

## TWO CONSECUTIVE COURSES OF rh-G-CSF-MOBILIZED PERIPHERAL BLOOD STEM CELLS FOR PRIMARY MARROW ALLOENGRAFTMENT FAILURE: CASE REPORT

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### ABSTRACT

We describe herein a case of bone marrow failure in a 53-year-old patient affected by Ph1-positive chronic myeloid leukemia who received an HLA-identical ABO-mismatched bone marrow transplant from a 56-year-old sibling donor. Hematopoietic recovery after marrow failure was obtained following two consecutive courses of rh-G-CSF-mobilized peripheral blood stem cell infusions. No potential risk factors associated with graft failure, excluding recipient and donor age, were documented, whereas a relatively high number of progenitor cells were necessary to overcome the host-versus-graft barrier in our patient. Therefore we suggest that growth factor-stimulated peripheral blood should be considered as the first choice for allogeneic stem cells in order to avoid primary graft failure with donors over 50 years of age.

Key words: PBSC transplantation, allogeneic graft failure, chronic myeloid leukemia

Graft failure remains a severe multifactorial complication after allogeneic bone marrow transplantation (BMT). Since both human recombinant growth factors and second marrow transplantation fail to restore hematopoiesis in a significant number of cases,<sup>1</sup> allogeneic peripheral blood stem cell (PBSC) transplantation is suggested as a new therapeutic approach for graft failure following BMT.<sup>2-4</sup>

Mobilization of PBSC can be safely induced by G-CSF in normal donors.<sup>5,6</sup> Similarly to the autologous setting, PBSC have been successfully employed in the last few years for allogeneic transplantation and represent a useful alternative to bone marrow.<sup>6</sup> Moreover, by increasing the size of the graft inoculum, PBSC reduce the risk of graft failure in patients who undergo transplantation with haploidentical *three-loci* mismatched T-cell-depleted bone marrow.<sup>7</sup> A speedier and sustained hemopoietic and immunologic recovery with a lower incidence of com-

plications is therefore expected with PBSC transplant.<sup>2-4,6</sup>

Theoretical and ethical problems concerning the donor over the unquestionable advantage of the recipient have restricted this procedure to selected situations, such as a second donation after a graft rejection, or failure and relapse after allogeneic BMT. In this regard, guidelines from the *Gruppo Italiano Trapianti di Midollo Osseo* (GITMO) for the utilization of rh-G-CSF-mobilized PBSC from healthy donors have been published recently.<sup>6</sup>

We describe herein a case of allogeneic graft failure that successfully recovered hematopoiesis after two consecutive harvesting courses and infusions of allogeneic rh-G-CSF-mobilized PBSC.

### Case Report

A 53-year-old man with Ph1-positive chronic

myeloid leukemia (CML) underwent BMT from an HLA-identical sibling thirty-six months after diagnosis. Progressive leukocytosis in spite of increasing dosages of hydroxyurea and  $\alpha$ -interferon suggested that the disease had entered an accelerated phase. Splenomegaly was mild and bone marrow histology showed the absence of myelofibrosis. Conditioning consisted of standard busulphan 16/mg/kg and cyclophosphamide 120 mg/kg (BuCy2). Cyclosporin-A (Cy-A) (1 mg/kg/day by continuous intravenous infusion) and short methotrexate (mtx) (Seattle protocol) were employed for graft-versus-host-disease (GVHD) prophylaxis. A 56-year-old HLA-identical AB0-mismatched (A1 donor, 0 recipient) sibling was harvested under general anesthesia, and  $0.8 \times 10^8$ /kg mononuclear cells (MNC),  $3.6 \times 10^6$ /kg CD34<sup>+</sup> cells,  $18 \times 10^4$ /kg CFU-GM and  $17.6 \times 10^6$ /kg CD3<sup>+</sup> lymphocytes (Table 1) were infused after depletion of red blood cells, granulocytes and platelets. The number of CD34<sup>+</sup> cells was measured with a direct immunofluorescence technique using the fluorescein-conjugated HPCA-2 MoAb (Becton Dickinson, Palo Alto, CA). An Epics Profile Analyzer (Coulter Electronics, Hialeah, FL) was used to identify positively staining cells. The recipient anti-donor titer of anti-A isohemagglutinin was low (1:32) before the transplant and declined to 1:1 during the course of subsequent events. No neutrophils were seen in the recipient smear at day +15. There were no signs of hemolysis. The patient was treated with rh-G-CSF (Granulokine, Roche) at a dosage of 10 mg/kg/day for 18 days without any improvement in neutrophilia. There was no evidence of acute GVHD or CMV infection, which was excluded by an immunofluorescence technique. Marrow

examination revealed very poor cellularity with no evidence of relapse. A diagnosis of graft failure was made and a stem cell mobilization program was started at day +33. Rh-G-CSF 10 mg/kg/day was administered to the donor for 5 consecutive days following written informed consent. Two large volume (12 L) leukaphereses were collected through antecubital veins with an AS-104 (Fresenius) cell separator and a C4Y device (Biofil) on days +5 and +6. A total of  $3.5 \times 10^8$ /kg MNC,  $16.6 \times 10^4$ /kg CFU-GM and  $6 \times 10^6$ /kg CD34<sup>+</sup> cells, together with  $119.2 \times 10^6$ /kg CD3<sup>+</sup> cells, were harvested (Table 1) and, after red blood cell depletion, were freshly infused into the recipient on day +37 from the first transplant. The patient was not conditioned further, but an additional cycle of intravenous Cy-A and Mtx was given for GVHD prophylaxis. Post-transplant rh-G-CSF was also administered. While the platelet count remained below  $10 \times 10^9$ /L, the absolute neutrophil count reached, respectively, 0.5 and  $1.0 \times 10^9$ /L at days +11 and +12 from PBSCT, with a maximum value of  $3.7 \times 10^9$ /L at day +14. Subsequently, the absolute neutrophil count fell below  $1.0 \times 10^9$ /L on day +66, indicating a second graft failure. A further course of rh-G-CSF was started in the donor with the same PBSC mobilization schedule. However, we increased the number of apheresis procedures (day +5, +6, +8 and +10) in order to improve the yield. The patient was infused with  $7.2 \times 10^8$ /kg MNC,  $10.1 \times 10^6$ /kg CD34<sup>+</sup> cells,  $47.2 \times 10^4$ /kg CFU-GM and  $225.3 \times 10^6$ /kg CD3<sup>+</sup> cells (Table 1). A neutrophil count of  $>1.0 \times 10^9$ /L was reached on day +14 from the second PBSCT and  $50.0 \times 10^9$ /L platelets on day +36. At day +300 neutrophil and platelet counts were  $5.1 \times 10^9$ /L and  $192 \times 10^9$ /L, respec-

Source	No. of apheresis procedures	MNC $\times 10^8$ /kg	CFU-GM $\times 10^4$ /kg	CD34 $\times 10^6$ /kg	CD3+ $\times 10^6$ /kg
Bone marrow harvest		0.8	18.0	3.6	17.6
1st PBSC collection course	2	3.5	16.6	6.0	119.2
2nd PBSC collection course	4	7.2	47.2	10.1	225.3

Table 1. Mononuclear cells (MNC), T lymphocytes, CFU-GM and CD34<sup>+</sup> cells collected from the same donor at 3 different time periods.

tively. A bone marrow examination showed good cellularity with no evidence of Ph1<sup>+</sup> metaphases. In addition, Western Blot analysis of the p210 protein was negative, while a signal of the bcr-abl gene rearrangement was documented at the PCR level.

Overall, the donor reported moderate back pain and headache; he never exceeded WBC above  $62.0 \times 10^9/L$  and his platelet level decreased to  $69.0 \times 10^9/L$  after the second cycle of apheretic procedures, although we reinfused autologous platelets to minimize this problem.

### Discussion

Graft failure is a multifactorial, life-threatening complication of HLA-matched BMT.<sup>1</sup> Potential causes of poor graft function may include major ABO incompatibility, mild immunosuppression, post-transplant infections and a low stem cell inoculum,<sup>1,8-10</sup> while it is unclear whether donor age may be an additional risk factor.<sup>9,10</sup> In this respect, as a result of improvements in the prevention and treatment of the major causes of mortality after BMT, an increasing number of patients older than 50 years are being transplanted, and an increasing use of donors beyond the age of 50 years is noted, since both recipient and HLA-identical sibling donor age are strongly correlated.<sup>10</sup>

This report documents successful hematopoietic engraftment after two consecutive primary

allograft failures using large doses of mobilized PBSC. Our patient received an ABO-mismatched transplant. However, major ABO mismatch appears to have no significant effect on the incidence of graft rejection if potential complications are anticipated and treated appropriately.<sup>8</sup> Moreover, donor stem cell proliferation does not appear to be inhibited by antidonor iso-hemoagglutinins.<sup>8</sup> It is of note that no sign of hemolysis was documented in our patient, that the titers of isohemagglutinin capable of agglutinating donor cells were low and that, as suggested, erythrocytes, granulocytes and platelets were removed from the graft prior to infusion.

Theoretically, mild immunosuppression due to the conditioning regimen might be considered to explain graft failure; however, a prospective randomized trial comparing BUCY2- and TBI-containing regimens demonstrated no difference in terms of engraftment speed or other clinical outcomes.<sup>11</sup> In addition, our patient received no further conditioning drugs prior the subsequent PBSC infusions.

Since a sufficient number of progenitor cells were infused and no signs of infection were observed, the etiology of graft failure in our patient is unclear. However, the course of events (Figure 1) demonstrates that definitive engraftment was probably due to the relatively higher numbers of CD34<sup>+</sup> cells and CFU-GM infused with the third transplant (about three times as many CFU-GM and little less than double the

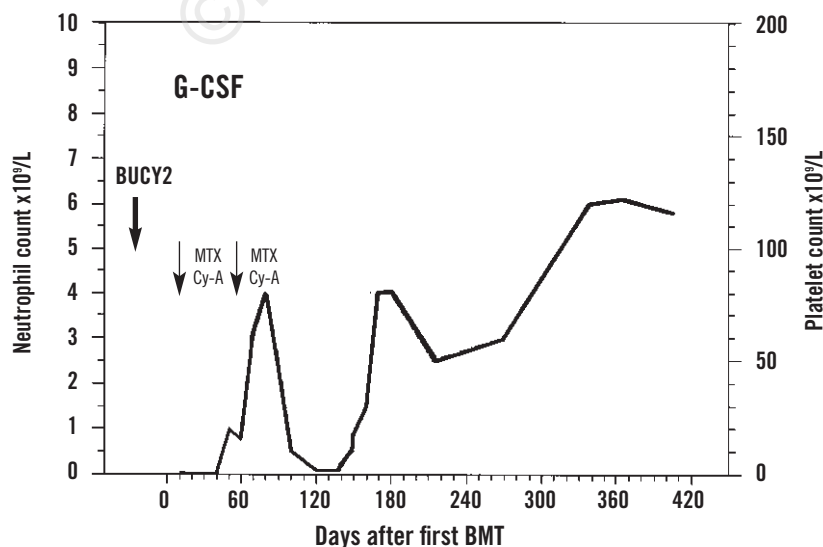


Figure 1. Neutrophil and platelet count in relation to stem cell transplantation, GVHD prophylaxis and growth factor administration.

CD34<sup>+</sup> cells, as compared

with marrow graft). On the other hand, a possible explanation for the engraftment failure observed after the first PBSC transplant may be the relatively low number of CFU-GM collected in spite of a satisfactory amount of CD34<sup>+</sup> cells harvested. This finding is in line with reported data which show that both the number of CFU-GM and CD34<sup>+</sup> cells infused influence hematopoietic reconstitution. Moreover, severe GVHD did not develop in our patient despite the high number of T cells infused along with PBSC. Conversely, both the number and the possibly different composition of peripheral T cells might have facilitated engraftment.

Finally, the role of donor age as a risk factor for graft failure is still unclear. Kernan *et al.* observed that donor age (or recipient age) may be a major factor associated with graft failure,<sup>10</sup> but this was not confirmed by others.<sup>1,9</sup> However, all these studies included mainly young patients and young donors, and the statistical methods used might have underestimated the role of donor age beyond 50 years. The CD34<sup>+</sup> peak in peripheral blood is inversely associated with donor age,<sup>5</sup> but PBSC grafts generally contain at least three times more progenitor stem cells than allogeneic marrow grafts.<sup>5</sup> Moreover, the progressive age-related decrease in hemopoietic cellularity to about 30-50 percent and the simultaneous reciprocal increase of the fat tissue fraction have been well documented.<sup>12</sup> Therefore donors over 50 years old might be expected to cause a high incidence of engraftment problems as a result of an insufficient number of harvested marrow progenitor cells, due to the limited availability of BM cells and the necessity of avoiding excessive trauma and hypovolemia in the donor.

Since (a) the amount of progenitor cells required for a complete engraftment remains questionable when the donor is over 50 years old, (b) a high number of hemopoietic progenitors can only be obtained from peripheral blood, and (c) mobilization and collection may theoretically be repeated in the same donor

within a relatively short period of time, we suggest that allogeneic rh-G-CSF-mobilized PBSC should be the modality of choice with donors over 50 years of age.

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