Experimental Hematology

The case for plerixafor to replace filgrastim as the optimal agent to mobilize peripheral blood donors for allogeneic hematopoietic cell transplantation

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Granulocyte colony-stimulating factor (G-CSF)-stimulated peripheral blood progenitor cells (G-PBs) from either a related or unrelated donor continue to be the preferred donor source for most allogeneic hematopoietic cell transplantation (HCT). Recently, the American Society for Blood and Marrow Transplantation has recommended marrow instead of G-PBs as an unrelated graft source due to its lower rate of chronic graft-versus-host disease (cGVHD). However, the use of marrow is limited by both clinical considerations (slower rate of engraftment and increased donor morbidity) and logistical considerations (use of operating room resources and increased physician utilization), so this recommendation has not been widely adopted. An optimal donor source would include the rapid engraftment characteristic and the low donor morbidity associated with G-PBs and a rate of cGVHD similar to or lower than that of marrow. Recent data suggest that plerixafor mobilized PBs (P-PBs) have the rapid engraftment characteristics of G-PBs in allogeneic HCT with less cGVHD. The biologic mechanism of the lower rate of cGVHD appears to be through mobilization of regulator natural killer cells and plasmacytoid dendritic cell precursors that are associated with lower acute and chronic GVHD compared with G-PBs and rapid engraftment characterized by rapid myeloid-repopulating capacity. We suggest that, based on the experience of the two Phase II clinical trials and the unique biology of plerixafor-mobilized donor product, it should be evaluated in Phase III trials as an approach to replacing G-CSF mobilization for allogeneic HCT. © 2018 ISEH – Society for Hematology and Stem Cells. Published by Elsevier Inc. All rights reserved.

Currently, the predominant approach to accessioning donor cells for hematopoietic cell transplantation (HCT) is the use of granulocyte colony-stimulating factor (G-CSF) treatment of the donor for a number of days, followed by leukapheresis of peripheral blood progenitor cells (G-PBs) [1]. Over 70% of adult allogeneic HCT procedures utilize G-PBs in the United States and Canada [2]. Studies have shown that allogeneic transplantation with unstimulated bone marrow (BM) allograft results in lower rates of acute and chronic graft-versus-host disease (aGVHD and cGVHD, respectively) and improved quality of life compared with G-PB grafts [1−3]. One of the definitive studies to confirm this conclusion was the prospective, randomized Phase III Blood and Marrow Transplant Clinical Trials Network Protocol 0201 (BMT CTN 0201) clinical trial that compared G-PBs with BM as a donor source for unrelated donor HCT. Although the overall survival was the same and the incidence of graft failure was significantly lower in patients receiving G-PBs, the incidence of cGVHD at 2 years in the G-PB group was significantly higher compared with BM. No significant difference in the incidence of aGVHD or relapse was observed. [4]. However, when the Center for Blood and Marrow Transplant Research investigated whether the study results influence the choice of donor source among HCT centers, they found no significant change in use of BM versus G-PB grafts. Ninety-two percent of respondents knew of the

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BMT CTN 0201 results, yet fewer than one-fifth of HCT physicians reported practice change [1] Observational data confirmed no discernible change in practice.

Recently, the American Society for Blood and Marrow Transplantation (ASBMT), in its "Choosing Wisely" recommendations, suggested based on a large consensus showing a higher rate of cGVHD with G-PB, that for unrelated donor HCT, that BM is the preferred source over PB [3]. However, procuring BM is an invasive procedure and is associated with risks of general anaesthesia. This recommendation is controversial for many centers and is challenging to implement. We will review the underlying reasons for the continued preference of G-PBs, their advantages and limitations, and discuss a newer agent, plerixafor, which mobilizes a unique blood product that is superior to G-PBs as an allograft.

Why does everyone use G-PBs as a donor source?

There is a strong case to use G-PBs as a donor source for allografting. G-PBs result in a much more rapid engraftment of neutrophils after HCT with potentially shorter hospitalizations after HCT [5]. As an example, the Canadian Blood and Marrow Transplantation Group (CBMTG) compared G-PBs with marrow as a donor source in related donor HCT and found in 228 adult patients that G-PBs had a more rapid time to neutrophil recovery and platelet recovery. The rates of aGVHD and cGVHD were not significantly different and the relapse rate was not different, with probability of survival at 30 months after HCT significantly better for G-PBs [5]. Not only did G-PBs result in more rapid engraftment, they eliminated a significant logistic problem for HCT centers, which is the need to schedule operating room time for a physician to harvest the marrow. Instead, the method paved the way for outpatient leukapheresis, primarily managed by qualified allied health workers. Most donors prefer G-PBs because marrow donors report increased fatigue and less energy 1 week after donation. Moreover, marrow donors report persistently moderate negative effects on quality of life and physical morbidity up to 1 month after donation compared with G-PB donors [6]. An additional factor that has facilitated the wide adoption of G-PBs as a donor source is the identical overall cost of G-PBs compared with marrow donor transplantation at 100 days after HCT [7]. Contrasting data showing poorer survival in children with G-PBs have limited its application in the pediatric population [8,9].

What are the limitations of G-PBs as an allogeneic donor graft?

Although G-CSF treatment presents some immediate risk to the donor, including splenomegaly with a an up to 1% estimated risk of splenic rupture [10] and bone pain [11], the main reason for the recommendation by the ASBMT to use marrow as a donor source over G-PBs is the risk to the recipient of cGVHD. There is now a wealth of data suggesting higher rate of cGVHD in both related and unrelated G-PB donor HCT [4,8,12−16]. The higher rate of cGVHD associated with G-PBs compared with marrow is also seen with alternative donor transplantations. When G-PBs are used as a donor source in related haploidentical donor transplantations treated with posttransplantation cyclophosphamide (PTCy); both grade 2−4 aGVHD (hazard ratio [HR], 0.45; $p < 0.001$) and cGVHD (HR, 0.35; $p < 0.001$) were higher with G-PB [17] compared with marrow as a donor source in PTCy transplantations.

There is a compelling argument to be made for minimizing cGVHD, which results in significant morbidity and mortality, decreased quality of life, lifelong demands on the health care system, and a shortened lifespan. European BMT leaders have therefore declared pre-emptive strategies to minimize or eliminate cGVHD as imperative [18]. The cost of cGVHD treatment is significant and lifelong. One study of the economic burden of cGVHD [19] estimated a worldwide loss of $25 billion U.S. dollars in lost wages from 43,750 years of foregone employment and an overall 10-year cost of $30.2 billion. The Canadian Institutes of Health Research-funded Applied Biomarkers in Late Effects of Childhood and Adolescent Cancer (ABLE) study on over 303 children evaluated the impact of cGVHD on the health care system. Using British Columbia provincial healthcare administrative datasets, the ABLE study found that children with cGVHD have a rate of physician utilization $1.9 \times$ higher and prescription rate $2.3 \times$ higher than pediatric BMT patients without cGVHD [20]. Further inflating chronic health care costs, management of cGVHD, with its multiorgan involvement, requires expert evaluation by a multidisciplinary team [21,22]. Therefore, any donor source that would minimize the development of cGVHD will have a significant impact on the outcome and cost of allogeneic HCT. Last, established protocols of mobilization generally require 4−6 days of daily G-CSF injections that may cause considerable inconvenience to donors with daily clinic visits to receive the injections and absence from work during the leukapheresis. Although long-acting G-CSF may overcome the need for multiple injections, a drug that can mobilize rapidly so that procurement of graft can occur on the same day is highly advantageous.

What about other donor sources?

Although umbilical cord blood has become an exciting source for infants and young children, it is limited in its application for adults due to the number of cells

infused per weight of the recipient required for sufficient engraftment [23]. Moreover, umbilical cord blood is also limited by the fact that the donor cannot be approached again for cells potentially required for treatment of infections, relapse, or rejection and the slower rate of engraftment [24].

One proposed alternative to G-PBs was G-CSF-stimulated bone marrow (G-BM) [25]. The CBMTG evaluated the efficacy of G-BM compared with G-PBs in a large Phase III study. Using a composite endpoint of extensive cGVHD, relapse/disease progression, and death, the study found no difference between G-BM versus G-PBs for the primary composite outcome. However, the cumulative incidence of overall cGVHD was lower with G-BM and there was no difference in the risk of relapse or progression. There was also no difference in secondary engraftment-related outcomes, such as time to first hospital discharge [6]. Despite these results, G-BM has not been adopted as standard of practice primarily due to the difficulty of operating room scheduling, the increased utilization of physician time to perform the marrow harvest, and the morbidity to the donor. We suggest that approaches that can give a similar or lower cGVHD rate as marrow or G-BM, more rapid engraftment of G-PBs or G-BM, and that could be accessioned through a leukaphereis approach would be optimal donor source for adult donors. The need for alternative mobilization approach or strategy is needed to replace G-PBs. A recent comprehensive review of stem cell mobilization concluded that there is currently no optimal stem cell mobilization regimen [26].

There are other established prophylactic approaches to lower cGvHD in addition to the type of donor source selected. These include ex vivo manipulation of the donor source including CD34 selection, TCRα/β and CD19 B cell depletion, and CD45RA T-cell depletion [27−29]. There are also in vivo depletion approaches including peri-transplantation ATG [30], alemtuzumab [31], posttransplantation rituximab [32], and PTCy [33,34]. At this point, it is unclear which of these approaches offers the optimal approach. In an attempt to answer this question, the open BMT CTN 1301 (PROGRESS II) trial is comparing three GvHD prophylaxis strategies on their ability to decrease cGvHD: 1. CD34 selected T-cell-depleted peripheral blood stem cell (PBSC) graft, 2. unmanipulated bone marrow graft followed by PTCy, or 3. an unmanipulated marrow graft with tacrolimus/methotrexate GVHD prophylaxis. Neither CD34 selection nor PTCy excludes possible future use of an optimal donor mobilization approach and in fact the combination of a source that already has an associated lower rate of cGvHD with one of these ex vivo or in vivo strategies may result in an even better outcome. Although there are a number of effective drug approaches to treat cGvHD, including sirolimus, mycophenolate mofetil, rituximab, extracorporeal photopheresis, ruxolitinib, ibrutinib, and imatinib [35,36], none has been used prophylactically to successfully decrease the incidence of cGvHD.

What donor product composition would give the best donor source?

In the last few years, several studies have performed correlative evaluation of the donor source in an attempt to identify which donor product components are most important in the development of cGVHD. Correlative studies from the prospective Phase III BMT CTN 0201 that evaluated unrelated donor BM versus G-PBs found that patients receiving a marrow donor with a higher median number of plasmacytoid dendritic cells (pDCs), naive CD8$^+$ T cells, or naive CD4$^+$ T cells had better 3-year overall survival, less aGVHD, and similar rates of relapse. Moreover, increased pDCs in the harvest was associated with fewer deaths resulting from GVHD or graft rejection, but not the incidence of either aGVHD or cGvHD. Interestingly, evaluations on G-PB grafts did not identify a donor cell subset significantly associated with overall survival, relapse, or GVHD [29]. Therefore, the study could not identify a donor cell population that was associated with the higher rate of cGvHD seen with G-PBs.

A similar analysis was performed by the CBMTG in a large Phase III randomized trial (CBMTG 0601) with HLA-identical sibling donors that compared the impact of G-PBs with G-BM on the development of cGvHD. In this trial, G-BM graft had a significantly lower frequency of cGvHD compared with G-PBs [37]. After a comprehensive analysis of donor immune cell composition, the study identified three donor cell populations that were associated with an increased incidence of cGvHD, including higher memory B cell, lower INFγ-producing CD4$^+$ T cells, and lower number of CD56^{bright} natural killer (NK) cells. A number of donor immune cell populations did not affect the development of cGvHD in this large study include CD8$^+$ T cells, regulatory T cells (T$_{reg}$s), pDCs, and myeloid dendritic cell. The study found that of these three populations associated with a higher rate of cGvHD, only lower numbers of CD56^{bright} regulatory NK (NK$_{reg}$) cells in G-PBs explained the underlying mechanism for the higher rate of cGvHD associated with G-PBs compared with G-BM [38]. Confirmation of the relative importance of this immune regulatory population is its correlation of low numbers of CXCR3$^+$CD56^{bright} NK$_{reg}$ cells with the onset of cGvHD in adults [39]. This population also expresses CD335 and is perforin negative. Currently, the pDC or pDC precursor (pre-pDC) populations appear to primarily correlate with development of aGVHD [40]. By contrast, we observed CD56^{bright} NK$_{reg}$ cells correlated with both a lower rate of aGvHD and cGvHD [38,39] similar to that

seen with plerixafor mobilized peripheral blood transplantation [40,41]. Although the large Phase III CBMTG study clearly identified that CD56^{bright} NK$_{reg}$ cells appeared to be the reason for the high rate of cGvHD associated with G-PBs, it was limited in that it only included matched related donors and the comparison group was G-BM rather than BM. It is possible that, when other studies include comparisons of unrelated donors or compare G-PBs with BM, other donor cell populations that correlate with cGvHD, such as B cells, may be implicated as a cause for the high rate of cGvHD seen with G-PBs. At present, NK$_{reg}$ cells are the only cell population clearly associated with the high rate of cGvHD in G-PBs, based on one large Phase III clinical trial, and we propose that mobilization strategies that increase NK$_{reg}$ cells and possibly pre-pDCs may result in a product that has a low rate of cGvHD. Other populations expanded in a P-PB donor product include B cells and overall NK cells [40−44].

There are a number of additional cell populations have been implicated in cGvHD either based on murine models or in humans. There is evidence that the number of T cells infused is important for development of cGvHD and that ex vivo CD3$^+$ T-cell depletion studies can have an impact on the development of aGvHD and cGvHD [45]. Similarly, T$_{reg}$s clearly appear to play a role in aGvHD [46]. Although they can alter murine cGvHD [47], their role in human cGvHD is much less clear. It has been difficult to establish a reproducible correlation between the numbers and/or function of T$_{reg}$s and cGVHD. Studies have shown that patients have markedly elevated numbers of T$_{reg}$s [48,49], decreased T$_{reg}$ numbers [50,51], and no correlation between T$_{reg}$ numbers and cGvHD [52,53]. One form of successful cGvHD therapy is low-dose interleukin-2 (IL-2) [54]. Low-dose IL-2 therapy causes a significant expansion of T$_{reg}$s, but that expansion does not correlate with therapeutic outcome, so causality is not apparent. Only when T$_{reg}$s are looked at in proportion to conventional CD4$^+$ T cells is a correlation of therapeutic response to low dose IL-2 observed [55]. Interestingly, the other population that is significantly expanded by low-dose IL-2 is CD25$^+$ CD56^{bright} NK$_{reg}$ cells [56]. The other population is that of naive CD4$^+$ T cells. There are data supporting that CD45RA$^+$ CD4$^+$ T cells are increased as a possible effector cell population and are associated with higher rates of cGvHD [57]. In our own experience, plerixafor did not affect CD45RA$^+$ CD4$^+$ T cells and we did not find it to be important in the CBMTG populations. Conversely, there are ex vivo strategies being pursued in clinical trials and such an approach may be combined with mobilization strategies in the future. Other immune cell populations that may affect cGvHD are regulatory B cells and M2 macrophages [58]. The impact of plerixafor on these populations is not known.

Is plerixafor the optimal mobilizing agent for a PB collection?

Plerixafor binds to and blocks the chemokine receptor type 4 (CXCR4) on stem cells, which results in migration of the stem cells into the bloodstream. There have now been two early-phase trials evaluating P-PBs as a donor source in allogeneic HCT. The first trial evaluated the donor source in 20 patients and found that all patients engrafted rapidly with neutrophils (median: day 10) and platelets (median: day 12). The study reported lower aGVHD (e.g., grade 2−4 aGVHD at 35%) and cGVHD (at 33%) rates relative to G-PB donor HCT and these rates were similar to marrow allograft [42]. A second more recent trial evaluated 33 patients and despite twofold higher numbers of CD3$^+$ and CD4$^+$ T cells contained in the P-PB grafts relative to G-PBs, recipients of P-PB allografts showed low rates of grade 2−4 aGVHD (21%) and cGVHD (35%) [40]. Most donors receiving plerixafor experienced some adverse effects, but all were mild. The most common grade 1 toxicities included light-headedness, nausea, bloating or flatulence, injection site discomfort or warm sensation, perioral paresthesias, loose stools, diaphoresis, and headache. The median time to neutrophil and platelet engraftment for recipients receiving P-PBs compared with G-PBs was equivalent. This was particularly interesting because CD34$^+$ cell doses in P-PBs were significantly lower compared with historical controls who had received 5 days of G-PBs. This may be partly due to the fact that plerixafor preferentially mobilized a unique CD34$^+$ interferon-alpha (IFNα)−producing pDC population that was CD34$^+$CD45RA$^+$CD123^{++}, characteristic of the pro-DC2 subpopulation [44]. These findings suggest that P-PBs have unique characteristics that result in lower rates of aGVHD and cGVHD.

At present, it is uncertain whether the relapse rate associated with the use of P-PBs is higher than for G-PBs. Of the two Phase II trials, in one trial, five patients relapsed between days 47 and 257 after transplantation in a a total of 25 patients. There was an overall 1-year survival of 73% [42]. The second study included 22 Phase I and 34 Phase II trial patients and allowed inclusion of reduced-intensity conditioning regimen [40]. In that study, there was a 45% overall 1-year survival rate with a 238 day median relapse-free survival. Although these are small numbers, four of five patients who received the reduced-intensity conditioning relapsed. Larger trials are needed to establish whether the relapse rate is different from that of G-PBs.

Does a plerixafor-mobilized donor product result in rapid engraftment and lower cGVHD?

Measurements of peripheral blood CD34$^+$ cells, in vitro myeloid colony-forming cells, 3- and 6-week long-term culture (LTC) cell outputs, and levels of circulating

human platelets (Table 1), as well as myeloid and lymphoid cells obtained in immunodeficient mice that received transplantations, all showed activities that were maximal 4 hours after plerixafor preceded by 4 days of G-CSF. Further, 3-week LTC outputs showed the highest concordance with the 3-week circulating human neutrophil levels obtained in mice that received transplantations [59]. Similar to the previously mentioned lack of correlation of CD34$^+$ cell dose with engraftment, the 3-week LTC assay appeared to offer a more specific predictor of neutrophil engraftment levels than conventional CD34$^+$ cell or colony-forming cell counts. Although mobilization was highest with G-CSF followed by plerixafor, it was not different from plerixafor alone but significantly higher than G-CSF alone.

A recent study evaluated P-PB for pre pDCs confirmed a lower rate of aGVHD [40]. The authors also showed that plerixafor mobilized a unique hematopoietic progenitor cell product that is enriched in pre-pDCs that produce high levels of IFNα. The study was too small to evaluate whether there was a direct correlation of the pre-pDCs with either aGVHD or cGVHD [44]. However, these data suggest the hypothesis that the relatively rapid onset of mobilization by plerixafor (hours vs. days by G-CSF) generates a novel PB graft that immunologically and phenotypically resembles cells harvested from bone marrow [40−44,59]. The resulting unique allograft contains immune subsets, including pre-pDCs and pDCs, which favorably modulate alloreactivity after transplantation, thus resulting in lower GvHD rates.

Recently, we hypothesized that an additional donor cell population, CD56^{bright} NK$_{reg}$ cells, may affect the development of cGVHD and that there is a selective increase in NK$_{reg}$ cells by plerixafor. Similar to earlier studies [40,60], we observed the peak action of plerixafor at 4 hours after administration and selected this time point for further analyses of plerixafor effect on CD56^{bright} NK$_{reg}$ cells. We found that plerixafor induced a significantly higher rise of CD56^{bright} NK$_{reg}$ cells in peripheral blood than 4 days of G-CSF alone or G-CSF followed by plerixafor. This result suggested that plerixafor effectively mobilized CD56^{bright} NK$_{reg}$ cells to the peripheral blood. Because the source of allograft influences the risk of GvHD, we examined the effect of plerixafor on the proportion of CD56^{bright} NK$_{reg}$ cells in the peripheral blood. We found a significant increase in the proportion of CD56^{bright} NK$_{reg}$ cells in peripheral blood relative to BM after plerixafor administration. The CBMTG clinical trial demonstrated that G-CSF-mobilized BM allograft was associated with a lower incidence of GvHD compared with G-CSF-mobilized PBs [38]. Moreover, a G-CSF-

Table 1. Comparison of donor sources mobilized by G-CSF and plerixafor

Donor Source	G-PBs vs. BM	G-BM vs. BM	P-PBs vs. BM	P-PBs vs. G-PBs
Clinical Features: Impact on Recipients				
Engraftment rate	Higher	Higher	Higher	Same
aGvHD	Higher	Same	Same	Lower
cGvHD	Higher	Same	Same	Lower
Leukemia relapse	Same	Same	Same	Same
Clinical Features: Impact on Donors				
Quality of Life	Higher	Same	Higher	Same
Hematopoietic Characteristics [59−61]				
In Vitro Characteristics				
CD34	+++	+	+++	++
CFC	++	++	++	Same
LTC (3 and 6 weeks)	+++	++	+++	Same
In vivo Engraftment in Immune Compromised Mice (at 3 Weeks)				
CD45	++	++	+++	+
Myeloid	++	ND	+++	+
B cells	Same	ND	+	Same
Platelets	++	ND	+++	++
Immunologic Characteristics [25,38−44,58]				
T cells	++	Same	+++	++
B cells	+	++	+++	++
NK cells	+	++	+++	+
Regulatory Populations Associated with Lower cGvHD [26,38,70,71]				
NK$_{reg}$ cells	−	Same	++	++++
Pre-pDCs and pDCs	In BM associated with lower aGvHD	Unknown	++	+++
T$_{reg}$s	−	Same	±	?

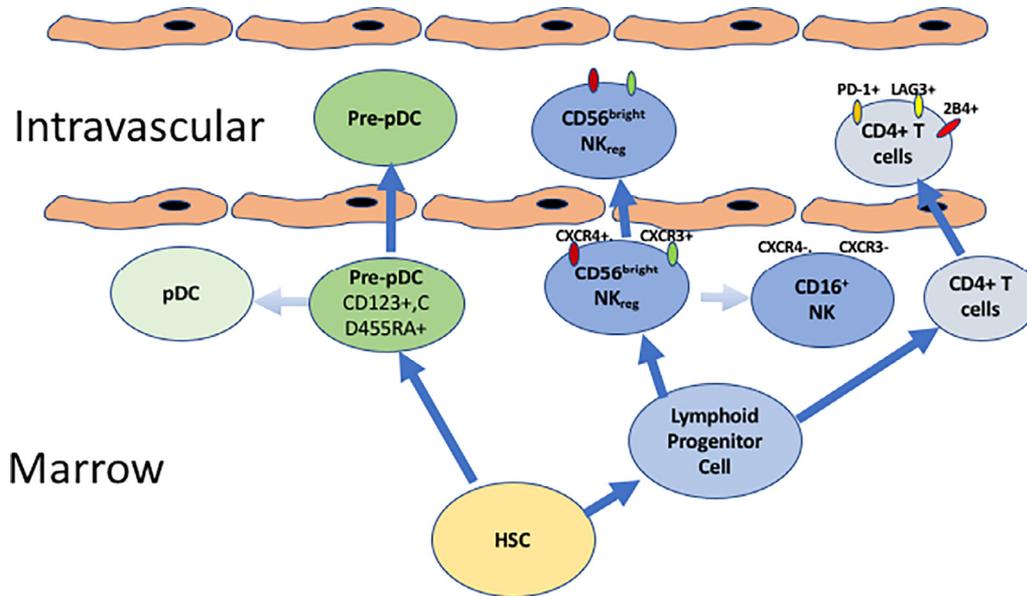ND=not done, ?=uncertain, −=lower, +=higher, ±=variable results in studies.

Figure 1. Proposed effect of plerixafor mobilization on donor immune cell components. Based on murine and human data, plerixafor appears to increase release of immature NK cells, T cells, B cells, and dendritic cell populations. Some of these populations, including CD56^{bright} NK$_{reg}$ cells and pre-pDCs, appear to be have regulatory immune function and decreased cGvHD (CD56^{bright} NK$_{reg}$ cells) and aGvHD (CD56^{bright} NK$_{reg}$ cells and pre-pDCs).

mobilized bone marrow allograft that contains higher number of CD56^{bright} NK$_{reg}$ cells is associated with lower frequency of GvHD [38]. In this study, plerixafor induced a significantly larger increase in CD56^{bright} NK$_{reg}$ cell population in the peripheral blood than G-CSF-stimulated BM allograft. These results suggested that plerixafor mobilized the highest proportion of CD56^{bright} NK$_{reg}$ cells to the peripheral blood compared with bone marrow. One limitation of this study is that we evaluated peripheral blood rather than a peripheral apheresis product and apheresis may alter graft composition.

Peripheral CD56^{bright} NK cells have a known regulatory function and are similar to decidual CD56^{bright} NK cells associated with immune tolerance necessary in the maternal−fetal graft between the uterus and placenta [61,62]. CD56^{bright} NK$_{reg}$ cells express a number of surface antigens including CD94/NKG2A, NCR1 (NKp46 or CD335), IL1RA, IL18RA, DNAM-1, CCL7, CXCR, and preferentially express granzyme K [63]. There appear to be a number of mechanisms by which CD56^{bright} NK$_{reg}$ cells modulate immune responses. The mechanism by which NK$_{reg}$ cells modulate CD4$^+$ T cells include cytotoxicity through granzyme K and granzyme B secretion as well as by NKGD−NKGD ligation and NCR−NCR ligation [64]. However, the non-cytolytic NK$_{reg}$ cells appear to be what we primarily observe and they have been described to reduce proliferation of CD4$^+$ T cells by secretion of adenosine, granzyme, and perforin. Interestingly, a recent study has shown that NK cells inhibit germinal center activity

and may depress follicular T-helper cell and B-cell interaction, resulting in decreased high affinity antibody production [64]. However, the exact mechanism by which CD56^{bright} NK$_{reg}$ cells modulate cGvHD at this point is unclear. Previously, it has been shown that CD16- CD56^{bright} NK cells population are the only NK or NKT populations that uniformly expressed trafficking molecules, including CXCR3 and CXCR4, which are necessary for homing into secondary lymphoid organs through high endothelial venules [65]. Therefore, we propose that plerixafor preferentially mobilizes the CD56^{bright} NK$_{reg}$ population (Figure 1).

Other immune cell populations that express the homing receptor CXCR4 include T cells, CD34$^+$ cells, NK cells, B cells, and pDCs. Other CXCR4 antagonists used to mobilize peripheral blood, including balixafortide [66] and BL-8040 [67], both appear to have similar mobilization patterns of T cells, B cells, CD34$^+$ cells, CD56$^+$ NK cells, and pDCs. In addition, in mice, CXCR4 blockade is associated with decreased PD-1$^+$LAG-3$^+$2B4$^+$CD4$^+$ T cells, suggesting that blockade of CXCR4 mitigates CD4$^+$ T-cell exhaustion [68]. Thymic T$_{reg}$s increase the expression of CXCR4 as they home to secondary lymphoid tissues, but little is known regarding the impact of plerixafor on this process [69] and the role of T$_{reg}$s in modulating cGVHD is variable. Overall, the rapid mobilization of immature lymphoid populations by plerixafor appears to result in a donor product that is more tolerant.

In this review, we have demonstrated that a single subcutaneous administration of plerixafor results in a

donor product that has a more rapid myeloid engraftment, minimal donor toxicity, and a low rate of both aGVHD and cGVHD with a similar relapse and engraftment rates compared with other graft sources. Moreover, there is a biological characteristic of P-PBs that explains the rapid engraftment and lower aGvHD and cGvHD rates. We suggest that the unique donor product mobilized by plerixafor meets criteria for ease of collection, rapid engraftment, and increased cell populations associated with cGvHD that will be superior to current donor collection strategies.

Acknowledgments

Conflict of interest disclosure

References

1. Khera N, Mau LW, Denzen EM, et al. Translation of clinical research into practice: an impact assessment of the results from the Blood and Marrow Transplant Clinical Trials Network Protocol 0201 on Unrelated Graft Source Utilization. Biol Blood Marrow Transplant. 2018;24:2204–2210.
2. Kollman C, Spellman SR, Zhang MJ, et al. The effect of donor characteristics on survival after unrelated donor transplantation for hematologic malignancy. Blood. 2016;127:260–267.
3. Bhella S, Majhail NS, Betcher J, et al. Choosing wisely BMT: American Society for Blood and Marrow Transplantation and Canadian Blood and Marrow Transplant Group's list of 5 tests and treatments to question in blood and marrow transplantation. Biol Blood Marrow Transplant. 2018;24:909–913.
4. Anasetti C, Logan BR, Lee SJ, et al. Blood and Marrow Transplant Clinical Trials Network. Peripheral-blood stem cells versus bone marrow from unrelated donors. N Engl J Med. 2012;367:1487–1496.
5. Couban S, Simpson DR, Barnett MJ, et al. Canadian Bone Marrow Transplant Group. A randomized multicenter comparison of bone marrow and peripheral blood in recipients of matched sibling allogeneic transplantations for myeloid malignancies. Blood. 2002;100:1525–1531.
6. Bredeson C, Leger C, Couban S, et al. An evaluation of the donor experience in the canadian multicenter randomized trial of bone marrow versus peripheral blood allografting. Biol Blood Marrow Transplant. 2004;10:405–414.
7. Couban S, Dranitsaris G, Andreou P, et al. Clinical and economic analysis of allogeneic peripheral blood progenitor cell transplantations: a Canadian perspective. Bone Marrow Transplant. 1998;22:1199–1205.
8. Eapen M, Horowitz MM, Klein JP, et al. Higher mortality after allogeneic peripheral-blood transplantation compared with bone marrow in children and adolescents: the Histocompatibility and Alternate Stem Cell Source Working Committee of the International Bone Marrow Transplant Registry. J Clin Oncol. 2004;22:4872–4880.
9. Khandelwal P, Millard HR, Thiel E, et al. Hematopoietic stem cell transplantation activity in pediatric cancer between 2008 and 2014 in the United States: a Center for International Blood and Marrow Transplant Research report. Biol Blood Marrow Transplant. 2017;23:1342–1349.
10. Stiff PJ, Bensinger W, Abidi MH, et al. Clinical and ultrasonic evaluation of spleen size during peripheral blood progenitor cell mobilization by filgrastim: results of an open-label trial in normal donors. Biol Blood Marrow Transplant. 2009;15:827–834.
11. Lambertini M, Del Mastro L, Bellodi A, Pronzato P. The five "Ws" for bone pain due to the administration of granulocyte-colony stimulating factors (G-CSFs). Crit Rev Oncol Hematol. 2014;89:112–128.
12. Schrezenmeier H, Passweg JR, Marsh JC, et al. Worse outcome and more chronic GVHD with peripheral blood progenitor cells than bone marrow in HLA-matched sibling donor transplantations for young patients with severe acquired aplastic anemia. Blood. 2007;110:1397–1400.
13. Schmitz N, Eapen M, Horowitz MM, et al. International Bone Marrow Transplant Registry; European Group for Blood and Marrow Transplantation. Long-term outcome of patients given transplantations of mobilized blood or bone marrow: A report from the International Bone Marrow Transplant Registry and the European Group for Blood and Marrow Transplantation. Blood. 2006;108:4288–4290.
14. Eapen M, Logan BR, Appelbaum FR, et al. Long-term survival after transplantation of unrelated donor peripheral blood or bone marrow hematopoietic cells for hematologic malignancy. Biol Blood Marrow Transplant. 2015;21:55–59.
15. Chu R, Brazauskas R, Kan F, et al. Comparison of outcomes after transplantation of G-CSF-stimulated bone marrow grafts versus bone marrow or peripheral blood grafts from HLA-matched sibling donors for patients with severe aplastic anemia. Biol Blood Marrow Transplant. 2011;17:1018–1024.
16. Eapen M, Logan BR, Confer DL, et al. Peripheral blood grafts from unrelated donors are associated with increased acute and chronic graft-versus-host disease without improved survival. Biol Blood Marrow Transplant. 2007;13:1461–1468.
17. Bashey A, Zhang MJ, McCurdy SR, et al. Mobilized peripheral blood stem cells versus unstimulated bone marrow as a graft source for T-cell-replete haploidentical donor transplantation using post-transplant cyclophosphamide. J Clin Oncol. 2017;35:3002–3009.
18. Bacigalupo A, Sica S, van Lint MT. Failure to effectively treat chronic graft-versus-host disease: a strong call for prevention. Haematologica. 2016;101:e214–e215.
19. Murphy E, Black N, Lamping D, et al. Consensus development methods, and their use in clinical guideline development: a review. Health Technology Assessment. 1998. Available from https://www.journalslibrary.nihr.ac.uk/hta/hta2030#.
20. Schultz KR, Zhang Y, Mary L, McBride ML. High rate of health care utilization associated with chronic graft-versus-host disease among Canadian childhood cancer survivors treated with blood and marrow transplantation. In: European Society for Blood and Marrow Transplantation meeting; March 18, 2018.
21. Crespo C, Pérez-Simón JA, Rodríguez JM, Sierra J, Brosa M. Development of a population-based cost-effectiveness model of chronic graft-versus-host disease in Spain. Clin Ther. 2012;34:1774–1787.
22. Jones CA Chapman DG, Fernandez L, Mesa OA, Peters C. Burden of cost in bronchiolitis obliterans syndrome (Bos): Predictions for the next decade. Value Health. 2015;18:A840.
23. Rodrigues CA, Sanz G, Brunstein CG, et al. Analysis of risk factors for outcomes after unrelated cord blood transplantation in adults with lymphoid malignancies: a study by the Eurocord-Netcord and lymphoma working party of the European group for blood and marrow transplantation. J Clin Oncol. 2009;27:256–263.

24. Miflin G, Russell NH, Hutchinson RM, et al. Allogeneic peripheral blood stem cell transplantation for haematological malignancies−an analysis of kinetics of engraftment and GVHD risk. Bone Marrow Transplant. 1997;19:9–13.

25. Frangoul H, Nemecek ER, Billheimer D, et al. A prospective study of G-CSF primed bone marrow as a stem-cell source for allogeneic bone marrow transplantation in children: a Pediatric Blood and Marrow Transplant Consortium (PBMTC) study. Blood. 2007;110:4584–4587.

26. Duong HK, Savani BN, Copelan E, et al. Peripheral blood progenitor cell mobilization for autologous and allogeneic hematopoietic cell transplantation: guidelines from the American Society for Blood and Marrow Transplantation. Biol Blood Marrow Transplant. 2014;20:1262–1273.

27. Saad A, Lamb LS. Ex vivo T-cell depletion in allogeneic hematopoietic stem cell transplant: past, present and future. Bone Marrow Transplant. 2017;52:1241–1248.

28. Busca A, Aversa F. In-vivo or ex-vivo T cell depletion or both to prevent graft-versus-host disease after hematopoietic stem cell transplantation. Expert Opin Biol Ther. 2017 Nov;17(11):1401–1415.

29. Waller EK, Logan BR, Harris WA, et al. Improved survival after transplantation of more donor plasmacytoid dendritic or naïve T cells from unrelated-donor marrow grafts: results from BMTCTN 0201. J Clin Oncol. 2014;32:2365–2372.

30. Walker I, Panzarella T, Couban S, et al. Pretreatment with anti-thymocyte globulin versus no anti-thymocyte globulin in patients with haematological malignancies undergoing haemopoietic cell transplantation from unrelated donors: a randomised, controlled, open-label, phase 3, multicentre trial. Lancet Oncol. 2016;17:164–173.

31. Ali R, Ramdial J, Algaze S, Beitinjaneh A. The role of anti-thymocyte globulin or alemtuzumab-based serotherapy in the prophylaxis and management of graft-versus-host disease. Biomedicines. 2017;5:E67.

32. Arai S, Sahaf B, Narasimhan B, et al. Prophylactic rituximab after allogeneic transplantation decreases B-cell alloimmunity with low chronic GVHD incidence. Blood. 2012;119:6145–6154.

33. Al-Homsi AS, Roy TS, Cole K, Feng Y, Duffner U. Post-transplant high-dose cyclophosphamide for the prevention of graft-versus-host disease. Biol Blood Marrow Transplant. 2015;21:604–611.

34. Mussetti A, Greco R, Peccatori J, Corradini P. Post-transplant cyclophosphamide, a promising anti-graft versus host disease prophylaxis: where do we stand? Expert Rev Hematol. 2017;10:479–492.

35. Cutler CS, Koeth J, Ritz J. Mechanistic approaches for the prevention and treatment of chronic GVHD. Blood. 2017;129:22–29.

36. Flowers ME, Martin PJ. How we treat chronic graft-versus-host disease. Blood. 2015;125:606–615.

37. Couban S, Aljurf M, Lachance S, et al. Filgrastim-stimulated bone marrow compared with filgrastim-mobilized peripheral blood in myeloablative sibling allografting for patients with hematologic malignancies: a randomized Canadian Blood and Marrow Transplant Group study. Biol Blood Marrow Transplant. 2016;22:1410–1415.

38. Kariminia A, Ivison S, Ng B, et al. CD56bright natural killer regulatory cells in filgrastim primed donor blood or marrow products regulate chronic graft-versus-host disease: the Canadian Blood and Marrow Transplant Group randomized 0601 study results. Haematologica. 2017;102:1936–1946.

39. Kariminia A, Holtan SG, Ivison S, et al. Heterogeneity of chronic graft-versus-host disease biomarkers: association with CXCL10 and CXCR3+ NK cells. Blood. 2016;127:3082–3091.

40. Schroeder MA, Rettig MP, Lopez S, et al. Mobilization of allogeneic peripheral blood stem cell donors with intravenous plerixafor mobilizes a unique graft. Blood. 2017;129:2680–2692.

41. Wong PPC, Kariminia A, Jones D, et al. Plerixafor effectively mobilizes CD56bright NK cells in blood, providing an allograft predicted to protect against GVHD. Blood. 2018;131:2863–2866.

42. Devine SM, Vij R, Rettig M, et al. Rapid mobilization of functional donor hematopoietic cells without G-CSF using AMD3100, an antagonist of the CXCR4/SDF-1 interaction. Blood. 2008;112:990–998.

43. Kean LS, Sen S, Onabajo O, et al. Significant mobilization of both conventional and regulatory T cells with AMD3100. Blood. 2011;118:6580–6590.

44. Waller EK. Mobilizing plasmacytoid dendritic cells. Blood. 2017;129:2600–2602.

45. Jakubowski AA, Petrlik E, Maloy M, et al. T cell depletion as an alternative approach for patients 55 years or older undergoing allogeneic stem cell transplantation as curative therapy for hematologic malignancies. Biol Blood Marrow Transplant. 2017;23:1685–1694.

46. Brunstein CG, Miller JS, McKenna DH, et al. Umbilical cord blood-derived T regulatory cells to prevent GVHD: kinetics, toxicity profile, and clinical effect. Blood. 2016;127:1044–1051.

47. McDonald-Hyman C, Flynn R, Panoskaltsis-Mortari A, et al. Therapeutic regulatory T-cell adoptive transfer ameliorates established murine chronic GVHD in a CXCR5-dependent manner. Blood. 2016;128:1013–1017.

48. Clark FJ, Gregg R, Piper K, et al. Chronic graft-versus-host disease is associated with increased numbers of peripheral blood CD4+CD25high regulatory T cells. Blood. 2004;103:2410–2416.

49. Rozmus J, Schultz KR, Wynne K, et al. Early and late extensive chronic graft-versus-host disease (cGVHD) in children is characterized by different Th1/Th2 cytokine profiles: findings of the Children's Oncology Group Study (COG), ASCT0031. Biol Blood Marrow Transplant. 2011;17:1804–1813.

50. Y1 Miura, CJ Thoburn, Bright EC, et al. Association of Foxp3 regulatory gene expression with graft-versus-host disease. Blood. 2004;104:2187–2193.

51. Zorn E, Kim HT, Lee SJ, et al. Reduced frequency of FOXP3+ CD4+CD25+ regulatory T cells in patients with chronic graft-versus-host disease. Blood. 2005;106:2903–2911.

52. Sanchez J, Casaño J, Alvarez MA, et al. Kinetic of regulatory CD25high and activated CD134+ (OX40) T lymphocytes during acute and chronic graft-versus-host disease after allogeneic bone marrow transplantation. Br J Haematol. 2004;126:697–703.

53. Meignin V, Peffault de Latour R, Zuber J, et al. Numbers of Foxp3-expressing CD4+CD25high T cells do not correlate with the establishment of long-term tolerance after allogeneic stem cell transplantation. Exp Hematol. 2005;33:894–900.

54. Koreth J, Matsuoka K, Kim HT, et al. Interleukin-2 and regulatory T cells in graft-versus-host disease. N Engl J Med. 2011;365:2055–2066.

55. Koreth J, Kim HT, Jones KT, et al. Efficacy, durability, and response predictors of low-dose interleukin-2 therapy for chronic graft-versus-host disease. Blood. 2016;128:130–137.

56. Hirakawa M, Matos TR, Liu H, et al. Low-dose IL-2 selectively activates subsets of CD4+ Tregs and NK cells. JCI Insight. 2016;1:e89278.

57. Greinix HT, Kuzmina Z, Weigl R, et al. CD19+CD21low B cells and CD4+CD45RA+CD31+ T cells correlate with first diagnosis of chronic graft-versus-host disease. Biol Blood Marrow Transplant. 2015;21:250–258.

58. de Masson A, Bouaziz JD, Le Buanec H, et al. CD24(hi)CD27+ and plasmablast-like regulatory B cells in human chronic graft-versus-host disease. Blood. 2015;125:1830–1839.

59. Miller PH, Nakamichi N, Knapp DJHF, et al. Quantitation of human cells that produce neutrophils and platelets in vivo obtained from normal donors treated with granulocyte colony-stimulating factor and/or plerixafor. Biol Blood Marrow Transplant. 2016;22:1945–1952.

60. Liles WC, Broxmeyer HE, Rodger E, et al. Mobilization of hematopoietic progenitor cells in healthy volunteers by AMD3100, a CXCR4 antagonist.. Blood. 2003;102:2728–2730.

61. Peralta CG, Han VK, Horrocks J, Croy BA, van den Heuvel MJ. CD56bright cells increase expression of {alpha}4 integrin at ovulation in fertile cycles. J Leukoc Biol. 2008;84:1065–1074.

62. Chantakru S, Wang WC, van den Heuvel M, et al. Coordinate regulation of lymphocyte-endothelial interactions by pregnancy-associated hormones. J Immunol. 2003;171:4011–4019.

63. Michel T, Poli A, Cuapio A, et al. Human CD56bright NK cells: An update. J Immunol. 2016;196:2923–2931.

64. Rydynski CE, Cranert SA, Zhou JQ, et al. Affinity maturation is impaired by natural killer cell suppression of germinal centers. Cell Rep. 2018;24:3367–3373.e4.

65. Campbell JJ, Qin S, Unutmaz D, et al. Unique subpopulations of CD56+ NK and NK-T peripheral blood lymphocytes identified by chemokine receptor expression repertoire. J Immunol. 2011;166:6477–6482.

66. Karpova D, Bräuninger S, Wiercinska E, et al. Mobilization of hematopoietic stem cells with the novel CXCR4 antagonist POL6326 (balixafortide) in healthy volunteers-results of a dose escalation trial. J Transl Med. 2017;15:2.

67. Abraham M, Pereg Y, Bulvik B, et al. Single dose of the CXCR4 antagonist BL-8040 induces rapid mobilization for the collection of human CD34^{+} cells in healthy volunteers. Clin Cancer Res. 2017;23:6790–6801.

68. Ramonell KM, Zhang W, Hadley A, et al. CXCR4 blockade decreases CD4+ T cell exhaustion and improves survival in a murine model of polymicrobial sepsis. PLoS One. 2017;12:e0188882.

69. Lee JH, Kang SG, Kim CH. FoxP3+ T cells undergo conventional first switch to lymphoid tissue homing receptors in thymus but accelerated second switch to nonlymphoid tissue homing receptors in secondary lymphoid tissues. J Immunol. 2007;178:301–311.

70. Shier LR, Schultz KR, Imren S, et al. Differential effects of granulocyte colony-stimulating factor on marrow- and blood-derived hematopoietic and immune cell populations in healthy human donors. Biol Blood Marrow Transplant. 2004;10:624–634.

71. Zhao XY, Wang YT, Mo XD, et al. Higher frequency of regulatory T cells in granulocyte colony-stimulating factor (G-CSF)-primed bone marrow grafts compared with G-CSF-primed peripheral blood grafts. J Transl Med. 2015;13:145.