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The Potential Role of Amifostine (Ethyol®) in Haematological Malignancies

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Autologous bone marrow transplantation (ABMT) has considerably developed in the past 20 years. In AML, the beneficial role of purging the graft with cyclophosphamide derivatives (4-HC or mafosfamide) has been strongly suggested by retrospective studies from the European Cooperative Group for Blood and Marrow Transplantation and by single institution studies. Also, gene marking experiments have clearly shown that tumour cells infused with unpurged marrow indeed recirculate and in some instances, induce or contribute to tumour recurrence. Amifostine protects normal progenitor cells without concomitantly protecting colony forming unit leukaemic progenitors (CFUL). In comparative *in vitro* studies, we have shown that pre-incubation of normal marrow contaminated by leukaemic progenitors with amifostine followed by mafosfamide, results not only in a protection of the more mature progenitors (CFUGM, BFUE), but also sensitises leukaemic progenitors, so that in the end, the therapeutic index of mafosfamide is increasing by 6 logarithms. In the clinical field, it has been shown in patients with breast cancer autografted with protection by amifostine results in a shortening of the duration of aplasia of about 10 days. A European randomised study evaluating amifostine in the context of autografting for acute leukaemia has just started. Copyright © 1996 Elsevier Science Ltd

Key words: acute leukaemia, autologous stem cell transplantation, purgery, amifostine, stem cell protection
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INTRODUCTION

DURING THE past 20 years, with the introduction of high-dose therapy and stem cell transplantation, considerable modification has occurred in the management of haematologic malignancies. Since our first report in 1977 [1–3], autologous bone marrow transplantation (ABMT) and, more recently, autologous blood stem cell transplantation (ABSCT) have been widely used to treat acute leukaemias (acute myelocytic leukaemia [AML] and acute lymphoblastic leukaemia [ALL]), lymphomas (non-Hodgkin's lymphoma [NHL] and Hodgkin's disease) and multiple myeloma (MM), resulting in significant improvement in disease-free survival (DFS) and overall survival. In 1994, more than 10 000 stem cell transplants were performed in Europe—6000 ABSCTs and approximately 4000 ABMTs for haematologic malignancies. Worldwide, approximately 20 000 autologous stem cell transplants are now performed for haematologic malignancies per year and this number is expected to increase rapidly.

However, major theoretic impediments to ABMT and ABSCT have been the potential contamination of the collected stem cells by residual tumour cells and the risk, posed by reinfusing them with a graft, of initiating tumour recurrence. Indeed, recent studies with high-resolution polymerase chain reaction have shown that both marrow and blood are frequently contaminated in leukaemias [4], lymphomas [5] and MM [6].

Gene-marking experiments have clearly shown that tumour cells infused with unpurged marrow indeed recirculate and, in some instances, induce or contribute to tumour recurrence [7, 8]. Retrospective clinical surveys from the European Cooperative Group for Blood and Marrow Transplantation (EBMT) have shown lower relapse incidences (RIs) in patients with AML autografted in first complete remission (CRI) with purged bone marrow as opposed to unpurged bone marrow, and have shown similar trends in ALL and follicular lymphomas [9, 10; EBMT Acute Leukemia Working Party data not shown]. Techniques to purge bone marrow are numerous and can be divided into two main categories: those relying on direct attempts to kill tumour cells (negative selection) and those relying on attempts to purify normal stem cells (positive selection).

Negative selection approaches have essentially consisted of cyclophosphamide (CY) derivatives such as 4-hydroperoxycyclophosphamide (4-HC) or mafosfamide. These derivatives have shown considerable efficacy, with an up to six-log tumour cell reduction. Our team and others have had considerable experience with CY derivatives. Despite a positive impact on RIs, the use of tumour cell purging with 4-HC and mafosfamide has not been widespread because of concern regarding delay in engraftment and possible increases in the risk of infection, duration of hospitalisation and cost. These potential drawbacks could occur due to alteration of the normal stem cell pool; indeed, such alteration appears to be more pronounced in

AML than in ALL or NHL [11–13]. Protecting the normal stem cell pool from CY-derivative injury *in vitro* with agents such as amifostine might solve the problem and further raises the possibility of increasing doses of CY derivatives for better bone marrow purging. Indeed, the protection conferred by amifostine to the normal stem cell during marrow purging with 4-HC has been shown in breast cancer patients with a 10-day reduction in duration of aplasia [14] and a reduction of platelet support in the group receiving protected marrow.

Negative selection approaches so far have essentially consisted of purifying normal stem cells, recognised by their $CD34^+$ antigen, in malignancies where tumour cells lack this antigen (i.e. NHL [15] and MM [16]). Pilot studies are in progress to investigate the possibilities of better defining normal stem cells by using combinations of immunological markers, such as $CD34^+$-lineage thy-1^+, thereby minimising the chance of tumour cell contamination of the graft.

Positive selection so far has been shown to result in a two- to four-log tumour reduction, suggesting that a combination of both positive and negative selection might be required.

Preliminary data in our institution indicate that *in vitro* treatment with mafosfamide of $CD34^+$ stem cell concentrates is feasible. Protection of $CD34^+$ normal stem cells by amifostine before negative selection with mafosfamide might enable better purging without jeopardising optimal engraftment. Below, we review the status of purging with mafosfamide and present the design of a randomised study conducted to assess the value of amifostine during purging with mafosfamide in acute leukaemias.

AUTOLOGOUS BONE MARROW TRANSPLANTATION FOR ACUTE LEUKAEMIA: THE 10-YEAR EXPERIENCE AT HÔPITAL SAINT-ANTOINE, PARIS, USING MARROW PURGED BY MAFOSFAMIDE

In the late 1970s, our team designed a programme of high-dose consolidation therapy consisting of total body irradiation (TBI) followed by ABMT with bone marrow (BM) purged by mafosfamine, a directly active congener of CY, in an effort to reduce or eliminate residual leukaemic cells that might contaminate the graft and later contribute to relapse [3, 13]. To purge the BM by mafosfamide, we used two techniques. In the first period, from January 1982 to January 1990, we adjusted mafosfamide doses to individual sensitivity of the normal colony-forming units-granulocyte/macrophage (CFU-GM) in each patient to reach the highest tolerable dose that would achieve a maximum antileukaemic activity without jeopardising BM engraftment. This dose was defined as the CFU-GM LD_{95} (95% lethal dose) on buffy coat BM cells, sparing $5 \pm 5\%$ CFU-GM. 95 patients had their BM treated according to this technique [16, 17]. In a second period, from January 1990 to January 1993, 30 patients had their BM Ficoll-Hypaque separated; the mononuclear cell fraction was adjusted to a final construction of 10^7 cells/ml and treated with a constant dose of 50 μg/ml of mafosfamide. Whatever the dose, the BM suspension was incubated with mafosfamide for 30 min in a water bath at 37°C, then immediately cooled and centrifuged at 4°C to block the action of the drug abruptly. After two washes, the BM cells were resuspended in irradiated autologous plasma (40 Gy) and TC 199 medium and finally frozen with 10% dimethylsulphoxide in Teflon-Kapton DF 1000 Gambro bags (Gambro Dialysatoren, GMBH, Germany), using a Nicool 316 programmed freezer (CFPO, Sassenage, France), following our freezing technique as previously published [18, 19]. The purged BM was then stored in the gas phase of liquid nitrogen at a temperature constantly below −190°C. Cell counts and CFU-GM evaluations were performed at all steps of the procedures. The number of residual progenitor cells after incubation with mafosfamide was known for each individual patient before ABMT.

A total of 125 adult patients with acute leukaemia (84 AML, 41 ALL) were autografted with BM purged by mafosfamide from January 1983 to January 1993. The median age of the patients was 33 years (range 16–55 years). The median length of follow-up was 64 months (range 3–126 months). At the time of ABMT, 64 AML patients were in CRI and 6 were in second complete remission (CR2). The median interval between achieving CR and autografting was five months (range 1.3–23 months). The pretransplant regimen consisted of CY (120 mg/kg) and TBI. The median initial richness in granulocyte/macrophage progenitors of all patients' harvested BM was 5.16×10^4 CFU-GM/kg (range $0.55–33 \times 10^4$ CFU-GM/kg). After mafosfamide purging, residual CFU-GM was 0.021×10^4 CFU-GM/kg (range $0–1.78 \times 10^4$ CFU-GM/kg). The probability of successful engraftment was significantly higher and time to engraftment significantly shorter in ALL patients. Of 33 patients grafted with BM containing no residual CFU-GM, those patients with AML ($n=22$) had platelet recoveries that were significantly longer than those for AML patients receiving BM with residual CFU-GM. At eight years, patients autografted in CRI had a leukaemia-free survival (LFS) of 58% (AML patients) and 56% (ALL patients), with a relapse incidence of 25% (AML) and 37% (ALL). Patients autografted in CR2 for AML had a LFS of 34% and a RI of 48% at five years. The incidence of late relapses was significantly higher in ALL patients. By multivariate analysis, four factors were found to influence engraftment favourably in addition to a diagnosis of ALL: younger age, ABMT performed in CRI, the adjusted dose technique of purging and a shorter interval from CR to ABMT. Two factors were correlated with a better outcome: LFS was significantly higher and transplant-related mortality significantly lower in patients who received richer BM, and RI was significantly lower in patients autografted within 150 days from CR. We drew the following conclusions from this experience: for patients autografted in CRI, a LFS of 58% (AML) and 56% (ALL) resembles the best results reported with allogeneic BMT using related donors, and the dose of marrow to be infused and how the bone marrow is purged are important parameters, not only for engraftment kinetics, but also for the outcome.

Contradictory observations have been made with unpurged bone marrow in AML [20] or with marrow purged by monoclonal antibodies in ALL [21], in which outcomes were worse in patients receiving higher doses of bone marrow. Our proposed interpretation of these observations is that the higher the dose infused, the better the outcome if concomitant bone marrow purging has been effective (e.g. if higher doses of marrow have not translated into higher numbers of tumour cells infused).

This is possibly the situation in AML, especially during CRI, when bone marrow purging is effective; in contrast, absence of bone marrow purging would mean more tumour cells infused. In ALL, the situation would be the same despite purging with monoclonal antibodies, the efficacy of which has unfortunately been very limited in this disease. We interpret these data as favouring bone marrow purging with mafosfamide at the

Table 1. Patients with acute leukaemia autografted with marrow purged by mafosfamide: factors influencing engraftment, multivariate analyses

Factor	Neutrophils RR*	Neutrophils *P* value	Platelets RR*	Platelets *P* value
Patient age > 33 years	0.4	0.0001	0.4	0.0003
Purging with mafosfamide at adjusted levels	2.16	0.006	2.33	0.008
Transplant in CR2	0.53	0.02	0.44	0.014
CR-ABMT > 5 months	0.66	0.05	0.6	0.04

* Relative risk for probability of engraftment.
CR2, second complete remission; CR-AMBT, complete remission-autologous bone marrow transplant.

highest possible level while sparing normal stem cells as much as possible, the basis of interest in protective agents such as amifostine. Table 1 lists the factors recognised by multivariate analysis to influence the kinetics of engraftment. Patients 33 years of age and older and those receiving more chemotherapy (interval from CR to ABMT > five months, patients in CR2) engraft more slowly. Figures 1 and 2 compare the speed of engraftment for neutrophils and platelets in AML and ALL patients, with faster kinetics in the latter. Figures 3 and 4 illustrate the impact of dose adjustment of mafosfamide to individual sensitivity of CFU-GM (late progenitors) as opposed to the use of a constant dose, which appears to be more toxic for both neutrophil and platelet recovery. Figure 5 shows the difference in terms of DFS according to the doses of purged bone marrow infused, with the better results in patients receiving the higher doses.

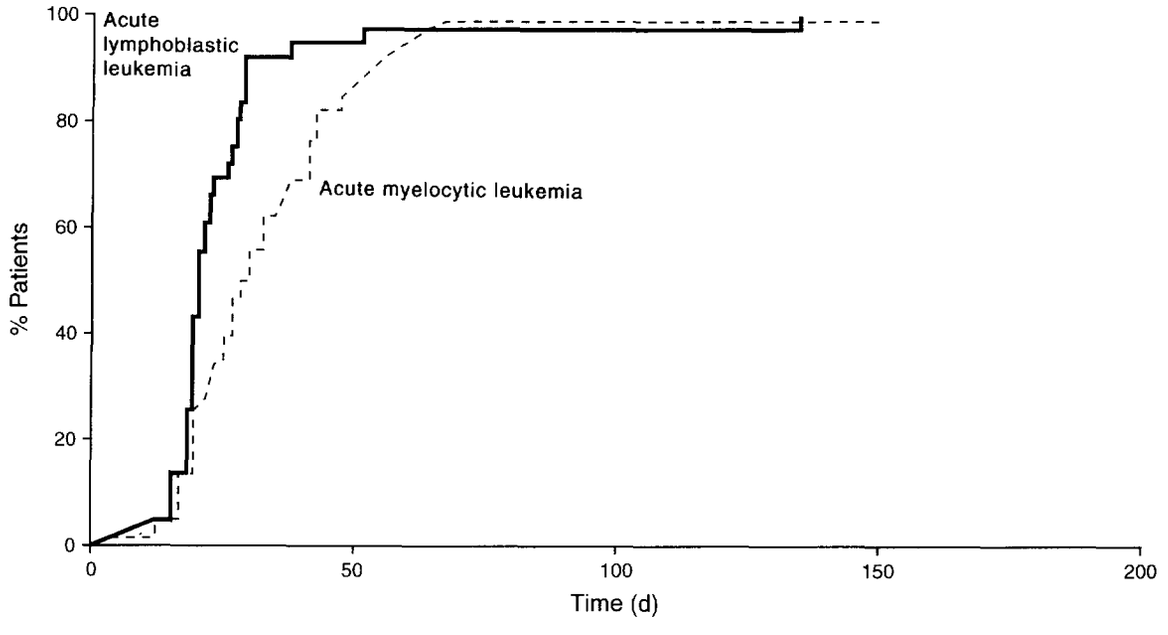
We currently continue autografting our adult patients with both AML and ALL as early as possible in CRI, collecting as much marrow as possible and purging it with mafosfamide. Our current research programme focuses on improving marrow purging through the use of protective agents, such as amifostine (ethyol®), in order to spare more immature normal haemopoietic progenitors and yet hopefully to increase further leukaemic progenitor killing with higher doses of mafosfamide. *In vitro* studies in our institution with amifostine indicate that this drug generates a six-log differential sensitivity to mafosfamide in favour of a better survival for CFU-GM in comparison to fresh leukaemic CFU.

EUROPEAN COOPERATIVE GROUP FOR BLOOD AND MARROW TRANSPLANTATION (EBMT) SURVEY ON PURGING IN ACUTE LEUKAEMIAS

Several EBMT surveys [9, 10] have shown better leukaemia-free probabilities in patients autografted in CRI with bone marrow purged by mafosfamide. In the 1990 survey, in particular, in 671 AML patients autografted in CRI, the relapse time was 35 ± 5% with purged bone marrow versus 47 ± 3% (P = 0.006) with unpurged bone marrow, and 29 ± 5% versus 50 ± 4% ($P < 0.0001$) when considering patients who received TBI as part of the pretransplant regimen. Results favouring bone marrow purging were even more impressive in patients autografted within six months (16 ± 6% versus 60 ± 6%, $P <$ 0.0001) and in slow responders who reached CRI in more than 40 days (20 ± 8% versus 61 ± 6%, $P = 0.001$). Figure 6A–C indicates the leukaemia-free survival following autologous purged and unpurged and allogenic BMT for AML patients in CR1 and CR2 and for ALL patients in CR1 as observed in the EBMT database in March 1995. A tentative explanation is that the efficacy of bone marrow purging is detectable in situations in which residual tumour persists, hence the need for bone marrow purging of the autograft (AML CR1 slow responders or early transplants), or in which the tumour is sensitive enough to chemotherapy so that the issue is not clouded by relapses originating from the body (ALL patients with good prognosis) [Figure 7].

AMIFOSTINE IMPROVES THE ANTILEUKAEMIC THERAPEUTIC INDEX OF MAFOSFAMIDE

A major limitation of bone marrow purging has been the cytotoxicity to normal BM progenitor cells, and hesitation to



Engraftment on neutrophils according to diagnosis

Figure 1. 125 Adult patients autografted for acute leukaemia: engraftment of neutrophils according to diagnosis.

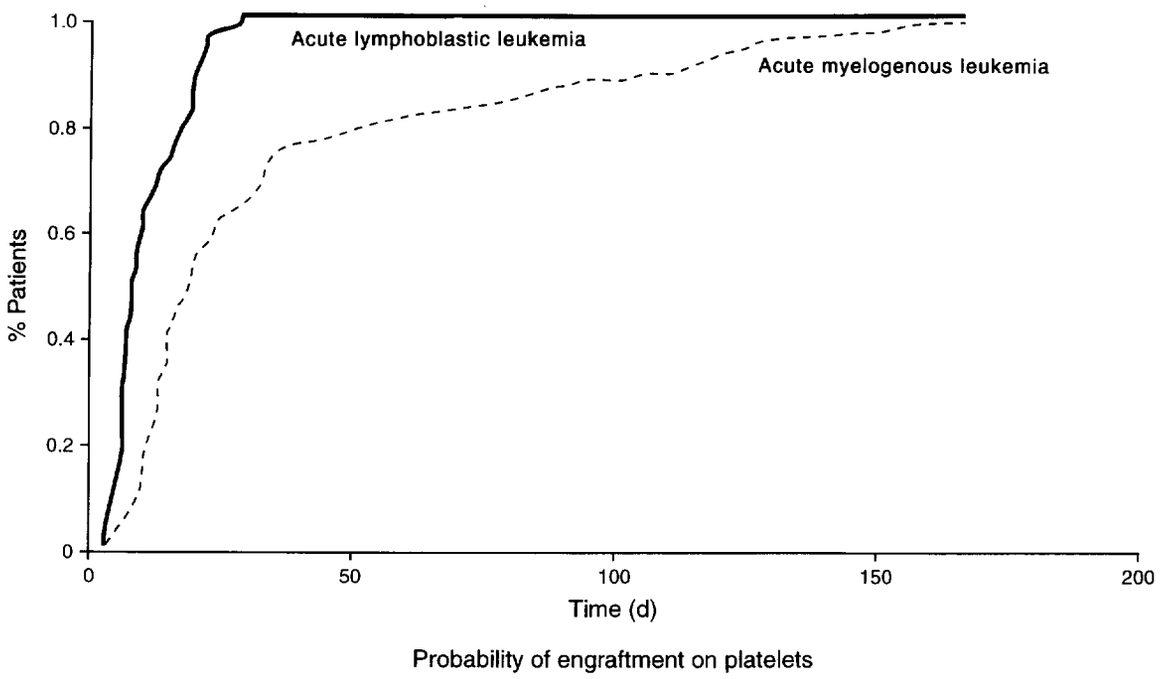


Figure 2. 125 Adult patients autografted for acute leukaemia: engraftment of platelets according to diagnosis.

induce graft failure or delay engraftment with too-aggressive purging and increased doses of mafosfamide. This concern has been essentially substantiated in AML [12, 13].

The key concern in introducing protective agents is making certain that while protecting normal progenitor cells, these agents do not concomitantly protect colony-forming unit-leukaemic progenitors (CFU-L). We studied simultaneously the sensitivity of normal early- and late-developing CFU-GM, burst-forming units-erythroid (BFU-E), long-term culture initiating cells (LTC-ICs) and CFU-L to increasing doses of mafosfamide, and found not only that normal progenitors were protected, but also that amifostine sensitises CFU-L to mafosfamide so that the combination of amifostine protection followed by mafosfamide purging increases the therapeutic index by six-log [22].

For example, Table 2 shows the respective survivals of normal GFU-GM, BFU-E, LTC-ICs and CFU-L with and without amifostine protection before purging with the constant dose of mafosfamide (50 μg/ml) widely used. Table 3 focuses on the LD_{95} dose of mafosfamide and indicates its modification and possible increase when protection by amifostine has first been used: amifostine essentially protects late-committed progenitors—i.e. placental-conditioned medium CFU-GM as well as CFU-GM and BFU-E grown on methylcellulose in the

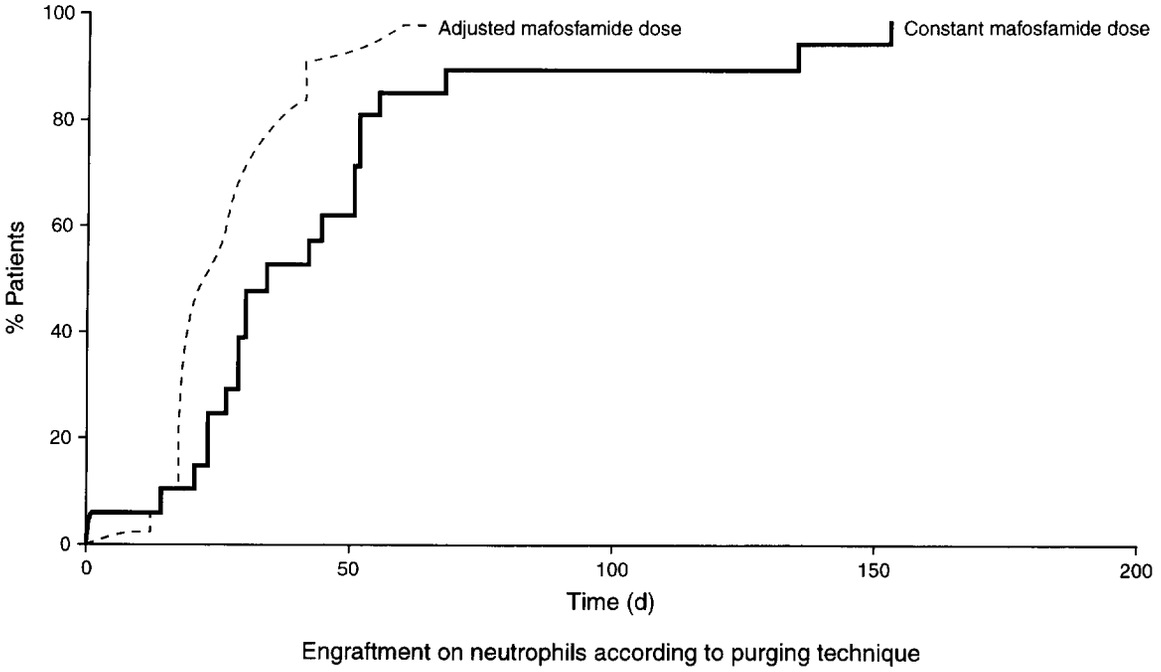


Figure 3. 125 Adult patients autografted for acute leukaemia: engraftment of neutrophils according to purging technique.

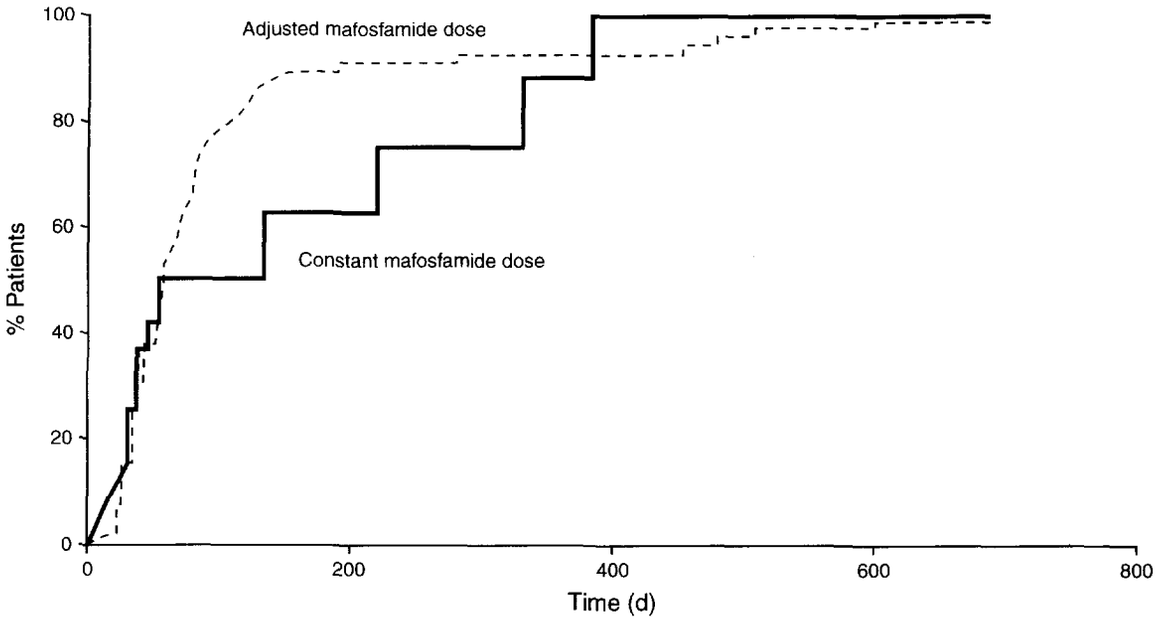


Figure 4. 125 Adult patients autografted for acute leukaemia: engraftment of platelets in relation to marrow purging techniques.

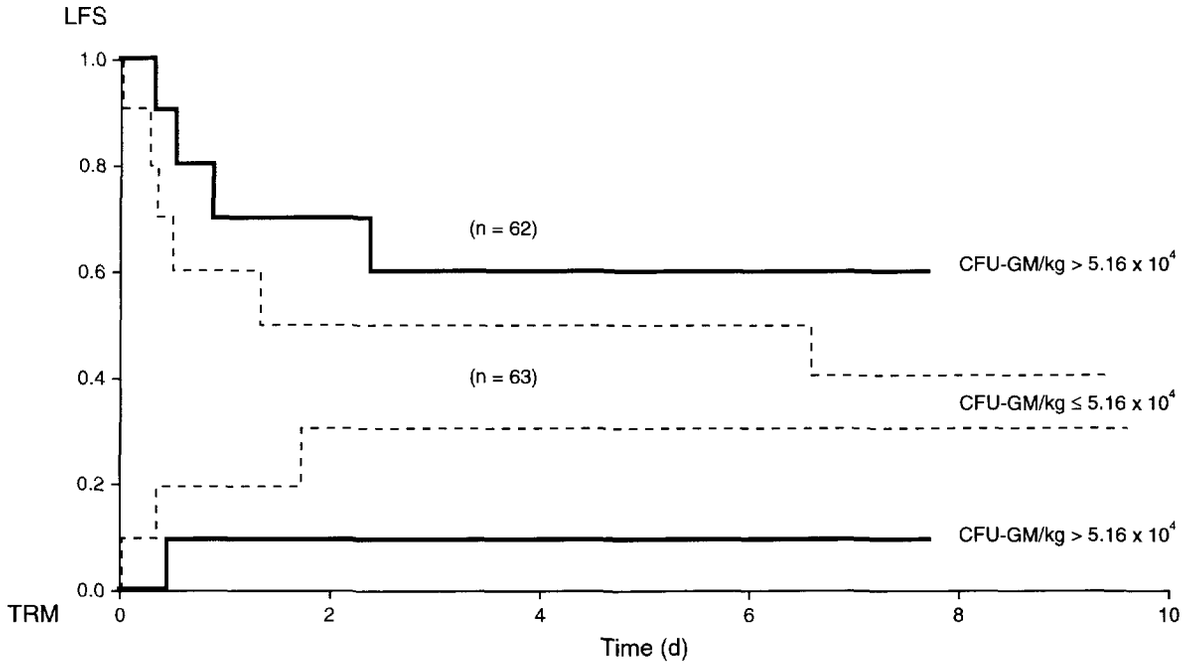


Figure 5. 125 Adult patients autografted for acute leukaemia: outcome (LFS, leukaemia free survival, TRM, transplant related mortality) in relation to the dose of marrow infused, evaluated in CFU-GM/kg before bone marrow purging.—, Patients who received the higher doses, i.e. above the median value: 5–16 × 10^4 CFU-GM/kg; ---, patients who received the lower doses, i.e. equal to or below the median value.

Table 2. Per cent viability of leukaemic and normal marrow progenitor cells after bone marrow purging with a fixed concentration of 50 μg/ml of mafosfamide

		CFU-GM			BFU-E		LTC-IC
	CFU-L	PCM	4R	5R	4R	5R	
No amifostine protection	0.01%	0.1%	10%	50%	5%	40%	70%
Amifostine protection	0.00001%	1%	30%	50%	30%	40%	70%

CFU-L, colony-forming unit-leukaemic progenitors; CFU-GM, CFU-granulocyte/virgule macrophage; PCM, placental conditioned medium; 4R and 5R, mixtures of four and five recombinant growth factors as described in the text; BFU-E, burst-forming unit-erythroid; LTC-IC, long-term bone marrow culture-initiating cells.

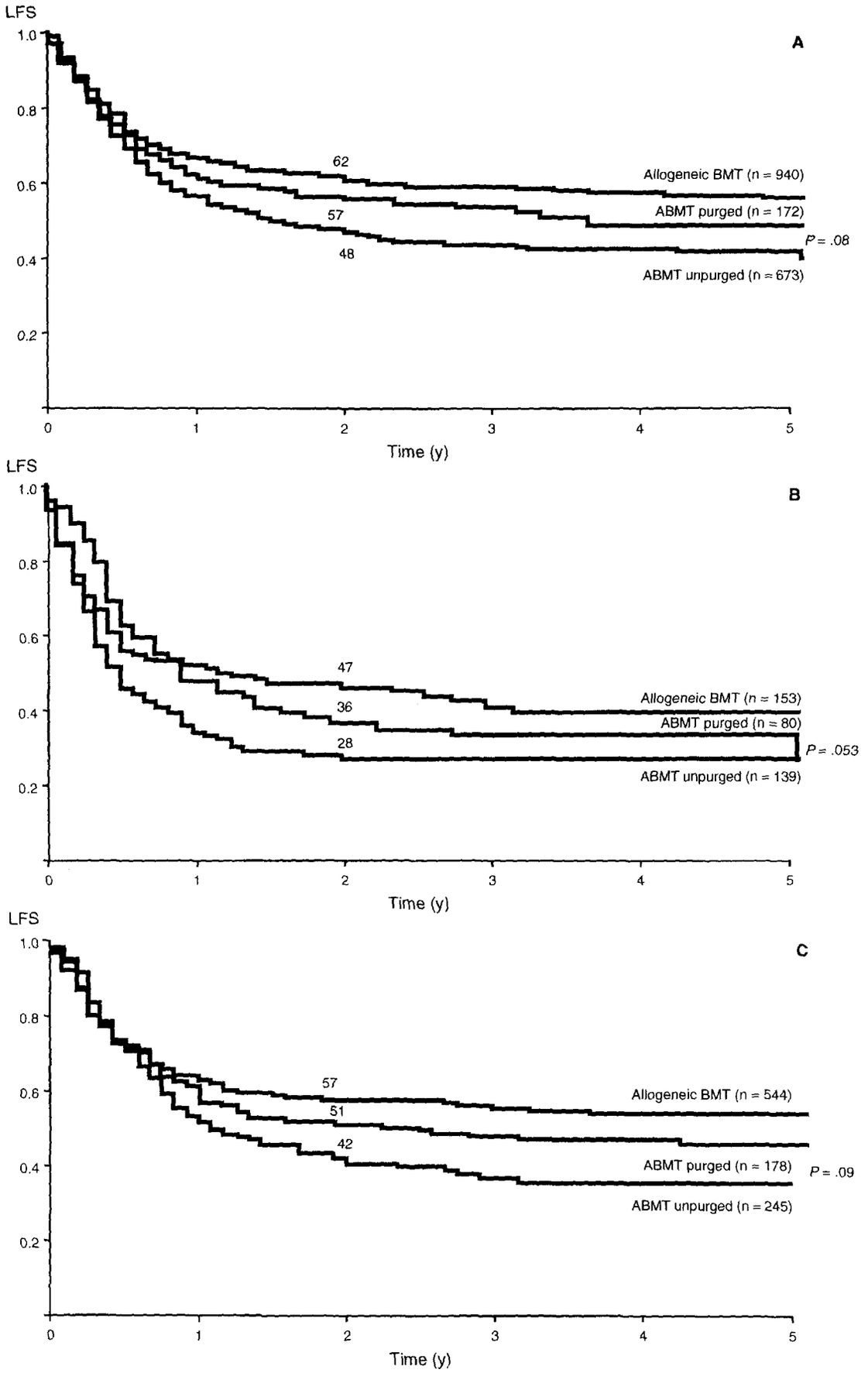


Figure 6. Bone marrow transplantation for acute leukaemia since January 1987. (A) First and (B) second remissions, post-total-body irradiation (TBI) and BU-CY. (C) First remission, post-TBI only. BU-CY, busulfan 16 mg/kg total + cyclophosphamide 120 mg/kg or 200 mg/kg total.

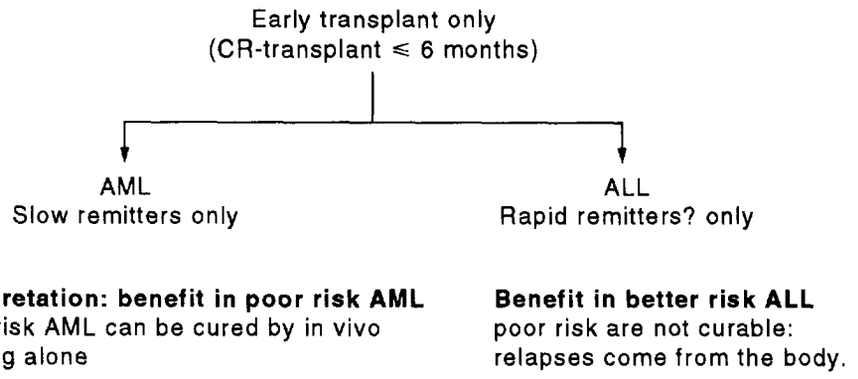


Figure 7. Patients with acute leukaemia who may benefit from purging: a tentative explanation.

presence of interleukin-3, granulocyte colony-stimulating factor, granulocyte-macrophage colony-stimulating factor and erythropoietin (a mixture of four recombinant growth factors referred to as 4R)—but has little or no influence on more immature progenitors such as LTC-IC, early CFU-GM and BFU-E grown in the presence of stem cell factor added to the same mixture of four recombinant growth factors described above (referred to as 5R). Because late progenitors are those that ensure immediate recovery from aplasia, this should clinically translate in faster kinetics of engraftment.

Table 3. *Amifostine protection of normal marrow progenitor cells from LD_{95} concentration* (μg/ml) *of mafosfamide*

	Mafosfamide	Amifostine→ Mafosfamide	
	LD_{95}	New LD_{95}	*P* value
Late progenitors			
PCM CFU-GM			
Mean ± S.E.M.	38 ± 14	42 ± 12	
Median (range)	38 (17–66)	39 (24–64)	0.056
n	14	14	
4R CFU-GM			
Mean ± S.E.M.	54 ± 13	66 ± 14	
Median (range)	50 (32–101)	60 (38–119)	0.031
n	5	5	
4R BFU-E			
Mean ± S.E.M.	48 ± 14	61 ± 11	
Median (range)	40 (11–91)	57 (28–93)	0.031
n	5	5	
Early progenitors			
5R CFU-GM			
Mean ± S.E.M.	89 ± 36	93 ± 41	
Median (range)	79 (28–136)	77 (37–164)	NS
n	10	10	
5R BFU-E			
Mean ± S.E.M.	69 ± 23	75 ± 26	
Median (range)	62 (29–99)	70 (32–117)	NS
n	10	10	
LTC-IC			
Mean ± S.E.M.	136 ± 56	139 ± 50	
Median (range)	118 (73–206)	132 (82–216)	NS
n	6	6	

LD_{95}, 95% lethal dose; PCM, placental conditioned medium; CFU-GM, colony forming units-granulocyte/macrophage; 4R and 5R, mixtures of four and five recombinant growth factors as described in the text; S.E.M., standard error of the mean; NS, not significant; BFU-E, burst-forming unit-erythroid; LTC-IC, long-term bone marrow culture-initiating cells.

RANDOMISED STUDIES

As indicated above, amifostine has been shown to shorten aplasia duration and reduce platelet support in patients with breast cancer autografted with marrow purged by 4-HC [14]. A randomised study currently underway follows these guidelines in patients with acute leukaemias. Patients with AML or ALL in CR1 or in CR2 will receive high-dose consolidation with BM purged by mafosfamide at a constant dose. They will be randomised either to have their marrow protected or not by amifostine prior to incubation with mafosfamide. Figure 8

Table 4. *Amifostine for autografting in acute leukaemia: inclusion criteria*

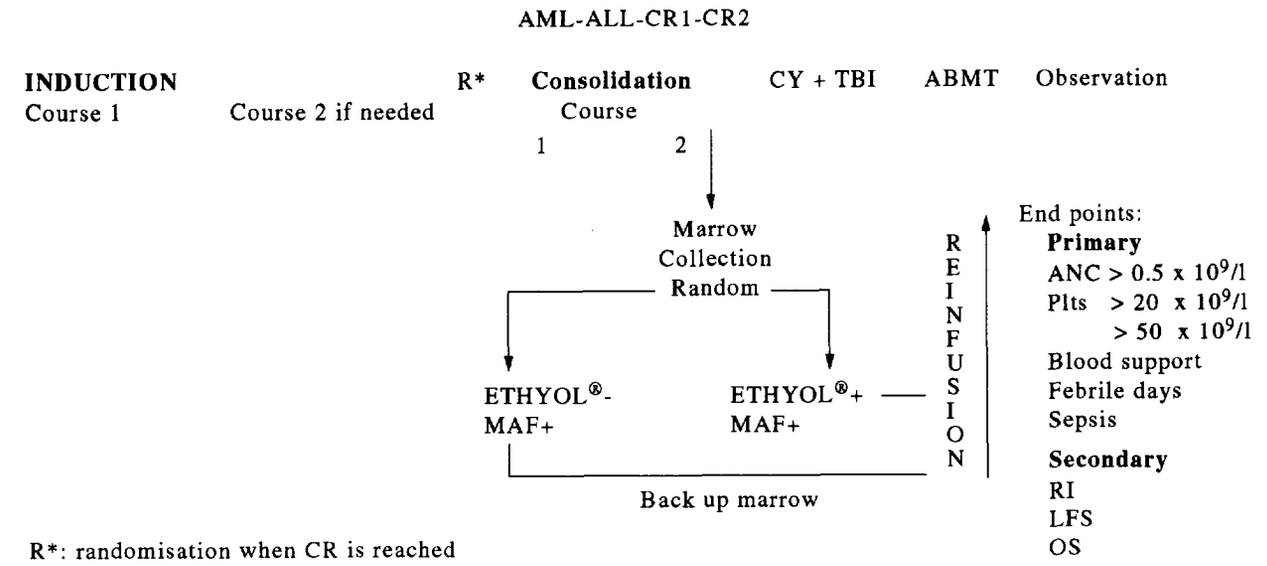
- Patients 10–60 years old
- Newly diagnosed (primary) AML/ALL
- One or two courses induction (free)
- Consolidation courses
  - two if CR1
  - one if CR2
- Interval CR-ABMT ≤ six months
- Preharvest evaluation
  - Blood: PMN > 1.5×10^9 cells/l
  - Platelets: > 100×10^9 cells/l
  - Marrow: cellularity > 10×10^6 cells/ml
    CFU-GM adequate
- No contraindication for an ABMT

AML, acute myelocytic leukaemia; ALL, acute lymphocytic leukaemia; CR1 and CR2, complete remissions 1 and 2; ABMT, autologous bone marrow transplant; PMN, polymorphonuclear cells; CFU-GM, colony-forming units-granulocyte/macrophage.

Table 5. *Amifostine for autografting in haematology: benefits expected*

Major
- Acceleration of engraftment
- Increase in purging efficiency

Side
- Protection against other toxicities:
- Immunological
- Intestinal cell crypt (melphalan)
- Pulmonary (cyclophosphamide)
- Lower risk of induced mutagenesis



CY, cyclophosphamide
TRI, total body irradiation
ABMT, autologous bone marrow transplant
ANC, absolute neutrophil count
Plts, platelets
RI, relapse incidence
LFS, leukaemia-free survival
OS, overall survival
MAF, mafosfamide

Figure 8. Marrow purging with mafosfamide: randomised study of protection with Ethyol®.

shows the general scheme of this study. Table 4 summarises the inclusion criteria; Table 5, the benefits expected; and Table 6, the primary and secondary end points. If amifostine does indeed improve engraftment in patients with purged marrow, the next questions will be whether purging can be increased and whether cytokines in addition to amifostine will result in a further acceleration of engraftment. Table 7 summarises the rationale for using mafosfamide in several *in vivo* and *in vitro* situations in the management of haematologic malignancies.

Table 6. Amifostine for autografting in acute leukaemia

Primary end points
- Polymorphonuclear cell recovery to 0.5 and 1 × 10^9 cells/l
- Platelet recovery to 20 and 50 × 10^9 cells/l
- Blood support
  Red cells
  Platelets
- Febrile days and documented sepsis

Secondary end points
- Transplant related mortality
- Relapse incidence
- Disease free survival
- Overall survival

Table 7. Amifostine for autografting in haematology, rationale

Protection for normal haematopoiesis	
Drugs	Situation
CY, 4-HC, mafosfamide	*In vivo*
Carmustine	CY-TBI
Melphalan	BU-CY
Busulfan?	BEAM-BEAC
Cytarabine?	*In vitro*
Etoposide?	Purging with 4-HC, mafosfamide

No protection for tumour
Leukaemic cell lines (REH, CEM-CCRF, MOLT4)
AKR mice (CFU-L sensitised)
Fresh CFU-L growth from individual patients (sensitised).

CY, cyclophosphamide; 4-HC, 4-hydroperoxycyclophosphamide; TBI, total body irradiation; BU, busulfan; BEAC, carmustine, etoposide, cytarabine, cyclophosphamide; CFU-L, colony-forming unit-leukaemic progenitors.

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