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Administration of erythopoietin and granulocyte colony-stimulating factor in donor/recipient pairs to collect peripheral blood progenitor cells (PBPC) and red blood cell units for use in the recipient after allogeneic PBPC transplantation

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Background and Objectives. It may be useful to reduce the exposure of transplant recipients to homologous blood. This may be achieved by procuring donor-derived red blood cell (RBC) units, collecting more peripheral blood progenitor cells (PBPC) with a combination of granulocyte colony-stimulating factor (G-CSF) + recombinant human erythropoietin (rHuEpo) and by administering rHuEpo post-transplantation.

Design and Methods. Eight ABO-compatible donors were treated with rHuEpo and intravenous iron to collect 12 RBC units for use in their recipients. PBPC were collected after mobilization with rHuEpo and G-CSF in the same donors. The recipients received G-CSF and rHuEpo post-transplantation. A control group of 10 donor/recipient pairs received G-CSF alone for PBPC mobilization and after the transplantation.

Results. Eighty-six out of 91 planned RBC units were collected in the donors without significant decrease in hematocrit because of a 4-fold increase in RBC production despite functional iron deficiency. After 2 leukaphereses, the cumulative yields of NC and CFU-GM were lower in the study group while those of BFU-E, CFU-Mix and CD34+ cells were similar. However, erythroid recovery was significantly accelerated in the study group.

Interpretation and Conclusions. Collection of 12 RBC units within 6 weeks is feasible with rHuEpo and intravenous iron; this strategy allows a dramatic reduction in recipient exposure to homologous blood; rHuEpo has no synergistic effect with G-CSF for mobilization of PBPC in normal donors and may even be deleterious; and rHuEpo in the recipient may enhance erythroid engraftment.

Key words: allogeneic hematopoietic stem cell transplantation, peripheral blood stem cells, RBC transfusion, erythropoietin, iron

The first series of allogeneic transplantation with peripheral blood progenitor cells (allo-PBPCT) were reported in 1995[1-3] and since then this procedure has become widely available. In comparison with allogeneic bone marrow transplantation (allo-BMT), allo-PBSCT is associated with faster hematologic recovery, reduced transfusion requirements, shorter hospital stay, and comparable incidences of acute graft-versus-host disease (aGVHD), transplant-related mortality and survival[4-6] although concerns have been raised about the trend towards an increased incidence of chronic GVHD (cGVHD).[5,6] Priming of PBPC in normal donors has been mainly achieved with filgrastim at a dose of 10 µg/kg/day[7] but there are indications that lenograstim mobilizes progenitors more efficiently than filgrastim on a weight-by-weight basis.[8,9]

Recombinant human erythropoietin (rHuEpo) used alone can induce a modest increase of progenitor cells in the peripheral blood of lymphoma patients[10] and autologous transplants have been performed with PBPC primed with rHuEpo alone although up to 14 leukaphereses were needed to reach the target of 6.5×10^8 mononuclear cells/kg.[11] Progenitor mobilization was enhanced by the association of G-CSF + rHuEpo compared to G-

CSF alone in a historical group, suggesting a synergy between the two growth factors in the autologous setting.[12] The apparent synergism between G-CSF and rHuEpo has been confirmed in a randomized trial of PBPC mobilization in women with ovarian carcinoma.[13] However, rHuEpo has never been used for allo-PBPC mobilization either alone or in association with G-CSF.

After allo-BMT, rHuEpo accelerates erythroid engraftment and produces some reduction in red blood cell (RBC) transfusion needs[14-20] but such an impact has not been shown after autologous transplantation whether rHuEpo is used alone[17,18,21] or in association with G- or GM-CSF.[22-24] rHuEpo has never been used in the setting of allo-PBPCT.

Collection and storage of autologous RBC is feasible before elective orthopedic or cardiac surgery. Randomized placebo-controlled trials have shown that rHuEpo increases the ability of such patients to donate up to 6 U of autologous blood within 3 weeks[25] or up to 5 U within 2 weeks.[26] Collection of up to 10 RBC units over a 5-week period was attempted with rHuEpo stimulation in bone marrow donors but only 18% of them were able to complete this program.[27] Nevertheless, the availability of these donor-derived RBC units decreased homologous blood needs after allogeneic BMT. These results have generally been obtained with oral iron supplements, but in one study, intravenous (i.v.) iron was shown to be superior to oral iron, at least in relatively anemic patients.[28] This superiority of i.v. over oral iron has also been demonstrated in dialysis or pre-dialysis patients treated with rHuEpo.[29-31] It is not known whether the use of i.v. iron would allow an intensive phlebotomy program over several weeks.

We, therefore, designed a prospective study of rHuEpo therapy in pairs of PBPC donors and their recipients. rHuEpo was given to the donor to facilitate blood donation and to increase PBPC mobilization, and to the recipient in order to accelerate erythroid engraftment. The aims of our study were: i) to evaluate the feasibility of collecting up to 12 autologous RBC units within 6 weeks with the use of rHuEpo and i.v. iron supplementation; ii) to investigate a possible additive or synergistic effect of rHuEpo and G-CSF on the mobilization of PBPC in normal donors; iii) to examine the possibility of avoiding post-transplantation exposure of the recipient to homologous blood; and iv) to study the effect of combined treatment with rHuEpo and G-CSF on engraftment of allogeneic PBPC and on the number of platelet and RBC transfusions.

Table 1. Patients' characteristics.

	Study group	*Control group*
Number of patients	8	10
Age (years) [median (range)]	45 (8-58)	34 (14-56)
Male/female	6 / 2	5/5
Diagnosis		
AML	5	1
CR1	3	1
refractory	2	0
MDS	0	3
RA-S	-	1
RAEB	-	2
CML (chronic phase)	1	2
NHL	1	2
CR2	0	1
PR1	1	0
PR2	0	1
SAA	1	1
Neuroblastoma	0	1

CR = complete remission; PR = partial remission; AML = acute myelogenous leukemia; CML = chronic myelogenous leukemia; MDS = myelodysplastic syndrome; RA-S = refractory anemia with ringed sideroblasts; RAEB = refractory anemia with excess of blasts; NHL = non-Hodgkin's lymphoma; SAA = severe aplastic anemia

Design and Methods

Patients and donors

Patient and donor pairs were included in the study group if they fulfilled the following inclusion criteria: (i) patient eligible for an allogeneic transplant by generally accepted criteria; (ii) family donor eligible for stem cell donation by leukapheresis; (iii) donor older than 18 years; (iv) donor available for an intensive program of autologous blood donation; (v) patient and donor ABO-compatible for donor red cell transfusions; (vi) informed consent signed by donor and patient. The protocol was approved by the Ethical Committee of the University of Liège. Patients treated in the same period but not included into the protocol were offered a standard PBPCT (control group). The patients' details are given in Table 1 and the donors' characteristics are displayed in Table 2. Patients with acute myeloid leukemia (AML) were in first complete remission except 2 patients in the study group with primary refractory disease.

Study design

Donor treatment. In the study group (n=8), donors were administered rHuEpo (Eprex®, kindly provided by Janssen-Cilag, Beerse, Belgium) subcutaneously at a dose of 600 U/kg twice weekly

Table 2. Donors' characteristics.

	Study group	*Control group*
Number	8	10
Male/female	4 / 4	8 / 2
Relationship to patient		
Sibling	5	10
Parent or child	3	0
HLA matching		
Identical	6	9
1 mismatch	2	1
ABO matching		
Match	7	4
Minor mismatch	1	1
Major mismatch	0	4
Major and minor mismatch	0	1
Age* (years)	35 (29-48)	41 (16-57)
Weight* (kg)	78 (52-119)	70 (52-122)
Hemoglobin* (g/dL)	14.4 (12.7-15.2)	13.1 (12.5-16.6)
Hematocrit* (%)	42.6 (38.5-45.2)	40.2 (36.1-53.5)
WBC* (x 10^9/L)	7.7 (4.2-10.0)	5.9 (3.2-12.7)
PLT* (x 10^9/L)	281 (171-371)	206 (156-326)

**Values are given as median (range). There is no statistically significant difference between the two groups.*

for 3 weeks before and 3 weeks after the transplant, except during the week of G-CSF administration (days –5 through –1) when the dose was 600 U/kg daily (total 15 doses). These donors also received 200 mg i.v. iron (Venofer®, Vifor, St. Gallen, Switzerland), given in 500 mL saline to compensate for blood donation, twice weekly for 3 weeks before and 3 weeks after the transplant. RBC units were collected at a rate of 1 unit twice weekly for 3 weeks before and 3 weeks after the transplant (total 12 units) if the hematocrit was ≥ 33%. In the control group (n=10), no rHuEpo was given and no RBC collection was done. In both groups allogeneic PBPC were primed with glycosylated rHuG-CSF (Granocyte®, kindly provided by Rhône-Poulenc-Rorer, Montrouge, France) 10 mg/kg/day subcutaneously from day –5 through day –1. Donors were followed up for 3 weeks following cessation of all procedures.

Patient treatment. Patients in the study group were treated with 200 U/kg/d i.v. rHuEpo until they reached an unsupported hematocrit ≥ 30% or for a maximum of 49 days. No iron supplements were given. Glycosylated rHuG-CSF (Granocyte®) was also administered intravenously at a dose of 5 µg/kg/d from day 1 until neutrophils were above 1×10^9/L for 3 consecutive days or above 10×10^9/L for 1 day. Patients in the control group were given glycosylated rHuG-CSF but did not receive rHuEpo. For patients with myeloid malignancies, Granocyte® was kindly provided by Rhône-Poulenc-Rorer.

Clinical care

All blood products were irradiated. Single-donor platelet transfusions were given if platelet counts decreased below 15×10^9/L and packed red blood cells when the hemoglobin level decreased below 9 g/dL. GVHD prophylaxis was carried out with cyclosporine with (n=15) or without (n=3) short methotrexate. Acute and chronic GVHD were diagnosed and graded according to the Seattle criteria. Acute GVHD was treated with corticosteroids.

Leukaphereses

PBPC were collected using the blood cell separator CS3000+ (Baxter-Fenwall Laboratories, Deerfield, IL, USA) or Spectra (Cobe BCT, Lakewood, CO, USA). Twelve liters of blood were processed per apheresis. Collections were performed on days -1 and 0. The harvest product obtained on day -1 was stored overnight at room temperature and both products were reinfused on day 0 through a double lumen Hickman catheter. In a few cases, 1 or 2 additional leukaphereses had to be performed and these cells were reinfused on the day of collection.

Laboratory analyses

CFC assays. Hematopoietic progenitors were grown in 0.9% methylcellulose in a commercially available medium (H4433, Terry Fox Laboratory, Vancouver, BC, Canada). Cultures were incubated at 37°C in a humidified 5% CO_2 atmosphere. Formation of colony-forming unit-granulocyte-macrophage (CFU-GM), burst-forming unit-erythroid (BFU-E) and colonies of multiple lineages (CFU-Mix) were scored on day 14.

Flow cytometry. Aliquots were incubated with phycoerythrin-conjugated monoclonal anti-CD34 (HPCA2, Becton-Dickinson, Palo Alto, CA, USA) for 20 min at 20°C, washed and fixed with 1% formaldehyde. A total of 1×10^5 cells was analyzed using a FACScan analyzer (Becton-Dickinson). The percentage of $CD34^+$ cells was defined with dot plot analysis using the whole nucleated cell population and the percentage of $CD34^+$ cells in the isotype control was subtracted to give the final percentage of $CD34^+$ cells. Data acquisition was performed with Cellquest software (Becton-Dickinson).

Miscellaneous analyses. Complete blood counts were determined in a Technicon H3 cell counter

(Bayer, Tarrytown, NJ, USA). Serum iron, total iron binding capacity (TIBC), transferrin saturation and serum ferritin were measured by standard methods. Serum soluble transferrin receptor (sTfR), a quantitative assay of erythropoietic activity, was assessed by ELISA as previously described.[32]

RBC production in the donor

The blood volume (BV) was estimated at baseline as the sum of the plasma volume (PV) and red cell mass (RCM) calculated from the following formulae based on the body surface area (BSA in m^2) and age (in years): PV (mL) = 1578 × BSA in men or 1395 × BSA in women; RCM (mL) = (1486 × BSA) - 825 in men or (1.06 × age) + (822 × BSA) in women.[33] The blood volume was assumed to remain constant and the RCM during treatment was calculated as follows: RCM (mL) = BV × Hct (in %) x 0.92/100. The production of red cells between 2 visits was therefore derived from the following formula: RBC production (mL) = (RCM at visit 2 – RCM at visit 1) + RC volume removed at visit 1. The latter always included the volume of red cells in the collected unit plus the volume of blood drawn for laboratory analyses.

Statistical analyses

Comparisons between the two groups were carried out with either Mann-Whitney U-tests or unpaired t-tests, usually after log transformation because of skewed distribution of the data. Times to hematopoietic recovery were studied by life table analyses and Wilcoxon rank tests were used to compare the two groups. This included times for neutrophils to reach 0.5, 1 or 2×10^9/L, platelets to reach 20, 25, 50, 100 or 150×10^9/L, hematocrit to reach 27%, 30% or a normal value, and reticulocytes to reach 0.5, 1 or 2%. Statistical analyses were done using Microsoft Excel (Microsoft Corp., Redmond, WA, USA) and Graphpad Prism (Graphpad Software, San Diego, CA, USA).

Results

In the donor

Tolerance and safety. Lenograstim injections were well tolerated with only mild bone pain in the majority of the donors, which resolved after paracetamol administration. One migrainous donor complained of headache during lenograstim administration. rHuEpo administration did not generate any significant side effects, and in particular no hypertension was noted. No serious adverse event was reported during or after the study period. Platelets decreased to $253\pm89\times10^9$/L in the study group and to $164\pm42\times10^9$/L in the control group after PBPC collections (p=0.0158) but recovered within one week.

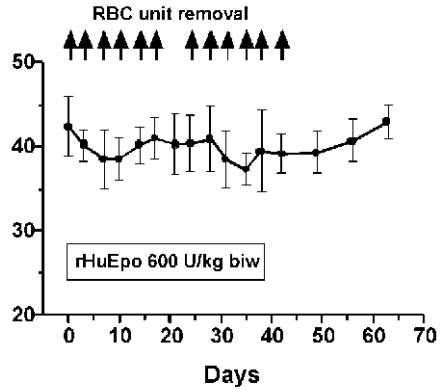

Figure 1. Hematocrit (mean ± SD) in the donor during and after rHuEpo stimulation.

Red blood cell collections. This study was designed to collect 12 RBC units from donors after stimulation with rHuEpo and i.v. iron supplementation. These units would then be available for the transplant recipient. In 4 donors the target was reached, 11 units were obtained from 1 donor, 10 units from 2 donors and 7 units from 1 donor. In this latter case, RBC collections were cancelled on day 3 post-transplant because of severe deterioration of the patient who died on day 4. Other reasons for cancelling RBC collections were a low hematocrit (<33 %) (n=3), headache (n=1) and the need for a 4th PBPC collection (n=1). We therefore managed to collect 86 of the expected 96 RBC units, or 86 out of 91 (95%) if those expected for the patient who died on day 4 are not taken into account.

Erythropoiesis. After a slight initial drop, donor hematocrit remained fairly stable during RBC collections and returned to baseline values within 3 weeks after the last phlebotomy (Figure 1). This stability despite intensive phlebotomies was achieved through a considerable expansion of erythropoietic activity, as illustrated by a more than 4-fold elevation in sTfR levels. Reticulocytes increased 5-fold during the first 10 days before decreasing progressively during the remaining course of rHuEpo therapy. After cessation of rHuEpo therapy, reticulocytes were even lower than at baseline, while sTfR levels had not normalized by 3 weeks. Daily RBC production was calculated as described

Table 3. Total PBPC collection yields after 2 leukaphereses (median [range]).

	Study group	*Control group*	*p*
NC $\times 10^8$/kg donor b.w.	4.83 [2.41-9.78]	9.57 [5.21-17.34]	0.0006
MNC $\times 10^8$/kg donor b.w.	4.80 [2.28-9.25]	9.37 [4.99-17.16]	0.0008
CFU-GM $\times 10^4$/kg donor b.w.	31.99 [8.13-80.45]	80.90 [33.00-322.68]	0.0164
BFU-E $\times 10^4$/kg donor b.w.	72.18 [21.35-138.69]	75.10 [10.78-242.39]	NS
CFU-Mix $\times 10^4$/kg donor b.w.	1.79 [0-20.11]	10.06 [0-33.63]	NS
CD34 $\times 10^6$/kg donor b.w.	4.79 [0.90-14.62]	10.75 [3.85-14.23]	NS

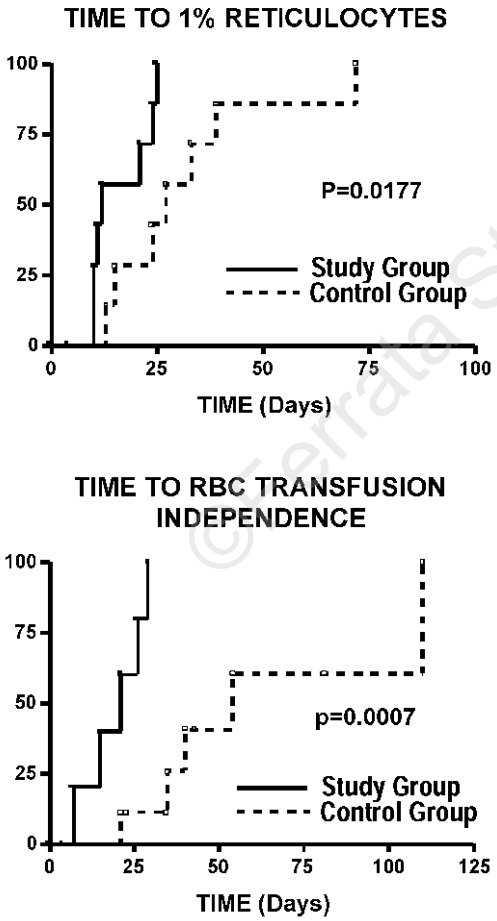

Figure 2. Erythroid engraftment in the recipient of the study group vs control group. A: Time to reach 1% reticulocytes. B: Time to RBC transfusion independence.

above. Compared to baseline (15±5 mL/d), there was an almost 4-fold increase in red cell production (63±8 mL/d) during the first 3-week period and this higher rate was maintained during the entire stimulation period. The considerable expansion of erythropoietic activity produced an enormous demand for iron and transferrin saturation rapidly dropped below 20% despite i.v. iron supplementation. Baseline ferritin ranged from 23 to 341 µg/L. Ferritin levels 3 weeks after cessation of rHuEpo and i.v. iron therapy (85±53 ng/mL) were not different from baseline values (97±106 ng/mL).

PBPC mobilization and collection. The administration of rHuEpo alone for 2.5 weeks was not associated with any significant increase of circulating white blood cells (WBC), CD34$^+$ cells or progenitor cell numbers. A steep rise of all these cell categories was observed after the start of G-CSF treatment. The concentrations of circulating CD34$^+$ cells, CFU-GM, BFU-E or CFU-Mix on the days of PBPC collections were not significantly different in the study group and the control group. The number of CD34$^+$ and progenitor cells in the PB tended to decrease below baseline (NS) 2 to 4 weeks after PBPC collections.

Leukaphereses were performed with the aim of collecting a minimum of 4×10^6 CD34$^+$ cells/kg of recipient body weight (b.w.). In the study group this target was reached after 1 leukapheresis in 1 donor, after 2 leukaphereses in 3 donors and after 3 leukaphereses in 2 donors. Four leukapheres were performed in 2 subjects for whom total yields reached only 1.62 and 3.64×10^6 cells/kg. In all but one donor at least 4×10^6 CD34$^+$ cells/kg donor b.w. were collected. In the control group the same goal was obtained after 1 leukapheresis in 1 donor, 2 leukaphereses in 7 donors, 3 leukaphereses in 1 donor and 4 leukaphereses in 1 donor. At least 4×10^6 CD34$^+$ cells/kg donor b.w. were procured from all donors. We compared the yields of the first 2 leukaphereses per kg of donor b.w. in the study group and control group (Table 3). Significant differences were found in the total yields of nucleated cells, mononucleated cells and CFU-GM with higher numbers obtained after priming with G-CSF alone, while BFU-E, CFU-Mix and CD34$^+$ cells yields were not statistically different.

In the recipient

Hematologic recovery post-transplant. WBC and platelet count recoveries were not statistically different between the control and study groups. Engraftment to an absolute neutrophil count (ANC) of 0.5×10^9/L was achieved after a median of 18

Table 4. RBC transfusion requirements post-transplant (median [range]).

Period (days)	*Study group*	*Control group*	*p*
Any origin			
0-56	12 [3-38]	13 [8-29]	NS
57-100	1 [0-17]	3 [0-11]	NS
Total	12 [4-39]	15 [9-33]	NS
Unrelated origin			
0-56	1 [0-28]	13 [8-29]	0.0185
57-100	0 [0-17]	3 [0-11]	NS
Total	2 [0-27]	15 [9-33]	0.026

Table 5. Origin of transfused red blood cells in the study group.

Patient	*No. of units collected*	*No. of units transfused (d 0-100)*	
		"Autologous"	*Unrelated*
1	12	12	27
2	12	4	0
3	11	11	3
4	12	12	4
5	10	10	28 *
6	10	9	0
7	7	°	°
8	12	10	0

**Until death on day 34. °Not evaluable because of early death on day 4 prior to any transfusion.*

days (range 9-21) in the study group and after a median of 20 days (10-25) in the control group. The median (range) time to an unsupported platelet count of 20×10^9/L was 28 days (12-116) in the study group vs 30 (12-49) for controls. The patient with the latest platelet recovery (day 116) was diagnosed as having post-transplant thrombocytopenic thrombotic purpura (TTP). RBC recovery was faster after transplantation of G-CSF + rHuEpo mobilized PBPC and administration of post-transplant rHuEpo than after transplantation of PBPC mobilized with G-CSF alone and no post-transplant rHuEpo. The median (range) times to ≥ 0.5% reticulocytes were 10 (7-24) vs 21 (12-37) days (p = 0.0323) and to ≥ 1% reticulocytes were 12 (10-25) vs 27 (13-72) days (p = 0.0177, Figure 2A) in the study group and control group, respectively. After excluding 3 patients who had severe bleeding or TTP in the early post-transplant period, RBC transfusion independence was achieved after a median of 21 (7-29) days in the study group vs 40 (21-110) days in the control group (p = 0.0007, Figure 2B).

Transfusion requirements. Comparative analysis of the 2 groups showed a trend towards fewer platelet transfusions being required in the study group. Between day 0 and day 30, a median (range) of 12 (4-31) vs 19 (3-24) platelet transfusions were required in the study and control groups, respectively (p=NS). There were no significant differences between the 2 groups in the total numbers of RBC units transfused either during or after the period of rHuEpo treatment (Table 4). However, the number of unrelated RBC units transfused was dramatically lower (2 vs 15, p = 0.026) in patients whose donors contributed to provide RBC. This difference was evident during the first 2 months after transplantation but the number of transfusions administered afterwards was low in both groups. Of the 7 evaluable patients in the study group, 3 received exclusively *autologous* RBC units and 4 were also transfused with homologous blood: these patients requiring homologous blood included 2 patients who received numerous transfusions because of prolonged severe gastrointestinal (GI) bleeding (Table 5). With the exception of these 2 patients, exposure to homologous blood was reduced by a median of 100% (73-100). Of the 86 units collected from the donors, 18 (21%) were not used.

Patients' outcome. One patient in the study group developed TTP with subsequent delay in recovery of RBC and platelet counts and another experienced severe GI bleeding because of grade i.v. acute GVHD. GVHD-associated severe GI bleeding was also encountered in one patient in the control group. Another patient in the control group developed TTP after full hematopoietic reconstitution. The incidence and severity of acute or chronic GVHD were not different between the two groups. Three patients in the study group died before day 100, one of vascular occlusive disease (VOD) and cardiac failure (day 4), one of acute GVHD (day 34) and one of lymphoma (day 67). Five control patients died before day 100. The causes of death were multiorgan failure (days 23 and 43), VOD (day 34), leukemia (day 59) and cerebral toxoplasmosis (day 81).

Discussion

We evaluated the concomitant use of rHuEpo and rHuG-CSF to mobilize allogeneic PBPC. Contrary to previous results in the autologous setting,[11] we did not obtain progenitor mobilization with rHuEpo alone in our donor population. Also contrary to previous observations of PBPC mobilization

after chemotherapy in cancer patients,[12,13] we did not observe any synergistic effect between rHuEpo and glycosylated rHuG-CSF. Rather, the combination of growth factors yielded similar numbers of BFU-E but lower numbers of NC, MNC and CFU-GM than priming with G-CSF alone. This combination had never been tested for allogeneic PBPC mobilization but it is likely that somewhat different cell populations are mobilized when growth factors are used alone in normal subjects or combined with chemotherapy in cancer patients. However, it is also possible that the preceding use of large doses of rHuEpo could somehow impair the subsequent capacity of growth factors to mobilize PBPC. We previously demonstrated that intensive treatment with rHuEpo was followed by a transient phase of profound inhibition of erythropoietic activity caused by erythroid marrow exhaustion.[34] This was also accompanied by the reduction of marrow and spleen CFU-GM. Whether the omission of previous rHuEpo therapy would restore the synergism between rHuEpo and G-CSF remains to be demonstrated.

Recombinant human erythropoietin increases the ability of patients to donate autologous RBC units[25,26] but the benefit is less evident in non-anemic patients[35] or those undergoing less intensive donation programs.[36] We present here the most intensive phlebotomy program so far published, with 12 RBC unit donations scheduled over 6 weeks, thus legitimizing the use of a high dose of rHuEpo. While the utilization of rHuEpo in normal donors before bone marrow collection has been reported[37,38] and appears safe, there is only one study, by Mitus *et al.* on the use of rHuEpo in allogeneic BMT donor/recipient pairs to facilitate collection of donor-derived RBC units available for use in the recipient after BMT.[27] Mitus *et al.* administered rHuEpo at a dose of 1,500 U/kg/wk and gave 300 mg elemental iron orally but were able to collect only a median of 6 units of RBC out of the 10 scheduled over 5 weeks. Only 2/11 donors managed to donate all the planned units while the remaining 9 gave between 4 and 8 units each, resulting in an overall success rate of only 65%. Administering a slightly lower dose of rHuEpo with i.v. iron supplementation, we were able to collect a median of 12 units of RBC and all donors provided at least 10 units, resulting in an overall success rate of 95% and only 3 units not taken because of low hematocrit. The lower RBC yield in Mitus' study is in part attributable to blood losses associated with bone marrow donation but to a larger extent is attributable to the relatively low iron status of several donors and the use of oral instead of i.v. iron. Indeed functional or absolute iron deficiency is the main cause of low response to rHuEpo and in renal failure[29-31] as well as in autologous blood donations[28] is best overcome by administration of i.v. iron rather than poorly absorbed oral iron. The efficiency of oral iron has even been questioned in autologous blood donors not receiving rHuEpo.[39] An identical program of rHuEpo 600 U/kg twice a week and collection of 6 RBC units over 3 weeks but with oral iron resulted in a mean rate of additional red blood cell production of 34 mL/day compared to 22 ml/day with placebo.[40] This 2.5-fold increase over basal erythropoiesis is much lower than the 4-fold increase over baseline and 48 mL/day of additional RBC production with our protocol. This implies that i.v. iron by itself has an impact that is comparable to that of rHuEpo in this setting.

Serum ferritin returned to baseline at the end of the period of observation, indicating that the dose of iron used (double that used in previous studies[28,36]) was appropriate for the additional demand for donated iron. In previous reports using oral[26] or i.v.[28,36] iron, ferritin was considerably decreased at the end of the iron supplementation period, so that it can be expected that it would further decrease after cessation of iron therapy. Therefore, only the doses given in our study are capable of maintaining iron stores, yet are not excessive so as to produce relative iron loading. Despite the apparent efficacy of i.v. iron in supporting rHuEpo-driven erythropoietic activity, transferrin saturation constantly below 20% is evidence that functional iron deficiency was limiting response to rHuEpo. It remains to be determined whether higher doses or different schedules of iron administration, e.g. total dose infusion at the beginning of the program, would correct this phenomenon. Despite this limitation, the hematocrit remained relatively stable throughout the phlebotomy program. This is in contrast with results from previous studies in which collection of only 6 units was associated with hematocrit decrements of 4 to 8%.[25,28,35] In a study of normal volunteers treated with oral iron and rHuEpo doses ranging from 750 to 3,000 U/kg/wk for 4 weeks and donating up to 11 units within 4 weeks, the hematocrit dropped by 10% and only 79% of the scheduled units were collected.[41]

Patients from both groups received G-CSF post-transplant and those in the study group were also treated with rHuEpo until an unsupported hematocrit ≥ 30% was reached. With the limitations of a small number of patients, we did not observe any

difference in post-transplant WBC and platelet recovery, but there was a trend towards fewer platelet transfusions being required in the study group. This is consistent with the observations of others who used rHuEpo without G-CSF after allogeneic BMT,[17,19,20,42] with some studies finding a favorable impact on platelet recovery and transfusion needs,[43] particularly with the association of rHuEpo and G-CSF.[44]

Erythroid recovery was accelerated in the study group, presumably in relation to the post-transplant rHuEpo administration (progenitor cell yields were similar in the 2 groups). Impaired erythropoietin response to anemia has been well documented in recipients of allogeneic BMT[45,46] and faster recovery of erythropoiesis and some reduction in RBC transfusion needs have been observed with the administration of rHuEpo.[14,17,19,20,42,43] Although time to RBC transfusion independence was shorter in the study group this was not associated with reduced transfusion requirements. It should be emphasized that 2 patients in the study group had exceptionally high RBC product consumption because of prolonged, severe bleeding. In addition, the trigger for RBC transfusions was relatively high compared to more recent practice. However, because of the large number of RBC units made available by the donors, exposure to homologous blood was significantly reduced in patients receiving these donor-derived RBC units, with 50% of evaluable patients not exposed to allogeneic RBC at all.

Our approach has several limitations. Administering i.v. iron, subcutaneous rHuEpo and G-CSF to a donor could theoretically increase the risk of cell donation, but we did not observe any side effects related to the erythropoietin or iron therapy. There are practical problems with keeping donors available for 6 weeks around the hospital and the inconvenience of almost 15 phlebotomy visits to the transfusion center is important. The logistic problems in organizing RBC collections in the donor may cause unwanted delays in the transplant procedure although this was not the case in our study. About 20% of RBC units collected were not used and this proportion could be even higher with the lower hemoglobin cut-off values currently used for transfusing RBC. The cost of rHuEpo and i.v. iron given to the donor as well as the cost of unused donor-derived RBC units add to the cost of standard allogeneic RBC transfusions, rendering the approach not cost-effective. The increased work load, cost and inconvenience to the donor may, therefore outweigh the benefits of the approach in practice.

Contributions and Acknowledgments

YB designed the study and wrote the paper. BS analyzed the data and wrote the paper. EB took care of the donors and collected the PBSC and RBC units. J-PS and SM collected and analyzed the data. MP co-ordinated the study. NS-L and J-MP performed the laboratory analyses on PBSC and RBC products. J-PS, GF and YB took care of the patients.

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Potential implications for clinical practice

The use of i.v. iron and rHuEpo makes collection of large numbers of autologous RBC units quite feasible. rHuEpo does not synergize with G-CSF for mobilization of PBPC in normal donors.

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