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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*†

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Abstract—*Twelve patients received a high dose combination chemotherapy regimen (TACC) consisting of cyclophosphamide* 45 mg/kg *i.v.* day 1–4, *ARA-C* 100 mg/m^2 *i.v. every* 12 hr day 1–4, 6 *thioguanine* 100 mg/m^2 *by mouth every* 12 hr day 1–4, *CCNU* 200 mg/m^2 *by mouth* day 2. *Of these* 12 *patients*, 8 *received cryopreserved autologous marrow and* 4 *received only supportive care.*

The patients were divided into 3 *groups:*

—Group 1 *consisted of* 4 *patients with solid tumours without bone marrow involvement.*

—Group 2 *consisted of* 4 *patients with drug resistant acute leukaemia in relapse.*

—Patients in groups 1 *and* 2 *received the TACC regimen followed by the infusion of cryopreserved marrow harvested at a time when bone marrow examination was normal. In these two groups, the doses of bone marrow infused ranged from* 0.5 *to* 2.2 $\times 10^8$ *nucleated bone marrow cells/kg and had been preserved for periods up to* 18 months. *Recovery to a WBC count of* 1000/mm^3 *occurred on* days 12–19 (*median* day 17) *and recovery to a platelet count of* 50,000/mm^3 *occurred on* days 9–28 (*median* day 15). *In group* 1, *one patient with Hodgkin's disease went into complete remission. The* 3 *other patients went into partial remission. In group* 2, *all* 4 *patients with acute leukaemia went into complete remission.*

—Group 3 *consisted of* 4 *patients with drug resistant acute leukaemia. These patients received the same high dose combination chemotherapy regimen without cryopreserved marrow. One patient went into a complete remission with a recovery to a WBC count of* 1000/mm^3 *on* day 30 *and recovery to a platelet count of* 50,000/mm^3 *on* day 35. *This patient relapsed on* day 69. *In* 2 *other patients, recovery to a WBC count of* 1000/mm^3 *occurred on* days 27 *and* 28 *with* 77% *and*

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9% residual circulating leukaemic cells. The last patient died on day 15 *with severe hypoplasia and persisting massive visceral leukaemic infiltration.*

—Groups 1+2 *(infusion of cryopreserved marrow) were compared to group* 3 *(no infusion of cryopreserved marrow), and group* 2 *was compared to group* 3 *(acute leukaemias).*

The results of this study support the following statements.

1. *Following high dose combination chemotherapy (TACC), the reinfusion of cryopreserved autologous marrow is beneficial in shortening by about 50% the duration of the aplasia, in all patients.*
2. *The TACC high dose combination chemotherapy without autologous bone marrow transplantation,*
   —does not induce irreversible aplasia in leukaemic patients,
   —does not eradicate leukaemia.

INTRODUCTION

AUTOLOGOUS bone marrow transplantation has been shown to reverse severe myelosuppression in experimental animals [1–10]. We have recently demonstrated in dogs a 100% preservation of stem cells, following periods of storage up to 5 months [11].

In man, autologous bone marrow transplantation may be of benefit in allowing higher dosage and more prolonged administration of chemotherapy and radiotherapy for the treatment of malignant diseases. It may also be used in an attempt to rescue a patient who has rejected an allogeneic bone marrow graft or who suffers from severe GVHD, following transplantation. However, few autologous bone marrow transplants have been reported in man [12–14] and the viability of frozen human stem cells is not yet fully established.

In 1976, we initiated a therapeutic trial, to test the feasibility of autologous bone marrow transplantation in man and to evaluate its contribution to the treatment of patients with acute leukaemias resistant to conventional chemotherapy [15, 16] and of patients with other drug-resistant malignancies without bone marrow involvement. Twelve patients received a high dose combination chemotherapy regimen (TACC) slightly modified from the original B.A.C.T. [17] developed to condition patients for allogeneic bone marrow transplantation. Of these 12 patients, 8 received cryopreserved autologous marrow, and 4 received only supportive care. We report here our results in these 12 patients.

MATERIALS AND METHODS

(1) *Patients*

The patients were divided into 3 groups.

Group 1 consisted of 4 patients with solid tumours without bone marrow involvement (1 nasopharyngeal carcinoma, 1 rhabdomyosarcoma, 1 Hodgkin's disease, 1 localized plasmacytoma of the liver). These patients received the TACC regimen followed by the infusion of autologous cryopreserved marrow.

Group 2 consisted of 4 patients with drug resistant acute leukaemia in relapse (2 acute myelocytic, 2 acute monoblastic leukaemias). These patients received the TACC regimen and cryopreserved marrow which had been harvested during their first remission.

Group 3 consisted of 4 patients with drug resistant acute leukaemia (1 acute lymphocytic, 1 acute myelocytic, 2 chronic myelocytic leukaemias in blast crisis). These patients received the same high dose combination chemotherapy regimen without cryopreserved marrow.

(2) *Bone marrow harvesting, freezing and storage*

To collect bone marrow cells from patients under general anaesthesia, we used the procedure described by Thomas *et al.* [18]. The total number of nucleated bone marrow cells collected was calculated, using a correction for dilution with media and peripheral blood based on the assumption that the number of nucleated cells present in excess of the peripheral blood leucocyte count represents bone marrow cells.

The fresh marrow suspensions was filtered in the surgery room and then divided into aliquots of 100 ml and stored in Hemoflex bags (catalogue No. 7450–2 Union Carbide Corp., Chicago Ill). Erythrocytes were not removed before freezing. The freezing solution which consisted of 20% dimethyl sulfoxide (DMSO) and 10% decomplemented human AB serum in TC 199 medium (Gibco Biocult—Glasgow, Scotland, UK), was prepared freshly and added to the bone marrow suspension in equal volume immediately prior to freezing.

Each bag was compressed between 2 flat aluminium plates so that temperature was uniformly distributed. The marrow was frozen in a Cryoson BV-4 biological freezing system (Cryoson—Midden Beemster, Holland), modified to respond to the heat of fusion, by increasing the release of nitrogen vapor. The rate of freezing was −1°C/min to fusion. It was then increased to −6°C/min down to −60°C at which temperature the bags were transferred to the gas phase of a liquid nitrogen freezer. The bags were stored at a temperature below −140°C (Fig. 1).

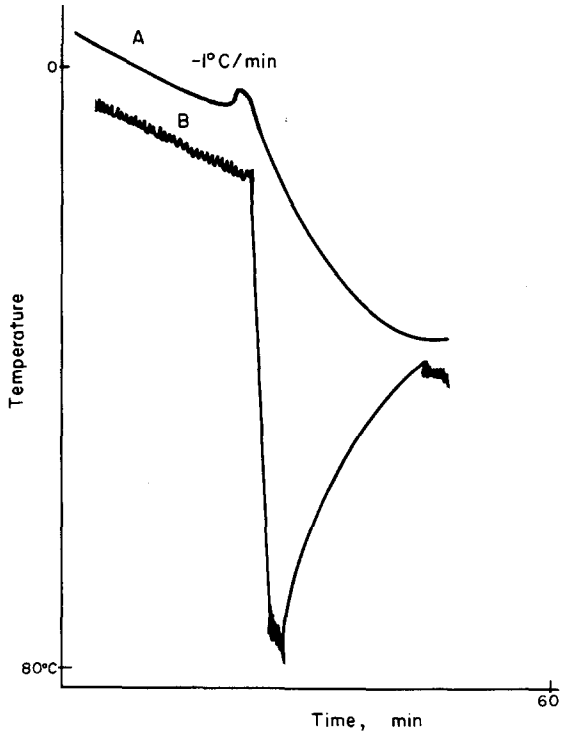

Fig. 1. Diagram of the freezing curves A: Temperature of the sample of bone marrow. B: Temperature inside the freezing chamber.

(3) *High dose combination chemotherapy (TACC)*

The basic 4 days course of TACC consisted of: cyclophosphamide 45 mg/kg i.v. day 1–4, ARA-C 100 mg/m^2 i.v. every 12 hr day 1–4, 6-thioguanine 100 mg/m^2 by mouth every 12 hr day 1–4 CCNU 200 mg/m^2 by mouth day 2.

One patient in group 1 (rhabdomyosarcoma) received a course of TACC extended to 5 days. The total doses were cyclophosphamide 225 mg/kg (18 g), ARA-C and 6-TG 900 mg/m^2 and CCNU 200 mg/m^2.

(4) *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 then administered by intravenous infusion without a filter, 48 hr after the last dose of cyclophosphamide, and at least 72 hr after the CCNU. The bags of bone marrow were administered 2 at a time to avoid renal damage from haemoglobinuria.

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

Except for the first patient, all patients were treated in a protected environment and received oral non-absorbable antibodies for bowel decontamination.

During the period of aplasia, the patients were supported with frozen red cells and platelets collected with the cell separator (Aminco—Silver Spring, MD) to maintain the platelets count above 30,000/mm^3. All blood products were irradiated with 2500 rad in a gammacell 1000 irradiator (Atomic Energy of Canada Ltd—Ottawa. Canada). Day 0 is the day of marrow infusion in groups 1 and 2 and the day on which marrow would have been transfused for patients in group 3.

RESULTS

Except for nausea and vomiting, the TACC regimen was well tolerated in all cases. There was no sign of cardiotixicity and no haematuria. No fat emboli were observed.

Two cases of *Staphylococcus aurens* sepsis developed in groups 1+2 and 3 cases of sepsis (1 *Staphlococcus*, 1 *E. Coli*, 1 *Candida albicans*) developed in group 3.

Renal function tests were performed routinely in all patients. They showed no damage in relation to haemoglobinuria.

Group 1 and group 2 were similar in term of haemopoietic reconstitution: bone marrow aspiration showed recovery of haemopoiesis with some myeloid precursors, a few erythroblasts and immature lymphoid cells between day 5 and 10. Megakaryocytes appeared between day 9 and 15. The kinetics of recovery of peripheral leucocytes were identical in all cases (Fig. 2). Recovery started between day 8 and day 10 (median day 9). Recovery to a WBC count of 1000/mm^3 occurred between day 13 and 19 (median day 17). The platelet count reached 50,000/mm^3 between days 9 and 28 (median day 15). Reticulocytes (0.1%)

Table 1. Recovery of peripheral blood cells and tumour response in patients treated with high dose combination chemotherapy (TACC) with and without cryopreserved autologous marrow. Day 0 is the day of marrow infusion in groups 1 and 2 and the day on which marrow would have been transfused for patients in group 3

Group	Diagnosis	Dose of marrow (10^8/kg)	Storage duration (months)	Time for recovery§ Leucocytes $>1000/mm^3$ (day)	PMN $>500/mm^3$ (day)	Platelets $>50{,}000/mm^3$ (day)	Reticulocytes $>0.1\%$ (day)	Number of platelet transfusions*	Tumour response‡
1	Nasopharyngeal carcinoma	2.2	1	17	17	15	16	2	PR
	Rhabdomyosarcoma	0.8	0.5	12	13	15	13	5	PR
	Hodgkin's disease	1.9	1.5	18	17	20	21	5	CR
	Plamacytoma	0.6	5	16	13	9	8	1	PR
2	A.M.L.	0.5	6	17	18	23	18	8	CR
	A.M.L.	1	18	19	22	14	15	5	CR
	A. Monoblastic L	1.4	3.	19	19	28	25	9	CR
	A. Monoblastic L	0.6	3.5	14	17	14	12	13†	CR
3	A.L.L.	No infusion of autologous marrow		30	29	33	27	18	CR
	A.M.L.			28	28	no	no	—	Failure
	CML in acute crisis			28 (9% blast cells)					
	CML in acute crisis			27 (77% blast cells)	no	no	26	—	Failure
					† on day 15				

*Number of platelet transfusions to maintain the platelet count above $30{,}000/mm^3$.
†Patient strongly immunized.
‡CR: Complete remission.
PR: Partial remission.
§Columns titled leucocytes, PMN, platelets and reticulocytes represent the time for these to return to the stated level.

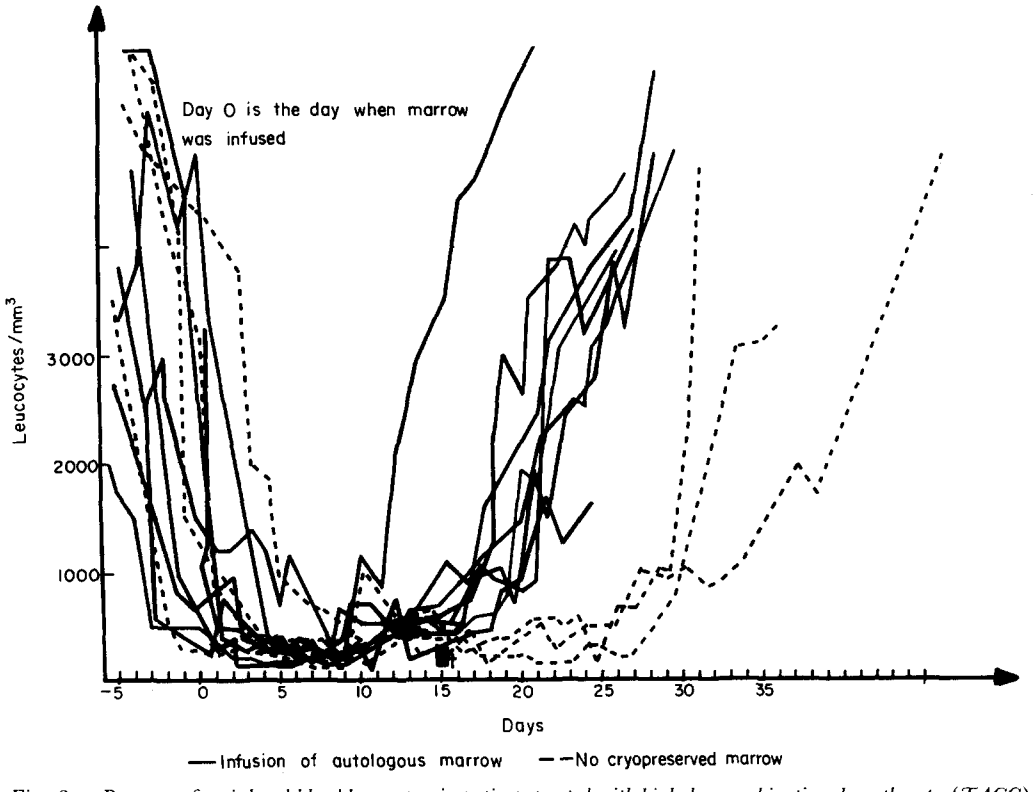

Fig. 2. Recovery of peripheral blood leucocytes, in patients treated with high dose combination chemotherapy (TACC) with and without infusion of cryopreserved autologous marrow.

appeared in the blood on days 8–25 (median day 16).

There was no relation between the speed of recovery and the dose of bone marrow infused.

In group 1, the patient with Hodgkin's disease achieved complete remission. The other patients achieved partial remission (50–75% tumour mass reduction).

The 4 patients with acute leukaemia (group 2) all achieved complete remission and for the 3 of them who relapsed, the duration of the remissions, although short (3, 3 and 5 months) paralleled the duration of the first remission during which the bone marrow had been harvested for cryopreservation (3, 6 and 8 months).

In contrast, in group 3, the marrow hypoplasia induced by the high dose combination chemotherapy appeared to last twice as long as the hypoplasia in groups 1 and 2.— One patient went into a complete remission (ALL) with a recovery to 1000 WBC/mm^3 on day 30 and a recovery to 50,000 platelets/mm^3 on day 35. This patient had unfortunately relapsed by day 69. Two other patients proved unexpectedly resistant to the TACC and slowly recovered to 1000 WBC/mm^3 on days 27 and 28 with 77 and 9% circulating leukaemic cells.

The last patient died on day 15. Post mortem examination demonstrated severe hypoplasia, with disseminated candidiasis, and persisting massive visceral leukaemic infiltration.

DISCUSSION

Previous attempts in man to prove the capacity of cryopreserved autologous marrow infusion to reverse drug or radiation induced myelosuppression, have been unsuccessful either because of spontaneous recovery of haemopoiesis after incomplete myelosuppression [19, 20] or because of delayed and partial engraftment [12].

In leukaemia, studies have suggested that autologous engraftment can occur after total body irradiation followed by the infusion of cryopreserved marrow collected during a remission period [13–21].

However, total body irradiation contributes to a high incidence of interstitial pneumonitis [Graw, personal communication] and, except for patients with leukaemia, is perhaps best avoided for patients with malignant disease. The TACC regimen that we used is closely related to the BACT initially designed to condition leukemic patients for allogeneic BMT. It includes CCNU, a long acting mye-

losuppressive nitrosourea, which causes a peak of myelotoxicity 4 weeks after its administration [23]. It has been generally assumed in the past that a drug combination such as TACC, if used without bone marrow transplantation, might succeed in eradicating leukaemic cells, but would also induce permanent aplasia. To the best of our knowledge, such a high dose combination chemotherapy has never previously been tested in leukaemia.

In solid tumours, recently Deisseroth reported the use of the BACT* regimen with and without cryopreserved marrow in patients with Burkitt's lymphoma [14]. With no marrow infusion, severe granulocytopenia (less than 100/mm^3) persisted for a median of 19 days and recovery to 500 granulocytes/mm^3 occurred 18–36 days after chemotherapy. In contrast, in autograft recipients, severe granulocytopenia persisted only for a median of 10 days and recovery to 500 granulocytes/mm^3 occurred 14–24 days after chemotherapy. These observations are in accordance with the results of the present study.

We draw the following conclusions:

(1) About the TACC regimen: The TACC high dose combination chemotherapy
—does not induce irreversible aplasia in leukaemic patients
—does not eradicate leukaemia.

(2) About autologous bone marrow transplantation: Combined with high dose combination chemotherapy, the reinfusion of cryopreserved autologous marrow is beneficial in shortening by about 50% the duration of the aplasia.

In patients with acute leukaemia resistant to conventional chemotherapy, it is possible that cryopreserved marrow harvested during the first remission provided enough stem cells with the ability to mature normally to induce another complete remission. However in view of the heterogeneity of our patients in groups 2 and 3 such a conclusion would unfortunately be premature.

*BACT: BCNU instead of CCNU (TACC).

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