Peripheral blood stem cell versus bone marrow allotransplantation: does the source of hematopoietic stem cells matter?

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Hematopoietic stem cells from 4 different sources have been or are being used for the reconstitution of lymphohematopoietic function after myeloablative, near-myeloablative, or nonmyeloablative treatment. Bone marrow (BM)–derived stem cells, introduced by E. D. Thomas in 1963,[1] are considered the classical stem cell source. Fetal liver stem cell transplantation has been performed on a limited number of patients with aplastic anemia or acute leukemia, but only transient engraftment has been demonstrated.[2] Peripheral blood as a stem cell source was introduced in 1981,[3] and cord blood was introduced as a source in 1988.[4] The various stem cell sources differ in their reconstitutive and immunogenic characteristics, which are based on the proportion of early pluripotent and self-renewing stem cells to lineage-committed late progenitor cells and on the number and characteristics of accompanying "accessory cells" contained in stem cell allografts. (Blood. 2001;98:2900-2908)

Introduction

This review article is an attempt to summarize our current knowledge on biologic and clinical differences between allogeneic peripheral blood stem cell transplantation (PBSCT) and bone marrow transplantation (BMT) regarding donor aspects and cell yield, posttransplantation characteristics, immunogenic–antimalignancy effects, engraftment across human leukocyte antigen (HLA) barriers, and feasibility of in vivo immunomodulation of stem cell allografts.

Cell yield, cell composition, and replicative potential of stem cell allografts

The percentage of $CD34^+$ cells among circulating total nucleated cells at steady state in healthy donors is 0.06%; it is 1.1% in the BM, an 18-fold difference in favor of the latter stem cell source. On the other hand, temporarily shifting hematopoietic progenitor cells, including stem cells, from extravascular BM sites into the circulating blood by using cytokine treatment dramatically increases the circulating stem cell concentration and easily compensates for the low baseline circulating $CD34^+$ cell concentration. When healthy donors are treated with recombinant human granulocyte colony-stimulating factor (rhG-CSF; 12 μg/kg per day) over 3 days, with another rhG-CSF dose given on the fourth day before the stem cell apheresis procedure, the mean peripheral blood (PB) $CD34^+$ cell concentration increases from 3.8×10^9/L to 61.9×10^9/L, a 16.3-fold increase over baseline. The increase in early $CD34^+38^-$ progenitor cells is, at 23.2-fold, even greater.[5] Three cytokines have been used so far under clinical conditions in healthy donors: rhG-CSF (5-20 μg/kg per day), recombinant human granulocyte-macrophage colony-stimulating factor (rhGM-CSF; 5-10 μg/kg per day), and Flt3 ligand (50 μg/kg per day). Whereas rhG-CSF treatment is considered the gold standard, a combination of rhG-CSF and rhGM-CSF has been investigated on a more experimental basis,[6,7] and Flt3 ligand alone has been tried in a phase 1 study only.[8] The addition of rhGM-CSF to rhG-CSF either does not increase or increases only moderately stem cell mobilization efficiency.[6,7] Flt3 ligand alone is a mild mobilizing cytokine (Table 1).

Cytokine regimens for mobilizing stem cells, rhG-CSF dose and schedule of administration, and the handling of apheresis products before transfusion vary among transplant centers. Most transplant groups use a 5-day rhG-CSF regimen, starting PBSC collections on the fifth day. Based on $CD34^+$ cell kinetic studies, a 4-day rhG-CSF regimen seems to provide comparable yields.[9] An rhG-CSF dose of 10 to 12 μg/kg per day is widely used in healthy donors; in a recent study published by Bensinger et al,[10] 16 μg/kg per day was given without compromising the donor's safety. PBSC or BM allografts either are transfused fresh into the recipient or are cryopreserved before transfusion. The latter approach has the advantage of being performed independently of the transplantation procedure, though cryopreservation and thawing can reduce the $CD34^+$ cell number in the transfusate by as much as 10% to 20%, mainly because of cell trapping during the thawing process. This is also true for lymphocyte subsets. There is, however, no indication of specific cell loss of any lymphocyte subset because of the freezing process.[5] Overall, no conclusive data exist to suggest that the different strategies for stem cell collection and processing mentioned above have a potential for any difference in transplantation outcome.

The cell composition of unmanipulated PBSC and BM allografts differs significantly. As shown by Ottinger et al[11] and by our group,[5] the total numbers of T cells, monocytes, and natural killer (NK) cells contained in a PBSC allograft are more than 10 times higher than those in a BM allograft, except for $CD3^+CD4^-CD8^-$ natural suppressor cells (PBSC:BM allograft ratio, 9:1) and $CD19^+$ cells (PBSC:BM ratio, 6:1) (Table 2). The absolute numbers of $CD34^+$ and $CD3^+$ cells contained in BM harvested in steady state are compared with those in rhG-CSF–mobilized PBSC or BM

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Table 1. CD34$^+$ cell mobilization efficiency of single or combined cytokine treatment in healthy donors

Cytokines	Dose	PB CD34$^+$ cell increase over baseline
rhG-CSF	5-20 μg/kg per day	16- to 23-fold[5]
rhG-CSF + rhGM-CSF	5-10 μg/kg per day	10- to 26-fold[6]
Flt3 ligand + G-CSF/GM-CSF	Up to 100 μg/kg per day	Doubles PB peak CD34$^+$ cell levels compared to G-CSF alone[12]

harvests (Table 3). Allografts of rhG-CSF–treated PBSCs contain 2 to 4 times more CD34$^+$ cells than do those from untreated BM. When a BM donor is treated for 3 days with rhG-CSF, the number of CD34$^+$ cells contained in the harvested BM averages 2.5×10^6/kg,[13] similar to that obtained by steady state BM harvest.

Although BM harvesting is usually a one-time, single-day procedure, PBSC harvesting extends over the stem cell mobilization period (usually 4 to 5 days) and the harvesting period (1 to 3 days). In our experience, the target CD34$^+$ cell transplantation dose of 4×10^6/kg is harvested during a single apheresis procedure in 59% of donors, 2 apheresis procedures in 31% of donors, and 3 procedures in 10% of donors.[14] BM harvesting under general anesthesia is limited by procedural risks (largely related to general or spinal anesthesia), which significantly increase with the donor's age and comorbid conditions. PBSC harvesting, on the other hand, can be performed safely on donors ranging from 1 year to the eighth decade. The principal limitation of the procedure is the need for venous access.

The replication potential of stem cells hypothetically may decrease with age and with the number of divisions those stem cells have undergone because of a progressive shortening of telomeres.[15] In addition, telomerase activity in CD34$^+$ cells decreases in intensity with age,[16] though the role of telomerase in stem cell biology is still unresolved.[17] Replication potentials, including telomere stability and telomerase activity, of cytokine-mobilized PBSC and steady state BM allografts may be different, but conclusive data to support this are missing. The short- and long-term engraftment potential of rhG-CSF–mobilized human PBSCs or steady state BM stem cells of healthy donors have been compared after xenogeneic transplantation into 60-day-old fetal sheep.[18] Mobilized PB CD34$^+$ or Lin$^-$ CD34$^-$ cells led to earlier engraftment. However, serial transplantation into secondary and tertiary recipients exhausted the repopulating ability of PB-derived CD34$^+$ cells or Lin$^-$ CD34$^-$ cell fractions faster than the repopulating ability of the BM-derived progenitor cells. These differences are not related to the cell phenotype but rather to the stem cell source—that is, BM or PB. The authors speculate that the relative frequency of long-term repopulating cells is lower in rhG-CSF–mobilized PB stem cell allografts than in BM allografts, which is, in the clinical setting, compensated for by transfusing much larger numbers of PB-derived cells. Nevertheless, intrinsic differences in the repopulating potentials of PB- or BM-derived progenitor cells cannot be ruled out. The longest surviving patient, who received a PBSC allograft in October 1993 from her HLA-matched sibling, shows sustained 100% donor chimerism with normal BM and PB cellularity.[19]

Potential risks and adverse effects of stem cell harvesting

Short-term adverse effects of stem cell harvesting

PBSC harvesting by single or multiple leukapheresis procedures avoids the risks associated with multiple marrow aspirations and general anesthesia and shortens donor recovery time. Mild to moderately severe bone pain, fatigue, headache, and, less commonly, nausea are expected in most donors.[20,21] Unexpected adverse events during rhG-CSF mobilization and PBSC collection have been reported, however. These include nontraumatic splenic rupture, iritis, acute gouty arthritis, anaphylactoid reaction, and cardiac ischemia.[22-27] The true incidence of these events is unclear, but they appear to be rare. This subject has been reviewed in detail elsewhere.[28] Moreover, 5% to 20% of donors may have inadequate peripheral venous access, and insertion of a central or femoral venous catheter may add to the risk and discomfort related to this procedure.[21,29] Overall, a complication rate of 1.1% has been reported recently by the International Bone Marrow Transplant Registry (IBMTR)/European Bone Marrow Transplant Group (EBMT) in a large sample of 1337 PBSC donors; no donation-related fatalities were reported.[29]

Unlike PBSC collection, BM harvesting has an extensive track record spanning more than 30 years. It is considered a safe procedure, though it is associated with considerable discomfort and a longer recovery time for the donor. Bortin and Buckner[30] reviewed 3290 cases of marrow donation and found an incidence of major (ie, life-threatening) complications of 0.27%. A survey of 493 marrow donations for the National Marrow Donor Program revealed 29 cases (5.9%) of acute complications and a mean recovery time of 15.8 days after donation,[31] largely attributed to postcollection pain and fatigue. A recent update from the IBMTR database revealed 2 fatalities among 7857 reported BM donations (0.02%) between 1994 and 1998.[29]

Long-term adverse effects of stem cell harvesting

Data on long-term follow-up of donors who underwent BM harvest or PBSC mobilization and collection procedure are scarce or nonexistent. One concern has been whether the short-term administration of rhG-CSF may trigger the development of malignancy, particularly acute myeloid leukemia, in otherwise healthy donors. The challenging biometrical and statistical problems involved in evaluating long-term risk in PBSC donors have been well characterized.[32] Small cohorts of healthy PBSC donors have been followed by different groups for variable (but usually fairly short) periods of time after donation, and the largest study on this issue published so far is from Cavallaro et al.[33] They reported a survey of 101 related donors who received rhG-CSF mobilization treatment before PBSC or granulocyte collection. Ninety-five of them were contacted successfully, with a median follow-up of 43 months (range, 35-73 months) after rhG-CSF administration. No cases of leukemia

Table 2. Immunocompetent cells contained in PBSC allografts and BM allografts

Cells $\times 10^6$/kg	PBSC allograft (n = 10)	BM allograft (n = 10)	PBSC:BM allograft
CD2$^+$	760 (383-1155)	54 (20-98)	14
CD3$^+$	701 (291-1082)	49 (19-95)	14
$TCR_{\alpha/\beta}$	663 (240-1064)	42 (18-86)	16
$TCR_{\gamma/\delta}$	26 (6-85)	2 (0-6)	13
CD3$^+$CD4$^+$	393 (145-587)	26 (9-51)	15
CD4$^+$CD45RA$^+$	188 (44-280)	11 (4-24)	17
CD4$^+$CD45RO$^+$	169 (18-296)	10 (1-22)	17
CD3$^+$CD8$^+$	236 (44-472)	18 (7-37)	13
CD3$^+$CD4$^-$CD8$^-$	45 (9-138)	5 (0-11)	9
CD19$^+$	93 (37-193)	15 (6-26)	6
CD45$^+$CD14$^+$	599 (216-1053)	25 (11-46)	24
CD16$^+$CD56$^+$CD3$^-$	77 (5-129)	6 (1-13)	13

Numbers in parentheses are ranges.
Modified from Ottinger et al.[11]

Table 3. CD34$^+$ and CD3$^+$ cell numbers contained in various stem cell allografts

	rhG-CSF-mobilized PBSC harvest	Steady state BM harvest	rhG-CSF-mobilized BM harvest
CD34$^+$ cells $\times$ 10^6/kg	7.3 (1.0-29.8)[10] (n = 81)	2.4 (0.8-10.4)[10] (n = 91)	2.5 (1.5-7.3)[13] (n = 29)
	7.6 (2.1-28)[14] (n = 112)	2.4 (0.8-25)[34] (n = 26)	
CD3$^+$ cells $\times$ 10^6/kg	279 (143-788)[10] (n = 81)	23.8 (5.4-347)[10] (n = 91)	16 (4-35)[13] (n = 21)
	294 (48-1376)[14] (n = 111)	24.1 (10-110)[34] (n = 26)	

Numbers in parentheses are ranges.

or myelodysplasia were detected in these donors, and hematologic values were within normal ranges in 70 in whom they were measured at a median follow-up of 40 months (range, 16-70 months). The results of this survey suggest that rhG-CSF mobilization treatment of healthy donors is safe and without any obvious adverse effects 3 years or longer after stem cell donation. In a survey performed by our group on 281 PBSC donors at a median follow-up time of more than 3 years after allogeneic PBSCT, 85% were willing to undergo another PBSC collection procedure for a family member, and 48% were willing to undergo it for a nonfamily member. No hematologic malignancy has been recorded in this donor cohort.[35]

Evaluation of long-term adverse effects of rhG-CSF exposure in healthy donors is particularly hampered by the fact that an underlying genetic predisposition for malignancy among siblings and blood-related family members cannot be ruled out. Studies on this issue are largely lacking. An association between specific HLA antigens and leukemia has been postulated,[36] and a follow-up survey of healthy related marrow donors suggests that their risk for leukemia may be higher than that of the general population.[37]

Posttransplantation characteristics

Seven clinical studies—6 randomized and 1 matched-pair analysis—have been published to date comparing the clinical short-term outcomes of allogeneic BMT and PBSCT.[10,38-42] Five of those 7 studies, including the most comprehensive multicenter trial by Bensinger et al,[10] were published in peer-reviewed journals. In addition, 3 registry analyses have been reported—2 interim analyses of ongoing multicenter trials from the EBMT[43] and the Canadian Bone Marrow Transplant Study Group,[44] both presented as abstracts at the 2000 Annual Meeting of the American Society of Hematology, and an extensive retrospective analysis of the IBMTR and EBMT databases published by Champlin et al[45] in this journal (Table 4).

It is the intent of this review to draw conclusions primarily based on data originating from the more mature clinical studies (as judged by study design, sample size, patient follow-up, and completeness of data presented).

Neutrophil and platelet reconstitution

Neutrophil and platelet reconstitution is uniformly reported to occur faster after allogeneic PBSCT than after BMT (Table 4). Reaching a neutrophil count greater than 0.5×10^9/L takes between 15 and 23 days for most BMT patients and between 12 and 19 days for most PBSCT patients. The respective intervals to reach a platelet count greater than 20×10^9/L are 17 to 25 days after BMT and 11 to 18 days after PBSCT. The differences in neutrophil and platelet reconstitution kinetics are statistically significant in all studies reported.

Table 4. Time to absolute neutrophil and platelet counts and treatment-related mortality after allogeneic PBSCT or BMT

Study	Study design, donor-recipient relationship	No. patients	Median days to ANC $> 0.5 \times 10^9$/L		Median days to platelet $> 20 \times 10^9$/L		Transplant-related mortality rates	
			BM	PBSC	BM	PBSC	BM (%)	PBSC (%)
Vigorito et al[38]	Randomized, related	37	18	16	17	12	77.8 (median FU, 631 d)	62.5 (median FU, 335 d)
Blaise et al[39]	Randomized, related	101	21	15	21 ($> 25 \times 10^9$/L)	13 ($> 25 \times 10^9$/L)	21 (at d 180)	23 (at d 180)
Ringden et al[40]	Matched-pair, unrelated	90	20	16	29 ($> 50 \times 10^9$/L)	23 ($> 50 \times 10^9$/L)	21 (at 1 y)	27 (at 1 y)
Heldal et al[41]	Randomized, related	61	23	17	21	13	0 (at d 100) 10 (at 1 y)	13 (at d 100) 17 (at 1 y)
Powles et al[42]	Randomized, related	39	23	17.5	18	11	31 (median FU, 57 d)	35 (median FU, 57 d)
Schmitz et al[43]	Randomized, related	350	15	12	20	15	No significant difference	No significant difference
Simpson et al[44]	Randomized, related	228	22	19	22	16	16* (at d 100)	7.5* (at d 100)
Champlin et al[45]	Retrospective registry analysis, related	824	19	14	25	18	28 (AL CR1) 30 (AL CR2)* 27 (CML CP1) 67 (CML AP/CP2)* (at 1 y)	18 (AL CR1) 13 (AL CR2)* 37 (CML CP1) 26 (CML AP/CP2)* (at 1 y)
Bensinger et al[10]	Randomized, related	172	21	16	19	13	30 (at 2 y)	21 (at 2 y)

AL indicates acute leukemia; CML, chronic myelogenous leukemia; CR, complete remission; CP, chronic phase; AP, accelerated phase; FU, follow-up.
*Statistically significant.

Transplant-related mortality

The retrospective registry analysis revealed significant differences in transplant-related mortality in favor of PBSCT in patients with advanced-stage leukemia (Table 4).[45] Similar findings were reported by the Canadian study.[44] Transplant-related mortality is known to be higher in patients with advanced leukemia, and it is among these patients that a benefit from PBSCT is more likely to emerge. However, the other trials, including the EBMT study,[43] did not show a favorable outcome for PBSCT, possibly because of patient selection (ie, lack of patients with advanced-stage disease).

Acute graft-versus-host disease

With the exception of the most recent EBMT trial update,[43] among the studies listed, the cumulative incidence of acute graft-versus-host disease (GVHD) was found to be statistically no different whether using PBSCs or BM stem cells for hematopoietic reconstitution (Table 5). Interestingly, the EBMT study (the largest prospective trial) is also the only study using 3 doses of posttransplant methotrexate as opposed to the customary 4 doses given for acute GVHD prophylaxis. After allogeneic BM transplantation, reduction to less than 80% of the scheduled dose of methotrexate has been reported to be a risk factor for the development of grades II-IV acute GVHD.[46]

Because the absolute T cell number is higher in unmanipulated PBSC allografts than in BM allografts by approximately 1 log, a higher incidence of acute GVHD might be expected in PBSC recipients. However, as outlined in more detail below, immunomodulatory effects of in vivo cytokine treatment and cell-to-cell interaction in the apheresis product conceivably could reduce the incidence of acute GVHD after allogeneic PBSCT despite the infusion of a larger T-cell dose. It has been pointed out that many of these studies lack the statistical power to detect a small difference in the incidence of acute GVHD, and the possibility that such a difference may indeed exist has been raised by a recent meta-analysis.[47]

Chronic graft-versus-host disease

The probability of chronic GVHD developing varies by report; 4 of 9 studies show a significantly higher probability of chronic GVHD after allogeneic PBSCT than after allogeneic BMT. This includes a retrospective registry analysis of 824 patients who underwent allotransplantation. In contrast, 5 studies do not show a statistically significant difference; this includes a large multicenter study published recently. As discussed by Bensinger et al[10] in more detail, the discrepancy in these findings may be accounted for by various factors, such as limited statistical power (eg, small number of patients eligible for evaluation, relatively short follow-up beyond day 100), type of acute GVHD prophylaxis regimen used, and in vivo immunomodulatory aspects of rhG-CSF administered for stem cell mobilization (outlined below). In addition, there is some inconsistency among investigators with regard to diagnosis and grading of chronic GVHD.[48] It is also conceivable that center-specific treatment modalities (ie, conditioning regimen, supportive care) play a role that may prove to be as relevant as the cellular composition of the allograft.

Relapse

The data reported show, with one exception,[42] no obvious difference in the relapse rates between allogeneic PBSCT and BMT. Because the potential graft-versus-leukemia (GVL) effect of PBSCT

Table 5. Incidence of acute and chronic GVHD after allogeneic PBSC or BM transplantation

	Acute GVHD grades II-IV (grades III-IV) (100-d probability)		Chronic GVHD	
Study	BM (%)	PBSC (%)	BM (%)	PBSC (%)
Vigorito et al[38]	19	27	53 overall	71 overall
			50 limited*	0 limited*
			50 extensive*	100 extensive*
			(median FU, 631 d)	(median FU, 335 d)
Blaise et al[39]	42	44	30 overall*	54.5 overall*
			73 limited*	37.5 limited*
			27 extensive*	62.5 extensive*
			(median FU, 20 mo)	(median FU, 20 mo)
Ringden et al[40]	20	30	85 overall	59 overall
			83 limited	82 limited
			17 extensive	12 extensive
			(median FU, 16 mo)	(median FU, 10 mo)
Heldal et al[41]	10	21	27	56
			75 limited	73 limited
			25 extensive	27 extensive
			(median FU, 27 mo)	(median FU, 27 mo)
Powles et al[42]	47	50	40 (2-y probability)	44 (2-y probability)
Schmitz et al[43]	39*	52*	53* (at 2 y)	74* (at 2 y)
	(16)*	(28)*		
Simpson et al[44]	40	40	39 (at 6 mo)	47 (at 6 mo)
			55 (at 1 y)	71 (at 1 y)
Champlin et al[45]	35 (19)	40 (13)	53*	65*
			51 limited*	49 limited*
			49 extensive*	51 extensive*
			(1-y probability)	(1-y probability)
Bensinger et al[10]	57 (12)	64 (15)	28	38
			(median FU, 20 mo)	(median FU, 20 mo)

NA indicates not available; FU, follow-up.
*Statistical significance.

is most prevalent in indolent hematologic malignancies such as chronic myelogenous leukemia (CML),[49,50] randomized studies are necessary to prospectively evaluate such an antimalignancy effect in subgroups of patients (Table 6).

Disease-free survival and overall survival

A higher probability for overall disease-free survival has been found in patients with more advanced disease[10] after allogeneic PBSCT than after allogeneic BMT, specifically in those with CML beyond first chronic phase and acute leukemia in second remission.[45] On the other hand, patients with low-risk disease (ie, patients with CML in first chronic phase and patients with acute leukemia in remission) have similar rates of disease-free survival irrespective of the stem cell source.[45] A matched-pair analysis trial[40] and 2 smaller randomized trials[38,39] lack evidence of a statistically significant difference in disease-free survival between patients who underwent transplantation with PBSCs and those who underwent it with BM. On the other hand, 2 of the larger randomized trials[10,44] show a significant difference in overall survival favoring PBSCT, largely because of lower transplant-related mortality in patients with advanced-stage disease (Table 6).

Immune reconstitution after PBSCT or BMT

The literature comparing immune reconstitution after allogeneic PBSCT or BMT is scarce because phenotypic and functional characterization of immune reconstitution are hampered by the potential development of GVHD and by its interference with immunosuppressive and myelosuppressive anti-GVHD, antifungal, and antiviral treatments. In a prospective sequential study, Ottinger et al[11] compared the reconstitution of lymphocyte subsets and their proliferative in vitro responses to mitogens and recall antigens in patients who underwent allogeneic PBSCT or BMT. The circulating numbers of naive $CD4^+CD45RA^+$ and memory $CD4^+CD45RO^+$ helper T cells, $CD19^+$ cells, and monocytes were significantly higher in patients who underwent PBSCT up to 11 months after transplantation, 6 months after transplantation, and 0 to 2 months after transplantation, respectively. Conversely, the reconstitution patterns of $CD3^+CD8^+$ and NK cells were no different between allogeneic PBSCT and BMT. In addition, in vitro proliferative responses to phytohemagglutinin, pokeweed mitogen, tetanus toxoid, and *Candida* were greater in PBSCT recipients than in BMT recipients. These differences in favor of PBSCT are explained, at least in part, by the transfer of donor immunocompetent cells. The higher $CD34^+$ cell count contained in PBSC allografts also may contribute to improved immune reconstitution, though there is no evidence of preferential lymphoid differentiation potential of circulating stem cells. In contrast to the findings by Ottinger et al,[11] findings from an uncontrolled study by DiPersio's group[51] revealed delayed recovery of NK cell numbers and functional NK activity after PBSCT.

Potential graft-versus-leukemia effects of PBSCT and BMT

The approximately 1 log higher number of T cells in unmanipulated PBSC allografts and the higher incidence of chronic GVHD seen in some studies after PBSCT may coincide with a more pronounced GVL effect. However, as shown above, there is no uniform correlation between stem cell source and relapse rates or disease-free survival rates. A difference in relapse rates in favor of PBSC transplants does emerge, however, when patients with leukemia are stratified by stage of disease.[10,45] This is probably because of a higher incidence of chronic GVHD after PBSC transplantation.[45] This difference may not necessarily translate into prolonged disease-free survival, in view of the morbidity and mortality associated with chronic GVHD.

The first direct evidence of a more pronounced antileukemic effect after PBSCT was provided by Elmaagacli et al.[50] In a prospective study, disease recurrence as demonstrated by sequential bcr-abl polymerase chain reaction assays and cytogenetic analyses was compared in patients in first chronic phase of CML who underwent allogeneic unmanipulated BMT or PBSCT. These patients had a significantly lower risk for molecular relapse after PBSCT than after BMT. Coinciding with molecular findings, the risk for cytogenetic relapse was significantly higher

Table 6. Relapse and leukemia-free survival rates after allogeneic PBSCT or BMT

	Relapse rate		Disease-free survival rate (overall survival rate)	
Study	BM (%)	PBSC (%)	BM (%)	PBSC (%)
Vigorito et al[38]	10.5 (median FU, 631 d)	11 (median FU, 335 d)	52 (at 1000 d)	58 (at 1000 d)
Blaise et al[39]	9 (median FU, 20 mo)	9 (median FU, 20 mo)	66 (at 2 y)	67 (at 2 y)
Ringden et al[40]	38 (1-y probability)	34 (1-y probability)	48 overall	46 overall
			70 (low risk)	63 (low risk)
			33 (high risk)	31 (high risk)
			(at 1 y)	(at 1 y)
Heldal et al[41]	30 (median FU, 36 mo)	3.2 (median FU, 34 mo)	NA	NA
Powles et al[42]	37* (2-y probability)	0* (2-y probability)	NA	NA
Schmitz et al[43]	No significant difference	No significant difference	No significant difference	No significant difference
Simpson et al[44]	NA	NA	(55 [at 2 y])*	(68 [at 2 y])*
Champlin et al[45]	11 AL CR1	14 AL CR1	61 AL CR1	70 AL CR1
	13 AL CR2	8 AL CR2	57 AL CR2*	77 AL CR2*
	1 CML CP1	2 CML CP1	74 CML CP1	63 CML CP1
	21 CML AP	13 CML AP	23 CML AP*	68 CML AP*
	(1-y probability)	(1-y probability)	(1-y probability)	(1-y probability)
Bensinger et al[10]	25 (at 2 y)	14 (at 2 y)	45 overall*	65 overall*
			72 (less advanced cancer)	75 (less advanced cancer)
			33* (more advanced cancer)	57* (more advanced cancer)
			(at 2 y)	(at 2 y)
			(54 [at 2 y])*	(66 [at 2 y])*

Abbreviations are explained in Tables 4 and 5.
*Statistical significance.

after BMT than after PBSCT. Interestingly, in a multivariate analysis, the stem cell source for transplantation was found to be the only independent predictor of molecular relapse. Neither acute nor chronic GVHD had an impact on the recurrence of molecular or cytogenetic disease.

Nevertheless, our current understanding of delivering an immune-mediated GVL effect requires the clinical or subclinical development of GVHD. An alternative hypothesis to support an improved antileukemic effect of allogeneic PBSCT may rely on the higher number of stem cells transfused and on faster achievement of complete donor chimerism with displacement of residual recipient-derived clonogenic tumor cells. This concept is based on stem cell competition rather than immunogenic causes.

Engraftment across HLA barriers

Experimental and, more recently, clinical data have shown that escalation of the hematopoietic progenitor cell dose contained in a stem cell allograft can overcome major genetic barriers, including a haploidentical 3-loci–mismatched transplant, with resultant stable engraftment and without GVHD.[52,53] Such a favorable clinical outcome depends on the conditioning regimen and on the cellular composition of the stem cell allograft using a maximal number of $CD34^+$ cells (median, 10×10^6/kg) and a minimal number of $CD3^+$ cells (mean, 3×10^4/kg). For reasons mentioned earlier, such a $CD34^+$ cell megadose concept is unique to the circulating stem cell source insofar as it can be realized only by using cytokine-mobilized PBSCs. When aggressively collected, the PBSC dose can be almost 1 log higher than that ordinarily harvested by multiple bone marrow aspiration.

Within the pluripotent and lineage-committed stem cells, there is a $CD34^+$ cell subset that facilitates engraftment by inducing tolerance (so-called veto cells).[52,54] The term veto relates to the ability of cells to neutralize cytotoxic T-lymphocyte (CTL)-p directed against their antigens.[55] Thus, when purified $CD34^+$ cells are added to bulk mixed lymphocyte reaction, they suppress CTLs against matched stimulators but not against stimulators from a third party.[54] The mechanism(s) mediating the veto activity of different veto cells is still not fully understood. The most potent veto cells known are the $CD8^+$ cytotoxic T lymphocytes. Very recently it was shown that their veto activity is dependent upon the simultaneous expression of both CD8 and FasL[56] leading to apoptosis of the effector cells. Considering that human $CD34^+$ cells do not express these molecules, apoptosis is likely mediated by other death ligands.[57] Dose escalation of the $CD34^+$ cell population with stem cell activity and $CD34^+$ cell subsets with veto activity in T-cell–depleted megadose PBSC allografts may account for their ability to overcome residual antidonor immune reactivity across major HLA barriers.

RhG-CSF–treated BM allografts

Attempts have been made to combine the advantages of PBSCT (fast cell recovery) with those of BMT (potentially low incidence of chronic GVHD) by using rhG-CSF–primed BM allografts. Initial studies that were focused on the kinetics of cell recovery after autologous PBSCT or rhG-CSF-primed BMT showed similar cellular reconstitution patterns.[58] Neutrophil and platelet recovery[13] or neutrophil recovery only[59] has been shown to be more rapid after rhG-CSF–primed BMT in patients who have undergone allogeneic transplantation than in historical steady state BMT controls. When comparing rhG-CSF–primed allogeneic BMT with rhG-CSF–mobilized allogeneic PBSCT in a sequential study, the time to neutrophil engraftment was identical in the 2 patient cohorts, whereas platelet engraftment occurred earlier after allogeneic PBSCT.[60]

Because rhG-CSF mobilizes lymphoid cells modestly,[14] hypothetically the expected incidence of chronic GVHD after cytokine-primed BMT should be in the same range as that for steady state allogeneic BMT and less than that for PBSCT. Indeed, in a sequential study by Serody et al,[60] the number of $CD3^+$ cells contained in rhG-CSF–primed BM allografts was found to be approximately 1 log less than the number in rhG-CSF–mobilized PBSC allografts, and the incidence of chronic GVHD was significantly less in the rhG-CSF–primed BMT cohort. However, the median follow-up time in the rhG-CSF–primed BMT cohort was much shorter (367 days vs 887 days), which makes these data difficult to interpret. Additionally, on the basis of the randomized data most recently published by Simpson et al[44] and Bensinger et al,[10] no difference in the incidence of chronic GVHD between recipients of steady state BM and recipients of rhG-CSF–mobilized PBSCs has been shown. Hypothetically, however, rhG-CSF could affect the cytokine secretion profile of the harvested BM and thus further decrease the incidence of GVHD.[61,62]

In 72 rhG-CSF–primed BM donors studied so far, no complications have been reported. The donor risk profiles under these circumstances must be evaluated in larger studies. Because these donors face the risks associated with both donation modalities (ie, marrow harvesting under general or spinal anesthesia and cytokine exposure), clear-cut clinical benefits in terms of patient outcome must be documented before this approach can be endorsed on a wider scale.

In vivo immunomodulation of PBSC allografts

Cytokine treatment of the donor is focused primarily on stem cell mobilization and peripheralization. Recent data also show an immunomodulatory effect of cytokine treatment on donor circulating lymphoid subsets. Cytokine-induced release and in vivo expansion of selected lymphoid subsets collected by apheresis are unique to the PBSC harvesting procedure, opening up the possibility for in vivo immunomodulation of stem cell allografts before harvesting. Clinical endpoints of in vivo allograft immunomodulation include improving immune reconstitution and graft-versus-malignancy effect and decreasing the incidence of severe GVHD. Three cytokines are used in healthy donors: rhG-CSF, rhGM-CSF, and Flt3 ligand.

RhG-CSF

RhG-CSF as used for stem cell mobilization is also known to modulate alloimmune responses by direct modification of T-cell function or by cell-mediated suppression of T-cell alloreactivity. Early experimental studies showed that pretreatment of donors with rhG-CSF polarizes donor T cells toward type-2 cytokine secretion (interleukin-4 [IL-4], IL-10) with reduced type-1 secretion (IL-2, interferon-γ), an effect that is associated with less severe GVHD.[63]

As shown recently by Arpinati et al[62] and Liu et al,[64] rhG-CSF treatment of healthy donors selectively increases the number of circulating T-helper (Th)2-inducing lymphoid dendritic cells (pre-DC2) ($CD4^+$IL-$3R\alpha^{++}CD11c^-$) 5-fold and more than 7-fold, respectively, but not the number of Th1-inducing myeloid dendritic cell precursors (pre-DC1) ($CD4^+CD11c^+CD13^+CD33^+$). Th2 polarization is initiated already in the rhG-CSF–treated donor before host alloantigen activation, resulting in an immunomodulated apheresis product. It is assumed that

after transfusion, host alloantigens further activate pre-DC2 and promote Th2 proliferation in recipients of transplants.[65]

Despite rhG-CSF–induced Th2 polarization, which is associated with the development of less severe GVHD, the GVL effect of rhG-CSF–mobilized PBSC allografts is maintained by preserving perforin-dependent donor CTL activity, as shown in a murine allogeneic transplant model.[66]

RhG-CSF treatment of healthy donors decreases NK cell function and the relative frequency of NK cell progenitors within the $CD34^+$ cell population contained in apheresis products.[67,68] RhG-CSF negatively affects the in vitro generation of cytotoxic effectors by IL-2 activation.[69] Posttranscriptional inhibition of tumor necrosis factor-α production could explain some of the down-regulatory effects of rhG-CSF on alloimmune responses.[70]

Mobilization of stem cells by rhG-CSF blocks the ability of donor monocytes and dendritic cells to produce IL-12, which plays a pivotal role in the initiation of protective Th1 immunity against bacteria, viruses, and fungi.[71,72] Antigen presentation in the absence of IL-12 promotes Th2 responses, thereby exerting a strong immunosuppressive effect in recipients of stem cell transplants. The data reported thus far on immunomodulation in rhG-CSF–mobilized apheresis products show a global impairment of immune function, a widely unknown negative aspect of rhG-CSF treatment in light of its highly efficient stem cell mobilization potential.

RhGM-CSF and Flt3 ligand

Apheresis products of healthy donors treated by a combination of rhG-CSF and rhGM-CSF contain significantly (more than 50%) fewer $CD3^+$, $CD4^+$, and $CD8^+$ cells than apheresis products from donors treated with rhG-CSF alone.[7,73] The same investigators reported a 25-fold higher number of activated $CD80^+$ dendritic cells contained in apheresis products treated by rhG-CSF + rhGM-CSF or GM-CSF alone than in those treated by rhG-CSF alone.[73] Flt3 ligand increases the numbers of myeloid $CD11c^+$ dendritic cell precursors (pre-DC1) (by 48-fold) and lymphoid $CD11c^-$ dendritic cell precursors (pre-DC2) (by 13-fold).[74] It remains to be shown whether rhGM-CSF or Flt3 ligand given alone or in combination with rhG-CSF in vivo modulates the immune response in a manner distinct from that shown for in vivo rhG-CSF treatment alone.

Monocyte-dependent T-cell inhibition

Human monocytes express G-CSF receptors, and rhG-CSF administration consistently induces peripheral blood monocytosis in healthy donors.[75] Not unexpectedly, cytokine-mobilized apheresis products contain a significantly higher proportion of $CD14^+$ monocytes than normal PB; the same is true, though to a lesser extent, for nonmobilized apheresis products, suggesting that the apheresis procedure itself enriches monocytes. RhG-CSF administration modulates the surface expression of effector cell molecules on human monocytes.[75] As shown by Mielcarek et al,[76] monocytes suppress alloantigen-induced T-cell proliferation mainly in a noncontact way. This T-cell inhibitory effect is independent of the type of cytokine used.[77] Therefore, besides the previously discussed rhG-CSF–induced alteration of the cytokine secretion pattern, this monocyte-dependent T-cell inhibitory effect may also contribute to the relatively low incidence of GVHD seen after allogeneic PBSCT.

Conclusions

Hematopoietic stem cells traffic constantly between extravascular marrow spaces and PB. Therefore, the quality of stem cells is not thought to be different between BM and PB stem cell pools. BM and PBSC allografts differ in their reconstitutive and immunogenic characteristics, which seem to be based on the proportion of early pluripotent and self-renewing stem cells to lineage-committed late progenitor cells and on the number of accompanying accessory cells, particularly T-cell subsets, contained in the stem cell allografts.

PBSC allografts contain a 3- to 4-fold higher number of $CD34^+$ cells and an approximately 10-fold higher total number of lymphoid subsets when mobilized with rhG-CSF. The potential risks of PBSC and BM harvesting are seemingly comparable, though the potential long-term effects of cytokine mobilization treatment are unknown. There are clear discrepancies of neutrophil and platelet reconstitution between the 2 transplantation modalities, in favor of allogeneic PBSCT. The incidence of acute GVHD is reported to be mostly similar. The issue of the incidence of chronic GVHD remains unsettled—it was found to be higher after PBSCT in the prospective and retrospective registry analyses but to be similar in 2 large prospective studies. Different cytokine and GVHD prophylaxis regimens may contribute to this discrepancy. In 2 major studies (prospective randomized and retrospective registry data), the disease-free survival rates were higher after PBSCT, especially in patients with advanced-stage disease. Data on immune reconstitution are in favor of PBSCT but discrepant for NK cell reconstitution. The significantly lower incidence of molecular and cytogenetic relapse in patients with CML is indicative of a more pronounced GVL effect after PBSCT. Whether this beneficial GVL effect holds up in more aggressive disease categories remains to be shown. The megadose concept of $CD34^+$ cells, including veto cells, contained in PBSC allografts allows crossing major HLA barriers. It has been proposed that RhG-CSF–primed BM allografts combine fast cellular reconstitution with an incidence of GVHD similar to steady state BMT, but the available data are inconclusive.

In addition to the use of cytokines to efficiently mobilize $CD34^+$ progenitor cells, other immunomodulatory aspects open up a new way to manipulate allogeneic PBSC grafts in vivo before transfusion into the patient. RhG-CSF has a global immunosuppressive effect on the PBSC allograft, including the shifting of the cytokine secretion profile to a Th2 type. Flt3 ligand and rhG-CSF are potent mobilization factors of distinct dendritic cell subsets in vivo that elicit distinct profiles of cytokines in T cells and immune responses in healthy persons. Therefore, hematopoietic growth factors should be studied further as potential adjuvants for in vivo modulators of immune response. Direct T-cell inhibition of monocytes preferentially collected in apheresis products also has an impact on the posttransplant immune response.

Does the stem cell source matter? Favorable disease-free survival rates after PBSCT, particularly in patients with advanced-stage cancer, give blood stem cell transplantation an advantage over BMT. It is foreseeable that allogeneic PBSCT will take advantage of in vivo stimulation and collection of cellular subsets, in addition to $CD34^+$ cells, that are beneficial to the patient. It is also conceivable that the stem cell product, together with accessory cells, will ultimately be tailored to the patient's disease characteristics and prognosis.

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