PCR-positivity in harvested bone marrow predicts relapse after transplantation with autologous purged bone marrow in children in second remission of precursor B-cell acute leukaemia

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Summary. Purging of autologous bone marrow (BM) grafts of children in second remission after a relapse of precursor B acute lymphoblastic leukaemia (ALL) in the BM has been carried out in our laboratory since 1987, initially by complement mediated cell lysis. This protocol was extended by performing an immunorosette depletion before lysis with complement. The aim of the present study was to assess by polymerase chain reaction the presence of residual leukaemic cells in the BM grafts before and after purging. The results were then correlated to clinical outcome. In 24/28 patients a PCR product was obtained by amplification of IgH and/or TcR junctional regions. BM before purging was available for analysis in 13 patients. We found that leukaemic cells could be detected in 8/13 (62%) of these grafts before purging. All these eight patients experienced a

relapse, regardless of whether the purging procedure had been successful (defined as achievement of PCR-negativity) or not. In contrast, none of the five patients with PCR-negative grafts before purging relapsed ($P = 0.0008$). One patient died due to transplant-related toxicity. Of the remaining 23 patients, nine patients received a PCR-positive BM graft after purging. All these nine patients experienced a relapse as compared to 6/14 whose BM was PCR-negative after purging ($P = 0.0072$). Two of eight PCR-positive BM grafts could be purged to PCR-negativity. Thus, improvements both in treatment of leukaemia and in purging efficacy are still needed.

Keywords: purging, acute lymphoblastic leukaemia, autologous bone marrow transplantation, polymerase chain reaction.

In approximately 70% of children with precursor B acute lymphoblastic leukaemia (ALL) long-term complete remission can be achieved with current chemotherapy (Pui, 1995). However, children experiencing a relapse in the bone marrow (BM) have a poor prognosis. Different chemotherapeutic regimens have resulted in a 5-year leukaemia-free survival of 20–30% in this group of patients (Behrendt *et al*, 1990; Henze *et al*, 1991; Culbert *et al*, 1991). Following high-dose chemotherapy combined with transplantation of allogeneic or autologous BM, leukaemia-free survival has

been achieved in 20–60% of cases, depending on the treatment protocol and the duration of first complete remission (Kersey *et al*, 1987; Sallan *et al*, 1989; Simonsson *et al*, 1989; Billett *et al*, 1993; Moussalem *et al*, 1995).

Since 1987, our laboratory has been involved in purging autologous BM grafts of children in second remission after a BM relapse of precursor B ALL. Initially, purging was performed by complement mediated cell lysis with CD9 and CD10 MoAb and baby rabbit complement. The purging protocol was then extended by introducing an immunorosette depletion with CD19 and CD22 MoAbs before complement lysis (Slaper-Cortenbach *et al*, 1990). For evaluation of the efficacy of purging, different assays for the detection of minimal residual disease in BM are used (Negrin, 1992).

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To date, PCR is the most sensitive technique to study involvement of BM and the effectiveness of purging. Amplification of antigen receptor gene rearrangements by PCR has been extensively applied for detecting small numbers of malignant cells in ALL in both retrospective and prospective studies, as reviewed recently (Campana & Pui, 1995). No consistent relationship between PCR results and clinical outcome was found in these reports. In some studies a single PCR-positive sample indicated an impending relapse, whereas in other studies no such relation was observed. In the present study, PCR analysis for the presence of residual leukaemic cells in autologous grafts of 28 patients with precursor B ALL both before and after the purging procedure was correlated with clinical outcome.

PATIENTS, MATERIALS AND METHODS

Cells. B-lineage ALL cell lines Nalm-6 and Nalm-16 were used, as well as the Burkitt's lymphoma cell lines Daudi and Ros-17 (gift of Professor Dr J. J. M. van Dongen, Rotterdam, The Netherlands). Cell line cells were cultured in RPMI-1640 medium (Gibco, Paisley, Scotland) supplemented with 10% (vol/vol) fetal calf serum (FCS; Bodinco, Alkmaar, The Netherlands). Only exponentially growing cells were used in the experiments. Mononuclear cells were isolated from normal human BM by density gradient centrifugation (Ficoll-Isopaque, Pharmacia, Uppsala, Sweden; $d = 1.077 \text{ g/cm}^3$). Remission BM was imitated by mixing 5% cell line cells with normal BM cells at a concentration of 20×10^6 cells/ml.

Monoclonal antibodies. Four B-cell MoAbs were employed during the purging procedure: CLB-thrombo/8 (CD9, IgG2a), CLB-cALLA/1 (CD10, IgG2a), CLB-B4 (CD19, IgG1) and CLB-B-ly/1 (CD22, IgG1). For formation of tetramolecular complexes (Lansdorp *et al*, 1986), rat anti-mouse IgG1 (CLB-RaMIgG1) and anti-glycophorin A (CLB-ery/1, IgG1) were also used. All MoAbs were produced in the Central Laboratory of the Netherlands Red Cross Blood Transfusion Service (CLB, Amsterdam, The Netherlands) and were tested for sterility, pyrogenicity and contamination with mycoplasma and viruses.

Clonogenic assay. The purging efficacy in model experiments, in which leukaemia contaminated remission BM was imitated by mixing cell line cells with normal BM, was determined in a limiting dilution assay as described (Bast *et al*, 1985).

Patients. Characteristics of the 28 children with a relapse of precursor B ALL in the BM are given in Table I. After primary diagnosis, induction, central nervous system (CNS) prophylaxis and maintenance therapy was given according to protocols from the Dutch Childhood Leukaemia Study Group (DCLSG ALL-V, ALL-VI, ALL-VII and ALL-VIII) which have been described previously (Van der Does-Van den Berg *et al*, 1989; Veerman *et al*, 1990; Reiter *et al*, 1995; van den Berg *et al*, 1995). After a first relapse, patients 1–13 were treated according to local protocol. After reinduction therapy and achievement of a second complete remission, intensive chemotherapy was given for 5 months followed by ABMT. BM was harvested approximately 1 month before ABMT. Conditioning therapy consisted of busulphan and

cyclophosphamide. The other patients were treated according to the DCLSG-ALL-Relapse-90 protocol. This regimen is based on the relapse protocol of the German BFM group (Henze *et al*, 1991). It includes high-dose chemotherapy for 5 months after attaining a second clinical remission followed by ABMT. In this group of patients the conditioning regimen before ABMT consisted of high-dose chemotherapy and total body irradiation.

Bone marrow processing and purging. BM was aspirated from the posterior iliac crests under general anaesthesia and collected into sterile bottles containing 20 ml of a Hepes-buffered Hanks' Balanced Salt Solution (HBSS) with 4000 U thromboliquine (Heparinum natricum, Organon Technika). The BM was pooled and a buffy coat was prepared. For patients 1–10, mononuclear cells were separated on Ficoll-Isopaque using an IBM-2991 blood cell processor centrifuge. The BM cells were washed twice and resuspended in RPMI-1640 medium supplemented with 10% PPF (purified protein fraction, CLB) and 3.8% tri-sodium citrate (TSC). Purging was done at a cell concentration of 20×10^6 to 25×10^6 mononuclear cells/ml. The cells were first incubated with CD9 and CD10 MoAbs at optimal concentrations at room temperature for 10 min. Then baby rabbit complement was added at a final concentration of 25% (vol/vol). The complement was obtained under sterile conditions from 6-week-old baby rabbits that were bred under specific pathogen-free conditions. The animals were bled and the rabbit serum was isolated and tested for viral and bacterial contamination. The cells were then incubated for another 60 min at room temperature, with shaking of the bag for optimal lysis. Then, the cells were washed twice to remove excess MoAbs, complement and cell debris. After the purging procedure, the BM cells were cryopreserved in Hepes-buffered HBBS, 3.8% TSC, 10% human serum albumin and 10% dimethyl sulphoxide (DMSO). For patients 11–28 a two-step purging procedure was used which consisted of an immunorosette depletion technique followed by one cycle of complement lysis (Slaper-Cortenbach *et al*, 1990). Patient erythrocytes were coated with tetramolecular complexes directed against glycophorin A on the erythrocyte and CD19 or CD22 on B cells. Before density gradient centrifugation, coated erythrocytes were added to buffy coat cells in a ratio of 10:1 target cells and immunorosettes were allowed to form for 15 min. Then a ficoll density separation was performed to isolate the immunorosette-depleted light-density cells. After washing the cells, complement mediated cell lysis was performed as described above.

Collection of patient material. Mononuclear cells (MNC) obtained at relapse and/or diagnosis were isolated from BM or peripheral blood (PB) and cryopreserved for sequence analysis. BM before purging was available for analysis in 13 patients. MNC were isolated both from PB obtained 1 d before BM harvest and from the harvested BM in a further 10 patients. Furthermore, approximately 10×10^6 cells were stored after the immunorosette depletion procedure and also after completion of the purging procedure, to assess the relative contribution of the two different purging techniques. Cells were cryopreserved in RPMI medium supplemented with 20% FCS and 10% DMSO. After thawing the stored

Table 1. Patient characteristics.

Patient	Age/sex	Time from diagnosis to first relapse (months)	Rearrangement*	Sensitivity of patient specific probe
1	5/M	31	TcR	10 ⁻⁵
2	2/M	27	TcR	10 ⁻⁴
3	2/M	37	TcR	10 ⁻⁴
4	14/M	26	IgH	10 ⁻⁴
5	5/F	9	IgH	10 ⁻³
6	13/F	31	IgH	UK
7	11/F	64	IgH	10 ⁻³
8	4/M	35	IgH	UK
9	8/F	42	IgH	10 ⁻⁴
10	8/F	70	TcR	10 ⁻³
11	7/F	85	IgH	10 ⁻⁵
12	7/M	86	TcR	10 ⁻⁴
13	5/F	28	TcR	UK
14	15/M	24	IgH+TcR	10 ⁻⁵
15	12/F	24	IgH	10 ⁻⁴
16	5/M	15	-	-
17	4/M	27	TcR	10 ⁻⁵
18	9/M	24	TcR	UK
19	5/M	42	IgH	10 ⁻⁴
20	5/M	30	TcR	10 ⁻⁶
21	1/M	33	-	-
22	3/M	48	IgH	UK
23	3/M	25	IgH	10 ⁻⁴
24	2/M	34	IgH	10 ⁻³
25	4/F	70	-	-
26	5/M	39	-	-
27	2/F	27	IgH	10 ⁻⁴
28	6/M	39	TcR	10 ⁻⁵

* When both IgH and TcR rearrangements were present, the rearrangement resulting in a probe that detected leukaemic cells with the highest sensitivity as determined with the dilution series is shown; age = age at diagnosis; M: male; F: female; -: no antigen receptor rearrangement could be amplified by PCR; UK: unknown, because no dilution series was available for these patients (DNA was extracted from glass slides instead of from frozen cells).

cells, the samples obtained after purging (except for patients 3, 4 and 15) were also subjected to two incubations of 1 h each at 37°C in RPMI medium supplemented with 100 µg/ml DNase (Sigma, type IV from bovine pancreas). Previous experiments had shown that this procedure resulted in optimal elimination of DNA from lysed cells that could give rise to false positive results in the PCR analysis (unpublished observations).

Polymerase chain reaction. DNA was isolated from the cell suspensions by proteinase K digestion and phenol/chloroform extraction as described previously (Steenbergen *et al*, 1995b). PCR reactions contained 1 µg genomic DNA (equivalent of approximately 10⁵ cells). Immunoglobulin heavy chain V_HDJ_H junctional regions (CDRIII) and T-cell receptor (TCR) Vδ2Dδ3 junctional regions were amplified by PCR as described (Steenbergen *et al*, 1995b). Briefly, CDRIII regions were amplified with a consensus Framework region 3 primer (FR3) and a consensus joining primer (JH21). For the amplification of TCR Vδ2Dδ3 rearrangements from

relapse and diagnosis samples, a semi-nested PCR approach was used. For analysis of serial dilutions and remission BM a single round of PCR was performed. To check DNA quality, all DNA samples were subjected to a PCR with primer pair PCO3–PCO4 which amplifies a 110 base pair product from the first exon of the human β-globin gene (Saiki *et al*, 1985). PCR products were separated according to size by electrophoresis on an acrylamide gel, re-amplified with the original primers and directly sequenced. ³²P-labelled patient-specific oligonucleotides were generated and hybridized to the PCR products by liquid hybridization (Steenbergen *et al*, 1995b). For semi-quantification of residual malignant cells, DNA from 10-fold serial dilutions of patient cells (always more than 90% leukaemic blasts as starting material) in PB MNC were subjected to the same protocol. Steenbergen *et al* (1995b) previously showed that this technique enables the monitoring of minimal residual disease with acceptable sensitivities (at least one malignant cell in 10⁴ normal cells could be detected for 85% of evaluable patients).

Statistical analysis. For statistical analysis of differences in purging efficacy using cell lines, Student's *t* tests and for comparison of patient groups Fisher's Exact Tests were done.

RESULTS

Purging efficacy determined in model experiments

The efficacy of the purging procedure was tested by simulating leukaemia-contaminated remission BM, using a mixture of 5% cell line cells and normal BM cells. Fig 1 shows that complement-mediated cell lysis with CD9 and CD10 MoAbs resulted in a 0.4–3.0 log depletion of clonogenic tumour cells, depending on the cell line used for the experiment. Addition of immunorosette depletion to the purging procedure resulted in a significantly increased elimination of leukaemic cells as is shown for cell lines Ros ($P < 0.005$) and Nalm-6 ($P < 0.025$). These results confirm and extend our earlier findings (Slaper-Cortenbach *et al*, 1990).

Fig 1. Remission BM was simulated by mixing 5% cell line cells with irradiated normal BM cells. For four cell lines the effect of complement-mediated cell lysis was tested (solid bars) and for Nalm-6 and Ros the effect of the two-step purging procedure was also determined (open bars) in clonogenic assays. Results are expressed as log depletion of clonogenic cells. Shown are average values of three or more experiments $\pm$ standard error of the mean (SEM). compl.: complement-mediated cell lysis; IR: immunorosette depletion.

Evaluation of treatment

Of the 28 patients included in this study, 10 patients (36%) remain in continuous complete remission after ABMT with a median follow-up of 23 months (range 13–76 months). The length of first complete remission in these 10 patients was >2 years. Patient 6 died due to transplant-related toxicity. Patient 25 relapsed before ABMT could be performed. The remaining 16 patients relapsed within the first 13 months after ABMT, except for patient 7 who relapsed after 53 months. The duration of first complete remission was 2 years or less in 5/16 patients who relapsed ($P = 0.1213$).

Tumour cell contamination at time of bone marrow harvest and correlation with clinical outcome

In 24/28 (86%) patients a PCR product was obtained by amplification of IgH and/or TCR junctional regions (Table I). Patient-specific probes were generated and hybridized to PCR products from serial dilutions of patient cells in PB MNC. When both IgH and TcR rearrangements were present, the rearrangement resulting in a probe that detected leukaemic cells with the highest sensitivity as determined with the dilution series is shown in Table I. Of probes derived from five patients the sensitivity could not be determined. For these patients, DNA was isolated from cells obtained from glass slides, so no dilution series could be made. A sensitivity equal to or higher than approximately one cell in 10^4 (10^{-4} , estimated from the results from the semi-quantitative PCR) was obtained with probes from 15/19 patients (80%).

For 13 patients BM before purging was available for analysis. Leukaemic cells were detected by PCR at the time of BM harvest in 8/13 patients (62%, Tables II and III). All these patients experienced a relapse as compared to 0/5 patients in whom no leukaemic cells were detected by PCR ($P = 0.0008$). PB was obtained the day before BM harvest from 10 patients. Tumour cells were detectable in the MNC isolated from PB of only two patients with heavily contaminated BM (1–10% leukaemic cells as estimated by the PCR results).

Results of in vitro treatment of bone marrow and correlation with clinical outcome

After completion of the purging procedure, residual leukaemic cells were detected in the BM of 9/24 patients (Tables II and III). All these nine patients experienced a BM

Table II. Detection of residual malignant cells in BM samples of patients whose BM was purged by complement lysis alone.

Patient	Pre-purge	After purge	Outcome after ABMT
1	ND	—	R (13)
2	+1	+	R (4)
3	+3	+	R (8)
4	ND	+	R (12)
5	ND	+	R (1)
6	ND	—	Tox.
7	ND	—	R (53) / CR (4)
8	ND	—	R (13) ^c
9	ND	—	CR2 (76)
10	ND	—	CR2 (68)

ND: not done; PCR data are expressed as + or —, indicating PCR positivity or negativity. Numbers indicate the level of positivity as estimated in a semi-quantitative PCR (+2 means 1:100 cells positive when compared with a dilution series, +3 means 1:1000 cells positive, etc.); Tox.: this patient died from transplant-related toxicity; ^c: the relapse of this patient was a CNS relapse; figures in parentheses indicate the time from ABMT to relapse (months) or the duration of follow-up for patients remaining in CR.

Table III. Detection of residual malignant cells in PBL and BM samples of patients whose BM was purged by the two-step procedure.

Patient	PB-MNC	BM pre-purge	After IR	After purge	Outcome after ABMT
11	ND	ND	ND	+	R (3)
12	ND	ND	ND	—	R (8)
13	ND	ND	ND	—	CR2 (61)
14	—	+3	ND	+	R (8)
15	ND	+4	+	+	R (9)
17	—	—	—	—	CR2 (33)
18	—	+	ND	—	R (6)
19	—	—	—	—	CR2 (25)
20	+1	+1	+	—	R (8)
22	—	+	+	+	R (5)
23	—	—	ND	—	CR2 (19)
24	—	—	—	—	CR2 (19)
27	+2	+2	+	+	R (3)
28	—	—	—	—	CR2 (13)

ND: not done; PCR data are expressed as + or —, indicating PCR positivity or negativity. Numbers indicate the level of positivity as estimated in a semi-quantitative PCR (+2 means 1:100 cells positive when compared with a dilution series, +3 means 1:1000 cells positive, etc.); IR: immunorosette depletion; figures in parentheses indicate the time from ABMT to relapse (months) or the duration of follow-up for patients remaining in CR.

relapse within the first year after ABMT. 15 patients received a PCR-negative graft. One patient died of transplant-related toxicity and was excluded from this analysis. A relapse (five BM and one CNS) occurred in only 6/14 patients whose

grafts were negative by PCR after purging ($P = 0.0072$). In two of these six patients, leukaemic cells were detected before purging, whereas in the other four patients no material was available for analysis of pre-purge BM.

Fig 2. Detection of residual leukaemic cells by PCR is shown for patients 20 (A) and 22 (B). In A, a dilution series is shown of material obtained at relapse diluted in normal mononuclear cells (MNC). PBL: peripheral blood lymphocytes obtained the day before bone marrow (BM) harvest; IR: immunorosette depletion.

The BM of four of the 10 patients whose BM was purged by complement alone was positive after purging. BM before purging could only be evaluated in two patients. The BM of both these patients remained PCR-positive after purging.

Samples after immunorosette depletion were collected from nine patients. The BM of five of these patients was negative by PCR before purging, whereas in four patients residual leukaemic cells could be detected. In the BM of all these four patients leukaemic cells were still detectable after immunorosette depletion and before complement-mediated cell lysis. Leukaemic cells were still detected in 5/14 patients after completion of the two-step purging procedure. PCR results were negative after purging in only 2/6 patients whose BM contained residual tumour cells before purging. However, these patients relapsed 6 and 8 months after transplantation. So, in summary, PCR positivity in the graft predicted relapse, whereas PCR negativity did not exclude relapse.

In patient 20, residual tumour cells were still detected after immunorosette depletion, but PCR was negative after the second purging step (Fig 2A). Fig 2B shows the results from patient 22, whose BM still contained residual leukaemic cells after both purging steps. This patient relapsed 5 months after ABMT. Immunophenotyping data were available for 21 patients. No correlation was observed between the ability to purge contaminated BM grafts to PCR negativity and the expression of CD9, CD10, CD19 and/or CD22.

DISCUSSION

The high incidence of relapse after autologous bone marrow transplantation using a purged graft in children with precursor B ALL can be due to several factors. The pretransplant conditioning regime may not have completely eliminated residual leukaemia in the patient, as a result of drug resistance or resistance to radiation therapy. The absence of a graft-versus-leukaemia effect (which may occur in allogeneic BM transplantation) may also influence the final outcome in these patients. Finally, inefficient purging and subsequent reinfusion of malignant cells into the patient may contribute to relapse.

The results from our study indicate that both the antileukaemic treatment and the purging procedure need to be optimized. Leukaemic burden in the pre-purge BM grafts correlated strongly with relapse. This confirms the results from the Minneapolis group (Uckun *et al*, 1993, 1992). In their studies, multiparameter flow cytometry and cell sorting were combined with a colony assay for leukaemic progenitor cells in a quantitative method for the detection of MRD. The pre-purge leukaemic burden correlated with the clinical outcome. Similar results were obtained by Seriu *et al* (1995). They reported the application of peripheral stem cell transplantation in five patients with precursor B ALL. Leukaemic cells were detected by PCR in peripheral blood stem cell autografts in 4/5 patients. Three of these four patients relapsed within 6 months after transplantation.

A strong suggestion that reinfusion of malignant cells that are present in autologous BM grafts contributes to relapse

was provided by Gribben *et al* (1991). In a group of adult patients with B-cell lymphoma, all BM grafts contained residual malignant cells at harvest as detected by polymerase chain reaction (PCR). After purging with monoclonal antibodies (MoAb) and complement, malignant cells could no longer be detected in BM grafts of 50% of the patients. Disease-free survival in this group was significantly more common than in those patients whose marrow still contained detectable residual lymphoma cells. For patients with acute myeloid leukaemia, neuroblastoma, and chronic myelogenous leukaemia, gene marking of the autologous marrow has shown that malignant cells from the graft were present at relapse (Brenner *et al*, 1993; Rill *et al*, 1994; Deisseroth *et al*, 1994). These studies suggest that elimination of malignant cells from the graft might increase disease-free survival. In our study, all patients whose marrow contained residual malignant cells after purging, experienced a relapse, as compared to 6/14 whose BM was PCR-negative after purging ($P = 0.0072$). However, this does not prove that leukaemic cells in the graft contributed to relapse. As suggested by others (Gribben & Nadler, 1995; Lazarus *et al*, 1993; Murgo *et al*, 1992), the inability to purge tumour cells from the graft might also reflect more resistant disease. However, the relation between drug resistance and, for example, resistance to complement mediated lysis, is not clear.

In cell line experiments, a 2.5–4.5 log depletion of malignant cells was achieved with our current purging protocol, as compared to 0.4–3.0 log depletion when complement lysis was applied without immunorosette depletion. Although the purging efficacy in autologous BM grafts could not be exactly determined by the semi-quantitative PCR assay, the estimated tumour contamination in BM grafts before and after purging suggests a considerable variety in purging efficacy among patients. For example, patient 20 had a large tumour burden (approximately 10%) before purging, which was reduced to PCR negativity after purging (with the patient specific probe, approximately one leukaemic cell could be detected among 10^6 normal cells). In contrast, tumour contamination of the BM at harvest of patient 15 was at the level of the detection limit (with the patient-specific probe, approximately one leukaemic cell could be detected among 10^4 normal cells) and remained PCR positive after the two-step purging procedure. A large variation in purging efficacy between patients has also been observed by others, either by immunofluorescence techniques (Garcia *et al*, 1994), PCR (Gribben *et al*, 1991) or colony assays (Uckun *et al*, 1992).

Heterogenous distribution of leukaemia in the BM (Hann *et al*, 1977; Martens *et al*, 1987) can be a cause of sample error and false-negative results by PCR or of an inaccurate estimation of leukaemic burden at the time of BM harvest. False-negative results can also be caused by ongoing antigen receptor gene rearrangements. As a consequence, patient-specific probes derived from the rearrangement at diagnosis might not hybridize with the amplified rearrangement from material obtained at relapse. However, from most of the patients analysed in this study, probes were derived from material obtained at first BM relapse and we have shown

previously that clonal changes did not occur from first relapse onward (Steenbergen *et al*, 1995a). False-positive results can be due to amplification of DNA from lysed cells. In order to prevent this, we developed a procedure of incubating thawed cells with high concentrations of DNase which reduces the risk of co-purification of DNA from lysed cells.

Garcia *et al* (1994) reported an improved, although not statistically significant, disease-free survival for ALL patients in whom at least a 1.5 log depletion of leukaemic cells was achieved by complement lysis as compared to cases in which purging was less efficient or in patients who received unpurged autologous BM. Results from their study are obscured by the fact that the purged group contained more patients transplanted in first complete remission (10/32) than patients in the unpurged group (2/14). Gilmore *et al* (1991) concluded that effective *in vitro* purging by complement-mediated cell lysis of remission BM in patients with both precursor B and T ALL appeared not to improve the outcome in high-risk patients transplanted in first remission. However, purge efficacy in this study was estimated by fluorescence techniques and Southern blotting which does not allow detection of <1% of tumour cells.

In conclusion, in this study remission BM of 62% of patients with precursor B ALL was contaminated with leukaemic cells despite 6 months of intensive chemotherapy. Regardless of whether the purging procedure was successful (defined by achievement of PCR negativity) or not, all these patients experienced a relapse after transplantation, indicating that the antileukaemic treatment was insufficient. If the results from this study are confirmed in a large prospective study, PCR analysis of the graft at BM harvest will be able to predict the clinical outcome after ABMT. Accordingly, the decision of whether ABMT should be performed can then be based on the outcome of this analysis. The number of patients whose BM was evaluated both before and after purging was too small to draw definite conclusions on the benefit of purging. The finding that only 25% of the PCR-positive grafts could be purged to PCR-negativity suggests that optimization of our purging procedure is also necessary.

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