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High Dose Chemotherapy and Autologous Bone Marrow Transplantation in Acute Leukemias, Malignant Lymphomas and Solid Tumors

A Study of 23 Patients*†

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Abstract—*Twenty-three adult patients with end stage and/or poor prognosis malignancies* (6 *solid tumors*, 6 *malignant lymphomas*, 11 *acute leukemias*) *were treated by high dose chemotherapy, with* (16 *patients*) *or without* (7 *patients*) *reinfusion of cryopreserved autologous marrow. Eighteen patients were treated by the TACC regimen* (*cyclophosphamide* 45 mg/kg days 1–4, *ARA-C* 100 mg/m^2 q 12 hr days 1–4, 6-*thioguanine* 100 mg/m^2 q 12 hr days 1–4, *CCNU* 200 mg/m^2 day 2) *and others received high dose combination chemotherapy regimens designed specifically for their anticipated tumor sensitivity. Additional radiotherapy was delivered in three cases. The results were analysed for toxicity, kinetics of recovery of hematopoiesis, and anti-tumor effects. All patients receiving cryopreserved marrow engrafted successfully and none died, although severe sepsis occured in six cases. In contrast, of the seven patients who did not receive cryopreserved marrow following the TACC regimen, three died from aplasia on days* 15, 24 *and* 33. *Recovery of leukocytes* (*WBC*) *and platelets in peripheral blood occurred twice as fast in patients with cryopreserved marrow: patients with solid tumors and malignant lymphomas recovered a WBC count of* 1000/mm^3 *and a platelet count of* 50,000/mm^3 *on day* 11, *regardless of the nature of the high dose therapy. Patients with acute leukemia had slightly delayed kinetics with recovery of leukocytes* (>1000/mm^3) *and platelets* (>50,000/mm^3) *occurring on day* 18. *Five of the six patients with solid tumors had a partial response* (*PR*) *on high dose therapy. Two patients with Hodgkin's disease achieved a complete remission, but the duration of the response was short* (6 *and* 14 weeks). *All four patients with non-Hodgkin's lymphomas went into complete remission* (*CR*) *and have remained free of disease without maintenance therapy for prolonged periods. All four patients with acute leukemias who received cryopreserved marrow went into complete remission and the duration of this CR paralleled the duration of the initial CR at the beginning of which the marrow had been harvested. One patient* (*AML*) *is still in CR* 32 months *after high dose therapy + autologous bone marrow transplantation* (*ABMT*). *Of the seven acute leukemia patients who did not receive cryopreserved marrow, only one had a CR of very short duration* (1 month), *and persisting, massive leukemic infiltration was demonstrated in five. These results demonstrate that ABMT is feasible in man and that it shortens the duration of aplasia following high dose therapy by about* 50%. *They also suggest that high dose therapy + ABMT should be included in the management of patients with acute leukemias, non-Hodgkin's lymphomas and some selected solid tumors.*

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INTRODUCTION

In a preclinical study, we demonstrated that canine stem cells, frozen by an optimum freezing technique, and stored for periods up to 6 months in liquid nitrogen, retain complete viability [1].

Consequent to this study, freezing and storage facilities for human marrow were developed in our University hospital and a therapeutic program was designed. The chief aim of this program was to answer the following questions:

(1) Is autologous bone marrow transplantation (ABMT) feasible in man?

(2) What is the benefit from high dose chemotherapy and infusion of cryopreserved marrow in term of antitumor effect, duration of aplasia and hematologic support?

(3) What are the side effects of these procedures? This question led to a careful evaluation of immune status.

We and others have demonstrated the reality of autologous grafting following the infusion of cryopreserved marrow [2–5]. Preliminary results of immunological studies after autologous bone marrow transplantation in man have been published elsewhere [6].

The purpose of this paper is to report the antitumor effect of high dose chemotherapy in 23 patients with acute leukemias, malignant lymphomas and solid tumors, who entered our therapeutic trial between July 1976 and September 1979.

In our opinion, these results may help to define the clinical situations in which autologous bone marrow transplantation can be of benefit. Furthermore, this paper provides additional data on the kinetics of recovery of hematopoiesis following infusion of cryopreserved marrow; these data corroborate and extend our previous observations [4].

MATERIALS AND METHODS

Patients (Tables 1–4)

This study concerned 23 adult patients: six with solid tumors, six with malignant lymphomas and eleven with end stage acute leukemias. At the time of high dose therapy, most of these patients were resistant to conventional therapy to which they had been previously and repeatedly submitted. However, one patient with an adenocarcinoma of the ovary stage III and five patients with malignant lymphomas were treated by high dose therapy on a "first line" basis in view of the poor prognosis by usual criteria.

Patients with solid tumors and malignant lymphomas had no marrow involvement as determined by marrow aspiration and multiple biopsies, and for this reason, were considered eligible for bone marrow harvesting and storage. Additional examinations of harvested marrow and bone marrow biopsies performed under general anesthesia confirmed the absence of marrow involvement. For these patients, the storage duration of marrow was relatively short (median: 2 weeks, range 2 week–6 months) since marrow harvesting was decided at a time when high dose therapy was deemed urgent.

All these patients received autologous cryopreserved marrow following high dose therapy.

Of the eleven patients with acute leukemias, four had marrow storage while in their first complete remission. In these patients, high dose therapy and autologous marrow was used at the time of relapse and therefore the duration of marrow storage was somewhat longer (median: 4 months, range 3–18 months). Seven patients with end-stage acute leukemias, for whom no autologous marrow was available, received high dose therapy alone. One additional patient (ALL) received a minimum dose of autologous marrow (0.23×10^8 nucleated BM cells/kg), followed by two doses of methotrexate (30 mg on day +2 and 20 mg on day +4) in an effort to further decrease the tumor cell load and its proliferation rate, to the advantage of the normal stem cell population predominating in the cryopreserved marrow. This patient did not engraft and since he was the only failure of grafting in our series, it was assumed that the methotrexate administered after infusion of cryopreserved marrow had destroyed it. Also, this patient was an hepatitis B virus antigen carrier.

This patient was classified for analysis with the group of patients who did not receive cryopreserved autologous marrow.

Bone marrow harvesting, freezing and storage

To collect bone marrow cells from patients under general anesthesia, we used the procedure described by Thomas *et al.* [7]. The technique for marrow freezing and storage was similar to that used in our canine preclinical model [1–8], and has been described extensively in previous publications [4].

Table 1. High dose combination chemotherapy and autologous bone marrow transplantation in patients with solid tumors.

Diagnosis	Age (years) and sex	Previous therapy*	High dose chemotherapy	Dose of marrow infused (10^8/kg)	Time to recovery to: Leukocytes $>1000/mm^3$ (days)	Time to recovery to: Platelets $>5.10^4/mm^3$ (days)	Platelet transfusions (number)†	Tumor response‡	Survival post ABMT (days)
1: Nasopharyngeal carcinoma	44 M	VCR C	TACC	2.2	17	15	2	PR completed to CR by additional radiotherapy	120
2: Rhabdomyo-sarcoma	28 M	Surgery VCR C AD ACT D	TACC 5§	0.8	12	15	5	PR	132
3: Localized plasmacytoma	50 M	Radiotherapy ALK	VCR: 1.5 mg/m^2 C: 180 mg/kg	0.6	16	9	1	PR 25% Decrease of monoclonal spike on day 17	1200+
4: Adenocarcinoma of ovary Stage III	27 F	None	VM: 26 100 mg/m^2 C: 170 mg/kg AD: 50 mg/m^2	1.1	11	7	1	PR By second look laparotomy. Completed to CR by additional chemotherapy	800+
5: Testicular choriocarcinoma Stage IV	37 M	Surgery VLB BLEO CIS PLA Radiotherapy	Methotrexate+ Leucovorin rescue: 150 mg/kg C: 100 mg/kg AD: 40 mg/m^2	0.45	11	10	1	Failure	107
6: Testicular choriocarcinoma Stage IV	22 M	Surgery VLB	VLB: 10 mg/m^2 Methotrexate+ Leucovorin rescue: 200 mg/kg C: 180 mg/kg AD: 50 mg/m^2	0.66	11	12	3	PR 75% Decrease of HCG excretion in urine	90

*All drugs previously administered at conventional dosage (VCR, Vincristine; C, Cyclophosphamide; AD, Adriamycin; ACT D, Actinomycin D; VLB, Vinblastine; ALK, Alkeran; CIS PLAT, *Cis* Platinum).
†To maintain platelet count above 30,000/mm^3.
‡PR: Partial remission.
§TACC 5: TACC extended to 5 days instead of 4 days.

Table 2. High dose combination chemotherapy and autologous bone marrow transplantation in patients with malignant lymphomas

Histopathologic classification	Age (years) and sex	Previous therapy*	High dose chemotherapy	Dose of marrow infused (10^8/kg)	Time to recovery to: Leukocytes >1000/mm^3 (days)	Time to recovery to: Platelets >5.10^4/mm^3 (days)	Platelet transfusions (number)†	Tumor response and duration (days)‡	Additional therapy post-AMBT to complete induction (days)	Survival post-ABMT (days)
7: Hodgkin's disease (nodular sclerosis)	22 M	Radiotherapy M.O.P.P. vinblastine	CCNU: 200 mg/m^2 Cyclophosphamide: 200 mg/kg AD: 60 mg/m^2	1.9	18	20	5	CR: 100	None	450
8: Hodgkin's disease (nodular sclerosis)	43 M	None	TACC	1	13	17	5	CR: 45	None	123
9: Diffuse histiocytic	55 F	None	TACC Double dose of CCNU	1.6	10	45§	18§	CR: 630	Localized irradiation to mesenteric root (25 Gy)	750+
10: Diffuse lymphocytic	29 M	None	TACC	1.2	12	10	3	CR: 250	Abdominal field irradiation (10 Gy)	550+
11: Nodular lymphocytic	32 M	None	TACC	1.25	11	10	2	CR: 700+	Localized irradiation to mesenteric root (30 Gy)	700+
12: Diffuse lymphocytic	42 M	Surgery	TACC	1.1	11	21	5	CR: 550+	None	550+

*All drugs previously administered at conventional dosage.
†To maintain platelet count above 30,000/mm^3.
‡CR: Complete remission.
§Received 2 consecutive doses of CCNU and marrow 48 hours only after the last dose, which probably accounts for the long lasting thrombocytopenia.

Table 3. High dose combination chemotherapy and autologous bone marrow transplantation (ABMT) in acute leukemia

Diagnosis	Age (years) and sex	High dose chemotherapy	Dose of marrow infused (10^8/kg)	Storage duration (months)	Time to recovery to: Leukocytes $>1000/mm^3$ (days)	Time to recovery to: Platelets $>5.10^4/mm^3$ (days)	Platelet transfusions (number)	Tumor response and duration (months)*	Survival post-ABMT (days)
13: AML	29 M	TACC	0.5	6	17	23	8	CR 3/6	105
14: A monoblastic leukemia	45 F	TACC	1.4	3	19	28	9	CR 5/8	152
15: A monoblastic leukemia	39 F	TACC	0.6	3.5	14	14	13	CR 3/3	136
16: AML	18 M	TACC Double dose of ARA-C	1	18	19	14	5	CR 32+/19	1000+

*CR: Complete remission. The first number indicates the duration of the remission following ABMT; the second number indicates the duration of the complete remission at the beginning of which marrow was harvested for cryopreservation.

Table 4. High dose combination chemotherapy (T.A.C.C.) without ABMT in end-stage adult acute leukemia

Diagnosis	Age (years) and sex	Time to recovery to: Leukocytes $>1000/mm^3$ (days)	Time to recovery to: Platelets $>5.10^4/mm^3$ (days)	Tumor response and duration (months)*	Survival post-TACC (days)
17: ALL	38 F	30	33	CR: 30	64
18: ALL	26 M	Died on day 33 No recovery		No visible blast cells in marrow on post-mortem examination	33
19: AML	39 M	28 (9% Blast cells)	No recovery	Failure	56
20: CML in acute crisis	48 M	27 (77% Blast cells)	No recovery	Failure	90
21: CML in acute crisis	41 F	Died on day 15 No recovery		Persisting massive visceral leukemic infiltration on post-mortem examination	15
22: CML in acute crisis	41 F	20	No recovery	Failure. Persisting leukemic population with 2 PHI chromosomes	150
23: A monoblastic leukemia	36 F	Died on day 24 No recovery		No visible blast cells on bone marrow smear. No post-mortem examination	24

*CR: Complete remission.

High dose therapy (Tables 1–4)

Eighteen patients including all eleven patients with acute leukemias, five of the six patients with malignant lymphomas and two patients with solid tumors, received the same high dose combination chemotherapy regimen, the TACC.

Fifteen of these 18 patients received the basic 4-day course of TACC consisting of: cyclophosphamide 45 mg/kg i.v. days 1–4, cytosine-arabinoside (ARAC) 100 mg/m^2 i.v. q 12 hr days 1–4, 6 thioguanine (6 TG) 100 mg/m^2 p.o.q 12 hr days 1–4, and CCNU 200 mg/m^2 p.o. day 2. The other three patients received a higher dosage of chemotherapy which consisted in one case (AML) of a double dose of ARAC, in one case (malignant lymphoma) of a double dose of CCNU, and in the last case (rhabdomyosarcoma) of a course of TACC extended to 5 days with total doses of cyclophosphamide 225 mg/kg (18 g), ARAC and 6 TG 900 mg/m^2, and CCNU 200 mg/m^2.

The five patients who did not receive the TACC regimen received other high dose drug combinations designed specifically to accommodate previously known resistances and expected sensitivities of their malignant tumors (1 localized plasmacytoma, 1 Hodgkin's disease, 1 adenocarcinoma of the ovary, 2 testicular tumors). None of these regimen included ARA-C or 6 TG (see tables for details).

In some instances, irradiation was delivered to localized fields, after chemotherapy, as soon as hematologic recovery had occurred.

Autologous engraftment

Frozen marrow was thawed rapidly in a water bath at 37°C and infused immediately, with no attempt to remove DMSO or destroyed red cells. The marrow cells were administered by intravenous infusion without a filter, 48 hr after the last dose of cyclophosphamide, and at least 72 hr after the CCNU, except for one patient (diffuse histiocytic lymphoma) who inadvertently received the marrow 48 hr only after CCNU. The bags of bone marrow were administered two at a time to avoid renal damage from hemoglobinuria.

Forced diuresis (4 l/m^2), initially started with the administration of cyclophosphamide, was continued until urine cleared of hemoglobin. Dexchlorpheniramine maleate was given to counteract the possible effects of histamine release associated with i.v. DMSO.

Except for one patient, all patients were

treated in a protected environment and received oral non-absorbable antibiotics for bowel decontamination.

During the period of aplasia, patients were supported with frozen red cells and platelets collected with a cell separator (Aminco, Silver Spring, MD) to maintain the platelet count above 30,000/mm^3. All blood products were irradiated with 2500 rads in a gammacell 1000 irradiator (Atomic Energy of Canada Ltd., Ottawa, Canada). Day 0 was defined as day of marrow infusion or the corresponding day for patients who did not receive marrow. The doses of bone marrow infused, calculated using a correction for dilution with media and peripheral blood, ranged from 0.5 to 2.2×10^8 nucleated cells/kg (median: 1.10^8/kg).

Evaluation of results

Patients were evaluated for toxicity, rate of recovery of hematopoiesis and antitumor effects. In evaluating the rate of recovery of hematopoiesis, malignant lymphomas were considered as solid tumors and the patients were divided into four groups.

Group 1. Patients with solid tumors treated by TACC + autologous marrow (seven patients). This group included one patient with a diffuse histiocytic lymphoma who inadvertently received cryopreserved marrow 48 hr only after a second dose of CCNU. This short interval was assumed to be responsible for the [9] unusually long period of thrombocytopenia. This patient was therefore excluded from the group for the evaluation of platelet recovery, but was still considered for leukocyte recovery.

Group 2. Patients with solid tumors treated by other drug combinations + autologous marrow (five patients).

Group 3. Patients with acute leukemias in relapse treated by TACC + autologous marrow (four patients).

Group 4. Patients with acute leukemias in relapse treated by TACC alone (seven patients).

The doses of marrow infused in groups 1, 2 and 3 were similar:

Group 1: median 1.2×10^8/kg (range 0.8–2.2×10^8/kg);

Group 2: median 0.66×10^8/kg (range 0.45–1.9×10^8/kg);

Group 3: median 0.8×10^8/kg (range 0.5–1.4×10^8/kg).

Blood counts and differentials were done daily. Bone marrow aspirations were examined on days 3, 5, 7, 11, 15 and in some cases weekly thereafter. In a few cases, bone marrow biopsies were also done weekly. The number of platelet transfusions required to maintain the platelet count above 30,000/mm^3 during the aplasia period was also recorded as an indirect measurement of the severity of the aplasia.

Statistical analyses were performed using the Wilcoxon rank sum test, to compare daily blood leukocyte counts and the numbers of platelet transfusions.

To evaluate antitumor effects, patients were staged prior to treatment and responses were assessed at the time of hematologic recovery, and again after additional radiotherapy when used. Complete remission (CR) was defined as complete disappearance of tumor by appropriate clinical, hematological, biological, radiographic and isotopic investigations. When necessary, a second look laparotomy was performed (one patient with an adenocarcinoma of the ovary and two patients with malignant lymphomas).

Partial response (PR) indicated a diminution in measurable tumor size by more than 50%. The duration of the partial responses was found to be unreliable and was not taken into account.

The remaining patients were considered as having achieved no response to therapy and were classified as failures.

RESULTS

Toxicity

Except for nausea and vomiting, the TACC regimen (18 cases), as well as the other drug combinations, were well tolerated. Six of the sixteen patients receiving autologous marrow and four of the seven control patients (group 4) developed documented sepsis during the period of aplasia.

None of the patients receiving high dose therapy and autologous marrow died directly or indirectly from the procedure, whereas three of the control patients died from sepsis while in aplasia. There was no sign of cardiotoxicity in these series. It should be mentioned, however, that one patient not included in this study (ALL in complete remission), who received the TACC regimen followed by autologous marrow in an effort to increase the duration of the remission, developed severe myocardial failure with pericardial effusion, leading to death 4 days after completion of chemotherapy. No fat emboli were observed.

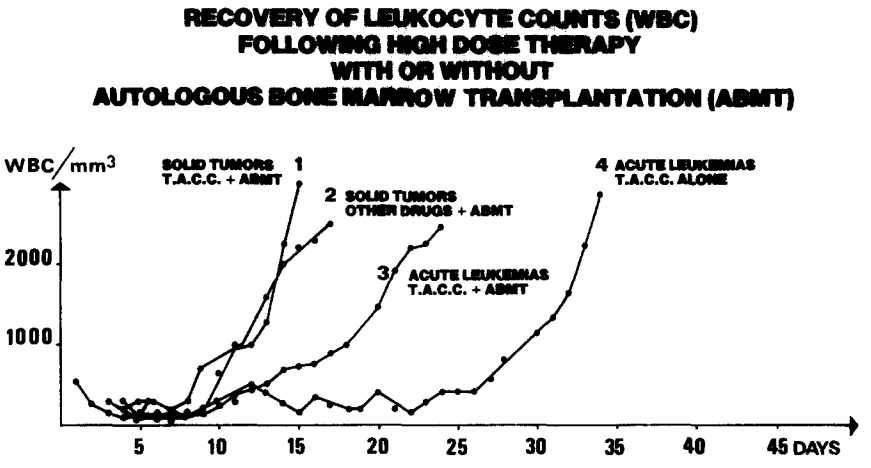

Fig. 1. Recovery of leukocyte counts (WBC) following high dose therapy with or without autologous bone marrow transplantation (ABMT). Median WBC/mm³ on each day, following: (1) TACC chemotherapy and ABMT in seven patients with solid tumors; (2) other high dose chemotherapy regimens and ABMT in five patients with solid tumors; (3) TACC chemotherapy and ABMT in four patients with end stage acute leukemia; (4) TACC chemotherapy in seven patients with end stage acute leukemia.

Renal function tests were performed routinely in all patients. They showed no damage resulting from hemoglobinuria nor was hematuria ever observed.

Kinetics of recovery of hematopoiesis (Fig. 1)

Overall, patients receiving cryopreserved marrow exhibited a similar pattern of hematologic reconstitution: bone marrow examinations showed recovery of hematopoiesis, with some myeloid precursors, a few erythroblasts and immature lymphoid cells between days 3 and 10. Megakaryocytes appeared between days 7 and 15. In the peripheral blood, recovery of leukocytes started between days 7 and 10 (median day 8) and recovery to a count of 1000/mm³ occurred between days 10 and 19 (median day 13). The platelet count reached 50,000/mm³ between days 9 and 28 (median day 14). Reticulocytes (>0.1%) appeared in the blood on days 8–25 (median day 13).

The comparison of peripheral blood leukocyte recovery in the four groups of patients showed that:

(a) Patients with solid tumors treated by TACC and ABMT (group 1 : 7 patients) had the same recovery pattern as patients with solid tumors treated by other high dose chemotherapy combinations + ABMT (group 2: 5 patients). In these groups combined (12 patients), the median day of leukocyte recovery to a count of 1000/mm³ was day 11 (range: days 10–18) and this was earlier than in patients with acute leukemias treated by TACC + ABMT (group 3: 4 patients) ($P < 0.05$ one-sided for days 11–15).

(b) Daily blood leukocyte counts in patients of groups 1 + 2, groups 1 + 2 + 3 and group 3 alone, were significantly higher than those of patients treated by TACC without marrow (group 4: 7 patients) ($P < 0.01$ one-sided for each day between day 10 and 25; $P < 0.05$ one-sided for each day between 10 and 25; $P < 0.01$ one-sided for each day between day 15 and 25). In group 4, the duration of bone marrow suppression induced by high dose therapy was twice as long as that observed in the three groups receiving cryopreserved autologous marrow.

The comparison of platelet counts in the peripheral blood showed a faster recovery to a count of 50,000/mm³ and a lower number of platelet transfusions for patients in groups 1 and 2, than for patients in group 3. The difference with group 4 patients was even more striking (Table 5). However, since six of the seven patients in group 4 never recovered a platelet count greater than 50,000/mm³, an evaluation of platelet recovery and needed support was not possible in this group.

A similar pattern of recovery was observed for reticulocytes which appeared in the peripheral blood (>0.1%) on days 11–16 (median day 13) in group 1, on days 8–21 (median day 10) in group 2, on days 8–21 (median day 12) in groups 1 + 2, and somewhat later, on days 12–25 (median day 16.5) in patients of group 3. These data are not statistically significant. In group 4, surviving

Table 5. Recovery of platelets and platelet support following high dose therapy with and without ABMT

Groups	Recovery to 50,000/mm³ day: median (range)		Number of platelet transfusions* median (range)	
1 (solid tumors TACC+ABMT)	15 (10–17)	11 (9–20)	2.5 (2–5)	3 (1–5)†
2 (solid tumors other drugs+ABMT)	10 (7–20)		1 (1–5)	
3 (acute leukemias TACC+ABMT)	18.5 (14–28)		8.5 (5–13)	
4 (acute leukemias TACC alone)	No recovery: 6 cases 1 recovery on day 33		Inevaluable >18	

*To maintain the platelet count above 30,000/mm³.
†These two values are statistically significant, $P<0.01$ one-sided by Wilcoxon rank sum test.

patients did not recover reticulocytes before day 26.

Thus the infusion of cryopreserved autologous bone marrow appears to accelerate the time to recovery of peripheral blood cells after high dose therapy. Furthermore, the recovery of peripheral blood cells is more rapid in patients with solid tumors, and is not related to the nature of the high dose therapy used.

Antitumor effect (Tables 1–4)

Solid tumors (other than malignant lymphomas) (Table 1). Table 1 indicates diagnosis, known resistances to drugs previously administered, high dose therapy used, tumor response and survival. Five of the six patients with solid tumors exhibited response. In two cases, additional radiotherapy (patient 1) or chemotherapy (patient 4) was given after full recovery of hematopoiesis and a complete response was then achieved. In all cases, the duration of the response was short, except in patient 4 (adenocarcinoma of the ovary) who is still free of disease 26 months post ABMT.

Malignant lymphomas (Table 2). The two patients with Hodgkin's disease went into complete remission, but the duration of the complete remission was short. All four patients (9–12) with non-Hodgkin's lymphomas had widespread infradiaphragmatic disease diagnosed by laparotomy which showed extensive involvement of the GI tract with multiple lumbar and aortic, celiac and mesenteric lymphadenopathies. In two cases (9 and 11), a "second look" laparotomy demonstrated complete remission, and biopsies of small residual lymphadenopathies of the mesenteric root were consistent with cicatricial fibrosis: additional radiotherapy was delivered to corresponding clipped areas. In the third case (patient 10), residual gastric tumor was visualized by fiberoptic endoscopy and biopsy-proven. Irradiation (1000 rads) was delivered to the abdominal field following recovery of hematopoiesis from high dose therapy, and complete remission was achieved. None of these patients received maintenance chemotherapy. Patients 9 and 10 relapsed 630 and 250 days post-ABMT. Patients 11 and 12 are still in unmaintained complete remission, 700 and 600 days post-ABMT.

Acute leukemias (Tables 3 and 4). All four patients treated with TACC+autologous marrow (Table 3) went into complete remission. The duration of this remission paralleled the duration of the initial remission at the beginning of which marrow had been harvested. Of particular interest was patient 16 whose marrow had been stored for 18 months before relapse. This patient has now been in complete remission for 32 months post-ABMT, with reinduction courses every month. These results contrasted with those of the seven patients treated with TACC without infusion of cryopreserved marrow, who had poor results (Table 4). Four survived the long aplasia period and only one (patient 17: ALL) went into a complete remission of very short duration. In five cases there was evidence for the absence of eradication of leukemia.

DISCUSSION

The efficacy of bone marrow cryopreservation to maintain hematopoietic stem cell viability, and the reality of autologous grafting following the infusion of cryopreserved marrow have been demonstrated in man [2, 4, 5, 10–14].

A preliminary report from our institution [4] on patients submitted to the TACC regimen has shown that infusion of cryopreserved marrow shortened the duration of aplasia by 50%. In this preliminary report, the median recovery of peripheral blood cells was observed on day 17 for leukocytes ($>1000/mm^3$), and on day 15 for platelets ($>50{,}000/mm^3$). No relation was demonstrated between the speed of recovery and the dose of bone marrow infused.

The present report indicates that the recovery of hematopoiesis is much faster in patients with solid tumors and malignant lymphomas than in those with leukemias. Indeed, in the former group, recovery of leukocytes ($>1000/mm^3$) and platelets ($>50{,}000/mm^3$) occurred on day 11, which accounts for the very low number of platelet transfusions required (median 3, range 1–5). Possible explanations for this faster recovery in patients with solid tumors include harvesting and infusion of greater numbers of CFUc (although doses of marrow by calculation of nucleated cells were similar in all groups), and/or shorter storage duration of marrow, hence better viability of stem cells, as reported recently in canine experiments [15]. Unpublished data from numerous institutions, including ours, favor the first hypothesis.

The similarity of the kinetics of recovery in patients treated by TACC and those treated by other high dose combination chemotherapy regimens not including the highly aggressive ARA-C–6 TG combination can be held as further evidence of the reality of autografting, with optimal kinetics of engraftment which cannot be further speeded up.

Whereas the results of kinetic studies demonstrate the value of the infusion of cryopreserved marrow as an adjunct to high dose therapy, in terms of hematologic support, very few indications are available at the present time as to the efficacy of high dose therapy *per se* for the treatment of human cancers. However, work in animal tumor systems has shown that higher doses of many anti-cancer drugs, including cyclophosphamide, ARA-C, adriamycin and methotrexate, have correspondingly greater anti-tumor effects [16–18], and a similar trend can reasonably be expected in man, with doses that would produce unacceptable bone marrow toxicity in unsupported patients.

Our report demonstrates the feasibility of high dose therapy thanks to autologous bone marrow transplantation and the relative safety of this technique, since none of the sixteen patients receiving cryopreserved marrow died from this procedure, although six developed severe sepsis while in aplasia.

In our department, high dose therapy was given to one patient at a time so as to allow optimal care and support. Consequently, the number of patients is small, although this paper covers our experience over a 3-year period. Evidently, the most suitable situations for which high dose therapy and ABMT may provide real benefit should be defined as early as possible. From our experience, it appears that most patients with solid tumors and Hodgkin's disease, although they responded well initially, had remissions of short duration. In contrast, all patients with non-Hodgkin's lymphomas of poor prognosis went into complete remission and have remained free of disease without maintenance therapy for long periods. These results are consistent with the NIH study which showed a high rate of complete remissions (7/9) and three prolonged complete unmaintained remissions in a group of nine patients with non-Hodgkin's lymphomas (6 Burkitt lymphomas, 3 diffuse histiocytic lymphomas) resistant to conventional therapy, and submitted to the BACT regimen* +ABMT [2]. Both our data and the data from NIH support the inclusion of high dose therapy and ABMT for the management of patients with non-Hodgkin's lymphomas.

All four patients with acute leukemias treated by TACC+ABMT in our department went into complete remission. The duration of this remission paralleled the duration of the initial remission at the beginning of which marrow was harvested.

In contrast, of the seven patients with end-stage acute leukemias who received the TACC regimen with marrow, only one (ALL) went into a complete remission of very short duration (1 month). Four patients survived the aplasia period which was twice as long as in patients receiving cryopreserved marrow, and three died from sepsis on days 15, 24 and 33. In five cases, these was clear evidence of massive persisting leukemic infiltration.

These results indicate that in patients with

*BACT: TACC with BCNU instead of CCNU.

acute leukemias, the value of high dose therapy + autologous marrow is not restricted to a mere increase in tumor cell killing. It is conceivable that, in addition to shortening the duration of post-chemotherapy aplasia, autologous marrow harvested at the time of remission supplies a large number of both normal stem cells and immunocompetent cells, which may somehow be responsible for a new complete remission.

We therefore believe that bone marrow from patients with acute leukemias of poor prognosis should be harvested and stored as soon as a complete remission is achieved. This marrow can either be used later, at the time of relapse, or immediately, while the patient is still in complete remission, to support a high dose consolidation chemotherapy regimen. This situation is somewhat analogous to allogeneic bone marrow transplantation for patients with acute leukemias in remission.

In conclusion, we believe that the results of our study support the following statements:

(1) Stem cells can be effectively conserved by cryopreservation;

(2) Autologous bone marrow transplantation (ABMT) is feasible in man;

(3) Following high dose chemotherapy, ABMT shortens the duration of the aplasia by 50%;

(4) High dose therapy + ABMT produces best results in patients with acute leukemias and non-Hodgkin's lymphomas;

(5) In end-stage acute leukemia, the combination of TACC + ABMT appears to produce a high rate of complete remission (CR). The duration of this CR parallels that of the initial remission during which marrow was harvested for cryopreservation;

(6) In non-Hodgkin's lymphomas, the combination of TACC + ABMT appears to produce a high rate of unmaintained long term remissions and complete cure is a possible goal;

(7) In patients with solid tumors, ABMT enables administration of high dose therapy. However, since a single course of high dose therapy is only partially effective, it may be more appropriate to postpone it until the end of a dose escalation program, at a time when complete remission has already been achieved by conventional therapy.

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REFERENCES

1. N. C. Gorin, G. Herzig, M. I. Bull and R. G. Graw, Long term preservation of bone marrow and stem cell pool in dogs. *Blood* **51,** 257 (1978).
2. F. R. Appelbaum, G. P. Herzig, J. L. Ziegler, R. G. Graw, A. S. Levine and A. B. Deisseroth, Successful engraftment of cryopreserved bone marrow in patients with malignant lymphoma. *Blood* **52,** 85 (1978).
3. J. M. Goldman, K. H. Th'ng, D. S. Park, A. S. D. Spiers, R. M. Lowenthal and T. Ruutu, Collection, cryopreservation and subsequent viability of haemopoietic stem cells intended for treatment of chronic granulocytic leukemia in blast cell transformation. *Brit. J. Haemat.* **40,** 185 (1978).
4. N. C. Gorin, A. Najman, Ch. Salmon, J. Y. Muler, J. C. Petit, R. David, J. Stachowiak, F. Hirsch Marie, Y. Parlier and G. Duhamel, High dose combination chemotherapy (TACC) with and without autologous bone marrow transplantation for the treatment of acute leukaemia and other malignant diseases. Kinetics of recovery of haemopoiesis. A preliminary study of 12 cases. *Europ. J. Cancer* **15,** 1113 (1979).
5. U. W. Schaefer, Transplantation of fresh allogeneic and cryopreserved autologous marrow in acute leukemia. *Exp. Hemat.* **5,** 101 (1977).
6. N. C. Gorin, J. Y. Muller, Ch. Salmon, J. M. Fine, P. Rouger, B. Fortier, J. C. Petit, O. Girard, G. Leblanc, Y. Parlier, A. Najman and G. Duhamel, Immunocompetence following autologous bone marrow transplantation. *Exp. Hemat.* **7,** suppl 5, 327 (1979).
7. E. D. Thomas and R. Storb, Technique for human marrow grafting. *Blood* **36,** 507 (1970).
8. N. C. Gorin, R. Elgjo, F. Stout and T. Knutsen, Long term preservation of canine bone marrow: *in vitro* studies. *Blood Cells* **4,** 419 (1978).
9. R. S. Weiner, S. A. Scudder and J. K. Lewis, Experimental isologous bone marrow transplantation after chemotherapy. *Exp. Hemat.* **5,** suppl No. 2, 44 (Abstract) (1977).

10. H. Kaiser, B. G. Leventhal, M. D. Wharam *et al.*, Cryopreserved autologous bone marrow transplantation in the treatment of selected pediatric malignancies. A preliminary report. *Transplast. Proc.* in press.
11. K. A. Dicke, K. B. McCredie, G. Spitzer, A. Zander, L. Peters, D. S. Verma, D. Stewart, M. Keating and E. E. Stevens, Autologous bone marrow transplantation in patients with adult acute leukemia in relapse. *Transplantation* **26,** 169 (1978).
12. J. M. Goldman, D. Catovsky, J. Hows, A. S. Spiers and D. A. Galton, Cryopreserved peripheral blood cells functioning as autografts in patients with chronic granulocytic leukaemia in transformation. *Brit. med. J.* **1,** 1310 (1979).
13. P. R. Graze, J. R. Wells, W. Ho, R. P. Gale and M. J. Cline, Successful engraftment of cryopreserved autologous bone marrow stem cells in man. *Transplantation* **27,** 142 (1979).
14. J. S. Tobias, R. S. Weiner, C. T. Griffiths, C. M. Richman, L. M. Parker and R. A. Yankee, Cryopreserved autologous marrow infusion following high dose cancer chemotherapy. *Europ. J. Cancer* **13,** 269 (1977).
15. F. R. Appelbaum, G. P. Herzig, R. G. Graw Jr., M. I. Bull, C. Bowles, N. C. Gorin and A. B. Deisseroth, Study of cell dose and storage time on engraftment of cryopreserved autologous bone marrow in a canine model. *Transplantation* **26,** 245 (1978).
16. W. R. Bruce, B. E. Meeker and F. A. Valeriote, Comparison of the sensitivity of normal hematopoietic and transplanted lymphoma colony forming cell to chemotherapeutic agents administered *in vivo*. *J. nat. Cancer Inst.* **37,** 233 (1966).
17. A. Goldin, Factors pertaining to complete drug induced remission of tumor in animals and man. *Cancer Res.* **29,** 2285 (1969).
18. H. E. Skipper and L. H. Schmidt, A manual on quantitative drug evaluation in experimental tumor systems. *Cancer Chemother. Rep.* **17,** 1 (1962).