Table 3. Phase two and phase two(a) PT test results.

	Phase two		*Phase two(a)*	
	Glass tubes BD Vacutainer®	Pet tubes Venoject® II exp 8/97	Glass tubes BD Vacutainer®	Pet tubes Venoject® II exp 8/97+30 days
Patients	52		18	
Average(s)	31.08	40.26	33.58	42.68
Difference	–9.2		–9.10	
±2 CV units	29.03-33.13		31.36-35.79	
t test	< 0.05		< 0.05	
Regression line	y = 0.65× + 5		y = 1.48× – 7.15	
r^2	0.91		0.99	

3. Phase one(a), performed in August 1997, compared Glass Tubes BD Vacutainer® with PET Tube Terumo Venoject® II expiring 10/98 but kept thirty days out of their pouch, which was the upper time limit recommended by the manufacturer. The PT tests results are summarized in Table 2.
4. Phase two(a), performed in August 1997, compared Glass Tubes BD Vacutainer® with PET Tube Terumo Venoject® II expiring in the same month and kept thirty days out of their pouch (since July). The PT tests results are summarized in Table 3.

In all the phases of the study the PET Tube Terumo Venoject® II gave longer values than Glass Tubes BD Vacutainer®, whatever the *age-end of storage* or storage condition. Our data showed that the difference was statistically significant and out of the 2CV limits (Tables 2 and 3). The difference was between 3 and 9 seconds which represents a difference of 10 to 30% from the glass tubes. Many variables have been reported to influence PT test results. Citrate concentration (3.2 vs 3.8%) has an impact which seems to be thromboplastin dependent.[2-5] Moreover both the temperature at which the sample is kept and the tube material influence the PT test.[6-8] In our study the main preanalytical variable was the tube material and its manufacturing process. Based on our data, further study is warranted because of the need for standardization in this field.

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Key words

Prothrombin time, oral anticoagulants, PET tubes, glass tubes

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Allogeneic peripheral blood stem cell transplantation in children with hematologic malignancies

Sir,

Over the past few years transplantation of allogeneic cytokine-mobilized peripheral blood stem cells (PBSCs) has increasingly been used instead of bone marrow to allow hemopoietic reconstitution after myeloablative therapy for hematologic malignancies.[1] Although available data do not indicate that a short course of granulocyte colony-stimulating factors (G-CSF) may cause untoward long-term effects, there is a theoretical concern about this issue. This fear has precluded routine use of PBSCs when the

donor is a minor. In this study, we analyzed the preliminary results of unmanipulated allogeneic PBSC transplantation performed in 15 children with hematologic malignancies as reported to the Italian Association for Pediatric Hematology and Oncology (AIEOP) - BMT registry.

From February 1995 to December 1998, 15 children (11 males and 4 females) aged 1-18 years (median 9 years) with hematologic malignancies received an allogeneic PBSC transplantation in 5 Italian AIEOP-BMT centers. Clinical and transplant details of the patients grafted with allogeneic PBSCs are reported in Table 1. Fifteen healthy donors aged 11 to 42 years (median 33 years) underwent G-CSF mobilization; 2 of them were younger than 18 years. Five donors were HLA-identical siblings, 8 HLA partially-matched fam-

Table 1. Characteristics and outcome of patients transplanted with allogeneic PBPCs.

No.	*Patient's sex/age*	*Donor's sex/age*	*Patient/donor weight (kg)*	*Diagnosis and disease status*	*Preparative regimens*	*Donor relationship*	*GvHD prophylaxis*	*Acute GvHD*	*Chronic GvHD*	*Outcome*
1	M/17	F/20	53/81	NHL - 2nd CR	BU (16 mg/kg) Thio (10 mg/kg) CTX (120 mg/kg)	HLA-id sibling	CSA	I	NA	Dead, d +127 (disease)
2	M/8	M/35	23/60	Common ALL, 2nd CR	VCR (4 mg/m^2) TBI 1,200 cGy CTX (120 mg/kg)	Phenotypically-id relative	CSA	IV	NA	Dead , d +47 (GvHD, ARDS)
3	F/16	M/24	90/80	Common ALL, 2nd CR	TBI 1200 cGy VP16 (60 mg/kg) CTX (100 mg/kg)	HLA-id sibling	CSA-MTX	III	Extensive	Alive, d +925
4	M/4	M/41	27/90	Hybrid-ALL, relapse	Thio (15 mg/kg) CTX (150 mg/kg)	1 Ag mm relative	CSA-MTX	III	NO	Dead, d +124 (disease)
5	M/9	F/38	24/85	NHL, 2nd CR	TBI 1,200 cGy VP16 (60 mg/kg) CTX (100 mg/kg)	1 Ag mm relative	CSA-MTX-PDN	III	NO	Alive, d +780
6	M/6	M/33	17/78	AML-M4, 1st CR	TBI 1,200 cGy Mel (140 mg/m^2)	1 Ag mm relative	CSA-MTX	I	NO	Alive, d +1517
7	M/10	M/42	52/87	NHL – relapse	TBI 1,200 cGy VP16 (60 mg/kg) CTX (100 mg/kg)	1 Ag mm relative	CSA-MTX-PDN	I	NA	Dead, d +25 (ARDS)
8	M/10	M/41	37/67	T-ALL, relapse	Thio (15 mg/kg) CTX (120 mg/kg)	1 Ag mm relative	CSA-MTX-ATG	III	NA	Dead, d +26 (MOF)
9	M/2	M/17	8/58	MDS	BU (16 mg/kg) CTX (120 mg/kg) Mel (120 mg/m^2)	HLA-id sibling	CSA	0	NA	Dead, d +69 (pulmonary infection)
10	M/6	M/20	25/68	T-ALL, relapse	TBI 1,200 cGy BU (4 mg/kg) Thio (10 mg/kg) CTX (120 mg/kg)	1 Ag mm relative	CSA-MTX	IV	NA	Dead, d +24 (GvHD)
11	M/13	F/11	38/45	Common ALL, relapse	BU (16 mg/kg) Mel (140 mg/m^2)	HLA-id sibling	MTX	NA	NA	Dead, d +9 (fungal infection)
12	F/7	F/35	30/82	Pre-B ALL, 3rd CR	TBI 1,200 cGy Thio (10 mg/kg) CTX (120 mg/kg)	MUD	CSA-MTX	II	Limited	Alive, d +689
13	F/1	F/29	8/78	MDS	Thio (8 mg/kg) Flud (90 mg/m^2) Mel (70 mg/m^2)	MUD	CSA-MTX-ATG	0	Limited	Alive, d +316
14	M/18	F/42	60/65	AML-M2, relapse	BU (8 mg/kg) Thio (15 mg/kg) Flud (120 mg/m^2)	1 Ag mm relative	CSA-MTX-Campath 1G	0	NO	Alive, d +119
15	F/16	M/25	96/102	Common ALL, relapse	BU (16 mg/kg) Thio (10 mg/kg) CTX (120 mg/kg)	HLA-id sibling	CSA	0	NA	Alive, d +92

Abbreviations: M, male; F, female; AML, acute myelogenous leukemia; ALL, acute lymphoblastic leukemia; MDS, myelodysplastic syndrome; NHL, non-Hodgkin's lymphoma; CR, complete remission; BU, busulphan; Thio, thiotepa; CTX, cyclophosphamide; TBI, total body irradiation; VCR, vincristine; VP-16, etoposide; Mel, melphalan; Flud, fludarabine; Ag mm, antigen(s) mismatched; MUD, matched unrelated donor; CSA, cyclosporine; MTX, methotrexate; PDN, prednisone; ATG, antithymocyte globulin; NA, not applicable; ARDS, adult respiratory distress syndrome; MOF, multiorgan failure.

ily members and 2 unrelated volunteers. All donors received G-CSF at doses between 10 and 12 µg/kg/day for 4-7 days. Leukapheresis was performed with a Fenwall CS3000 (Baxter, Deerfield, IL, USA) cell separator. One to 3 leukapheresis procedures yielded a median of 14.4×10^8 nucleated cells/kg of recipient body weight (BW), containing between 3.2 and 62.9×10^6 $CD34^+$ cells/kg BW (median 10.4), 9 to 284.2×10^4 CFU-GM/kg BW (median 74) and 1.9 to 17.6×10^8 $CD3^+$ cells/kg BW (median 3.7), (see Table 2 for further details). Eight recipients received products that had been frozen and thawed before infusion. Mobilization and leukapheresis procedures were generally tolerated well. Mild bone pain was reported by the 2 pediatric donors. The adult donors had various complaints, including mild to moderate bone pain (n=10), headache (n=5) and general fatigue (n=3); 1 donor experienced fever. A transient moderate increase of lactate dehydrogenase and alkaline phosphatase was observed in 1 pediatric donor and 3 adults.

Fourteen patients engrafted and the median time to an absolute neutrophil count (ANC) greater than 0.5×10^9/L was12 days (range 10-18 days). One patient who died soon (day +9) after transplantation, failed to reach this threshold. An unsupported platelet count of 50×10^9/L was achieved by 10 out of 15 patients in a median of 15 days (range 11-50 days). All surviving patients had complete hematologic chimerism as documented by DNA analysis.

Grade II-IV acute graft-versus-host disease (GvHD) occurred in 7 patients (50%). In 7 evaluable patients, chronic GvHD was scored as absent in 4 children, limited in 2 and extensive in 1 patient.

Toxic complications of transplantation were evaluated according to Bearman's score.[2] All patients but two (86%) developed grade II-IV regimen-related toxicity (RRT) in at least one organ system and 5 (33%) patients had life-threatening or fatal toxicity (grades III-IV RRT). The median time to discharge was day +33 (range 9-78 days). Six of the 15 (40%) patients died of transplant-related complications; 2 patients relapsed 64 and 101 days post-transplant respectively, and, subsequently, died of disease progression. As of March 1999, 7 patients are alive in continued complete remission between 92 and 1517 days post-transplant (median follow-up 689 days, see Table 1).

Even though several studies have reported on the use of allogeneic PBSC transplantation in large series of adult patients,[3,4] few clinical data are available on this procedure in children.[5-8] In this regard, our findings confirm the reluctance of many transplant centers to consider PBSC mobilization when the donor is a minor. In fact, only 2 of the 15 donors were younger than 18 years, the remainder being adult family members or unrelated volunteer donors.

The theoretical possibility that acute GvHD could occur in PBSC transplant recipients with greater incidence and severity than after marrow transplantation has not been confirmed in clinical studies.[3] It has been postulated that acute GvHD following PBSC transplantation may be related to the genetic disparity between donor and recipient rather than to the number of infused T cells. In keeping with these observations, it is tempting to speculate that the high incidence of acute GvHD grades II to IV (50%) observed in our series may be related to the fact that 10 of the 15 patients (67%) were grafted from partially matched family donors or unrelated donors, whereas the number of infused T cells was similar to the other adult PBSC series. Transplant-related mortality and severe RRT occurred at a very high frequency in our population (40% and 33% respectively) compared to in other adult or paediatric PBSC cohorts.[4,5] However, these rates are similar to those reported using partially matched related donor transplants or matched unrelated donors.[9] Approximately half of patients are alive disease free, and only 2 patients have relapsed. Follow-up of our study group is too limited to draw definitive conclusions about the efficacy of the procedure to cure the underlying disease. However, our results are encouraging if we consider that two thirds of patients were grafted with advanced diseases or received transplants from donors other than HLA-identical siblings.

To conclude, despite the limited number of patients, these preliminary results indicate that allogeneic PBSC transplantation is feasible in children and may be considered as an alternative to bone marrow for allograft also in pediatric patients. Further prospective controlled studies are needed to evaluate

Table 2. Stem-cell product characteristics.

No.	G-CSF	Aph no.	Aph vol.	Yield of peripheral blood leukapheresis N cells $\times10^8$/kg	$CD34^+$ $\times10^8$/kg	CFU-GM $\times10^8$/kg	$CD3^+$ $\times10^8$/kg
1	10	1	14	17.6	9.1	284.27	2.21
2	10	3	4	28.34[a]	18.8[b]	25.0[c]	17.67[d]
3	10	2	6	10.10	8.6	234.71	3.78
4	10	2	6	14.45	20.44	83.0	6.54
5	10	2	5	17.44	12.58	70.10	5.41
6	10	2	6	40.61[e]	62.97[f]	90.0[g]	17.0[h]
7	10	2	6	9.23	10.4	12.5	2.45
8	12	1	ND	26.9	28.0	ND	6.54
9	10	1	5	18.7	19.5	9.0	9.2
10	12	1	8	14.0	3.2	80.0	2.8
11	10	1	7	8.0	7.0	30.0	1.9
12	12	1	10	10.8	4.6	78.0	2.7
13	10	1	7	12.5	25.0	65.0	3.0
14	12	1	10	8.0	6.9	ND	2.3
15	12	2	10	18.2	25.0	65.0	3.0

Abbreviation: Aph no.: no. of apheresis procedures; Aph vol: total apheresis volume (L); N cells: nucleated cells; ND, not determined.
[a]Nucleated cells transplanted ($\times10^8$/kg): 11.1. [b]$CD34^+$ cells transplanted ($\times10^6$/kg): 10.8. [c]CFU-GM transplanted ($\times10^4$/kg): ND. [d]$CD3^+$ cells transplanted ($\times10^8$/kg): 7.1. [e]Nucleated cells transplanted ($\times10^8$/kg): 15.5. [f]$CD34^+$ cells transplanted ($\times10^6$/kg): 21.8. [g]CFU-GM transplanted ($\times10^4$/kg): 50.0. [h]CD3+ cells transplanted ($\times10^8$/kg): 7.54.

whether the advantages of fast engraftment and avoidance of general anesthesia outweigh the theoretical risk of donor leukemogenesis related to a short course of G-CSF and whether PBSC transplant offers advantages in terms of clinical outcome over marrow transplantation in children with leukemia.

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Key words

Peripheral blood stem cells, allogeneic transplantation in children, G-CSF priming

Appendix

Patients' data for this report were contributed by the following institutes and physicians: Department of Pediatrics, University of Turin (R. Miniero, A. Busca); Department of Pediatrics, IRCCS Policlinico S. Matteo, University of Pavia (F. Locatelli, F. Bonetti); Department of Pediatrics, University of Bologna (A. Pession, R. Rondelli); Department of Pediatrics, University of Milan, Monza (C. Uderzo, A. Balduzzi); Department of Pediatrics, Ospedale Pausilipon, Naples (M. Ripaldi).

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4.2 Nippon mutation in a non-Japanese patient with hereditary spherocytosis

Sir,

The erythrocyte protein 4.2 (P4.2) is a major component in the erythrocyte skeletal network that participates directly in band 3-cytoskeleton linkage.[1,2] The main isoform has an apparent molecular weight of 72 kD on sodium dodecylsulphate polyacrylamide gel electrophoresis (SDS-PAGE). P4.2 absence has seldom been observed in hereditary spherocytosis (HS).[3] Since the first description in 1974, most HS patients with absent red blood cell P4.2 have been found in Japan. A band 4.2 gene (*EPB4.2*) point mutation (GCT→ACT: Ala→Thr at codon 142) defining the Nippon allele has frequently been shown.[4] This occurs in the heterozygous state in around 3% of healthy subjects and is specific to the Japanese population.[5] We report the first case of HS associated with 4.2 Nippon mutation in a non-Japanese patient.

The proband (a 30-year-old female) was born in a small mountain village in central Italy. Her family has no Japanese ancestry and have lived there for several generations. Although consanguinity between parents cannot be formally demonstrated, it is strongly suspected. The proband showed splenomegaly and had suffered from moderate hemolytic anemia since birth. The pink test, an osmotic fragility test, was increased. Based on these findings, moderate HS was diagnosed (Figure 1). Her parents and siblings were clinically and hematologically normal (Figure 1). Red cell P4.2 was completely missing in the proband on SDS-PAGE (Figure 2A).[6] Linkage analysis eliminated ankyrin and band 3 genes as candidate genes.[7,8] The thirteen exons of the EPB4.2 gene were amplified by PCR using primers reported by Takaoka *et al.*[9] and submitted to single-strand conformational polymorphism (SSCP) analysis. In the proband, nucleotide sequence analysis of the *EPB4.2* exon 3 revealed a single base substitution at codon 142 (GCT→ACT: Ala→Thr) in the homozygous state. This mutation defines the Nippon allele. Digestion with *Hga* I confirmed the mutation in the proband and demonstrated that the parents and a sister were heterozygotes. Western blot analysis of red cell P4.2 revealed, as reported in the Japanese patients, a minimal trace of an immunoreactive P4.2 doublet of 74 and 72 kD when a large number of proteins were loaded onto SDS-PAGE (Figure 2B). Protein analysis in the other family members was normal except for the presence of a 74 kD peptide on Western blotting in the parents