Many mechanisms mediating mobilization: an alliterative review
Jonathan Hoggatt and Louis M. Pelus

Introduction
Hematopoietic stem cells (HSCs) reside within specialized bone marrow niches, ‘tethered’ through adhesion molecule interactions to a complex of stromal cells, endosteal lining osteoblasts, and mesenchymal stem cells within a matrix of collagens, fibronectin, and proteoglycans, where they produce mature cells that ultimately exit the marrow and enter the peripheral blood. HSCs and hematopoietic progenitor cells (HPCs) also traffic to the peripheral blood [1–6], leaving open niches that can be repopulated by transplanted HSCs [7]. On the basis of observations that increased circulating HPCs were found in patients after chemotherapy [8,9], we now know that this natural trafficking of HSCs and HPCs can be modulated, allowing for directed mobilization [3,4]. Mobilization is widely used clinically to acquire HSCs for transplantation. G-CSF-mobilized HSCs and HPCs are associated with more rapid engraftment, shorter hospital stay [14–17], and in some circumstances, superior overall survival compared with bone marrow [18]. The mobilization process is not well understood and many mechanisms mediating the effect have been proposed. This alliterative review focuses on recent advances and controversies in defining the mechanisms responsible for G-CSF-induced mobilization.

Purpose of review
Blood cell production is maintained by hematopoietic stem cells (HSCs) that reside in specialized niches within bone marrow. Treatment with granulocyte-colony stimulating factor (G-CSF) causes HSC egress from bone marrow niches and trafficking to the peripheral blood, a process termed ‘mobilization’. Although the mobilization phenomenon has been known for some time and is utilized clinically to acquire HSC for transplant, the mechanisms mediating HSC release are not completely understood. We discuss recent advances and controversies in defining the mechanisms responsible for G-CSF-induced mobilization.

Recent findings
New reports define a role for resident monocytes/macrophages in maintaining niche cells, which is diminished after G-CSF treatment, suggesting a new mechanism for mobilization. Although osteoblasts have been reported to be a primary component of the HSC niche, new results suggest a unique niche composed of innervated mesenchymal stem cells. Modulating bioactive lipid signaling also facilitates mobilization, and may define a future therapeutic strategy.

Summary
Hematopoietic mobilization by G-CSF is primarily mediated by alterations to the bone marrow niche by both direct and indirect mechanisms, rather than directly altering HSC function. Further understanding of the processes mediating mobilization will advance our understanding on the cellular and molecular components of the HSC niche.

Keywords
granulocyte-colony stimulating factor, hematopoietic stem cell, microenvironment, mobilization, niche

CXCR4 controls (hematopoietic) cell circulation
The CXCR4 receptor (CXCR4) and its ligand stromal cell-derived factor-1α (SDF-1α) form the most widely explored hematopoietic niche interaction regulating HSC and HPC retention and trafficking. Hematopoietic cells express CXCR4 and are chemoattracted to and retained within the bone marrow by SDF-1α [19–21].

Granulocyte-colony stimulating factor (G-CSF) (Neupogen) is widely used clinically to mobilize HSCs and HPCs for transplantation. G-CSF-mobilized HSCs and HPCs are associated with more rapid engraftment, shorter hospital stay [14–17], and in some circumstances, superior overall survival compared with bone marrow [18]. The mobilization process is not well understood and many mechanisms mediating the effect have been proposed. This alliterative review focuses on recent findings that add to our understanding of the G-CSF mobilization response, but also introduces new controversies and questions.
Genetic knockout of either CXCR4 [22] or SDF-1α [23] is embryonic lethal in mice, with a failure to populate the bone marrow niche during development. Conditional deletion of either CXCR4 [24] or SDF-1α [25] results in a substantial hematopoietic cell egress from the bone marrow and impaired marrow retention of gene-deleted HSCs and HPCs after transplantation [25*,26].

Although numerous reports support a key role for the CXCR4/SDF-1α axis in hematopoietic cell retention/trafficking/mobilization within the bone marrow niche, the predominant source of SDF-1α is still not clear. Osteoblasts [27], reticular cells found in endosteal and vascular niches [28], endothelial cells, and bone itself [29,30] all produce/express SDF-1α. Recently, Mendez-Ferrer et al. [31] described a novel population of nestin+ mesenchymal stem cells (MSCs) that express high levels of SDF-1α mRNA and suggest they form a unique hematopoietic niche. Early reports demonstrated that osteoblast SDF-1α is reduced after G-CSF treatment [30,32,33], suggesting that osteoblast-derived SDF-1α was a key regulator of hematopoietic cell retention/mobilization. However, other studies question the importance of osteoblast-derived SDF-1α [28,31**,34]. Christopher et al. [35] recently showed reduced SDF-1α production in Col2.3-expressing osteoblasts with no reduction in Col2.3-negative stromal cells, suggesting that reduced osteoblast SDF-1α is a common mechanism of cytokine-induced mobilization. However, Mendez-Ferrer et al. [31**], using a similar approach, showed substantially reduced SDF-1α in nestin+ MSC relative to a similar population of stromal cells described by Christopher et al. [35], although a direct comparison to defined osteoblasts was not made. This is similar to findings that a mesenchymal precursor cell may form the hematopoietic niche [36*]. Overall, these results suggest that heterogeneous cell populations expressing SDF-1α and other supportive factors create unique niches, each of which can be manipulated to facilitate mobilization. Defining the specific niche(s) responsible for SDF-1α production and HSC retention will aid in the development of future therapeutic strategies.

Osteoblasts, osteoclasts, osteomacs, and other operators

Osteoblasts express signaling molecules in addition to SDF-1α that may regulate HSC function and niche retention [37–40]. Targeting the interaction between osteoblast vascular cell adhesion molecule-1 (VCAM-1) and HSCs expressing very late antigen-4 (VLA-4) with antibodies against VLA-4 [41,42] or VCAM-1 [43,44], or a small molecule inhibitor of VLA-4 (BIO5192) [45], results in mobilization. Osteoblasts also express significant amounts of osteopontin (OPN), and HSCs adhere to OPN via β1 integrins [46]. Intriguingly, OPN also negatively regulates HSC pool size within the marrow niche [46,47], and OPN knockout mice show enhanced endogenous and G-CSF-induced mobilization [48].

Hematopoietic mobilization with G-CSF results in the suppression of niche osteoblasts [30,33,49**], with increased osteoblast apoptosis [33] and a characteristic osteoblast ‘flattening’ [30], and decreased endosteal niche expression of retention molecules. This suppression has been reported to result from altered sympathetic nervous system (SNS) signaling to osteoblasts [30] (discussed below); however, recent reports also suggest an alternate, perhaps parallel, pathway involving bone marrow macrophages. Winkler et al. [49**] reported that the marrow niche contains supportive endosteal lining ‘osteomacs’, and that G-CSF treatment results in a trafficking and reduction of these osteomacs, causing osteoblast suppression. Macrophage depletion using Maf transgenic mice or by treatment with clodronate-loaded liposomes (Clo-lip) resulted in hematopoietic mobilization. The osteomac population of cells was characterized as F4/80+Ly-6G+CD11b+, largely based on findings that this was the predominate population depleted after Clo-lip treatment.

Two recent reports also suggest a role for resident macrophages/microcytes in mobilization. Chow et al. [50**] also demonstrated that macrophage depletion with Clo-lip results in mobilization; however, in slight contrast to osteomacs, these cells were defined as Gr-1negative F4/80+CD115+CD169+. In an animal model where CD169+ cells are specifically depleted, mobilization was enhanced. The authors suggest that CD169+ macrophages express a soluble, yet to be identified, factor that supports niche cells, specifically nestin+MSC. Similarly, Christopher et al. [51**] utilized G-CSF receptor (G-CSFR) knockout mice crossbred to mice expressing G-CSFR under the control of the CD68 (macrosialin) promoter, which restricts G-CSFR expression to the

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**Key points**

- Granulocyte-colony stimulating factor (G-CSF) reduces resident monocytes/macrophages, causing niche suppression and mobilization.
- The niche consists of a heterogeneous population of cells that can coordinateably be altered during mobilization.
- Sphingosine-1-phosphate, endocannabinoids, and other bioactive lipids mediate hematopoietic stem cell retention and trafficking to and from the bone marrow.
- The mechanisms regulating G-CSF-induced mobilization alter multiple, parallel mechanistic pathways, suggesting combinatorial therapies should be explored.
monocyte/macrophage lineages showing that treatment of these mice with G-CSF reduced marrow macrophages, coincident with HPC mobilization. Intriguingly, we previously reported that treatment of mice with an anti-Gr-1 antibody to deplete neutrophils significantly reduced the G-CSF mobilization response [52]; yet, the animal model used by Christopher et al. showed mobilization to G-CSF despite neutropenia. As noted by Christopher et al., the antibody we used to deplete neutrophils (clone RB6–8C5) would also target monocyte and osteomac populations, possibly accounting for the differing effects seen. However, if this were the case, one would expect increased mobilization because of depletion of these populations, not decreased mobilization. In addition, the anti-Gr-1 antibody would not target the CD169+ macrophages [50**]. More likely, mobilization is a complex process coordinated by a balance between cell populations in the bone marrow rather than specifically a function of a single cell lineage. Thus, model systems that target specific populations would be sufficient to induce mobilization, but not necessarily exclusive. Clearly, the cellular players and factors warrant further exploration.

As a prime example of cellular balance, osteoblasts and osteoclasts regulate bone formation/bone resorption, within the bone marrow niche. Kollet et al. [53] reported that RANK ligand treatment, which increases osteoclast activity, produces a moderate mobilization of HPCs. Similar results are also seen in an independent report [54*]. Correspondingly, stress models such as bleeding or lipopolysaccharide treatment increased the number of osteoclasts that was coincident with HPC mobilization. G-CSF mobilization was decreased after inhibition of osteoclasts, by treatment with calcitonin or using a PTP gene knockout model, further suggesting osteoclast involvement in G-CSF-mediated mobilization. The authors proposed that osteoclast-derived proteolytic enzymes, such as cathepsin K, degrade important niche interaction components including SDF-1α and OPN [53]. In a more recent study, they demonstrated reduced osteoclast maturation and activity in CD45 knockout mice that correlated with reduced mobilization to RANK ligand and G-CSF [55].

In contrast, an earlier report demonstrated that although G-CSF increases osteoclast number and bone resorption in both BALB/c mice and humans, the increase in osteoclasts did not occur until 10–15 or 6–8 days, respectively, after treatment with G-CSF [56], a finding also observed by others using similar systems [32,57]. As G-CSF mobilization is typically evaluated after 4–5 days, the importance of osteoclasts to G-CSF-induced mobilization remains unclear. Furthermore, treatment of mice with bisphosphonates, which inhibit osteoclast activity and/or number, prior to G-CSF administration does not result in impaired mobilization [49**,56], and in fact, in one case, bisphosphonate treatment increased mobilization [49**]. Of note, the endosteal bone surface, particularly underneath resorbing osteoclasts, is a significant source of extracellular calcium, and studies by Adams et al. [58] demonstrated that HSC expression of calcium-sensing receptors mediates chemotraction to soluble Ca\(^{2+}\). Calcium-sensing receptor knockout mice had reduced marrow HSC content and increased peripheral blood HSCs, perhaps suggesting that increased G-CSF mobilization seen in zoldronate-treated mice resulted from reduced calcium retention signaling within the niche. Although increased osteoclast activity can clearly induce mobilization, their role in G-CSF-mediated mobilization is not sufficiently defined and may not be a primary mechanism of mobilization. Figure 1 summarizes some of the key cellular components involved in HSC retention and subsequent mobilization following G-CSF treatment.

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**Hypoxia harbors hematopoiesis**

Hematopoietic stem cells reside in hypoxic niches within the bone marrow [59–61], and HSCs that reside in hypoxic niches have greater hematopoietic repopulating ability [62*]. Stabilization of the transcription factor hypoxia inducible factor 1-α (HIF-1α) is a known physiological response to hypoxia, and HIF-1α has been shown to upregulate erythropoietin (EPO) production [63], numerous cell proliferation and survival genes [64–66], the angiogenic vascular endothelial growth factor (VEGF) [67], and other genes. The hypoxic niche may maintain HIF-1α activity thereby maintaining HSCs [68], a hypothesis supported by the fact that hypoxic conditions expand human HSC [69] and HPC populations [70–72] in vitro. In response to G-CSF, HIF-1α expression is increased [73*] and both the hypoxic environment and HIF-1α expand within the marrow compartment [74], with increased VEGF production; however, marrow vascular density and permeability are not increased [62*]. In addition, HIF-1α is stabilized in peripheral blood HSCs acquired from G-CSF mobilized donors [75]. HIF-1α also increases SDF-1α production [76] and CXCR4 expression [77], suggesting that hypoxia may be a physiological regulator of this important signaling axis. Of note, one report suggests that decreased osteoblast SDF-1α expression and reduced CXCR4 expression on bone marrow cells can be facilitated by osteoclasts activated by the hypoxia-mimetic CoCl\(_2\) in vitro [54*]. Recently, a population of marrow-derived very small embryonic-like stem cells was reported to mobilize as a result of intermittent hypoxia, coincident with an increase in plasma SDF-1α [78*], further suggesting that hypoxia may regulate HSC retention/trafficking.

HIF-1α also prevents hematopoietic cell damage caused by reactive oxygen species (ROS) [79], suggesting that
the hypoxic niche helps maintain HSC lifespan. In slight contrast, another report demonstrated that enhanced c-Met activity promotes mobilization by activating mTOR and increasing ROS production in HSCs and HPCs \[80\]/, whereas inhibition of mTOR with rapamycin reduced HSC mobilization \[80\]/81\], suggesting that a small amount of ROS may be necessary for optimal mobilization. Knockout of the thioredoxin-interacting protein gene results in increased mobilization under stress conditions \[82\], suggesting a role for oxygen tension and ROS in mobilization.

Nervous niche
The marrow microenvironment is highly innervated \[83\], and catecholamine signaling can alter numerous physiological processes of immune cells \[84\]. A seminal study by Katayama et al. \[30\] demonstrated that G-CSF mobilization was reduced in chemically sympathectomized mice, mice treated with the β-blocker propanolol, or mice genetically deficient in the gene for dopamine β-hydroxylase (Dbh), an enzyme that converts dopamine into norepinephrine, demonstrating that mobilization...
requires peripheral β2-adrenergic signals. This study also demonstrated that G-CSF attenuated osteoblast function, via the SNS, resulting in osteoblasts having a marked flattened appearance. Intriguingly, β2-adrenergic signaling can regulate osteoclast differentiation [85] and nestin+ MSCs are highly innervated [31], perhaps suggesting that SNS signaling alters niche cell components through multiple mechanisms. In addition to the niche, human CD34+ cells express β3-adrenergic and dopamine receptors that are upregulated by G-CSF [86], and neurotransmitters serve as direct chemottractants to hematopoietic cells [86] and increase CXCR4 expression [87]. Epinephrine treatment also results in mobilization [86].

Signaling from β2-adrenergic receptors regulate circadian oscillations mediating norepinephrine release, CXCR4 expression, and SDF-1α production, leading to rhythmic egress of hematopoietic cells from the niche [88,89]. Both β2 and β3-adrenergic signals cooperate to regulate G-CSF-induced mobilization, although knockout of both only partially blocks mobilization [90] and does not abrogate mobilization by Clo-lip [50], suggesting multiple/parallel pathways regulating mobilization. β2-Adrenergic signaling also upregulates osteoblast vitamin D receptors (VDRs) and it was recently demonstrated that expression of VDR is necessary for the G-CSF-induced suppression of osteoblast function and that HSC mobilization is reduced in VDR knockout mice [91]. VDR is also regulated by circadian rhythms [92], demonstrating further complexity in interconnected mobilization mechanisms.

**Fatty acids for the future**

Studies exploring the role of SNS signaling in G-CSF-mediated mobilization began in mice defective in ceramide galactosyltransferase (CGT), an enzyme that synthesizes galactocerebrosides, major lipid components of myelin sheaths and are important for proper nerve conduction [30]. Mice deficient in the related enzyme galactocerebrosidase (GALC) have a defective niche in which transplanted HSCs fail to engraft [93]. Sphingosine-1-phosphate (S1P) and ceramide, which affect a wide variety of cellular processes, are part of the GALC metabolic pathway [94]. HSCs and HPCs express the S1P receptor S1P1 [95], and S1P1 signaling alters CXCR4/SDF-1α signaling and chemotaxis [95–97]. S1P directs trafficking of immature B cells [98] and trafficking of HSCs and HPCs from blood, bone marrow, and lymph tissues [1]. A recent report by Ratajczak et al. [99] suggests that plasma S1P increases following G-CSF administration and directs peripheral chemotraction, facilitating mobilization. An increase in S1P in peripheral blood coordinated with a decrease in marrow was recently reported, suggesting the formation of an S1P gradient driving mobilization [100], or perhaps even a dual lipid action mediated by both SIP and ceramide concentrations in peripheral blood and bone, respectively [101]. If SIP signaling through S1P1 regulates G-CSF mobilization, then co-treatment with an S1P1 antagonist, like FTY720, would be expected to reduce G-CSF mobilization. This was indeed reported in one case [100]; however, others report no decreases in G-CSF mobilization in FTY720-treated mice [1,102]. Further studies evaluating the role of SIP and ceramide will aid in determining their functional importance in hematopoietic mobilization, and whether agents modifying S1P signaling, like the SIP agonist SEW2871, can be utilized as an adjunctive therapeutic agent in mobilization.

In addition to the sphingolipids, other bioactive lipids have the capacity to regulate hematopoiesis, including eicosanoids. We recently reported that prostaglandin E2 (PGE2) regulates CXCR4 expression on HSCs and HPCs and facilitates their chemotraction to SDF-1α and homing [103]. Like many lipid systems, homeostasis is maintained by a balance of signaling among eicosanoids with several of the eicosanoids acting in opposing manners. We also showed that agonism of cannabinoid receptors, receptors for the eicosanoid-related endocannabinoids, acts in an opposing fashion to PGE2 signaling, decreasing adhesion molecule expression and CXCR4, and enhancing G-CSF mobilization [104]. Two separate reports by Jiang et al. [105,106] have also demonstrated that cannabinoid signaling mediates mobilization, and that endocannabinoids are expressed in bone marrow and increase HPC migration and proliferation in vitro. Another report demonstrated that cannabinoid mobilize myeloid-derived suppressor cells, with a possible role for endogenous G-CSF production [107]. It is interesting to note that cannabinoid receptors are physically associated with β2-adrenergic receptors and modulate adrenergic signaling [108,109], possibly suggesting a SNS/cannabinoid mechanistic link. With the abundance of FDA-approved compounds modulating eicosanoid signaling, additional studies exploring their role in mobilization are likely to lead to new therapeutic strategies.

**Conclusion**

Although significant progress has been made in defining the mechanisms mediating mobilization by G-CSF, a complete understanding remains elusive. Do osteoblasts comprise the important hematopoietic niche suppressed by G-CSF administration, or is it innervated nestin+ MSCs, or a heterogeneous population of cells forming several unique niches? Experimental animal models designed to explore mobilization have significantly advanced the field, but manipulation of a specific system, although sufficient to mimic or block mobilization by G-CSF, may not tell the whole story. Although both genetic
alteration of the SNS or macrophages can mediate mobilization, neither regulates the niche exclusively during mobilization and other parallel or intersecting pathways are likely involved. Combination therapies manipulating multiple mechanistic pathways should be explored to not only maximally mobilize hematopoietic cells for transplant, but also possibly to mobilize other potential therapeutically efficacious cell populations.

Acknowledgements
This work was supported by the NIH grants HL089669 and HL098305 (to L.M.P.J.; H.J. is supported by the training grant HL007910.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
**• of outstanding interest
Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 301–304).

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This seminal study describes a population of ‘ostemacs’, which are macrophages that form a layer over endosteal osteoblasts. This report demonstrates that osteoclasts provide support to osteoblasts, and when macrophages are ablated using Mafa mice or Clo-lip treatment, osteoblasts are suppressed and HSC mobilize. Importantly, treatment with G-CSF results in migration of the osteomacs away from the endosteal layer, causing osteoblast suppression and HSC mobilization. The authors also show that when osteoclasts are inhibited using zolindornate treatment, G-CSF mobilization is not attenuated, and in fact increases.


This report, like the one above by Winkler et al., also demonstrates that a resident macrophage population regulates the HSC niche. The authors describe a slightly different phenotype than the previously described osteomacs, and use an elegant lineage-tracing model to demonstrate that the CD169不能™ macrophages are specifically involved. The authors also suggest that a soluble factor produced by the macrophages supports niche cells, specifically nestin™ MSCs.


This group developed a sophisticated animal model in which the G-CSF receptor was only expressed on CD68-expressing monocytes. The authors demonstrate that G-CSF signaling only on monocytes is sufficient to mediate mobilization, and like similar reports, demonstrate that monocytes/macrophages produce a soluble factor which increases SDF-1x production by osteoblasts.


This study demonstrates in in-vitro assays that osteoclasts mobilize. The authors also show that when osteoclasts are inhibited using Mafia mice or Clo-lip treatment, osteoblasts are suppressed and HSC mobilize. Importantly, treatment with G-CSF results in migration of the osteomacs away from the endosteal layer, causing osteoblast suppression and HSC mobilization. The authors also show that when osteoclasts are inhibited using zolindornate treatment, G-CSF mobilization is not attenuated, and in fact increases.

Hemato poiesis

78 Ghanbari SA, Dayyat EA, Khalifa A, et al. Intermittent hypoxia mobilizes bone marrow–derived very small embryonic-like stem cells and activates development-tional transcriptional programs in mice. Sleep 2010; 33:1439–1446. While studying sleep apnea, the authors used a model of exposure to intermittent hypoxia and demonstrate that hypoxia increases SDF-1α in plasma and mobilizes very small embryonic like stem cells from the bone marrow to peripheral blood.


80 Tesio M, Golan K, Corso S, et al. Enhanced c-Met activity promotes G-CSF-induced mobilization of hematopoietic progenitor cells via ROS signaling. Blood 2011; 117:419–428. The report evaluated the mechanisms governing hematopoietic mobilization, with an interest on stress-induced mobilization as an endogenous response for host defense and repair. This report demonstrates that stress-induced mobilization, or G-CSF treatment, result in c-Met activation, causing quiescent, nonmotile HPCs to migrate. This effect requires ROS activity, demonstrating that at a least a small level of ROS is needed for mobilization.


82 Jeong M, Piao ZH, Liu R, et al. Basic helix–loop–helix proteins contribute to the maintenance of a functional hematopoietic stem cell niche. Cell Stem Cell 2008; 2:364–376. This report follows on from previous studies by the authors and demonstrates that vitamin D receptor (VDR) knockout mice have impaired mobilization to G-CSF-stimulated chemotaxis - plasma sphingosine-1-phosphate is a major chemoattractant that directs hematopoietic progenitor cell egress and mobilization. Using a genetic knockout of the VDR, this study demonstrates that VDR is a mediator of SNS signaling to the niche.

83 Kawamori Y, Katayama Y, Asada N, et al. Sphingosine 1-phosphate receptor agonists mediate hematopoietic stem and progenitor cell egress and mobilization via the major receptor S1P1 and by SDF-1 inhibition in a p38/Akt/mTOR dependent manner. Blood 2009; 116:555–63. This report demonstrates that VDR knockout mice have impaired mobilization to G-CSF stimulation - plasma sphingosine-1-phosphate is a major chemoattractant that directs hematopoietic progenitor cell egress and mobilization. Using a genetic knockout of the VDR, this study demonstrates that VDR is a mediator of SNS signaling to the niche.
