Cancer Chemother Pharmacol (1989) 25: 213–218

Cancer Chemotherapy and Pharmacology

Haematological recovery following high-dose cyclophosphamide with autologous bone marrow transplantation*

Christopher Twelves[1], Robert Souhami[1], Peter Harper[2], and Anthony Goldstone[3]

[1] Department of Oncology, University College & Middlesex School of Medicine, London, U. K.
[2] Clinical Oncology Unit, Guy's Hospital, London SE1 9RT, U. K.
[3] Department of Haematology, University College Hospital, London W1E 6AU, U. K.

Summary. A total of 31 patients with previously untreated small-cell carcinoma of the lung were treated with very-high-dose cyclophosphamide, using autologous bone marrow transplantation (ABMT) to assist haematological recovery. The period of neutropenia was shorter with 40 mg/kg cyclophosphamide × 4 (7 patients) than when the dose of cyclophosphamide was increased to 50 mg/kg × 4 (11 patients), despite ABMT 2 days after chemotherapy in each group. In all, 13 patients were treated with 50 mg/kg cyclophosphamide × 4, with infusion of bone marrow delayed to day 4, 6 or 8 after chemotherapy to determine the contribution of ABMT to haematological recovery. The period of neutropaenia was increased when marrow was returned 6 days following chemotherapy, confirming that ABMT contributed to haematological recovery after this schedule of treatment. A total of 11 patients had a second cycle of 50 mg/kg cyclophosphamide × 4 after recovery from the first cycle of high-dose chemotherapy. The period of myelosuppression was greater with the second cycle of chemotherapy, although ABMT was carried out during both cycles. The results show that ABMT contributes to haematological recovery when the dose of cyclophosphamide is high enough to produce prolonged hypoplasia. The increased myelosuppression observed after a second high-dose treatment in spite of ABMT suggests either that both transplanted and endogenous marrow activity contribute to recovery of myelopoiesis or that there is residual damage to marrow stroma after the first cycle of treatment. The data indicate the necessity of carefully assessing the role of ABMT in haematological recovery with high-dose chemotherapy regimens.

Introduction

Clinical and laboratory evidence suggests that the cytotoxic activity of alkylating agents is dose-related [18, 22]. Regimens using very-high-dose alkylating agents have been introduced into clinical practice, often using autologous bone marrow transplantation (ABMT) to shorten the period of myelosuppression [10]. ABMT was first used to accelerate recovery of the peripheral blood count following the administration of nitrogen mustard [2] and has been shown to shorten the period of aplasia associated with melphalan [13] and multi-agent regimens containing a nitrosourea, cytosine arabinoside, cyclophosphamide and 6-thioguanine [1, 6]. However, in most situations where ABMT is used, there is little firm evidence of its value because there is no "marker" to confirm engraftment. ABMT is an expensive, invasive procedure that carries the theoretical risk of reinfusion of malignant cells. Nevertheless, if ABMT shortens the period of aplasia by only a few days, this might be of value in reducing the toxicity of high-dose chemotherapy, particularly in older patients.

We have previously reported the treatment of a substantial number of patients with small-cell carcinoma of the lung (SCCL) using high-dose cyclophosphamide as initial chemotherapy, usually as a single agent [22, 23]. In the present report we describe our experience of the haematological toxicity of cyclophosphamide, with particular reference to the role of ABMT in recovery from myelosuppression.

Methods

A total of 31 patients with histologically proven SCCL were treated with one or two cycles of high-dose chemotherapy plus ABMT. They were fit with no other significant disease and had not received previous chemotherapy or radiotherapy. A bone marrow aspirate and trephine was free of tumour in all patients as assessed by light microscopy. With patients under general anaesthetic, bone marrow was harvested by multiple aspirations from the iliac crest and sternum and then cryopreserved [11]. In patients receiving two cycles of high-dose chemotherapy, marrow was harvested before the first cycle and divided into halves for reinfusion after each treatment. Chemotherapy started 1 or 2 days after marrow harvest. All patients who entered these studies consented after full discussion of the procedures.

Patients received either 40 mg/kg i.v. cyclophosphamide, on 4 successive days (total dose, 160 mg/kg) or 50 mg/kg on 4 successive days (total dose, 200 mg/kg). High-dose cyclophosphamide was given over 30 min with mesna (2-mercapto ethane sulphonate) to prevent

* This work was supported by a grant from the Cancer Research Campaign and by the Leukaemia Research Fund
Offprint requests to: R. Souhami

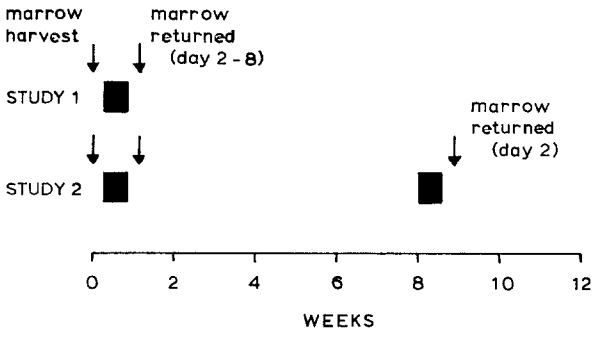

Fig. 1. Plan of two studies

urothelial toxicity. In the first study (Fig. 1), 20 patients were treated with a single cycle of high-dose chemotherapy consisting of cyclophosphamide alone at 160 (7 patients) or 200 mg/kg (13 patients). In the second study, 11 patients received two cycles of high-dose chemotherapy with 200 mg/kg cyclophosphamide, the second cycle being given 4 weeks after haematological recovery from the first. After completing chemotherapy, patients in both studies received mediastinal irradiation (40 Gy in 20 fractions) once their blood count had returned to normal.

In both studies, most patients had marrow reinfused 48 h after chemotherapy was completed, which is designated day 2. However, an attempt was made to evaluate the role of ABMT by delaying marrow return to days 4, 6 and 8 in sequential groups of patients. ABMT and marrow cryopreservation is expensive and time-consuming and carries the risk of reinfusing malignant cells. On the other hand, shortening the period of aplasia is desirable in this elderly population; we therefore considered it important to know whether or not ABMT was necessary. The time interval was increased beyond day 4 because there was no evidence of lengthening of the period of aplasia. The interval was increased to day 8 but was not further extended when it became apparent that there was a trend towards increasing duration of myelosuppression at this point (see below).

During the period of neutropaenia patients were barrier-nursed and, if pyrexial, received broad-spectrum antibiotics. When the platelet count fell below 20×10^9/l, platelet transfusions were given. We define severe

Table 1. Treatment groups

Group	Chemotherapy	Day of marrow return	Patients (n)	Mean age, years (range)	Mean marrow yield, 10^8 cells/kg (range)
1	Cyclophosphamide 160 mg/kg (cycle 1)	2	7	54 (39–58)	1.6 (1.1–2.4)
2	Cyclophosphamide 200 mg/kg (cycle 1)	2	11	50 (38–65)	1.2 (0.5–2.6)
3	a) Cyclophosphamide, 200 mg/kg	4	3	53 (47–63)	1.8 (1.3–2.2)
	b) Cyclophosphamide, 200 mg/kg	6	8	52 (39–61)	1.4 (0.8–2.3)
	c) Cyclophosphamide, 200 mg/kg (cycle 1)	8	2	58 (51–65)	1.15 (0.8–1.5)
4	Cyclophosphamide, 200 mg/kg (cycle 2)	2	11	53 (38–65)	1.1 (0.8–1.5)

Table 2. Duration (days) of neutropaenia and thrombocytopaenia in relation to the day of the ABMT and chemotherapy regimen

Chemotherapy	Day of marrow return	Mean neutrophils $<500 \times 10^9$/l days (range)	Mean platelets $<50 \times 10^9$/l, days (range)
Cyclophosphamide 160 mg/kg (cycle 1)	2	9.6 (6–13)	1.7 (0–6)
Cyclophosphamide 200 mg/kg (cycle 1)	2	10.6 (6–16)	6.3 (0–18)
Cyclophosphamide 200 mg/kg (cycle 1)	4	12.0	4.8
Cyclophosphamide 200 mg/kg (cycle 1)	6	13.7 (11–15)	9.7 (8–15)
Cyclophosphamide 200 mg/kg (cycle 1)	8	14.5	9.5
Cyclophosphamide 200 mg/kg (cycle 2)	2	14.8 (8–23)	14.5 (5–26)

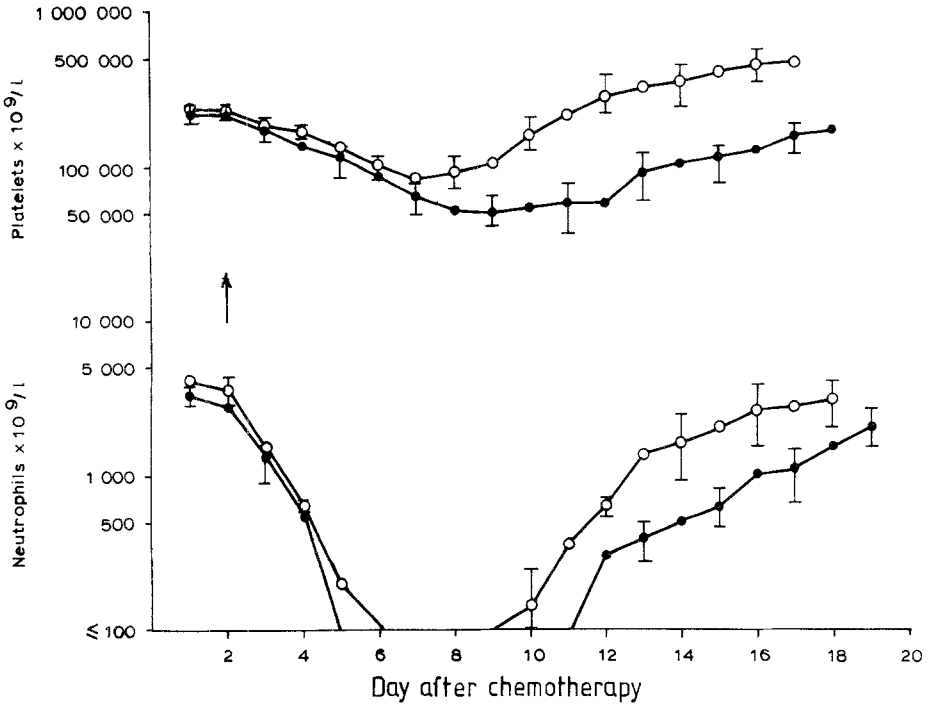

Fig. 2. Haematological recovery after cyclophosphamide (160 or 200 mg/kg, cycle 1) plus ABMT on day 2. Mean platelet and neutrophil counts (± 1 SEM) in patients treated with 160 (*open circles*) or 200 mg/kg cyclophosphamide (*closed circles*) and ABMT on day 2 after chemotherapy (*arrow*)

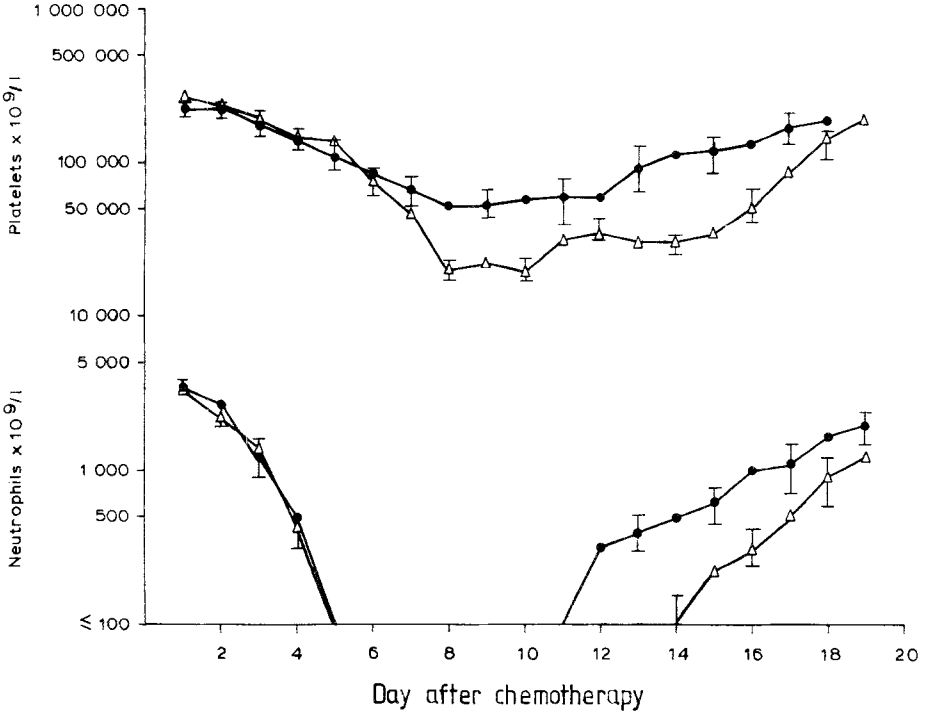

Fig. 3. Haematological recovery after cyclophosphamide (200 mg/kg, cycle 1) plus ABMT on day 2 or 6. Mean platelet and neutrophil counts (± 1 SEM) in patients treated with 200 mg/kg cyclophosphamide plus ABMT either on day 2 (*closed circles*) or day 6 (*open triangles*) after chemotherapy

neutropaenia as a neutrophil count of $<0.5\times10^9$/l and severe thrombocytopaenia as a platelet count of $<50\times10^9$/l. The blood count was considered to have recovered when there was a sustained rise above these levels without blood product support.

Results

The groups of patients that form the basis of this analysis are shown in Table 1. The mean duration of neutropaenia and thrombocytopaenia are shown in Table 2, and these groups were compared using Wilcoxon's rank-sum test. The pattern of haematological recovery of each group is also compared in Figs. 2–4 as the mean neutrophil and platelet count (±1 SEM) on each day following chemotherapy.

Cyclophosphamide at 160 and 200 mg/kg plus ABMT on day 2

In groups 1 and 2 an increase in cyclophosphamide dose from 160 to 200 mg/kg caused an increase in both the

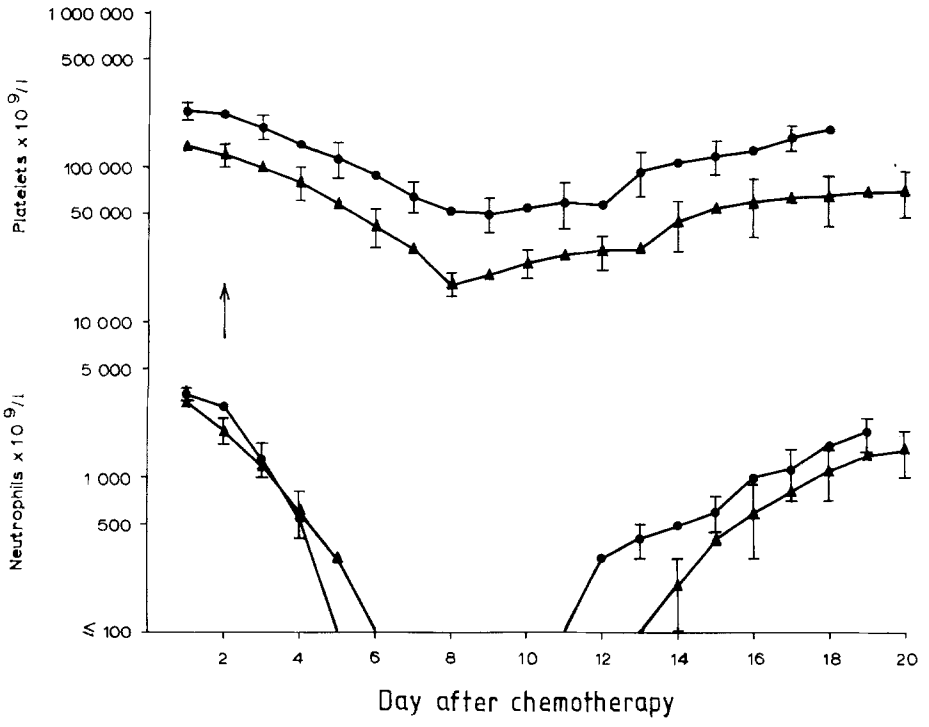

Fig. 4. Haematological recovery after cyclophosphamide (200 mg/kg, cycles 1 and 2) plus ABMT on day 2. Mean platelet and neutrophil counts (±1 SEM) in patients treated with 200 mg/kg cyclophosphamide on cycle 1 (*closed circles*) and cycle 2 (*closed triangles*) plus ABMT on day 2 after chemotherapy (*arrow*)

severity and the duration of myelosuppression (Fig. 2). The neutrophil and platelet counts fell at the same time in the two groups. The period of neutropaenia was similar at 9.6 and 10.6 days, respectively ($P > 0.05$), but the duration of thrombocytopaenia was increased significantly from 1.7 to 6.3 days ($P < 0.05$) at the higher dose, although both groups had ABMT on day 2 (Table 2).

Cyclophosphamide at 200 mg/kg with variable marrow delay

In patients receiving 200 mg/kg cyclophosphamide (groups 2 and 3), the timing of marrow reinfusion was varied after the end of chemotherapy to investigate the effect on haematological reconstitution. In the two groups who underwent ABMT on day 2 or day 6 the counts started to fall at the same time, but myelosuppression was more severe, with much lower nadir platelet counts and later recovery of both neutrophil and platelet counts, when marrow reinfusion was delayed to day 6 (Fig. 3). The duration of neutropaenia increased from 10.6 to 13.7 days ($P < 0.05$) and that of thrombocytopaenia, from 6.3 to 9.7 days ($P > 0.05$) in the group undergoing ABMT on day 6 (Table 2). Although the numbers of patients in whom marrow was returned on days 4 and 8 were small, there was a trend towards prolonged neutropaenia with increasing marrow delay. The pattern for thrombocytopaenia was less consistent (Table 2).

Cyclophosphamide at 200 mg/kg plus ABMT on day 2, cycles 1 and 2

A total of 11 patients (group 4) received a second cycle of 200 mg/kg cyclophosphamide plus ABMT 1 month after recovery from the first treatment. The neutrophil and platelet counts before treatment were lower before the second cycle than before initial chemotherapy (Fig. 4). In spite of ABMT using marrow harvested before the initial chemotherapy and returned on day 2 in both cycles, there was greater haematological toxicity with the second treatment. The period of neutropaenia increased from 10.6 to 14.8 days ($P > 0.05$) and the duration of thrombocytopaenia, from 6.3 to 14.5 days ($P < 0.05$) (Table 2).

Treatment deaths

There was one death due to infection; the patient died on day 18 after the first cycle of 200 mg/kg cyclophosphamide plus ABMT on day 8.

Non-haematological toxicity

There were no complications following marrow harvest. After high-dose chemotherapy nausea and vomiting were mild, but all patients had complete hair loss and a transient cyclophosphamide-induced rash. None developed macroscopic haematuria or subsequent urinary tract complications. Most patients had mild or moderate mucositis during the period of aplasia. There was no clinical evidence of heart failure or dysrhythmia. Serial ECGs and echocardiograms were normal in the first 13 patients, after which they were not routinely carried out. There was no radiological evidence of acute pulmonary toxicity.

Discussion

There is considerable and growing interest in the treatment of a variety of tumours with chemotherapy of sufficient intensity to cause profound myelosuppression. Treatment trials are being carried out in leukaemia [4, 9], non-Hodgkin's lymphoma [19], Hodgkin's disease [15], small-cell carcinoma of the lung and a number of other solid tumours [21]. In these studies ABMT is used to minimise the period of hypoplasia, but there have been very few studies assessing the necessity for this complex and expensive procedure. The contribution of ABMT to

haematological recovery has often been assumed but seldom demonstrated. Appelbaum et al. [1] and Gorin et al. [6] demonstrated that the period of aplasia was shortened by ABMT when a multi-drug protocol was used. With single-agent chemotherapy, two studies have assessed the contribution of ABMT. McElwain et al. [13] showed that ABMT shortened the period of hypoplasia produced by 140 mg/m^2 melphalan. By contrast, Smith et al. [20] found that ABMT did not influence recovery from the relatively brief period of aplasia caused by 7 g/m^2 cyclophosphamide given over 12 h.

Assessments of the need for ABMT are necessary not only because of the expense of marrow harvest and cryopreservation but also because ABMT carries the risk of reinfusing small numbers of malignant cells even when infiltration cannot be detected in the marrow. The question posed is not solely whether ABMT is essential for marrow recovery to occur at all, but whether it contributes to a shortening of the period of aplasia to such an extent that the clinical benefits outweigh the disadvantages. The answer to this question will vary with the type of chemotherapy used, the nature of the disease being treated, the likelihood of marrow contamination and the age of the population. Older patients have a reduced marrow proliferative capacity [12] and may benefit considerably from even a few days' reduction in the duration of hypoplasia.

The present series of clinical studies illustrates this complexity. A single disease was treated using one drug cyclophosphamide, given on one or two occasions, as initial treatment in a uniformly selected group of previously untreated patients. We instituted a programme of delay in return of the marrow in groups of patients. This was designed to assess the contribution of ABMT to haematological recovery without exposing the patients to the risk of prolonged aplasia that might have resulted from withholding ABMT entirely.

Our first finding is that ABMT contributes to haematological recovery when cyclophosphamide is used as a single agent at very high doses. The duration of myelosuppression was prolonged when marrow reinfusion was delayed from day 2 to day 6. This finding contrasts with that of Smith et al. [20], who showed that ABMT did not aid recovery after 7 g/m^2 was given over 12 h. Schuler et al. [17] have shown that when cyclophosphamide is given over a 4-day period, there is a successive increase in plasma alkylating activity on each day. A possible explanation is that cyclophosphamide induces hepatic enzymes responsible for its own activation. The 4-day cyclophosphamide schedule is more myelosuppressive than the same dose as a single injection which made it possible to demonstrate the effect of ABMT. This study enabled us to determine a threshold dose and schedule of cyclophosphamide above which ABMT contributes to haematological recovery and below which the degree and duration of neutropaenia and thrombocytopaenia is insufficient for ABMT to exert a discernible influence.

Although ABMT contributed to recovery after 200 mg/kg cyclophosphamide, the period of hypoplasia was longer than that observed with 160 mg/kg. Cyclophosphamide has a short half-life [8], and the increase in dose would not cause it to persist in the circulation until the time of ABMT and thus interfere with engraftment. The more probable explanation of the prolongation is that haematological recovery is mainly dependent on endogenous marrow after the lower dose of cyclophosphamide. With a higher dose of cyclophosphamide, myelosuppression is more severe, and the transplanted marrow then begins to contribute to recovery of myelopoiesis.

Although ABMT accelerated haematological recovery after the first cycle of 200 mg/kg cyclophosphamide, the second cycle of chemotherapy caused more prolonged neutropaenia and thrombocytopaenia than the first despite ABMT, which on both occasions involved equal quantities of marrow harvested before the first treatment. After both cycles of treatment (Table 1), the number of nucleated cells reinfused was sufficient for engraftment [7]. Bone marrow is viable for several months when stored [16], and a significant difference in the efficacy of marrow frozen briefly and that stored for several weeks is unlikely.

In patients receiving a double autograft, the pre-treatment peripheral blood counts returned to normal limits before the second treatment but were still lower than before the initial chemotherapy. This suggests that the marrow had not recovered fully and that toxicity to endogenous marrow is likely to be greater with the second cycle of high-dose chemotherapy. Recovery from hypoplasia after the second treatment may thus depend to a greater extent on the transplanted marrow than that following the first treatment, when endogenous marrow makes a greater contribution. An alternative explanation for the more prolonged myelosuppression after a second cycle of chemotherapy is residual damage to the host marrow stroma following the initial treatment. In vitro animal studies have shown that cyclophosphamide causes prolonged stromal and stem cell damage [14], which becomes evident after a second marrow insult [5]. Erythropoiesis requires both marrow stem cells and stroma [3], and a "soil" effect on the marrow stroma may impair normal function of the second autograft. Haematological recovery after high-dose chemotherapy with AMBT is probably a complex process involving the endogenous marrow and its stroma as well as transplanted marrow.

There has been a considerable increase in the use of high-dose chemotherapy and ABMT in malignant disease but little firm evidence either that ABMT accelerates haematological recovery or that high-dose chemotherapy is clinically beneficial [10]. We have shown that ABMT contributes to marrow recovery after 200 mg/kg cyclophosphamide given over 4 days in a relatively elderly population of patients with SCCL, but the value of ABMT must be assessed in each clinical setting. The clinical benefit of either high-dose chemotherapy or a second cycle of high-dose treatment must be balanced against the increased myelosuppression that may result despite ABMT.

References

1. Appelbaum FR, Herzig GP, Ziegler JL, Graw RG, Levine AS, Deisseroth AB (1978) Successful engraftment of cryopreserved autologous bone marrow in patients with malignant lymphoma. Blood 52: 85
2. Clifford P, Clift RA, Duff JK (1961) Nitrogen-mustard therapy combined with autologous marrow infusion. Lancet I: 687

3. Dexter TM, Allen TD, Lajtha LG (1977) Conditions controlling the proliferation of haemopoietic stem cells in vitro. J Cell Physiol 91: 335
4. Dicke KA, Spitzer G (1986) Clinical studies of autografting in acute lymphocytic leukaemia. Clin Oncol 15: 85
5. Fried W, Barone J (1980) Residual marrow damage following therapy with cyclophosphamide. Exp Haematol 8: 610
6. Gorin NC, Najman A, Salmon CH, Muller JY, Petit JC, David R, Stachowiak J, Hirsch Marie F, Parlier Y, Duhamel G (1979) High dose combination chemotherapy (TACC) with and without autologous bone marrow transplantation for the treatment of acute leukaemia and other malignant diseases. Eur J Cancer 15: 1113
7. Gorin NC, David R, Stachowiak J, Salmon C, Petit JC, Parlier Y, Najman A, Dulhamel G (1981) High dose chemotherapy in adult leukaemias, malignant lymphomas and solid tumours. A study of 23 patients. Eur J Cancer 17: 557
8. Graham MI, Shaw IC, Souhami RL, Sidau B, Harper PG, McLean AEM (1983) Decreased plasma half-life of cyclophosphamide during repeated high-dose administration. Cance Chemother Pharmacol 10: 192
9. Linch DC, Burnett AK (1986) Clinical studies of ABMT in acute myeloid leukaemia. Clin Oncol 15: 167
10. Linch DC, Goldstone AH (1984) Autologous bone marrow transplantation. Br J Haematol 58: 1
11. Linch DC, Knott LJ, Patterson KG, Cowan DA, Harper PG (1982) Bone marrow processing and cryopreservation. J Clin Pathol 35: 186
12. Mauch PM, Botnick LE, Hannon EC, Obbagy J, Hellman S (1982) Decline in bone marrow proliferative capacity as a function of age. Blood 60: 245
13. McElwain TJ, Hedley DW, Burton G, Clink HM, Gordon MY, Jarman M, Juttner CA, Millar JL, Milsted RAV, Prentice G, Smith IE, Spence D, Woods M (1979) Marrow autotransplantation accelerates haematological recovery in patients with malignant melanoma treated with high-dose melphalan. Br J Cancer 40: 72
14. Molineux G, Xu C, Hendry J, Testa NG (1986) A cellular analysis of long term haemopoietic damage in mice after repeated treatment with cyclophosphamide. Cancer Chemother Pharmacol 18: 11
15. Phillips GL, Reece DE (1986) Clinical studies of autologous bone marrow transplantation in Hodgkin's disease. Clin Oncol 15: 151
16. Ragab AH, Gilkerson E, Myers M (1977) Factors in the cryopreservation of bone marrow cells from children with acute lymphocytic leukaemia. Cryobiology 14: 125
17. Schuler U, Ehringer G, Wagner T (1987) Repeated high-dose cyclophosphamide administration in bone marrow transplantation. Exposure to activated metabolites. Cancer Chemother Pharmacol 20: 248
18. Shorthouse AJ, Jones JM, Steel GG, Peckham MJ (1982) Experimental combination and single-agent chemotherapy in human lung tumour xenografts. Br J Cancer 46: 35
19. Singer CRJ, Goldstone AH (1986) Clinical studies of ABMT in non-Hodgkins lymphoma. Clin Oncol 15: 105
20. Smith IE, Evans BD, Harland SJ, Millar JL (1983) Autologous bone marrow rescue is unnecessary after very high-dose cyclophosphamide. Lancet I: 76
21. Souhami RL, Peters W (1986) High-dose chemotherapy in solid tumours in adults. Clin Oncol 15: 219
22. Souhami RL, Harper PG, Linch D, Trask C, Goldstone AH, Tobias JS, Spiro SG, Geddes DM, Richards JDM (1983) High-dose cyclophosphamide with autologous marrow transplantation for small cell carcinoma of the bronchus. Cancer Chemother Pharmacol 10: 205
23. Souhami RL, Finn G, Gregory WM, Birkhead BG, Buckman R, Edwards D, Goldstone AH, Harper PG, Spiro SG, Tobias JS, Geddes D (1985) High-dose cyclophosphamide in small cell carcinoma of the lung. J Clin Oncol 3: 958

Received 30 January 1989/Accepted 1 June 1989