



Published in final edited form as:

Curr Stem Cell Rep. 2018 December ; 4(4): 273–281. doi:10.1007/s40778-018-0141-9.

Peripheral blood stem cell mobilization; a look ahead

Louis M. Pelus[†] and Hal E Broxmeyer

Department of Microbiology & Immunology, Indiana University School of Medicine, 950 W Walnut Street, R2-301, Indianapolis, IN 46202

Abstract

The purpose of review: Mobilized peripheral blood is the predominant source of stem and progenitor cells for hematologic transplantation. Successful transplant requires sufficient stem cells of high enough quality to recapitulate lifelong hematopoiesis, but in some patients and normal donors, reaching critical threshold stem cell numbers are difficult to achieve. Novel strategies, particularly those offering rapid mobilization and reduced costs, remains an area of interest.

This review summarizes critical scientific underpinnings in understanding the process of stem cell mobilization, with a focus on new or improved strategies for their efficient collection and engraftment.

Recent findings: Studies are described that provide new insights into the complexity of stem cell mobilization. Agents that target new pathways such HSC egress, identify strategies to collect more potent competing HSC and new methods to optimize stem cell collection and engraftment are being evaluated.

Summary: Agents and more effective strategies that directly address the current shortcomings of hematopoietic stem cell mobilization and transplantation and offer the potential to facilitate collection and expand use of mobilized stem cells have been identified.

Keywords

Peripheral blood stem cell mobilization; Hematopoietic stem cells; stem cell collection; HSC egress; high engrafting stem cells; hypoxic collection

Introduction

Hematopoietic cell transplantation (HCT) has been used for over 50 years to successfully treat hematologic disease. The use of autologous and allogeneic hematopoietic stem cells (HSC) for HCT has expanded beyond hematological malignancies and bone marrow failure syndromes to non-malignant hematologic disorders and immunological diseases. With the reemergence of HSC based gene therapy strategies and the use of less myelotoxic

[†] To whom all correspondence should be directed. Impelus@iupui.edu.

Conflict of Interest

Louis M Pelus and Hal E Broxmeyer declare that they have no conflict of interest.

Human and Animal Rights and Informed Consent

This article does not contain any studies with human or animal subjects.

preparative regimens HCT is positioned to expand even further. Successful transplantation requires HSC in sufficient quantity and quality to recapitulate lifelong hematopoiesis. There are three potential sources of HSC for clinical utility; bone marrow, mobilized peripheral blood and umbilical cord blood [see [1] for a historical perspective]. Each source varies in cellular characteristics with potential advantages and disadvantages for clinical use.

Currently, the predominant source of HSC for transplant is peripheral blood collected by apheresis after a multi-day regimen of granulocyte colony-stimulating factor (G-CSF), a process termed peripheral blood stem cell mobilization (PBSCM), [2;3]. While highly successful, G-CSF regimens can be associated with lifestyle disruptive and stressful morbidities [4;5] and in some cases more serious life-threatening toxicities [6]. In high risk individuals myocardial infarction and cerebral ischemia can result from the thrombophilic effects of G-CSF.

The dose of CD34+ cells infused in a mobilized peripheral blood stem cell transplant (PB SCT) is an important predictor of neutrophil and platelet recovery, and serves as a biomarker of potential stem cell engraftment. An optimal autologous mobilized PBSC graft requires a minimum of 2×10^6 CD34+ cells per kg patient body weight to provide for rapid and sustained multilineage engraftment, with 5×10^6 being optimal. For allogeneic transplant, a CD34+ cell dose of $\sim 4.5 \times 10^6$ per kg is associated with improved survival without increased incidence of acute or chronic graft versus host disease. Despite its success for most patients, poor mobilization rates as high as 40% are observed [7] and often require multiple apheresis, resulting in increased patient stress, added clinical resources, and higher costs [8;9].

Recently, the small molecule AMD3100 (plerixafor), a CXCR4 antagonist, shown to mobilize alone and with G-CSF [10–12] was clinically validated [13;14] and approved by the FDA specifically for use in combination with G-CSF for patients who fail to mobilize a minimum CD34+ cell graft using G-CSF alone. However, addition of plerixafor to the multiday G-CSF regimen adds significant cost and has restricted its universal use. Based on the potential benefit of a single day mobilization and apheresis procedure, administration of plerixafor as a standalone agent has been explored clinically, however the level of mobilization was not clinically effective, being significantly lower than G-CSF [15–17]. Moreover, a significant number of donors fail to mobilize sufficient cells even after multiple apheresis sessions or dose escalation and infusion [17]. Thus, the development of novel alternative strategies, particularly those that offer rapid mobilization and reduced costs remains an area of interest. Better understanding of mechanisms of mobilization may lead to more effective strategies and is an area of significant investigation.

Paradigmatic mobilization mechanisms?

Mechanisms underlying the process of HSPC mobilization have been studied for several decades but still remain unclear. What is clear is that G-CSF does not mobilize hematopoietic stem and progenitor cells (HSPC) by directly acting on them. This is supported by pharmacokinetic studies that indicate a two compartment model. Since HSPC are held within supportive and regulatory marrow niches through interactions with stromal

cells, mechanism studies logically focused on the hematopoietic niche. Early studies demonstrated a need for neutrophils for mobilization with G-CSF or the combination of G-CSF plus the chemokine GRO β [18;19] and a dramatic reduction in concentration of the chemotactic chemokine stromal cell derived factor-1 (SDF-1/CXCL12) in bone marrow associated with increased marrow proteases [20–22]. This led to the hypothesis of protease mediated changes in retention mechanisms and alteration of SDF-1 gradient in favor of migration of untethered cells to the periphery as the primary mechanism of G-CSF-induced PBSC mobilization. Several proteases including neutrophil elastase, cathepsin G, matrix metalloproteases and plasmin are increased and the endogenous protease inhibitors serpin A1 and A3 are reduced in marrow after mobilization by G-CSF. Later, proteases were shown to cleave other HSPC retention factors including integrins and c-kit. Despite the clear evidence in favor of proteases and proteolysis of retention mechanisms as a potentially important and perhaps common mechanism in HSPC mobilization, at least by G-CSF, the identity of the specific proteases involved and their targets remain poorly defined. Conflicting data on the role of individual proteases has come primarily from studies using knockout mice and selective enzyme inhibitors, but these may result from inherent redundancy in the models used [23–25]. Overall, findings support that G-CSF administration results in a highly proteolytic marrow environment and a role for proteases that interfere with the SDF1/CXCR4 and other retention axes. Exactly which protease(s) is involved, whether disruption of these pathways is necessary in all settings and how this facilitates transit of HSPC out of marrow is not clear.

The SDF-1/CXCR4 axis has been the most widely studied and best characterized retention pathway implicated in HSPC mobilization and plays a central common role in a number of mobilization strategies, e.g., G-CSF, Flt-3 ligand, SCF. This led to the development of plerixafor, a CXCR4 antagonist, for clinical use in patients who mobilize poorly to G-CSF. Following on from plerixafor, additional CXCR4 antagonists, including POL636 [26–28], BKT140 [29;30], LY2510924 [31–33], TG-0054 [34], and ALT-1188 [35] are in preclinical and/or clinical development, although what their benefit above plerixafor might be is yet to be determined. NOX-A12 an anti-SDF-1 Spiegelmer, a first in class mirror-image oligonucleotide inhibitor of SDF-1 mobilizes HSPC [36]. In addition, anti CXCR4 nanobodies have also been shown to mobilize HSPC [37]. These agents have the potential to replace G-CSF or improve current G-CSF based mobilization strategies. Their potential for routine clinical use remains to be determined.

The bioactive phospholipid sphingosine-1-phosphate (S1P) [38] and the complement cascade [39] have been implicated in HSPC mobilization. Proteins involved in niche interactions can be cleaved by C5-mediated proteolysis in bone marrow and membrane attack complex (MAC)-mediated increase in S1P levels favoring migration to the periphery. Mobilization studies in mice deficient in sphingosine kinase 2 support a role for plasma S1P in HSPC egress [40]. Moreover, administration of a S1P agonist prior to AMD3100 enhanced mobilization was further increased by G-CSF. Interestingly S1P agonism was unable to increase mobilization alone or with G-CSF, pointing to a critical role of CXCR4 antagonism for mobilization in this setting [41]. This enforces a central role for the SDF-1/CXCR4 axis in the process of mobilization.

Integrins are transmembrane glycoproteins that mediate cell-cell/matrix interactions. The $\alpha 4\beta 1$ integrin VLA4 and counter ligand VCAM-1 pair serve as a HSPC retention mechanism. Interruption of this axis by antibodies or genetic manipulation leads to HSPC mobilization (see [42]). A selective VLA4 inhibitor BIO5192 has been developed and shown to mobilize HSPC and in combination with G-CSF and plerixafor [43]. However, it is unclear whether this mobilization strategy is being developed and or if combinations of these agents for mobilization is economically efficient and viable.

A number of natural and synthetic polysaccharides are able to mobilize HSPC, including sulfated polysaccharides and modified glycosaminoglycans (reviewed in [44]). Of particular note, the synthetic octasaccharide EP80031 mobilizes HSPC alone and in combination with G-CSF and/or AMD3100 [45] and uridine diphosphate glucose (UDP-Glc) mobilizes high engrafting HSC when used in combination with G-CSF [46]. Advances in oligosaccharide synthesis and development of more potent compounds may lead to therapeutic utility of this class of compounds.

Proteasome inhibitors have been shown to be particularly effective in the treatment of patients with Multiple Myeloma (MM). In preclinical studies, combining the proteasome inhibitor bortezomib with G-CSF or AMD3100 was more effective in mobilizing HSPC than either agent alone. In a recent phase II trial, combination of bortezomib with cyclophosphamide and G-CSF resulted in enhanced CD34+ cell yield allowing 85% of MM patients to mobilize a sufficient PBSC graft in one apheresis [47].

Prostaglandin E2 (PGE2) signaling through its EP4 receptor has been shown to enforce retention of HSPC in bone marrow, and inhibition of PGE2 synthesis has been linked to HSPC mobilization alone and in combination with G-CSF. In particular, the non-steroidal anti-inflammatory drug (NSAID) meloxicam has been shown to mobilize HSPC in mice, monkeys and man [48]. Moreover, the meloxicam mobilized graft led to faster neutrophil and platelet recovery compared to a graft mobilized without NSAID. Since NSAID enhanced G-CSF mobilization occurred in CXCR4 knockout mice, it appears to be independent of the changes in the SDF-1/CXCR4 axis induced by G-CSF [48]. In a single center clinical study, addition of meloxicam to chemotherapy/G-CSF mobilization in poorly mobilizing patients with MM, increased peripheral hematopoietic CD34+ cell levels and reduced the need for plerixafor rescue, and significantly lowering the overall mobilization costs [49].

It has been known for some time that stresses such as exercise and ACTH can mobilize HPC [50]. Recently, a link was made between neurotransmitters and osteoblasts, cells known to support HSPC retention and to be reduced in activity following administration of G-CSF, and egress of HSPC [51]. Osteoblasts do not express G-CSF receptors, rather these receptors are expressed on sympathetic neurons that innervate bone marrow. While G-CSF administration does not increase norepinephrine, it does prevent its reuptake leading to higher tissue level [52]. Mobilization in mice occurs following norepinephrine administration where it binds to stromal cells resulting in reduction in niche retention mechanisms, including SDF-1, SCF and VCAM [51;53;54]. The potential role of adrenergic receptor agonists as clinical mobilizers remains to be tested.

New experimental pathways

The pathways described above serve to place the state of the field in perspective. It is not our intent to exhaustively review the field of peripheral blood stem cell mobilization or to exhaustively present the numerous pathways, mechanisms and agents that have shown activity to mobilize hematopoietic stem and/or progenitor cells; these have been recently extensively reviewed in several excellent publications [55–59]. Rather, we will discuss several new perhaps non paradigmatic experimental pathways that may lead to new or improved strategies for HSPC mobilization.

Neuropeptide Y

One protease of interest not discussed above is dipeptidylpeptidase 4 (DPP4/CD26), a serine exopeptidase that cleaves N-terminal dipeptides with alanine or proline in the penultimate position. DPP4 exist both as a membrane bound protease as well as in soluble form. Reduced mobilization response to G-CSF is observed in CD26 knockout mice or mice treated with a selective pharmacologic DPP4 inhibitor [60;61]. Since DPP4 is expressed on HSPC and can cleave and inactivate SDF-1, and as described, reduced marrow SDF-1 is a hallmark observed after administration of G-CSF, it was hypothesized that cleavage of SDF-1 by CD26 on HSPC plays an essential role in HPC trafficking, likely through cleavage of SDF-1 thereby reducing bone marrow retention [62]. However, evidence showing a direct association between CD26 and disruption of SDF-1 signaling in vivo during G-CSF administration was lacking, as was definitive studies on repopulating HSC.

Recently, proof was provided that mobilization of repopulating HSC by G-CSF is in fact reduced by inhibition of DPP4/CD26, however, using chimeric mice created by transplanting bone marrow from wild-type or CD26 knockout mice into syngeneic wild-type or CD26 knockout recipient mice, it was found that HSPC-intrinsic CD26 expression was not required for HSPC egress in response to G-CSF but rather mobilization was dependent on CD26 expression on stromal cells [63]. Moreover, G-CSF associated degradation of SDF-1 occurred equally in wild-type and CD26 knockout mice or mice treated with a DPP4 enzyme inhibitor, as determined by MASS Spectrometry. Following G-CSF administration CD26 was only increased on a subpopulation of sinusoidal endothelial cells (EC) that form the mechanical barrier between the peripheral blood and marrow and regulate hematopoietic trafficking [64]. Since HSPC must transmigrate across the EC barrier to enter the peripheral circulation whether EC CD26 regulated HSPC egress was evaluated. In monolayer EC transmigration models, G-CSF increased EC CD26 expression and activity leading to enhanced HSPC transmigration, but blocking DPP4 activity prevented transmigration.

Since optimal mobilization was dependent on CD26 but not mediated through cleavage of SDF-1, protein databases were searched for proteins involved in leukocyte trafficking and possessing a putative CD26 cleavage site. This search identified the neurotransmitter neuropeptide-Y (NPY), a ligand with cognate receptors on marrow stromal cells including EC and which has been reported to regulate immune cell and bone homeostasis and be produced by both nerve fibers and endothelium[65–68]. Mass spectrometry confirmed that CD26 cleaved full length NPY into a NPY_{3–36} truncated form. NPY interacts with several G-protein coupled receptors (NPYR1–5), preferentially binding NPYR1, while NPY_{3–36}

preferentially binds NPYR2 and NPYR5. Each of these receptors was found to be expressed by sinusoidal EC [63]. In the monolayer EC transmigration model, HSPC transmigration that is blocked by inhibiting DPP4 activity was reversed by addition of NPY₃₋₃₆. Administration of NPY₃₋₃₆ restored normal HSPC mobilization in CD26 knockout mice or mice treated with a DPP4 inhibitor, and this restoration of response was blocked by selective antagonists of the NPY2 and NPY5 receptors. Mice genetically deficient in NPY also showed poor mobilization to G-CSF that could be restored by administration of NPY₃₋₃₆. Since NPY receptors are known to regulate vascular integrity, bone marrow sinusoidal permeability was evaluated. Using live animal imaging, G-CSF enhanced sinusoidal permeability that could be blocked by a DPP4 inhibitor that was reversed by NPY₃₋₃₆. Truncated NPY augmented endothelial barrier permeability by downregulating adherence junction molecules VE-cadherin and CD31 that widened the gap between vascular ECs. This resulted in greater HSPC transmigration.

These studies provide new insights into the complexity of stem cell mobilization. Alteration of retention axes represent only one part of the mobilization process but activation of additional steps are required for optimal activity. These studies now show that ECs act as gatekeepers regulating HSPC egress and that enzymatic regulation of NPY by CD26 acts as the open/close signal. The ability to target NPY receptors with ligands and antagonists in vivo make them an attractive new target for regulating HSPC trafficking. Treatment of mice with NPY₃₋₃₆ but not full length NPY significantly enhanced HSPC mobilization by AMD3100 which mobilizes independently of CD26 [63], supporting the concept of regulation of vascular permeability as a common feature associated with HSPC marrow egress and a potential broadly applicable strategy for HSPC mobilization.

GRO β plus AMD3100

With the goal of developing a safe, rapid single day mobilization regimen, combination mobilization with the CXCR4 antagonist AMD3100/plerixafor and the CXCR2 ligand GRO β , previously shown to mobilize HSPC in mice and rhesus monkeys [18;69;70] and recently also in man [70] was explored. While the CXCR4 signaling axis has been a focus of mechanism of HSPC mobilization, particularly associated with the action of G-CSF the chemokine ligands of the CXCR2 receptor, notably GRO β and IL8, induce rapid HSPC mobilization. The mechanism of action of these chemokines has not been linked to the SDF-1/CXCR4 axis, but instead to metalloprotease-9 (MMP-9), which has the potential to nonselectively degrade many types of cell-stromal-matrix interactions. Genetic models and antibody neutralization studies indicate that rapid mobilization by these ligands is dependent on MMP-9 [18;69;71] mediated through the CXCR2 receptor expressed on neutrophils but not expressed on HSPC.

Single combined injection of GRO β plus AMD3100 mobilized more HSPC in 15 minutes than the number of HSPC mobilized by G-CSF in a 4 day regimen in mice [70]. Extensive biochemical, molecular and genetic evidence all confirmed crosstalk between these neutrophil receptors. Stimulation of release of MMP-9 from neutrophil granules through stimulation of CXCR2 by GRO β was greatly enhanced by antagonizing CXCR4 receptors on the same cells. These studies also identified a negative signaling pathway as a result of

antagonism of the CXCR4 receptor, at least on the MMP-9 protease. In this regard it is possible that the degradation of SDF-1 observed during G-CSF based regimens may release this negative signal as well, allowing increased MMP-9 release that contributes to mobilization. The rapid and robust kinetics of GRO β plus AMD3100 mobilization was not associated with the histological changes commonly observed following G-CSF mobilization, e.g., osteolineage cell flattening, reduction of adhesion and chemoattractant molecules, and localization of accessory cell populations. When intra vital microscopy was applied, there was an increase in vascular permeability with the GRO β plus AMD3100 combination mobilization within 5 minutes. Importantly, this increased vascular permeability was completely blocked if mice were treated with an anti-MMP-9 antibody. On histological examination of marrow sections increased nucleated cells within the sinusoid lumens could also be seen as early as 5 minutes. These findings strongly suggest an effect of MMP-9 on EC barrier integrity. It remains to be determined how this is accomplished.

Functional comparison of the hematopoietic grafts mobilized by GRO β plus AMD3100 versus G-CSF in transplant studies were quite revealing. The GRO β plus AMD3100 combination mobilized a higher engrafting and competitive HSC population than G-CSF. While both GRO β [72] and AMD3100 [10] alone have shown enhanced engraftment compared to G-CSF, these studies only compared whole peripheral blood mononuclear populations and although suggestive are not definitive assessments of HSC function and could be due solely to a graft containing greater numbers of HSC. However, in studies comparing HSC number in the GRO β plus AMD3100 versus G-CSF mobilized grants, the GRO β plus AMD3100 graft actually contained fewer phenotypically define HSC. Competitive transplants using highly purified HSC from mice mobilized with GRO β plus AMD3100 or G-CSF and transplanted with the exact same number of HSC showed the HSC from GRO β plus AMD3100 mobilized mice to be twice as competitive as those from mice mobilized with G-CSF, and indicate that GRO β plus AMD3100 mobilizes a distinct highly engraftable HSC population (heHSC) with superior competitiveness [70]. RNA sequencing of these heHSC indicated that they had a distinct transcriptome compared to HSC mobilized by G-CSF. Intriguingly, in gene set enrichment analysis, these cells showed a transcriptome that mirrors young fetal liver HSC.

Other chemokines/chemokine pathways have been explored as HSPC mobilizers. A genetic variant BB10010 of the chemokine macrophage inflammatory protein-1 (MIP-1) mobilized HPC in mice but was without activity in patients [73]. A rationally designed SDF-1 analog that downregulates CXCR4 rapidly mobilized neutrophils and HPC when used alone and synergized with G-CSF [74;75], but did not mobilize HSC in mice [76]. In addition, an alternate ligand of the CXCR2 receptor GRO γ had no activity to mobilize HSPC [76]. Given that plerixafor (AMD3100) is an approved drug and GRO β has already been administered in man it is expected that clinical testing of combination mobilization with GRO β plus AMD3100 should be forthcoming. Both compounds have shown the same pattern of mobilizing fewer HSPC than G-CSF as standalone mobilizers in both mice and man. Since synergistic mobilization of both HPC and repopulating HSC by GRO β plus AMD3100 is seen in the mouse model, this suggests a similar effect will be seen in man.

In terms of quality of mobilized cells, one wonders how the HSC and HPC are organized within the marrow with regards to their capacity to be mobilized. It is known that there is a large store of HPC within the marrow [77], many more than are likely needed in non-stressed conditions, and this may also apply to HSC. There is also a large reserve of marrow polymorphonuclear neutrophils (PMN), that can be characterized as younger or older PMN depending on the timing of their production, such that the older PMN, those which are produced earlier are the PMN that are first released into the blood [78]. A question is if this concept of first in the marrow (e.g. produced) versus first out to the blood for PMN, also may apply to the marrow stores of HSC and/or HPC when these cells are mobilized. If such a first-in, first-out scenario applies to HSC/HPC are these kinetics different for the different HSPC mobilizing procedures, and is the combination of GRO- β plus AMD3100/Plerixafor that appears to mobilize a more potent population of competing HSC reflecting this kinetic hierarchy.

Optimal collections of HSC

The optimal collection of mouse BM and human cord blood HSC has been underestimated [79;80]. Oxygen is an important factor in the stem cell microenvironment. Upon immediate collection of these cells in atmospheric (ambient) air levels of ~21% O₂, there is an induced differentiation of the HSC to HPC. This is due to the ambient air induced production of reactive oxygen species (ROS) that involves a p53-mitochondrial permeability transition pore opening-cyclophilin D axis, and also involves hypoxia inducing factor 1- α and the hypoxamir miR210. If these cells are instead collected and processed at much lower oxygen levels of hypoxia (~3% O₂) or at ambient oxygen levels but in the presence of cyclosporine A [79], or with combinations of anti-oxidants and/or epigenetic enzyme inhibitors [81], one can collect significantly more HSC than if the cells were collected/processed as usually done in ambient air. This increase in collected HSC is at the expense of HPC, but these hypoxia collected cells are potent engrafting cells. Whether such procedures will enhance the actual numbers of HSC collected after peripheral blood mobilization especially for patients that do not mobilize well, remains to be determined as does the engrafting capabilities of these increased numbers of mobilized HSC. Such studies are underway.

Summary

The need for more effective mobilization strategies in hard to mobilize patients, to reduce resources and costs of mobilization regimens, and to reduce patient fear of pain and inconvenience of multiday regimens, lie at the heart of current studies in understanding and targeting mechanisms of HSPC mobilization. Targeting the gateway to HSPC release with agonists of the NPY receptors on EC, rapid mobilization of high engrafting HSC by the combination of GRO β and plerixafor and utilization of agents that mitigate oxygen shock are new strategies that can be used alone and in combination or in combination with other procedures to acquire more HSC or better engrafting HSC for transplant.

The benefit of rapid mobilization with safe and standalone inexpensive agents, and efficacy in hard to mobilize populations is well recognized and new compounds and strategies in development may ultimately address these issues. Strategies that can enhance HSPC homing

and engraftment and mobilize a hematopoietic graft with enhanced engraftment capabilities may reduce the need to attain higher numbers of HSPC.

Several approaches have been taken to enhance homing/engraftment of cord blood HSPC including short term exposure of the grafts to inhibitors of DPP4 [82;83], pulsing with prostaglandin E2 [84;85], glucocorticoids [86], inhibiting HDACs [87] or hyperthermia treatment [88] and some have been successful in clinical testing [89] as has been treatment of recipients of single cord HCT with the DPP4 inhibitor Januvia® [90;91]. Enhancing homing and engraftment of mobilized hematopoietic grafts has not been extensively studied. However in a recent study, using mobilized PBSC, pulse exposure to PGE2 enhanced homing/engraftment of transduced HSC in patients undergoing gene therapy [92]. Given the strategies outlined above it will be interesting to see how effective and how far these strategies can push the concept of minimal cells numbers for transplant and expand the use of PBSCT particularly in the area of gene therapy where transduction protocols adversely affect HSC homing and engraftment.

It should be noted that mobilization procedures may also be useful in additional contexts. AMD3100 has been shown to ameliorate cigarette smoke induced emphysema-like manifestations in mice [93] and use of G-CSF plus AMD3100 in canines with x-linked severe combined immunodeficiency disease (SCID-X1) has enhanced *in vivo* gene therapy approaches to treat SCID-X1 [94]. Thus, more mechanistic insight into mobilization processes may be of relevance to more than just that for use of HSPC for HCT.

Acknowledgements

References in this review from the co-authors were supported by the National Institutes of Health Grants HL096305, AG046246, CA182947, DOD PR140433 (to LMP), and HL056416, HL112669, DK109188 HL139599, DK106846 (to HEB).

References

1. Panch SR, Szymanski J, Savani BN, Stroncek DF. Sources of Hematopoietic Stem and Progenitor Cells and Methods to Optimize Yields for Clinical Cell Therapy. *Biol Blood Marrow Transplant* 2017; 23(8):1241–1249. [PubMed: 28495640]
2. D'Souza A, Lee S, Zhu X, Pasquini M. Current Use and Trends in Hematopoietic Cell Transplantation in the United States. *Biol Blood Marrow Transplant* 2017; 23(9):1417–1421. [PubMed: 28606646]
3. 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 Transplant* 2016; 51(6):778–785. [PubMed: 26901703]
4. Pulsipher MA, Chitphakdithai P, Miller JP, Logan BR, King RJ, Rizzo JD et al. Adverse events among 2408 unrelated donors of peripheral blood stem cells: results of a prospective trial from the National Marrow Donor Program. *Blood* 2009; 113(15):3604–3611. [PubMed: 19190248]
5. Pulsipher MA, Chitphakdithai P, Logan BR, Shaw BE, Wingard JR, Lazarus HM et al. Acute toxicities of unrelated bone marrow versus peripheral blood stem cell donation: results of a prospective trial from the National Marrow Donor Program. *Blood* 2013; 121(1):197–206. [PubMed: 23109243]
6. Tigue CC, McKoy JM, Evens AM, Trifilio SM, Tallman MS, Bennett CL. Granulocyte-colony stimulating factor administration to healthy individuals and persons with chronic neutropenia or

cancer: an overview of safety considerations from the Research on Adverse Drug Events and Reports project. *Bone Marrow Transplant* 2007; 40(3):185–192. [PubMed: 17563736]

- 7 • Giralt S, Costa L, Schriber J, Dipersio J, Maziarz R, McCarty J et al. Optimizing autologous stem cell mobilization strategies to improve patient outcomes: consensus guidelines and recommendations. *Biol Blood Marrow Transplant* 2014; 20(3):295–308.

This provides an excellent summary of the discussion and consensus of a panel of experts to discuss data availability for peripheral blood mobilization and the transplantation of these mobilized cells. This information was used to devise guidelines for optimized mobilization

. [PubMed: 24141007]

8. Hosing C, Smith V, Rhodes B, Walters K, Thompson R, Qazilbash M et al. Assessing the charges associated with hematopoietic stem cell mobilization and remobilization in patients with lymphoma and multiple myeloma undergoing autologous hematopoietic peripheral blood stem cell transplantation. *Transfusion* 2011; 51(6):1300–1313. [PubMed: 21575005]
9. Shaughnessy P, Chao N, Shapiro J, Walters K, McCarty J, Abhyankar S et al. Pharmacoeconomics of hematopoietic stem cell mobilization: an overview of current evidence and gaps in the literature. *Biol Blood Marrow Transplant* 2013; 19(9):1301–1309. [PubMed: 23685251]
10. 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(8):1307–1318. [PubMed: 15837815]
11. 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(8):2728–2730. [PubMed: 12855591]
12. Liles WC, Rodger E, Broxmeyer HE, Dehner C, Badel K, Calandra G et al. Augmented mobilization and collection of CD34+ hematopoietic cells from normal human volunteers stimulated with granulocyte-colony-stimulating factor by single-dose administration of AMD3100, a CXCR4 antagonist *Transfusion* 2005; 45(3):295–300. [PubMed: 15752146]
13. DiPersio JF, Micallef IN, Stiff PJ, Bolwell BJ, Maziarz RT, Jacobsen E et al. Phase III prospective randomized double-blind placebo-controlled trial of plerixafor plus granulocyte colony-stimulating factor compared with placebo plus granulocyte colony-stimulating factor for autologous stem-cell mobilization and transplantation for patients with non-Hodgkin's lymphoma. *J Clin Oncol* 2009; 27(28):4767–4773. [PubMed: 19720922]
14. DiPersio JF, Stadtmauer EA, Nademanee A, Micallef IN, 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(23):5720–5726. [PubMed: 19363221]
15. Devine SM, Vij R, Rettig M, Todt L, McGlauchlen K, Fisher N et al. Rapid mobilization of functional donor hematopoietic cells without G-CSF using AMD3100, an antagonist of the CXCR4/SDF-1 interaction. *Blood* 2008; 112(4):990–998. [PubMed: 18426988]
16. Pantin J, Purev E, Tian X, Cook L, Donohue-Jerussi T, Cho E et al. Effect of high-dose plerixafor on CD34(+) cell mobilization in healthy stem cell donors: results of a randomized crossover trial. *Haematologica* 2017; 102(3):600–609. [PubMed: 27846612]
- 17 • Schroeder MA, Rettig MP, Lopez S, Christ S, Fiala M, Eades W et al. Mobilization of allogeneic peripheral blood stem cell donors with intravenous plerixafor mobilizes a unique graft. *Blood* 2017; 129(19):2680–2692.

This report notes that plerixafor results in rapid stem cell mobilization regardless of route of administration in clinical trials (clinicaltrials.gov as #NCT00241358 and #NCT00914849) with a “novel” cellular graft composition and favorable recipient outcomes

. [PubMed: 28292947]

18. Pelus LM, Bian H, King AG, Fukuda S. Neutrophil-derived MMP-9 mediates synergistic mobilization of hematopoietic stem and progenitor cells by the combination of G-CSF and the chemokines GRObeta/CXCL2 and GRObetaT/CXCL2delta4. *Blood* 2004; 103(1):110–119. [PubMed: 12958067]

19. Singh P, Hu P, Hoggatt J, Moh A, Pelus LM. Expansion of bone marrow neutrophils following G-CSF administration in mice results in osteolineage cell apoptosis and mobilization of hematopoietic stem and progenitor cells. *Leukemia* 2012; 26(11):2375–2383. [PubMed: 22543963]
20. 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(7):687694.
21. 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(6):657–664. [PubMed: 16715089]
22. 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(2):187–196. [PubMed: 12531874]
23. Levesque JP, Liu F, Simmons PJ, Betsuyaku T, Senior RM, Pham C et al. Characterization of hematopoietic progenitor mobilization in protease-deficient mice. *Blood* 2004; 104(1):65–72. [PubMed: 15010367]
24. Richter R, Jochheim-Richter A, Ciuculescu F, Kollar K, Seifried E, Forssmann U et al. Identification and characterization of circulating variants of CXCL12 from human plasma: effects on chemotaxis and mobilization of hematopoietic stem and progenitor cells. *Stem Cells Dev* 2014; 23(16):1959–1974. [PubMed: 24708339]
25. 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(12):4779–4788. [PubMed: 9389694]
26. 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(24):8396–8404. [PubMed: 17010618]
27. 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(21–22):944–951. [PubMed: 18725320]
28. Karpova D, Brauninger S, Wiercinska E, Kramer A, Stock B, Graff J et al. Mobilization of hematopoietic stem cells with the novel CXCR4 antagonist POL6326 (balixafortide) in healthy volunteers—results of a dose escalation trial. *J Transl Med* 2017; 15(1):2. [PubMed: 28049490]
29. 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(9):2158–2166. [PubMed: 17525235]
30. 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(2):469–479. [PubMed: 24246358]
31. Peng SB, Zhang X, 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(2):480–490. [PubMed: 25504752]
32. 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(13):3581–3588. [PubMed: 24727324]
33. Cho BS, Zeng Z, Mu H, Wang Z, 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(2):222–232. [PubMed: 26031918]
34. 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* 122, 905 2013.
35. Rettig MP, Ghobadi A, Holt M, Meier S, Ritchet J, Tahirovic Y et al. ALT-1188: A new CXCR4 antagonist in development for mobilization of HSPCs. *Blood* 122, 891 2013.

36. Vater A, Sahlmann J, Kroger N, Zollner 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(1):150–157. [PubMed: 23588307]
37. 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. *Proc Natl Acad Sci U S A* 2010; 107(47):20565–20570. [PubMed: 21059953]
38. Ratajczak MZ, Lee H, Wysoczynski M, Wan W, Marlicz W, Laughlin MJ et al. Novel insight into stem cell mobilization-plasma sphingosine-1-phosphate is a major chemoattractant that directs the egress of hematopoietic stem progenitor cells from the bone marrow and its level in peripheral blood increases during mobilization due to activation of complement cascade/membrane attack complex. *Leukemia* 2010; 24(5):976–985. [PubMed: 20357827]
39. Lee HM, Wu W, Wysoczynski M, Liu R, Zuba-Surma EK, Kucia M et al. Impaired mobilization of hematopoietic stem/progenitor cells in C5-deficient mice supports the pivotal involvement of innate immunity in this process and reveals novel promobilization effects of granulocytes. *Leukemia* 2009; 23(11):2052–2062. [PubMed: 19657368]
- 40 •. Adamiak M, Chelvarajan L, Lynch KR, Santos WL, Abdel-Latif A, Ratajczak MZ. Mobilization studies in mice deficient in sphingosine kinase 2 support a crucial role of the plasma level of sphingosine-1-phosphate in the egress of hematopoietic stem progenitor cells. *Oncotarget* 2017; 8(39):65588–65600.
- The results provide support for a role for S1P gradients in blood plasma during the mobilization process, and suggest that small molecule inhibitors of sphingosine kinase 2 and S1P-degrading enzyme S1P lyase could be used as compounds that might facilitate mobilization of HSPCs
- . [PubMed: 29029455]
41. Juarez JG, Harun N, Thien M, Welschinger R, Baraz R, Pena AD et al. Sphingosine-1-phosphate facilitates trafficking of hematopoietic stem cells and their mobilization by CXCR4 antagonists in mice. *Blood* 2012; 119(3):707–716. [PubMed: 22049516]
42. Rettig MP, Anstas G, DiPersio JF. Mobilization of hematopoietic stem and progenitor cells using inhibitors of CXCR4 and VLA-4. *Leukemia* 2012; 26(1):34–53. [PubMed: 21886173]
43. 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(7):1340–1343. [PubMed: 19571319]
44. Hoggatt J, Speth JM, Pelus LM. Concise review: Sowing the seeds of a fruitful harvest: hematopoietic stem cell mobilization. *Stem Cells* 2013; 31(12):2599–2606. [PubMed: 24123398]
45. Di GF, 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(4):491–499. [PubMed: 22180429]
- 46 •. 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(8):3420–3435.
- This study demonstrates that a nucleotide sugar, UDP-glucose, which is released into extracellular fluids in stress can mobilize HSC and HPC. This might provide an alternative strategy to improve the yields of HSPCs in poorly-mobilizing allogeneic and autologous donors
- . [PubMed: 23863713]
47. Niesvizky R, Mark TM, Ward M, Jayabalan DS, Pearse RN, Manco M et al. Overcoming the response plateau in multiple myeloma: a novel bortezomib-based strategy for secondary induction and high-yield CD34+ stem cell mobilization. *Clin Cancer Res* 2013; 19(6):1534–1546. [PubMed: 23357980]
- 48 ••. Hoggatt J, Mohammad KS, Singh P, Hoggatt AF, Chitteti BR, Speth JM et al. Differential stem- and progenitor-cell trafficking by prostaglandin E2. *Nature* 2013; 495(7441):365–369.
- Prostaglandin (PGE)2 is a regulator of hematopoiesis and enhance HSC expansion. This study demonstrates that inhibition of endogenous PGE2 by non-steroidal anti-inflammatory drugs

(NSAIDs) results in egress in HSC from mouse bone marrow through E-prostanoid 4 (EP4) receptor signaling, effects confirmed in non-human primates and healthy human volunteers, and that grafts mobilized with NSAIDs had superior repopulating ability and long-term engraftment capability

. [PubMed: 23485965]

49. Jeker B, Novak U, Mansouri TB, Baerlocher GM, Seipel K, Mueller BU et al. NSAID treatment with meloxicam enhances peripheral stem cell mobilization in myeloma. *Bone Marrow Transplant* 2018; 53(2):175–179. [PubMed: 29058701]
 50. Barrett AJ, Longhurst P, Sneath P, Watson JG. Mobilization of CFU-C by exercise and ACTH induced stress in man. *Exp Hematol* 1978; 6(7):590–594. [PubMed: 211045]
 51. Katayama Y, Battista M, Kao WM, Hidalgo A, Peired AJ, Thomas SA et al. Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. *Cell* 2006; 124(2):407–421. [PubMed: 16439213]
 52. Lucas D, Bruns I, Battista M, Mendez-Ferrer S, Magnon C, Kunisaki Y et al. Norepinephrine reuptake inhibition promotes mobilization in mice: potential impact to rescue low stem cell yields. *Blood* 2012; 119(17):3962–3965.
- It was previously reported by this group that G-CSF induced mobilization of hematopoietic stem cells (HSCs) was controlled by peripheral sympathetic nerves via signaling of norepinephrine (NE). The main point of this paper is that blockage of NE reuptake has the potential to serve as a novel therapeutic target to increase yields of HSCs in patients
- . [PubMed: 22422821]
53. Mendez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. *Nature* 2010; 466(7308):829–834. [PubMed: 20703299]
 54. Mendez-Ferrer S, Battista M, Frenette PS. Cooperation of beta(2)- and beta(3)-adrenergic receptors in hematopoietic progenitor cell mobilization. *Ann N Y Acad Sci* 2010; 1192:139–144. [PubMed: 20392229]
 55. Hopman RK, DiPersio JF. Advances in stem cell mobilization. *Blood Rev* 2014; 28(1):31–40. [PubMed: 24476957]
 56. Domingues MJ, Nilsson SK, Cao B. New agents in HSC mobilization. *Int J Hematol* 2017; 105(2):141–152. [PubMed: 27905003]
 57. Ratajczak MZ, Suszynska M. Emerging Strategies to Enhance Homing and Engraftment of Hematopoietic Stem Cells. *Stem Cell Rev* 2016; 12(1):121–128. [PubMed: 26400757]
 58. Bendall L Extracellular molecules in hematopoietic stem cell mobilisation. *Int J Hematol* 2017; 105(2):118–128. [PubMed: 27826715]
 59. Yosupov N, Haimov H, Juodzbalys G. Mobilization, Isolation and Characterization of Stem Cells from Peripheral Blood: a Systematic Review. *J Oral Maxillofac Res* 2017; 8(1):e1.
 60. Christopherson KW, Cooper S, Hangoc G, Broxmeyer HE. CD26 is essential for normal G-CSF-induced progenitor cell mobilization as determined by CD26^{-/-} mice. *Exp Hematol* 2003; 31(11):1126–1134. [PubMed: 14585379]
 61. Christopherson KW, Cooper S, Broxmeyer HE. Cell surface peptidase CD26/DPPIV mediates G-CSF mobilization of mouse progenitor cells. *Blood* 2003; 101(12):4680–4686. [PubMed: 12576320]
 62. Christopherson KW, Hangoc G, Broxmeyer HE. Cell surface peptidase CD26/dipeptidylpeptidase IV regulates CXCL12/stromal cell-derived factor-1 alpha-mediated chemotaxis of human cord blood CD34⁺ progenitor cells. *J Immunol* 2002; 169(12):7000–7008. [PubMed: 12471135]
 63. Singh P, Hoggatt J, Kamocka MM, Mohammad KS, Saunders MR, Li H et al. Neuropeptide Y regulates a vascular gateway for hematopoietic stem and progenitor cells. *J Clin Invest* 2017; 127(12):4527–4540.

This report defines ECs as gatekeepers of the trafficking of HSCs/HPCs and mechanistically identified a CD26-mediated neurotransmitter neuropeptide Y axis with the potential to pharmacologically target trafficking of HSCs/HPCs in homeostasis and during stress

. [PubMed: 29130940]

64. 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(7599):323–328. [PubMed: 27074509]
 65. Bedoui S, Lechner S, Gebhardt T, Nave H, Beck-Sickinger AG, Straub RH et al. NPY modulates epinephrine-induced leukocytosis via Y-1 and Y-5 receptor activation in vivo: sympathetic co-transmission during leukocyte mobilization. *J Neuroimmunol* 2002; 132(1–2):25–33. [PubMed: 12417430]
 66. Straub RH, Schaller T, Miller LE, von HS, Jessop DS, Falk W et al. Neuropeptide Y cotransmission with norepinephrine in the sympathetic nerve-macrophage interplay. *J Neurochem* 2000; 75(6):2464–2471. [PubMed: 11080198]
 67. Ekblad E, Edvinsson L, Wahlestedt C, Uddman R, Hakanson R, Sundler F. Neuropeptide Y co-exists and co-operates with noradrenaline in perivascular nerve fibers. *Regul Pept* 1984; 8(3):225–235. [PubMed: 6379758]
 68. Zukowska-Grojec Z, Karwowska-Prokopczuk E, Rose W, Rone J, Movafagh S, Ji H et al. Neuropeptide Y: a novel angiogenic factor from the sympathetic nerves and endothelium. *Circ Res* 1998; 83(2):187–195. [PubMed: 9686758]
 69. 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 CXCR4 chemokine GROβ. *Blood* 2001; 97(6):1534–1542. [PubMed: 11238087]
 - 70 ••. Hoggatt J, Singh P, Tate TA, Chou BK, Datari SR, Fukuda S et al. Rapid Mobilization Reveals a Highly Engraftable Hematopoietic Stem Cell. *Cell* 2018; 172(1–2):191–204.
- The CXCR2 agonist GROβ in combination with the CXCR4 antagonist AMD3100/Plerixafor mobilize HSCs within hours rather than days needed for G-CSF. This effect occurs through synergistic signaling on neutrophils and leads to mobilization of HSC with more potent engraftment capacity
- . [PubMed: 29224778]
71. Pruijt JF, Fibbe WE, Laterveer L, Pieters RA, Lindley IJ, Paemen L et al. Prevention of interleukin-8-induced mobilization of hematopoietic progenitor cells in rhesus monkeys by inhibitory antibodies against the metalloproteinase gelatinase B (MMP-9). *Proc Natl Acad Sci U S A* 1999; 96(19):10863–10868. [PubMed: 10485917]
 72. Fukuda S, Bian H, King AG, Pelus LM. The chemokine GROβ mobilizes early hematopoietic stem cells characterized by enhanced homing and engraftment. *Blood* 2007; 110(3):860–869. [PubMed: 17416737]
 73. Broxmeyer HE, Orazi A, Hague NL, Sledge GW Jr., Rasmussen H, Gordon MS. Myeloid progenitor cell proliferation and mobilization effects of BB10010, a genetically engineered variant of human macrophage inflammatory protein-1α, in a phase I clinical trial in patients with relapsed/refractory breast cancer. *Blood Cells Mol Dis* 1998; 24(1):14–30. [PubMed: 9516378]
 74. Merzouk A, Wong D, Salari H, Bian H, Fukuda S, Pelus LM. Rational design of chemokine SDF-1 analogs with agonist activity for the CXCR4 receptor and the capacity to rapidly mobilize PMN and hematopoietic progenitor cells in mice. *Letters in Drug Design & Discovery* 2004; 1:126–134.
 75. Pelus LM, Bian H, Fukuda S, Wong D, Merzouk A, Salari H. The CXCR4 agonist peptide, CTCE-0021, rapidly mobilizes polymorphonuclear neutrophils and hematopoietic progenitor cells into peripheral blood and synergizes with granulocyte colony-stimulating factor. *Exp Hematol* 2005; 33(3):295–307. [PubMed: 15730853]
 76. Hoggatt J, Pelus LM. Hematopoietic stem cell mobilization with agents other than G-CSF In: Kolonin MG, Simmons PJ, editors. *Stem Cell Mobilization; Methods and Protocols*. New York: Springer; 2012 p. 49–67.

77. Broxmeyer HE, Cooper S, Lasky LA, De SF. Identification of a massive reserve of hematopoietic progenitors in mice. *Stem Cells Dev* 2005; 14(2):105–110. [PubMed: 15910237]
78. Broxmeyer HE, Van ZG, Schultz EF, Koltun LA, LoBue J, Gordon AS. Glass wool separation of rat polymorphonuclear leukocytes according to adherence and maturity. *J Reticuloendothel Soc* 1975; 18(2):118–124. [PubMed: 1185726]
79. Broxmeyer HE, O'Leary HA, Huang X, Mantel C. The importance of hypoxia and extra physiologic oxygen shock/stress for collection and processing of stem and progenitor cells to understand true physiology/pathology of these cells ex vivo. *Curr Opin Hematol* 2015; 22(4):273–278. [PubMed: 26049746]
- 80 ••. Mantel CR, O'Leary HA, Chitteti BR, Huang X, Cooper S, Hangoc G et al. Enhancing Hematopoietic Stem Cell Transplantation Efficacy by Mitigating Oxygen Shock. *Cell* 2015; 161(7):1553–1565.
- This study demonstrates that there are more hematopoietic stem cells (HSCs) in mouse bone marrow and human cord blood than previously realized, and that ablating the phenomenon of extra physiologic oxygen shock stress (EPHOSS) by collecting and processing cells in low oxygen (3%) one can collect 2–5 times more HSCs
- . [PubMed: 26073944]
81. Cai Q, Capitano M, Huang X, Guo B, Cooper S, Broxmeyer HE. Combinations of antioxidants and/or of epigenetic enzyme inhibitors allow for enhanced collection of mouse bone marrow hematopoietic stem cells in ambient air. *Blood Cells Mol Dis* 2018; 71:23–28. [PubMed: 29422232]
- 82 ••. Broxmeyer HE, Hoggatt J, O'Leary HA, Mantel C, Chitteti BR, Cooper S et al. Dipeptidylpeptidase 4 negatively regulates colony-stimulating factor activity and stress hematopoiesis. *Nat Med* 2012; 18(12):1786–1796.
- Cell surface Dipeptidylpeptidase (DPP)4/CD26, was found to truncate and decrease the activities of GM-CSF, IL-3 and SDF-1/CXCL12. By inhibiting the DPP4 activity with small molecules such as Diprotin A (ILE-PRO-ILE) or sitagliptin/Januvia one can enhance the functional activities of GM-CSF, IL-3, and SDF-1 and enhance recovery of stressed hematopoiesis
- . [PubMed: 23160239]
83. Christopherson KW, Hangoc G, Mantel CR, Broxmeyer HE. Modulation of hematopoietic stem cell homing and engraftment by CD26. *Science* 2004; 305(5686):1000–1003. [PubMed: 15310902]
84. Hoggatt J, Singh P, Sampath J, Pelus LM. Prostaglandin E2 enhances hematopoietic stem cell homing, survival, and proliferation. *Blood* 2009; 113(22):5444–5455. [PubMed: 19324903]
85. North TE, Goessling W, Walkley CR, Lengerke C, Kopani KR, Lord AM et al. Prostaglandin E2 regulates vertebrate haematopoietic stem cell homeostasis. *Nature* 2007; 447(7147):1007–1011. [PubMed: 17581586]
- 86 •. Guo B, Huang X, Cooper S, Broxmeyer HE. Glucocorticoid hormone-induced chromatin remodeling enhances human hematopoietic stem cell homing and engraftment. *Nat Med* 2017; 23(4):424–428.
- This paper reports the identification of glucocorticoid (GC) hormone signaling as an activator of CXCR4 expression on human cord blood (CB) HSCs and HPCs, mediated by activated glucocorticoid receptor binding with the glucocorticoid response element in the CXCR4 promoter and recruitment of the SRC-1-p300 complex promoting H4K5 and H4K6 histone acetylation resulting in enhanced engraftment of short-term treated human CB in immune deficient mice
- . [PubMed: 28263313]
- 87 •. Huang XX, Guo B, Broxmeyer HE. Neutralizing negative epigenetic regulation by HDAC5 enhances human hematopoietic stem cell homing and engraftment. *Nat Commun* 2018; 9(1):2741

This report provides additional means to enhance the expression of CXCR4 on human cord blood hematopoietic stem cells (HSCs) and their engraftment in immune deficient mice. Inhibition of HDAC5 increased acetylated p65 levels in the nucleus, with activation of the NF- κ B signaling pathway via TNF- α , important for enhancing the homing and engraftment of mobilized peripheral blood HSCs

. [PubMed: 30013077]

88. Capitano ML, Hango G, Cooper S, Broxmeyer HE. Mild Heat Treatment Primes Human CD34(+) Cord Blood Cells for Migration Toward SDF-1 α and Enhances Engraftment in an NSG Mouse Model. *Stem Cells* 2015; 33(6):1975–1984. [PubMed: 25753525]
89. Cutler C, Multani P, Robbins D, Kim HT, Le T, Hoggatt J et al. Prostaglandin-modulated umbilical cord blood hematopoietic stem cell transplantation. *Blood* 2013; 122(17):3074–3081. [PubMed: 23996087]
90. Farag SS, Srivastava S, Messina-Graham S, Schwartz J, Robertson MJ, Abonour R et al. In vivo DPP-4 inhibition to enhance engraftment of single-unit cord blood transplants in adults with hematological malignancies. *Stem Cells Dev* 2013; 22(7):1007–1015. [PubMed: 23270493]
91. Farag SS, Nelson R, Cairo MS, O’Leary HA, Zhang S, Huntley C et al. High-dose sitagliptin for systemic inhibition of dipeptidylpeptidase-4 to enhance engraftment of single cord umbilical cord blood transplantation. *Oncotarget* 2017; 8(66):110350–110357. [PubMed: 29299152]
92. Genovese P, Schirotti G, Escobar G, Tomaso TD, Firrito C, Calabria A et al. Targeted genome editing in human repopulating haematopoietic stem cells. *Nature* 2014; 510(7504):235–240. [PubMed: 24870228]
93. Barwinska D, Oueini H, Poirier C, Albrecht M, Bogatcheva N, Justice M et al. AMD3100 ameliorates cigarette smoke-induced emphysema-like manifestations in mice. *Amer J Physiol Lung Cell Molec Physiology*, 2018 5 10. doi: 10.1152/ajplung.00185.2018. [Epub ahead of print].
94. Humbert O, Chan F, Rajawat YS, Torgerson TR, Burtner CR, Hubbard NW et al. Rapid immune reconstitution of SCID-X1 canines after G-CSF/AMD3100 mobilization and in vivo gene therapy *Blood Adv* 2018; 2(9):987–999. [PubMed: 29720491]