of 1.75gm/m^2 with one hour between each infusion and using Mesna for urothelial protection, a protocol first reported in patients with multiple myeloma [19]. The patients had non-Hodgkin's lymphoma, multiple myeloma, ovarian or breast carcinoma and most had extensive previous chemotherapy and/or BM involvement.

Whilst our initial recommendation was that a minimum dose of 30-50 x 10^4 CFU-GM/kg was required for safe HR in AML [15], we now have evidence that lower doses are satisfactory in other diseases [20], and are prepared to autograft with as few as 15-20 x 10^4 CFU-GM/kg. We have also found that peak PB CFU-GM levels of 1000/ml almost always lead to collection of more than 15-20 x 10^4/kg [161. With Cy 4gm/m^2 28 of 37 mobilizations (76%) were successful by these criteria. We have analysed this group to delineate the parameters which predict the correct time to begin apheresis, the optimum frequency of apheresis, the number of aphereses, and identify patients whose mobilization is destined to be unsuccessful because of previous myelosuppressive therapy, BM involvement, or individual variation in haemopoietic biology. This may help to avoid both weekend apheresis and unnecessary apheresis in non-responders.

The CFU-GM assay takes 14 days and is of course not available to direct collection. The rise in WCC and platelets is the best current predictive parameter, but imperfect, and we are actively investigating the levels of CD34 positive cells as a rapid and potentially more accurate guide to apheresis. Preliminary data is encouraging, with an excellent correlation between CD34 and CFU-GM [20]. We generally begin apheresis early at a WCC of 0.9-1.0 x 10^9/1 because three AML patients g receiving CFU-GM doses of 62, 88 and 119 x 10^4/l showed rapid granulocyte recovery but slow and incomplete platelet recovery. In two of these patients apheresis was started late, at the end of the rapid recovery phase when counts were back to normal. The incomplete HR observed in these patients suggests that distinct subpopulations of haemopoietic stem cells may enter the PB at different times during recovery. Late in recovery there may be many committed progenitor cells, but relatively few pluripotent stem cells.

The mean day post Cy on which the WCC = 1.0 was 15 + 0.4 (range 12 - 19). In an attempt to avoid weekend apheresis and cryopreservation the Cy is usually given on a Monday so that collection usually starts on the following Tuesday or Wednesday. However, five of the seven patients who had not received previous chemotherapy showed vigorous recovery on days 12 and 13, and they should probably receive Cy on a Tuesday or Wednesday.

Table II shows the CFU-GM yield as a percentage of the total yield for each patient related to the WCC at the time of apheresis in the 28 patients receiving Cy 4gm/m^2 who had successful collections. Delaying the

commencement of apheresis until the WCC reaches 1.0 x 10^9/l will only reduce the yield by 8.9%.

The peak CFU-GM/ml occurred at a WCC between 1.0 and 3.5 x 10^9/l in 82% of mobilizations, and before a WCC of 1.0 in only one of 28 mobilizations. In that case high levels persisted for many days and the two aphereses performed when the WCC was <1.0 contributed only 14% of the CFU-GM yield.

Other factors investigated for the success of Cy mobilization included previous chemotherapy, BM involvement and the rate of rise of the WCC during recovery. As previously reported [16], a rapid rise in the WCC from 1.0 - 3.0 x 10^9/l ($p<0.005$) and a past exposure to chemotherapy ($p<0.007$) correlated to the peak CFU-GM level, although 5/13 heavily pre-treated patients had peak CFU-GM levels >1000/ml. Patients whose WCC took more than 5 days to rise from 1.0 to 3.0 x 10^9/l never had adequate collections by our criteria. In contrast with the previous report, BM involvement did not correlate with peak CFU-GM levels, probably because more patients in this expanded study had worthwhile tumour response to the mobilising chemotherapy.

Apheresis beyond day 19 (into the next weekend) was performed in 11/28 successful mobilizations. Because the CFU-GM level usually remains elevated for 7 days after Cy [16] it is probably reasonable to delay these collections until the next Monday. At present we sometimes perform collections on Saturday mornings, but can usually avoid Sundays. Our present recommendations for PBSC mobilization using Cy 4gm/m^2 are summarised in Table III.

Cyclophosphamide 7GM/M^2

Although our results with this increased dose of Cy are preliminary, the outcome in three patients who had unsuccessful mobilizations with the lower dose is encouraging (table IV). In each case the higher dose produced higher yields, and the clinical toxicity was no different.

We have also compared the yields in nine pre-treated patients with myeloma. In 5 patients six mobilizations were performed using 4gm/m^2, whereas in another four patients five mobilizations were performed using 7gm/m^2 (table V). Whilst the data is preliminary and the numbers are small, the higher dose produced >15-20 x 10^4 CFU-GM/kg in all five mobilizations, compared with two of six with the lower dose ($p<0.05$).

CONCLUSIONS AND FUTURE DIRECTIONS

Remission induction and consolidation chemotherapy in AML can reliably produce high levels of PBSC in patients in remission. Cyclophosphamide 4gm/m^2 can achieve the same result in most patients with lymphoma, multiple myeloma and cancer of the breast and ovary. A higher dose of cyclophosphamide (7gm/m^2) may be more effective.

Further refinements such as the use of synergistic combinations of haemopoietic growth factors with myelosuppressive chemotherapy for

mobilization as pioneered by the Milan group [21], the development of better means of quantitating PBSC, better measurement of malignant contamination, and double autotransplantation, may lead to safer mobilizations and more effective autotransplantation.

ACKNOWLEDGEMENT

The authors acknowledge the grant support of the National Health and Medical Research Council of Australia, the Anti- Cancer Foundation of the Universities of South Australia and the Baxter Healthcare Corporation, Illinois, USA.

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TABLE 1

Peripheral Blood Stem Cell Mobilization in Acute Myeloid Leukaemia

Regimen	No	MNC $x10^8$/kg mean ± SE	CFU-GM $x10^4$/kg mean ± SE
DAT	41	3.0 ± 0.3	91.9 ± 13.9
737	5	5.0 ± 1.5	95.7 ± 43.8
HIDAC-37	3	5.8 ± 2.2	38.2 ± 14.2
DAT (consolid[a])	9	4.8 ± 0.5	81.9 ± 10.9
525	7	4.2 ± 0.9	56.0 ± 9.3

TABLE 2

CFU-GM Yield as Percent of Total, Related to WCC

WCC ($x10^9$/L)	GM yield (%)	No of aphereses
0.6-0.9	8.9	21
1.0-1.9	17.7	46
2.0-2.9	26.4	33
3.0-3.9	26.0	18
> 4.0	31.5	11

TABLE 3

$4GM/M^2$ Cyclophosphamide: Recommendations

* No previous Rx: give Cy on Tuesday
* Previous Rx: give Cy on Monday
* CBE, CD34, CFU-GM day 7, 10 and daily from 13 or 14
* Start apheresis when WCC > 1.0 x 10^9/l or when platelets rise rapidly: eg 40-90 in 1 day
* Daily apheresis if WCC and/or platelet increase >20%
* Apheresis on consecutive days when WCC reaches 2.0; even if WCC/platelets not rising

TABLE 4

Comparison: cyclophosphamide 4GM/M^2 & 7GM/M^2

Diagnosis	Previous Rx	CFU-GM yield after Cyclo 4gm/m^2	7gm/m^2
NHL	CHOP/MACOP B	15.0x10^4/kg	27.2x10^4/kg
NHL	CHOP	6.8x10^4/kg	47.0x10^4/kg
Ca breast	CMF	14.5x10^4/kg	72.4x10^4/kg

TABLE 5

Multiple Myeloma: Comparison CY 4GM/M^2 & 7GM/M^2

UPN	Cy 4gm/m^2 CFU-GM yield	UPN	Cy 7gm/m^2 CFU-GM yield
MM001	2.6 x 10^4/kg	MM007	29.8 x 10^4/kg
MM003	65.3	MM008	35.9
MM004	8.2	MM008	31.7
MM004	8.4	MM009	15.3
MM005	65.6	MM010	368.7
MM006	4.3		

THE ROLE OF STEM CELL MOBILIZATION IN AUTOLOGOUS BLOOD STEM CELL TRANSPLANTATION (ABSCT)

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INTRODUCTION

The concentration of hemopoietic precursor cells (CFU-GM, BFU-E, CFU-GEMM) in the circulating blood is only about 1/10 to 1/20 of that in the marrow (1). Therefore, it is widely believed that hemopoietic precursor cells have to be mobilized from extravascular sites into the circulation to guarantee a sufficient and safe blood stem cell autograft. Kessinger et al.(2) on the other hand, reported on 10 patients with advanced malignant disease involving the marrow whose hemopoietic function after marrow ablative therapy was fully restored with non-mobilized stem cells. The question of using mobilized or non-mobilized stem cells for transplantation purposes addresses the quality of hemopoietic reconstitution rather than its feasibility.

METHODS

Quality of Hemopoietic Reconstitution

Hemopoietic reconstitution after myeloablative therapy encompasses two aspects which are of clinical relevance:

- the increment of peripheral cell concentration per time
- shortening the duration of total aplasia following myeloablation and stem cell transplantation

Increment of Peripheral Cell Concentration Per Time

When we reported our first successful case of ABSCT (3), the speed of hemopoietic reconstitution after autologous transfusion of chemotherapy primed stem cells was by far faster than expected from autologous bone marrow transplantation (ABMT). Meanwhile, there is abundant clinical evidence that both transplant modalities, ABSCT and ABMT, are different in their reconstitutive pattern. One possible explanation for this is that the transfusion of blood derived stem cells combines early pluripotent as well as more mature committed stem cells, which requires less time to reach maturation than the compartment transit time needed by immature stem cells in order to differentiate

and to develop (4). Those blood derived committed stem cells with limited or no self-renewal capacity give rise to a first and early transient peak which is followed by hemopoietic reconstitution originating from early pluripotent stem cells. The interference of both curves might result in a dip of peripheral cell concentration which is seen in some patients depending on the total number of cells and/or stem cells transfused (Figure 1). In the undisturbed system it can be assumed that less than 10% of the stem cells undergo a cell cycle. The majority of cells are in a resting phase (5,6). It is known from experiments in mice that an increased utilization of peripheral granulocytes results in a recruitment of stem cells from their resting state into a cell cycle (7). Therefore, one can assume that a blood stem cell autograft transfused contains a higher percentage of hemopoietic precursor cells in cycle with a high replication and differentiation probability.

Another aspect which might contribute to a steep increase in cell concentration after ABSCT is due to the fact that about ten times more cells are transfused into the patient than what a marrow autograft contains (Table 1).

The phenomenon described is not necessarily dependent upon the use of the various mobilizing techniques but might be enforced by them. Unfortunately, data on the reconstitutive characteristics of mobilized versus non-mobilized stem cell autografts from randomized trials are not yet available.

Shortening the Duration of Total Aplasia

Shortening the duration of total aplasia after ABSCT is the most crucial aspect of hemopoietic reconstitution, thus diminishing the risk of early death due to severe infections. As schematically shown in Figure 2, recombinant human (rh) GM-CSF i.v. application (16 ug/kg/day) after ABMT accelerates leukocyte and granulocyte recovery compared with controls, but does not significantly shorten the duration of total aplasia (8). The expansion of a transplanted stem cell pool is not affected much by GM-CSF application, whereas the transfusion of large amounts of differentiating and developing cells serves as cell support with limited or non-self-renewal capacity. This bridges the early post-transplant period from the day of transplant to the start of "true" cell recovery. The role of early pluripotent stem cells at this period is not as much clinically relevant but maintains hemopoietic function.

In a non-randomized clinical trial, we compared the kinetics of hemopoietic reconstitution of 20 patients with standard risk AML undergoing autologous transplantation of chemotherapy primed blood stem cells with 23 patients with the same stage of disease undergoing autologous transplantation of a Mafosfamide purged marrow graft. As shown in Figure 3, the white blood cell reconstitution following myeloablative therapy and ABSCT is initiated much earlier from day 1 on ($p=0.0001$). The median time to reach 1.000/ul WBC or 500/ul PMN was 10 resp. 14 days after ABSCT ($p=0.0001$) versus 28 resp. 42 days following ABMT ($p=0.0001$)(9).

Cell and CFU-GM Numbers Harvested and Transfused

In a series of 15 patients with advanced stage Hodgkin's disease (sensitive relapse), we compared the efficacy of the two different stem cell mobilization techniques with non-mobilization using cell yield per apheresis, number of aphereses, and cells needed for a sufficient and safe stem cell autograft as parameters.

Chemotherapy priming consisted of ara-C and Daunorubicin, and rhGM-CSF (Behring Werke, Marburg, Germany) was given as continuous i.v. infusion (250 ug/m^2/day). The MNC yield was not significantly different, but the CFU-GM yield during GM-CSF application exceeded that after chemotherapy priming by a factor 5 (Table 2).

As shown in Table 3 the median number of stem cell aphereses in those chemotherapy primed patients was 13, but only 6 when using CSF priming. We also performed 6 stem cell aphereses on each of 3 patients where we did not mobilize stem cells at all. We attempted to keep the total number of MNC transplanted per kg body weight in the same range between 5.75 and 6.4 x 10^8. Again, the CFU-GM numbers were found highest in the CSF primed patient group, whereas there was no difference in those numbers when giving back chemotherapy primed or non-mobilized cells.

It is obvious that the GM-CSF approach is the most efficient in terms of number of aphereses and CFU-GM yield per apheresis. Unfortunately, patient numbers are too small to draw any definite conclusions.

Side Effects of Chemotherapy and CSF Priming

Chemotherapy priming, using a rather moderate ara-C/Daunorubicin maintenance treatment or single dose Cyclophosphamide (4 g/m^2), resulted in a transient myelosuppression with leukocyte counts below 500/ul lasting between 1 and 3 days. Fever usually resolved when leukocytes began to rise.

The side effects of GM-CSF priming (250/ug/m^2/day) were transient bouts of fever up to 39C seen in all patients. One patient experienced severe axillary phlebothrombosis 7 days following subclavian catheter implantation (Hickman catheter) on the same side and 5 days following start of GM-CSF application. A local increase in cell concentration at the opening of the catheter could account for this side effect.

Reconstitutive Potential of Mobilized Versus Non-Mobilized Stem Cell Autografts

The reconstitutive ability of the various stem cell autografts, whether chemotherapy, cytokine, or non-mobilized, were not significantly different in our series of 15 advanced Hodgkin's lymphoma patients. Again, the number of cases studied is too small to allow a precise statistical evaluation.

Risk of Mobilizing Clonogenic Tumor Cells

Mobilization of hemopoietic precursor cells does not necessarily exclude a concomitant mobilization of clonogenic tumor cells. In acute leukemias, this aspect has to be kept in mind when comparing the disease-free

survival with that after ABMT. In our comparative AML study mentioned above (9), when comparing the purged marrow autograft with the non-purged blood stem cell autograft, the disease free survival (DFS) in ABSCT patients was inferior although statistically not different (36% probability DFS versus 59% probability DFS, p=0.33). In the group of 23 ABMT patients, no relapses have occurred later than 9 months post transplant to date. In the group of ABSCT patients, a DFS plateau cannot be clearly recognized. As proposed by Andreeff et al.(10), recruitment of myeloid leukemic cells into cell cycle by cytokines can be used to increase cell killing by administering timed cell cycle specific chemotherapy (i.e. ara-C). This concept could combine an efficient way to eliminate "minimal residual disease" with mobilization of "normal" stem cells.

Possible Parameters Influencing Efficacy of Various Mobilization Techniques

As to our present knowledge the efficacy of the various stem cell mobilization techniques might depend upon:

- the concentration of hemopoietic precursor cells available at the beginning of the first stem cell apheresis (CFU-GM, CD34+, CD34+/33-)
- the quality of the microenvironment
- the marrow tumor cell infiltration
- the type of stem cell priming (chemotherapy versus cytokine)
- the specificity of CSF (GM-CSF, IL-3, or a combination)
- the time sequence of aphereses (one day versus two or more days)

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TABLE 1

Median number of mononuclear cells (NC) and CFU-GM transfused per kg b.w.
AML= acute myelogenous leukemia; CR = complete remission

	MNC x 10^8	CFU-GM x 10^4
AML (CR 1) peripheral blood (n = 20)	8.0 (1.7-14)	2.4 (0.2-4.1)
Hodgkin's disease peripheral blood (n = 12)	6.0 (1.7-22.7)	0.9 (0.6-4.3)
AML (CR 1) bone marrow (n = 23)	0.5 (0.06-1.3)	0.14 (0.006-1.0) 4-HC purged

Table 1. Median number of mononuclear cells (MNC) and CFU-GM transfused per kg b.w.
AML = acute myelogenous leukemia; CR = complete remission

TABLE 2

Median number of mononuclear cells (MNC) and CFU-GM per apheresis in patients with advanced stage Hodgkin's disease

	MNC x 10^9	CFU-GM x 10^4
rh GM-CSF (Behring) (n = 4)	4.9 (2.8-9.9)	25.4 (11.5-169)
chemotherapy (ara-C/ Daunorubicin or Cyclophosphamide) (n = 8)	3.7 (2.4-7.1)	5.4 (1.4-139)

TABLE 3

Median number of aphereses per patient, mononuclear cells (MNC) an CFU-GM transfused per kg b.w.

	no.aphereses	MNC x 10^8	CFU-GM x 10^4
chemotherapy primed (n = 8)	13	5.9 (1.7-22.7)	0.75 (0.6-4.3)
GM-CSF primed (n = 4)	6	5.75 (4.0-6.7)	2.2 (0.9-3.0)
no priming (n = 3)	6	6.4 (0.9-10.2)	1.0 (0.45-3.0)

FIGURE 1
Schematic course of white blood cell (WBC) reconstitution following autologous blood stem cell transplantation. A first transient peak originating from more mature hematopoietic precursor cells is followed by reconstitution form the early pluripotent stem cells.

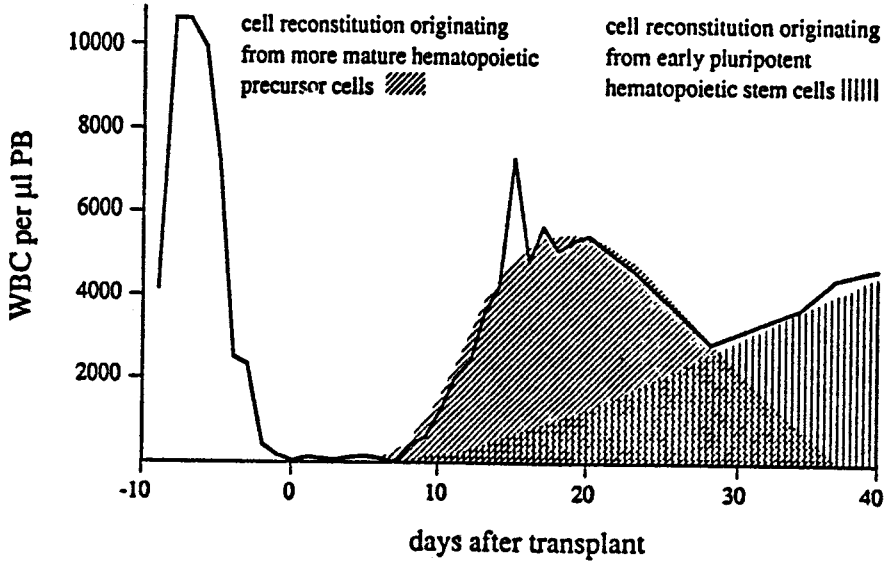


FIGURE 2
Schematic course of white blood cell (WBC) reconstitution following autologous blood stem cell transplantation (ABSCT) or autologous bone marrow transplantation (ABMT).

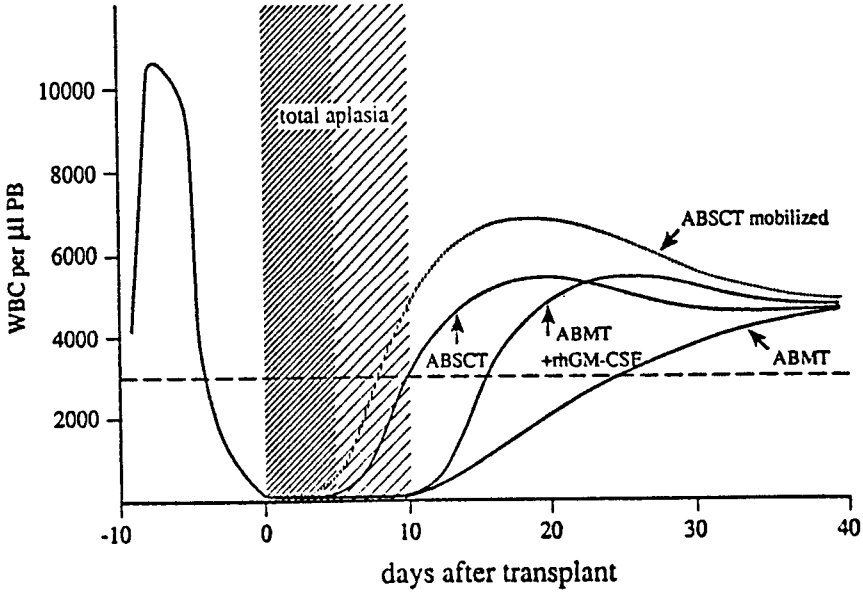
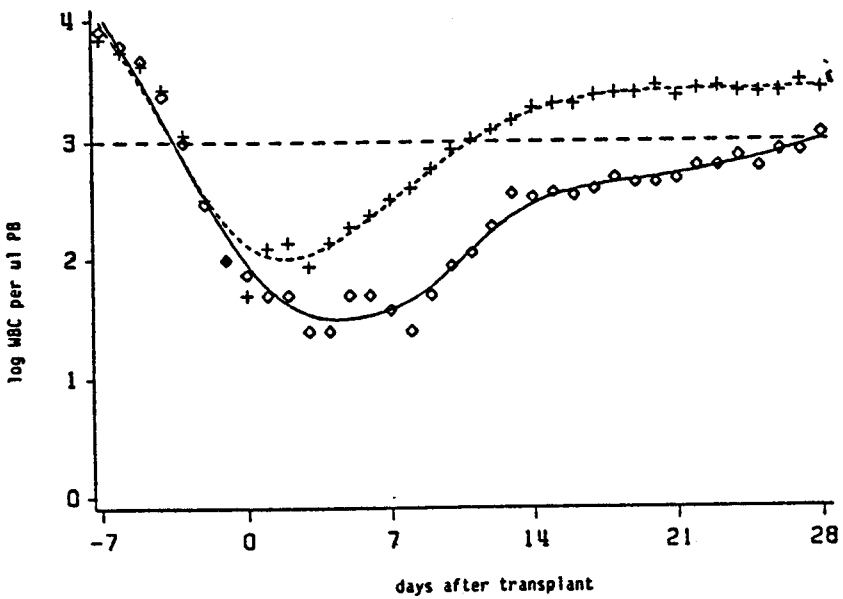


FIGURE 3
Course of peripheral white blood cell (WBC) concentration following myeloablative therapy and autologous transplantation of blood derived (---) versus bone marrow derived ( ) stem cells on a daily blood count basis. Day 0 = day of transplantation.



FREQUENCY OF DETECTION OF SUSPECTED LYMPHOMA CELLS IN PERIPHERAL BLOOD STEM CELL COLLECTIONS

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INTRODUCTION

We have previously reported preliminary data which suggested that peripheral blood stem cell (PBSC) harvests of cancer patients whose marrow was known to be involved with tumor appeared not to be contaminated with tumor in the majority of cases (1). Similar results have been reported in a study of 5 patients with non-Hodgkin's lymphoma (NHL), all of whom had a germ-line configuration of T cell receptor and immunoglobulin heavy chain genes in their PBSC harvests although two of the five had a monoclonal rearrangement evident in the bone marrow (2). Horning et al. (3) have reported that the incidence of circulating lymphoma cells detected by gene rearrangement analysis is related to the histologic subtype, stage of disease and clinical status.

We have recently reported that PBSC harvests of breast cancer patients appeared to be less likely to be contaminated with tumor cells than the corresponding bone marrow (4). This report describes our current experience with the application of long term cultures to detect tumor cells in the PBSC harvests of patients with non-Hodgkin's lymphoma (NHL) and Hodgkin's disease (HD).

MATERIALS AND METHODS

Bone Marrow

Cells, fat, and particulate material collected during the filtering process of freshly harvested marrow were scraped from the screens and placed in HBSS. They were then processed for initiation of cultures as reported previously (5). Flasks (25 CM2) containing $2x10^7$ cells were incubated at 37C for NHL samples. HD samples were incubated for 7 days at 37C and then moved to 33C for the remainder of the study. Flasks were demidepopulated weekly. Weekly cellularities and cytospins were performed on the NHL samples because they produced non-adherent cell populations. Flasks from HD samples were examined by trypsinizing the adherent layers and making

cytospins. All cytospin preparations were coded and examined by the pathologist for morphologically abnormal cells.

Peripheral Stem Cells

Apheresis (PBSC) samples were washed once with calcium and magnesium free Hank's balanced salt solution (HBSS) and processed for initiation of cultures as described previously (5). Flasks (25 CM2) with $2x10^7$ cells in 10 ml medium were cultured in a 5% CO_2 in air atmosphere in a 37C incubator. Cytospin preparations were made from the cultures on a weekly basis, blind coded, and examined by the pathologist for morphologically abnormal cells.

Cytologic and Immunochemical Techniques

Cells obtained directly from the harvest screens and cells harvested from the long-term cultures were spun onto precleaned glass slides using a cytospin centrifuge (Shandon Southern, London, UK). The slides were air dried and stained with Wright's stain. Slides were also fixed for 2 min in ice-cold acetone and stained with an avidin-biotin immunoperoxidase technique using antibodies to surface markers.

Flow Cytometry

Cultured cells were aliquoted at 10^6 cells/tube and washed 2X with fluorescence buffer (FB) (phosphate buffered saline, pH 7.4, 5% FCS and 0.05% NaAzide) and incubated for 30 min at 4C with saturating concentrations of directly labelled monoclonal antibodies conjugated either with phycoerythrin (PE) or fluorescein. The cells were washed 3X with FB and analyzed on an EPICS C flow cytometer (Coulter) using Coulter software. Background fluorescence was determined by incubating an aliquot of cells with directly conjugated class matched myeloma controls. Antibody combinations utilized were CD15-FITC/CD38-PE, CDl5FITC/CD25-PE, and CD25-FITC/CD13-PE. DNA analysis was performed utilizing standard techniques (6). Briefly, an aliquot of 10^6 cells was added to 1 ml Vindelov's solution and incubated for 2 hr at 40C. The nuclei were then analyzed on a Coulter EPICS C flow cytometer utilizing normal peripheral blood lymphocytes as a diploid standard.

Molecular Probing Techniques

Cultured cells were subjected to molecular probing for immunoglobulin heavy chain (JH) and T-cell receptor (CT,3) gene rearrangements as described previously (5).

Karoytyping Techniques

Five ml of the primary culture were combined with 5 ml of fresh media in a new flask and incubated overnight at 37C. 0.2 ml of Colcemid (10 ug/ml, Irvine Scientific) were added for 45 min at 37C. The cells were then pelleted, gently resuspended in 3 ml of 0.7% sodium citrate and incubated at 37C for 25 min. After centrifugation, the pellet was resuspended in 3 ml of fixative (3

parts methanol to 1 part glacial acetic acid) . The cells were again centrifuged and then resuspended in 3 ml of fresh fixative and left overnight at 40C. Before preparing the slides, the cells were fixed and spun again. Cells were dropped on cold, wet slides and aged overnight in a 65C oven. The slides were stained with buffered Wright's stain and the metaphases scored for numerical and structural abnormalities.

Nude and SCID Mouse Studies

Nude (Balb/c, Life Sciences Inc.) mice were injected subcutaneously and intraperitoneally and CB17 SCID mice were injected intraperitoneally with 5×10^6 to 1×10^7 cells from cultures growing BerH2 and IRac positive cells. Animals were monitored weekly for circulating abnormal cells using peripheral blood smears. Animals were euthanized and autopsied eight months following injection of the human cells.

RESULTS AND DISCUSSION

The relative frequencies of detection of suspected tumor cells in the PBSC versus bone marrow harvests of all patients with solid tumors, NHL and HD, are presented in Table 1.

The results demonstrated that the frequency of suspected tumor cells in the apheresis harvests of solid tumor patients was lower than in bone marrow harvests. However, this difference was not statistically significant (Table 1). When the solid tumors other than breast cancers, i.e. melanoma, ovarian carcinoma, neuroblastoma and a small number of sarcomas were excluded, the frequency of suspected tumor cells in the breast cancer apheresis harvests (19%) was significantly lower ($P=0.03$) than for bone marrow (46%) (4) . The frequency of suspected lymphoma cells in the apheresis harvests of patients with intermediate and high grade lymphomas was significantly lower than for bone marrow. Although the numbers were also lower for low grade lymphoma, the difference was not statistically significant because of the small sample size. When the data are combined for all NHL patients, the frequency of suspected tumor cells in the apheresis harvests was significantly lower than in the bone marrow harvests.

The converse was true of patients with Hodgkin's disease (HD), for whom the apheresis harvests showed a significantly greater frequency of morphologically abnormal cells when placed in culture than cultured bone marrow harvests. Initially, we were concerned that the culture techniques applied to apheresis harvests might be less sensitive than when applied to bone marrow (1). Consequently, we have performed calibration studies for a breast cancer cell line (MCF-7), T cell line (CEM) and B cell line (RAJI) added to normal apheresis harvests in a manner similar to recent calibration studies performed using bone marrow (7). The results showed that there was not a significant difference in the ability to detect breast tumor cells (4) or lymphoma cells (data not shown) in the cultures of bone marrow or apheresis harvests.

These observations are conditional due to some reservations concerning the methods employed to confirm that the suspected tumor cells were indeed tumor cells. Although morphological criteria are probably adequate for breast cancer (4), more specific criteria must be employed for the lymphomas. In NHL, it is possible to employ molecular probing to demonstrate that the suspected tumor cells match the phenotype of the original tumor (8). Unfortunately, in Hodgkin's disease, there are no markers specific for the ReedSternberg cell, which is usually identified on the basis of its unique morphology in tissue sections. However, it is uncertain whether morphological features can be used to identify these cells in cultures (9). The exact nature of the morphologically abnormal cells from HD patient apheresis harvests is puzzling. Morphologically, the abnormal cells are first evident after 4-6 weeks of culture as sporadic clusters of cells among a majority of histiocytes mixed with small- to medium-sized mononuclear cells. Such cultures have an easily detectable population of these cells by eight weeks. The cells are large and occasionally binucleated, with prominent nucleoli and abundant basophilic cytoplasm (Fig. 1A). They stain immunocytochemically with Ber-H2 (anti-CD30) antibody in a characteristic paranuclear Golgi and membranous pattern (Fig. 1B). They also stain with anti-IRAC antibody kindly provided by Dr. Hsu Ming Hsu (10,11). They occasionally also stain with LeuM1 (CD15) and CD13. Immunocytochemical staining of early cultures suggests that these cells develop from small BerH2 positive mononuclear cells which are present as early as 3-4 weeks, but not distinguishable morphologically from other mononuclear cells in the cultures. These cells did not grow over an eight month period when transplanted subcutaneously into nude or intraperitoneally into nude or SCID mice. Intracerebral transplantation has not yet been attempted (12). Although these cells morphologically and immunocytochemically resemble the ReedSternberg cells seen in sections, and are very similar in these respects to the Hodgkin's cell line, HD1, kindly provided by Dr. Hsu Ming Hsu, the lack of definitive criterion for identifying Reed-Sternberg cells leaves their exact nature an open question.

A high incidence of Epstein-Barr virus (EBV) genomes has been reported in Hodgkin's disease (13). Our study is further complicated by the observation that some cells in these cultures share morphological and phenotypic markers which are generated in culture by herpes (particularly Epstein-Barr) virus-infected cells. Continuous maintenance of most of the cultures which contain Ber-H2 cells early for periods of 10-20 weeks has demonstrated that morphologically abnormal cells become the majority population in the cultures. This permits the harvesting of sufficient cells for flow cytometric phenotyping (6 patients) and molecular biological analysis (5 patients) (Table 2).

We have some evidence from studies of a leukemic cell line, Croco II (14) that lymphoblastoid cells can coexist with cells having a monocytoid (CD15 positive) population, and possibly the latter are important for the continued growth of the former. This suggests the possibility that, in our apheresis harvests from HD patients, a minority population of CD30+, CD15+

and/or CD13+ myeloid or dendritic cells early in the cultures (4-6 weeks) might promote the later outgrowth, possibly by factor production (IL6?) (15), of B lymphoid cells in the presence of EBV. Other factors such as IL-1 (10) or IL-5 (16) might be involved. A similar outcome could also occur if a bipotential precursor cell initially differentiated mostly to CD15+ cells and later B lymphoid cells. Also, we cannot exclude that a hybrid cell could potentially produce this same result (9,17,18).

In an attempt to resolve the question as to the significance of morphologically abnormal cells in apheresis harvests particularly of HD patients we have attempted a preliminary analysis as to whether there are any clinical correlates to the presence of these cells. on the basis of a simplistic interpretation of the data in Table 1, the clinical outcome of high dose therapy with autologous rescue by using apheresis harvests would be predicted to be better than for bone marrow for patients with breast cancer and NHL, and worse for HD. It is too early to evaluate the results in breast cancer.

We have very limited preliminary data concerning NHL (Figure 2). These results suggest that recipients of apheresis harvests have a better outcome than recipients of bone marrow. Currently, there is no difference in outcome for apheresis positive and negative HD recipients but the follow-up is short (1 year).

CONCLUSIONS

We have employed culture techniques to evaluate the frequency of suspected tumor cells in patient bone marrow and apheresis harvests. In patients with breast cancer and non-Hodgkin's lymphoma, suspected tumor cells are observed significantly less frequently in the apheresis harvests than in bone marrow harvests. This does not appear to be due to any differences in the sensitivity of the culture technique as applied to these harvests. In contrast, morphologically abnormal cells are observed more frequently in the apheresis harvests of patients with Hodgkin's disease than in their bone marrow harvests. Cultures of such apheresis harvests initially contain morphologically abnormal cells which resemble ReedSternberg cells and which express myeloid (monocyte- or dendritic cell-associated) markers. Later, such cultures generate clonal B lymphoid populations which are almost certainly derived by culture selection and promotion of clonal dominance of Epstein-Barr virus infected polyclonal or oligoclonal B cells. Whether this has any relevance to HD in vivo is unclear. The observation of suspected tumor cells in marrow harvests of breast cancer or non-Hodgkin's lymphoma patients is associated with a poor prognosis. It is too early to evaluate if the differences in frequency of such cells in apheresis harvests versus bone marrow is associated with differences in outcome. However, there is a hint that non-Hodgkin's lymphoma patients with marrow involvement who receive PBSC transplants may do better than those who receive marrow. A randomized trial to evaluate this possibility appears justified.

ACKNOWLEDGEMENTS

This research was supported in part by an Imogene Jacobs Memorial Grant from the American Cancer Society. We thank Dr. Hsu Ming Hsu for kindly making available the IRAC antibody and HD cell lines. We also thank Don Daley, Molly Lang and all members of the UNMC Transplant Team for valuable assistance in conducting these studies.

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TABLE 1

Harvest Type	Primary Tumor Type			
	Solid Tumors	Non-Hodgkin's Lymphoma		Hodgkin's Disease
		Intermed./High Grade	Low Grade	
Bone Marrow	23/62 (31%)	24/61 (39%)	2/4 (50%)	3/58 (5%)
Apheresis	6/28 (21%)[a]	1/11 (9%)[b]	0/6 (0%)[c,d]	14/38 (37%)[e]

[a]Not significantly different from bone marrow (P=0.11); [b]significantly lower than bone marrow (P<.05); [c]not significantly different from bone marrow (P=.13); [d]for all non-Hodgkin's lymphomas combined, 1/17 (6%) positive apheresis harvests is significantly lower than 26/65 (40%) for bone marrow (P=.005); [e]significantly greater than for bone marrow (P=.0001).

TABLE 2

Phenotypic Marker	Patient Sample ID#					
	1	2	3	4	5	6
CD15+	3	0.5±0.3	0	2±1	1±0.7	10±2
CD38+	22	78±7	70	40±7	28±5	53±1
CD25+	ND	0	ND	ND	ND	5±4
CD13+	ND	ND	ND	ND	ND	9±4
CD15+/ CD38+	2	8±3	0	3	0	16±6

a. Molecular biologic studies of patients with ID#'s 3, 4, and 5 all showed a monoclonal B cell population but comparison of different cultures from the same apheresis harvest showed they were derived by positive selection of dominant clones from a polyclonal or oligoclonal source. All were positive for EBV genome using the Bam HIW probe.

b. Cytogenetic studies of patients with ID#'s 3 and 6 showed normal karyotypes with random chromosome rearrangements.

c. Cells from patients with ID#'s 1 and 6 failed to grow intraperitoneally in SCID mice. One mouse developed a thymoma suspected to be of mouse origin.

FIGURE 1

Morphologically abnormal cells in cultured apheresis harvests from a Hodgkin's disease patient's apheresis harvest.
A. Wright's-Giemsa stain. B. Immunocytochemical staining with Ber-H2 (anti-CD30) antibody.

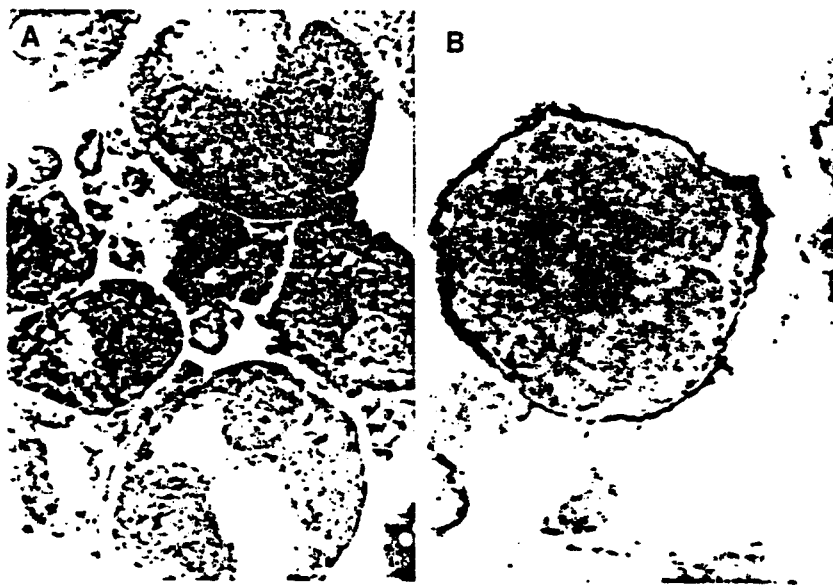
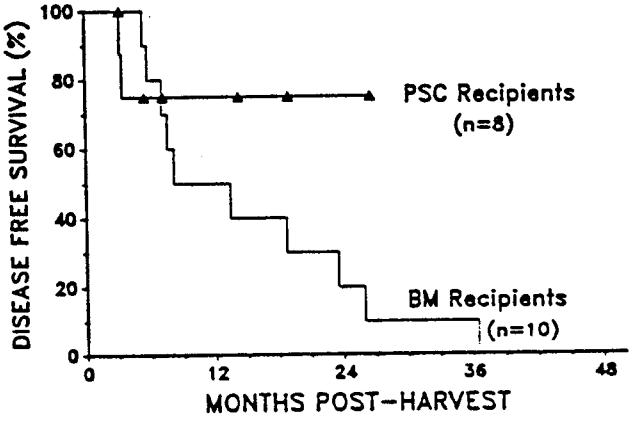


FIGURE 2

Disease-free survival of patients with non-Hodgkin's lymphoma involving their bone marrow receiving either bone marrow (BM) or peripheral blood stem cell (PSC) transplants.



TREATMENT OF CHILDHOOD ACUTE LEUKEMIAS AND LYMPHOMA WITH HIGH-DOSE CHEMOTHERAPY AND PERIPHERAL BLOOD STEM CELL AUTOGRAFTS

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INTRODUCTION

Progress is being made in the treatment of childhood leukemias and non-Hodgkins lymphoma (NHL) but the salvage of patients relapsing from the disease on first-line chemotherapy is very difficult. One potential approach to overcoming drug resistance is the use of dose-intensive therapies. However, the results of autologous as well as allogeneic bone marrow transplantation (BMT) in these patients are generally poor.

Once restricted to patients who are not the candidates for BMT, high-dose chemotherapy and autografts with peripheral blood stem cells (PBSC) have become viable therapeutic procedures for stem cell rescue. These procedures are increasingly used for the treatment of patients with high-risk leukemia or NHL as an alternative to the use of autologous BMT, possibly eliminating the risk of leukemic contamination in the graft. Both the absence of graft-versus-host disease (GVHD) and the rapid recovery of granulopoiesis make PBSC autografts (PBSCT) far safer than allogeneic BMT. However, the use of PBSCT in children with acute leukemias or NHL is quite limited and the value of this approach in producing long-term survival of these patients needs clarification. The ideal condition in which to test the efficacy of PBSCT is the salvage therapy for patients relapsing shortly after the initiation of chemotherapy.

We have developed a PBSCT program for the treatment of children with cancer. We have demonstrated that PBSC can be safely collected from children and that PBSCT is an useful procedure in reducing the period of cytopenia following marrow-updated results of a study in 31 children with high-risk acute leukemias or NHL are presented in this paper.

PATIENTS AND METHODS

Patients

Full description of the procedure and preliminary results of the clinical trial have been published elsewhere(1-5). In a multi-center trial underway since 1987, we have treated a total of 31 children with acute leukemias or NHL with PBSCT at transplant centers listed in Table 1. The characteristics and clinical outcomes of these patients are shown in Table 2. None of these patients had HLA-compatible marrow donors. The risks of the treatment protocols were explained in detail and a consent form was obtained from the guardians. The mean age of the patients was 10 years (range, 1-15 years); 18 had acute lymphoblastic leukemia (ALL), 8 had acute non-lymphoblastic leukemia (ANLL) and 5 had NHL. Four patients underwent PBSCT at the 2nd or 3rd relapse.

Thirteen of the 27 patients transplanted in complete remission (CR) were associated with one or more very high-risk (VHR) features: relapse within 18 months of first-line therapy (8), multiple relapses (2), or primary resistance to induction therapy (3). In the 10 relapsed patients, median duration of the preceding remission was 6 months (range, 2-16 mo). Because all these patients had heavily pretreated diseases, we did not rely on drugs such as vincristine, prednisolone, 6-MP, cyclophosphamide or low to intermediate-dose methotrexate (MTX), Instead, attempts to reinduce and maintain complete remissions included 1) combined use of mitoxantrone, 1-asparaginase plus cytosine arabinoside (CA) or 2) high-dose CA (HD-CA) regimen (2.0 g/m^2 x 10), with or without daunorubicin (30 mg/2 x ^{2}. The potential of these regimens to prolong CR before PBSCT was satisfactory and no patient developed relapse after being transferred into the PBSCT protocol. Furthermore, we found that in children, the HD-CA regimen has a marked mobilization effect on PBSC in patients with both myeloid and lymphoid malignancies.

A high-risk group (HR) included two with late relapse and 12 first CR patients associated with two or more high-risk features (WBC count >100 x $10^9/L$ massive organ involvement at presentation: leukemia/lymphoma syndrome, T- or B-phenotype, infant). After the induction of remission, the patients were removed from conventional protocols to be placed on a cyclic mode of intensive therapy administered every 4-6 weeks. They received a minimum of one course of consolidation chemotherapy, the goal being the mobilization of PBSC and the reduction of tumor load prior to PBSCT. All patients received central nervous system (CNS) prophylaxis with a minimum of 5 courses of intrathecal administration of MTX (15 mg/m^2), CA (30 mg/m^2) and hydrocortisone (60 mg/m^2) before PBSCT.

Collection and Storage of PBSC

The patients underwent apheresis for the collection of PBSC by a Fenwall CS 3000 cell separator (Fenwall Lab., IL), using regular program "3" in the phase of bone marrow recovery after chemotherapy as reported elsewhere

(1-3). The procedure was started when the blood cell counts were rising rapidly after chemotherapy and when there were no visible tumor cells in the bone marrow. Based on our experience, the yield of PBSC by apheresis can be predicted by the magnitude of the increment speed of monocyte and platelet counts, although quantitation is difficult.

In small children, the extracorporeal line was primed with 400 mL of leukocyte-depleted donor red blood cells after regular priming with normal saline to prevent rapid blood volume change at the very beginning of apheresis. Acid-citrate-dextrose was used as an anticoagulant and calcium gluconate (15-25 mL) was administered continuously using a pump during apheresis to prevent hypocalcemia. Generally the morbidity related to the PBSC harvest was small. Apheresis-derived cells were subsequently fractionated on a discontinuous gradient of 40% and 60% Percoll. Cells recovered in the interface were then frozen using a Cryo-Med programmed freezer and stored in the liquid phase of liquid nitrogen, No leukemia purging procedure was used. Details of the hematopoietic progenitor assay in methylcellulose with a low leukocyte conditioned medium have been reported. Triplicate cultures were incubated and CFU-GM, BFU-E and CFU-mix were scored on day 14.

The number of CFU-GM harvested in the first apheresis at Tokushima was 4.6-fold higher than that in the second, which was performed 1 to 4 days later (n=23). The number of CFU-GM collected after the first course of consolidation therapy was also compared with those obtained in apheresis performed after subsequent courses of chemotherapy: the yield was 9.2-fold higher in the first procedure (n=18). Hence, we now perform two aphereses in the initial collection trial and then each one per subsequent chemotherapeutic course. The mean number of CFU-GM collected in 12 patients younger than five years was 9.2 x 10^5/kg body weight, and 1.7 x 10^5/kg in those older than 10 years (n=15). Thus, a larger proliferative capacity of hematopoiesis may exist in smaller children compared with their older counterparts.

Cytoreductive Regimen and Transplant Procedures

An autograft with PBSC lacks the Graft-vs-Leukemia effect. The available chemotherapy regimens including total body irradiation (TBI) are highly toxic on growing children. However, it still is inadequate to eradicate the clonable leukemic cells in many cases when used in autografts. In an effort to achieve a greater antileukemic effect, we developed a multi-drug combination chemotherapy without TBI as a conditioning regimen. After the dose-finding trial, the "MVAC" regimen became the standard in the current study. It consists of MCNU 250 mg/m^2 on day -8, and 200 mg/m^2 on day -3, CA (2.0 g/m^2) and VP-16 (200 mg/m^2) b.i.d. on days -7 through -4, and cyclophosphamide (50 mg/kg) on days -2 and -1.

Patient management protocols differed between the participating centers. At the University Hospital in Tokushima, the patients were cared for in single-bed rooms with a laminar air flow facility, but received no gut sterilization with non- absorbable antibiotics. All received prophylactic acyclovir 15 mg/kg/day by mouth or vein. Cytomegalovirus (CMV) prophylaxis

consisted of 2.5-5.0 g alkylated hyperimmune CMV globulin preparation intravenously once a week. Leukocytes were removed from all blood products by the use of cotton wool-packed filter columns (Imugard IG-400Y, Terumo Co., Tokyo) before transfusion to prevent the occurrence of CMV pneumonitis and transfused lymphocyte-mediated GVHD.

Thirty-six hours after completion of the cytoreductive regimen, the cells were rapidly thawed at 37C and promptly infused into the patients through a central venous catheter without any additional post-thaw washing manipulation (day 0). No further specific antileukemia therapy was given after PBSCT.

RESULTS

Toxicities of the Procedure

In the initial dose-finding study five patients developed reversible interstitial pneumonitis secondary to the conditioning regimens used. The protocol was altered to reduce dosages in a subsequent study and the toxicity was limited to self-subsiding nausea, vomiting, mucositis and diarrhea. Despite a simplified infection control protocol without the use of strict isolation technique, the incidence of severe infections was low and no confirmed episodes of CMV infection were observed. All febrile episodes were treated successfully with parenteral antibiotic therapy and there were no deaths related to infectious complications. One patient who underwent PBSCT at relapse died of intracranial bleeding due to thrombocytopenia; this was the only procedure-related mortality.

Hematological Engraftment

We observed that the log of CFU-GM content infused per kg body weight of the patients treated at Tokushima, but not the number of nucleated cells, was significantly related to the recovery of granulopoiesis (unpublished data). Early hematological engraftment was not seen in six patients who received 0.5×10^4, 0.7×10^4, 0.9×10^4, 1.5×10^4, 2.1×10^4, and 2.2×10^4 CFU-GM/kg. In these patients prolonged thrombocytopenia was observed but without life-threatening hemorrhages, and subsequent gradual recovery from the hematopoiesis followed.

In 14 patients who received more than 1×10^5 CFU-GM/ g, the mean number of days required to achieve an AGC of 0.5×10^9/L was 10.7 (range, 6-15), and a platelet count of 50×10^9/L was reached by 18 days (9-46). All patients showed transient and clinically insignificant decreases in the number of once recovered granulocytes and platelets 3-7 weeks post-transplant.

Clinical Results

The interval from the diagnosis or relapse to PBSCT varied from 2 to 18 months, depending on the progenitor yields by leukapheresis. Patients who had a refractory disease tend to require a longer pre-transplant consolidation for the collection of PBSC than those remaining in the 1st CR, in whom only one

or two aphereses are required for the collection of enough PBSC for safe autografts.

The study results were updated August 1, 1990. After PBSCT, 3 of the 4 patients transplanted at relapse developed recurrence of leukemia 20 days, 3 and 3 months post-transplant and subsequently died. The remaining patient died in CR due to intracranial bleeding at 3 months. Among 13 VHR group patients, a local recurrence of the disease developed in 2 cases: one in the mediastinum at 2 months post-transplant, and in the CNS at 7 months in the other patient with chronic CNS leukemia. Two patients with ANLL developed systemic relapse at 4 and 11 months, respectively. The remaining nine children are surviving disease-free 2, 3, 3, 7, 9, 10, 16, 26, and 32 months, with low performance and nutritional status (Figure 1).

In the HR group, four patients developed systemic relapse 2, 3, 5, and 7 months after PBSCT, but nine have survived for 2, 3, 4, 4, 5, 15, 16, 18, and 29 months, After relapse remission was not obtained with chemotherapy in six patients and five of them died with overwhelming leukemia. One is alive with refractory disease. Three patients were reinduced with chemotherapy and are alive in complete remission.

When the time period between the initial diagnosis or relapse to PBSCT was analyzed, 8 of the 9 relapsed patients underwent PBSCT within 5 months of the diagnosis or relapse (Figure 2). On the other hand, most of the surviving patients underwent PBSCT after 5th month. Based on these data, since April of 1990 a minimum of four consolidation chemotherapy treatments are given before PBSCT.

DISCUSSION

This series is the largest updated report of high-dose chemotherapy and PBSCT in the treatment of childhood leukemias and NHL. We have demonstrated that this therapeutic approach is associated with a low rate of severe complications and can be performed in a regular pediatric ward without strict isolation techniques when a larger number of PBSC are infused.

The superiority of this procedure over current intensified chemotherapeutic regimens or ABMT in terms of the risk of leukemic contamination and the ability of inducing a long-term survival of patients can be assessed with more confidence in patients relapsed early on chemotherapy than those in the first CR. In the Dutch study of 164 children with relapsed ALL, no survivors as reported in those who relapsed within 24 months of diagnosis (7). Even with intensified therapy, the long-term survival rate in these children is low at best, (8,9) whereas 30% of the children with late marrow relapse achieved long-term survival (10). In this setting, our very preliminary data compare favorably with those of previous studies with ABMT or intensified chemotherapy, However, we used different preparative regimens in this study and statistical evaluation of survival is not possible due to the small number of patients and shorter period of follow-up. A randomized clinical trial

is needed to define the role of current therapeutic options in these patients; an expanded group-wide trial is ongoing.

PBSCT as a primary upfront therapeutic regimen, short-term induction therapy followed by consolidation* and marrow ablative therapy with PBSCT, may shorten the treatment period and limit the exposure of patients to the deleterious effects of chemotherapeutic drugs. On the other hand, hematopoietic stem cells have limits on their proliferative capacity and repeated cytotoxic chemotherapy also leads to a reduced potential of stem cells (11). The quality of life-long, stable reconstitution of hematopoiesis after PBSCT has not yet been proven. At the present time the application of PBSCT during an initial remission should be limited to patients at very high risk of subsequent relapse.

Interestingly we detected no essential difference between the VHR and HR groups in the leukemia relapse rate after PBSCT. A substantially higher incidence of relapse than was expected was noted in the HR patients. The only predictiVe factor for remission appeared to be the duration of pre-transplant consolidation therapy. When relapse occurs after PBSCT, it is not possible to determine the exact origin of the leukemic cells; whether relapse is caused by residual leukemic cells in the patient or leukemic cells in the infused graft. However, we think that residual leukemic cells which escape the conditioning regimen are primarily responsible for inducing most relapses. In our patients, the time and dose-intensity of pre-transplant consolidation therapy would affect subsequent relapse after PBSCT. The observed time effect on relapse after PBSCT may not reflect only the patient selection bias. This was also true for VHR patients, for whom long-term survival or cure with currently available chemotherapy alone is unlikely.

Patients who relapse after PBSCT are very unlikely to become long-term survivors due both to the resistance of leukemia cells to subsequent chemotherapy and to the diminished marrow reserve capacity after PBSCT. Intensification of the conditioning regimen to prevent relapse without increasing appeared to be limited. Hence, more intensified and prolonged pre-transplant consolidation is required both for the control of the disease before PBSCT and collection of a sufficient amount of PBSC; this may result in the decrease of post-transplant relapse by decreasing the tumor bulk subjected to high-dose chemotherapy and PBSCT.

In conclusion, the preliminary study obviously needs to be replicated with a larger number of patients and prospective research design. Nevertheless, the data justify the incorporation of PBSCT in the design of salvage protocol for children with leukemias or NHL. A randomized clinical trial is needed to define the role of current therapeutic options which include intensive chemotherapy, allogeneic or autologous BMT, and PBSCT in children relapsing from leukemia/NHL. Application of this procedure as part of the initial therapy for patients with high-risk features should be regarded as highly experimental and deserving of further clarification, The establishment of the criteria to identify the patients most likely to benefit from PBSCT is of major interest.

ACKNOWLEDGEMENT

The authors are grateful to many physicians and the nursing staff involved in the care of these patients, the physicians who referred patients for treatment in our program, and to Drs. T. Masaoka, S. Asano, M. Ohira, R. Hosoya, R. Onishi, M. Harada and T. Fujimoto for valuable suggestions. This work was supported by grants from the Ichiro Kanehara Foundation, Osaka Cancer Research Association, Kudoh Foundation for Scientific Research, Tokyo Biochemical Research Foundation, Children's Cancer Association of Japan, Japanese Foundation for Multidisciplinary Treatment of Cancer, Mochida Memorial Foundation for Medical and Pharmaceutical Research, Kowa Life Science Foundation, Uehara Memorial Foundation, Inoue Foundation for Science, Grant-in-aid for Cancer Research from the Ministry of Health and Welfare (1-44) and the Ministry of Education, Science and Culture of Japan (01010017). Authors' affiliations: Department of Pediatrics, University Hospital of Tokushima, Tokushima; Jikei University School of Medicine, Tokyo: Fukushima Medical College, Fukushima; University of Akita, Akita; National Medical Center Hospital, Tokyo; University of Kobe, Kobe; Kawasaki Medical School Hospital, Kurashiki; and Shikoku Cancer Center Hospital, Matsuyama, Japan.

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FIGURE 1

Actual disease-free survival after PBSCT for 13 patients with very high-risk (VHR) acute leukemias or non-Hodgkin's lymphoma (----) and 14 patients associated with high-risk (HR) features (....) treated with high-dose chemotherapy without TBI. Refer to the text for the definition of risk factors.

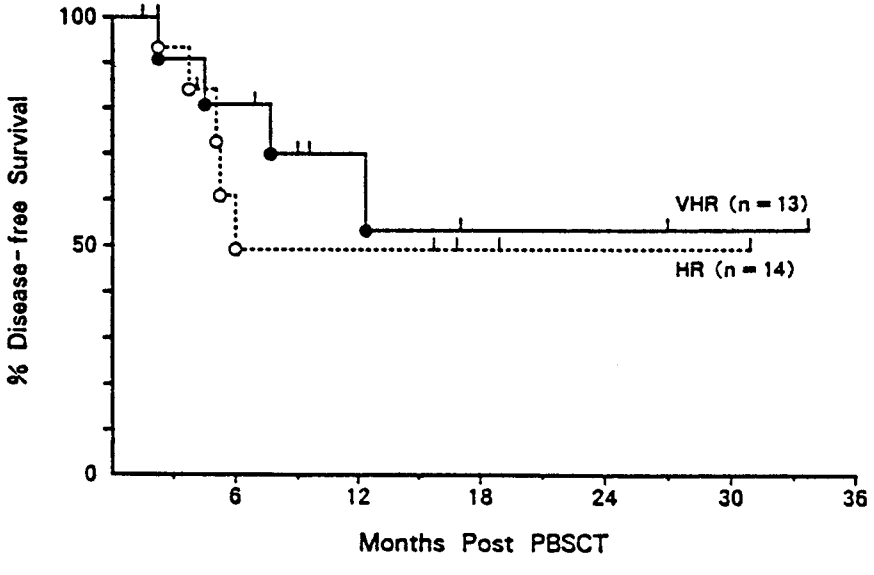

FIGURE 2

Retrospective analysis of the time period between the initial diagnosis or relapse to PBSCT was performed in patients relapsed after PBSCT (left) and those remaining in CR (right). Closed bar indicates the data for VHR patients and open bar for HR patients. Surviving patients received more consolidation than those relapsed after PBSCT but there was no essential difference in the observed time effect on relapse between VHR and HR group.

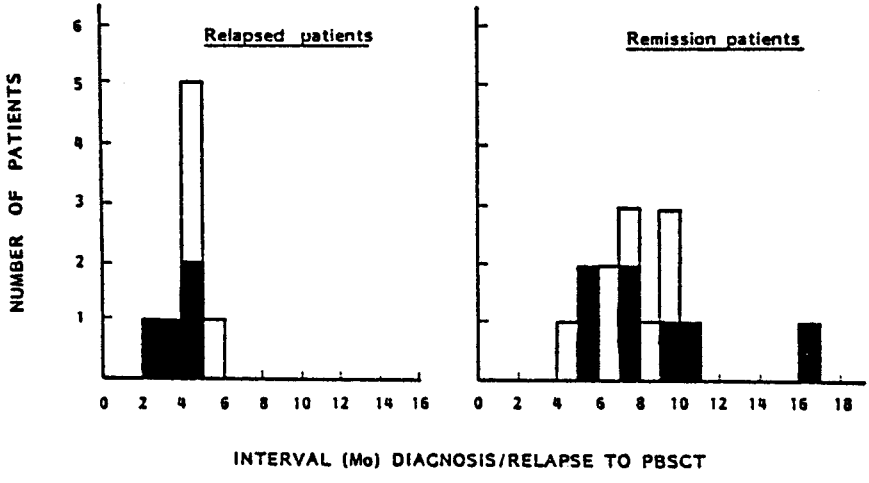

TABLE 1

Participating Institutions and Investigators in this study.

INSTITUTION	INVESTIGATOR
Jikei University School of Medicine, Tokyo	Y. Hoshi, H. Uchiyama
National Medical Center Hospital, Tokyo	T. Matsushita
University of Kobe Kobe	R. Murakami, K. Sano, Y. Kosaka K. Ikeda, T. Maeda
University of Akita Akita	A. Watanabe, K. Ishikawa
Fukushima Medical College Fukushima	A. Kikuta, M. Katayose, M. Watanabe
Kawasaki Medical School Hospital, Kurashiki	A. Yokobayashi, T. Morita
Shikoku Cancer Center Hospital Matsuyama	T. Shimokawa, T. Koyama, T. Suzue
University of Tokushima Tokushima	Y. Takaue, T. Abe, K. Matsunaga S. Saito, T. Watanabe, Y. Kawano, A. Hirao, S. Azegawa, M. Hirose, T. Ninomiya, Y. Kuroda

TABLE 2

Characteristics and clinical course of the 27 patients who underwent high dose chemotherapy and peripheral blood stem cell autografts.

UPN	Age/Sex	Dx/Status	Interval Dx/Relapse to PBSCT	Regimen	No. of Cells Infused: Cells (x10⁹)	No. of Cells Infused: CFU-GM (x 10⁴)	Days to Reach: ANC 0.5K	Days to Reach: Platelet 20K	Days to Reach: Platelet 50K	Duration of CR in months
VERY HIGH-RISK: n=13										
UT-001	5/F	T-NHL/2nd CR	2	m MVAC	3.2	35.6	10	13	15	2
UT-002	13/F	ANLL/1st CR*	4	Bu + CY	21.0	1.5	18	55	195	11
UT-003	3/F	T-ALL/1s CR*	5	m MVAC	14.3	203	6	10	12	32+
UT-007	10/M	ALL/2nd CR	16	Bu+MCNU	13.2	2.2	29 (CSF)	320	427	26+
UT-009	3/F	ANLL/2nd CR	3	MVAC	18.0	200	9	10	13	4
UT-011	10/F	ALL/4th CR	4	MVAC	19.2	9.1	19	80	7	7
UT-012	10/M	ALL/2nd CR	10	MVAC	38.0	46.4	13	-	14	16+
UK-001	14/F	T-ALL/2nd CR	4.5	MVAC	13.4	10.0	14		30	10+
JU-003	2/M	ANLL (M7)/2nd CR	5.5	MVAC	2.8	16.4	34	69	87	9+
UA-002	9/F	ANLL (M2)/1st CR*	11	Bu+CY+CA	5.2	26.8	11		110	7+
FMC-003	6/M	ALL/2nd CR	12	MVAC	21.4	34.4	14	25	45	3+
UT-021	8/M	ALL/2nd CR	7	MVAC	28.3	109	10	8	14	3+
UT-022	8/M	ALL/4th CR	7	MVAC	16.0	8.2	11	14	34	2+
HIGH-RISK GROUP: n=14										
UT-005	11/M	T-NHL/1st CR	9	Bu+MCNU	20.8	32	13	14	16	29+
UT-010	12 mo	ALL/1st CR	4	MVAC	3.5	144	7	8	9	7
JU-001	2/M	B-ALL/1st CR	11	MVAC	6.9	36.0	17	6	14	18+
JU-002	3/M	B-NHL/1st CR	10	MVAC	8.3	80.0	11	10	12	16+
UT-013	13/F	B-NHL/1st CR	4	MVAC	33.5	54.3	10	-	25	2
UT-014	12/M	ANLL (M4)/1st	4	MVAC	24.2	30.0	11	-	16	16+
UT-016	13/M	ALL/2nd CR	4	MVAC	13.0	20.0	11	-	16	3
NMC-001	15/F	ALL/1st CR	18	MVAC	27.3	80	63		89	5
UT-018	6/F	T-ALL/1st CR	5	MVAC	2.2	156	10		11	5
UT-019	12/M	ALL/1st CR	6	MVAC	6.7	2.0	20	49	55	5+
JU-004	2/F	B-NHL/1st CR	6	MVAC	5.1	15.6	19	10	18	4+
JU-005	15/F	ANLL (M3)/1st CR	9	MVAC	15.3	20.4	34	13	65	4+
UT-020	15/F	ALL/2nd CR	6	MVAC	6.7	0.6	25 (CSF)	-	-	3+
UT-023	3/F	ANLL (M3)/1st CR	7	MVAC	1.4	22.6	14	23	38	2+

*Patients with primary resistant leukemia to first-line induction therapy. Abbreviations used for unique patient numbers (UPN) are: UT, the University of Tokushima; JU, Jikei University School of Medicine; UK, University of Kobe; UA, University f Akita; NMC, National Medical Center Hospital; FMC, Fukushima Medical College.

PERIPHERAL BLOOD STEM CELL TRANSPLANTATION IN PATIENTS WITH ACUTE MYELOID LEUKEMIA

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INTRODUCTION

High dose chemotherapy alone or combined with Total Body Irradiation (TBI) followed by autologous bone marrow transplantation (AutoBMT) has become a standard form of treatment for patients with acute myeloid leukemia (AML) (1,2). Peripheral blood stem cells (PBSC) can also be used to reconstitute hematopoiesis after a supra-lethal therapy. Thus, autologous blood stem cell transplantation (ABSCT) may be used as an alternative to AutoBMT for the treatment of hematological malignancies (3).In AML patients, the use of ABSCT instead of ABMT could present two hypothetical advantages which will be discussed in this paper (faster engraftment and lower risk of relapse) and which have to be demonstrated before this expensive technique is more widely used.

PATIENTS AND METHODS

Hematological Reconstitution

Hematological reconstitution occurs within 3-4 weeks following AutoBMT. Without bone marrow purging, granulocytic (polymorphonuclear cells, PMN > 500/mm^3) is usually seen 20 to 30 days after transplantation but platelet engraftment (platelet count > 50,000/mm^3) may be more delayed in some patients (more than 30 days) (4,5). The predictive value of the number of CFU-GM cells infused has not been clearly established, even if a CFU-GM threshold in respect to engraftment (10^5/kg) has been identified in some studies (6).

After different methods of marrow purging, hematological reconstitution appears to be slower than after unpurged ABMT and may exceed one month to achieve more than 500 PMN/mm^3 and two months for platelet engraftment (2). This delayed engraftment obviously increases the risk of infectious complications and thus morbidity or mortality due to the transplantation procedure.

Peripheral blood stem cells collected by leukaphereses after induction or consolidation chemotherapy have been demonstrated to be able to rapidly reconstitute hematopoiesis. This hematological reconstitution is stable for several weeks or years and its quality can be assayed using long term culture techniques (7). In the retrospective study performed by the France Auto Greffe Group in 72 patients (61 of them had acute leukemia), the median time to recover 500 PMN/mm^3 and 50,000 platelets/mm^3 was 14 days and 26 days (3). The granulocytic engraftment was not influenced by the type or status of leukemia at the time of transplantation, or by the type of conditioning regimen but only by the number of CFU-GM cells infused. It was very rapid in most cases and was due to high numbers of mature progenitors (mostly CFU-GM) which are collected in AML patients after induction chemotherapy (8).

A "cell-dose" effect has also been clearly established for granulocytic engraftment either in multicentric or monocentric studies (9) but the minimum threshold of CFU-GM cells to be infused to ensure a safe and rapid engraftment is not yet well defined, (varying from 15 x 10^4/kg to 50 x 10^4/kg). These discrepancies may be explained (at least in part) by variations in the CFU-GM assay. The predictive value of the number of CD 34 positive cells which are present in the leukaphereses products is presently under investigation but some recent data suggest that this parameter could be of limited value to predict either platelet or granulocytic engraftment : in a series of 55 patients undergoing ABSCT for different malignancies, the mean time to recover 500 granulocytes/mm^3 was 13.7 days for patients transplanted with more than 19 x 10^6 CD-34 positive cells/kg and not statistically different from that observed for patients transplanted with less than 19 x 10^6 CD-34 positive cells (15.1 days) (10).

Following ABSCT, platelet engraftment may sometimes be delayed or unstable. This seems to occur more frequently when low numbers of CFU-GM cells (that probably correlates with low numbers of CFU-Mega) are infused. In some patients, it is not infrequent to observe a double wave of engraftment with an early reconstitution occurring 2-3 weeks after ABSCT followed by a drop in the platelet count lasting 4-8 weeks (due to insufficient marrow production) (11). A second (and stable) wave of engraftment is usually seen in those patients, but it cannot be established if this is due to "endogenous" or "exogenous" (present in the leukaphereses products) primitive stem cells. However, in these patients with delayed engraftment, the duration of thrombocytopenia following ABSCT doesn't seem to be longer than that observed after purged AutoBMT (12).

Risk of Relapse

The fact that ABSCT may decrease the risk of relapse when compared with "purged" or "unpurged" AutoBMT implies (or admits the principle) that leukemic relapses (or most of them) may be due to the reinfusion of clonogenic leukemic cells. This hypothesis, which is presently the main rationale for using ABSCT instead of AutoBMT, has not yet been proved. If this hypothesis is accepted, it remains to demonstrate that PBSC are less contaminated by residual

leukemic cells than bone marrow cells. That has not been reported in patients with AML. Castagnola et al reported in a patient with AML that the karyotypic abnormality present at initial diagnosis was found in 30% of the metaphases analysed from the leukaphereses products collected after induction chemotherapy when the patient entered complete remission (13). However, using a combination of culture and cytogenetical studies, To et al were unable to find any difference between peripheral blood and bone marrow (14). Thus, if "in vitro" studies fail to detect any differences in the leukemic contamination of peripheral blood (collected after chemotherapy) and bone marrow, clinical results following ABSCT or AutoBMT need to be compared.

In acute myeloid leukemia, more than 100 patients have been transplanted worldwide, and the retrospective analysis of these cases seems to indicate that the results of ABSCT do not differ significantly to those after AutoBMT. We have now transplanted 11 patients with AML in second CR after a conditioning regimen of Busulfan (4 mg/kg/day x 4) associated with Cyclophosphamide or Melphalan or using the combination of total body irradiation (TBI) and Cyclophosphamide (120 mg/kg). one patient died early, six patients had leukemic relapse, occurring one year following transplantation and finally four patients are still alive in complete remission with a median follow-up from ABSCT of two years. These results did not differ significantly from those reported after AutoBMT for AML patients in second CR (15). More recently, the France Auto Greffe reported data for 26 patients who had undergone ABSCT for AML in first complete remission after conditioning regimens consisting of either TBI (13 patients) or Busulfan (13 patients) (16). Two patients died early from veno-occlusive disease or interstitial pneumonitis, 13 patients had leukemic relapse occurring 3 to 16 months (median = 6) after ABSCT and 11 patients are still alive in first complete remission, with a median follow-up of two years (3 to 55 months). The estimated chance of surviving without disease at two years was 34%. These results did not differ significantly from those recently reported in AML patients undergoing ABSCT in first complete remission (17,18) For example, in the series of 22 patients reported by Juttner et al, two patients died early, ten patients had leukemic relapse and ten other patients are still alive in remission with a median follow-up of approximately 4 months (17).

The results of AutoBMT in AML patients have been extensively reported. Considerable variations have been observed in the published series (2,19,20). However, in most prospective studies, the estimated chance of surviving without disease at 2-3 years seems to be similar to that recently reported after ABSCT. In the study reported by Korbling et al (1990), there was no statistical difference between AutoBMT and ABSCT. However, these results concern a series of 23 patients undergoing ABSCT which was retrospectively compared to a historical group of 23 patients who received mafosfamide-purged AutoBMT. These results need to be confirmed by prospective randomized studies. Such a study is now in progress within the BGMT group, where the patients are randomized to receive either ABSCT or unpurged AutoBMT after a conditioning regimen of Busulfan and Melphalan.

In an intermediate analysis performed in April 90, 27 patients had received either AutoBMT (n 14) or ABSCT (n 13). The median follow-up was similar in both groups (16 months). The number of patients who died during the transplant procedure (1/14 versus 1/3), who had leukemic relapse (3/14 versus 3/13) or who remain alive in continuous complete remission was similar after either AutoBMT or ABSCT (21). Thus, it seems probable that the results of ABSCT and purged or unpurged AutoBMT will be followed by a similar risk of recurrence of the underlying disease.

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AUTOGRAFTING FOR PATIENTS WITH CHRONIC MYELOID LEUKEMIA IN BLASTIC CRISIS: PROMISING RESULTS ACHIEVED WITH INTENSIVE CHEMOTHERAPY, PERIPHERAL BLOOD STEM CELL COLLECTION AND HIGH-DOSE CHEMO-RADIOTHERAPY

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ABSTRACT

Six patients underwent autologous peripheral blood stem cell transplantation (APBSCT) for chronic myeloid leukemia in blastic crisis. All patients received a conventional chemotherapy consisting of 4 - demethoxydaunorubicin (Idarubicin), intermediate dose Ara-C (Cytarabine) and VP-16-213 (Etoposide). When the patients had reached a peripheral WBC count $<1000/mm^3$ after aplastic phase, we started to perform the peripheral stem cell collection by leukapheresis. In four out of 6 patients, 15% to 30% of these cells were CD34+/CD33- and by chromosomal analysis were found to be Philadelphia chromosome (Ph) negative. These four patients subsequently achieved a remission while the other two patients were unresponsive. All patients in remission were treated with Cyclophosphamide and Total Body Irradiation followed by reinfusion of APBSCT. The day after the reinfusion, Cyclosporin A was started at 1.5 mg/kg in continuous infusion for 28 days in an attempt to cause transient acute graft versus host disease which may have an autologous graft versus leukemia effect. When patients' peripheral blood counts and bone marrow had recovered, all four patients who were Ph-negative received recombinant alphaInterferon (RhU-alpha INF) (6 million units twice weekly and than 6 million units weekly). Results: All these four patients are currently Ph negative and survive without reoccurrence of Ph chromosome for 3+, 6+ and 12+ months. This approach seems to be effective in obtaining Ph negative "remissions" in these patients. CyA and interferon may be contributing to the continued Ph negative state. We conclude that APBSCT combined with highly intensive cytoreductive treatment and followed by CyA and high-dose Interferon could be an interesting treatment modality for patients with chronic myeloid leukemia in blastic crisis and is worthy of future exploration.

INTRODUCTION

Treatment of chronic myelogenous leukaemia (CML) remains unsatisfactory and has had little impact on the overall survival. Only a small group of patients who underwent allogeneic Bone Marrow Transplant have had a slight survival advantage. The disease progresses to an accelerated phase or blastic crisis (BC) at an annual rate of approximately 25% and is always fatal (1,2). Treatment of BC with intensive chemotherapy has been disappointing and only a few patients with lymphoid transformation have slight survival advantage (3).

We have previously observed that two patients in "myeloid" BC-CML achieved a second chronic phase after receiving a combination of Idarubicin, intermediate dose of Cytarabine and Etoposide. We have also observed that, during the recovery phase after the cytotoxic chemotherapy, there was a substantial increase in the number of circulating hematopoietic progenitor cells, further the cells were predominantly Ph-negative. In this study, we have evaluated the use of these PBSC for hematopoietic engraftment after high-dose chemoradiotherapy protocols.

Recently, a syndrome similar to Graft versus host disease (GVHD) has been reported to occur in mice after receiving Cyclosporin A (CyA) (4). This autologous GVHD can have antitumor activity and recently a few investigators have exploited this phenomena in clinical trials. In addition, recent studies seem to demonstrate that RhU-alpha INF has no specificity in inhibiting the formation of colonies by acute myeloid leukaemia blasts as opposed to normal hemopoietic precursor cells. However, RhU-alpha INF markedly reduces the self-renewal capacity of leukemic myeloblasts. This observation suggests that RhU-alpha INF might be better in prolonging remission rather than inducing remission in patients with leukemia (5). On the basis of these observations, we decided to employ a schema involving the use of combination chemotherapy (Idarubicin, Cytarabine and Etoposide); at appropriate time when PBSC showed Ph negative status, PSC were harvested, patients were then given high-dose chemoradiotherapy with PBSC "rescue". CyA and RhU-alpha INF were given to control the minimal residual disease (MRD).Results of our new protocol are briefly detailed in this study.

PATIENTS AND METHODS

Six patients in BC-CML, entered this study (table 1). The planned therapy and the risks of the procedure were explained in detail to the patients. All patients agreed to be part of this study. Median age at the time of autografting was 40 years (range, 3067). No prior chemotherapy regimens were used to treat the blastic phase of the disease. The blastic phase was characterized as "myeloid" in three patients, "erythroid" in one patient and "lymphoid" in two patients using standard methods of morphologic, cytochemical and monoclonal antibody analysis (6). Activity of TdT was identified by an immunofluorescent - antibody technique.

Cytoreductive Therapy

A central venous catheter was inserted 12 hours before starting inductive therapy. The patients were maintained in single room, received allopurinol, antiemetics, oral nonadsorbable antibiotics, antimycotics as prophylactic treatment. Urine output was maintained at 150 mL/h. The induction therapy consisted of Idarubicin (6 mg/m^2/d for five days), IV; Cytarabine (600 mg/m^2/d for five days) infused in 1000 mL of 5% dextrose, I.V., for 2 hours; Etoposide (150 mg/m^2/d for three days) in 250 mL of 5% dextrose, over a period of 2 hours.

Stem Cell Collection

Peripheral stem cell collection was started immediately after hemopoietic recovery following inductive chemotherapy when the patient had reached a peripheral WBC of $< 1000/mm^3$. The technical aspects of collection and cryopreservation of cells has been described elsewhere (7). Briefly, the total number of nucleated cell collected in each patient varied between 0.5 x10^9/kg body weight and 0.8 x 10^9/kg body weight (median 0.7). Autologous bone marrow cells were harvested and treated with ASTA-Z "adjusted dose" technique (8) only to be used in case the PBSC fails to engraft by 30 days.

High-Dose Chemoradiotherapy

The four patients achieving Ph negativity by analysis and clinical remission (table II), received cyclophosphamide at the dosage of 60 mg/kg/d for two consecutive days and 24 hrs later, total body irradiation (TBI) (10Gy, in single dose) was delivered with a (60) Co source, 3-m source-axis distance, with the patient in the lateral position, half dose per side. Dose rate at the reference point (perineum, between thighs) was 5-9 cGy/min. Dose homogeneity throughout the patient's body was t 15%, with mean lung dose (at surface) t 8% the reference dose. The day after TBI (day 0), the cryopreserved PBSC were reinfused through a Hickman central venous catheter. Blood counts were monitored daily. Done marrow specimens were taken every two months for morphological and cytogenetic evaluations.

Cyclosporin A was started on the day of PBSC infusion and was continued for twenty-eight days at the dosage of 1.5 mg/kg/day, in continuous intravenous infusion. Patients took the drug properly. RhU-alpha INF was begun after high-dose therapy and CyA when WBC and platelets were > 3 x 10^3/mmc and 10 x 10^4/mmc, respectively. RhU-alpha INF was given at the dosage of 6 million units/d, IV or SC twice weekly and than 6 million unit weekly. Cytogenetic analysis was carried out according to standard techniques (9,10). The chromosomes were analysed using a QFQ banding method and classified according to International System for Human Cytogenetic Nomenclature (ISNC) (1985).

RESULTS

Six patients have been treated on this protocol. Two patients did not achieve a remission. Four patients were responsive to the induction protocol. Remission was usually achieved in six-weeks after the induction therapy (table 1). Cytogenetic evaluation, did not show Ph-chromosome positive cells at 3+, 6+ and 12+ months in the mitotic cells of all patients (table 2). Median duration of neutropenia (PMN < 500 x 10^6/mmc) and thrombocitopenia (platelets < 30 x 10^9/mmc) after autografting was 16 days (range, 10-54) and 30 days (range, 15-70), respectively. The marrow was hypocellular with normal cell distribution and no relationship was found between cellularity and Ph-negative metaphases.

Toxicity

Induction Therapy. The induction therapy was well tolerated with no major extrahematological toxicity. Nausea and vomiting were mild, severe mucositis was observed in all patients. One patient had interstitial pneumonia combined with pseudomonas aeruginosa septicemia. The patient responded to aggressive treatment which included the use of IV Aztreonam and IV Acyclovir.

High Dose Chemoradiotherapy. The high-dose therapy was relatively uncomplicated. All patients developed Gram negative infections responsive to broad spectrum antibiotic therapy (in association with granulocyte recovery). One patient developed candida albicans infection during neutropenic phase but he responded to treatment with amphotericin B.

Cyclosporin A. GvHD of the skin developed in all four responsive patients at a median time of 12 days (range 9-15) after the start of CyA infusion. An erythematous maculopapular rash affected the extremities and/or the face, ears and the upper trunk. Grade II GVHD was confirmed histologically by skin biopsy in two patients. The rash resolved spontaneously in all patients one - two weeks after the onset.

No patient had diarrhea or increased serum bilirubin suggestive of extracutaneous GVHD. In two patients we observed gingival hyperplasia in association with skin rash, this resolved spontaneously in both patients at the end of CyA infusion.

Alpha Interferon. The patients were able to receive the programmed dose of RhU alpha INF. All patients had headache and fever, controlled by antipyretic drugs.

DISCUSSION

For patients with CML, the only available chance of avoiding fatal blastic transformation is the allogeneic BMT. This procedure is limited by age and the availability of an HLA matched donor. Recently, several centers have reported follow-up of patients who received allogeneic marrow transplants while still in chronic phase, and prolonged disease free survival has occurred in

40-60% (11-17). The complications of BMT such as infection and GVHD, remain a major problem, but the long-term probabilities of benefit may outweigh the risks for young patients with a compatible donor. For those without a matched donor, the outlook is less hopeful. Various methods are being utilized to prevent graft rejection and GVHD when mismatch transplants are performed but transplantation of mismatched marrow still has a high risk of failure and/or complications. Also the idea of possible donation through marrow banks still remains very difficult. One possibility of helping such patients might come from the development of a better treatment of BC, which is able to induce a Ph-negative remission. The inductive approach employed by our team, appears promising and, the peripheral stem cells collected immediately after post-chemotherapeutic aplasia, demonstrated to be CD34+/CD33- and Ph negative. As suggested by a few investigators, the expansion of Ph chromosome clone(s) in CML might have a proliferative advantage over the proliferation of "putative" normal Ph negative hemopoietic stem cells but this proliferative advantage might in certain circumstances be reversible (18-20). Although in most patients, Ph-negative cells cannot be routinely identified, a wide spectrum of data, including recent in vitro cytogenetic studies of the bone marrow findings spontaneously and after treatment, suggest that the Ph negative cells are still present, albeit in suppressed state, in the marrow and peripheral blood of newly diagnosed patients with CML (19-24). This suggests that treating patients before the onset of blastic transformation with high dose chemoradiotherapy followed by autografting with hemopoietic cells collected at diagnosis or, better, in post-aplastic phase after an intensive chemotherapy protocol, might facilitate the restoration of Ph-negative hemopoiesis.

In an attempt to evaluate the theory's clinical applications, we have treated six patients with CML in BC and four are now Ph negative alive and well. At this point it is urgent to evaluate a greater number of patients.

ACKNOWLEDGEMENTS

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HIGH DOSE THERAPY AND AUTOLOGOUS PERIPHERAL STEM CELL TRANSPLANTATION FOR PATIENTS WITH RELAPSED LYMPHOMAS AND BONE MARROW METASTASES

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INTRODUCTION

Some patients with lymphomas that have progressed after potentially curative chemotherapy are eligible for high dose therapy followed by autologous bone marrow transplantation (ABMT). Approximately a third of patients treated with this strategy have experienced long-term event free survival (1,2). If, however, the patient is eligible for high dose therapy but has evidence of lymphomatous bone marrow involvement, ABMT usually is not performed for fear of introducing tumor cells during the transplant that could re-establish the malignancy. To make administration of potentially curative high dose therapy feasible for these patients, we have substituted autologous peripheral stem cell transplantation (PSCT) for ABMT to facilitate recovery of marrow function.

PATIENTS AND METHODS

From February 1986 until March 1990, 57 patients with relapsed lymphomas and bone marrow metastases were treated at the University of Nebraska Medical Center with high dose therapy and PSCT. Their median age was 36 years with a range of 18 - 53 years. Forty-one patients were males. Twenty-seven patients had Hodgkin disease, twenty-nine patients had non-Hodgkin lymphoma, and one patient had an unclassifiable lymphoma. All patients had tumor involvement in bone marrow as determined by light microscopic examination of bone marrow biopsies or, in two instances, by gene probe analysis at the time of entry into this study.

Peripheral stem cells were collected with apheresis using a discontinuous apheresis device at a time when myelopoiesis was in a steady state. If after six four-hour aphereis procedures (performed no more often than five times weekly), 6.5×10^8 mononuclear cells had not been collected, the procedures were continued until that number was reached. Ten percent DMSO by volume was used as a cryoprotectant, the cells were frozen in a controlled rate freezer, and stored in the vapor phase of liquid nitrogen.

The high dose therapy administered to these patients varied according to the histologic type of lymphoma being treated. Twenty-six patients with Hodgkin disease received CBV: carmustine 300 mg/m^2 etoposide 125 - 150 mg/m^2 for six doses given every 12 hours, and cyclophosphamide 1.5 g/m^2 for four days. One patient with Hodgkin disease and five patients with non-Hodgkin lymphoma received BECH: carmustine 300 mg/m^2, cyclophosphamide 2.5 g/m^2 for two days, hydroxyurea 1.5 g/m^2 every six hours for 12 doses, and etoposide 150 mg/m^2 every 12 hours for six doses. Ten patients with non-Hodgkin lymphoma received BEAC: carmustine 300 mg/m^2, cyclophosphamide 35 mg/kg for four days, etoposide 100 mg/m^2 every 12 hours for eight doses and cytarabine 100 mg/m^2 every 12 hours for eight doses. Fourteen patients with non-Hodgkin lymphoma received CY-TBI: cyclophosphamide 60 mg/kg for two days followed by 12 Gy total body irradiation (TBI) given in five fractionated doses. One patient with non-Hodgkin lymphoma received cytarabine 18 g/m^2 in six divided doses, cyclophosphamide 90 mg/kg, and 9 Gy TBI given in five fractionated doses.

On the day of the transplant, the cryopreserved cells were transported to the patients bedside, thawed in a 37C water bath, and immediately infused intravenously without washing or filtration.

RESULTS

Recovery of marrow function could be assessed for all but 12 patients. Four patients could not be evaluated for granulocyte recovery of 0.5 x 10^8/1 because of early death on days 25, 29, 35 and 37 after PSCT. Eleven patients could not be evaluated for red cell or platelet recovery (time after transplant to become independent of transfusion support) because of early death (days 22, 25, 29, 31, 35, 37, 38) or progression of disease in marrow and elsewhere with or without additional marrow toxic therapy on days 93, 102. 123, 305). One patient, who had recovered 0.5 x 10^9/1 granulocytes, died of sepsis on day 116 without becoming totally independent of red cell and platelet transfusions. For the 53 evaluable patients, the median time to reach 0.5 x 10^9/1 granulocytes was day 31 after PSCT (range, day 11 - day 157). For the 45 patients evaluable for red cell and platelet transfusion independence, the median time to reach red cell transfusion independence was day 26 (range, day 7 - day 259), and the median time to reach platelet transfusion independence was day 25 (range, day 8 - day 199).

Patients who received TBI experienced the slowest recovery. Although there was evidence of reappearance of white blood cells at a median of 8 days (range, 4 - 11 days) after transplant and reappearance of granulocytes at a median of 12 days (range, 7 - 18 days) after PSCT, the median time to reach 0.5 x 10^9/1 granulocytes was 49 days (range, 30 - 157 days) . The median time to independence from red cell transfusion was 75 days (range 25 - 259 days) after PSCT and the median time to independence from platelet transfusion was 76 days (range 21-199) days. Twelve of these fifteen patients experienced a complete response, making persistence of tumor in the marrow an unlikely

reason for the slow engraftment. A recent change in our peripheral stem cell collection parameters for patients receiving TBI as part of their planned high dose therapy (no fewer than 8 four-hour collections and no fewer than 8 x 10^8 mononuclear cells/kg patient weight) has resulted in a markedly shorter engraftment period.

Evaluable patients treated with CBV had recovery of 0.5 x 10^9/l granulocytes at a median of 30 days (range, 11-60 days) , were independent of red cell transfusions at a median of 22 days and received their last platelet transfusion at a median of 25 days after PSCT. Those evaluable patients who received BEAC recovered 0.5 x 10^9/1 granulocytes at a median of 25 days (range, 19 - 43 days) after PSCT. They were independent of platelet transfusions at a median of 15 days and independent of red cell transfusions at a median of 17 days after PSCT. Those evaluable patients who received BECH recovered 0.5 x 10^9/l granulocytes at a median of 32 days after PSCT and were independent of red cell transfusions at a median of 32 days after transplant and were independent of platelet transfusions at a median of 24 days after PSCT.

Following high dose therapy and PSCT, early toxic death prevented assessment of tumor response for 7 (12%) of the patients. Of fifty evaluable patients, thirty-two (64%) had a clinical complete response, 11 patients had a partial response, and 7 patients failed to respond. The four year actuarial event free survival (survival without tumor progression) for these 57 patients was 30% and their projected survivorship at four years was 48% (Figure 1). Patients who were event free at the time of this report had been followed for a median of 471 days after PSCT.

DISCUSSION

Peripheral stem cell transplantation functioned as an in vivo marrow purging technique for these 57 patients with lymphomatous marrow involvement who received high dose therapy. They experienced an actuarial event free survival of 30% at four years, an outcome very similar to lymphoma patients with no marrow involvement who have received the same high dose therapy and ABMT at our institution. To determine if long term survival would be better with a purged ABMT or PSCT, a randomized prospective trial would be needed.

CONCLUSION

Fifty-seven patients with relapsed lymphoma and marrow metastases at the time of peripheral stem cell harvesting experienced a 30% actuarial event free survival four years after PSCT. Autologous peripheral stem cell transplantation can be considered for patients with relapsed lymphomas who are candidates for high dose therapy but have lymphomatous involvement of their bone marrow.

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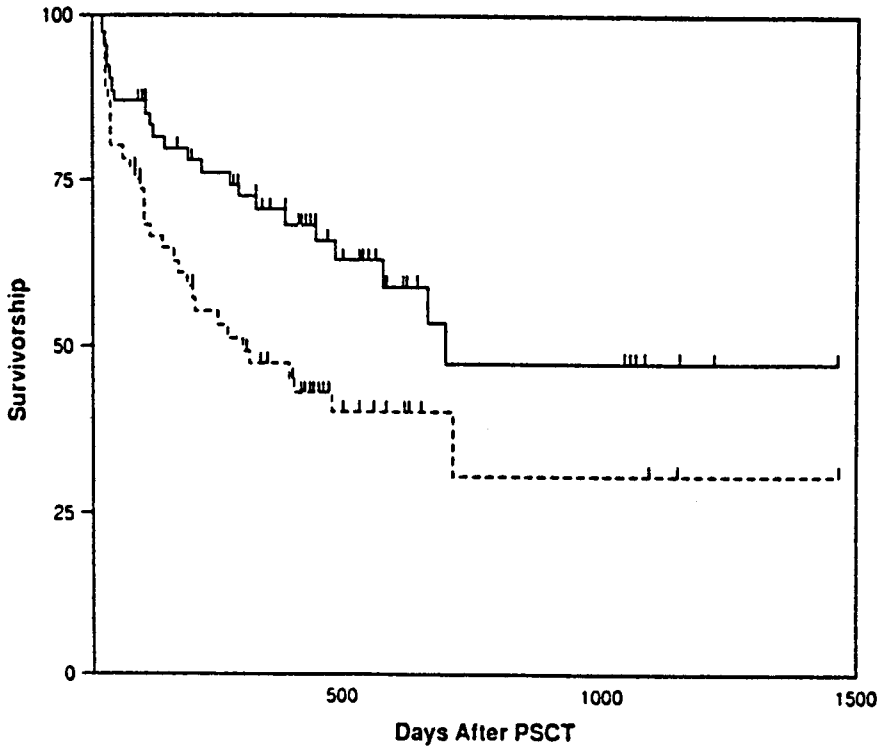
FIGURE 1

Actuarial survival (----) and event free survival (----) for 57 patients with lymphomatous marrow involvement at the time of peripheral stem cell harvest.



AUTOLOGOUS BLOOD STEM CELL TRANSPLANTATION (ABSCT) IN HIGH RISK MYELOMA

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INTRODUCTION

The prognosis of high risk myeloma (HRM) treated with conventional chemotherapy remains extremely poor, with a median survival of less than one year. Recently, there has been considerable interest in intensification myeloablative radio-chemotherapy followed by transplantation of stem cells derived either from allogenic (1) or from autologous bone marrow (2,3), or, more recently, from peripheral blood (4-7). Several considerations suggest the use of autologous blood stem cell transplantation (ABSCT) rather than allogenic (BMT) or autologous bone marrow transplantation (ABMT) in HRM. BMT is limited by the low incidence of myeloma in patients under 50 years of age. A substantial tumor mass reduction can be achieved following ABMT, but the graft remains generally contaminated with the malignant cells, and relapse occurs most of the time within one year post-transplant. In contrast, the hope of a lower likelihood of the graft containing neoplastic cells compared with autologous marrow graft represents the main reason to consider transplants of peripheral blood stem cells (PBSC). Furthermore, the rapid hematopoietic recovery following ABSCT decreases the risk of post-transplant morbidity and mortality, also allowing ABSCT in older patients. Nevertheless, the possibility of performing ABSCT is sometimes limited in HRM in case of difficulty to achieving sufficient yields of PBSC, even after stem cell mobilization with high dose chemotherapy, especially in patients refractory to previous treatments (8, 9).

We report here our experience in collecting blood stem cells and their use for autografting in previously untreated HRM patients.

PATIENTS AND METHODS

Patients (Table I)

Eleven consecutive patients (6 men and 5 women) were treated with high dose chemotherapy within a few weeks following diagnosis, both for tumor load reduction and for PBSC mobilization. Their median age was 58 years

(ranging from 45 to 64), eight being over 55 years of age. They all presented at diagnosis a Stage III myeloma in the Durie-Samon classification. Myeloma protein was IgG in 7 and IgA in 1, while two patients had a light chain myeloma, and another one a non-secretory myeloma. All patients were highly disabled by various painful fractures, five even being bedridden and requiring intensive nursing. Written informed consent was obtained from all patients prior to therapy. Eight patients received high dose melphalan (HDM) in a single i.v. bolus: 140 mg/m^2 in the first five patients, but the dose was decreased to 100 mg/m2 in the following three patients because of a high rate of morbidity in two out of the first five. The last three patients (No. 9-11) received a chemotherapy regimen (CAMVP) proposed by Bell et al (5), consisting of cyclophosphamide 4 g/m^2, adriamycin 100 mg/m^2, methylprednisolone 5g, and vincristine 2mg. All eleven patients achieved a profound aplasia within one week following chemotherapy; they were all nursed in laminar air flow rooms and received gut decontamination. Supportive care included blood products and broad spectrum antibiotics when needed. The median length of aplasia in the whole group was 29 days (ranging from 14 to 90) but was significantly shorter after CAMVP than after HDM (mean 12 days versus 34 days). One patient (No.5) died of disseminated aspergillosis after an extremely slow recovery from aplasia. Patient No. 4 recovered still slower (90 days) and did not achieve a rebound phase allowing leukopheresis; she needed supportive transfusions over more than six months, but she is now alive and well without further therapy with a two-year follow-up. The other patients achieved either complete remission (CR: 5 patients) or partial remission (PR: 4 patients) at the hematopoietic recovery phase. But one patient died of progressive disease two weeks after leukophereses.

Methods

CFU-GM Assay. CFU-GM assay was performed both from bone marrow samples collected before high dose chemotherapy in all patients but one, and from leukopheresis products, using the technique we have previously described (10). Determination of CD34+ cells by immunocytofluometry using a FacStar (Becton-Dickinson, Mountain View, Ca.) was also performed in bone marrow before chemotherapy and in leukapheresis products of the last three patients (No. 9-11) in accordance with the technique of Sowala et al (11).

Leukaphereses. Leukaphereses were started at the rebound phase following aplasia, when the WBC count reached 1 x 10^9/l, including monocytosis and myelocytosis, and when platelets were usually but not always > 100 x 10^9/l. Leukaphereses were performed with a continuous flow cell separator (Fenwall CS 3000). Mononucleated cells (MNC) were then sedimented with Plasmagel and directly frozen in plastic bags (Gambro DF 700) in a 10% DMSO-Albumin solution at a final concentration of 4 x 10^7 cells/mi. On average, 4.5 leukaphereses (ranging from 3 to 9) were performed in nine of the eleven patients.

Detection of Residual Tumor Cells in Leukophereses. Light microscopy, immunocytochemical studies, immunofluorescent labelling and

analysis for aneuploidy were systematically used to detect eventual tumor cells in the graft. Immuno-cytochemical studies were performed on MNC smears, using the APAAP technique in accordance with Eiber et al (12). Monoclonal antibodies coupled with fluorescein were mixed with MNC suspended in RPMI 1640 buffered by bicarbonate at pH 7.4 for immunofluorescent labelling and analyzed for red and green fluorescence on the FacStar. Analysis for aneuploidy was performed in leukopheresis products of patients in whom aneuploidy had been initially observed in bone marrow before treatment, according to the technique described by Vindelov (13).

ABSCT. Six out of eleven patients underwent transplantation using PBSC. The average time from collection of PBSC to ABSCT was 3.5 months (ranging from 2-6 months), with none of the patients undergoing chemotherapy during this period. All patients received the same conditioning regimen: total body irradiation (TBI), 10 Gy in 6 fractions, and HDM 140 mg/m^2 x 2 days. The first two patients were transplanted with both PBSC and marrow cells harvested after leukopheresis because of a number of blood CFU-GM/kg inferior to our "standard" values at that moment ($>10 \times 10^4$ /kg body weight). The following patients were transplanted only with PBSC. The plastic bags containing PBSC (average number: 7) were thawed and infused i.v. one by one over an entire period of about three hours. A diuretic regimen had been previously instituted to avoid hypervolemia. All patients were nursed in the same conditions as during the first aplasia.

RESULTS (Table II)

In all patients but two, the number of marrow CFU-GM measured before mobilization chemotherapy (mean 260/ml for 2×10^5 CMN plated, range 205-358) was comparable to that of our controls (225 ± 50/ml). In the two other patients it was substantially lower: 59 and 73 CFU-GM/ml, respectively. But even in these two patients, the number of CD34 + cells was always normal (ranging from 0.7 to 1.6% of total MNC) compared to our standard values (1+ 0.2%).

Nine patients underwent leukaphereses. The average number of CFU-GM collected per patient was 11.1×10^4/kg body weight (ranging from 0.12 to 30). It was correlated with the length of aplasia, which was also inversely proportional to the intensity of the rebound phase, rather than with the initial number of marrow CFU-GM, except in case No. 8. It was not possible with any of the techniques used to detect residual tumor cells in leukopheresis products. The average number of blood CFU-GM injected for transplant measured after thawing was 14.5×10^4/kg (ranging from 3.4 to 30). Two patients also received marrow CFU-GM (2.9 and 1×10^4/kg respectively). No patient died during aplasia following the conditioning regimen; the extra-medullary toxicity was bearable, despite severe digestive mucositis. The average length of post-transplant neutropenia was short (mean 12 days, range 8-16) as compared with that observed in leukemia. On the other hand, the length of thrombopenia $<20 \times 10^9$/l was longer (mean 33 days, ranging from

14 to 60) and was well correlated with the number of CFU GM infused. Some patients therefore needed supportive transfusions for more than two months.

Of the two patients transplanted both with PBSC and with marrow cells, one achieved CR (defined by the absence of monoclonal component in serum and in concentrated urine, and of detectable plasma cells on bone marrow samples) but unfortunately died ten months later of an intercurrent sepsis shock occurring after renal lithiasis; the second one had minimal residual disease, remained well for 14 months post-transplant, then relapsed and died of progressive disease 22 months post-transplant. Of the four patients transplanted with PBSC alone, one died on day 45 post-transplant of procedure-related complications, even though he had recovered hematopoietically and had been in CR. The other three achieved CR and are still alive and well 8, 20, and 28 months post-transplant respectively, even though one had a biological relapse 24 months post-transplant and is now responding well to a light ambulatory chemotherapy. The overall survival time for all transplanted patients to date is 18 months post. transplant (ranging from 8 to 28 months) and 22 months from diagnosis (ranging from 11 to 33 months).

DISCUSSION

Compared with other diseases, the frequent difficulty in achieving large amounts of PBSC after mobilization chemotherapy, thus limiting the possibility of performing ABSCT in some cases, represents a specific problem of HRM. In a preliminary trial in patients heavily pretreated with alkylating agents and resistant to conventional chemotherapy, Laporte et al (8) obtained yields of blood CFU-GM which were 24 times less than those observed in patients with acute leukemia (14, 15). In a more recent study, Ventura et al (9) also achieved such low yields in a series of patients with advanced myeloma resistant to both alkylants and conventional regimen (VAD). The eventual role of extensive and repeated prior chemotherapy in exhausting the pool of pluripotent stem cells, analogous to the findings of Mauch et al (16) in murines, must then be questioned. In order to avoid such cell damage, it appears preferable to collect PBSC as soon as possible after diagnosis, following a mobilization chemotherapy in yet untreated patients. But, in our study, where eleven previously untreated patients were clinically managed under the use of largely identical criteria, as well as in the comparable study of Bell et al (5), the yields of PBSC had a wide range, and it is debatable whether drug sensitivity of the hematopoietic system varies considerably from one patient to the other. Even if, as we observed in our study, alkylating agents are probably the best drugs to achieve a drastic reduction of the myeloma tumor load, they are also probably more damaging to the hematopoietic system than others, and their use as mobilizing agents must be questioned, since their impact on general morbidity may also be considerable.

Furthermore, besides the risk of cell damage by drug toxicity, hematopoiesis is also frequently disregulated in steady state myeloma (17). In two patients in this study, and in several others not reported here, we

encountered, prior to any treatment, values of bone marrow CFU-GM in the range of 10-70 versus 150-300 CFU-GM /2 x 10^5 MNC in the standard bone marrow culture system after stimulation with placental cell supernatant. In this instance, the yield of the whole harvest by 3-5 cytaphereses might be below or little above 1 x 10^4 CFU-GM/kg body weight, as observed in our patient No. 8. Whether such low cell quantities have the capacity to reconstitute hematopoiesis completely and in a sustained way is highly questionable.

Different mechanisms could account for this disregulation of hematopoiesis. It may as well be partially mediated by natural killer (NK) cells via different cytotoxins (18), as it could be the consequence of the secretion by tumor cells of inhibiting factors, like tumor necrosis factor (TNF) (19) or interferon (20). Such inhibitory factors could act either directly on hematopoietic precursors or on accessory cells, like monocytes, which participate in the regulation of the clone formation response of CD34+ progenitors (21). Direct determination of CD34+ cell rates revealed effectively the amazing fact that the immature cell fraction is of normal size and that these cells can be readily stimulated in the correct cellular environment, while the activation process itself, which involves accessory cells, is disturbed (22). These data might thus underlie the concept of an inhibition of the hematopoiesis arising at the CD34+ level.

The hope of a lower likelihood of tumor cells residual in graft represents the main reason to consider ABSCT in myeloma. In fact, we never found residual plasma tumor cells in our study, but we did not use, as did others, anti-idiotype antibodies (5) and/or Ig gene rearrangements (5, 6), which are more sensitive techniques than those we used. Five out of our six transplanted patients were in CR after ABSCT, the last one having achieved a tumor mass reduction >90%. It is worthwhile to note that three of these patients were already in CR and the three others in PR (75% or greater tumor mass reduction) at the time of transplantation. ABSCT acts in this instance as the second phase of a two part therapeutic process: 1) prime reduction of tumor load and mobilization of PBSC; 2) achievement of the tumor load reduction by reinforcement of the treatment with supralethal radio-chemotherapy. Although the rate of morbidity and mortality of such a therapeutic process is relatively high, the whole average survival time two times longer to date than that obtained by conventional chemotherapies may favor such aggressive treatment in poor prognosis myeloma, even in older patients. It should be interesting to compare the rate of relapses and of deaths following ABSCT with that of similar series of patients transplanted with autologous marrow cells, in order to clinically confirm a lower likelihood of contamination of PBSC graft with residual tumor cells. Further studies are required.

CONCLUSION

In conclusion, ABSCT, when feasible, appears to be a very interesting new therapeutic approach in poor prognosis myeloma, even in patients over 60 years of age. The cardinal problem remains in collecting sufficient amounts of

PBSC in all cases. Mobilization of PBSC with chemotherapy associated with hematopoietic growth factors (rh GM-CSF or rh G-CSF), as proposed by Gianni et al in solid tumors (23), could favor the yield of PBSC. Nevertheless, one should be cautious in using here rh GM-CSF, which is an activator of synthesis of interleukine-6, known to play an important role in the growth of myeloma tumors (24).

ACKNOWLEDGEMENTS

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TABLE 1

High risk myeloma: Clinical and biological data.

Patients	1	2	3	4	5	6	7	8	9	10	11
Age	58	64	62	54	45	60	62	63	55	59	60
Sex	M	M	F	F	F	M	F	M	F	M	M
Stage	III A	III B	III B	III B	III A	III B	III A	III B	III B	III A	III B
Ig subtypes	IgA-K	IgG-K	IgG-K	IgG-L	non excret.	light chain	IgG-K	IgG-L	IgG-L	IgG-K	light chain
Pre-CT BM CFU GM/ml	358	244	329	211	318	N.D.	179	59	205	73	236
Mobilization CT	HDM 140mg	HDM 140mg	HDM 140mg	HDM 140mg	HDM 140mg	HDM 100mg	HDM 100mg	HDM 100mg	CAMVP	CAMVP	CAMVP
Length of granulopenia (days)	28	22	14	90	42	25	18	34	14	8	13
Post CT status	PR	CR	PR	CCR 24 mo.	Dead	CCR 20 mo.	CR	PR 18 mo.	CR	CR	PR
Nb of leucapheresis	9	4	5	-	-	5	4	4	4	3	3
PB CFU GM x 10^4/kg b.w. collected	7	7.4	30	-	-	4.75	28	0.12	3.4	34	1.2

CT = chemotherapy
CR = complete remission
CCR = continuous complete remission
PR = partial remission
PB = peripheral blood
BM = bone marrow
N.D. = not done

TABLE 2

ABSCT in high risk myeloma.

Patients	1	2	3	7	9	10
Type of TX	PBSC + BM	PBSC + BM	PBSC	PBSC	PBSC	PBSC
Conditioning regimen	Fractionated TBI (10 Gy in 6 fractions) + HDM 140 mg x 2 days					
Nb of CFU GM x 10^4/kg b.w. infused	7 (PB) + 2.9 (BM)	7.4 (PB) + 1 (BM)	30	28	3.4	11.4
Length of granulopenia post TX < 0.5 x 10^9/l (days)	20	16	10	10	14	11
Length of thrombopenia post TX < 20 x 10^9/l (days)	60	60	14	18	30	16
Status post TX	PR	CR	CR	CR	CR	CR
Follow up	Relapse 14 mo.	CCR	Biological relapse 24 mo.	CCR	CCR	Dead of procedure-related complications 1.5 mo.
Clinical outcomes post TX	Dead 22 mo.	Dead of sepsis shock 10 mo.	Alive and well 28 mo.	CCR 20 mo.	CCR 8 mo.	

TX = transplantation

PB = peripheral blood

BM = bone marrow

MACROPHAGE COLONY-STIMULATING FACTOR IN AUTOLOGOUS BONE MARROW TRANSPLANTATION

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ABSTRACT

In order to study the effects of recombinant human granulocyte macrophage colony stimulating factor (rhGM-CSF) on hematopoietic reconstitution, it was administered to patients undergoing autologous bone marrow transplantation (ABMT) in Seattle. The data indicate that rhGM-CSF was well tolerated and associated with earlier neutrophil recovery, fewer infections and shorter initial hospital stays. Survival was not improved, however, less regimen-related toxicity was observed. This suggests that further escalation of chemo-radiotherapy may be possible in patients who receive rhGM-CSF after ABMT.

INTRODUCTION

Long term survival of patients with lymphoid neoplasia undergoing autologous bone marrow transplantation (ABMT) is significantly limited by relapse. Increased cytotoxic chemo- radiotherapy has been shown to reduce relapse rates in patients with lymphoma, however, survival was limited by increased regimen-related toxicity (1). The most common regimen-related toxicities in ABMT include veno-occlusive disease of the liver, pneumonitis, hemorrhage, and infection. A reduction of regimen-related toxicity may allow an increase in the maximum tolerated dose levels of chemo-radiotherapy.

Recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) is a protein necessary for the survival, proliferation and maturation of myeloid cells. It also enhances the functional activation of mature neutrophils and monocytes (2). Animal trials indicate that rhGM-CSF is associated with earlier neutrophil recovery after ABMT (3,4). Phase I/II trials in patients undergoing ABMT were consistent with animal trials in that earlier neutrophil recovery was observed. As a consequence, the incidence of infection was lower in patients who received rhGM-CSF compared to historical controls (5-8). Somewhat surprisingly, in one trial, patients who received rhGM-CSF

(5-8). Somewhat surprisingly, in one trial, patients who received rhGM-CSF had substantially lower bilirubin and creatinine levels than historical control patients (5). In an attempt to reduce marrow transplant-related toxicity a series of phase I, II and III trials were done in Seattle exploring the use of rhGM-CSF in patients with lymphoid malignancy undergoing ABMT.

PHASE I TRIAL RESULTS

RhGM-CSF-was administered to 15 patients as a 2 hour intravenous infusion from day 0 (the day of marrow infusion) to day 13 at doses ranging from 15 ug/m^2/day to 240 ug/m^2/day (9). Toxicity ascribed to rhGM-CSF was minimal consisting of abdominal, chest and bone pains in 20% of the patients. No patients discontinued therapy because of toxicity. Results indicated that patients who received > 60 ug/m^2/day of rhGM-CSF had earlier neutrophil and platelet recovery, fewer febrile days and earlier hospital discharge when compared to historical control patients (see Table 1).

PHASE II TRIAL RESULTS

When rhGM-CSF was discontinued on day 13 after marrow infusion, a temporary reduction in ANC to below 500/m^3 occurred in the majority of the patients who had reached an ANC of 500/m^3 by this time. A number of these patients required reinstitution of antibiotics for recurrent fevers. Therefore, additional patients were treated with 240 ug/m^2 of rhGM-CSF by 2 hour IV infusion from day 0 to 20 in an attempt to obtain higher neutrophil counts before discontinuation of rhGM-CSF therapy. None of the subsequent patients had a decrease of their ANC to below 500/mm^3 after initially achieving an ANC > 500/mm^3 (10).

As part of the phase II trial, we explored the affect of rhGM-CSF at a dose of 240 ug/m^2 by continuous infusion from day 0 to 20 in 3 patients. Neutrophil recovery was comparable to patients receiving rhGM-CSF by the 2 hour infusion schedule, however, platelet recovery was significantly delayed in all 3 patients.

Overall, results of neutrophil recovery, platelet recovery, number of febrile days and day of discharge in patients who received rhGM-CSF by 2 hour infusion from day 0 to 20 were the same as in patients who received rhGM-CSF from day 0 to 13 at doses > 60 ug/m^2/day shown in Table 1. The only difference was that patients no longer had a temporary reduction in ANC to below 500/mm^3 after discontinuation of rhGM-CSF. The course of rhGM-CSF was not extended to greater than 20 days because progenitor cell assays performed on patients who received rhGM-CSF suggested that even the additional 7 days of rhGM-CSF therapy from day 14 to 20 ray temporarily reduce the number of committed progenitor cells (11).

LONG TERM FOLLOW UP RESULTS

Results of the phase I and II trials indicated that rhGM-CSF was associated with a reduction in ABMT-related toxicity. This resulted in earlier discharge times than in historical controls. The patients entered into phase I and II trials (n=28) have now been followed > 1 year. Disease free survival is greater than 40% which is comparable to historical control patients. There was no evidence of late graft failure. These results suggest that rhGM- CSF can reduce regimen-related toxicity of ABMT without affecting survival, relapse or long term hematopoietic reconstitution.

PRELIMINARY RESULTS OF THE PHASE III TRIAL

In order to provide more definitive results concerning the role of rhGM-CSF in ABMT a prospective double blind placebo controlled trial in patients with lymphoid malignancy undergoing ABMT was initiated. Preliminary results of patients who received rhGM-CSF suggested that toxicity was minimal, earlier neutrophil recovery, platelet recovery, less infection and earlier discharge times were observed compared to prospective control patients (see Table 2). Of importance, mucositis was less and bilirubin levels were lower in patients who received rhGM-CSF (12).

DISCUSSION

Multiple trials have suggested that rhGM-CSF stimulates earlier neutrophil recovery after ABMT (5-8). Results in Seattle are also consistent with this (9,10,12), however, another important question is whether rhGM-CSF may benefit patients by stimulating cells other than myeloid cells, either directly or indirectly. The majority of trials, including trials described here, suggest that rhGM-CSF reduces ABMT-related morbidity by reducing days of infection, thus allowing earlier hospital discharge. Additionally, some trials suggest that jrhGM-CSF is associated with lower bilirubin and creatinine levels and less mucositis (5,12). The level of bilirubin generally correlates with the severity of veno- occlusive disease. Additional trials in patients undergoing ABMT at high risk of developing regimen-related toxicity will be necessary before conclusions regarding the effect of rhGM-CSF on veno-occlusive disease and mucositis can be made. It is possible that benefit from rhGM-CSF may be derived from effects on non- hematopoietic cells and studies addressing this are needed. Since preliminary evidence suggests that rhGM-CSF is associated with less regimen-related toxicity, phase I trials with rhGM-CSF administration in patients undergoing ABMT receiving higher dose chemo-radiotherapy regimens are being designed.

In conclusion, evidence suggests that rhGM-CSF is associated with induction of earlier neutrophil recovery and less non-hematopoietic ABMT morbidity which results in shorter initial hospital duration. However, it is unlikely that rhGM-CSF will significantly improve survival of patients undergoing ABMT unless regimen-related toxicity can be sufficiently reduced by rhGM-CSF to allow further escalation of maximum tolerated preparative regimen dose levels to reduce relapse rates.

ACKNOWLEDGEMENT

Supported by PHS Grant Nos. CA 18029, CA 18221, CA 09515, CA 26828, and CA 47748, awarded by the National Cancer Institute, DHHS; Grant NO. CCA-8510/019; awarded by the US-Spain Joint Committee for Scientific and Technological Cooperation; HL 36444 from the National Heart, Lung and Blood Institute, DHHC; and funding from Behringwerke, AG; Hoechst-Roussel Pharmaceuticals, Inc., Somerville, NJ; and Immunex Corp, Seattle.

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Table 1

Comparison of patients who received ≥ 60 μg/m² of rhGM-CSF (n=22) to historical control patients (n=86)

rhGM-CSF dose μg/m²/day	Day ANC[b] ≥ 1000/mm³	Day platelet transfusion independent[b]	Number of days T≥38°C (Day 0-30)	Day of discharge[b]
0[a]	29 ± 11	38 ± 20	12 ± 7	41 ± 25
≥ 60	22 ± 4	29 ± 9	6 ± 3	29 ± 9

[a] matched historical control patients who underwent ABMT prior to the start of the study

[b] mean value ± standard deviation

Table 2

Preliminary analysis of randomized, placebo controlled trial of rhGM-CSF in autologous BMT patients treated in Seattle (median values) [12].

	Placebo (n=13)	rhGM-CSF (n=17)	p value*
Day ANC > 100	11	11	NS
Day ANC >1000	23	19	0.09
Day platelet independent	29	21	0.007
Day discharged	36	27	0.009
Maximum bilirubin	6.2	2.5	0.06

* Wilcoxon Rank Sum

MODIFICATION OF THE ABSOLUTE NEUTROPENIA AFTER HIGH-DOSE THERAPY WITH GROWTH FACTORS AND PERIPHERAL BLOOD CELLS

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INTRODUCTION

Modification of High Dose Therapy Toxicity by Recombinant Growth Factors and Autologous Bone Marrow Transplantation

An important issue in high-dose chemotherapy is the modification of its toxicity by administration of growth factors plus autologous bone marrow transplantation (ABMT) or by mobilized peripheral blood. To illustrate the effects of recombinant growth factors in what we have termed "an in vivo depleted granulocytic progenitor state," we provide a brief description of the hematopoietic effects of granulocyte colony-formulating factor (G-CSF) and its impact on fever in patients with Hodgkin's disease we treated using CBV. The doses of CBV and the doses and methods of recombinant human G-CSF (rh-GSF) are described elsewhere. From Table 1, it is obvious that the difference in recovery to 1000 granulocytes/ul was remarkably faster. in patients receiving recombinant human GSA (rh-GSA) 14 days faster than in control patients. Recovery to 500 granulocytes/ul was 9 days faster in patients receiving rh-GSA but to 100 granulocytes/ul was only 4 days faster.

The importance of the length of time for which patients had less than 100 granulocytes/ul, which we have called the "absolute neutropenia," and its relation to morbidity in these chemotherapy regimens has been underemphasized. It should also be clarified that the important factor is the duration of neutropenia, not leukopenia. Patients with counts of several hundred leukocytes per milliliter of peripheral blood, but without neutrophils are regularly febrile. The clinical problem is that within a few days of patients' becoming absolutely neutropenic they develop a fever. The median onset of fever is 4 days after the ABMT that accompanies growth factor administration or 2 days after developing absolute neutropenia. The proportion of patients with fever rises rapidly thereafter. By the time most patients have recovered to 100 granulocytes/ul (absolute granulocyte count [AGC] of 100/ul), despite

its being hastened with rh-GSA, 95% are already febrile. So, although recombinant G-CSF or granulocyte-macrophage colony-stimulating factor (GM-CSF) dramatically shortens the time needed for full neutrophil recovery, the effect is concentrated on the terminal part of the process and not on the more important early component (1-3). However, even this late effect could be clinically significant if patients did not lose their fever until they had recovered to 500 granulocytes/ul of blood, that is, if infection did not resolve until the neutrophil mass had recovered to levels reflected by peripheral-blood counts of 500 or more granulocytes/ul. However, in analyzing the resolution of fever in 39 patients who received rh-GSF post ABMT for Hodgkin's Disease, 70-80% have resolution by the time the AGC reaches 100/ul; the rest generally lose their fevers in the next few days. A small proportion, however, maintain persistent fevers even with adequate neutrophils, probably because of catheter infection. Five hundred neutrophils in each microliter of blood are not needed to resolve infection; instead, probably just the early immigration of tissue neutrophils is necessary. Therefore, in the "CFU-GM or progenitor-depleted circumstance", recombinant growth factors do not affect the early part of hematopoietic recovery sufficiently to prevent the neutropenic episode. The 6-8 day-long phase of absolute neutropenia persists with these intense therapies, no matter what dose of recombinant factors, method of administration, or timing relative to ABMT we use.

DISCUSSION

Peripheral Blood Cell Infusion Effects on Morbidity and Recovery After High Dose Therapy

The rapid hematopoietic recovery that follows the administration of peripheral blood after high-dose chemotherapy has sparked interest recently. Some even claim that this strategy can totally ameliorate absolute neutropenia (4,5). We conducted a study to validate these claims. Peripheral blood cells were collected during the neutrophil upswing following neutropenia induced by cyclophosphamide and adriamycin at doses of $3G/m^2$ and $50mg/m^2$ respectively. Marrow was collected from patients and divided into two aliquots. To determine whether peripheral-blood cells collected in this fashion hastened the early part of neutrophil recovery, we evaluated its effect on hematopoietic recovery, when administered with bone marrow and growth factors. During cycle 1, we gave the patients marrow and GM-CSF following CVP (cyclophosphamide $5.25G/m^2$, etoposide $1200mg/m^2$ and cisplatin $165mg/m^2$). During the second cycle at exactly the same dose, we added peripheral blood to the postchemotherapy infusion and growth factor to see whether it enhanced recovery compared with marrow and growth factor alone. Thus, the first cycle served as an internal control.

Table 2 illustrates that in an individual patient the median time of recovery to an AGC of 100/ul was not affected by the addition of peripheral blood. That is, the period of early recovery remained 6-8 days long. The terminal part of recovery (to 500 granulocytes/ul) was shifted minimally, but

the difference was of no clinical significance. The overall number of documented infections was not reduced in the second cycle, and patients were febrile for approximately 7 days in each cycle. The median duration of thrombocytopenia (<20,000 and 50,000) was however reduced by the peripheral blood infusion.

Thus, in our two studies of unselected patients we were unable to ameliorate the absolute neutropenia. However, other techniques in which more progenitor cells might be mobilized remain to be explored (6).

ACKNOWLEDGEMENTS

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TABLE 1

HEMATOPOIETIC RECOVERY

	Bolus infusion		
	rhG-CSF	Control	P value
>100	9(8-19)	13(7-34)	0.103×10^{-4}
>500	13(10-31)	22(10-51)	0.189×10^{-2}
>1,000	16(11-33)	30(14-61)	0.125×10^{-5}
>50,000	27.5	32	0.370

TABLE 2

Recovery to 100 AGC/mm from day 1 of Chemotherapy

Cycle #1	Cycle #2
13	15
15	15
15	17
16	15
16	16
16	16
18	15
19	18
20	16
20	18
21	20

CAN MAXIMAL DOSE CHEMOTHERAPY WITH MARROW GROWTH FACTORS REPLACE AUTOLOGOUS BONE MARROW TRANSPLANTATION?

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SUMMARY

A tabular summary of the maximal dose given both with and without a bone marrow transplant for 15 chemotherapeutic drugs is presented as support for the hypothesis that high-dose chemotherapy may not be myeloablative. Although many of these agents are conventionally given in conjunction with bone marrow, the information presented suggests there is no conclusive evidence that the maximal tolerated dose of chemotherapeutic drugs given singly or in combination, without bone marrow, would result in irreversible myelosuppression in man. Based upon this hypothesis, and assuming cumulative marrow damage from these agents can be avoided, continued exploration of repetitive courses of high-dose combination chemotherapy alone or in conjunction with bone marrow growth factors is suggested as an alterative to autologous bone marrow transplantation for malignancies.

INTRODUCTION

Recent observations have brought into question the veracity of the long held dogma that high-dose chemotherapy as used in the setting of autologous or allogeneic bone marrow transplantation is myeloablative (i.e. irreversible pancytopenia would result if "stem cells" from the marrow or peripheral blood were not reinfused to restore hematopoiesis). The advent of bone marrow transplantation as a methodology to facilitate dose escalation of myelosuppressive agents has eliminated concern of pancytopenia as a dose limiting toxicity for these agents. Unfortunately and inevitably, a new dose limiting toxicity arises in other organs such as the mucosa, lung, liver, etc. The successes of high-dose chemotherapy used in conjunction with bone marrow transplantation in overcoming drug resistance, coupled with technical and financial issues surrounding the use of bone marrow transplantation has spurred on the exploration of high-dose chemotherapy without bone marrow

support. In this setting supportive care as used with bone marrow transplantation (i.e. transfusions, anti-infectives, analgesics and parenteral nutrition) allows recovery of hematopoiesis from bone marrow "stem cells" presumably not effected by the high-dose therapy.

HYPOTHESIS

The table displays the results of a comprehensive literature search undertaken to determine the maximal dose reported, both with and without bone marrow support, for those chemotherapeutic agents used as part of bone marrow transplant preparative regimens. As seen from the table, many chemotherapeutic drugs given as single agents in high-doses without bone marrow do not result in irreversible myelosuppression. With certain cycle--specific agents (i.e. busulfan, carmustine, dacarbazine, and thiotepa) the maximum dose given to date is significantly less without bone marrow infusion than with. But, as no formal dose escalations without bone marrow transplantation have been done for these agents, it cannot therefore be concluded they are indeed myeloablative, and that recovery of marrow function would not occur if the maximal tolerated dose were given without marrow support. As would be predicted from experimental evidence, none of the phase-specific agents listed (i.e. amsacrine, cytarabine, etoposide, hydroxyurea, and teniposide) are myeloablative (35-37). Of interest, and not predicted from theory, is that most of the cycle-specific agents can be given at the maximal tolerated dose without marrow.

It is possible that even if high-dose single agent therapy is not myeloablative, multi-drug combinations may be. There is no experimental evidence to support this conjecture, indeed, combination chemotherapy does not necessarily result in a prolongation of the time for marrow recovery as compared to a single myelosuppressive agent, as suggested by Price and Hill (36,37). One high-dose combination of cyclophosphamide, etoposide and cisplatin has been given without bone marrow (38,39). As none of the individual agents in this combination are myeloablative, it is not unexpected that the combination is also not myeloablative. Other combinations have not been evaluated.

In experimental systems repeated doses of radiation and certain cycle-specific agents (i.e. busulfan, carmustine, melphalan) appear to cause progressive loss of bone marrow function either by damaging "stem cells", the marrow microenviroment or both (40-42). Thus, it is possible that some recovery of peripheral blood counts following high-dose therapy with these agent may occur despite irreversible damage to the marrow (40). These agents would not be myeloablative in the traditional sense as an initial recovery would occur. Repeated doses, without marrow, could eventually lead to total aplasia, whereas marrow infusion would presumably prevent this, assuming the damage is limited to the "stem cells". Alternatively, if the microenvironment is damaged, repeated doses, even if given with marrow, would likely lead to aplasia. In the scenario where combined "stem cell" and microenvironment

damage occurred, marrow infusion would be expected to offer an advantage, unless the microenvironment damage takes precedent, in which case the use of bone marrow would be expected to offer a short term advantage only, and no long term advantage.

The use of human recombinant bone marrow growth factors in conjunction with high-dose therapy may offer an alterative to bone marrow transplantation. Talmadge et al have demonstrated that recombinant granulocyte and granulocyte-macrophage colony stimulating factor can protect mice from "lethal" doses of cyclophosphamide or total body radiation (43). Also, a single case has been reported of a patient with lymphoma who 2 received the BEAM transplant regimen (BCNU 250 mg/m^2, etoposide 800 mg/m^2, cytarabine 800 mg/m^2, melphalan 140 mg/m^2) without marrow, followed by recombinant human granulocyte-macrophage colony stimulating factor (44). The patient had identical hematopoietic recovery to concomitant controls receiving mafosfamide purged autologous marrow and no bone marrow growth factor after transplant. Neihardt et al have demonstrated accelerated recovery using recombinant human granulocyte colony stimulating factor following up to three courses of high-dose cyclophosphamide, etoposide, and cisplatin (45). As many bone marrow growth factors appear to operate on differentiated cells, the use of cellular support in combination with growth factors may offer an additional advantage by supplying cells capable of promptly responding to the factor rather than requiring recovery from "stem cells" which would prolong the duration of neutropenia and increase the risk of infection (46). This approach needs further evaluation to determine if it is superior to the use of bone marrow growth factors alone.

One critical question that needs exploration in experimental animal systems and man is: can the use of bone marrow growth factors ameliorate the progressive marrow "stem cell" / microenvironment damage seen after repetitive doses of radiation and certain cycle-specific agents? Repetitive courses of high-dose chemotherapy with bone marrow growth factors could then be safely utilized to enhance the therapeutic effect. This approach, at present, has only been sparingly applied with autologous bone marrow transplantation for a variety of reasons. Other advantages of using bone marrow growth factors instead of autologous marrow include elimination of the concern of reinfusing tumor cells, as even with "purged" grafts it has not yet been technically possible to demonstrate all clonogenic tumor cells are actually eliminated. In addition, bone marrow growth factors may augment effector cell anti-tumor response and possibly decrease the risk of tumor progression or recurrence as commonly occurs following autologous bone marrow transplantation (47). Based upon the evidence presented it is suggested that continued exploration of high-dose combination chemotherapy with bone marrow growth factors is warranted as a therapeutic modality for the treatment of cancer.

ACKNOWLEDGEMENT

Supported in part by the Children's Cancer Research Fund of the University of Minnesota and grant NIH-NCI PO1CA-21737-10. I thank Drs. Bruce Blazar, Norma Ramsay and Roger Herzig for constructive comments.

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TABLE 1

Maximum dose of chemotherapeutic agents given either with or without a bone marrow transplant (mg/m^2/course)

Agent	type *a*	Stand. dose *b*	Max.Dose w/o BMT	(ref)	Max.Dose w/ BMT	(ref)
amsacrine (m-AMSA)	P	120	1200	(1)	1000	(2)
busulfan	C	6	180*c*	(3)	509*e*	(4)
carboplatin	C	300	2100	(5)	2400	(6)
carmustine (BCNU)	C	225	750	(7)	2850	(8)
cyclophosphamide	C	2000	7000	(9)	8000*e*	(10)
cytarabine (ARA-C)	P	1000	54000	(11)	36000*e*	(12)
dacarbazine (DTIC)	C	1000	2500*e*	(13)	*10500e*	(14)
diaziquone (AZQ)	C	40	224	(15)	295	(16)
etoposide	P	172	4800*e*	(17)	3500	(18)
hydroxyurea	P	7500	27700*f*	(19)	23100*f*	(20)
ifosfamide/mesna	C	9000	18000	(21)	*12000*e	(22)
melphalan	C	40	100,254*d*	(23,24)	260	(25)
mitomycin	C	6	90*c*	(26)	90,120*d*	(27,28)
teniposide (VM26)	P	67	1000	(29)	*450* c,e	(30)
thiotepa	C	60	120,270*d*	(31,32)	1575	(33)

a) C = cell cycle specific agent, P = phase specific agent

b) Standard dose given per course, except for busulfan which is dose per day. Data from Cancer Principles and Practice of Oncology, DeVita VT, Hetman S, Rosenberg SA (eds), 3rd edition, J B Lippincott Company, Philadelphia, 1989, pp 352-354, except for thiotepa from reference #34.

c) dose converted from mg/kg (mg/m2 = 30 x mg/kg).

d) If only one patient received the highest dose the next highest dose is given if significantly different.

e) given in combination with other chemotherapeutic agents of total body irradiation.

f) The dose per m2 was estimated by dividing total dose given to adult patients by 1.73

SUBCUTANEOUS ADMINISTRATION OF RECOMBINANT HUMAN GRANULOCYTE-MACROPHAGE COLONY STIMULATING FACTOR (rhGM-CSF) IN AUTOLOGOUS BONE MARROW TRANSPLANTATION

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INTRODUCTION

Colony stimulating factors (CSFS) are glycoproteins that control several functions of the hematopoietic progenitor cells [1]. The genes for 4 human myeloid CSFs have been cloned and their recombinant proteins produced. These include granulocyte colony-stimulating factor (G-CSF) [2], granulocyte-macrophage colony-stimulating factor (GM-CSF) [3], macrophage colony-stimulating factor (M-CSF) [4] and interleukin-3 (IL-3) [5].

Recently, a rapidly expanding literature has reported on the therapeutic effects of G-CSF and GM-CSF in patients with cancer with and without chemotherapy, AIDS, congenital agranulocytosis, cyclic neutropenia, idiopathic neutropenia, aplastic anemia, myelodysplasia and autologous bone marrow transplantation (reviewed in [6]). The availability of these hemopoietic hormones for clinical use represents a great promise in reducing chemotherapy-induced myelosuppresion, one of the major toxicities related to anti-cancer therapy, and to shorten the critical period following bone marrow transplantation. We report here our experience with rhGM-CSF In autologous bone marrow transplantation (ABMT).

PATIENTS AND METHODS

Nine patients who underwent autologous bone marrow transplantation (ABMT) for refractory or relapsed malignancy received rhGM-CSF. Written informed consent was obtained from all patients before their entry into the study. The clinical characteristics of the nine patients are listed in Table 1. Two patients with ALL had their bone marrow purged with mafosfamide (ASTA Z 7557 kindly provided by Dr. Sinderman from Asta Werke, FRG) at 80 ug/20 x 10^6, cells [7].

A double lumen right atrial indwelling catheter was placed and patients were housed in laminar air flow rooms. Antimicrobial prophylaxis consisted

of nystatin orally six times a day and Bactrim until one day before the transplant. Prophylactic acyclovir was given for patients with positive serology to herpes virus. Patients with negative serology for cytomegalovirus received CMV negative blood products. Patients who developed fever while neutropenic were cultured and started on a ceftazidime/amikacin protocol. Vancomycin and Amphotericin-B were added if needed. Single donor platelet transfusions were administered for platelet counts less than 20,000/uL or active bleeding. Packed red blood cells were transfused to maintain the hematocrit above 30%. All blood products were irradiated with 2,500 rads. Total parenteral nutrition was given depending on oral compliance for adults and from the beginning of the conditioning chemotherapy in the pediatric cases.

rhGM-CSF

Recombinant human granulocyte-macrophage colony stimulating factor (rhGM-CSF) was kindly provided by Schering-Plough and was reconstituted in 1 ml sterile water. rhGM-CSF was administered at 10 ug/kg as a daily subcutaneous injection from day 1 post-ABMT up to 14 days or until granulocytes reached 500/uL for two consecutive days.

Laboratory Assessment

Daily blood counts, including white cell differential, platelet count, reticulocyte count and hematocrit were performed during the hospitalization. Electrolytes, renal and liver function tests, PT and PTT were obtained three times a week. Bone marrow aspiration and biopsy were performed at days + 1; +8; +15; +22.

RESULTS

Eight patients were evaluable for response to treatment. Patient #6 died because of sepsis on day 11 without evidence of recovery of her blood counts and no evidence of toxicity that might have been related to rhGM-CSF. Four patients reached 500 granulocytes/ul before day 14 (Table 2) and a platelet count > 50,000/uL not related to platelet transfusions was observed between 2 and 12 days after the recovery of granulocytes. In the peripheral blood immature granulocytic and monocytic forms with toxic granulations appeared first and no eosinophilia was evident. However, this was a conspicuous finding in bone marrow biopsies until four weeks after the transplant. Two patients (#5 & #8) reached 500 granulocytes/uL with some delay (day + 32 and + 26). These cases received a low number of bone marrow mononuclear cells and bone marrow in patient #8 was purged with mafosfamide (ASTA Z 7557).

Toxicity of rhGM-CSF

At the dose of 10 ug/kg subcutaneous daily injection, toxicity was mild and well tolerated. It consisted of flu-like symptoms, muscular pain, water and salt retention and fever. Due to this side effect it was difficult to differentiate

infectious fever vs fever related to GM-CSF and empiric antibiotic therapy was started while the patients were neutropenic.

DISCUSSION

High-dose combination chemotherapy with ABMT as salvage therapy has been shown to be effective in selected cases of hematologic malignancies and solid tumors [8]. This procedure lacks the complication of the graft-vs-host phenomenon, usually associated with allogeneic transplants, however, patients go through a period of profound cytopenias which carries high morbidity due to infection and the need of platelet transfusions to prevent bleeding.

CSFs offer an attractive new modality to shorten this critical period and to reduce the incidence of infections, antibiotics use, platelet transfusions and hospital days. Clinical studies with rhGM-CSF and rhGM-CSF following bone marrow transplantation have recently been reported [9,10,11].

We investigated the clinical effects of rhGM-CSF given at a fixed dose by subcutaneous daily injection. A reproducible finding has been the acceleration of granulocytic recovery, however, little or no impact was observed on the megakaryocytic sector.

In our patients, median time to achieve an absolute granulocyte count >500/uL was 13.5 days (range 9-32). Recovery was sustained In all cases and no drop in counts was observed upon cessation of rhGM-CSF administration. Two patients in our group failed to obtain a rapid recovery in their counts despite of rhGM-CSF administration. This could be due to a low number of bone marrow mononuclear cells in the inoculum and in addition one of them received a purged graft. Failure of rhGM-CSF in some cases of severe aplastic anemia have been reported [12] and in one study no difference was observed between patients receiving rhGM-CSF and historical controls when purged bone marrows were transplanted for patients with acute lymphoblastic leukemia [13]. These observations address the possibility that a minimum of stem cells is needed to elicit a positive response to rhGM-CSF.

In our experience rhGM-CSF can be safely given to patients by subcutaneous daily Injection with mild and tolerable toxicity, however, controlled randomized trials need to be performed to establish the true Impact of rhGM-CSF In the clinical outcome of ABMT.

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TABLE 1

Patient	sex/age	dx	Induction	bmnc/kg	purged (Asta-Z)
1	f/ 7	ES	CBV	2.2×10^8	no
2	m/23	HD	CBV	1.4×10^8	no
3	f/22	HD	CBV	2.3×10^8	no
4	M/ 9	ALL	BEAC	3.0×10^8	no
5	m/20	HD	CBV	0.6×10^8	no
6	f/45	NHL	BEAC	2.5×10^8	no
7	m/41	ES	vic	4.5×10^8	no
8	m/26	ALL	BEAC	1.9×10^8	yes
9	f/ 9	ALL	BEAC	6.8×10^8	yes

HD = Hodgkin's Disease. NHL = Non-Hodgkin Lymphoma. ALL = Acute lymphoblastic Leukemia. ES = Ewing's Sarcoma.
CBV = Cyclophosphamide 6g/m2, BCNU 300 mg/m2, VP-16 750mg/m2.
BEAC = BCNU 300 mg/m2, VP-16 800 mg/m2, Ara-C 800 mg/m2, Cyclophosphamide 140 mg/kg. VIC = VP-16 1250 mg/m2, ifosfamide 7.5 g/m2, Carboplatinum 1 g/m2.
bmnc: bone marrow nucleated cells

TABLE 2

PATIENT #	day ANC >500/uL	day platelet count >50,000/uL
1	+12	+14
2	+17	+28
3	+11	+16
4	+14	+22
5	+32	+38
6	-	-
7	+9	+12
8	+26	+40
9	+13	+20

ANC = absolute neutrophile count

PERIPHERAL BLOOD PROGENITOR CELLS (PBPC): TWO PROTOCOLS USING GM-CSF POTENTIATED PROGENITOR CELL COLLECTION

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INTRODUCTION

Myelosuppression remains the dose-limiting toxicity for many combination chemotherapy regimens. For many malignancies, optimal responses are only achieved with dose intensive therapy, resulting in unacceptable myelotoxicity. The concept of removal and storage of sufficient numbers of hematopoietic stem cells to re-establish normal marrow function has been explored in both animal models and human trials. In lymphomas and Hodgkin's disease, high dose chemotherapy with autologous marrow "rescue" for patients failing standard therapy appears to result in prolonged disease-free survival compared to treatment with "salvage" chemotherapy regimens (1-5). Standard techniques for marrow harvest and reinfusion provide virtually all patients adequate recovery of neutrophils, platelets, and red cells. Despite the fact that organ toxicities are generally dose limiting in the ABMT setting, the major source of the 4-25% mortality remains infection or bleeding during the 3-4 week period of profound myelosuppression. The superior response rates of selected solid tumors to dose intensive chemotherapy (with marrow autograft support) has led to strategies designed to limit the morbidity of the procedure. More rapid hematologic reconstitution should substantially reduce mortality, morbidity, and the expense of high dose therapy.

The observation that the peripheral circulation of normal dogs and humans contained hematopoietic progenitor cells (6,7) was subsequently followed by the demonstration that human peripheral blood progenitor cells (PBPC) could be collected via established techniques for harvesting platelets (8). The discovery that selected cancer patients treated with cyclophosphamide and doxorubicin had a significantly increased concentration of PBPC at the time of leukocyte recovery (9) led to the observation that the initial depletion of the peripheral blood CFU-GM was followed by a rebound of 4-fold median increase above the baseline level. This phenomena has been confirmed in

patients receiving chemotherapy for lung, ovary, breast and other solid tumors (10-14). Although mononuclear cells harvested from peripheral blood during the chemotherapy rebound phenomenon increased the capacity to reconstitute hematopoiesis in dogs and man, 7- 10 leukaphereses were required for adequate stem cell collection. The finding that GM-CSF will further increase the absolute number of circulating CFU-GM enhanced by chemotherapy rebound (15) led to the realization that this augmentation may facilitate collection of adequate numbers of peripheral blood progenitor cells with fewer leukaphereses. Potentially, this may significantly increase the feasibility of PBPC autografting following high dose chemotherapy. Gianni et al have reported that a more rapid engraftment (8 days) was achieved if both autologous marrow cells and peripheral blood progenitor cells were used together in patients with breast cancer or NBL (16).

Given that peripheral blood progenitor cells appear to be a reliable source of stem cells for reconstitution of myelopoietic function, we have piloted two studies using either GM-CSF stimulated PBPC alone, or in combination with bone marrow support to rescue the myelosuppression of high dose chemotherapy. The objectives were to determine the time to neutrophil (500/ul) and platelet recovery (20,000/ul) after either PBPC or PBPC & marrow reinfusion, and the number of peripheral blood progenitor cells (CFU-GM/hour leukapheresis) that can be harvested by leukapheresis following GM-CSF, with or without chemotherapy rebound. An additional question was whether, like rhG-CSF (17), GM-CSF could reduce the oral toxicity of chemotherapy.

PATIENTS AND METHODS

Patients with solid tumors who were eligible for Phase I/II high dose therapy had a routine bone marrow harvest. Subsequently, GM-CSF (5ug/kg/bid) was administered and PBPCs were collected twice between days 5-7. Following high dose chemotherapy, both PBPC and marrow autogafts were returned to the patient.

In a second study, eligible patients with metastatic breast cancer underwent four cycles of induction chemotherapy with doxorubicin, 5-FU and methotrexate (AFM). Backup bone marrow was harvested following recovery from cycle 2. During cycles 3 and 4, GM-CSF (5ug/kg/d) was administered IV continuous infusion days 6-15, and peripheral blood stem cells were collected in two pheresis settings between days 14-18 of each cycle. Following high dose cyclophosphamide, thiotepa and carboplatin (CTCb), all PBPC collections (4 total) were reinfused.

Comparison was made with breast cancer patients treated with the same CTCB regimen but autogafted with marrow only (STAMP). Tumor involvement of bone marrow rendered a patient ineligible for any of the three studies.

RESULTS

Twelve evaluable patients have completed induction therapy for breast carcinoma and received intensification C-TCb with PBPC support only (Table 1). Myelopoietic recovery was incomplete in one patient who required bone marrow reinfusion day +107 for low platelets, and recovered > 20,000/ul platelets by day +134. Compared to 29 patients with responding breast cancer receiving identical intensification and bone marrow support alone, these patients had a significantly more rapid hematopoietic reconstitution. The time to ANC > 500/ul, platelets > 20,000/ul and discharge from hospital was reduced by a median of 7, 11 & 14 days, respectively. There was no evidence that GM-CSF reduced the mucositis in cycles 3 and 4 of AFM induction chemotherapy, although a cumulative dose effect could not be ruled out. Patients are followed to determine the time course of peripheral blood count normalization, and in selected patients, the marrow cellularity post-high dose chemotherapy with PBPC rescue.

The 12 evaluable patients receiving both marrow and PBPC reinfusions had a substantial reduction in their transfusion requirements when compared to 29 patients receiving marrow alone.

Compared with the STAMP CTCb patients, the time to re-engraftment of platelets > 20,000/ul, neutrophils > 500/ul, and discharge from the hospital were reduced by a median of 4, 7 and 11 days, respectively. These times to reingraftment correlated inversely with the number of PBPC and marrow cells reinfused. There were no clinical instances of delayed platelet count return.

DISCUSSION

Mobilization of PBPCs by GM-CSF with or without chemotherapy appears to facilitate collection of adequate numbers of peripheral blood progenitor cells with fewer leukaphereses, enhancing the feasibility of PBPC autografting. Clearly, granulocyte reconstitution may be faster when PB are used alone, or in combination, possibly due to reinfusion of a larger number of committed mononuclear cells. Adequacy and speed of recovery appears related in part to the number of stem cells reinfused. The larger number of CFU-GM required for peripheral blood progenitor cells reinfusion compared to autologous bone marrow transplantation cells may reflect a lower ratio of pluripotent to committed stem cells in peripheral blood or to the few stromal cells transplanted.

We continue to investigate the optimal numbers of PBPC for reinfusion post intensification chemotherapy, and improved methods of stem cell collection. Subsequent breast cancer patients receiving autografts with PBPC alone will receive GM-CSF post reinfusion.

ACKNOWLEDGEMENT

Supported in part by U.S. Public Health Service Grant P01CA-38493 and a grant from the Mather's Foundation. We wish to acknowledge the excellent clinical efforts of the house staff of the Beth Israel Hospital and the Brigham and Women's Hospital and the nursing staffs of 12W at the Dana Farber Cancer Institute and the Beth Israel Hospital. We also wish to acknowledge the excellent secretarial help of Judy McBreen and Janet Ullah. Authors' affiliations: the Departments of Medical Oncology, Biostatistics, and Tumor Immunology, Dana-Farber Cancer Institute (DFCI), and the Division of Medical Oncology and Department of Surgery, Beth Israel Hospital (BIH); Harvard Medical School, Boston, MA 02115.

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TABLE 1

	BM		PBPC & BM		PBPC	
# Patients	29		12		12	
Histologies	Breast		Solid Tumors		Breast	
# leukapheresis	0		2		4	
Days from Reinfusion to:						
ANC > 500/ml	21	(10-44)	17	(12-27)	14	(10-26)
Plts > 20000/ml	23	(10-81)	16	(10-24)	12	(8-15;134)
Hospital days	38	(26-93)	27	(21-36)	24	(19-34)

THE ROLE OF MONOCYTES IN THE STIMULATION OF PROGENITOR CELLS

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INTRODUCTION

Determination of CFU-GM in the mononuclear cell fraction (MNC) is most commonly used as a parameter for the viability of grafting material. The short term assay measures the most advanced progenitor cells of the granulo-monocytic line, which after stimulation go readily into final differentiation. They may account for the initial wave of peripheral white blood cells (WBC) which follows the aplastic phase after high dose chemotherapy or ablative treatment and transplantation; long term recovery depends on more immature cells with self-regeneration potential (1), which cannot be directly identified in man currently. Practical experience shows however, that complete and long term engraftment takes place if the graft contains a sufficient number of CFU-GM, which appears to imply that in this case also a sufficient number of repopulating cells are present.

All immature hematopoietic cells carry the surface marker CD34 (2). Highly specific monoclonal antibodies can be used for direct enumeration of this cell pool and for its separation from mature cells. Berenson and Bernstein have shown, that successful autografting can be achieved by use of this fraction instead of the total MNC in baboons and man (3), although it seems that in this case the aplastic phase lasts longer than after grafting total bone marrow MNC or cytapheresis products, the latter leading to most rapid recovery (4). Precise reasons are not known for dependence of the duration of the aplastic phase on the cellular composition of the graft. Progenitor cells might be more advanced in maturity than those in the bone marrow, but this would not explain a prolonged aplastic phase if similar rates of identical progenitors devoid of mature cells are transplanted.

Most of the complex events during hematopoietic recovery are not accessible for direct study in the human body, but better understanding of regulation mechanisms at the cellular level may ultimately help to improve insight into this process. Our current work concentrates on study of the size of CD34+ cell fractions in grafting material from different origin, on the

correlation between CD34+ cell rates and colony yield after stimulation, and on the stimulation mechanism for final differentiation.

Range of CD34+ Cell Fractions in Normal Bone Marrow, Cytapheresis Products and Fetal Blood

The size of the CD34+ cell fraction in the MNC can be reliably determined by cytofluorimetric analysis details of the method are described elsewhere (5,6). Briefly, cells are incubated with the monoclonal antibody HPCA-1 (My10, BD), washed, incubated with FITC-conjugated second antibody (goat-anti mouse IgG), washed and analyzed in a fluorescence activated flow cytophotometer. The positive cell fraction, representing all cells with fluorescence intensity above the background, necessitates correction for cells that are fluorescent due to nonspecific direct binding with the second antibody.

Bone marrow MNC were evaluated in 13 healthy volunteers (age 20-64, both sexes); cells were gained in 10 cases by aspiration from the iliacal crest, while in 3 cases small pieces of spongious bone were obtained from surgery; the bone spots were flushed with RPMI medium. After Ficoll separation an average rate of 1.02 % 0.6 CD34 cells were found in aspirates (table 1) ; it was noted, that rates in all bone flushes were in the higher range, possibly indicating that in aspirates the relative rate of immature cells is diminished by admixture of mature cells from simultaneously removed blood. Umbilical cord blood contains CD34+ cells at slightly lower concentrations than bone marrow aspirates of the adult, while the rate in normal adult blood is below the detection level (0.1 % of MNC).

After mobilization treatment of adults with high doses of Endoxan or Melphalan, CD34+ levels in cytapheresis products are found in the measurable range; rates change in the same individual from day to day during the rebound phase of recovery. This has an interesting practical implication: the result of direct determination is available on the same day, and therefore the decision on continuation of cytapheresis can be made according to the actual level of progenitor cells.

Correlation Between CD34+ Cells and CFU-GM Yield

If in normal bone marrow MNC the rate of CFU-GM is plotted against the rate of CD34+ cells in each sample, both turn out to be strictly proportional for the whole dose range (Fig. 1A). The same is observed in cord blood MNC (Fig. 1B). In both cases, there is moderate scatter. In cytapheresis products this correlation can also been found, although with more scatter, which is generated by daily fluctuations in cytapheresis products from the same individual (Fig. 1C).

In a total of 20 cases (Table 1, A and B) the correlation between the CD34 + cell rate and CFU-GM yield (b = 241 CFU-GM/ $2x10^3$ CD34+ cells) is highly significant (r= 0.85). The values collected from cord blood are well in line with those of adults. This indicates, that regardless of the source of the

cells, always a constant fraction of all immature cells gives raise to a colony of granulo-monocytic differentiation.

Since the absolute number of CD34+ cells is known, it becomes evident that in total MNC regularly one in 8 to 9 hematopoietic cells forms a day 10 CFU-GM under optimized stimulation and growth conditions (GMCSF + IL3 and Placental Conditioned Medium gave equivalent colony yields). This relation appears to have theoretical and practical implications and we call it in the following "Progenitor Stimulation Rate" (PSR).

While it is evident, that only a fraction of all CD34+ cells, which contains hematopoietic cells of all stages of development, will give raise to colonies of differentiated cells, it is astonishing, that this fraction is relatively small. *A priori* different reasons may account for this restriction. Only the progenitors committed to the granulomononcytic lineage respond to this kind of stimulation.It is conceivable that at any given moment a constant rate of more immature progenitors and precursors advance to the state where stimulation for terminal differentiation is possible. Alteratively, the immature cells themselves may not be limiting, and instead mature cells may act as regulators of progenitor stimulation.

Are Mature Cells Playing a Role in Terminal Progenitor Stimulation?

In order to distinguish between these possibilities, the bulk of mature cells is separated from the immature hematopoietic cell fraction by flow sorting according to FITC fluorescence intensity (Fig. 2). The positively stained cell fraction consists mainly of CD34+ cells, but some accessory cells, i.e. nonspecifically stained lymphocytes and monocytes, are still included as stated earlier (5).

If the positively stained fraction is put into culture, about fifty times more colonies per cell are obtained than in total MNC (Tab. 2), evidently reflecting the enrichment in progenitor cells. It is interesting to determine the PSR under this condition since enrichment in CD34+ cells is 82 fold, it actually decreases from initially 10.5% to 6.4%, i.e. only one in about 16 CD34+ cells forms a clone now! This indicates that the composition of the cell population is relevant for clonal outgrowth of progenitors at otherwise identical stimulation and growth conditions.

If purification is continued by purging for monocytes using the monoclonal antibody Mo2 and complement, extremely few colonies are seen at day 7 beside clusters, corresponding to a progenitor stimulation rate of 0.02%, or one out of 4500 cells forms a GM colony. If in turn the original cell population is reconstituted from the separated fractions, the PSR is restored to near initial values (Tab. 2). This suggests, that monocytes play a modulating role in intact MNC fractions, and at physiologic concentrations produce a homeostatic regulation of progenitor stimulation.

Consistent results in several experiments confirmed, that if highly enriched CD34+ fractions are essentially depleted from monocytes, their growth rate is strongly depressed ; if purified monocytes are added now, CFU-GM yields increase proportionally, as we showed before (7); at higher

monocyte rates, a plateau is achieved, that may correspond to the physiological range of homeostatic regulation, while To and Juettner (1984) have demonstrated, that exceedingly high monocyte concentrations decrease the colony yield.

Colony Growth Kinetics of Highly Purified CD34+ Cells

If cultures of highly enriched and monocyte depleted CD34+ cells are observed for extended periods, interesting events can be noted. During the first week of incubation, most cells maintain their initial size, round shape and light diffraction in spite of GM-CSF + IL3 or PCM being present; only clusters form, and few reach colony size. During the second week rapid colony growth starts and arrives at a maximum during the third week. Flow-sorted cells, in comparison, who still contain mature monocytes, show explosive growth during the first week already, which continues during the second week. Total MNC start growing early and reach their maximum during the first week (Fig. 3).

If the cells in cultures from the monocyte-depleted progenitors are analyzed, it seems that the begin of rapid growth coincides with de-novo formation of mature monocytes in the scarce colonies.

This suggests, that growth factors like GM-CFS + IL3 or PCM (termed ct CSF) are not sufficient for stimulation of CFU-GM progenitors, and that presence of mature monocytes is crucial, as is depicted in the following scheme (Fig. 4). In situations where monocytes are initially absent, they may exert a trigger function.

Although recovering hematopoiesis happens in a much more complex framework within complete three dimensional marrow microenvironment, analogous events happen with similar kinetics : after high dose chemotherapy has killed nearly all mature blood cells, there is an aplastic phase. Evidently resting progenitor cells survive, but do not proliferate initially. The begin of the rebound phase of recovering hematopoiesis is heralded characteristically by a massive monocyte peak.

Extrapolation of our data would then suggest, that growth factors produced by the activated reticuloendothelial system alone cannot stimulate the progenitors until first mature monocytes stemming from few primed advanced progenitors arrive. Once triggered, a snow ball effect on advanced progenitors gives rise to more mature monocytes and adjacently other peripheral blood cells.

While this interpretation is tentative and necessitates systematic in-vivo analysis, a fortuitous clinical observation may support this contention: in a patient treatment with GM-CSF during the aplastic phase had to be discontinued prior to the begin of recovery of mature peripheral WBC; the rebound phase came at the usual time and intensity, as seen without growth factor treatment, while in cases where this treatment was continued until recovery started, always strongly increased rebound and recovery of CFU-GM in cytapheresis was seen (8) and own unpublished observations).

CONCLUSION

The notion that the progenitor stimulation rate is constant in unmodified MNC fractions of different origin implies, that direct CD34+ cell rate determination gives equivalent information to CFU-GM determination in short term culture; the practical advantage, that it can be determined immediately, may make it a desirable alternative to conventional colony assays for clinical situations that necessitate fast decisions.

The suggested role of monocytes as regulators of progenitor stimulation may modify the interpretation of transplantation data: the duration of the aplastic phase may not only depend on the content of advanced progenitors in the graft, but also on the amount of viable mature monocytes. This may be one reason why recovery is faster after transplantation with peripheral blood cells, as well gained by cytapheresis in adults or as cord blood, (9), which both contain many more monocytes than bone marrow aspirates.

Finally, this model would provide an explanation why supportive treatment with recombinant rHu GM-CFS can only be effective in the presence of mature monocytes; it has been shown in vitro, that monocytes after stimulation with GM-CSF transform into macrophages (10), which supposedly contribute other indispensable factors (u-CFS) for regulated progenitor stimulation, thus adapting blood maturation to the actual need.

ACKNOWLEDGEMENTS

The expert technical assistance of M. Baerezung and J. Bachorz is highly appreciated. We thank N. Haerrig for typing the manuscript.

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TABLE 1

CD34+ cell rates and CFU-GM yield. A) bone marrow, a) flushes, b) aspirates; and B) umbilical cord blood.

	Case no.	% CD34+	d10 CFU-GM/2x10^{5}MNC
A a)	1	1.9	361
	2	2.0	540
	3	1.9	534
b)	1	1.1	255
	2	0.6	231
	3	0.9	138
	4	1.7	485
	5	1.7	225
	6	0.6	194
	7	0.5	211
	8	1.0	127
	9	0.7	38
	10	1.4	330
	Total :	1.02	228
B	1	0.3	76
	2	0.7	198
	3	0.8	113
	4	0.9	121
	5	0.9	153
	6	0.3	45
	7	0.4	112
	Total :	0.61	116.9

TABLE 2

Progenitor cell stimulation rate (PSR) during purification of bone marrow MNC. After Ficoll separation, cells were stained and flow sorted by FAC Star. Colonies were counted on day 7. For reconstitution, the purified and the unstained cell fraction were mixed in appropriate concentrations in order to reproduce the original composition.
* After purging, cells were put into culture without further analysis.

	% CD34+	CD34+/dish	CFU-GM/dish	CFU Enrichment	% CFU/CD34+	PSR
BM-MNC Total	1.1	2.200	232	1	10.5	9.5
Flow sorting a) stained b) unstained	90 0 (3)	18.000 /	1.150 63	50 /	6.4 /	15.7 /
Monoc.* Purge	90- 100	18.000- 20.000	4	(-)	>0.02	4.500- 5.000
Reconst.	1.1	2.200	211	0.9	9.6	10.4

FIGURE 1

Correlation between the rate of CD34+ cells in the MNC fraction, as determined by immunofluorescence activated cytofluorimetry (HPCA/FAC Star), and day 10 CFU-GM yield in short term culture after stimulation by 0.1 ml PCM or 10 ng/ml GM-CSF + 10 U IL3.

A) Bone marrow aspirates;

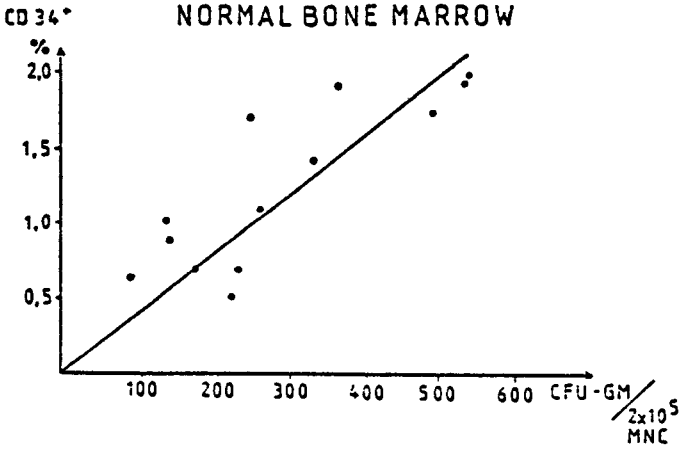
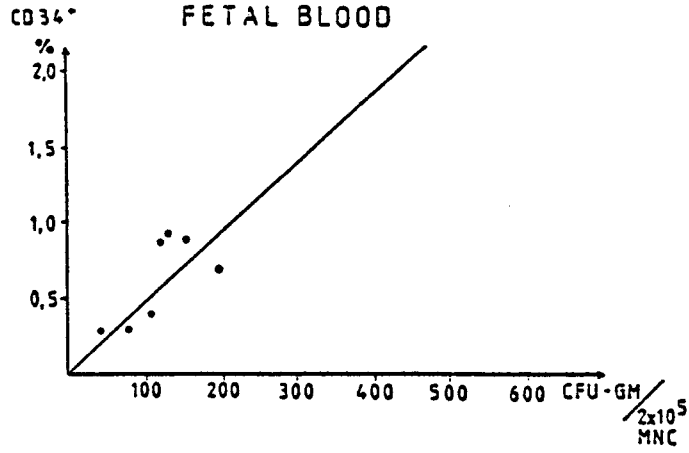


FIGURE 1 cont'd

B) Umbilical cord blood; and C) Cytapheresis products (samples gained from the same individual at subsequent days are connected by lines).



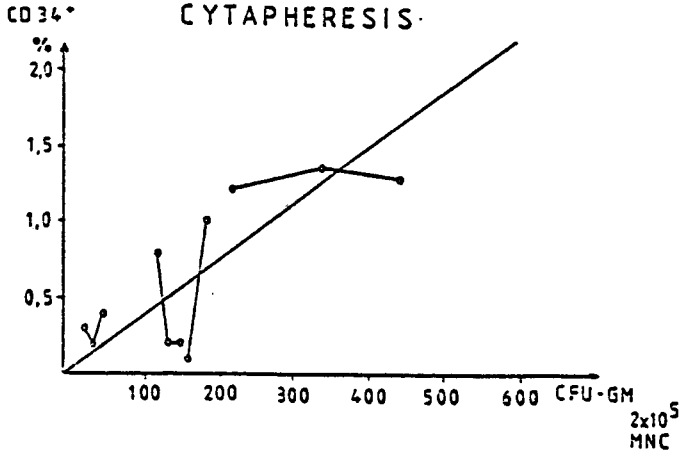


FIGURE 2

Flow sorting of bone marrow MNC after immunostaining with moAB My 10 and goat anti-mouse FITC. CD34+ cells are shaded, mature cells are symbolized as open circles. Black stars symbolize CFU-GM; their density per dish reflects the relative amount of colonies per CD34+ cells present in the culture.

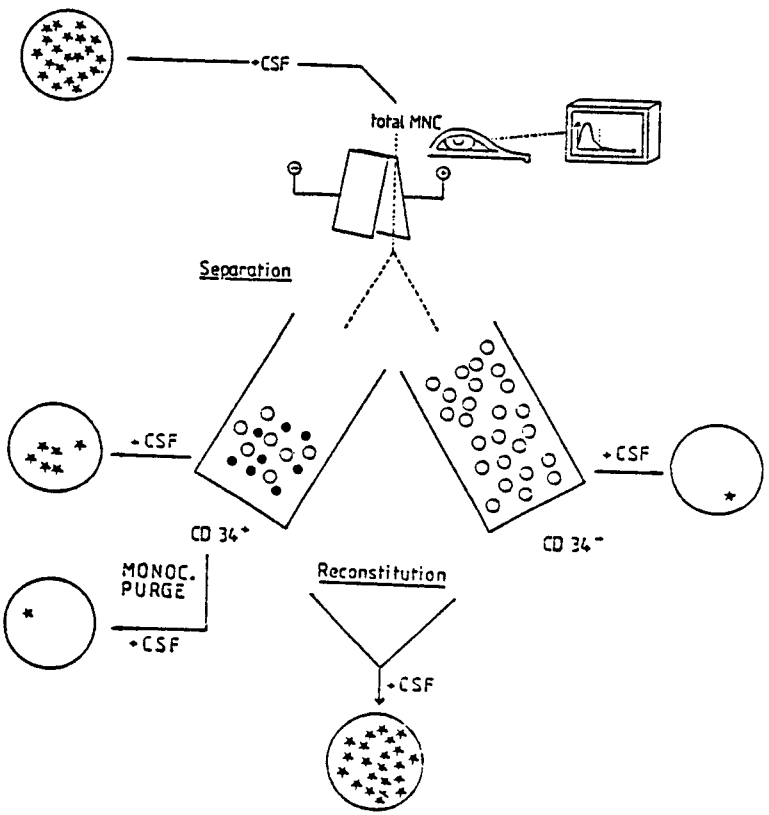


FIGURE 3

Kinetics of CFU-GM in 1) total bone marrow MNC; 2) flow sorted, highly CD34+ enriched fluorescence-positive cells; and 3) in sorted and monocyte depleted cells (designated CD34+ purged). CFU-GM rates per 2 x 10^5 cells of the respective fraction.

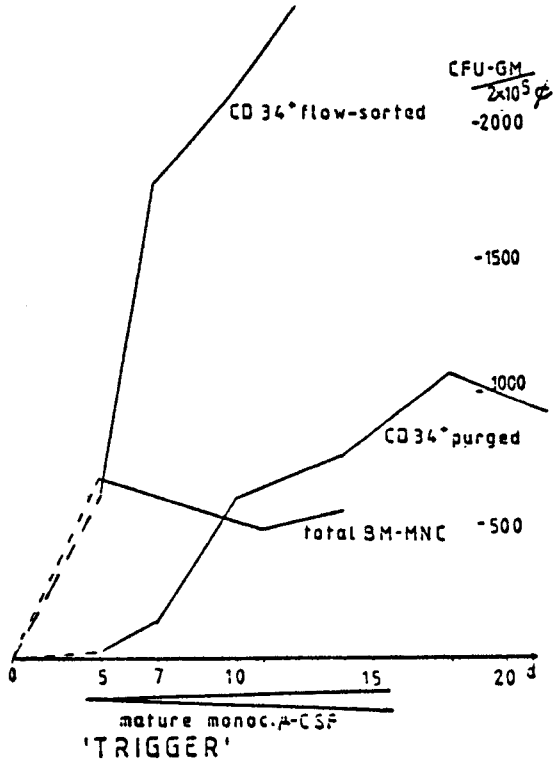
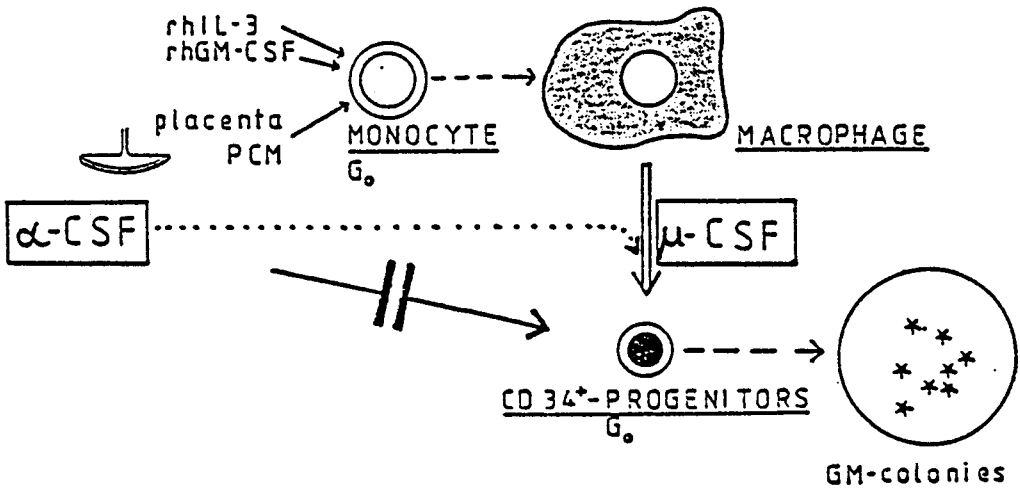


FIGURE 4

Schema of stimulation of advanced progenitor cells for terminal differentiation by GM-CSF + IL3 and placental conditioned medium. The latter, termed alpha-CSF, activate monocytes, and only in conjunction with monokines (termed u-CFS) stimulation of progenitors takes place, thus permitting regulation.



USE OF INTERLEUKIN-2 AFTER AUTOLOGOUS BONE MARROW TRANSPLANTATION

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SUMMARY

Graft Versus Leukemia Effects

One of the most important ways in which an allogeneic bone marrow transplantation (BMT) produces a lower risk of leukemic relapse, compared with chemotherapy alone, is by exerting a graft-versus-leukemia (GVL) effect. In this phenomenon, alloreactive T lymphocytes in the donor graft recognize recipient-specific allo-antigens on the leukemic blasts and eradicate the cells that bear them. Evidence for GVL in animal models is now very strong (1- 3), but its existence in man continues to be inferred from three lines of evidence (4). First, patients developing graft-versus-host disease (GVHD) are less likely to relapse than those who do not suffer this complication. Second, patients receiving non- reactive grafts from identical twins have a higher incidence of relapse than do recipients of MHC-identical but alloreactive siblings. Third, in some centers and for some disease categories, depletion of T cells from donor marrow is associated with an increase in the risk of leukemic relapse. This close link between graft alloreactivity and a GVL effect is unfortunate, because it seems to imply that GVHD is a necessary consequence of a "curative" allograft. Thus, attempts have been made to reduce the risk of relapse in transplant patients, either by inducing GVHD or by deliberately increasing its severity. Unfortunately, like most attempts to use one disease to cure another, this approach has not proved successful (5).

Mechanisms of GvL Effects

A more attractive way of capitalizing on the GVL effect is to try to separate it from GVHD after allografting or to try and generate such an activity after autografting, when GVHD is absent. There are two ways in which this might be done. The first depends on the existence of clones of T cells that recognize leukemia-specific antigens in the context of recipient MHC antigens. Previous efforts to find leukemia-specific antigens in man have failed. More recently, however, some leukemic cells have been found to possess unique

proteins formed by chromosomal translocations in which genes encoding two normal proteins become fused (e.g., t(9;22) in ALL/CML and (1;19) in ALL). If these unique proteins are processed and presented by the leukemic cell, there might be T-cell clones in the donors or patient's marrow that could recognize cells expressing such peptides (6). Expansion of these clones ex vivo with T-cell growth factors such as IL2 and reinfusion of the expanded clones into the patient would be expected to produce a specific GVL action.

The second approach to separating GVL from GVHD is to identify cytotoxic effector lymphocytes that can distinguish normal from malignant cells but are not alloreactive and are MHC unrestricted. An example of such effector cells are activated killer (AK) lymphocytes (7). since these cells do not depend on recognition of alloantigens for their activity, they would not produce GVHD and they might be inducible not only after allogeneic transplantation but after autologous BMT (ABMT) as well.

AK Cells in the Antileukemic Response

For the past five years, we have been attempting to identify such MHC-unrestricted, allo-nonreactive killer cells in peripheral blood after ABMT. We would like to determine if the AK cells' activity can be increased in vitro and in vivo by using stimulatory cytokines, such as interleukin-2 (IL-2), and if such treatment is likely to confer any benefits to the patients so treated.

Our data indicate rapid recovery of CD16+ and CD56+ AK cells after ABMT. These lymphocytes not only kill the classical NK target, K562, they are also able to destroy herpes virus-infected target cells and to inhibit the clonogenic growth of leukemic blasts, while having little effect on normal myeloid progenitor cells (9). The AK cells also spontaneously secrete cytokines such as tumor necrosis factor (TNF) and gamma-interferon (Y-IFN) (11). Interestingly, these cells are not produced during the recovery phase from chemotherapy and may develop as a consequence of the immune dysregulation associated with the deranged pattern of lymphocyte subset regeneration observed after both autologous and allogeneic bone marrow transplantation (9). In other words, the pattern of lymphoid recovery after ABMT already contains elements that may provide an antileukemic effect beyond that produced by chemotherapy alone. It is possible that this antileukemic effect contributes to the lower relapse rate associated with autografting compared to chemotherapy - most strikingly in the treatment of acute myeloid leukemia (AML) in second remission.

Activated killer cells regenerating after ABMT are not maximally stimulated, however. Addition of IL-2 to these cells in vitro markedly augmented their cytotoxic effector function and increased by two logs or more the quantity of Y-IFN and TNF produced (10). These observations imply that IL-2 administered in vivo might produce beneficial immunomodulation with antileukemic effects.

Specificity of AK Cells

It is important, however, to determine whether the apparent ability of these AK cells to distinguish between normal and malignant target cells is genuine. Most AK cells lack CD3 and therefore lack the only known antigen specific receptor present on cytotoxic effector cells. If AK cells cannot genuinely discriminate between normal and malignant targets, then there seems little purpose in further increasing their activity by infusing IL-2.

Fortunately, there are at least two mechanisms by which AK cells could discriminate between normal marrow and malignant clonogenic blast cells. The first relates to the cytokines these lymphocytes produce. A number of groups have now shown that normal myeloid clonogenic progenitors are much less readily inhibited by the combination of TNF and Y-IFN than are malignant myeloid clones (12,13). More importantly, perhaps, there are differences in the cell adhesion molecules used by AK cells to bind normal and malignant progenitors, so that in vivo AK cells may selectively bind to and therefore selectively destroy malignant blasts rather than normal progenitor cells. The AK subset with the most potent cytotoxicity against normal and malignant clonogenic cells is CD2 positive (Fig. 1). CD2 is a cell adhesion molecule (the sheep erythrocyte receptor) that binds the ligand LFA3 (CD58). It appears that LFA3 is critically important for the binding of activated killer cells to normal myeloid progenitor cells; preincubation of normal progenitors with anti-LFA3 antibody entirely blocks their susceptibility to killing (Fig. 2). By contrast, killing of malignant myeloid blasts by CD2+ effector cells is largely unaffected by antibody to LFA3 (Fig. 2). Killing of these cells can be prevented only if the target cells are coated with antibodies to another cell adhesion system involving the heterodimer LFA1 and its ligands, ICAM1 and ICAM2 (Fig. 2).

What is the relevance of this difference in binding to selective killing of malignant blasts in vivo? Since LFA3 (the CD2 ligand) is also present on human erythrocytes and erythroid progenitors, there will be competition for any interaction involving CD2+ effector cells and LFA3+ myeloid progenitor cells. Because interaction of AK cells with normal myeloid progenitors appears largely dependent on CD2 binding to LFA3, LFA3-bearing erythroid cells in the marrow will compete for the CD2 molecule on AK cells. This will reduce the binding and subsequent destruction of normal myeloid progenitors. Since the binding of malignant clonogenic cells can occur even when the CD2-LFA3 interaction is blocked, binding and killing of these cells should continue unimpeded. Although we have no evidence that this mechanism operates in vivo, the effect can be demonstrated clearly in vitro, where addition of free erythrocytes reduces still further the killing of normal progenitors by AK cells, but leaves killing of malignant blasts unaffected.

IL-2 Immunomodulation After ABMT

If we accept that there are reasonably sound theoretical reasons for giving IL-2 after ABMT, the next issue is whether such administration is safe or whether the combined toxicities of marrow transplantation and IL-2 would overwhelm the patient. Starting in January 1987, we undertook a phase I/II

trial of IL-2 in 17 patients who had received chemotherapy or autografts. The toxicity findings are summarized in Tables 1 and 2. In general, IL-2 was well tolerated when given as two 5-day courses repeated twice. Almost all patients developed fever >38C and nausea, and about half became significantly, but transiently, hypotensive. No patient needed treatment on an ITU. There was one death in this series, a patient who received prolonged infusion of IL-2 early in the study and developed interstitial pneumonitis that progressed despite termination of the IL-2 infusion.

These doses of IL-2 produced a high level of immune modulation, characterized by increased numbers of CD56+, CDl6+, or CD8+ AK cells (15). There was increased direct cytotoxicity against cells infected with herpes viruses and, most importantly, against both autologous and allogeneic leukemia, so that colony and cluster formation by malignant cells was inhibited by up to 95% (15). It was also possible to augment production of the "antileukemic" cytokines TNF and Y-IFN. Serum Y-IFN levels rose sharply during infusion of IL-2 and although a rise in TNF could not be detected in the serum, lymphocytes cultured from these patients showed a greatly increased production of TNF in vitro if they were obtained during IL-2 infusion (16).

One residual concern about IL-2 infusion in these patients was that the cytokines, particularly TNF and Y-IFN, would not only inhibit the growth of malignant myeloid progenitor cells but would also damage the engrafting normal progenitors. In fact, we found that the neutrophil count rose significantly during IL-2 infusion, an effect that could not be attributed solely to "demargination." Instead, we found that hematopoietic growth factors were also induced by IL-2. Thus, IL-3 and GM-CSF could both be detected in circulating lymphoid cells as transcripts and as protein; there was also a rise in serum levels of GM-CSF during infusion (17). Despite production of IL-3, however, platelet levels fell slightly during IL-2 infusion, for reasons that are not yet clear. In addition, IL-2 infusion modified the humoral immune response, although not in the way originally anticipated. IL-2 acts directly as a B cell growth factor; it also induces other B- cell growth and differentiation factors such as IL-4 (see below) and IL-6. Surprisingly, however, infusion of IL-2 did not augment the recovery of humoral immunity. Instead, total IgG levels in these patients fell and, more importantly, IL-2 infusion completely abrogated their ability to respond to both primary and secondary (recall) antigens such as KLH and tetanus toxoid (18). IL-2- induced humoral immunosuppression is therefore profound and may contribute to the observed increase in nosocomial infection rates in all patients receiving IL-2 infusion. At present we do not know why suppression of humoral immunity occurs.

These studies showed that IL-2 could be given safely after ABMT and could induce or enhance effector mechanisms that would be predicted to exert a GVL effect and reduce relapse rates; however, there were too few patients to allow firm conclusions about clinical efficacy. Accordingly, in May 1989, in collaboration with Glaxo-IMB, we undertook a multicenter study in which patients were randomized to receive IL-2 or no IL-2 on entering second remission of AML. IL-2 was to be given before and after autografting to 200

years; would have had an 80% power of demonstrating a 20% difference in survival induced by IL-2.

Sadly, in November 1989, the Glaxo Board of Directors decided to terminate all clinical trials of all cytokines, thus curtailing our efforts to learn whether or not IL-2 has any role in improving the outcome of ABMT. This decision has been particularly frustrating because of evidence that an "immunomodulatory" approach to ABMT after AML may indeed be appropriate (19). For example, a recently published study of the effects of BCG (bacille Calmette-Guerin) over a 10-year period showed that this weak immunomodulatory agent had beneficial effects (20). Even though BCG has limited immunologic efficacy, patients receiving the vaccine showed an improved long-term disease-free survival over those who were not so treated.

We continue to believe that immunomodulation will play an important role after ABMT, although at present this conviction has no firm basis. Until the clinical efficacy of IL-2 is established, it would seem worthwhile to attempt to improve the immunologic efficacy of IL-2 infusion while reducing its toxicity. One way to achieve this aim may be to manipulate those mechanisms responsible for the downregulation of IL-2-induced lymphocyte activation. An example of such a homeostatic mechanism involves IL-4, a cytokine induced during IL-2 infusion. If endogenous IL-4 activity is neutralized by monoclonal antibody in patients receiving IL-2, then the half-life of AK cell function is greatly prolonged. Moreover, secretion of cytotoxic cytokines such as TNF and Y-IFN can be augmented 100-fold or more by neutralizing endogenous IL4 (Fig. 3). If infusion of IL-2 were combined with infusion of antibody to IL-4 or IL-4 receptors it might be possible to reduce IL-2 dosage and simplify regimens of IL-2 administration. This approach is being investigated.

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TABLE 1

Toxicity in Two Patients Receiving IL-2 in Escalating Doses

Pt. no.	Pt. Age/Sex	Disease (FAB subtype)	Prior treatment	Total duration if IL-2 treatment (days)	Total IL-2 dose ($\mu g\ m^2$)	Adverse effects
1	67 M	AML (M4)	MACE	11[b]	1800	fever, nausea, vomiting, diarrhea bronchospasm hypotension
2	45 M	AML M3	Autologous BMT	12[b]	2700	fever, rigor nausea, vomiting, dyspnea pneumonitis hypotension death

AML, acute myeloid leukemia; MACE, MetAMSA, cytosine arabinoside, etoposide.
[a]Administered by daily infusion over 6 hr.
[b]Infusion interrupted because of toxicity.

TABLE 2

Toxicity Associated with Ten Courses of IL2 Given by Short Course Fixed-Dose Infusion After Cytotoxic Chemotherapy or BMT

Pt. no.	Age/ sex	Disease (FAB subtype)	Prior treatment	Total duration of IL2 treatment (days)	Daily IL2 dose ($\mu g\ m^2$)	Total IL2 dose ($\mu g\ m^2$)	Adverse effects
			After chemotherapy				
3	20 M	AML (M3)	HD ara-C	3	160	480	Fever
			HD ara-C	3	320	960	Fever
4	33 M	AML (M4)	HD ara-C	3	175	525	Fever
			HD ara-C	3	350	1050	Fever, nausea
5	45 M	AML (M3)	Mito/ara-C	3	315	945	Fever, nausea
			Mito/ara-C	3	580	1740	Fever, nausea
			Mito/ara-C	5	475	2375	Fever, rigors, nausea, vomiting, peripheral edema
6	30 M	AML (M4)	Mito/ara-C	5[a]	600	2500	Fever, rigors nausea, vomiting hypotension
7	18 M	AML (M2)	MACE	5	630	3150	Fever, nausea
			MACE	5	630	3150	Fever, nausea, skin rash
8	58M	MM	Autologous BMT	3	160	480	fever nausea skin rash
			Autologous BMT	5	315	1575	fever, nausea arm swelling skin rash peripheral edema
9	29F	AML M6	Autologous BMT	3	195	585	fever, rigors myalgia
			Autologous BMT	2[a]	390	600	fever, rigors myalgia chest pain

AML, acute myeloid leukemia; MM, multiple myeloma; HD ara-C, high-dose cytarabine; Mito/ara-C, mitozantrone plus cytarabine; MACE, MetAMSA, cytosime arabinoside, etoposide.
[a]Course interrupted because of toxicity.

TABLE 2 cont'd

10	33F	AML M7	Autologous BMT	4	480	1920	fever myalgia, nausea
11	58M	MM	Autologous BMT	5[a]	525	2500	fever, rigors nausea, vomiting diarrhea, confusion hypotension
12	55F	MM	Autologous BMT	5	600	3000	fever nausea, vomiting peripheral edema
			Autologous BMT	5	600	3000	fever nausea, vomiting peripheral edema
13	22F	AML M6	Allogeneic BMT	5	700	3500	fever, rigors nausea, vomiting

FIGURE 1

Peripheral blood lymphocytes and PBL either CD2+ depleted or enriched for CD2+ cells were prepared and incubated with IL2. The lymphocytes were then co-cultured with normal marrow or malignant blast cells before assessment of direct cytotoxicity or inhibition of colony growth. Results shown are mean $\pm$ SD of 5 experiments. Cytotoxicity is illustrated as % CR^{51} release at an effector:target ratio of 100:1 and colony inhibition is shown as a percentage of control culture colonies (no lymphocytes added) after co-culture with PBM-LAK, with CD2+ LAK or with CD2-LAK all added at 10:1 effector:target ratio. Control cultures of normal marrow produced 64 $\pm$ 7 colonies while control cultures of malignant blasts produced 29 $\pm$ 5 colonies. Paired t testing shows that CD2+ effectors are significantly more effective than CD2-effectors in both direct cytotoxicity ($p<0.001$ for normal and malignant cells) and colony inhibition ($p<0.01$ for normal cells $p<0.02$ for malignant cells).

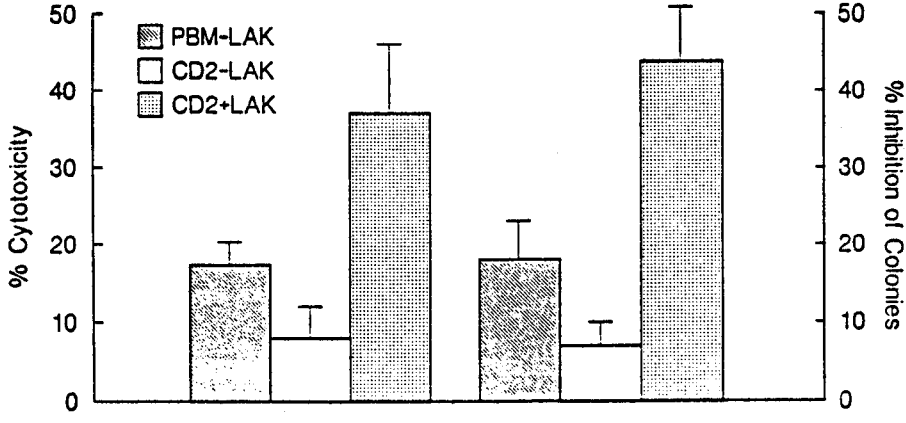


FIGURE 2

Lymphocyte depleted remission marrow or malignant blast cells were cultured alone, with CD2+ AK cells at 10:1 or with CD2+ AK cells and antibodies to LFA1 or LFA3. Colonies produced in the presence of AK cells are quoted as a percentage of control colonies in each experiment produced by marrow (mean number of colonies = 73 $\pm$ 11) or by blast (mean number of colonies = 26 $\pm$ 5) cultured alone. Data shown are the means + SEM of seven experiments for normal marrow and patient blasts. T testing showed: 1) significant inhibition of normal ($p = 0.04$) and malignant colony formation ($p < 0.001$) by CD2+ LAK cells; 2) Greater inhibition of malignant than normal cells by CD2+-AK effectors ($p = 0.01$). 3) Abrogation of significant inhibition of normal colony growth only by anti-LFA3, while inhibition of malignant colony cell growth was abrogated only by anti-LFA1.

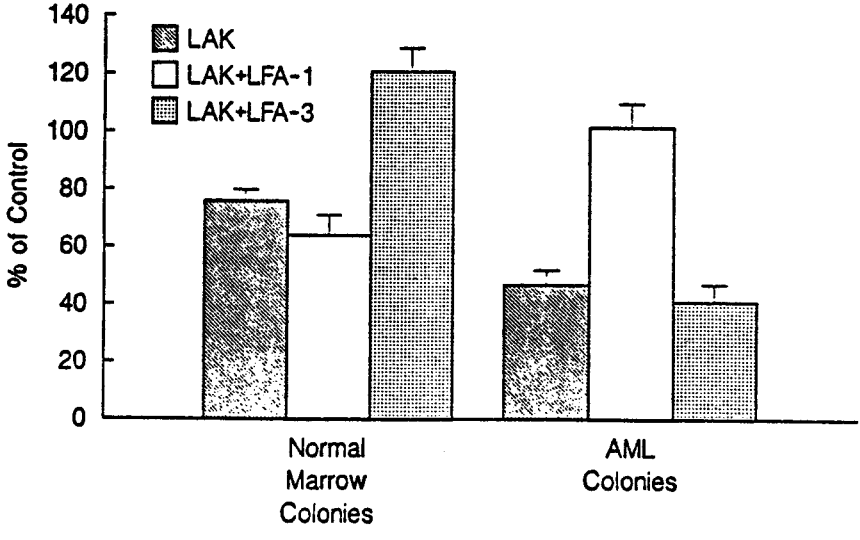
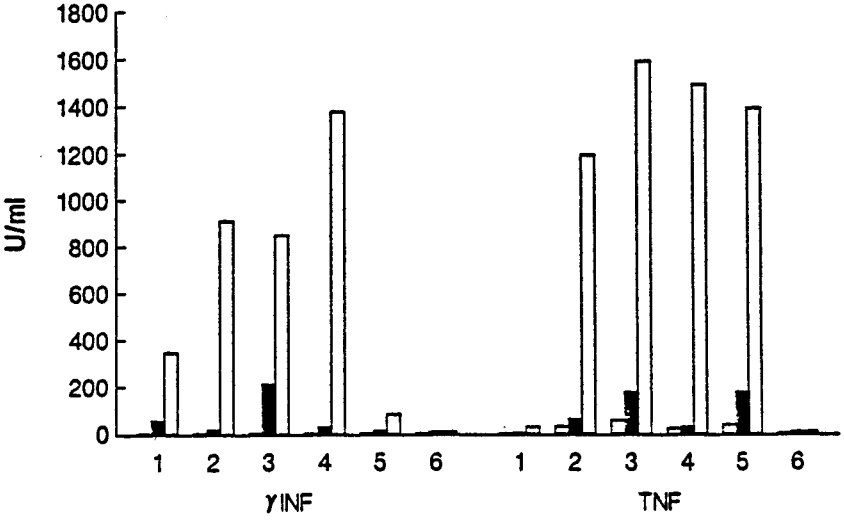


FIGURE 3

Blood mononuclear cells from 6 patients (4 post BMT, 2 renal cell carcinoma) were cultured at 2.5 x 10^6 (4 post BMT, 2 bars), with 500 U/ML IL2 (solid bars) or with IL2 and anti-IL4 antibody at 10 ng/ml (open bars). The gamma-interferon and TNF content of the supernatants were measured after 48 hr of culture. Addition of anti-IL4 antibody alone or of normal mouse Ig at 10 ug/ml to patient or normal control cells did not modify background cytokine secretion.



MEETING REPORT; AUTOTRANSPLANTS: NOW AND IN THE FUTURE

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DISCUSSION

The Fifth International Symposium: Autologous Bone Marrow Transplantation was held in Omaha, Nebraska from 22-25 August 1990. It was enthusiastically attended by about 250 clinical scientists from 12 countries. Here, we briefly summarize some of the presentations, discussions, concepts and controversies from the meeting.

The first sessions considered results of autotransplants in acute lymphoblastic leukemia (ALL) and acute myelogenous leukemia (AML). These data indicate 40 to 60 percent three- to five-year leukemia-free survival in adults with ALL in first remission and in adults and children with AML in first remission. These outcomes may be comparable to chemotherapy results when data are adjusted for subject-selection, prognostic factors for relapse and treatment failure and for time-to-treatment bias (time-censoring). Reasons for treatment failure are likewise comparable, mostly leukemia relapse. Some data presented suggest that persons with AML transplanted after longer intervals in first remission had fewer relapses. It was argued that is because post- remission chemotherapy given during this interval eradicates covert leukemia cells (termed minimal residual leukemia) in the subject and consequently in the harvested bone marrow. However, a more likely explanation is subject-selection and time-censoring. This interpretation is supported by the absence of a dose-response relationship in adults with ALL or AML receiving post-remission chemotherapy.

The rationale for autotransplants in leukemia is that increasingly intense therapy increases responses, and possibly cures. Is this correct? Part of the answer lies in analyses of data from twin transplants. Here, the reinfused bone marrow is normal. Also, immune-mediated anti-leukemia mechanisms associated with HLA-identical sibling transplants do not operate. Data from twin transplants in first remission or chronic phase indicate about 35 percent relapses in adults with ALL and about 60 percent in children and adults with AML. These rates are lower than autotransplants in ALL but comparable in

AML suggesting that more intense therapy increases cures in the former but not the latter.

There was considerable discussion regarding the possible role of in vitro bone marrow treatment in autotransplants for acute leukemia. As discussed, most relapses in AML are explained by persisting leukemia in the subject. Consequently, there is little rationale for this approach. Nevertheless, a large survey from the EBMTG Registry reported fewer relapses in persons with AML in first remission, but only in those where the interval between remission and autotransplant exceeded six months. Curiously, the rate was lower than that in twin transplants after a comparable interval in remission. These data suggest that an effect of in vitro treatment on decreasing relapse is not because leukemia cells are removed from the reinfused bone marrow. Perhaps in vitro treatment alters post-transplant immune recovery favoring graft-versus-leukemia reactions. Again, this seems unlikely since most data suggest an immune suppressive effect of this treatment.

In contrast to the situation in AML, it may be possible to evaluate in vitro treatment in adults with ALL in first remission. Here, twin data suggest that at least some relapses develop from reinfused leukemia cells. Data from the EBMTG Registry show no decrease in relapses in this setting. Perhaps this is because current in vitro treatment is not effective.

Results of autotransplants in more advanced acute leukemia, such as ALL or AML in second or third remission is less satisfactory with lower leukemia-free-survival and more frequent relapses. Here, there is also concern whether results of autotransplants are superior to chemotherapy or whether selection biases are responsible for these apparent differences. The high relapse rates preclude any meaningful analysis of efficacy of in vitro treatment. Some regimens seemed extraordinary effective in second remission. Curiously, these were less effective in first remission where greater efficacy is expected. These data suggest that small numbers and selection biases are likely responsible for these exceptional results.

Some data were presented regarding autotransplants in chronic phase chronic myelogenous leukemia (CML). Here, there was less than 10 percent three- to five-year leukemia- free-survival with conventional autotransplants. Data from autotransplants using bone marrow cells grown in vitro were also presented. By now, several recipients relapsed tempering these authors' enthusiasm. Results of autotransplants of blood cells from conventionally treated subjects or of blood or bone marrow cells from persons receiving interferon are too limited in numbers and follow-up for meaningful analysis.

The next important area discussed was autotransplants in lymphomas. Several forms of non-Hodgkin lymphomas such as lymphoblastic lymphoma, diffuse large-cell and nodular-mixed lymphomas were considered. Although the focus was on advanced intermediate and high-grade lymphomas, the possible role of autotransplants in low-grade lymphomas was also discussed. In advanced intermediate and high-grade lymphoma, two- to three-year progression-free survival was 20 to 60 percent. This range of outcomes likely reflects small numbers of subjects and selection biases. There was consensus

that better results are achieved in subjects with less advanced disease, notably those still responding to chemotherapy. As anticipated, persons with prior partial remission achieving complete remission following pre-transplant therapy had superior progression-free survival to non-responders. Also, persons with favorable prognostic factors for chemotherapy responded best to autotransplants. No specific pre-transplant regimen produced clearly superior results. Nor was it certain that in vitro bone marrow treatment was necessary or effective (vide infra).

The issue in intermediate- and high-grade lymphomas is whether seemingly improved results with autotransplants reflect inclusion of a greater proportion of subjects already cured (or curable) with chemotherapy and time-to- treatment bias. In an extensive literature review of about 1000 subjects with advanced intermediate- and high-grade diffuse large-cell lymphoma, we estimated minimal two-year disease-free survival of three percent with chemotherapy versus about 15 percent with autotransplants. This difference is unlikely to be explicable by time-censoring. Since other selection biases may operate, a randomized comparison is needed. Several trials sponsored by cooperative groups are in progress. Preliminary data from the only trial now analyzable shows no difference in progression-free survival. A similar question pertains to persons with high-grade B-cell lymphomas, especially Burkitt lymphoma and adults with poor-risk lymphoblastic lymphoma. There was considerable controversy whether results of chemotherapy in the latter group are so poor as to justify autotransplants as part of initial therapy. (Results of chemotherapy in children with these diagnoses are sufficiently good to preclude such a strategy.)

Several other important issues arose in regard to autotransplants for lymphomas. One disappointment is that cooperative trials to evaluate early autotransplants in adults with high-risk lymphoblastic lymphoma or diffuse large cell lymphoma are accruing subjects very slowly (or not at all). Consequently, the issue of optimal timing of autotransplants here is unlikely to be answered soon. Another issue was whether in vitro treatment prevents relapse. One study in diffuse large-cell lymphoma showed a correlation between in vitro growth of (presumably) tumor cells from the bone marrow pre-transplant and relapse post-transplant. However, most relapses were in sites of prior disease rather than in the bone marrow. The question then is whether bone marrow involvement is merely a prognostic factor or whether it causes relapse; perhaps because contaminating lymphoma cells migrate to sites of prior disease. Only a randomized trial can answer this question. Also unknown is whether in vitro bone marrow treatment is effective in persons with low grade lymphoma receiving autotransplants. Data here were not convincing. Also disturbing in low grade lymphomas was the observation that few additional subjects received remissions with more intensive pre-transplant therapy.

One interesting study compared results of autotransplants and HLA-identical sibling transplants in subjects with non- Hodgkin lymphomas and Hodgkin disease. These data indicate increased relapses in autotransplant

recipients. This is consistent with relapse from reinfusing lymphoma cells, absence of a graft-versus lymphoma effect or both.

Another important topic was the role of autotransplants in breast cancer. The sequence of these studies, involving almost 1000 subjects, began with subjects with advanced metastatic disease and progressing to women relapsing after conventional treatment. Some transplants are now performed in women with early breast cancer at high risk for relapse such as those with locally advanced or inflammatory breast cancer, or with operable stage 2 or 3 disease with ten or more positive lymph nodes or other adverse prognostic factors (HER2/NEU amplification, aneuploidy, high S-phase fraction, estrogen-receptor negative). Most trials of high- dose therapy now combine three or more alkylating drugs including platinol or carboplatin followed by a bone marrow autotransplant. Sometimes blood cells are given instead of, or combined with, the bone marrow graft. In several recent trials, hematopoietic growth factors, such as granulocyte or granulocyte-macrophage colony stimulating factor (G- or G/M- CSF) were given post-transplant to accelerate hematopoietic recovery.

There are several important questions in autotransplants for breast cancer. First, is there a relationship between increasing doses and response and/or cure? Here, results of conventional trials are contradictory. It is argued that those failing to detect a relationship investigate relatively small dose ranges. Also, few studies escalate doses of alkylating drugs such as those used for autotransplants. A more difficult issue is whether increased responses translate into increased long-term disease-free survival (cures). Data from several autotransplant studies are consistent with a dose-response relationship in breast cancer; women with advanced disease unresponsive to conventional doses achieve remission with more intensive therapy. However, most of these responses are brief. Not surprisingly, response rates are higher in women with less advanced disease. These persons are also more likely to respond to conventional therapy. The greatest difficulty is to evaluate the efficacy of autotransplants in early breast cancer. There are several problems here. One is that some subjects are already cured or could be cured with less intensive chemotherapy or hormone treatment. Another issue is whether women with stage 2 or 3 breast cancer with a sufficiently poor prognosis to justify intensive therapy can be identified accurately. These issues engender considerable debate. Although early transplants have better results, it is increasingly difficult to prove an advantage over conventional therapy. Clearly efficacy in early breast cancer can only be critically analyzed in a randomized trial.

Autotransplants in advanced breast cancer raise additional issues. Here, the debate is whether responses reflect subject selection and whether any women with advanced disease will be cured. Most, but not all, participants were convinced that the 20 percent complete responses in this setting were superior to conventional therapy. However, there are not yet sufficient numbers of subjects with sufficient follow-up to be certain of cure. These issues are similar too those discussed regarding autotransplants in lymphomas.

Another question in autotransplants for breast cancer is the possible role of bone marrow contamination in causing relapse. About 20 percent of histologically normal bone marrow are shown to contain breast cancer cells by sensitive techniques such as staining with monoclonal antibodies or cell culture. Are these cells capable of causing relapse and do they do so under current conditions of autotransplants? Data presented suggest a complex relationship if anything. Two studies reported increased relapses in sites of prior disease in persons receiving autotransplants containing these cells. These data suggest that either the reinfused cells home to these sites or that bone marrow involvement is a correlate but not the direct cause of relapse. A similarly complex relationship is reported in lymphomas. These data raise the issue of a possible role for in vitro bone marrow treatment in autotransplants for breast cancer. These issues can only be answered in controlled trials.

SUMMARY: TRANSPLANTATION FOR LYMPHOMAS

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DISCUSSION

Although much progress has been made in the initial treatment of patients with Hodgkin's disease and non-Hodgkin's lymphoma, there are still approximately 30% of the Hodgkin's patients and 50 - 60% of the non-Hodgkin's patients who cannot be cured with standard therapy options now available. High-dose chemo/radiotherapy with bone marrow or peripheral stem cell transplantation offers one method to overcome the resistance of the cell by dose escalation of the agents along with amelioration of the myelotoxicity by the infusion of hematopoietic stem cells. There have also been many great strides in the area of transplantation for lymphomas as outlined in the talks presented at this Fifth International Autologous Bone Marrow Transplantation symposium.

Transplantation has now been performed for a number of years for patients with relapsed Hodgkin's disease. As this symposium has pointed out, there are a number of different preparative regimens employed in this patient population utilizing various agents which may have different intensities and side effects. The regimen to which others have been compared to is the CBV regimen originally developed at the M.D. Anderson Cancer Center consisting of cyclophosphamide 6 gm/M, carmustine 300 mg/M, and etoposide 750 mg/M. The results of 128 patients treated with this program and followed for a minimum of 30 months were presented at this meeting as combined results from the patients treated at the University of Nebraska Medical Center and M.D. Anderson. The major prognostic factors identified in this protocol were the number of prior chemotherapies, Karnofsky performance status at the time of transplantation, and tumor bulk. The overall disease-free survival in this patient cohort was 23% with a median follow-up of 44 months. For those patients with only one prior chemotherapy, transplantation with CBV produced a disease-free survival curve of 40% during the same time interval.

A number of different variations have now been tested on this patient population including different chemotherapeutic agents, augmented doses of the same chemotherapeutic agents, double transplants, and additional supportive care measures such as growth factors. Several of the talks at this symposium discussed different chemotherapeutic agents useful for transplantation; however,

it is unclear at this time what the optimal conditioning regimen for Hodgkin's disease transplantation is. Also, more is not necessarily better, as demonstrated with the initial augmented CBV trials which had a 21% toxic death rate. However, as the use of agents has continued to be developed such as infusional VP-16 with cisplatin and decreased BCNU (CBViP), the toxic death rate has become more acceptable with perhaps an increase in progression free survival, although the follow-up time is much shorter with this study. The use of tandem or double transplants in this disease has also been of interest. It is difficult to get a large number of patients through a double transplant program due to toxicity and sometimes financial considerations; however, the results are certainly interesting.

The non-Hodgkin's lymphomas are a somewhat diverse group of diseases; therefore, transplantation in this disease must often be tailored to special circumstances. Transplantation for the lowgrade NHLs has only been used in a select number of cases over the past few years. Due to the relative newness of this approach, the follow-up may not be adequate to insure that patients transplanted for this disease are indeed free of disease. Several studies utilizing purged marrow or peripheral stem cells have been outlined in the symposium. Although the final outcomes are as yet unknown, several features can be discussed. Patients with minimal disease prior to the transplant seem to perform better with lower toxicity rates and longer disease free survival intervals. The transplantation of patients with histologically transformed disease is somewhat controversial with some studies showing worse results in this patient cohort, while others show no effect as long as the disease is minimal at the time of transplantation. Much longer follow-up in these studies will be need to give us the results of ABMT in low-grade NHL.

Although ABMT has been utilized for intermediate and high grade NHL for a longer period of time, the optimal timing and conditioning regimen have not been identified as of yet. As in ABMT for Hodgkin's disease, a number of different agents are currently being reported for transplantation in NHL. Augmentation with growth factors is also being intensely investigated. Several studies presented in this symposium also discuss the optimal timing for the transplant in this disease. Identification of the patients who are likely to relapse following initial induction therapy may allow these patients to receive high-dose chemotherapy with ABMT in first CR as was discussed in the lymphoblastic lymphoma section. The use of ABMT in these highly selected patients may greatly improve their prognosis.

Although the treatment of patients with newly diagnosed Hodgkin's disease and non-Hodgkin's lymphoma has greatly improved over the past two decades, the conventional therapy for patients who fail their initial management is less satisfactory especially in the non-Hodgkin's lymphomas. Strides as presented here for transplantation in these diseases have greatly expanded the ability to salvage patients after their initial chemotherapeutic failure. Although much more research will be needed to identify the best conditioning regimen and supportive measures for ABMT in lymphomas, the underlying premise of dose intensification has certainly been verified in a certain percentage of the

patients. Our goals for the future should be to improve upfront therapies in order to decrease the failures needing ABMT, to increase the effective drug delivery without increasing the non-myelsuppressive toxicities, and to improve the supportive care measures used during ABMT to decrease the morbidity and mortality associated with this procedure.

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