CD34 Selected Cells for the Treatment of Poor Graft Function after Allogeneic Stem Cell Transplantation

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A B S T R A C T

Poor graft function (PGF) is characterized by pancytopenia and a hypoplastic marrow, with complete donor chimerism, usually without severe graft-versus-host disease (GVHD). We report 41 patients with PGF, treated with granulocyte colony-stimulating factor–mobilized CD34 selected cells, at a median interval from transplant of 140 days, without conditioning and without GVHD prophylaxis. Donors were HLA matched siblings (n = 12), unrelated donors (n = 18), or mismatched family members (n = 11). The median number of infused $CD34^+$ cells was 3.4×10^6/kg. The rate of trilineage recovery was 75%: 83% for HLA matched siblings and 72% for unrelated and mismatched family members ($P = .3$). The cumulative incidence of acute grade II GVHD was 15%, and no patient developed de novo chronic GVHD. The actuarial 3-year survival is 63%: 76% and 25% for patients with or without trilineage recovery. These data confirm the role of $CD34^+$ selected cells from the same donor in the treatment of PGF and warrant the request for a second donation also when the donor is unrelated.

INTRODUCTION

Poor graft function (PGF) is a severe complication that occurs after allogeneic hematopoietic stem cell transplantation (HSCT) in 5% to 27% of patients [1-3]. Several factors have been shown to predict PGF, such as donor type, HLA matching, ABO incompatibility, cell dose, stem cell source, graft-versus-host disease (GVHD), viral infections, intensity of conditioning regimen, and the use of myelotoxic agent, such as ganciclovir [2-5]. The pathogenesis of PGF is probably multifactorial and includes immunologic issues as well as abnormalities in bone marrow microenvironment, number of progenitor cells, and type of the underlying disease [2-6].

PGF is defined as severe cytopenia of at least 2 cell lines and/or transfusion requirement in the presence of a hypoplastic/aplastic bone marrow, with full donor chimerism, and in the absence of severe acute or chronic GVHD [7,8] or relapse [1,2]. PGF should not be mistaken for rejection, in which chimerism is mixed or 100% host. Primary PGF occurs early after transplant, whereas secondary PGF occurs after the patient has experienced a certain degree of hematologic recovery. PGF may be treated with granulocyte colony-stimulating factor (G-CSF) [9] or with G-CSF and thrombopoietin, as recently reported in 3 patients [10]. However, a significant proportion of patients do not respond to growth factors and remain cytopenic. In this cohort of poor/nonresponders, an efficient treatment option is the infusion of donor $CD34^+$ selected peripheral blood stem cells (PBSCs) without further conditioning [1]. In this report we update our previous experience and report 41 patients with PGF treated with $CD34^+$ selected hematopoietic progenitors.

METHODS

Patients

We studied 41 patients with secondary PGF after allogeneic HSCT who received a boost of $CD34^+$ selected PBSCs without prior conditioning and without GVHD prophylaxis at median interval of 140 days from the first transplant. Clinical characteristics are shown in Table 1.

At the time of the CD34 infusion, the median neutrophil count was 1.44×10^6/L (range, 0 to 3.2), the median platelet count was 21×10^9/L (range, 5 to 193), and the median hemoglobin concentration was 8.9 g/dL (range, 6.9 to 11). We infused a median number of 3.45×10^6/kg.

$CD34^+$ selected cells (range, .05 to 22.5). The median age of patients was 37 years (range, 18 to 62) (Table 1).

Poor Graft Function

We defined PGF as follows: (1) 2 or 4 cytopenic lines for at least 2 consecutive weeks beyond day +14 from allogeneic HSCT (hemoglobin <10 g/dL), neutrophil count $<1.0 \times 10^9$/L, platelet count $<30 \times 10^9$/L and/or (2) transfusion requirement, in the presence of a hypoplastic-aplastic bone marrow, with full donor chimerism and (3) without grades III to IV acute GVHD [7,8] and (4) in the absence of relapse [1].

Boost Infusion

G-CSF mobilized peripheral blood cells were collected by standard procedures from the original donor. $CD34^+$ selection was performed by immune-magnetic separation using the CliniMACS Device (Miltenyi Biotec, Bologna Italy). This device allows depletion of 3 log of T cells: the final product contained between 2.5×10^3 and 10×10^3 $CD3^+$ cells/kg of a recipient's body weight. $CD34^+$ selected cells were infused intravenously, without conditioning regimen and without GVHD prophylaxis (Table 1).

Chimerism

Chimerism was assessed on marrow cells using the microsatellite technique (short-tandem-repeat PCR) [11].

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Table 1
Clinical Characteristics of 41 Patients Receiving Boost CD34$^+$ Selected Cells

Characteristic	Value
No. of patients	41
Conditioning before CD34$^+$ PB	None
GVHD prophylaxis before CD34$^+$ PB	None
Median age, yr (range)	37 (18-62)
Diagnosis	ALL n = 7, AML n = 8, CML n = 8, MFI n = 4, NHL n = 6, SAA n = 4
Donor type	HLA id n = 12, MUD n = 18, family mm n = 11
Source	BM n = 32, PB n = 9
Conditioning regimen at first HSCT	Myeloablative, n = 25 (58%)
CMV before CD34$^+$ cell infusion	30 (73%)
TMA before CD34$^+$ cell infusion	8 (19%)
GVHD before CD34 cell infusion	Grade 0/I n = 36 (78%); grade II n = 5 (12%)
Conditioning regimen before CD34$^+$ cells	None
GVHD prophylaxis after CD34$^+$ cells	None
Median CD34$^+$ infused (range)	3.45 × 10^6/kg (.05-22.5)
Median CD3$^+$ cells infused (range)	5.6 × 10^3/kg (2.5-10)
Median days from first allogeneic HSCT (range)	140 d (48-374)
Median platelet count at CD34$^+$ infusion (range)	21 × 10^9/L (5-193)
Median neutrophil count at CD34$^+$ infusion (range)	1.44 × 10^9/L (0-3.2)
Median Hb level at CD34$^+$ infusion (range)	8.9 g/dL (6.9-11)
Acute GVHD after infusion	Grade II-III n = 9, grade IV none
Median follow-up (range)	1245 d (94-4151)

PB indicates peripheral blood; ALL, acute lymphoblastic leukemia; AML, acute myeloid leukemia; CML, chronic myeloid leukemia; MFI, myelofibrosis idiopathic; NHL, non-Hodgkin lymphoma; SAA, severe aplastic anemia; MUD, matched unrelated donor; family mm, family mismatch; BM, bone marrow; CMV, cytomegalovirus; Hb, hemoglobin.

Response Criteria

Complete hematologic response or trilineage recovery was defined as achieving hemoglobin >10 g/dL, absolute neutrophil count >1 × 10^9/L, and platelets >100 × 10^9/L. Partial response or partial recovery was defined as transfusion independence, without a complete hematologic recovery.

Statistical Analysis

The NCSS 9 package (Kaysville, UT) was used for the chi-square tables, cumulative incidence (CI) rates, Student *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 nonrelapse mortality, the competing risk was relapse-related mortality.

RESULTS

Graft-versus-Host Disease

The CI of acute GVHD grade II after CD34 infusion was 15% (Figure 1A). Three patients had moderate chronic GVHD before and after CD34$^+$ cell infusion.

Response

The CI of trilineage recovery was 75% (Figure 1B). A partial response was seen in 3 patients, with an overall response rate of 83% (34/41 patients); 7 of 41 patients (17%) were considered nonresponders. The median time for complete hematologic recovery to occur was 101 days (range, 13 to 994) from CD34$^+$ PBSC boost infusions (Figure 1B).

The median platelet count on days 0, +30, +60, and +100 from CD34$^+$ cell infusions were as follows, respectively: 21 × 10^9/L (range, 5 to 193), 51 × 10^9/L (range, 10 to 246), 65 × 10^9/L (range, 5 to 304), and 82 × 10^9/L (range, 1 to 274). We could find no association between trilineage recovery and clinical characteristics such as age, sex, underlying diagnosis, and disease status before transplant. Similarly, the dose of CD34$^+$ PBSCs infused (<3.3 versus 3.3 × 10^6/kg) appeared to have no impact on hematologic recovery (78% versus 72%, respectively, $P = .6$). There was a nonsignificant trend for a greater chance of trilineage recovery in siblings (83%) as compared with unrelated and family mismatched donors (72%) ($P = .3$); the timing was nearly identical (103 and 105 days, respectively). Patients who received a myeloablative conditioning regimen at first transplant had a borderline greater chance of trilineage recovery (87% versus 61%, $P = .06$).

Survival

With a median follow-up of 1245 days (range, 94 to 4151) from the infusion of CD34$^+$ cells, 28 patients (68%) survived disease free, all having achieved a durable trilineage recovery. The current actuarial 3-year survival rate is 63% (Figure 1C).

There was a strong impact, in univariate analysis, of hematologic recovery on survival: 76% versus 25% for patients with or without trilineage recovery ($P < .0001$) (Figure 1D). In multivariate analysis, trilineage recovery was the strongest predictor (relative risk, .01; $P = .0001$) followed by sibling donors (relative risk, .13; $P = .03$).

Thirteen patients died. Relapse-related death was diagnosed in 8 of 41 patients (19%) and nonrelapse mortality in 5 of 41 patients (12%): 2 due to pre-existing chronic GVHD and 3 to infections, in patients who did not recover hematopoiesis.

DISCUSSION

We report 41 patients who received infusion of CD34$^+$ selected PBSCs from the original donors, without conditioning regimen and without GVHD prophylaxis, for the treatment of PGF. The procedure is safe, with a low risk of grade II acute GVHD (15%) and no grades III to IV or de novo chronic GVHD: 3 patients had moderate chronic GVHD, which did not worsen after infusion of CD34$^+$ cells. The primary objective of intervention in patients who are cytopenic, transfusion dependent, and often infected is a lack of severe side effects and of GVHD, despite the lack of GVHD prophylaxis. It should be noted that all patients were infused without conditioning regimen, due to the demonstration of full donor chimerism, and this may have been a relevant factor to reduce side effects to a minimum, together with a very low number of infused CD3$^+$ cells, with a median of less than 10 × 10^3/kg. The latter observation is in keeping with the efficacy of the separation device currently used worldwide.

In a previous study we reported 20 patients, with 75% trilineage recovery [1]. In the present study we included 21 newly treated patients and updated the 20 previously reported: the median follow-up is now 1245 days (range, 94 to 4151). The overall CI of trilineage recovery is 75%, therefore confirming, in this larger series of patients, our previous results. Hematologic recovery was durable in all patients we evaluated so far. Two recent reports have addressed the issue of PGF and its treatment with CD34$^+$ selected donor stem cells [12,13], showing 50 patients with a chance of hematologic improvement of, respectively, 72% and 81%.

The overall 3-year actuarial survival, in our study, was 63%, with a significant impact on survival of hematologic recovery: 76% for patients in complete remission and 25% for

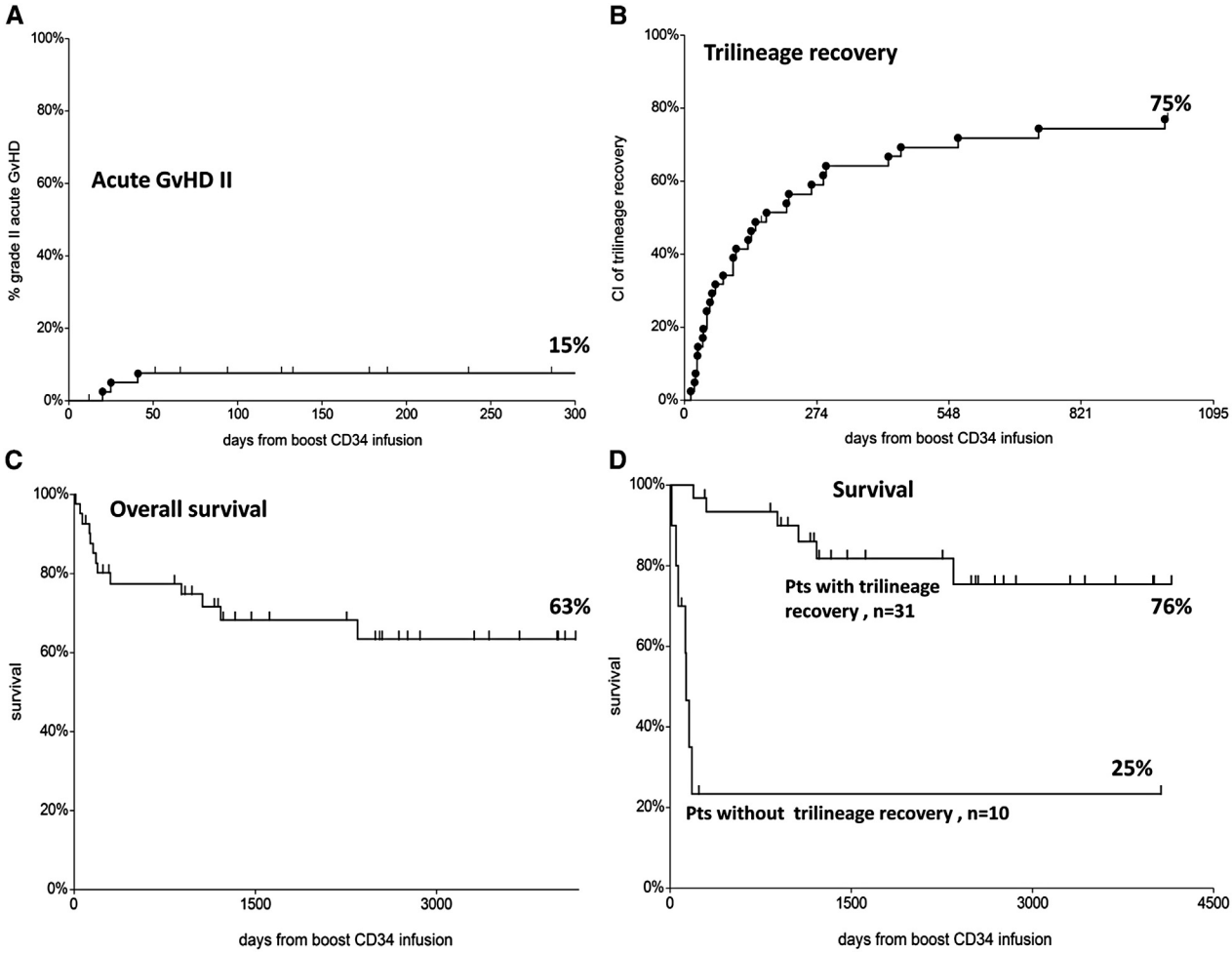

Figure 1. (A). CI of acute GVHD grade II; no patient had grades III to IV. (B) CI of trilineage recovery. (C) Actuarial survival. (D) Actuarial survival in patients stratified for trilineage response: a significant advantage for patients with trilineage recovery.

patients with partial hematologic recovery (achieving transfusion independency without trilineage recovery) ($P < .0001$). In multivariate analysis this was also the strongest predictor of survival, followed by the use of $CD34^+$ cells from HLA identical sibling donors. We did not find an association of trilineage recovery with the underlying disease, HLA matching, donor recipient gender and age, ABO compatibility, or history of previous GVHD. In addition in our cohort, the probability of recovery was not influenced by the dose of $CD34^+$ cells, ranging from .05 to 22 $\times$ 10^6/kg; recovery could also be seen with a low dose of $CD34^+$ cells. The median time to trilineage recovery from $CD34^+$ boost infusion was 101 days, although platelet counts started to rise on day +30 and progressively increased thereafter, similar to that recently described [12,13]. The relatively long time to achieve complete trilineage recovery may be due to the complex nature of these patients, often with multiple infections and cytotoxic treatment. Indeed, the large majority (73%) had cytomegalovirys reactivation and were therefore treated with ganciclovir and/or foscarnet before $CD34^+$ cell infusion, and thus at the time of PGF. In addition, almost 20% had a diagnosis of transplant-associated microangiopathy preinfusion; transplant-associated microangiopathy is known to cause prolonged cytopenia.

When pooling together 2 recent reports [12,13] with the present study (Table 2), there are 91 patients with PGF treated with boost $CD34^+$ without conditioning and without GVHD prophylaxis exhibiting some strong similarities. First, there was a high proportion of patients with myelofibrosis. Although we are unaware of the total denominator, it is

Table 2
Studies with $CD34^+$ Boost Infusions in Patients with PGF

	Askaa et al. 2014 [12]	Klyuchnikov et al. 2014 [13]	This Study	Total or Average
Year of publication	2014	2014	2014	
No. of patients	18	32	41	91
Myelofibrosis	6	14	4	24 (26%)
Interval transplant boost	113	150	140	134
$CD34^+$ cell dose $\times$ 10^6/kg	3,7	3,4		3,55
$CD3^+$ cell dose $\times$ 10^3/kg	11	9	5.6	8.5
Hematologic recovery	72%	81%	75%	76%
Stable hematologic recovery	Yes	Yes	Yes	Yes
GVHD grades III-IV	2	4	0	6 (7%)
De novo chronic	0	0	0	0
Median follow-up, d	1072	900	1245	1072.5
Actuarial 3-yr survival	40%	45%	63%	49%

unlikely that 26% of allogeneic transplants are composed of patients with myelofibrosis. Indeed, at our own center, during the time interval when our 41 patients were diagnosed with PGF, the proportion of myelofibrosis of all allogeneic transplants was 7% (81/1206), suggesting that myelofibrosis is at high risk for PGF patients, as already suggested by others. The second similarity is the almost identical interval between transplant and boost infusion, with a very narrow range (113 to 140 days), suggesting we are reporting the same type of PGF. The number of infused $CD34^+$ cells is also almost identical, as well as the lack of correlation between cell dose and recovery. Of interest, once hematologic recovery has occurred, it was defined as stable in all 3 studies. The rate of acute grades III to IV GVHD is somewhat higher in 2 studies, but we are talking of small numbers. We did not calculate the average time to hematologic recovery, only because in 1 study it was given as initial recovery and in our study as full recovery; however, the overall proportion of patients showing recovery is almost identical.

These data bring additional evidence for the effectiveness of $CD34^+$ selected cells from G-CSF mobilized peripheral blood of the original donor in patients with PGF. The low rate of GVHD in the absence of GVHD prophylaxis is reassuring. Finally, the high rate of trilineage recovery also with $CD34^+$ cells from unrelated donors may be relevant for registry policies.

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