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REVIEW

Novel agents and approaches for stem cell mobilization in normal donors and patients

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In spite of the safety and efficiency of the classical mobilization protocols, recombinant human G-CSF ± chemotherapy, there is still a considerable amount of mobilization failures (10–30%), which warrant novel agents and approaches both in an autologous and an allogeneic transplant setting. Attempts to improve CD34+ yields by using several cytokines and growth factors as adjuncts to G-CSF could not change the standard approaches during the last decade, either because of inefficiency or the adverse events encountered with these agents. As a long-acting G-CSF analog, pegfilgrastim has the advantages of an earlier start of apheresis, reduction in the number of apheresis procedures as well as a reduced number of injections as compared with unconjugated G-CSF. However, dosing and cost-effectiveness especially in cytokine-only mobilizations require further investigation. As interactions between hematopoietic stem cells and the BM microenvironment are better understood, new molecules targeting these interactions are emerging. Plerixafor, which started its journey as an anti-HIV drug, recently ended up being a popular stem cell mobilizer with the ability of rapid mobilization and gained approval as an adjunct to G-CSF for poor mobilizers. At present, it is challenging to search for the best approach by using the available drugs with appropriate timing to provide sufficient CD34+ yield after an initial mobilization attempt, and in a cost-effective manner thereby avoiding further mobilization attempts and exposure to chemotherapy. Approaches not only for increasing stem cell yield, but also aiming to improve the quality of graft content and the associated transplantation outcomes are promising areas of research.

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INTRODUCTION

The most common mobilization protocols today are cytokines alone or cytokines after chemotherapy. Recombinant human G-CSF is reliable, with predictable mobilization efficiency and managable toxicities such as bone pain, low-grade fever and headache. However, G-CSF requires 5–6 days of administration and may be associated with rare serious side effects such as spontaneous splenic rupture, thrombosis, flare of autoimmune disease and precipitation of sickle crisis.[1] Recombinant human G-CSF commonly administered at 10 μg/kg/day for 4 days and PBPCs are collected by apheresis from day 5 onwards and G-CSF continued until the last day of apheresis. Randomized studies demonstrated similar outcomes when a twice daily (5 μg/kg/12 h) dosage of filgrastim was compared with a once daily (10 μg/kg/day) dosage.[2] However, there is controversy regarding the efficiency of twice daily, high-dose G-CSF administration versus once daily administration. Most studies have reported higher CD34+ cell yields when G-CSF 10 and 12 μg/kg/12 h were compared with G-CSF 10 μg/kg/day in healthy donors and patients, respectively.[3,4] Disease-specific intensive chemotherapy plus G-CSF followed by PBPC mobilization is an effective approach in lymphoma patients who require salvage therapy. The anti-CD20 MoAb, rituximab, commonly included in the salvage protocol in non-Hodgkin's lymphoma (NHL), does not seem to have any adverse effects on PBPC mobilization, even though the fact that the chemotherapy stimulation may overcome the inhibition of rituximab cannot be ruled out.[5–8] On the other hand, high-dose CY (3–4 g/m^2) plus G-CSF is commonly used in patients with plasma cell myeloma (PCM) who fail prior mobilization attempts with growth factor alone or who are assumed to have a high tumor load before transplantation. However, use of CY for mobilization in myeloma does not seem to improve response rates and time to progression after high-dose therapy and autologous hematopoietic cell transplantation (AHCT).[9] On the other hand, the studies have clearly demonstrated the superiority of CY plus G-CSF mobilization over G-CSF alone in PCM patients who received regimens containing lenalidomide, which is shown to interfere with mobilization on prolonged exposure. Thus, initial use of CY plus G-CSF for stem cell mobilization is recommended for patients who have received >4 cycles of lenalidomide-containing therapy.[10–12]

Mobilization with chemotherapy plus G-CSF yields 2- to 6- fold more CD34+ cells as compared with G-CSF alone. However, unpredictable timing of apheresis, requirement for hospitalization, the risk of febrile neutropenia and transfusion of blood products and associated costs are the drawbacks for using chemomobilization especially for sole mobilization purposes. Optimal timing of G-CSF administration after chemotherapy has not been defined conclusively. In our recent randomized study, we demonstrated similar efficiency in mobilizing CD34+ cells when G-CSF was administered late (day 7) or early (day 4) after chemotherapy in patients with PCM and lymphoma. Additionally, late administration of G-CSF was cost-effective.[13] Both glycosylated (lenograstim) and unglycosylated (filgrastim) G-CSF with or without chemotherapy have been used with similar success in PBPC mobilization and are equally well-tolerated in an autologous setting and also in

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Table 1. High-risk patients for mobilization failures

Older age
Extensive BM involvement with malignancy
Prior radiotherapy
Prior treatment with alkylating agents
Prior exposure to fludarabine
Prior prolonged exposure to lenalidomide
Multiple chemotherapy regimens

healthy allogeneic donors.[14,15] In spite of the safety and efficiency of the classical mobilization protocols, there is still a considerable amount of mobilization failures (10–30%), which suggests that novel PBPC mobilization methods are required (Table 1).[16–18]

ATTEMPTS TO IMPROVE PBPC MOBILIZATION

Historically, attempts to increase mobilization efficiency concentrated on using high doses of G-CSF or combining G-CSF with other cytokines and growth factors some of which are currently used in other indications. However, either because of inefficiency or adverse events (AEs), these agents could not become a part of the standard mobilization. The conflicting initial results about the superiority of high-dose G-CSF (16–32 μg/kg/day) in mobilizing CD34+ cells and the reported increased toxicity prevented this approach to be the preferred salvage regimen. Although the improved PBPC mobilization with high-dose G-CSF (10 or 12 μg/kg/12 h) in healthy allogeneic donors avoided them from having to undergo more apheresis sessions, this was associated with increased toxicity and significantly higher cost.[3,4,19–21] GM-CSF is approved for PBPC mobilization; however, it mobilizes fewer CD34+ cells than G-CSF and mobilization regimens combining GM-CSF with G-CSF with or without chemotherapeutic agents have not been shown to have substantial benefits over regimens that use G-CSF alone.[22–26] On the other hand, GM-CSF changes the profile of the types of cells mobilized in healthy donors, as decreased mobilization of T cells and natural killer cells, and increased mobilization of CD4+CD25+ regulatory T cells with GM-CSF as compared with G-CSF, has been observed, which is consistent with decreased acute GVHD. However, the higher doses required to mobilize adequate CD34+ cells cause greater toxicity. Therefore, GM-CSF is rarely used today for PBPC mobilization in patients and healthy donors.[27–29] Recombinant human EPO, in non-randomized studies, has been shown to potentiate the mobilization effect of G-CSF after a priming chemotherapy in patients with gynecological cancers, but this benefit could not be reproduced in randomized studies.[30–33] In a recent study of 28 patients with PCM, recombinant human EPO improved the mobilization efficiency of G-CSF after chemotherapy and reduced the costs of PBPC mobilization and AHCT. Although the results were not statistically significant, patients treated with G-CSF plus recombinant human EPO achieved a higher number of CD34+ cells per kg with lower number of apheresis as compared with patients receiving G-CSF alone. After transplantation, significantly fewer febrile neutropenia attacks, fewer days on antibiotics and a shorter hospital stay were noted in the G-CSF plus recombinant human EPO group.[34] There is evidence that EPO-stimulated grafts are enriched for cells known to have beneficial effects on angiogenesis and endothelial repair, and reinfusion of such grafts is associated with reduced transplantation-related toxicity.[35] Randomized studies have demonstrated improvement in stem cell mobilization efficiency when adding recombinant human SCF (rhSCF, ancestim) to filgrastim with or without chemotherapy in hematological and non-hematological malignancies. The combination of rhSCF and filgrastim exerts a sustained mobilization effect that the increased number of peripheral blood (PB) CD34+ cells persists longer than does the effect of filgrastim alone (13 days versus 7 days).[36–39] Moreover, 20–38% of the prior failed mobilizers with hematological malignancies or solid tumors could be rescued by addition of rhSCF to G-CSF with or without chemotherapy.[40–42] Herbert *et al*.[43] suggests the use of rhSCF and high-dose filgrastim regimen as a first-line mobilization strategy in patients with indolent lymphoproliferative disease who had prior exposure to fludarabine. Despite the efficacy of rhSCF, its use was not approved by the US Food and Drug Administration (FDA) owing to occasional occurrence of severe anaphylactoid reactions as a result of mast cell degranulation.[44] rhSCF has received approval for marketing in Australia, Canada and New Zealand for use in combination with filgrastim for mobilization of hematopoietic stem (HSCs) and progenitor cells with/without mobilizing chemotherapy. Recombinant human TPO (rhTPO), a full-length glycosylated molecule identical to endogenous TPO, not only promotes the proliferation and maturation of cells of megakaryocytic lineage, but also expands and induces the mobilization of hematopoietic progenitors to the PB.[45] Both non-randomized and randomized studies demonstrated that rhTPO safely and effectively augmented the number of PBPCs mobilized with G-CSF with or without chemotherapy in patients with breast cancer and various hematological malignancies.[46–48] However, delayed action, intravenous mode of administration and potential risk of excessive thrombocytosis and associated thrombosis, as well as induction of antibodies against endogenous TPO, appear to be disadvantages related to rhTPO in this setting. On the other hand, the TPO-receptor agonists (romiplostim and eltrombopag) recently approved by the US FDA for treatment of chronic refractory immune (idiopathic) thrombocytopenic purpura may have an effect similar to that of TPO on mobilization, providing potential for an additional option for patients who are difficult to mobilize using standard approaches.[49,50]

Table 2. Agents investigated as adjunct to G-CSF for PBPC mobilization

Growth factors
GM-CSF
Recombinant human EPO
Recombinant human SCF
Recombinant human TPO
Parathyroid hormone
Recombinant human growth hormone
Chemokine axis mobilizers
AMD3100
GRO-β analogs (SB-251353)
Other small molecules and peptides
Very late antigen-1 antibodies
Retinoic acid receptor-α agonists
TPO-receptor agonists

In recent years, several cytokines and chemokines have been investigated that may prove useful for amplifying yields of CD34+ cells without introducing additional toxicity (Table 2). In this review, we will focus on studies using the G-CSF analog, pegylated filgrastim, as a candidate growth factor for PBPC mobilization and briefly touch on chemokine axis mobilization, focusing mainly on the CXCR4 antagonist, plerixafor, which recently received approval for stem cell mobilization. We will also summarize some of the investigational agents, which are yet in preclinical and phase-I clinical trials.

PEGFILGRASTIM

Pegylated G-CSF (pegfilgrastim, Neulasta; Amgen Inc., Thousand Oaks, CA, USA) is currently approved by the US FDA for prevention of prolonged neutropenia after chemotherapy for non-myeloid

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Table 3. Phase-II studies of PBPC mobilization with chemotherapy plus pegfilgrastim

	Number of patients, indication	*Pegfilgrastim dose, schedule*	*Onset of apheresis, median (range)*	*Total CD34+ yield $\times 10^6$/kg, median (range)*	*Number of apheresis, median (range)*	*Patients (%) achieving the target*
Steidl[54]	12, PCM	12 mg, 3 days after CY	13 (11–15)	7.4 (4.9–38)	1 (1–2)	100%, $\geqslant 4 \times 10^6$/kg
Fruehauf[55]	26, PCM	12 mg, 1 day after CAD	13 (10–20)	9.7 (4.9–40.5)	2 (1–4)	88%, $\geqslant 7.5 \times 10^6$/kg
Isidori[56]	25, lymphoma	6 mg, 3 day after IEV	14 (13–16) peak PB34+ reached	8.7 (1.78–17.3)	1	96%. $\geqslant 2 \times 10^6$/kg
Putkonen[58]	38, PCM, lymphoma, CLL	6–18 mg, 1 day after chemotherapy	10 (10–18)	4.9 (1.6–27)	1 (1–3)	79%, $\geqslant 4 \times 10^6$/kg (single), $\geqslant 6 \times 10^6$/kg (tandem)
Simona[59]	38, lymphoma	6 mg, 1 day after ESHAP	10 (8–12)	9.42 (2.4–47.6)	1	83%, $\geqslant 5 \times 10^6$/kg

Abbreviations: CAD = CY, adriamycin, dexamethasone; ESHAP = etoposide, cytarabin, cisplatin, methyl prednisolone; IEV = ifosfamide, epirubicine, etoposide.

Table 4. Different dosing schedules of pegfilgrastim and comparison with filgrastim

	Patients and method	*Median onset of apheresis*	*Median CD34 + yield $\times 10^6$ /kg*	*Target achieved by patients (%)*	*Comment*
Bruns[60]	PCM ($n = 45$) Non-randomized study Pegfil 6 mg versus Pegfil 12 mg versus Fil 8 μg/kg/day; started on day 4 of CY	12 versus 13 versus 15	10.2 versus 7.4 versus 8.6	$\geqslant 4 \times 10^6$/kg All patients	Earlier onset of apheresis in pegfilgrastim, no difference in CD34+ cell yield
Russel[61]	NHL ($n = 90$), most heavily pretreated patients Randomized study Pegfil 6 mg versus Pegfil12 mg versus Fil 5 μg/kg/day; started 1d after ICE	14 versus 12.5 versus 14	4.9 versus 4.4 versus 5.1	$\geqslant 2 \times 10^6$/kg 69 versus 59 versus 72 $\geqslant 5 \times 10^6$/kg 41 versus 45 versus 56	No difference between groups
Tricot[62]	PCM ($n = 237$) Pegfil 6 mg on day 6 and day 13 versus Fil 2×5 μg/kg started after DT-PACE	ND	14.5 versus 10.0	$\geqslant 15 \times 10^6$/kg 71.7 versus 17.7 $\geqslant 5 \times 10^6$/kg 89.1 versus 71.9	Significant difference on first day and first three apheresis yields

Abbreviations: DT-PACE = dexamethasone, thalidomide, cisplatin, adriamycine, CY, etoposide; ICE = ifosfamide, carboplatin, etoposide; ND = not determined.

malignancies.[51] Pegfilgrastim is characterized by the covalent binding of a monomethoxypolyethylene glycol molecule to the N-terminal methionine residue of filgrastim. The pegylation of filgrastim produces a larger molecule of about 39 kDa with an increased elimination half-life owing to slow rate of renal elimination and masked proteolytic cleavage sites. Its plasma half-life of 33 h is substantially longer than the 4- to 6-h half-life of unconjugated G-CSF. This property allows a single s.c. dose of 6 mg pegfilgrastim to result in clinically effective serum levels over about 2 weeks until neutrophil recovery. When the number of granulocytes increases, pegfilgrastim is eliminated by cellular uptake through the G-CSF receptor and intracellular degradation, as well as by cleavage through neutrophil elastase.[52,53]

Pegfilgrastim's potential in PBPC mobilization is currently being explored. Phase-II clinical studies have demonstrated that chemotherapy followed by pegfilgrastim is able to mobilize a sufficient number of PBPCs in patients with PCM and lymphoma (Table 3).[54–59] The targeted number of CD34 + cells could safely be collected from 79–100% of patients after mobilization with single-dose (6–18 mg) pegfilgrastim usually administered 1–3 days after chemotherapy. The results were not significantly different from historical controls mobilized with 5–10 μg/kg/day filgrastim. In two of the studies, pegfilgrastim was associated with a 2 days earlier start of apheresis as compared with filgrastim.[54,55] In the other studies, a higher number of patients mobilized with pegfilgrastim reached the target CD34 + cell collection in fewer aphereses.[58,59] Both the mobilization efficiency of a single 6-mg, s.c. pegfilgrastim dose after chemotherapy and the time to neutrophil and platelet engraftment following AHCT appear to be comparable to filgrastim cohorts.

Different doses of pegfilgrastim with or without chemotherapy have been investigated in PBPC mobilization. Studies could not reveal a correlation between the amount of pegfilgrastim administered after cytotoxic chemotherapy and the PB CD34 + cell concentration (Table 4).[60–62] A single dose of 6 mg pegfilgrastim was equally potent as 12 mg for mobilization and harvest of PBPCs after chemotherapy in patients with PCM and lymphoma. Pegfilgrastim in either dose was associated with a more rapid leukocyte recovery and an earlier performance of the first apheresis procedure in comparison with unconjugated G-CSF in PCM patients. Tricot *et al.*[62] in their tandem transplant study, compared twice a day 5 μg/kg filgrastim and two doses of pegfilgrastim 6 mg on day 6 and day 13 after chemotherapy in 237 PCM patients. The percentage of patients who achieved the targeted CD34 + cells in the first 3 days of apheresis and the median number of CD34 + cells collected on the first day of apheresis were significantly higher in the pegfilgrastim group as compared with the filgrastim group. The better results obtained in this study seem to be the result of the second booster injection of pegfilgrastim that might have improved the plasma G-CSF

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concentrations, which tend to parallel the recovery of neutrophils. In contrast to the lack of dose dependency of mobilization after chemotherapy, steady-state mobilization requires higher doses of pegfilgrastim.[63] A pilot study involving 19 PCM patients demonstrated that successful stem cell mobilization, similar to filgrastim, for both single and tandem transplantation could be achieved with single-dose 12 mg pegfilgrastim without prior chemotherapy. The median time to reach the target PB CD34+ cell count of 15×10^6/L was 3 (2–4) days.[64] Because clearance of pegfilgrastim depends mainly on the number of PB neutrophils, higher doses of pegfilgrastim may be required to provide effective serum G-CSF levels.

In healthy volunteers, administration of 100 or 300 μg/kg pegfilgrastim has been shown to induce a sufficient increase of CD34+ cells in PB, with peak concentration of CD34+ cells detected between day 3 and day 4.[65] Phase-I/II studies have reported that mobilization of stem cells with pegylated G-CSF in normal donors is safe and feasible, and a single 12-mg dose results in mobilization characteristics similar to those of standard G-CSF, restoring hematopoiesis in allogeneic transplant recipients after myeloablative conditioning. In 80% of donors, sufficient PBPC yields were achieved by a single apheresis.[65,66] Mobilization with pegfilgrastim results in enhanced expansion of tolerogenic APCs and augmentation of regulatory T-cell activity that in turn reduces GVHD. Pegylated G-CSF or G-CSF conjugated to FLT3 ligand results in the expansion and activation of donor invariant natural killer/T cells, which significantly augment CD8+ T-cell-mediated cytotoxicity and GVL effects after transplantation. As a result, pegylation of G-CSF further separates GVHD and GVL, offering potential clinical advantages in the allogeneic transplant setting.[67]

Pegfilgrastim is well-tolerated, with an AE profile similar to that of unconjugated G-CSF. Bone pain is the most common AE and incidence is ranging from 3 to 20%, which is not higher than filgrastim.[56–62] A case of splenic rupture that may not have been related to pegfilgrastim was reported in one trial.[68]

As a long-acting G-CSF analog, earlier start of apheresis, reduction in the number of apheresis procedures as well as reduced number of injections may increase the compliance of patients and add cost-effectiveness to pegfilgrastim during chemotherapy plus cytokine mobilizations. However, mobilization with 12 mg pegfilgrastim is required in cytokine-only mobilizations and this approach is not cost-effective when compared with unconjugated G-CSF. Therefore, routine use of pegfilgrastim at high doses is not justified unless a clear superiority of pegfilgrastim over unconjugated G-CSF could be demonstrated.

CHEMOKINE AXIS MOBILIZATION

The BM microenvironment is critical in hematopoietic stem cell (HSC) trafficking. The hematopoietic progenitor cells are anchored in the BM by cytokines (such as CXCR4), which interact with SDF-1 (stromal cell derived factor-1, CXCL12) and adhesion molecules (such as very late antigen-1, VLA-1), which interact with vascular cell adhesion molecule-1.[69] During stem cell mobilization by G-CSF, the release of neutrophil proteases and the subsequent formation of a highly proteolytic environment in the BM results in the cleavage of these key molecules (mainly vascular cell adhesion molecule-1 and SDF-1). The interaction of HSCs with different types of stromal cells in the endosteal and endothelial niches not only has an important role in regulating HSC trafficking, but also affects their self-renewal, proliferation and differentiation capacity.[70] SDF-1 is a CXC chemokine constitutively produced in BM by stromal cells, mainly osteoblasts. Owing to accumulating evidence showing that SDF-1/CXCR4 signaling is the key step in HSC mobilization, there has been an emerging interest in the new molecules, which regulate the CXC chemokine axis in the BM.[71–73] Some of the important chemokines and their presumed mechanism of action are summarized in Table 5. Except AMD3100, all others are in their early phases of investigation.[74–76] Preclinical experience revealed some important characteristics of the chemokine axis mobilization of HSCs. The hallmark of chemokine axis mobilization is rapid mobilization, which occurs in minutes to hours after a single-dose administration. A highly synergistic effect in the mobilization of HSCs was obtained when GRO-β, AMD3100, CTCE002 or MIP-1α was each added as a single bolus agent at the end of a standard G-CSF mobilization regimen. A synergistic effect was also observed when chemokines were combined with each other. Grafts mobilized by chemokine axis contained cells with a greater engraftment potential and induced greater chimerism in murine transplantation models. An analog of GRO-β, SB-251353, when combined with G-CSF in rhesus monkeys was shown to greatly increase the mobilization of stem cells and progenitor cells in comparison with G-CSF alone. In a murine PB transplantation model, long-term repopulating cells mobilized by SB-251353 alone or in combination with G-CSF demonstrated enhanced neutrophil and platelet engraftment as compared with long-term repopulating cells mobilized by G-CSF alone.[77,78]

Table 5. Potential agents targeting the chemokine axis in the stem cell niche

	Receptor	*Mechanism of action*
Macrophage inflammatory protein-1α (MIP-1α, CCL3)	CCR1, CCR2	Unknown
SDF-1α peptide analogs CTCE-0021, CTCE-0214 Met-SDF-1β AMD3100	CXCR4	Alteration of SDF-1/CXCR4 signaling
IL-8 (CXCL8) GRO proteins GRO-β (CXCL2) SB-251353	CXCR2	Neutrophil-dependent upregulation of matrix metalloproteinase-9 activity, may ultimately impact the SDF-1/CXCR4 axis

PLERIXAFOR

Plerixafor (AMD3100, Mozobil; Genzyme Corporation, Cambridge, MA, USA) is a bicyclam molecule, which selectively and reversibly antagonizes CXCR4, and disrupts its interaction with SDF-1, thereby releasing HSCs into the circulation.[79,80] In the earlier phase-I studies, AMD3100 was administered s.c. at doses of 40–240 μg/kg to healthy volunteers, resulting in a dose-dependent, 4- to 10-fold increase in CD34+ cells, beginning within 1 h after injection, peaking at 9 h and declining to baseline by 24 h. Furthermore, when combined with G-CSF, AMD3100 synergistically increased the yield of CD34+ cells.[80–83] The results from phase-II studies with plerixafor added to G-CSF for PBPC mobilization from patients supported the data obtained from healthy subjects. Flomenberg *et al.*[84] in a sequential mobilization design in which 25 patients with NHL and PCM served as their own controls, showed that plerixafor and G-CSF mobilized more CD34+ cells per day of apheresis than G-CSF alone (4.4 versus 3–3.5 fold) in fewer apheresis days. Other studies reported that plerixafor used in conjunction with G-CSF enhanced the number of CD34+ cells circulating in the PB, with 95–100% of patients achieving the minimum number ($\geqslant 2 \times 10^6$/kg) of target CD34+ cells in a median of 1–2 apheresis days (Table 6).[85–88] The proportion of patients achieving $\geqslant 5 \times 10^6$ CD34 cells per kg was lower when refractory/relapsed and heavily pretreated patients were included in the studies. But even the heavily pretreated patients had the median 2.5-fold increase in PB

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Table 6. Phase-II Clinical trials with plerixafor plus G-CSF

	Stewart[85]	*Cashen*[86]	*Stiff*[87]	*Gazitt*[88]
Patient diagnosis (*n*)	NHL (8) PCM (14)	HL (22)	NHL (23)[a] PCM (26)[a]	NHL (10)[a]
Median fold increase in PB CD34 cells/μL after plerixafor	2.9 (2.0–7.6)	3.2 (1.7–5.7)	2.5 (1.3–6.0)	2–6
Median (range) apheresis days	1 (1–5)	2 (1–5)	2 (1–5)	1–2
Median (range) collected total CD34 cells $\times 10^6$/kg	9.2	6.6 (0.9–10.4)	5.9 (1.5–22.5)	3.0 (1.99–6.99)
$\geqslant 2 \times 10^6$ CD34 cells/kg, *n* (%)	22 (100)	21 (95)	47 (95.9)	10 (100)
$\geqslant 5 \times 10^6$ CD34 cells/kg, *n* (%)	20 (90.9)	15 (68)	11 (22.4)	1 (10)

[a]Included heavily pretreated patients.

Table 7. Phase-III clinical trials of PBPC mobilization with plerixafor plus G-CSF

	PCM study[89]		*NHL study*[90]	
	Plerixafor+G-CSF (n = 148)	*Placebo+G-CSF (n = 154)*	*Plerixafor+G-CSF (n = 150)*	*Placebo+G-CSF (n = 148)*
Achieved primary endpoint (%)	71.6	34.4	59.3	19.6
Achieved min. collection (%)	95.9	92.9	86.7	47.3
Fold increase in PB CD34/μL	4.8	1.7	5.0	1.4
Median number of apheresis days to collect the target	1	4	3	NE[a]
Median (range) collected CD34 cells $\times 10^6$/kg	10.96 (0.66–104.57)	6.18 (0.11–42.66)	5.69 (0.03–29.22)	1.98 (0.06–15)
Failed mobilization (%)	0	4.6	7.3	38.5

Abbreviation: PCM = plasma cell myeloma.
[a]Median time was not estimable (NE) as <50% of patients reached the target within four apheresis days.

CD34+ cells and could proceed with high-dose therapy and AHCT.[87,88]

Two multicenter, randomized, placebo-controlled studies demonstrated that addition of plerixafor to the G-CSF regimen in PCM and NHL patients resulted in a greater efficacy in PBPC mobilization than was seen with a regimen of G-CSF alone (Table 7).[89,90] The patients received G-CSF 10 μg/kg s.c. daily for 4 days. On the evening of day 4, patients received either placebo or plerixafor (240 μg/kg, s.c.). Patients underwent apheresis on day 5, 10–11 h after plerixafor administration, and continued to receive the evening dose of either placebo or plerixafor, followed by the morning dose of G-CSF, up to the time point when the primary target dose of CD34+ cells per kg was collected. The proportion of patients from whom $\geqslant 6 \times 10^6$ CD34+ cells per kg were collected in $\leqslant 2$ days of apheresis served as the primary endpoint in the PCM study,[89] and the proportion of patients from whom $\geqslant 5 \times 10^6$ CD34+ cells per kg were collected in $\leqslant 4$ days of apheresis was the primary endpoint in the NHL study.[90] These phase-III studies demonstrated that addition of plerixafor to G-CSF resulted in a significantly higher probability of achieving the optimal CD34+ cell target for tandem transplantation in PCM patients, and both the optimal and the minimal CD34+ cell target in NHL patients in fewer apheresis procedures and without any additional toxicity. PBSCs mobilized by plerixafor and G-CSF resulted in prompt and durable engraftment after AHCT. Mainly based on the results of these studies, the US FDA and the European Medicines Evaluation Agency approved plerixafor for use in combination with G-CSF to mobilize PBPCs for collection and subsequent AHCT in patients with NHL and PCM who previously failed mobilization with G-CSF alone.[91] Although the current indication for plerixafor mobilization of PBPCs is restricted to PCM and NHL, studies also report significant improvement in PBPC mobilization in patients with Hodgkin's lymphoma with G-CSF plus plerixafor when compared with historical outcomes with G-CSF alone.[86,92] Plerixafor combined with chemotherapy and G-CSF in a recent open-label, multicenter trial of 40 patients with PCM and NHL also proved to be a feasible method of stem cell mobilization. However, further studies are warranted to evaluate the exact timing of incorporating plerixafor into chemomobilization.[93]

High-dose CY and G-CSF has been the preferred salvage mobilization regimen for PCM patients who failed the first cytokine-only mobilization attempt. Additionally, in our institution, we usually administer CY and G-CSF for myeloma patients who cannot achieve at least a partial remission after the induction therapy assuming that this method will purge the contaminating tumor cells *in vivo*. However, use of CY to mobilize patients with PCM does not appear to improve outcome.[9] There is lack of sufficient information on direct comparison of mobilization with G-CSF and plerixafor to mobilization with chemotherapy and G-CSF. In a retrospective comparison, both G-CSF plus plerixafor and CY plus G-CSF resulted in similar numbers of cells collected as well as costs of mobilization and clinical outcomes.[94] Rescue stem cell mobilization with G-CSF and plerixafor can be offered in patients who only require rescue mobilization without any need for further tumor reduction. Thus, a promising approach with growth factor and patient-adapted use of plerixafor has recently been suggested to be superior to chemotherapy and growth factor for autologous PBPC mobilization.[95]

Several attempts have been initiated recently to build up an algorithm to incorporate plerixafor into clinical practice in a proper way to improve clinical outcomes. The preemptive use of plerixafor using the PB CD34+ cell count on day 4 of G-CSF administration and the collection target to decide between continuing G-CSF only or adding plerixafor to the mobilization regimen may potentially reduce the percentage of failure in first-line mobilizations. This approach may also increase the percentage of optimal collections, avoiding treatment delays, morbidity and costs associated with second mobilization attempts.[96] A recent study demonstrated that the quantity of CD34+ cells collected on day 1, rather than the PB CD34+ cell count, might identify patients unlikely to achieve adequate stem cell collection

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Table 8. Pharmacokinetic characteristics of plerixafor

Dosage, route	240 μg/kg actual body weight, s.c.
Peak plasma concentration	30–60 min
Plasma half-life	4 h (3–5)
Distribution	Up to 58% bound to plasma proteins Mainly extravascular fluid space
Metabolism	Not related to cytochrome-P450
Elimination	Renal
Median maximum increase in PB CD34+cells/kg from baseline	4.2 fold (3–5.5)
Time to maximum increase in PB CD34+cells/kg	10 h
Special consideration in renal failure	Cr Cl >50 mL/min, 240 μg/kg (max. 40 mg/day) Cr Cl $\leqslant 50$ mL/min, 160 μg/kg (max. 27 mg/day)

for AHCT, and suggested that patients who collect $<0.70 \times 10^6$ CD34+ cells per kg on day 1 could be considered for treatment modifications such as adding plerixafor.[97]

Adverse events

Plerixafor is well-tolerated and AEs are usually mild and transient. The most common AEs observed in $\geqslant 10\%$ of patients and more frequently with plerixafor plus G-CSF than with placebo plus G-CSF were diarrhea, nausea, vomiting, flatulence and injection-site reactions (erythema, edema), fatigue, arthralgia, headache, dizziness and insomnia. Severe AEs were rare and included hypotension and dizziness after drug administration, and thrombocytopenia after apheresis.[89–92] No evidence of tumor cell mobilization could be demonstrated after plerixafor in PCM and NHL patients.[98] However, plerixafor is not recommended for PBPC mobilization in leukemia patients. No case of splenic rupture due to plerixafor has been reported to date. However, plerixafor combined with G-CSF should be used with caution in patients with splenomegaly.[91] Like other chemokine axis mobilizers, plerixafor results in rapid mobilization of PBPC, which peaks at approximately 10 h (Table 8). Plerixafor clearance is proportionally reduced and plasma half-life is increased in subjects with mild-to-severe renal impairment. A plerixafor dose reduction to 160 μg/kg in patients with a creatinine clearance value $\leqslant 50$ mL/min is expected to result in exposure similar to that in patients with normal to mildly impaired renal function, and efficient mobilization of CD34+ cells at this dose level has been demonstrated.[83,91,99] The limited post-marketing experience suggests that plerixafor mainly at a dose of 160 μg/kg can be safely and efficiently conjugated to G-CSF for PBPC mobilization in patients with advanced-stage renal failure on hemodialysis.[100,101]

Normal donors

In accordance with the initial studies of plerixafor in healthy volunteers, the pivotal study by Devine *et al.*[102] demonstrated safe and successful mobilization of healthy matched sibling donors with plerixafor as a single agent for allogeneic PBPC transplantation. Donors were treated with single-dose plerixafor 240 μg/kg s.c. and leukapheresis was initiated 4 h later. Overall, 67% (16/24) of the donors collected the target cell count on the first day and 92% (22/24) collected the target cell count after 1 or 2 days of apheresis. Healthy donors tolerated the drug well, without any cumulative toxicity in donors who required the second day of plerixafor administration. The duration of neutrophil and platelet engraftments after transplantation was similar to mobilization with G-CSF alone.[102] The PBPCs mobilized by plerixafor in combination with G-CSF seem to have a greater capacity for reconstituting BM as compared with those mobilized with G-CSF alone.[103,104] Studies demonstrated that greater numbers of CD3+ and CD4+ cells per kg were contained within the plerixafor-mobilized allografts as compared with the G-CSF-mobilized allografts. On the other hand, plerixafor addition to G-CSF increased the number of precursor DCs in the graft, without any polarization toward Th1- or Th2-type cytokines.[88,105] These observations may explain the prompt engraftment without any significant increase in the incidence of acute or chronic GVHD in patients who received lower number of CD34+ cells and the greater number of T cells in the plerixafor-mobilized allografts after the myeloablative regimen.[102] Stem cell mobilization from healthy donors for allogeneic PBPC transplantation needs further evaluation in clinical trials.

Plerixafor addition to G-CSF has undoubtedly increased the number of patients who could proceed with high-dose therapy and AHCT. Its combination with other growth factors such as single-dose pegfilgrastim or multiple cytokine combinations may further enhance the yield of PBPC mobilizations.[106] Plerixafor incorporation in first-line mobilization protocols in patients who are predicted to be poor mobilizers will eliminate the need for further mobilization attempts and the cost-effectiveness of such approaches should be clarified. The use of plerixafor in allogeneic stem cell donors is as yet in its early phases.

INVESTIGATIONAL AGENTS

Parathyroid hormone

Osteoblasts are important regulatory components of the stem cell niche and can be targeted as a means to increase the stem cell number. Signaling pathways, which regulate hematopoietic self-renewal, are stimulated upon activation of the parathyroid hormone (PTH) receptor on osteoblasts. PTH is FDA-approved for treatment of osteoporosis in postmenopausal women, and men who are at high risk of fracture. Primary hyper-parathyroidism has been shown to be associated with increased circulating BM-derived progenitor cells.[107] In mice, PTH was shown to mobilize hematopoietic progenitor cells without depletion of the BM.[108] A recent phase-I study demonstrated that 11 of 20 patients who had failed previous mobilization attempts achieved the mobilization criterion with PTH combined with G-CSF on the last 4 days of treatment. PTH was well-tolerated at high doses, but further research is required to establish the efficacy and safety of PTH as a mobilizing agent.[109]

VLA-4 antibodies

Antibodies against VLA-4 have been shown to mobilize HSCs in mice and primates. The anti-VLA-4 antibody, natalizumab, which is approved by the FDA for treatment of patients with multiple sclerosis and Crohn's disease, was found to induce a sustained and modest increase in circulating CD34+ cells.[17,110] The combination of α4-integrin blockade with natalizumab and CXCR4 blockade with AMD3100 demonstrated additive effects on stem cell mobilization.[111] The small-molecule inhibitor of VLA-4, BIO5192, which caused a 30-fold increase in the mobilization of murine hematopoietic stem and progenitors over basal levels in mice, is promising. An additive affect on PBPC mobilization was observed when AMD3100 was combined with BIO5192. Furthermore, the combination of G-CSF, BIO5192 and AMD3100 enhanced mobilization by 17-fold as compared with G-CSF alone.[112] These studies provide evidence for the utility of small-molecule inhibitors of VLA-4 either alone or in combination with G-CSF or AMD3100 for mobilization of HSCs and progenitor cells. However, its role in clinical PBPC mobilization has yet to be explored.

Growth hormone

Additional strategies to make stem cell collection more efficient include expansion of HSCs in the BM, which are then available for

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release into PB upon concomitant use of a mobilizing agent such as G-CSF. In a pilot study, patients with relapsed or refractory hematological malignancies who had failed a first mobilization attempt with chemotherapy plus G-CSF were remobilized with chemotherapy, G-CSF (5 μg/kg/day, 5 days) and recombinant human growth hormone (100 μg/kg/day, maximum daily dose of 6 mg). This combination resulted in efficient mobilization and collection of $\geqslant 5 \times 10^6$ CD34+ cells per kg in 87% of these poor mobilizers, with a median of three apheresis.[113] Although no evidence suggesting tumor growth stimulation could be detected in this study, increased risk of tumor growth through recombinant human growth hormone-induced insulin-like growth factor-1 secretion still remains major concern, which has to be clarified in a larger patient population and longer observation.

Retinoic acid receptor-α agonists

Investigators have demonstrated that pretreatment of mice with the retinoic acid receptor-α-specific agonist, VTP195183, increased the numbers of immature granulocyte progenitors in the BM and subsequently synergized to enhance the G-CSF-mediated mobilization of HSCs and progenitor cells. This supported the novel approach to improve G-CSF-induced mobilization by accelerating granulocyte maturation in the BM. The same group of investigators also demonstrated in a phase-I study that all-*trans* retinoic acid could be safely combined with G-CSF in PCM and lymphoma patients for stem cell mobilization.[114,115]

TPO-receptor agonists

The TPO-receptor agonists, orally active small-molecule eltrombopag and s.c. administered peptide romiplostim, interact with the TPO receptor (c-Mpl) leading to increased platelet production. Contrary to rhTPO, they do not have the potential to induce antibody production to endogenous TPO and may have an effect similar to that of rhTPO on CD34+ cell mobilization to PB, and in the future, may be potential adjuncts to G-CSF in poor mobilizers.

CONCLUSION

As stem cell interactions with the BM microenvironment are better understood, new mobilizing agents targeting pathways in regulating stem cell trafficking are emerging. A patient-adapted approach in initial stem cell mobilization to prevent further mobilization attempts and to decrease the time to transplantation and costs, as well as morbidity of remobilization, is encouraging. Combining various cytokines, which target different molecular targets in the stem cell niche, not only for increasing stem cell yield but also to improve the quality of graft content and the associated transplantation outcomes are promising areas of research.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

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