

New agents in HSC mobilization

Mélanie J. Domingues^{1,2} · Susan K. Nilsson^{1,2} · Benjamin Cao^{1,2} 

Received: 8 November 2016 / Accepted: 22 November 2016 / Published online: 30 November 2016
© The Japanese Society of Hematology 2016

Abstract Mobilized peripheral blood (PB) is the most common source of hematopoietic stem cells (HSC) for autologous transplantation. Granulocyte colony stimulating factor (G-CSF) is the most commonly used mobilization agent, yet despite its widespread use, a considerable number of patients still fail to mobilize. Recently, a greater understanding of the interactions that regulate HSC homeostasis in the bone marrow (BM) microenvironment has enabled the development of new molecules that mobilize HSC through specific inhibition, modulation or perturbation of these interactions. AMD3100 (plerixafor), a small molecule that selectively inhibits the chemokine receptor CXCR4 is approved for mobilization in combination with G-CSF in patients with Non-Hodgkin's lymphoma and multiple myeloma. Nevertheless, identifying mobilization strategies that not only enhance HSC number, but are rapid and generate an optimal “mobilized product” for improved transplant outcomes remains an area of clinical importance. In recent times, new agents based on recombinant proteins, peptides and small molecules have been identified as potential candidates for therapeutic HSC mobilization. In this review, we describe the most recent developments in HSC mobilization agents and their potential impact in HSC transplantation.

Keywords Mobilization · CXCR4 · Integrin · AMD3100 · G-CSF

Introduction

Hematopoietic stem cell transplantation (HSCT) is a critical procedure in the successful treatment of many blood disorders and malignancies including leukaemia, lymphoma and myeloma [1–4]. Over 68,000 HSCT are performed each year worldwide, with mobilized peripheral blood (PB) being the predominant source of HSC for both autologous and allogeneic transplants [5]. Recombinant granulocyte colony stimulating factor (G-CSF; filgrastim/lenograstim) is the most common mobilization agent and is administered daily for up to 6 days, either alone or in conjunction with chemotherapy (Reviewed in [6]). Alternatively, use of the PEGylated variant of G-CSF (Pegfilgrastim), which has a significantly longer half-life, eliminates the need for daily dosing (Reviewed in [7]). An important factor that predicts the success of long-term hematopoietic reconstitution is the number of CD34⁺ hematopoietic stem and progenitor cells (HSPC) in the graft. The minimum collection threshold required for transplantation is 2×10^6 CD34⁺ per kg body weight, although graft doses $>5 \times 10^6$ CD34⁺ per kg are associated with faster recovery (Reviewed in [8]). Nevertheless, despite G-CSF administration, approximately 5–30% of patients fail to reach this minimum threshold [9, 10] and as a consequence leads to significantly increased costs associated with greater resource utilization such as mobilization agents and antibiotics, transfusion and apheresis procedures as well as hospitalization times [11]. In recent times, novel strategies specifically targeting the interactions within the BM stem cell niche have been developed, such as the small molecule AMD3100

✉ Benjamin Cao
ben.cao@csiro.au

¹ CSIRO Manufacturing, Commonwealth Scientific and Industrial Research Organisation, Bag 10, Clayton South, VIC 3169, Australia

² Australian Regenerative Medicine Institute, Monash University, Clayton, VIC 3800, Australia

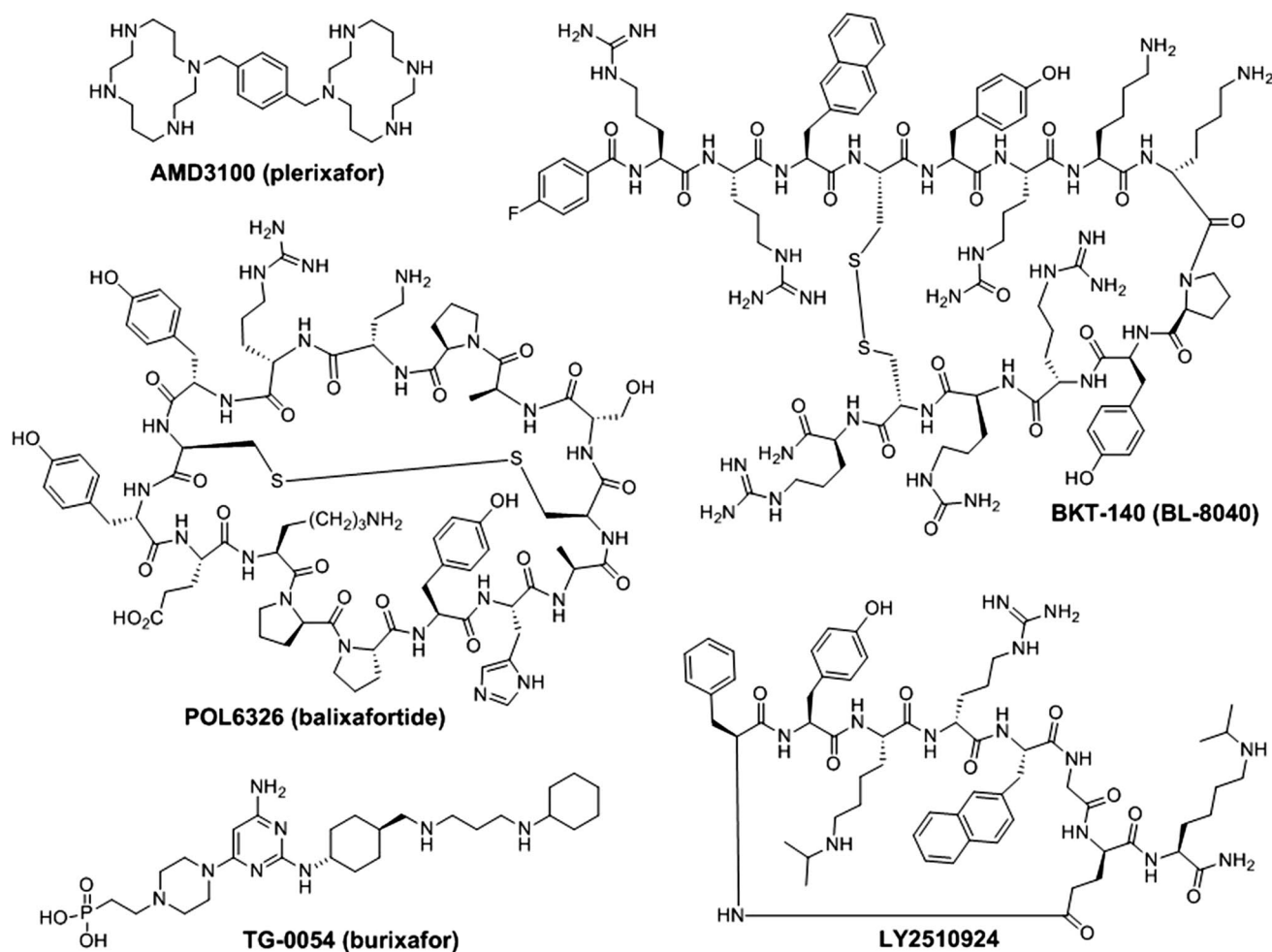


Fig. 1 Chemical structures of AMD3100 and other CXCR4 antagonists in clinical development for HSC mobilization

(plerixafor/Mozobil[®]), which is a selective CXCR4 antagonist [12–14]. Clinically, AMD3100 is not sufficiently effective when used alone and is currently only indicated for “rescue mobilization” in combination with G-CSF, when G-CSF alone has failed to mobilize the minimum threshold of CD34⁺ cells. Consequently, the development of novel, rapid and more effective mobilization agents as alternatives to current strategies remains an area of growing interest. This review describes the different classes of HSC mobilization agents that are currently in clinical and pre-clinical development and their potential influence on traditional mobilization strategies.

Inhibitors of the CXCR4/SDF-1 α axis

HSC express the chemokine receptor CXCR4 and are in part retained in BM through the interaction with stromal-derived factor-1 α (SDF-1 α), a secreted chemokine synthesized by several cell types including osteoblasts, nestin-positive

(Nes⁺) mesenchymal stromal cells (MSC), CXCL12 abundant reticular (CAR) cells, endothelial cells and leptin receptor-positive (Lepr⁺) perivascular cells (Reviewed in [15–17]). The CXCR4/SDF-1 α axis is the most well-studied target for HSC mobilization [18, 19] and many inhibitors/modulators that perturb this interaction have been reported to mobilize HSC in animal models and humans. The canonical CXCR4 antagonist, AMD3100 (Fig. 1), is a small molecule bicyclam drug that is currently used in combination with G-CSF for clinical HSC mobilization in patients with NHL and multiple myeloma (MM) who have previously failed to mobilize with G-CSF alone [20–23]. Originally developed as an anti-HIV drug (Reviewed in [24]), AMD3100 remains the only CXCR4 antagonist approved by the Food and Drugs Administration (FDA). Since the adoption of the first-in class AMD3100 for clinical HSC mobilization, several other diverse classes of CXCR4 antagonists have been identified that are currently in various stages of clinical and pre-clinical development for HSC mobilization as well as anti-cancer and anti-HIV applications.

Table 1 Summary of HSC mobilization agents tested in clinical studies

Agent	Company	Mechanism/target(s)	Stage of development
POL6326 (balixafortide)	Polyphor	CXCR4 antagonist	Phase I/II completed for HSC mobilization (NCT01841476) Phase II completed for acute myocardial infarction (NCT01905475) Ongoing Phase I for metastatic breast cancer (NCT01837095)
TG-0054 (burixafor)	TaiGen Biotechnology	CXCR4 antagonist	Phase I/II completed (NCT01458288) Phase I/II trials for AML(NCT01838395)
BKT140 (BL8040)	Biokine Therapeutics and BioLine Rx	CXCR4 antagonist	Phase I for mobilization (NCT01010880) Phase II for AML. (NCT01838395) Phase II for metastatic pancreatic cancer (NCT02826486)
NOX-A12	NOXXON Pharma	Anti-SDF-1	Phase I completed Phase II completed for MM (NCT01521533) Phase II for relapsed CLL (NCT01486797)
Bortezomib	Millennium Pharmaceuticals	Proteasome inhibitor, downregulation of VLA4/VCAM-1 axis	In phase I clinical trial for MM Clinical trial for mobilization in combination with AMD3100
Groß (SB-251353)	GlaxoSmithKline	CXCR2 agonist, induction of MMP-9 secretion	Phase II trial completed for HSC mobilization
PTH (teriparatide)	Eli Lilly	PTH receptor agonist, expansion of BM HSC	Phase I/II completed for HSC mobilization
CDX-301 (rhFLT3L)	Celldex	FLT3 agonist	Phase I clinical trial completed
LY2510924	Eli Lilly	CXCR4 antagonist	Phase I completed
Natalizumab	Biogen	VLA-4 antagonist	Clinical study in MS patients
Meloxicam	Boehringer Ingelheim	Non-steroidal anti-inflammatory drug	Phase II (NCT02003625)
Eltrombopag	GlaxoSmithKline	TPO receptor agonist	Mobilization in MM (NCT01286675)
ALX-0651	Ablynx	Anti-CXCR4 Nanobody	Phase I completed (NCT01374503)

Currently, there are several promising CXCR4 antagonists that are in advanced clinical development, including POL6326, TG-0054, BKT-140 and LY2510924 (Fig. 1). POL6326 is a macrocyclic peptide that has been demonstrated to be efficacious in mice and humans when used as a monotherapy [25–27] and has been the subject of several clinical studies (Table 1). POL6326 was found to be well tolerated when given as an intravenous infusion over 2 h, with maximum mobilization observed after approximately 6–8 h [28]. These promising studies concluded that the minimum threshold of CD34⁺ cells could be achieved with a single apheresis after only one dose of POL6326 and may be beneficial to patients and donors with contraindications to G-CSF. Interestingly, there are no reports on the effects of POL6326 in combination with G-CSF despite extensive reports of CXCR4 antagonists capable of synergistically augmenting HSC mobilization when used with G-CSF. Unlike POL6326, the small molecule CXCR4 inhibitor TG-0054 (Burixafor) [29] and the cyclic peptide

BKT-140 [30] have been tested with G-CSF and shown to synergistically augment HSC mobilization when given as a single dose after a standard course of G-CSF, results which were corroborated in human volunteers and patients with haematological malignancies [31–34]. Other promising CXCR4 antagonists in development for HSC mobilization include the cyclic peptide LY2510924 [35–37] and the small molecule ALT-1188 [38]. Notably, ALT-1188 has been shown to effectively mobilize murine HSPC when given as a single dose and synergistically when used in combination with G-CSF [38]. Impressively, ALT-1188 plus G-CSF mobilized significantly more HSPC than G-CSF plus AMD3100, suggesting ALT-1188 could significantly improve current mobilization strategies [38]. However, the efficacy in human HSC mobilization remains to be determined. Together, these promising studies highlight POL6326, TG-0054, BKT-140 and ALT-1188 as rapid and effective mobilization agents that could replace G-CSF, and in the case of TG-0054, BKT-140 and ALT-1188, could

also be used to improve current G-CSF-based mobilization strategies, which will be particularly beneficial in poor G-CSF mobilizers.

Novel modulators of CXCR4/SDF-1

The successful development of inhibitors against CXCR4/SDF-1 interactions based on small molecules, peptides and antibodies in clinical and pre-clinical studies for HSC mobilization (Reviewed in [39, 40]) has prompted the development of other classes of inhibitors with greater pharmacokinetic stability and superior in vivo efficacy. New structural classes of CXCR4/SDF-1 inhibitors may also assist in illuminating new mechanisms into HSC mobilization owing to the complexity of the CXCR4/SDF-1 axis (Reviewed in [41]). An interesting new class of SDF-1 inhibitors is the “aptamer” or “Spiegelmer” family of drugs [42]. Spiegelmers are a class of artificial mirror-image RNA oligonucleotide drugs constructed using non-natural L-ribose units and have been of substantial clinical interest since the age-related macular degeneration aptamer drug Macugen® (Pfizer) was first approved by the FDA in 2005 (Reviewed in [42, 43]). NOX-A12 (olaptased pegol) is a PEGylated Spiegelmer that specifically targets SDF-1 and has been shown to induce rapid mobilization of murine HSPC either alone or synergistically with G-CSF [44, 45]. In human trials, NOX-A12-induced mobilization resulted in significant numbers of human CD34⁺ cells that persisted in the blood for up to 4 days at the highest dose tested, which was attributed to its long plasma half-life (~38 h) [45]. The sustained mobilization of CD34⁺ cells may be desired to allow time for collection via apheresis but also drew speculation to its potential benefits towards chemosensitization of haematological cancers (Table 1) [43, 45, 46]. Should NOX-A12 perform favourably in clinical studies, it may open doors for the development of other target-specific Spiegelmer drugs for HSC mobilization.

Another interesting new class of drugs are the single-domain antibodies or “nanobodies”, which unlike conventional antibodies, are antigen specific, heavy-chain-only antibody fragments (Reviewed in [47]). Nanobodies have garnered significant interest as potential therapeutics owing to their ease of production as recombinant proteins, small size, high solubility, good thermal stability and good in vivo tissue penetration (Reviewed in [48]). The single-domain CXCR4-specific nanobody referred to as “L8” mobilized equivalent numbers of CD34⁺ cells in Cynomolgus monkeys and with similar kinetics to AMD3100 [49]. L8 is a bivalent nanobody constructed by coupling two llama-derived monovalent nanobodies designated 238D2 and 238D4 with a short 20 amino acid peptide linker, which results in significantly increased affinity to CXCR4 [49]. However, while a

phase I clinical trial assessing the bivalent nanobody (now ALX-0651) was initiated in healthy volunteers, the trial has been abandoned for undisclosed reasons. In a separate study, fully human shark antibody-mimicking protein scaffolds termed “i-bodies” that target CXCR4 were found to inhibit HIV infection, cell migration and leukocyte recruitment but did not mobilize HSPC in either mice or humanized xenograft mouse models [50]. Similar to CXCR4 i-bodies, anti-CXCR4- and anti-SDF-1-blocking antibodies also fail to mobilize HSC [51] and support the notion that direct inhibition of CXCR4/SDF-1 chemotaxis by CXCR4 antagonists is only partially responsible for HSC mobilization and likely requires perturbation of CXCR4-dependent downstream signalling (Reviewed in [52]). Indeed, additional mechanisms for AMD3100-mediated HSC mobilization has been attributed to ROS signalling and CXCR4-dependent SDF-1 release [53] as well as AMD3100-induced reduction of BM vascular integrity and increased vascular permeability [54]. As such, complete understanding of the detailed downstream effects that are mediated by CXCR4 inhibition will aid in improving future developments of HSC mobilization agents based on CXCR4 antagonists.

Up until recently, all described CXCR4 antagonists are from non-natural origins. In 2015, a naturally occurring 16-mer amino acid peptide fragment derived from human serum albumin termed “EPI-X4” (LVRYTKKVPQVSTPTL) was discovered and revealed to be a potent CXCR4 antagonist and rapid mobilizer of murine HSPC [55, 56]. EPI-X4 is produced from the abundantly present serum albumin precursor through enzymatic digestion by the aspartyl proteases Cathepsin D and E [55]. Intriguingly, while all the prerequisites for the generation of EPI-X4 are ubiquitously available throughout the body, its presence and endogenous regulatory role, if any, on native BM HSC remains to be determined. Nevertheless, EPI-X4 has been demonstrated to reduce basal CXCR4 signalling activity and, therefore, also behaves as an inverse agonist, suggesting endogenous CXCR4 signalling may not be restricted to SDF-1 [55]. Of note, G-CSF induces HSC mobilization through dynamic changes in the BM microenvironment mediated in part by secretion of the proteolytic enzymes cathepsin K or cathepsin G, which are produced by osteoclasts and neutrophils, respectively [51, 57, 58]. Thus, whether G-CSF can modulate the release of cathepsin D and E, which subsequently produce EPI-X4 within BM or whether EPI-X4 is involved in the complex cascade of events that follow G-CSF mobilization is not known. Furthermore, EPI-X4 has a very short plasma half-life of ~17 min, which may limit its utilization as a therapeutic. However, the identification of several synthetic derivatives, specifically the dimeric derivative designated “WSC02” ((IVRWSKKVPCVS)×2) has been shown to possess superior plasma stability compared to EPI-X4 peptide and

exhibited enhanced suppression of HSC migration towards SDF-1 [55]. These promising preliminary studies featuring EPI-X4 and its more stable synthetic derivatives warrant further investigation in their therapeutic potential [59].

Cytokines, growth factors and hormones

Although G-CSF and its related derivative, PEGylated G-CSF are the most common recombinant proteins used in clinical mobilization [60], other recombinant proteins based on cytokines, growth factors and hormones have surfaced as potential mobilization agents. Early studies and clinical trials using recombinant human stem cell factor (rhSCF; aneastim) [61, 62], which binds the c-kit receptor expressed by HSC, showed strong synergism with G-CSF and although approved in multiple countries, it has not been commonly utilized owing to adverse allergic reactions (Reviewed in [63]). Similarly, recombinant human thrombopoietin (rhTPO), which binds its receptor c-Mpl, has also been demonstrated to enhance G-CSF and chemotherapy-induced mobilization [64, 65] but its clinical efficacy was not deemed sufficient for further development (Reviewed in [66]). Subsequent development of synthetic non-peptidic small molecule TPO receptor agonists identified the orally bioavailable thrombocytopenia drug eltrombopag (Fig. 3), which is currently undergoing clinical testing in combination with G-CSF for mobilization of patients with MM (Table 1). The CXC chemokine Gro- β and its truncated human recombinant analogue SB-251353 effectively and rapidly mobilize stem and progenitors when used alone or synergistically in combination with G-CSF in mice and rhesus monkeys [67, 68]. HSC mobilization with Gro- β was found to be dependent on metalloproteinase-9 (MMP-9) and the collected PB HSC were shown to have enhanced homing and long-term reconstitution potential compared to G-CSF-mobilized HSC [67, 68]. Nevertheless, the efficacy of Gro- β in human HSC mobilization remains to be determined.

Parathyroid hormone (PTH), the primary regulator of calcium homeostasis and bone remodelling, has also been shown to be critical in controlling HSC function via PTH receptor activation on osteoblasts [69]. Daily injections of PTH for 5 weeks followed by G-CSF treatment led to enhanced HSC mobilization compared to G-CSF alone [70]. PTH treatment alone does not mobilize, instead it promotes the expansion of BM HSC thereby increasing the number of HSC available to mobilize when using a standard mobilization regimen [70]. Furthermore, PTH treatment also expands HSPC in transplant recipients and may be useful for improving BM recovery post-transplant [70]. However, due to lack of clinical efficacy there have been no further developments of PTH for HSC mobilization or for improving BM recovery post-transplant [71–73].

One of the more promising cytokines in clinical development is the human recombinant FMS-like tyrosine kinase-3 ligand (rhFLT3L) termed “CDX-301”. Pre-clinical studies in mice showed a daily course of CDX-301 over 10 consecutive days effectively mobilized murine HSPC when used alone or synergistically in combination with a single dose of AMD3100 [74]. Importantly, the combination of CDX-301 and AMD3100 mobilized significantly greater numbers of HSC compared to G-CSF plus AMD3100 [74]. The transplant of blood mobilized using CDX-301 plus AMD3100 into lethally ablated mice was also shown to be associated with a survival advantage [74]. Moreover, the CDX-301 plus AMD3100 combination effectively mobilized regulatory T cells (T_{reg}), which may have protective effects against graft vs host disease (GvHD) after allogeneic transplantation (Reviewed in [75]). Indeed, transplantation of irradiated recipients with blood mobilized with CDX-301, either alone or in combination with AMD3100 correlated with enhanced survival in an allogeneic transplant setting [74]. A subsequent phase 1 clinical trial showed CDX-301 was well tolerated in healthy volunteers and the optimal mobilization of CD34⁺ cells occurred using a 10-day course, with all patients treated with this regimen achieving the minimum threshold of PB CD34⁺ cells for transplantation [76]. While CDX-301 did not produce significantly greater T_{reg} numbers in the PB of patients, the study does raise important considerations as to whether accompanying immune cells such as T_{reg} can effect transplant outcomes. In any case, these promising studies warrant further testing to determine whether CDX-301 either alone or in combination with AMD3100 (or other CXCR4 antagonists) could be used as an effective alternative to G-CSF-dependent mobilization regimens. However, like G-CSF, the major drawback of CDX-301, particularly in healthy donors, is the unavoidable 5–10-day course of daily injections required to elicit optimal mobilization.

Integrin antagonists

HSC express several integrin subtypes that are known to modulate HSC function and retention in BM (Reviewed in [77]), with VLA-4 (α_4) being the most well-established integrin target for mobilization. The integrin α_4 , which associates with both β_1 and β_7 subunits, mediates HSC retention via adhesion to niche-derived VCAM-1, fibronectin and thrombin-cleaved osteopontin (tcOPN) (Reviewed in [77, 78]). Inhibition of α_4 using the blocking antibody natalizumab, a drug currently used for the treatment of multiple sclerosis (MS) and Crohn’s disease, effectively mobilized murine and non-human primates when used alone or in combination with G-CSF or SCF [79]. Subsequent clinical studies in MS patients showed natalizumab

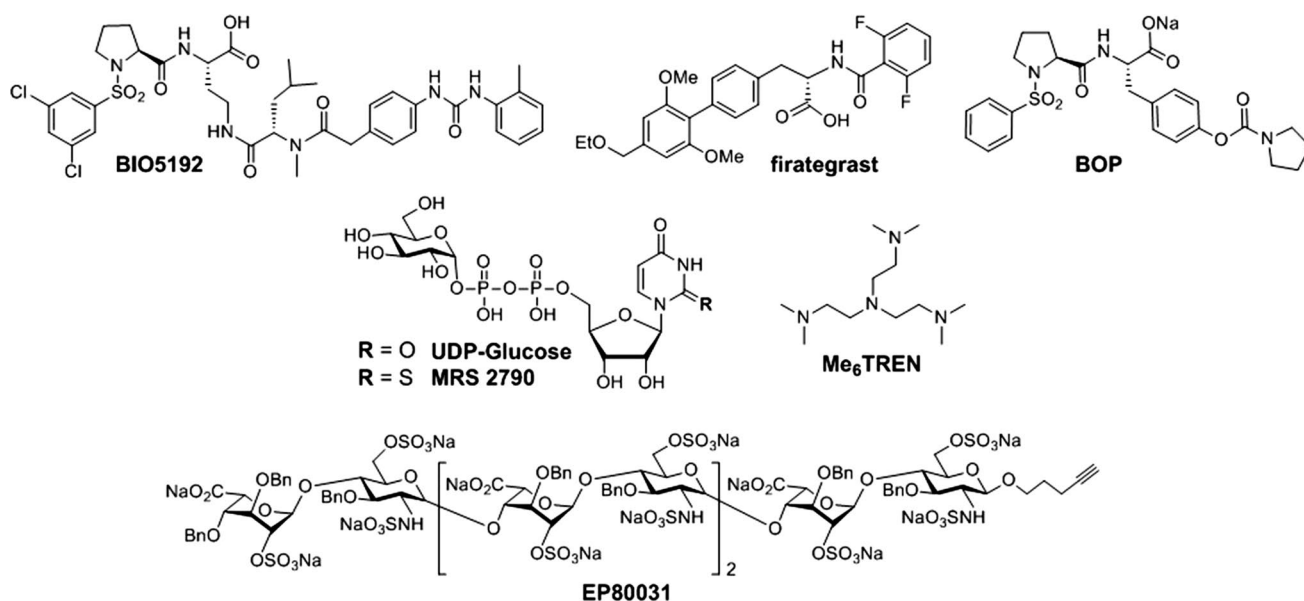


Fig. 2 Chemical structures of promising experimental agents for HSC mobilization

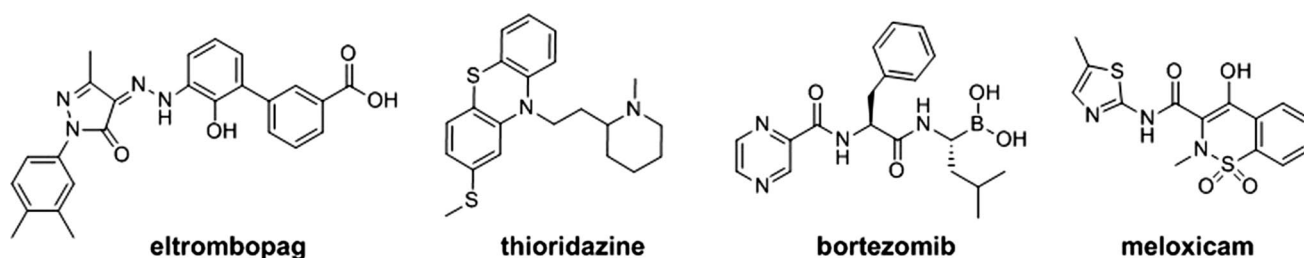


Fig. 3 Chemical structures of repurposed drugs for HSC mobilization

effectively mobilized CD34⁺ cells in humans [80, 81] but its association with development of the potentially fatal condition progressive multifocal leukoencephalopathy (PML) [82] has prevented its use in clinical mobilization. To address the issues associated with natalizumab-induced prolonged inhibition of VLA4, the highly potent and selective small molecule $\alpha_4\beta_1$ antagonist BIO5192 (Fig. 2) was developed and shown to mobilize HSPC alone and in combination with G-CSF and/or AMD3100 [83] without the sustained leukocytosis that can lead to PML [84]. Nevertheless, BIO5192 is not being pursued for clinical development. However, similar studies using the orally bioavailable dual $\alpha_4\beta_1/\alpha_4\beta_7$ antagonist firsategrast (SB-683699) (Fig. 2), which is currently in clinical development for treatment of MS, will be tested in due course for efficacy in HSC mobilization in murine models [85].

In addition to $\alpha_4\beta_1$, HSC express the related $\alpha_9\beta_1$ integrin, which also binds to VCAM-1 and tOPN [86]. However, unlike $\alpha_4\beta_1$ which is ubiquitously expressed by all leukocytes, the expression of $\alpha_9\beta_1$ is largely restricted to

HSPC and HSC [87]. A single dose of the dual $\alpha_4\beta_1/\alpha_9\beta_1$ integrin antagonist “BOP” (Fig. 2) was shown to mobilize HSPC and HSC in a rapid and transient manner [87]. Of note, inhibition of $\alpha_9\beta_1$ was identified to be important for HSC mobilization, while $\alpha_4\beta_1$ was predominantly involved in WBC mobilization [87]. Using a fluorescent analogue of BOP termed “R-BC154” [88], human and murine HSC were found to bind BOP through endogenously primed/activated integrins within the endosteal BM, the region near bone where HSC with superior homing potential and enhanced proliferative capacity reside [89]. These observations are consistent with the greater amount of divalent metal cations (Mn^{2+} , Mg^{2+} and Ca^{2+}) present near bone and the high dependency of integrin activity on these specific cations [87]. Small molecule integrin antagonists like BOP, which can take advantage of the enhanced integrin activity near the bone/BM interface to enable preferential targeting and mobilization of potent endosteal HSC may lead to grafts that benefit long-term transplant outcomes. The integrin antagonists BIO5192, firsategrast or

BOP may prove to be effective and rapid alternatives to G-CSF mobilization, especially when used in combination with AMD3100 or other CXCR4 antagonists described in this review. Furthermore, since these small molecule integrin antagonists are expected to be used as a single-dose, short-acting, transient mobilization agent, they are unlikely to lead to the development of PML, although further long-term safety studies are required.

Carbohydrates: polysaccharides, synthetic mimetics and nucleotide sugars

Several natural and synthetic polysaccharides have been shown to mobilize HSC, including sulfated polysaccharides such as fucoidan and sulfated colominic acid, betafectin, and modified glycosaminoglycans (Reviewed in [90]). A synthetic heparin sulphate mimetic termed “EP80031” has also been shown to mobilize potent long-term HSC when used alone or with G-CSF and/or AMD3100 [91]. Interestingly, EP80031 is an octasaccharide that contains an alkyne-functionalized linker, making it amenable to Cu(I)-catalysed “click chemistry” [92], and also possesses benzyl ether-protecting groups, suggesting the hydroxyl functionality of EP80031 is not required for biological activity (Fig. 2). In any case, the clinical development of EP80031 or related compounds for mobilization can be complicated by the well-known challenges related to the scalable and economical production of pure and structurally defined oligosaccharides in large quantities (Reviewed in [93]). However, with advancements in automated synthesis of oligosaccharides, such endeavours have become more feasible (Reviewed in [94]). Thus, this proof-of-concept study highlights the potential of synthetic oligosaccharide mimetics for therapeutic mobilization.

The natural nucleotide sugar uridine diphosphate glucose (UDP-Glc), normally involved in metabolism and a precursor to glycogen synthesis, was recently shown to selectively mobilize HSC when used alone or in combination with G-CSF [95]. Of note, sorted PB HSC from UDP-Glc-mobilized mice possessed superior long-term engraftment potential compared to phenotypically equivalent HSC harvested from G-CSF-mobilized blood, which is consistent with previous reports indicating G-CSF-mobilized HSC have reduced capacity to sustain long-term hematopoiesis [87, 96]. Although UDP-Glc was not associated with any observable toxicity, high doses were required for effectiveness. Consequently, it has been speculated that the more potent analogue MRS 2690 (Fig. 2) [97] may elicit similar effects at lower doses and thus represents a better compound for clinical development. In any case, these encouraging results warrant further assessment into this class of compounds in efforts to identify more

potent and effective derivatives that mobilize long-term repopulating HSC.

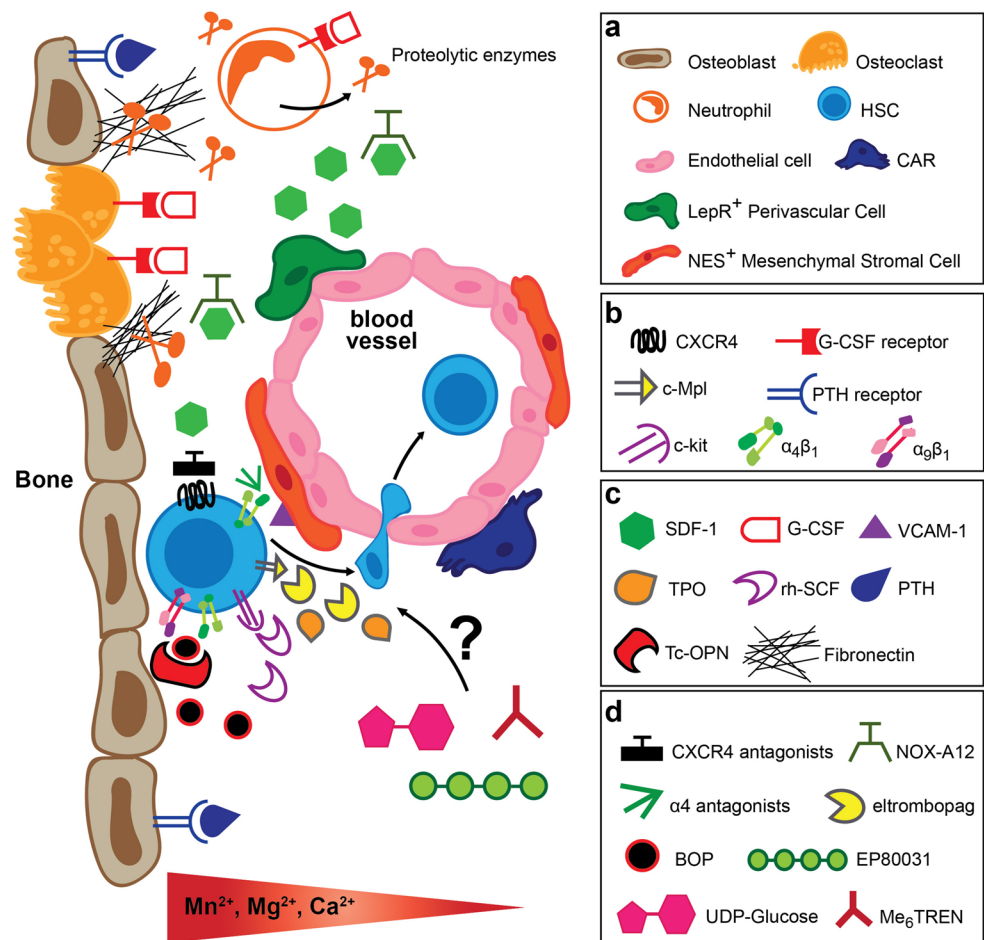
Repurposed drugs for mobilization

Repositioning or repurposing existing drugs for new indications has several advantages as the repurposed drug is faster and cheaper to develop and is less likely to fail in clinical trials due to toxicity [98]. Several existing drugs have been assessed for HSC mobilization including the anti-psychotic drug thioridazine (Fig. 3) [99], the thrombocytopenia drug eltrombopag, the osteoporosis drug teriparatide (rhPTH) and the MS drug natalizumab as described earlier. Bortezomib (Velcade[®]) (Fig. 3), a proteasome inhibitor used in the treatment of MM, has been reported to mobilize HSPC when used alone or in combination with either AMD3100 or G-CSF in murine models [100] and has also undergone early clinical studies [101, 102]. In addition, the non-steroidal anti-inflammatory drug (NSAID) meloxicam (Fig. 3), has also been shown to mobilize HSC and HSPC in mice, non-human primates and healthy human volunteers [103]. Furthermore, meloxicam-mobilized grafts lead to faster recovery of neutrophils and platelets in transplant recipients and suggest this regime would be highly beneficial to patients undergoing HSCT [103]. Based on these promising results, a clinical trial has been initiated to test meloxicam with G-CSF in MM patients (Table 1) and should these trials prove successful, NSAID-induced HSC mobilization with G-CSF would be a simple method for improving transplant outcomes, particularly because meloxicam is administered orally.

Novel mobilization agents from random screening

In most cases, the identification of potential mobilization agents (e.g. CXCR4 antagonists, integrin antagonists, cytokines, etc.) is based on our existing understanding of interactions that regulate HSC function, maintenance and retention with the BM. An alternative strategy is the application of unbiased screening of compounds, which has the benefit of potentially unravelling new molecular targets and the discovery of new classes of biologically active compounds. While unbiased screening has proven successful in the identification of StemReginin 1 (SR1) for ex vivo expansion of human CD34⁺ cells [104], it is more difficult and costly for HSC mobilization applications as such phenotypic screens are dependent on in vivo assays. Nevertheless, the novel small molecule mobilization agent Me₆TREN (Fig. 2) was identified in a compound screen through phenotypic analysis of murine HSPC in PB of treated mice [105]. Further analysis showed Me₆TREN

Fig. 4 Summary of HSC mobilization agents and their therapeutic targets within the BM microenvironment. *Inset figure legend* refers to: **a** cellular components of the BM niche; **b** receptors; **c** extracellular matrix molecules and/or ligands; **d** mobilization agents



mobilizes more HSPC than AMD3100 or G-CSF, synergizes with G-CSF and sustains HSPC levels in PB for up to 3 days after a single dose [105]. Although the direct target of Me₆TREN was not elucidated, it is speculated the mechanism is distinct to AMD3100 based on their differing mobilization kinetics [105]. Nevertheless, Me₆TREN-induced mobilization was shown to occur through activation of MMP-9 and disruption of the CXCR4/SDF-1 axis, which are downstream effects that have also been attributed to AMD3100 [106]. While Me₆TREN has yet to be tested in humans, its clinical development would see it used as either a single-agent replacement of G-CSF or for augmenting G-CSF mobilization for both autologous and allogeneic HSCT.

Summary

Since the approval of G-CSF and AMD3100 for HSC mobilization in PB HSCT, significant effort has been made to identify novel, rapid and effective mobilization agents as alternatives to G-CSF or for augmentation of current mobilization regimes. Several candidates based on recombinant

proteins, synthetic and endogenous peptides, small molecules and carbohydrates as well as more unique classes of agents such as Spiegelmers and nanobodies have been identified with promising therapeutic potential (Summarized in Fig. 4). The diverse class of agents investigated so far provides opportunities to identify novel mechanisms that regulate HSC trafficking and mobilization. Indeed, it is appreciated that HSC mobilization is not only mediated by direct inhibition of adhesive HSC niche interactions but is also attributed to perturbation of signalling pathways, changes in BM vascular integrity and permeability and modulation of other niche constituents. Thus, identification of agents that can collectively influence these mechanisms may provide substantial improvements to existing HSC mobilization methods and subsequent transplant outcomes. In addition, current and future therapeutic development of HSC mobilization agents should also consider the entire “mobilized blood product”, which comprises other cell types in addition to HSC and progenitors when evaluating the success of a lead candidate. For example, in the allogeneic transplant setting, mobilization strategies that can provide enhanced HSPC yield in addition to balancing the numbers of accompanying effector T cells and regulatory

T cells may lead to improved transplant outcomes through modulation of graft-versus-leukaemia or abrogation of graft-versus-host effects, respectively. Despite the recent advancements in HSC mobilization agents, no new drugs have been approved for use in this context since the clinical adoption of AMD3100. However, with several candidates in clinical development (Summarized in Table 1) and encouraging pre-clinical data from new agents and repurposed drugs, the outlook is promising.

Compliance with ethical standards

Conflict of interest The authors have no conflicts to declare.

References

- Philip T, Guglielmi C, Hagenbeek A, Somers R, Vanderlelie H, Bron D, et al. Autologous bone-marrow transplantation as compared with salvage chemotherapy in relapses of chemotherapy-sensitive non-Hodgkin's Lymphoma. *N Engl J Med*. 1995;333:1540–5.
- Attal M, Harousseau JL, Stoppa AM, Sotto JJ, Fuzibet JG, Rossi JF, et al. A prospective, randomized trial of autologous bone marrow transplantation and chemotherapy in multiple myeloma. *N Engl J Med*. 1996;335:91–7.
- Schmitz N, Pfistner B, Sextro M, Sieber M, Carella AM, Haenele M, et al. Aggressive conventional chemotherapy compared with high-dose chemotherapy with autologous haematopoietic stem-cell transplantation for relapsed chemosensitive Hodgkin's disease: a randomised trial. *Lancet*. 2002;359:2065–71.
- Child JA, Morgan GJ, Davies FE, Owen RG, Bell SE, Hawkins K, et al. High-dose chemotherapy with hematopoietic stem-cell rescue for multiple myeloma. *N Engl J Med*. 2003;348:1875–83.
- Niederwieser D, Baldomero H, Szer J, Gratwohl M, Aljurf M, Atsuta Y, et al. Hematopoietic stem cell transplantation activity worldwide in 2012 and a SWOT analysis of the Worldwide Network for Blood and Marrow Transplantation Group including the global survey. *Bone Marrow Transpl*. 2016;51:778–85.
- Bendall LJ, Bradstock KF. G-CSF: from granulopoietic stimulant to bone marrow stem cell mobilizing agent. *Cytokine Growth Factor Rev*. 2014;25:355–67.
- Piedmonte DM, Treuheit MJ. Formulation of Neulasta (R) (peg-filgrastim). *Adv Drug Deliver Rev*. 2008;60:50–8.
- To LB, Levesque JP, Herbert KE, Winkler IG, Bendall LJ, Hiwase DK, et al. Mobilisation strategies for normal and malignant cells. *Pathology*. 2011;43:547–65.
- Bensinger W, DiPersio JF, McCarty JM. Improving stem cell mobilization strategies: future directions. *Bone Marrow Transpl*. 2009;43:181–95.
- To LB, Levesque JP, Herbert KE. How I treat patients who mobilize hematopoietic stem cells poorly. *Blood*. 2011;118:4530–40.
- Gertz MA. Current status of stem cell mobilization. *Brit J Haematol*. 2010;150:647–62.
- Liles WC, Broxmeyer HE, Rodger E, Wood B, Hubel K, Cooper S, et al. Mobilization of hematopoietic progenitor cells in healthy volunteers by AMD3100, a CXCR4 antagonist. *Blood*. 2003;102:2728–30.
- Devine SM, Flomenberg N, Vesole DH, Liesveld J, Weisdorf D, Badel K, et al. Rapid mobilization of CD34 + cells following administration of the CXCR4 antagonist AMD3100 to patients with multiple myeloma and non-Hodgkin's lymphoma. *J Clin Oncol*. 2004;22:1095–102.
- Broxmeyer HE, Orschell CM, Clapp DW, Hangoc G, Cooper S, Plett PA, et al. Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. *J Exp Med*. 2005;201:1307–18.
- Wilson A, Trumpp A. Bone-marrow haematopoietic-stem-cell niches. *Nat Rev Immunol*. 2006;6:93–106.
- Mendez-Ferrer S, Scadden DT, Sanchez-Aguilera A. Bone marrow stem cells: current and emerging concepts. *Ann NY Acad Sci*. 2015;1335:32–44.
- Morrison SJ, Scadden DT. The bone marrow niche for haematopoietic stem cells. *Nature*. 2014;505:327–34.
- Aiuti A, Webb IJ, Bleul C, Springer T, Gutierrez-Ramos JC. The chemokine SDF-1 is a chemoattractant for human CD34+ hematopoietic progenitor cells and provides a new mechanism to explain the mobilization of CD34+ progenitors to peripheral blood. *J Exp Med*. 1997;185:111–20.
- Kim CH, Broxmeyer HE. In vitro behavior of hematopoietic progenitor cells under the influence of chemoattractants: stromal cell-derived factor-1, steel factor, and the bone marrow environment. *Blood*. 1998;91:100–10.
- De Clercq E. Potential clinical applications of the CXCR4 antagonist bicyclam AMD3100. *Mini-Rev Med Chem*. 2005;5:805–24.
- Uy GL, Rettig MP, Cashen AF. Plerixafor, a CXCR4 antagonist for the mobilization of hematopoietic stem cells. *Expert Opin Biol Ther*. 2008;8:1797–804.
- DiPersio JF, Stadtmauer EA, Nademanee A, Micallef INM, Stiff PJ, Kaufman JL, et al. Plerixafor and G-CSF versus placebo and G-CSF to mobilize hematopoietic stem cells for autologous stem cell transplantation in patients with multiple myeloma. *Blood*. 2009;113:5720–6.
- Pusic I, DiPersio JF. Update on clinical experience with AMD3100, an SDF-1/CXCL12-CXCR4 inhibitor, in mobilization of hematopoietic stem and progenitor cells. *Curr Opin Hematol*. 2010;17:319–26.
- De Clercq E. The bicyclam AMD3 100 story. *Nat Rev Drug Discov*. 2003;2:581–7.
- DeMarco SJ, Henze H, Lederer A, Moehle K, Mukherjee R, Romagnoli B, et al. Discovery of novel, highly potent and selective beta-hairpin mimetic CXCR4 inhibitors with excellent anti-HIV activity and pharmacokinetic profiles. *Bioorg Med Chem*. 2006;14:8396–404.
- Robinson JA, DeMarco S, Gombert F, Moehle K, Obrecht D. The design, structures and therapeutic potential of protein epitope mimetics. *Drug Discov Today*. 2008;13:944–51.
- Schmitt S, Weinhold N, Dembowski K, Neben K, Witzens-Harig M, Braun M, et al. First results of a phase-II study with the new CXCR4 antagonist POL6326 to mobilize hematopoietic stem cells (HSC) in multiple myeloma (MM). *Blood*. 2010;116:359–60.
- Karpova D, Brauninger S, Wiercinska E, Kraemer A, Stock B, Graff J, et al. Potent Stem Cell Mobilization with the Novel CXCR4 Antagonist POL6326-Results of a Phase IIa Dose Escalation Study in Comparison to G-CSF. *Blood*. 2015;126:511.
- Huang YH, Liu YC, Yen CF, Chen HC, Chen SJ, King CHR, et al. Rapid mobilization of murine hematopoietic stem and progenitor cells with TG-0054, a novel CXCR4 antagonist. *Blood*. 2009;114:1374–5.
- Abraham M, Biyder K, Begin M, Wald H, Weiss ID, Galun E, et al. Enhanced unique pattern of hematopoietic cell

- mobilization induced by the CXCR4 antagonist 4F-Benzoyl-TN14003. *Stem Cells*. 2007;25:2158–66.
31. Chung DT, Chang LW, Huang YH, Tsai CY, Hsu CH, King CHR, et al. TG-0054, a novel and potent stem cell mobilizer, displays excellent PK/PD and safety profile in phase I trial. *Blood*. 2009;114:357.
 32. Schuster MW, Hagog N, Jalilizeinali B, Funkhauser S, Yohannan MS, Sadler J, et al. Rapid mobilization Of CD34+ progenitor cells with TG0054-03, a novel CXC chemokine receptor 4 (CXCR4) antagonist. *Blood*. 2013;122:905.
 33. Nagler A, Shimoni A, Avivi I, Rowe JM, Beider K, Hardan I, et al. BKT140 is a novel CXCR4 antagonist with stem cell mobilization and antimyeloma effects: an open-label first human trial in patients with multiple myeloma undergoing stem cell mobilization for autologous transplantation. *Blood*. 2010;116:2260.
 34. Peled A, Abraham M, Avivi I, Rowe JM, Beider K, Wald H, et al. The high-affinity CXCR4 antagonist BKT140 is safe and induces a robust mobilization of human CD34+ cells in patients with multiple myeloma. *Clin Cancer Res*. 2014;20:469–79.
 35. Peng SB, Zhang XY, Paul D, Kays LM, Gough W, Stewart J, et al. Identification of LY2510924, a novel cyclic peptide CXCR4 antagonist that exhibits antitumor activities in solid tumor and breast cancer metastatic models. *Mol Cancer Ther*. 2015;14:480–90.
 36. Galsky MD, Vogelzang NJ, Conkling P, Raddad E, Polzer J, Roberson S, et al. A phase I trial of LY2510924, a CXCR4 peptide antagonist, in patients with advanced cancer. *Clin Cancer Res*. 2014;20:3581–8.
 37. Cho BS, Zeng ZH, Mu H, Wang ZQ, Konoplev S, McQueen T, et al. Antileukemia activity of the novel peptidic CXCR4 antagonist LY2510924 as monotherapy and in combination with chemotherapy. *Blood*. 2015;126:222–32.
 38. Rettig MP, Ghobadi A, Holt M, Meier S, Ritchey J, Tahirovic Y, et al. ALT-1188: A new CXCR4 antagonist in development for mobilization Of HSPCs. *Blood*. 2013;122:891.
 39. Debnath B, Xu S, Grande F, Garofalo A, Neamati N. Small molecule inhibitors of CXCR4. *Theranostics*. 2013;3:47–75.
 40. Vela M, Aris M, Llorente M, Garcia-Sanz J, Kremer L. Chemokine receptor-specific antibodies in cancer immunotherapy: achievements and challenges. *Front Immunol*. 2015;6:12.
 41. Hoggatt J, Pelus LM. Mobilization of hematopoietic stem cells from the bone marrow niche to the blood compartment. *Stem Cell Res Ther*. 2011;2:1–13.
 42. Keefe AD, Pai S, Ellington A. Aptamers as therapeutics. *Nat Rev Drug Discov*. 2010;9:537–50.
 43. Vater A, Klusmann S. Turning mirror-image oligonucleotides into drugs: the evolution of Spiegelmer therapeutics. *Drug Discov Today*. 2015;20:147–55.
 44. Scheller M, Schwoebel F, Vossmeier D, Leutz A. Rapid and efficient mobilization of murine hematopoietic stem and progenitor cells with Nox-A12, a new Spiegelmers (R)-based CXCR4/SDF-1(CXCL12) antagonist. *Blood*. 2011;118:1290–2995.
 45. Vater A, Sahlmann J, Kröger N, Zöllner S, Lioznov M, Maasch C, et al. Hematopoietic stem and progenitor cell mobilization in mice and humans by a first-in-class mirror-image oligonucleotide inhibitor of CXCL12. *Clin Pharmacol Ther*. 2013;94:150–7.
 46. Ludwig H, Weisel K, Petrucci MT, Leleu X, Cafro AM, Laurent G, et al. Final results from the phase IIa study of the anti-CXCL12 Spiegelmer[®] Olaptosed Pegol (NOX-A12) in combination with bortezomib and dexamethasone in patients with multiple myeloma. *Blood*. 2014;124:2111.
 47. Hassanzadeh-Ghassabeh G, Devoogdt N, De Pauw P, Vincke C, Muyldermans S. Nanobodies and their potential applications. *Nanomedicine-Uk*. 2013;8:1013–26.
 48. Wesolowski J, Alzogaray V, Reyelt J, Unger M, Juarez K, Urrutia M, et al. Single domain antibodies: promising experimental and therapeutic tools in infection and immunity. *Med Microbiol Immunol*. 2009;198:157–74.
 49. Jahnichen S, Blanchetot C, Maussang D, Gonzalez-Pajuelo M, Chow KY, Bosch L, et al. CXCR4 nanobodies (VHH-based single variable domains) potently inhibit chemotaxis and HIV-1 replication and mobilize stem cells. *P Natl Acad Sci USA*. 2010;107:20565–70.
 50. Griffiths K, Dolezal O, Cao B, Nilsson SK, See HB, Pflieger KDG, et al. i-Bodies, human single domain antibodies that antagonize chemokine receptor CXCR4. *J Biol Chem*. 2016;291:12641–57.
 51. Petit I, Szyper-Kravitz M, Nagler A, Lahav M, Peled A, Habler L, et al. G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. *Nat Immunol*. 2002;3:687–94.
 52. Angelopoulou MK, Tsirkinidis P, Boutsikas G, Vassilakopoulos TP, Tsirigotis P. New insights in the mobilization of hematopoietic stem cells in lymphoma and multiple myeloma patients. *Biomed Res Int*. 2014;2014:835138.
 53. Dar A, Schajnovitz A, Lapid K, Kalinkovich A, Itkin T, Ludin A, et al. Rapid mobilization of hematopoietic progenitors by AMD3100 and catecholamines is mediated by CXCR4-dependent SDF-1 release from bone marrow stromal cells. *Leukemia*. 2011;25:1286–96.
 54. Itkin T, Gur-Cohen S, Spencer JA, Schajnovitz A, Ramasamy SK, Kusumbe AP, et al. Distinct bone marrow blood vessels differentially regulate haematopoiesis. *Nature*. 2016;532:323–8.
 55. Zirafi O, Kim K-A, Ständker L, Mohr Katharina B, Sauter D, Heigle A, et al. Discovery and characterization of an endogenous CXCR4 antagonist. *Cell Rep*. 2015;11:737–47.
 56. Zirafi O, Hermann PC, Munch J. Proteolytic processing of human serum albumin generates EPI-X4, an endogenous antagonist of CXCR4. *J Leukocyte Biol*. 2016;99:863–8.
 57. Kollet O, Dar A, Shvitiel S, Kalinkovich A, Lapid K, Sztainberg Y, et al. Osteoclasts degrade endosteal components and promote mobilization of hematopoietic progenitor cells. *Nat Med*. 2006;12:657–64.
 58. Levesque JP, Hendy J, Takamatsu Y, Simmons PJ, Bendall LJ. Disruption of the CXCR4/CXCL12 chemotactic interaction during hematopoietic stem cell mobilization induced by GCSF or cyclophosphamide. *J Clin Invest*. 2003;111:187–96.
 59. Buske C, Kirchhoff F, Munch J. EPI-X4, a novel endogenous antagonist of CXCR4. *Oncotarget*. 2015;6:35137–8.
 60. Kim MG, Han N, Lee EK, Kim T. Pegfilgrastim vs filgrastim in PBSC mobilization for autologous hematopoietic SCT: a systematic review and meta-analysis. *Bone Marrow Transpl*. 2015;50:523–30.
 61. Shpall EJ, Wheeler CA, Turner SA, Yanovich S, Brown RA, Pecora AL, et al. A randomized phase 3 study of peripheral blood progenitor cell mobilization with stem cell factor and filgrastim in high-risk breast cancer patients. *Blood*. 1999;93:2491–501.
 62. Stiff P, Gingrich R, Luger S, Wyres MR, Brown RA, LeMaistre CF, et al. A randomized phase 2 study of PBPC mobilization by stem cell factor and filgrastim in heavily pretreated patients with Hodgkin's disease or non-Hodgkin's lymphoma. *Bone Marrow Transpl*. 2000;26:471–81.
 63. Herbert KE, Prince HM, Ritchie DS, Seymour JF. The role of ancestim (recombinant human stem-cell factor, rhSCF) in hematopoietic stem cell mobilization and hematopoietic reconstitution. *Expert Opin Biol Ther*. 2010;10:113–25.
 64. Somlo G, Sniecincki I, ter Veer A, Longmate J, Knutson G, Vuk-Pavlovic S, et al. Recombinant human thrombopoietin in combination with granulocyte colony-stimulating factor enhances

- mobilization of peripheral blood progenitor cells, increases peripheral blood platelet concentration, and accelerates hematopoietic recovery following high-dose chemotherapy. *Blood*. 1999;93:2798–806.
65. Linker C, Anderlini P, Herzig R, Christiansen N, Somlo G, Bensinger W, et al. Recombinant human thrombopoietin augments mobilization of peripheral blood progenitor cells for autologous transplantation. *Biol Blood Marrow Transpl*. 2003;9:405–13.
 66. Kuter DJ. The biology of thrombopoietin and thrombopoietin receptor agonists. *Int J Hematol*. 2013;98:10–23.
 67. King AG, Horowitz D, Dillon SB, Levin R, Farese AM, MacVittie TJ, et al. Rapid mobilization of murine hematopoietic stem cells with enhanced engraftment properties and evaluation of hematopoietic progenitor cell mobilization in rhesus monkeys by a single injection of SB-251353, a specific truncated form of the human CXC chemokine GRO beta. *Blood*. 2001;97:1534–42.
 68. Fukuda S, Bian HM, King AG, Pelus LM. The chemokine GRO beta mobilizes early hematopoietic stem cells characterized by enhanced homing and engraftment. *Blood*. 2007;110:860–9.
 69. Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, et al. Osteoblastic cells regulate the haematopoietic stem cell niche. *Nature*. 2003;425:841–6.
 70. Adams GB, Martin RP, Alley IR, Chabner KT, Cohen KS, Calvi LM, et al. Therapeutic targeting of a stem cell niche. *Nat Biotechnol*. 2007;25:238–43.
 71. Ballen KK, Shpall EJ, Avigan D, Yeap BY, Fisher DC, McDermott K, et al. Phase I trial of parathyroid hormone to facilitate stem cell mobilization. *Biol Blood Marrow Transpl*. 2007;13:838–43.
 72. Ballen K, Mendizabal AM, Cutler C, Politikos I, Jamieson K, Shpall EJ, et al. Phase II trial of parathyroid hormone after double umbilical cord blood transplantation. *Biol Blood Marrow Transpl*. 2012;18:1851–8.
 73. Yu EW, Kumbhani R, Siwila-Sackman E, DeLelys M, Preffer FI, Leder BZ, et al. Teriparatide (PTH1-34) treatment increases peripheral hematopoietic stem cells in postmenopausal women. *J Bone Miner Res*. 2014;29:1380–6.
 74. He S, Chu JH, Vasu S, Deng YC, Yuan SZ, Zhang JY, et al. FLT3L and Plerixafor combination increases hematopoietic stem cell mobilization and leads to improved transplantation outcome. *Biol Blood Marrow Transplant*. 2014;20:309–13.
 75. Beres AJ, Drobyski WR. The role of regulatory T cells in the biology of graft versus host disease. *Front Immunol*. 2013;4:163.
 76. Anandasabapathy N, Breton G, Hurley A, Caskey M, Trumfheller C, Sarma P, et al. Efficacy and safety of CDX-301, recombinant human Flt3L, at expanding dendritic cells and hematopoietic stem cells in healthy human volunteers. *Bone Marrow Transpl*. 2015;50:924–30.
 77. Levesque J-P, Winkler IG. Cell adhesion molecules in normal and malignant hematopoiesis: from bench to bedside. *Curr Stem Cell Rep*. 2016;2:356–67.
 78. Oostendorp RAJ, Dormer P. VLA-4-mediated interactions between normal human hematopoietic progenitors and stromal cells. *Leuk Lymphoma*. 1997;24:423–35.
 79. Craddock CF, Nakamoto B, Andrews RG, Priestley GV, Papayannopoulou T. Antibodies to VLA4 integrin mobilize long-term repopulating cells and augment cytokine-induced mobilization in primates and mice. *Blood*. 1997;90:4779–88.
 80. Jing D, Oelschlaegel U, Ordemann R, Holig K, Ehninger G, Reichmann H, et al. CD49d blockade by natalizumab in patients with multiple sclerosis affects steady-state hematopoiesis and mobilizes progenitors with a distinct phenotype and function. *Bone Marrow Transpl*. 2010;45:1489–96.
 81. Zohren F, Toutzaris D, Klarner V, Hartung HP, Kieseier B, Haas R. The monoclonal anti-VLA-4 antibody natalizumab mobilizes CD34 + hematopoietic progenitor cells in humans. *Blood*. 2008;111:3893–5.
 82. Bloomgren G, Richman S, Hotermans C, Subramanyam M, Goelz S, Natarajan A, et al. Risk of Natalizumab-associated progressive multifocal leukoencephalopathy. *N Engl J Med*. 2012;366:1870–80.
 83. Ramirez P, Rettig MP, Uy GL, Deych E, Holt MS, Ritchey JK, et al. BIO5192, a small molecule inhibitor of VLA-4, mobilizes hematopoietic stem and progenitor cells. *Blood*. 2009;114:1340–3.
 84. Leone DR, Giza K, Gill A, Dolinski BM, Yang W, Perper S, et al. An assessment of the mechanistic differences between two integrin alpha(4)beta(1) inhibitors, the monoclonal antibody TA-2 and the small molecule BIO5192, in rat experimental autoimmune encephalomyelitis. *J Pharmacol Exp Ther*. 2003;305:1150–62.
 85. Rettig MP, Anstas G, DiPersio JF. Mobilization of hematopoietic stem and progenitor cells using inhibitors of CXCR4 and VLA-4. *Leukemia*. 2012;26:34–53.
 86. Grassinger J, Haylock DN, Storan MJ, Haines GO, Williams B, Whitty GA, et al. Thrombin-cleaved osteopontin regulates hemopoietic stem and progenitor cell functions through interactions with alpha9beta1 and alpha4beta1 integrins. *Blood*. 2009;114:49–59.
 87. Cao B, Zhang Z, Grassinger J, Williams B, Heazlewood CK, Churches QI, et al. Therapeutic targeting and rapid mobilization of endosteal HSC using a small molecule integrin antagonist. *Nat Commun*. 2016;7:11077
 88. Cao B, Hutt OE, Zhang Z, Li SH, Heazlewood SY, Williams B, et al. Design, synthesis and binding properties of a fluorescent alpha(9)beta(1)/alpha(4)beta(1) integrin antagonist and its application as an in vivo probe for bone marrow haemopoietic stem cells. *Org Biomol Chem*. 2014;12:965–78.
 89. Grassinger J, Haylock DN, Williams B, Olsen GH, Nilsson SK. Phenotypically identical hemopoietic stem cells isolated from different regions of bone marrow have different biologic potential. *Blood*. 2010;116:3185–96.
 90. Hoggatt J, Speth JM, Pelus LM. Concise review: sowing the seeds of a fruitful harvest: hematopoietic stem cell mobilization. *Stem Cells*. 2013;31:2599–606.
 91. Di Giacomo F, Lewandowski D, Cabannes E, Nancy-Portebois V, Petitou M, Fichelson S, et al. Heparan sulfate mimetics can efficiently mobilize long-term hematopoietic stem cells. *Haematologica*. 2012;97:491–9.
 92. Meldal M, Tornøe CW. Cu-catalyzed azide-alkyne cycloaddition. *Chem Rev*. 2008;108:2952–3015.
 93. Boltje TJ, Buskas T, Boons GJ. Opportunities and challenges in synthetic oligosaccharide and glycoconjugate research. *Nat Chem*. 2009;1:611–22.
 94. Seeberger PH, Werz DB. Automated synthesis of oligosaccharides as a basis for drug discovery. *Nat Rev Drug Discov*. 2005;4:751–63.
 95. Kook S, Cho J, Lee SB, Lee BC. The nucleotide sugar UDP-glucose mobilizes long-term repopulating primitive hematopoietic cells. *J Clin Invest*. 2013;123:3420–35.
 96. Yeoh JS, Ausema A, Wierenga P, de Haan G, van Os R. Mobilized peripheral blood stem cells provide rapid reconstitution but impaired long-term engraftment in a mouse model. *Bone Marrow Transpl*. 2007;39:401–9.
 97. Ko H, Fricks I, Ivanov AA, Harden TK, Jacobson KA. Structure-activity relationship of uridine 5'-diphosphoglucose analogues as agonists of the human P2Y(14) receptor. *J Med Chem*. 2007;50:2030–9.

98. Ashburn TT, Thor KB. Drug repositioning: identifying and developing new uses for existing drugs. *Nat Rev Drug Discov.* 2004;3:673–83.
99. Chigaev A, Sklar LA, Schrader R, Stephens N, Raissy H, Winter SS. The evaluation of thioridazine as a hematopoietic progenitor cell mobilizing agent in healthy human subjects. *J Clin Pharmacol.* 2015;55:512–6.
100. Ghobadi A, Rettig MP, Cooper ML, Holt MS, Ritchey JK, Eisenberg L, et al. Bortezomib is a rapid mobilizer of hematopoietic stem cells in mice via modulation of the VCAM-1/VLA-4 axis. *Blood.* 2014;124:2752–4.
101. Giglio G, Romito S, Carozza F, Musacchio M, Antuzzi G, Gigli R, et al. Successful mobilization of peripheral blood stem cells with bortezomib plus high-dose cyclophosphamide plus G-CSF in a light chain myeloma patient after failure with Total Therapy 2. *Int J Hematol.* 2009;90:81–6.
102. Abhyankar S, Lubanski P, DeJarnette S, Merkel D, Bunch J, Daniels K, et al. A novel hematopoietic progenitor cell mobilization regimen, utilizing bortezomib and filgrastim, for patients undergoing autologous transplant. *J Clin Apher.* 2016;6:559–63.
103. Hoggatt J, Mohammad KS, Singh P, Hoggatt AF, Chitteti BR, Speth JM, et al. Differential stem- and progenitor-cell trafficking by prostaglandin E-2. *Nature.* 2013;495:365–9.
104. Boitano AE, Wang JA, Romeo R, Bouchez LC, Parker AE, Sutton SE, et al. Aryl hydrocarbon receptor antagonists promote the expansion of human hematopoietic stem cells. *Science.* 2010;329:1345–8.
105. Zhang J, Ren XL, Shi W, Wang SH, Chen HX, Zhang BW, et al. Small molecule Me6TREN mobilizes hematopoietic stem/progenitor cells by activating MMP-9 expression and disrupting SDF-1/CXCR4 axis. *Blood.* 2014;123:428–41.
106. Lee HM, Wysoczynski M, Liu R, Shin DM, Kucia M, Botta M, et al. Mobilization studies in complement-deficient mice reveal that optimal AMD3100 mobilization of hematopoietic stem cells depends on complement cascade activation by AMD3100-stimulated granulocytes. *Leukemia.* 2010;24:573–82.