Leukemia (2001) 15, 50–56

www.nature.com/leu

Purified autologous grafting in childhood acute lymphoblastic leukemia in second remission: evidence for long-term clinical and molecular remissions

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Autologous transplantation is a treatment option for relapsed childhood acute lymphoblastic leukemia (ALL) in second complete remission (CR2) when a suitable donor is not available. In an attempt to prevent relapses originating from graft leukemic contamination, the experimental protocol of *in vitro* purification of leukapheretic products with monoclonal antibodies (MoAbs), previously reported for adults, was adopted in 11 of 12 consecutive patients (median age, 9 years) with B cell precursor ALL in CR2 after late relapse (median, 37; range, 31–51 months after the onset) enrolled between July 1997 and July 1999 at a single pediatric center. At a median of 12 days after the mobilizing chemotherapy followed by G-CSF, a median of 13.9 (range, 5.9–18.7) $\times$ 10^6 $CD34^+$ cells/kg were collected from each patient and a median of 7.5 (range, 4.1–12.6) $\times$ 10^6 $CD34^+$ cells/kg underwent the purification procedure. The first step of immunorosetting allowed a one-log reduction of the total cell count, by eliminating more than 90% of the $CD11b^+$ cells; the second step, performed after incubation with anti-CD19 MoAbs, allowed the depletion of 99% (range, 93–100) of the $CD19^+$ cells, kept within the magnetic field of the immunodepletion column, with a median recovery of 73% (range, 55–87) of the collected $CD34^+$ cells. Molecular analysis assessed the *in vitro* eradication of detectable leukemic cells. A median reinfusion of 5.2 (range, 3.2–9.1) $\times$ 10^6 $CD34^+$ cells/kg for each patient (median viability, 90%), after conditioning with the 'TBI-VP16-CY' regimen, allowed prompt engraftment and immunological reconstitution; no patients experienced severe transplant-related toxicity or major infections. One patient relapsed 7 months after transplantation, while 10 patients are alive in clinical and molecular remission, at a median follow-up of 29 months (range, 15–40) (2-year EFS, 89%, s.e. 9). In conclusion, the procedure proved to be reproducible for pediatric purified autografting, highly efficient concerning stem cell recovery and depletion of leukemia-lineage specific cells, and promising in terms of final outcome. *Leukemia* (2001) **15,** 50–56.
Keywords: acute lymphoblastic leukemia (ALL); relapse; autologous transplantation; purging; $CD34^+$ negative selection

Introduction

Hematopoietic cell transplantation is widely used in treating relapsed childhood acute lymphoblastic leukemia (ALL). Whenever a suitable related donor is not available, autologous transplantation could be an alternative to unrelated donor transplantation (URDT), whose times, costs and transplant-related mortality (TRM) are still too high, at least for those patients who relapse late after diagnosis and whose outcome could be acceptable even with chemotherapy only.[1–7] Relapse is the most frequent cause of failure after autologous transplantation, lacking the antileukemic effect of an allograft, and can originate either from graft contamination or from residual disease in the patient, possibly resistant to a supposed myeloablative conditioning regimen.[8] Historical purging of bone marrow progenitors was not shown to improve outcome in autologous transplantation; on the other hand, large amounts of circulating progenitor cells (CPC) collectable from peripheral blood (PB) ensure prompt engraftment and allow for promising new purging procedures, which role is still to be assessed.[9,10]

In an attempt to reduce relapse after autologous transplantation, by possibly preventing at least those relapses originating from leukemic blasts contaminating the graft, the experimental protocol of *in vitro* purification of leukapheretic products with monoclonal antibodies (MoAbs), previously reported for adults by Rambaldi *et al*,[11] was adopted. The aim of this study was to demonstrate the feasibility and efficacy of CPC collection, double step purification, and reinfusion in pediatric B cell precursor ALL in second complete remission (CR2), after late relapse. The efficacy of *in vitro* leukemia eradication was assessed by molecular evaluation of minimal residual disease (MRD) and the final outcome was evaluated.

Patients and methods

Eligibility criteria

Patients under 18 years with B cell precursor ALL in CR2, after late (30 months after the onset) isolated or combined relapse, who did not have a compatible related donor, and did not present absolute contraindication for transplantation, were eligible for the protocol, after obtaining informed consent from parents or guardians.

Mobilization and collection

The mobilizing regimen consisted of 'DIAVV', a five-drug chemotherapy (dexamethazone 5 mg/m^2 for 7 days, idarubicine 10 mg/m^2 and cytosine-arabinoside 3 g/m^2 on day 1, vincristine 1.5 mg/m^2 and vepeside 450 mg/m^2 on day 2) followed by G-CSF (10 g/kg s.c. daily from day 5) adopted to consolidate remission and mobilize CPCs.[12] From day 10, the absolute number of $CD34^+$ cells was monitored daily in the PB, by flow cytometry with fluorescein isothiocyanate-conjugated murine MoAbs against human CD34 antigen and a FACScan analyzer (Becton Dickinson, Mountain View, CA, USA). Once a threshold of 0.05 $\times$ 10^9 $CD34^+$ cells/l was reached, the leukapheresis was performed through a central venous catheter and a temporary peripheral vein access, by means of a continuous flow cell separator (COBE Spectra, Lakewood, CO, USA), and approximately 2.5 patient-blood volumes were processed to obtain at least 5 $\times$ 10^6 $CD34^+$ cell/kg; if necessary, a second leukapheresis was performed the following day.[13] In order to prevent clotting, ACD was used at a ratio of 1:12 for children weighing 25 kg or more; for children under 25 kg, 1:16 ACD combined with heparin 50 U/kg was

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Received 19 April 2000; accepted 26 September 2000

used, and the separator was initialized with a compatible filtered and irradiated RBC unit, suspended in albumin, up to the patient's hematocrit, to avoid transient hypovolemia, due to the volume sequestered in the separator.

Purification

About 1–3 × 10^6 $CD34^+$ cell/kg were to be stored unmanipulated for rescue, in case of purification procedure failure or of non-engraftment. The purification consisted of a double step procedure, as described by Rambaldi *et al*;[11] at each step, cells were sampled for molecular and immunophenotypic analyses. The first step aimed at reducing cell counts, by incubation of the apheresis product with filtered irradiated compatible RBC, incubated in chromium chloride and coated with murine MoAbs anti CD11b (hybridoma cell line OKM1, IgG2b, CRL 8026, obtained from the American Colture Collection ATCC, Rockville, MD, USA); the coupling process of rosette formation allowed the removal of $CD11b^+$ cells (monocytes, granulocytes, natural killer lymphocytes) by gradient sedimentation through Ficoll–Hypaque. The second step consisted of a positive selection of possible contaminating blasts, by incubation of CPCs, obtained after immunorosetting, with panB anti-CD19 MoAb (clone HD37, IgG1; kindly provided by Dr Moldenhauer, Deutsches Krebsforschugszentrum, Heidelberg, Germany), conjugated to goat anti-mouse iron-dextrane microbeads (Miltenyi Biotec, Bergisch Gladbach, Germany). B lineage cells were kept within the immunomagnetic depletion column, inserted in a SuperMacs device (Miltenyi Biotec) and negative selection of the B-depleted CD34 cells was allowed. The two steps were estimated to cost about 400 US$ and 1500 US$, respectively, and to require a 6-h work-time for one operator.[11]

Cryopreservation, conditioning regimen and infusion

The final eluated product, $CD34^+$-enriched and virtually leukemia-free, was cryopreserved in DMSO, at a final concentration of 10%, without further manipulation, regardless of the nucleated cell concentration, until thawing at the bedside at the time of reinfusion, when cell viability was tested as per standard methods.[14–16] Patients were conditioned with total body irradiation (TBI: 12 Gy divided in six doses, twice daily, days −7, −6, −5), vepeside (VP16: 60 mg/kg, day −4) and cyclophosphamide (CY: 50 mg/kg, days −3, −2).[17] Patients were managed according to standard practice, as previously reported.[18]

Molecular evaluation of MRD

MRD was evaluated by molecular analysis of the junctional regions of rearranged T cell receptor (TCR) or genes or of the fusion gene transcripts PBX1/E2A and ETV6/CBFA2, generated by the t(1;19) and t(12;21) translocations respectively.[19–21] Specific patterns of recombination of TCR or genes were identified at relapse by Southern blot analysis, according to standard techniques. The TCR or gene rearrangements were subsequently amplified by polymerase chain reaction (PCR) with V or V family primers and J or J general primers, respectively. The PCR products were cloned in pMos vector (Amersham, Buckinghamshire, UK) sequenced to define the precise nucleotide sequence of the junctional regions and leukemia patient-specific oligonucleotides probes were synthesized. Reaction products were identified by hybridization studies with DNA oligonucleotide probes labeled with -^{32}P-ATP.[22] The presence of the fusion transcripts PBX1/E2A and ETV6/CBFA2 was assessed by reverse-transcription PCR, as previously described.[20,21] Leukapheretic peripheral blood and subsequent marrow aspirates sampled before collection and every 3 months up to 1 year after transplantation were analyzed.

Post-transplant follow-up

Myeloid engraftment was considered to occur on the first of 3 consecutive days with an ANC 0.5 × 10^9/l, and platelet engraftment the first of 5 consecutive days with a platelet count 50 × 10^9/l, sustained without transfusional support. PB lymphocyte subsets were periodically monitored by labelling cell samples with directly conjugated MoAbs, including anti-CD19, -CD3, -CD4, -CD8, -CD16 and -CD56, subsequently analyzed on a FACScan. EFS was determined using the Kaplan–Meier product limit estimate expressed with its standard error (s.e.).[23] Patients were considered at risk from the day of transplantation until they experienced an event, ie non-leukemic death, relapse, or second tumor diagnosis, whichever occurred first. Patients who did not experience an event were censored at the time of last follow-up, on 31 August 2000.

Results

Accrual

Between July 1997 and July 1999, at a single pediatric center, 12 consecutive patients (seven males; median age, 9 years) were eligible for autologous transplantation for B cell precursor ALL in CR2, after late relapse (30 months after diagnosis). One patient (female, 8 years, medullary relapse 43 months after the onset) did not mobilize enough CPCs, underwent unmanipulated bone marrow autograft and relapsed 17 months after transplantation. All data reported from now and in the Tables refer to the 11 of 12 patients who mobilized enough stem cells and actually underwent purified CPC autologous transplantation. Patient characteristics are reported in Table 1. At disease onset, all 11 children had been enrolled either in the standard (two patients) or intermediate (nine patients) risk LLA-91 or LLA-95 frontline protocols of the 'Associazione Italiana di Ematologia e Oncologia Pediatrica' (AIEOP).[24] When relapse (six medullary, three combined, two extramedullary) occurred, at a median of 37 months (range, 31–51) after the onset, a second remission was obtained at a median time of 45 days (range, 9–76) with BFM-like protocols, according to AIEOP guidelines.

Mobilization, collection and depletion by anti-CD19 magnetic microbeads

At a median time of 60 days (range, 42–151) after CR2 was achieved, CPCs were mobilized by the five-drug chemotherapy 'DIAVV', which was well tolerated, in nine patients, and by the BFM-like block 3, included in the AIEOP-LLA-91 protocol, administered for the underlying disease, in the other two patients.[24] One (six patients) or two (five patients) leu-

Table 1 Characteristics and outcomes of the 11 pediatric patients (seven males) with B cell precursor ALL in CR2 undergoing autologous transplantation with purified CPCs

	Median	*Range*
Age (years)	9	5–16
Weight (kg)	31	21–56
Months diagnosis–relapse	37	31–51
Site of relapse	6 medullary, 3 combined, 2 extramedullary	
Mobilization regimen	9 DIAVV,[a] 2 block 3[b]	
Day of mobilization	12	10–17
$CD34^+ \times 10^6$/kg collected	13.9	5.9–18.7
$CD34^+ \times 10^6$/kg stored[c]	4.6	1.7–8.9
$CD34^+ \times 10^6$/kg processed	7.5	4.1–12.6
$CD34^+ \times 10^6$/kg purified	5.2	3.2–9.1
Viability %	90	71–95
Months 2nd CR–transplant	4	3–6
Conditioning regimen	TBI, VP16, CY[d]	
Day with ANC 1.0×10^9/l	13	11–16
Day with PTL 50×10^9/l	27	13–49
Status	10 alive in CR, 1 dead in relapse	
Months of follow-up[e]	29	15–40

[a]DIAVV: dexamethazone 5 mg/m^2 for 7 days, idarubicine 10 mg/m^2 and cytosine-arabinoside 3 g/m^2 on day 1, vincristine 1.5 mg/m^2 and vepeside 450 mg/m^2 on day 2, followed by G-CSF 10 g/kg from day 5 daily until collection.
[b]Block 3: dexamethazone 10 mg/m^2 for 7 days, cytosine-arabinoside 3 g/m^2 twice daily on days 1, 2, vepeside 150 mg/m^2 on days 3, 4, 5, followed by G-CSF 10 g/kg daily until collection.
[c]An amount of CPCs was stored unmanipulated as a rescue in case of purification procedure failure or of non-engraftment.
[d]TBI: 12 Gy divided in six doses, twice daily for 3 days, VP16: vepeside 60 mg/kg, CY: cyclophosphamide 50 mg/kg for 2 days.
[e]The follow-up refers to the 10 patients alive in CR.

kaphereses, yielding a median of 13.9 (range, 5.9–18.7) $\times 10^6$ $CD34^+$ cells/kg, were performed at a median time of 12 days (range, 10–17) after the beginning of the mobilizing regimen. No child required sedation to perform the apheresis. A median of 7.5 (range, 4.1–12.6) $\times 10^6$ $CD34^+$ cells/kg underwent the purification procedure and a median of 4.6 (range, 1.7–8.9) $\times 10^6$ $CD34^+$ cells/kg were stored unmanipulated. Absolute cell counts yielded by the purification procedure are reported in Table 2. The first step of immunorosetting allowed a one-log reduction of the total cell count, by eliminating more than 90% of the $CD11b^+$ cells; the second step in the column, performed after incubation with anti-CD19 MoAbs, allowed the depletion of 99% (range, 93–100) of $CD19^+$ cells, with a median recovery of 73% (range, 55–87) of $CD34^+$ cells, and a five-fold enrichment. A median amount of 5.2 (range, 3.2–9.1) $\times 10^6$ $CD34^+$ cells/kg in the final purified product for each patient was frozen; the viability rate was 90% (range, 71–95) at thawing.

Post-transplant follow-up

Purified CPCs were reinfused at a median of 26 days (range, 12–39) after collection, after all patients had been conditioned with TBI, VP16, CY. Fever, occurring in all patients and lasting 3 to 9 days, and I–II WHO mucositis, occurring in eight patients, were the only early complications. All patients reached myeloid engraftment at 13 days (range, 11–16) and platelet engraftment at 27 days (range, 13–49) after infusion, with no need for any back-up infusion. At a median time of 9 days (range, 12–34), nine patients developed an 'engraftment syndrome', consisting of diffuse skin erythema, possibly associated with fever,[25] which required treatment with steroids for a median of 26 days (range, 9–47). Median duration of hospitalization after infusion was 19 days (range, 15–28); within 100 days after the first discharge, a median of 10 days (range, 3–44) was spent in hospital, either in the ward (two patients only were readmitted after discharge, one for Herpes zoster i.v. treatment and the other for fever, possibly related to TBI), or in the outpatient department. One patient developed a cardiomyopathy, requiring mild diuretic treatment, which was discontinued after 6 months. No patient experienced major infections. B and T lymphocyte counts normalized in the second semester after transplantation, despite persistent alteration of the CD4/CD8 ratio beyond the first year (Figure 1). One patient relapsed 7 months after transplantation and died of disease progression, while 10 patients are alive in CR at a median follow-up of 29 months (range, 15–40), with Lanski or Karnofsky scores of 100% for all patients. The 2-year-EFS estimate was 89% (s.e., 9).

Molecular evaluation of MRD

The efficacy of the procedure in terms of *in vitro* eradication of the disease and complete remission maintenance was

Table 2 Purification by immune-rosettes (CD11b) and B cell specific magnetic microbeads (CD19) on leukapheresis from 11 patients with B cell precursor ALL in CR2

	Apheresis	*Immunorosettes (anti-CD11b)*	*D-column (anti-CD19)*
Total cells ($\times 10^9$)	20.873 (7.590–53.915)	4.970 (1.075–13.675)	4.136 (0.652–13.260)
Cell loss (%)		76.9 (58.5–86.1)	81.7 (63.0–92.5)
$CD34^+$ ($\times 10^9$)	0.307 (0.111–0.647)	0.222 (0.102–0.433)	0.223 (0.089–0.417)
$CD34^+$ (%)	1.7 (0.7–2.6)	6.2 (2.8–15.7)	9.1 (1.9–24.1)
$CD34^+$ recovery (%)		74.9 (51.5–92.9)	73.4 (54.6–86.7)
$CD34^+$ enrichment fold		3.7 (2.2–6.6)	5.3 (1.5–10.7)
$CD11b^+$ ($\times 10^9$)	15.629 (4.213–46.043)	1.856 (0.007–8.889)	1.724 (0.006–8.818)
$CD11b^+$ loss (%)		91.7 (67.8–100.0)	92.7 (65.1–100.0)
$CD19^+$ ($\times 10^9$)	0.065 (0.002–0.270)	0.010 (0.000–0.057)	0.001 (0.000–0.009)
$CD19^+$ loss (%)		73.5 (44.9–100.0)	99.0 (92.8–100.0)
$CD3^+$ ($\times 10^9$)	4.548 (1.909–10.474)	2.290 (0.384–5.558)	2.056 (0.327–4.862)
$CD3^+$ loss		51.9 (25.2–79.9)	56.9 (29.9–82.9)

Mean absolute counts and ranges are reported.

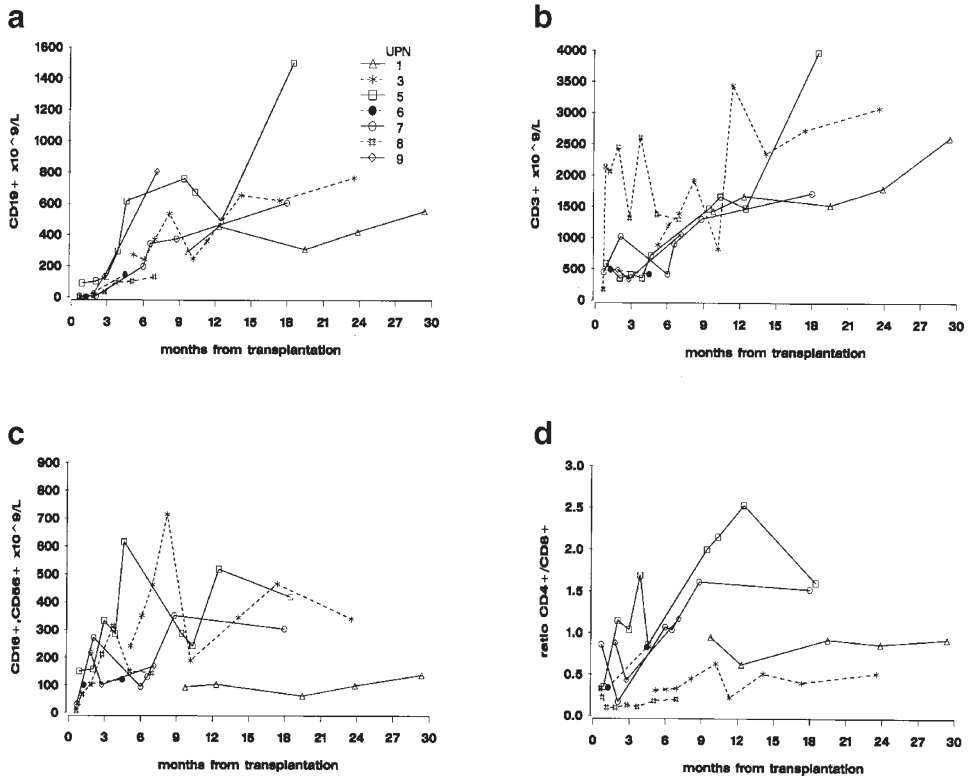

Figure 1 Lymphocyte subsets in peripheral blood periodically sampled after transplantation in seven patients. (a) Absolute counts of $CD19^+$ cells; (b) absolute counts of $CD3^+$ cells; (c) absolute counts of $CD16,CD56^+$ cells; (d) $CD4^+/CD8^+$ cell ratio.

assessed by MRD molecular analysis. Since no relapse sample was available for patient No. 4 and no marker was found for patient Nos 5 and 9, eight of 11 patients were analyzed. The markers analyzed were the leukemia-specific DNA sequence amplified from the rearranged TCR and chain genes (V I(V 5)-J 1.3/2.3, V I(V 3)-J 1.3/2.3, V II-J 1.3/2.3, V 2-D 3) in six patients and the fusion transcripts PBX1/E2A and ETV6/CBFA2 in two patients. As shown in Table 3, all CD19-purified CPCs were judged as PCR-negative, within the sensitivity limits of our analysis (between 10^{-3} and 10^{-5}). Interestingly, MRD was not detectable in the whole unmodified apheretic product of the two patients (Nos 2 and 3) who were MRD positive in the bone marrow just before mobilization and collection (Figure 2). However, the leukemic contamination was clearly shown in the unwanted B-lymphocytes, kept within the magnetic field of the depletion column. Bone marrow aspirates after transplantation were constantly negative in all but one patient (No. 2) up to clinical relapse, occurring 7 months after transplantation.

Discussion

In the present study an innovative purification procedure for pediatric autografting was proved feasible, reproducible and highly efficient in terms of recovery of $CD34^+$ cells, depletion

Table 3 Molecular evaluation of minimal residual disease (MRD) in the collected circulating progenitor cells and in the bone marrow

Patient No.[a]	*Molecular marker*	*Sens*[b]	*MRD of circulating progenitor cells*			*Pre*[c]	*MRD in the bone marrow months after transplantation*					*Follow-up*
			Apheresis	*Wasted*	*Purified*		*1*	*3*	*6*	*9*	*12*	
1	PBX1/E2A	$10^{-4/-5}$	–	–	–	–	–	–	–	–	–	40+
2	V I(V 3)-J 1.3/2.3	10^{-3}	–	+	–	+	+	+				Died
3	V I(V 5)-J 1.3/2.3	10^{-4}	–	+	–	+	–	–	–		–	35+
6	V II-J 1.3/2.3	$10^{-3/-4}$	–	–	–	–	–	–	–		–	31+
7	ETV6/CBFA2	10^{-4}	–	–	–	–	–	–	–	–	–	28+
8	V I(V 3)-J 1.3/2.3	10^{-4}	–	–	–	–	–	–	–	–	–	23+
10	V 2-D 3	10^{-4}	–	–	–	–	–	–	–	–		16+
11	V 2-D 3	$10^{-3/-4}$	–	–	–	–	–	–	–	–		15+

[a]MRD analysis was possible in eight of 11 patients, since no relapse sample was available for patient No. 4 (CCR, 35+) and no marker was found for patient Nos 5 and 9 (CCR 32+, 21+).
[b]Sensitivity (tested by serial dilution).
[c]'Pre-transplantation' results refer to bone marrow samples analyzed before mobilization and collection.

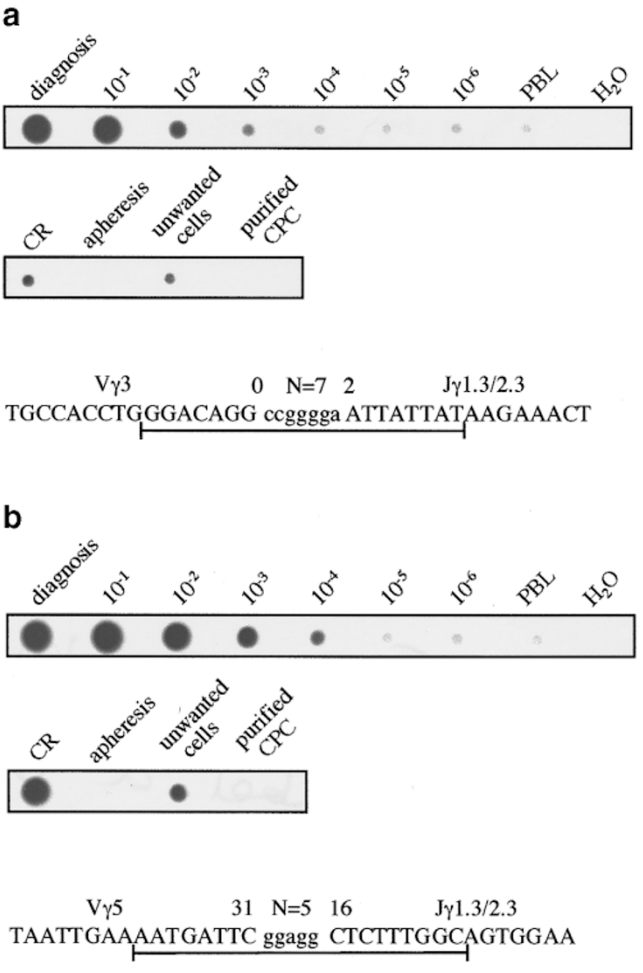

Figure 2 Detection of MRD by PCR analysis at V -J 1.3/2.3 junctions of the TCR chain gene in the two patients (patient No. 2: a; patient No. 3: b) who were MRD positive in the bone marrow at the time of CPCs collection. DNA samples were extracted from (1) bone marrow aspirates performed just before the mobilizing therapy administered for CPCs collection, when patients were in complete hematological remission (CR), (2) unmanipulated collected CPCs (apheresis), (3) cells coated with anti-CD19 magnetic microbeads and retained within the magnetic column (unwanted cells), negatively selected eluated purified cells (purified CPCs). The sensitivity of the PCR was checked by serial log dilution of the patient DNA obtained at the diagnosis of relapse (diagnosis) with DNA from a normal donor (upper lane in the panels). PCR products were blotted on to nylon membranes and hybridized with the indicated clonospecific probe labeled with -^{32}P-ATP.

of leukemia-lineage specific cells and eradication of molecularly detectable leukemic cells. The well-tolerated mobilizing regimen allowed 11 of 12 consecutive patients to mobilize enough stem cells for collection, manipulation and back-up, and the conditioning regimen with TBI-VP16-CY did not cause relevant toxicity or death. The outcome of long clinical and molecular remissions obtained in 10 of 11 patients transplanted for B cell precursor ALL in CR2 after late relapse (2-year EFS, 89%, s.e. 9; follow-up: median, 29 months, range, 15–40) highlighted a possible role for autologous transplantation in late relapsed ALL. The efficient and relatively inexpensive method of purification by leukapheresis for transplantation, reported by Rambaldi *et al* for adults, was preferred because of its favorable characteristics.[11] A procedure of negative selection was required, since ALL cells may express the CD34 antigen, thus preventing the adoption of CD34$^+$ cell positive selection, widely adopted in autografting purging for most solid tumors.[26] Since the high cellularity of leukapheresis would have jeopardized the efficacy of the selection, monocytes, granulocytes, and natural killer cells, which are not crucial for engraftment in the autologous setting, were depleted in the first step, with an acceptable loss of stem cells (about 25%). The aspecific reduction of the total cellularity increased the efficacy of the second step, which yielded a virtually leukemia-free product. The high specificity of the MoAb anti-CD19 was utilized for the most crucial aim of getting rid of possible leukemic blasts by whole lineage elimination. More than 70% of the collected stem cells were ultimately included in the eluate by negative selection, with a five-fold enrichment. The purification procedure did not alter the quality of the graft, since all patients promptly engrafted, and allowed immunological reconstitution, similar to that reported elsewhere.[27,28] The MRD molecular analysis assessed the leukemia eradication from the grafts, at least in the two patients who were MRD positive. The blast positivity in the two patients' wasted fraction, despite negative leukapheresis, highlighted a relative limit of the technique, which virtually allowed detection of one leukemic cell out of 10^3–10^5 normal cells, according to each marker probe sensitivity. However, the leukemic contamination was clearly shown in the unwanted B-lymphocytes, kept within the magnetic field of the depletion column. Tumor residual burden in the patient before collection and before transplant are likely to influence the final outcome.[29] Interestingly, the only relapsed patient was one of the two who had detectable MRD before mobilization (Figure 2) and before transplantation (not shown). The conditioning regimen including TBI plus two drugs was adopted after results from the Dana Farber Institute.[10] No patient died of transplant-related toxicity. Despite the fact that most failures after autologous transplantation are due to relapses, strikingly only one of our 11 patients relapsed; anyway since the longer the duration of CR1, the later a subsequent relapse may occur,[30,31] a long-term follow-up is needed to assess results and conclusions must be cautious, due to the small sample.

The time from diagnosis to relapse and the site of relapse, as well as unfavorable translocations and immunophenotypes, are among main prognostic factors for EFS, and are usually adopted as the basis for relapsed patient stratification.[4,30] Most relapsed patients are treated with hematopoietic cell transplantation; when a suitable related donor is not available, URD (either cord or volunteer), or autologous transplantation can be adopted.[5–7] The still high TRM after URDT is counterbalanced by the absence of valid alternatives in early relapsed ALL, while in late and isolated relapses a high TRM seems less acceptable, as even chemotherapy reports an acceptable outcome, ranging from 30–40% in medullary or combined relapses, to 40–70% in extramedullary relapses.[2–4,31–40] Whether a child with ALL achieving CR2 after late relapse, and lacking a suitable donor, should receive chemotherapy with or without autologous graft remains controversial. Autologous transplantation, allowing earlier discontinuation of treatment, but causing more worrying late effects, so far did not seem to significantly improve chemotherapy outcomes, as shown in the retrospective matched paired analyses conducted in 1995 by the BFM group.[36] Pediatric autologous transplantation within AIEOP reported in 1995 a 3-year EFS of 49% for 12 patients with late relapse, regardless of site, and in 1998 an 8-year DFS of 24% for 40 patients with late marrow relapse and of 69% for 33 patients with extramedullary relapse,

regardless of time, with purging performed in 64% of the patients.[18,37] Although the efficacy of purging procedures, either pharmacologic or, more recently, immunologic, has never been tested in prospective randomized trials, its role in reducing the relapse rate is suggested by some reports, dealing with autologous grafts immunologically purged with anti-CD10 and anti-CD19 and complement.[41] In 1993, Billet *et al*[10] reported a 3-year EFS of 53% ± 7% for 51 patients relapsed later than 24 months after diagnosis, and Maldonado *et al*[42] in the Spanish group reported a 6-year EFS of 56% for 23 late marrow-relapsed patients. Concerning URDT as a possible alternative to autografting in ALL, the comparative analysis by Weisdorf *et al*[5] showed no superiority between the two procedures. The ultimate efficacy of novel approaches for chemotherapy and the two transplantation techniques remains uncertain, since retrospective studies, not addressing factors influencing patients' allocation to either procedure, are not conclusive in terms of relapse reduction for chemotherapy or autologous transplantation, TRM reduction for URDT, and final EFS assessment.

In conclusion, the proposed experimental protocol achieved its primary aim of demonstrating the feasibility and safety of the purification procedure in pediatric B cell precursor ALL in CR2 after late relapse. Moreover the efficacy of *in vitro* leukemia eradication was assessed by molecular analyses. In terms of EFS, the ultimate outcome of this small series is very promising, when compared to historical estimates. Besides the graft purification, to avoid the contribution to subsequent relapses caused by residual disease in the patient, future perspectives will deal with stem cells as possible vehicles for gene transfer and post-transplant immunologic therapy for autologous recipients to generate an antileukemic effect and possibly extend eligibility for autologous transplantation to early relapsed ALL.

Acknowledgements

The authors wish to thank the 'Comitato Maria Letizia Verga per lo studio e la cura della leucemia del bambino' and the 'Fondazione Tettamanti' for their continuous support, Miss Sara Vaghi for secretarial assistance and Miss Joanna Upton for linguistic consultancy. This work was partially supported by grants from the Associazione Italiana Ricerca sul Cancro (AIRC) and Ministero Università Ricerca Scientifica e Tecnologica (MURST) (to AB).

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