A boost of CD34+-selected peripheral blood cells without further conditioning in patients with poor graft function following allogeneic stem cell transplantation

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Background and Objectives. A proportion of patients develop poor graft function (PGF) following an allogeneic hemopoietic stem cell transplant (HSCT). It is uncertain whether a boost of donor marrow or blood cells is beneficial in terms of trilineage recovery and non-relapse-related mortality (NRM).

Design and Methods. The aim of this study was to compare outcomes in patients with PGF and full donor chimerism following an allogeneic HSCT who did or did not receive a boost of donor stem cells. The study included patients with primary PGF - i.e. those failing to achieve sustained graft function- and secondary PGF - i.e. those developing PGF after complete hematologic recovery. We studied 54 patients with PGF: 20 patients received no further donor cell infusion (group A), 14 received a boost of unmanipulated marrow or blood cells from the original donor, without further conditioning (group B), and 20 received donor cells after CD34 selection without conditioning (group C). The three groups were comparable for disease phase, patients' age, donor type, primary or secondary PGF, full donor chimerism and duration of PGF.

Results. Trilineage recovery was seen in 40%, 36% and 75% of the patients in, respectively, groups A, B and C (p=0.02). In multivariate Cox analysis trilineage recovery was more frequent in patients with secondary PGF (RR of complete recovery 2.82, p=0.01) and in patients receiving CD34+-selected cells (RR of complete recovery 3.0; p=0.007). There was no effect of donor type on hematologic recovery. The rate of NRM was 55%, 64%, 20% in groups A, B and C, respectively (p=0.06) and was highly correlated with trilineage recovery (RR 0.36, p<0.0001). PGF was the primary cause of death in 30%, 21% and 10% of the patients in the three groups, graft-versus-host disease (GVHD) in 5%, 36%, and 10%.

Interpretations and Conclusions. In patients with poor graft function (a) a boost of CD34+-selected peripheral blood donor cells is associated with a high chance of trilineage recovery and a low risk of acute GVHD; (b) a boost of unmanipulated donor cells does not seem to offer a survival advantage over no infusion of cells; and (c) NRM is lower when using peripheral blood cells for the boost. These data may be useful when discussing second stem cell donations for patients with poor graft function.

Key words: allogeneic hemopoietic stem cell transplantation, CD34+ selection, poor graft function, boost stem cell infusion.

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Poor graft function (PGF) remains an important complication following allogeneic hematopoietic stem cell transplantation (HSCT). It occurs in 5-27% of patients[1-4] and is associated with considerable morbidity and mortality related to infections and hemorrhagic complications. Graft function may be poor as a result of slow or incomplete recovery of blood counts (primary PGF) or decreasing blood counts after successful and prompt hematopoietic engraftment (secondary PGF).[3] Several factors may determine primary or secondary PGF in HSCT recipients. These factors include disease status at transplantation, prior alloimmunization, intensity of the conditioning regimen,[4,5] hematopoietic stem cell dose,[3,6] histocompatibililty,[6] donor type,[3] sex match between donor and recipient, T-cell content of the graft,[7] intensity of immunosuppression, graft-versus-host disease (GvHD),[3] viral infections such as cytomegalovirus[3,8] and human herpes virus-6[9] and myelosuppressive drugs such as gancyclovir.

There are various options for the management of graft failure. The use of granulocyte colony-stimulating factor (G-CSF) may be considered for neutropenia, because G-CSF is readily available and usually effective in the short term. Recombinant erythropoietin can also be useful to improve hemoglobin levels[10] but has no effect on platelet counts.[10,11] Second transplants have been used,[12,13] although in heterogeneous groups of patients and with different preparative regimens: in a review of 82 second HSCT for PGF, 41% of patients developed grade III-IV GVHD and the estimated 3-year survival was 53%.[13] An alternative treatment option is to administer a boost of unmanipulated donor cells.[14,15] Remberger *et al.* reported successful hematopoietic recovery in the majority of 20 patients with PGF who were treated with an additional dose of donor cells without conditioning: the probability of grade II-IV acute GVHD and chronic GVHD was 31% and 50%, respectively, with a 3-year survival of 43%.[2,14,15] In order to reduce the risk of acute and chronic GvHD, CD34+ selection has been used in some cases.[16,17] One case of prolonged primary PGF, lasting over 2 years, with 98% donor chimerism, was recently

reported:[17] this patient was successfully treated with a boost of CD34+-selected donor cells without previous conditioning. Other cases have been reported in the pediatric setting.[18,19] Here we report the outcome of 34 patients with PGF who were treated with a second infusion of unmanipulated or CD34+-selected allogeneic stem cells, without additional conditioning, and a matched group of 20 patients with PGF who received no additional infusion of donor cells.

Design and Methods

Poor graft function

PGF was diagnosed in patients with two or three cytopenic lines (Hb<10 g/dL, neutrophil count <1.0×10⁹/L, platelet count <30×10⁹/L) for at least 2 consecutive weeks beyond day +14 post-transplant, with transfusion requirement, associated with hypoplastic-aplastic bone marrow, in the presence of complete donor chimerism and in the absence of severe GVHD and relapse. A very small number of patients (n=5) had one cytopenic cell line. The overall median duration of PGF was 104 days.

Chimerism

Chimerism studies were performed until 1994 on marrow cells, using cytogenetics for sex-mismatched pairs and erythrocyte markers.[20] Thereafter chimerism was assessed on marrow cells by a microsatellite technique (short-tandem repeat polymerase chain reaction).[1] In the late 1990s, when separation procedures became available, CD3+ and CD3- cells on peripheral blood were also assessed.

Primary and secondary PGF

Primary PGF was diagnosed as the failure to achieve trilineage recovery to the levels outlined above. Secondary PGF was diagnosed in patients who had previously fulfilled the criteria for trilineage recovery after HSCT.

Treatment groups

There were three treatment groups: 20 controls (group A), allografted in the period 1998-2004, received no boost donor stem cells, because such cells were unavailable or because of the decision of the attending physician; 14 patients grafted between 1980 and 2005 received unmanipulated cells (group B); 20 patients, grafted between 1998 and 2004 received CD34+-selected stem cells (group C). The clinical characteristics of these three groups are outlined in Tables 1 and 2. CD34+-selection was performed by immunomagnetic separation using the CliniMACS Device (Miltenyi Biotec). This device allows the removal of over 3 logs of T cells: we therefore infused between 1×10⁵ and 1×10⁴ CD3+ cells /kg of the recipient's body weight.

Group A: control group

Twenty patients underwent an allogeneic stem cell transplant from a family donor (n=10) or an unrelated donor (n=10). The source of stem cells was bone marrow in 15 patients and peripheral blood in five patients. All these patients developed primary (n=12) or secondary (n=8) PGF at a median interval of 63 days after transplantation (range, 47-241 days). The median duration of PGF was 138 days (range, 78-734 days). At diagnosis all three cell lines were involved in five patients, whereas two cell lines were involved in 14 patients. In patients with primary PGF, at 50 days after transplant, the median neutrophil count was 2.3×10⁹/L (range 0.6-9), the median platelet count was 22×10⁹/L (range 10-35) and the median hemoglobin concentration was 9 g/dL (7.5-10). In patients with secondary PGF the median neutrophil count, platelet count and hemoglobin concentration were respectively 2×10⁹/L, 21×10⁹/L and 9 g/dL at the time of diagnosis. During this period, patients were given supportive treatment, such as growth factors (G-CSF, recombinant human erythropoietin) and transfusion of red blood cells and/or platelets.

Table 1. Clinical data of patients.

	Group A	Group B	Group C	p
Type of boost donor cell infusion	*No cells*	*Unmanipulated*	*CD34+-selected*	
Number	*20*	*14*	*20*	
Age median (range)	31 (16-48)	32.5 (23-47)	37 (22-57)	
Age > 33 years (median)	45%	35%	55%	0.5
Gender males/females	15/5	11/3	12/8	0.4
Diagnosis				
Acute leukemia	5	6	6	
Chronic myeloid leukemia	11	4	8	
Other	4	4	6	
Advanced leukemia (>1 CR)	7 (35%)	6 (46%)	10 (50%)	0.6
Donor type				
HLA identical sibling	8	10	7	
Family mismatched	2	1	5	
Unrelated	10	3	8	0.1
Stem cell source BM/PB*	15/5	11/3	17/3	0.8
PGF type: primary	12	7	9	
PGF type: secondary	8	5	11	0.6

*Other: non-Hodgkin's lymphoma, severe aplastic anemia, idiopathic myelofibrosis, myelodysplastic syndrome; >1CR: beyond first complete remission; PB: peripheral blood; BM: bone marrow; PGF: poor graft function; HSCT: hematopoietic stem cell transplant; *first HSCT.*

Group B: unmanipulated cells

In this group of 14 patients, seven developed primary PGF and seven developed secondary PGF at a median of 70 (range, 43-1037) days after transplantation. The median duration of PGF, before infusion of cells, was 105 days (range, 20-317). All these patients received a boost of unmanipulated stem cells from the original donor at a median of 121 days (range, 25-1162) after the first transplant. At the time of second infusion, the median neutrophil count was 1.2×10⁹/L (range, 0-3), the median platelet count was 15×10⁹/L (range, 7-125) and the median hemoglobin concentration was 9.7 g/dL (7.7-11). The median age of this group was 32.5 years (range, 23-47). At the time of boost infusion seven of 12 patients tested were

Table 2. Graft function and outcome.

Type of boost donor cell infusion	Group A No cells	Group B Unmanipulated	Group C CD34+-selected	p[a]
Number of patients	20	14	20	
PGF: primary/secondary	12/8	7/7	9/11	0.5
Interval Tx -secondary PGF	63	70	92	0.4
Days : median (range)	(47-241)	(43-1037)	(47-1064)	
Patients with ≥ 2 cytopenic lines at diagnosis of PGF	19 (95%)	11 (78%)	19 (95%)	0.2
CMV positive at boost cell infusion		7	13	0.3
PB counts at the time of boost infusion				
Neutrophil count	2.1	1.2	1.5	0.5
×10⁹/L-median(range)	(0.2-9)[b]	(0-3)	(0-3.2)	
Platelet count	21	15.5	17	0.7
×10⁹- median (range)	(10-35)[b]	(7-125)	(5-193)	
Hemoglobin gr/dL	9	9.7	9.4	0.3
median (range)	(7-10)[b]	(7.7-11)	(7.4-11.7)	
Duration of PGF				
Before Infusion	138	105	97	0.9
median (range)	(78-734)[d]	(20-317)	(15-1115)	
Interval 1st HSCT-boost infusion	–	121	153	0.6
		(25-1162)	(67-1188)	
Acute GvHD III-IV				
at time of infusion	0[b]	0	0	–
after boost donor cell infusions	0[b]	3/14	0/20	0.06
Chronic GvHD				
at time of infusion	–	8/10[3]	9/16[c]	0.3
after boost donor cell infusions	–	6/6[3]	12/19[c]	0.1
Stem cell source PB/BM	–	7/7	19/1	0.002
Cell dose×10⁸/kg		3.75 (1-40)	9.2 (3-25.1)	
PB				
Cells dose×10⁸/kg	–	8.3 (1.54-40)	9.9 (3-25.1)	0.4
CD34×10⁶/kg	–	na	2.57 (0.7- 31.4)	0.6
BM				
Cells dose×10⁸/kg	–	2.1 (1-3.9)	5.35	
CD34×10⁶kg	–	na	1.14	
Follow up days	274	84.5	696	
median (range)	(100-3371)	(2-4134)	(98- 2345)	
Status alive/dead	9/11	4/10	13/7	0.1

CMV: cytomegalovirus; GvHD: graft vs host disease; na: not available; [a]: p values represent differences between group B and group C; [b]: at time of diagnosis of PGF; [c]: evaluable patients; [d]: median overall duration of PGF in patients not receiving boost donor cells.

positive for cyclomegalovirus antigenemia (median, 3.5 positive cells) and were treated with ganciclovir and foscarnet alone or in combination. Eleven patients had family donors and three patients had an unrelated donor. Donor cells were obtained from bone marrow (n=7) or peripheral blood after G-CSF stimulation (n=7). The median dose of infused cells was 8.3×10⁸/Kg peripheral blood stem cells (range, 1.54-40) and 2.1×10⁸/Kg for bone marrow stem cells (range, 1-3.9). One patient received anti-CD52 and another received antithymocyte globulin before infusion as preparative regimens. In one female patient blood counts did not recover after chemotherapy given to treat leukemia relapse; the patient had good recovery following a boost infusion after 125 days, but died of relapse on day +307 after a second infusion. Another female patient was given two boost infusions of unmanipulated stem cells after an interval of 49 days and died 76 days later of extensive GVHD and infection.

Group C: CD34 selected cells

In this group of 20 patients, nine patients with primary PGF and 11 with secondary PGF received a boost of CD34+-selected stem cells at a median of 154 days after the first transplant. Secondary PGF was diagnosed at a median of 92 days after the transplant (range, 47-1064). PGF lasted a median of 97 days (range, 15-1115) before patients received a boost of donor cells. At the time of the stem cell infusion, the median neutrophil count was 1.5×10⁹/L (range, 0-3.2), the median platelet count was 17×10⁹/L (range, 5-193) and the median hemoglobin concentration was 9.4 g/dL (range, 7.4-11.7). Thirteen of the 20 patients were positive for cytomegalovirus antigenemia and were all treated with ganciclovir and foscarnet alone or in association; Epstein-Barr virus infection was documented in two patients, both treated with cidofovir. Eight patients had a suspected thrombotic thrombocytopenic purpura-like syndrome, treated with defibrotide or plasma exchange or both. After donor apheresis of G-CSF-mobilized peripheral blood, a median of 9.9×10⁸/Kg cells (range, 3-25.1) were obtained and a median of 2.57×10⁶/Kg CD34+-selected cells were infused (range, 0.7-31.4). In one case, marrow was used as the stem cell source and 1.14×10⁶/Kg CD34 cells were infused. One patient received antithymocyte globulin before the stem cell infusion.

Hematologic response

Blood counts required to define a patient as having cell lineage recovery were as follows: neutrophils ≥ 2×10⁹/L, platelets ≥100×10⁹/L and hemoglobin ≥10 gr/dL. Patients were also scored for transfusion independency and analyzed for hematologic recovery of one, two or three cells lines at day +30, +50, +100 and thereafter following a boost donor cell infusion. The best response for the three cell lines was recorded as the best response beyond day +100 during the follow-up period.

Statistical analysis

The NCSS package was used for the χ^2 tables, cumulative incidence (CI) rates, Student's T-test and Mann-Whitney test. When calculating the CI of trilineage recovery, the competing risk was death due to any cause. When calculating the CI of non-relapse mortality, the competing risk was relapse-related mortality.

Results

Unmanipulated or CD34+-selected cells

Data on graft funtion, cells infused and outcome are presented in Table 2. The two treated groups were comparable for severity of PGF, duration of PGF before infusion and interval from first transplant to boost donor infusion. They were also comparable for pre-infusion acute and chronic GVHD.

Hematologic recovery

Table 3 shows the timing and quality of hematologic recovery in the three groups of patients. At 30 days after boost infusion most patients were still cytopenic (82% and 80% in patients receiving unmanipulated or CD34+-

Table 3. Proportion of patients with cell lineage recovery at different times after boost cell infusion.

	Day +30	Day +50	Day +100	Best
Recovery of cell lines				
Group A°				
0-1	10 (50%)	10 (50%)	10 (50%)	11/20(55%)
2	0	0	1 (5%)	1/20 (5%)
3	0	0	0	8/20 (40%)
Group B				
0-1	9/11* (82%)	6/9* (67%)	1/6* (17%)	6/14 (43%)
2	1/11* (9%)	2/9* (22%)	3/6* (50%)	3/14 (21%)
3	1/11* (9%)	1/9* (11%)	2/6* (33%)	5/14 (36%)
Group C				
0-1	16/20 (80%)	11/20 (55%)	7/20 (35%)	3/20 (15%)
2	3/20 (15%)	5/20 (25%)	6/20 (30%)	2/20 (10%)
3	1/20 (5%)	3/20 (15%)	7/20 (35%)	15/20(75%)

*Results are given as percentage of patients recovering 0-1, 2 or 3 cell lines. *evaluable patients ; °group A: from diagnosis of PGF .*

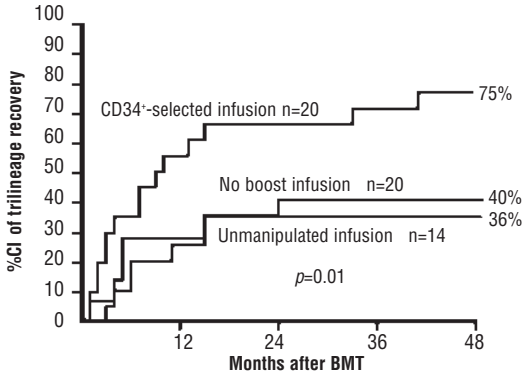

Figure 1. Cumulative incidence (CI) of trilineage recovery in the three groups. Recovery was significantly better in patients receiving CD34-selected boost donor cells.

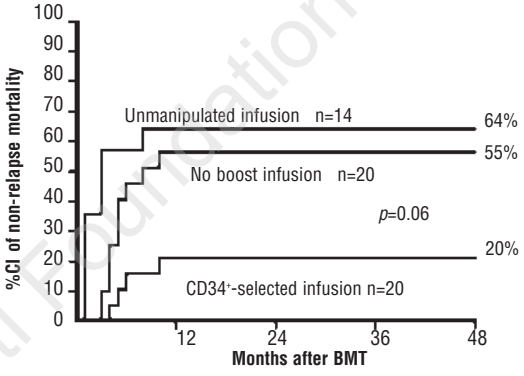

Figure 2. Cumulative incidence (CI) of non-relapse mortality (NRM) in the three groups. NRM was significantly lower in patients receiving CD34-selected boost donor cells.

selected cells, respectively). At day +50 these figures were reduced to 67% and 55% respectively, and by day +100 there had been further reductions to 17% and 35%. Conversely, the proportion of patients with trilineage responses increased from 9% and 5% on day +30 to 33% and 35% on day +100 (Table 3). Best hematologic response was as follows: trilineage recovery was recorded in 40% of patients in the control group, 36% in the group receiving unmanipulated cells and 75% in those receiving CD34$^+$-selected cells (p=0.01). Bilineage recovery was recorded in 5%, 21% and 10%, respectively (p=ns) and no recovery occurred in 55%, 43% and 15%. The cumulative incidence of trilineage recovery in the three groups is illustrated in Figure 1.

Graft-versus-host disease

Acute GVHD grade III-IV occurred in 21% and 0% of patients receiving unmanipulated or CD34$^+$-selected cells, respectively (p=0.06) (Table 2). Four patients in the group receiving an unmanipulated boost infusion died of complications of acute GVHD. Acute GVHD grade I-II was documented in 54% and 70% of the patients in group B and C, respectively, after infusion. Chronic GVHD developed in 18/25 patients who survived more than 100 days after the boost infusion: 6/6 evaluable patients in group B and 12/19 in group C. Three patients of groups B and C, all of whom received peripheral blood stem cells as their boost infusion, developed extensive chronic GVHD and died of complications related to GVHD after 257, 297 and 2345 days.

Factors predictive of hematologic recovery

Hematologic recovery was not related to the patient's age (</≥ the median: 33 years), sex, disease phase (early/advanced), duration of PGF (</≥ the median: 101 days), number of cytopenic lines at diagnosis of PGF (0-1 vs 2-3), ABO compatibility (yes/no), and donor type (matched sibling/ alternative donor). The use of CD34$^+$-selected cells as compared to unmanipulated cells was associated with a greater chance of trilineage recovery (75% vs 36%; p=0.02). The use of peripheral blood stem cells versus bone marrow was also associated with a significantly higher rate of trilineage recovery (73% vs 14%, p=0.02). The rate of trilineage recovery was 69% in patients with secondary PGF compared to 36% in patients with primary PGF (p=0.09). In multivariate Cox analysis the use of CD34 selected cells (RR of complete recovery 3.0; p=0.007) and secondary PGF (RR of complete recovery 2.82, p=0.01) were the two significant predictors of trilineage recovery.

Survival and non-relapse mortality

The cumulative incidence of NRM was 55% in group A, 64% in group B and 20% in group C (p=0.06)(Figure 2). Cumulative NRM was strongly influenced by the quality of hematologic recovery: only patients with recovery of all three cell lines had a low NRM (7%) (Figure 3) , whereas patients with no or partial recovery (one or two cell lines) had NRM rates exceeding 60% (Figure 3) (p<0.001). The rate of NRM was higher in patients with primary PGF than in those with secondary PGF (p=0.04). In multivariate Cox analysis testing the effect of type of cell infusion (CD34$^+$-selected vs unmanipulated and no infusion), donor type (related vs unrelated), patient's age (< vs ≥ 33

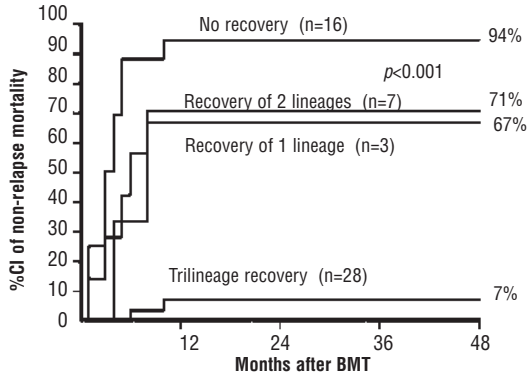

Figure 3. Cumulative incidence (CI) of non-relapse mortality (NRM) according to whether patients attained hematologic recovery of 3 cell lines (complete), 2 cell lines, or 0-1 cell lines. Only patients with trilineage recovery had a low rate of NRM.

years), disease status (first complete remission vs more advanced), original source of stem cells (bone marrow vs peripheral blood) and type of PGF (primary vs secondary, CD34+-selected cell infusion emerged as the only significant predictor (RR 0.32, p=0.03). When achievement of trilineage recovery was also entered into the model, this remained the only variable predicting a significantly lower risk of NRM (RR 0.36, p<0.0001). The actuarial 5-year survival was 45% in group A, 29% in group B and 65% in group C (p=0.01). Causes of death are shown in Table 4. The most frequent cause of death in the control group, who received no infusion of donor cells, was poor graft function (30%), whereas it was GVHD (36%) in group B patients, who received unmanipulated stem cells. Among the group C patients, who received CD34+-selected cells, equal numbers of death were due to graft failure, GVHD and relapse.

Discussion

When PGF develops after an allogeneic stem cell transplant, in the presence of full donor chimerism, it is unclear whether patients should or should not be given a boost infusion of donor cells, and whether they should receive a conditioning regimen before the boost infusion. In this study we show that a boost of CD34+-selected peripheral blood donor cells, without further preparation, is associated with a higher chance of trilineage recovery and better survival when compared to a boost of unmanipulated donor cells ; the latter produces similar results as no infusion of donor cells.

Because the causes of PGF, despite full donor chimerism, may be multi-factorial, including viral infections, GVHD and medications, it was rewarding to see trilineage recovery in many patients receiving CD34+- selected peripheral blood cells, without the need for pre-boost conditioning: this occurred over a period of several months, and was seen in a greater proportion of patients given CD34+-selected peripheral blood cells than in patients receiving unmanipulated cells. In the first 100 days, the proportion of patients with trilineage recovery was similar in both boosted groups, but thereafter the group receiving CD34+-selected cells showed significantly better recovery: at 12 months complete response was recorded in 60% of this group and in 30% of the group of patients who received unselected cells. The best response was finally assessed in 75% vs 36%, respectively.

Table 4. Distribution and causes of death in the three groups of patients.

	Group A	*Group B*	*Group C*	*Total*
PGF	6/20(30%)	3/14(21%)	2/20(10%)	11/54(20%)
Acute GVHD	0	4/14(29%)	0	4/54(7%)
Chronic GVHD	1/20(5%)	1/14(7%)	2/20(10%)	4/54(7%)
Relapse	0	1/14(7%)	2/20(10%)	3/54(6%)
Other*	4/20(20%)	1/14(7%)	1/20(5%)	6/54(11%)

Other: acute respiratory distress syndrome, idiopathic pneumonia, viral acute hepatitis, multi organ failure.

These results can also be compared with those in patients with PGF who did not receive a boost infusion of donor cells, usually because the donor was unavailable. Hematologic recovery was slower in this group, with no patient showing complete recovery at 100 days. Nevertheless, eight patients in this group did eventually have a complete trilineage response and recovered their marrow function. The primary cause of death in this group was complications of aplasia, with 30% dying of infections and hemorrhages. When comparing the cumulative incidence of survival in this group with that of patients boosted with unmanipulated cells, the two curves had a similar shape, because the group receiving unmanipulated cells had problems with GVHD and patients died due to this complication. Therefore deaths due to aplasia in patients not receiving cells were balanced by deaths due to GVHD in patients receiving unmanipulated cells: indeed NRM was significantly lower only in patients given CD34 selected cells.

Univariate analysis of factors predictive of hematologic response identified only three factors: the use of CD34+-selected cells, the use of peripheral blood stem cells rather than bone marrow cells and the diagnosis of secondary PGF rather than primary PGF. It was interesting to note that the duration of PGF had no impact on response, nor did donor type (related or unrelated). Therefore, given the choice, mobilized peripheral blood stem cells may be preferred over marrow for a second donation. Multivariate analysis confirmed that the use of CD34+-selected cells and secondary PGF were significant predictors of response.

As noted, patients with PGF are exposed to complications of prolonged aplasia: the final end-point of any treatment strategy aimed at improving PGF is mortality due to causes other than leukemia relapse. When factors predicting NRM were assessed in a Cox analysis, again infusion of CD34+-selected cells emerged as the most relevant predictor. However, when trilineage recovery was entered into the model, then achievement of trilineage recovery became the only significant variable: patients with tri-line-

age recovery had a significantly lower NRM, suggesting that it is not enough to have partial recovery of one or two cell lines in order to prevent non-relapse-related deaths.

Therefore when an allografted patient presents with poor graft function, and full donor chimerism, the aim should be to achieve complete trilineage recovery: it seems that the most effective way of doing this is to harvest G-CSF-primed cells from the original donor and select CD34$^+$ cells for the infusion. Although the number of patients in this study is relatively small, the demonstrated high chance of success, the encouraging survival and the low risk of GVHD, suggest, in our opinion, that this procedure could also be used when the donor is unrelated.

It is not clear how long to wait before calling the donor and asking him or her for a second donation of cells: probably the sooner the better, but one needs to make sure that the cytopenia is not transitory. We believe that 2 or 3 weeks of severe cytopenia beyond day +14 or after the diagnosis of secondary PGF should be a time frame within which the attending physician may seriously think of calling the donor, if related, or activating the donor registry, if unrelated. It should be realized that it may take an additional 1 to 4 weeks from calling the donor to actually having the cells available for infusion: by then the patient may have been cytopenic for 3-7 weeks. One criticism of this strategy is that many co-factors can influence blood counts, such as viral infections[8,9] and GVHD,[21] and that treatment of the latter should be the attending physician's primary focus.

We are not arguing against this approach: we believe a second infusion of donor cells is not to be considered an alternative , but rather an adjunct of treatment for transplant-related complications, and cytopenia is a common additional problem. In our opinion, the high success rate shown in the present study warrants the use of CD34$^+$-selected donor cells, without further conditioning, for patients with primary or secondary poor graft function in the presence of full donor chimerism.

AL: retreived information, analyzed the data and wrote the manuscript; GP: responsible for laboratory hematology analyses: MP, AP: performed the CD34 cell selection; BB, RO: in charge of the data base; CDG, FG, DO: in charge of the inpatient transplant care; AMR, AD, SB: responsible for the out-patient transplant care; TL: in charge of the unrelated transplants; ET: performed CMV/EBV monitoring; FF: in charge of cell therapy; MTvL: in charge of the identical sibling transplants; EP: provided clinical supervision; AB: designed the study and reviewed the manuscript. The authors declare that they have no potential conflicts of interest. *Funding. This work was partly supported by the Fondazione CARIGE Genova, the Associazione Italiana Ricerca contro il Cancro (A.I.R.C.) Milano and the Associazione Ricerca Trapianto Midollo Osseo (A.RI.T.M.O.) Genova.*

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