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REVIEW

Mobilization of hematopoietic stem cells from the bone marrow niche to the blood compartment

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Abstract

The vast majority of hematopoietic stem cells (HSCs) reside in specialized niches within the bone marrow during steady state, maintaining lifelong blood cell production. A small number of HSCs normally traffic throughout the body; however, exogenous stimuli can enhance their release from the niche and entry into the peripheral circulation. This process, termed mobilization, has become the primary means to acquire a stem cell graft for hematopoietic transplant at most transplant centers. Currently, the preferred method of HSC mobilization for subsequent transplantation is treatment of the donor with granulocyte colony-stimulating factor. The mobilizing effect of granulocyte colony-stimulating factor is not completely understood, but recent studies suggest that its capacity to mobilize HSCs, at least in part, is a consequence of alterations to the hematopoietic niche. The present article reviews some of the key mechanisms mediating HSC mobilization, highlighting recent advances and controversies in the field.

Introduction

Higher organisms have the remarkable capacity to produce and maintain adequate numbers of blood cells throughout their entire lifespan to meet the normal physiological requirements of blood cell turnover, as well as to respond to needs for increased blood cell demand as a consequence of injury or infection. At the center of lifelong blood cell production is the hematopoietic stem cell (HSC), with the capacity to give rise to all mature circulating blood cell types. Regulation of HSC function is a highly complex process involving not only intrinsic cues within the HSC themselves, but signaling from the surrounding microenvironment in which they reside. It

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was first postulated by Schofield that defined local microenvironments created specialized stem cell niches that regulated HSCs [1]. Bone marrow is the primary HSC niche in mammals and is composed of stromal cells and an extracellular matrix of collagens, fibronectin, proteoglycans [2], and endosteal lining osteoblasts [3-6]. HSCs are thought to be tethered to osteoblasts, other stromal cells, and the extracellular matrix in this stem cell niche through a variety of adhesion molecule interactions, many of which are probably redundant systems.

Disruption of one or more of these niche interactions can result in release of HSCs from the niche and their trafficking from the bone marrow to the peripheral circulation, a process termed peripheral blood stem cell mobilization. Mobilization can be achieved through administration of chemotherapy [7-9], hematopoietic growth factors, chemokines and small-molecule chemokine receptor inhibitors or antibodies against HSC niche interactions [10-12].

The process of mobilization has been exploited for collection of hematopoietic stem and progenitor cells (HSPCs) and is widely used for hematopoietic transplantation in both the autologous and allogeneic settings. Mobilized peripheral blood hematopoietic stem cell grafts are associated with more rapid engraftment, reduction in infectious complications and, in patients with advanced malignancies, lower regimen-related mortality [13-15] compared with bone marrow grafts. In many transplantation centers, mobilized HSC grafts are now the preferred hematopoietic stem cell source used for human leukocyte antigen-identical sibling transplants as well as for matched related and unrelated donor transplants [16,17]. Granulocyte colony-stimulating factor (G-CSF), granulocyte–macrophage colony-stimulating factor and – more recently, for patients who fail to mobilize with a G-CSF or granulocyte–macrophage colony-stimulating factor – plerixafor (AMD3100) are the only US Food and Drug Administration-approved agents for mobilizing HSCs. Despite the clinical prevalence of peripheral blood stem and progenitor cell mobilization, the mechanisms orchestrating the release of these cells from the hematopoietic niche are still not completely understood. In the following sections, we

highlight some of the key mechanistic findings concerning HSPC mobilization, with an emphasis on the effects of mobilizing agents on bone marrow niche interactions.

CXCR4/SDF-1α: the paradigm of mobilization

The most explored HSC niche interaction is between the CXC4 chemokine receptor (CXCR4) and its ligand, stromal cell-derived factor 1α (SDF-1α). SDF-1α is produced by osteoblasts [18], a specialized set of reticular cells found in endosteal and vascular niches [19], endothelial cells and bone itself [20,21], and high levels of SDF-1α were observed recently in nestin-positive mesenchymal stem cells [22]. HSPCs express CXCR4 and are chemoattracted to and retained within the bone marrow by SDF-1α [23-25]. Genetic knockout of either CXCR4 [26] or SDF-1α [27] in mice is embryonically lethal, with a failure of HSPCs to traffic to the bone marrow niche during development. In addition, conditional CXCR4 knockout in mice results in a substantial egress of hematopoietic cells from the bone marrow [28] and impaired ability of CXCR4 knockout HSPCs to be retained within the bone marrow after transplantation [29].

Many agents reported to mobilize HSCs have been shown to disrupt the CXCR4/SDF-1α axis. Most notably, the CXCR4 antagonist AMD3100 (Plerixafor; Mozobil™, Genzyme Corporation, Cambridge, MA, USA) mobilizes HSPCs [30-35]; and similarly, the CXCR4 antagonists T140 [36] and T134 [37] are both capable of mobilization. Partially agonizing CXCR4 with SDF-1α mimetics including (met)-SDF-1β [38], CTCE-0214 [39], and CTCE-0021 [35] also mobilizes HSCs through CXCR4 receptor desensitization and/or downregulation of surface CXCR4 expression. Intriguingly, these agents that directly disrupt the CXCR4/SDF-1α axis lead to rapid mobilization of HSPCs – that is, hours after treatment – in contrast to other mobilization agents like G-CSF, which take several days to maximally mobilize HSPCs.

Despite the abundance of evidence supporting a key role for the CXCR4/SDF-1α axis in HSPC retention/trafficking/mobilization, it is still not clear which population of cells within the bone marrow niche is the predominate source of SDF-1α. Some studies have demonstrated that SDF-1α production by osteoblasts is reduced after G-CSF treatment [21,40,41], and seminal work by Katayama and colleagues suggests that this reduction in osteoblast SDF-1α is at least partly mediated by the sympathetic nervous system [21]. Notwithstanding the fact that decreased levels of SDF-1α production by osteoblasts are routinely seen following G-CSF administration, however, other studies have questioned the relative importance of osteoblast-derived SDF-1α in HSC maintenance and mobilization [19,22,42]. A recent study by Christopher and colleagues indicated that reduction in osteoblast production of SDF-1α is a common mechanism

of cytokine-induced HSC mobilization and showed a specific reduction in SDF-1α production in Col2.3-expressing osteoblasts with no reduction in Col2.3-negative stromal cells [43]. Mendez-Ferrer and colleagues, however, showed, using a similar approach, a substantial decrease in SDF-1α in a novel population of nestin-expressing mesenchymal stem cells [22], relative to a similar population of stromal cells described by Christopher and colleagues [43], although a direct comparison with defined osteoblasts was not made. Future studies are clearly required in order to define the specific niche cells responsible for SDF-1α production and HSC retention, and may identify specific targets for future HSC therapies.

There is more to an osteoblast than SDF-1α

Osteoblasts are important HSC regulators [3-6], and express numerous signaling molecules in addition to SDF-1α that regulate HSC function and retention in the bone marrow niche. Osteoblasts express vascular cell adhesion molecule 1 (VCAM-1), and targeting the interaction between very late antigen 4 (VLA-4) and VCAM-1 with either antibodies against VLA-4 [44,45], antibodies against VCAM-1 [46,47], or a small molecule inhibitor of VLA-4 (BIO5192) [48] results in HPSC mobilization. In addition, the Eph–ephrin A3 signaling axis increases adhesion to fibronectin and VCAM-1, and disruption of this signaling axis *in vivo* with a soluble EphA3-Fc fusion protein mobilizes HSPCs [49].

Osteoblasts also express significant amounts of osteopontin, and HSPCs adhere to osteopontin via β_1 integrins, such as VLA-4 [50]. Osteopontin is a negative regulator of HSC pool size within the bone marrow niche [50,51], and knockout of osteopontin in mice results in endogenous HSPC mobilization and increases the mobilization response to G-CSF [52]. Future therapies that target osteopontin may not only increase the HSC pool size available for hematopoietic mobilization, but may also act to untether the expanded HSCs from the bone marrow niche, resulting in significantly enhanced HSC mobilization.

Mobilizing regimens of G-CSF are associated with suppression of niche osteoblasts [21,41,53], with increased osteoblast apoptosis [41] and osteoblast flattening [21], resulting in significant decreases in endosteal niche expression of many of the above-mentioned retention molecules. This suppression has been reported to be the result of altered sympathetic nervous system signaling to osteoblasts [21]. A recent report by Winkler and colleagues demonstrated that G-CSF treatment results in the reduction of endosteal-lining osteomacs, which results in suppression of osteoblasts [53]. This osteomac population of cells is F4/80+ Ly-6G+ CD11b+ and provides a yet to be determined positive supporting role for osteoblasts.

When osteomacs are depleted using Mafia transgenic mice or by treatment of mice with clodronate-loaded liposomes, significant mobilization of HSPCs was observed. These findings support a mechanistic role for osteoblasts in mediating G-CSF-induced mobilization, independent of the sympathetic nervous system, and highlight that multiple mechanisms may be responsible for the mobilizing effects of G-CSF.

What about osteoclasts?

Osteoblasts and osteoclasts regulate/coordinate bone formation and bone resorption, respectively, within the bone marrow niche. A report from Kollet and colleagues suggested that osteoclasts can mediate HSPC mobilization [54], and proposed a model where the balance between osteoblasts and osteoclasts is required for homeostatic maintenance of the stem cell niche and HSPC pool size. In their model, increased osteoblasts – for example, after parathyroid hormone administration [3] – increase the stem cell pool size and adherence in the niche, whereas increased osteoclasts degrade the niche – facilitating release and egress of HSPCs.

A role for osteoclasts in mobilization was shown by treating mice with RANK ligand, which increased osteoclast activity that correlated with a moderate increase in hematopoietic progenitor cell (HPC) mobilization [54]. Similarly, bleeding mice or treating them with lipopolysaccharide, two models of physiological stress, resulted in an increase in the number of bone marrow niche osteoclasts as well as HPC mobilization. Inhibition of osteoclasts, either by treatment with calcitonin or using a genetic knockout model of PTP_{ε} in female mice, resulted in a reduced HPC mobilization response to G-CSF compared with controls, further suggesting that osteoclasts were involved in G-CSF-mediated mobilization. The authors proposed that osteoclast-derived proteolytic enzymes, such as cathepsin K, degraded important niche interaction components including SDF-1α and osteopontin, thereby facilitating mobilization [54]. A more recent study by the same laboratory demonstrated reduced osteoclast maturation and activity in CD45 knockout mice, which correlated with reduced mobilization to RANK ligand and G-CSF [55], providing an additional link between osteoclast activity and HSPC mobilization.

In contrast to studies showing that increased osteoclasts enhance HPC mobilization, an earlier report by Takamatsu and colleagues demonstrated that while G-CSF treatment increases osteoclast number and bone resorption in both BALB/c mice and humans, the increase in osteoclasts did not occur until 10 to 15 days or 6 to 8 days, respectively, after treatment with G-CSF [56] – a finding that has also been observed by other groups using similar systems [40,57]. Since HSPC mobilization by G-CSF is typically evaluated after 4 to 5 days, the importance of osteoclasts to HSPC mobilization in response to G-CSF treatment remains unclear. Furthermore, treatment of mice with bisphosphonates, which inhibit osteoclast activity and/or number, prior to G-CSF administration does not result in an impaired HSPC mobilization response [53,56]; in fact, in one case, bisphosphonate treatment increased mobilization by G-CSF [53]. These studies suggest that while osteoclasts elicit mechanisms that can induce hematopoietic stem and progenitor mobilization, their role in clinical HSC mobilization with G-CSF is not sufficiently defined and may not be a primary mechanism of mobilization.

The endosteal surface of bone, particularly at the site of resorbing osteoclasts, is a significant source of soluble extracellular calcium within the bone marrow niche. Studies by Adams and colleagues demonstrated that HSCs express calcium-sensing receptors and are chemoattracted to soluble Ca^{2+} [58]. When the gene for the calcium-sensing receptor was knocked out, mice had reduced HSC content within the bone marrow niche and increased HSCs in peripheral blood. Moreover, calcium-sensing receptor-knockout HSCs failed to engraft in hematopoietic transplantation experiments. These results suggest that Ca^{2+} at the endosteal surface is an important retention signal within the hematopoietic niche and that pharmacologic antagonism of the HSC calcium-sensing receptor may represent a possible strategy for HSPC mobilization.

Oxygen regulation of hematopoietic stem cell mobilization

The bone marrow hematopoietic niche has been shown to be hypoxic [59,60]. HSCs that reside in hypoxic niches have also been shown to have greater hematopoietic-repopulating ability than those that do not [61]. A known physiological response to hypoxia is stabilization of the transcription factor hypoxia inducible factor 1α (HIF-1α). HIF-1α has been shown to upregulate erythropoietin production [62], numerous cell proliferation and survival genes [63-65], the angiogenic vascular endothelial growth factor [66], and other genes. It has also been suggested that the hypoxic bone marrow niche maintains HIF-1α activity, thereby maintaining stem cells [67] – a hypothesis supported by the fact that hypoxic conditions expand human HSCs [68] and HPC populations [69-71] *in vitro*. In response to G-CSF, both the hypoxic environment and HIF-1α expand within the bone marrow compartment [72] and increase production of vascular endothelial growth factor A; however, bone marrow vascular density and permeability are not increased [61]. HIF-1α also increases production of SDF-1α [73] and CXCR4 receptor expression [74], suggesting that hypoxia may be a physiological regulator of this important signaling axis within the hematopoietic niche.

HIF-1α has recently been reported to prevent hematopoietic cell damage caused by overproduction of reactive oxygen species [75], suggesting that the hypoxic niche helps maintain the long lifespan of HSCs. However, some small degree of reactive oxygen species signaling may be necessary for HSC mobilization. A recent report demonstrated that enhanced c-Met activity promotes HSPC mobilization by activating mTOR and increasing reactive oxygen species production in HSPCs [76], while inhibition of mTOR with rapamycin reduced HSC mobilization [76,77]. Genetic knockout of the gene for thioredoxin-interacting protein also results in increased HSPC mobilization under stress conditions [78], suggesting a role for oxygen tension and reactive oxygen species in regulation of hematopoietic stem and progenitor mobilization. These findings clearly warrant additional exploration.

Control of the bone marrow niche by the nervous system

It has been known for some time that there is dynamic interaction between the bone marrow niche and the nervous system. Studies by Katayama and colleagues demonstrated that HSPC mobilization by G-CSF requires peripheral β_2-adrenergic signals [21], showing that G-CSF mobilization was reduced in chemically sympathectomized mice treated with 6-hydroxydopamine, in mice treated with the β-blocker propanolol, or in mice genetically deficient in the gene for dopamine β-hydroxylase (Dbh), an enzyme that converts dopamine into norepinephrine. They also showed that treatment with the β_2-adrenergic agonist clenbuterol reversed the phenotype of Dbh knockout mice [21]. Intriguingly, G-CSF attenuated osteoblast function via the sympathetic nervous system resulting in osteoblasts having a marked flattened appearance. The effects of nervous system signaling can also be mediated directly on HSCs, as human CD34+ hematopoietic cells express β_2-adrenergic and dopamine receptors that are upregulated after G-CSF treatment [79]. Neurotransmitters serve as direct chemoattractants to HSPCs, and treatment with norepinephrine results in HSC mobilization [79]. Norepinephrine treatment of mice has also been shown to increase CXCR4 receptor expression [80], perhaps suggesting that adrenergic signaling could directly affect CXCR4/SDF-1α signaling in HSPCs. Additional studies directly assessing effects of neurotransmitter signaling in HSPCs will help to further define the role of the nervous system in hematopoietic regulation.

Not only does the sympathetic nervous system affect HSC mobilization during stress situations, but it also regulates HSC trafficking via a circadian rhythm [81,82]. β_3-Adrenergic stimulations demonstrate regular oscillations controlling norepinephrine release, CXCR4 expression, and SDF-1α production, leading to rhythmic release

of HSPCs from the bone marrow niche. Intriguingly, while optimal mobilization occurs in the morning in mice (Zeitgeber time 5), HSC mobilization circadian control is inverted in humans, with peak mobilization occurring later in the evening [81]. Mobilization by both G-CSF and AMD3100 is affected by circadian control of the CXCR4/SDF-1α axis. Recently, it was demonstrated that β_2-adrenergic signaling upregulates the vitamin D receptor on osteoblasts; that expression of this receptor is necessary for the G-CSF-induced suppression of osteoblast function; and that vitamin D receptor knockout mice have reduced HSC mobilization [83]. Intriguingly, vitamin D receptor is an important regulator of extracellular calcium and HSPC localization [84] and the receptor is also regulated by circadian rhythms [85], possibly suggesting additional interconnected mobilization mechanisms. Further assessment of the role of nervous system signaling and vitamin D receptor signaling on other niche cells, particularly mesenchymal stem cells, should be performed.

Conclusion

There has been significant progress in understanding the mechanisms of action of G-CSF and other stimuli that increase HSPC trafficking/mobilization. As described in the present review, however, there is currently an abundance of proposed mechanisms that may be responsible for mobilization. This raises the question of whether the proposed mechanisms, be they HSPC intrinsic or manifested through the bone marrow niche, truly represent alternate and independent means to mobilize or enhance egress of HSPCs from bone marrow to the circulation, or whether we have not yet found the unifying mechanism.

Intriguingly, many of the proposed mechanisms of mobilization converge on the CXCR4/SDF-1α pathway (Figure 1). Alterations of the osteoblast/osteoclast balance result in a reduction of SDF-1α production and/or degradation of SDF-1α by proteases. Signaling from the sympathetic nervous system, stimulated by G-CSF, can alter the osteoblast/osteoclast balance leading to reduced CXCR4/SDF-1α signaling and HSPC mobilization. Circadian rhythms act to reduce niche SDF-1α production and HSPC CXCR4 expression in an oscillating manner, suggesting that clinical mobilization should be performed at the trough of SDF-1α and CXCR4 expression (early night for humans) and perhaps suggesting that clinical transplantation should be performed at the peak of expression (early morning in humans). The hypoxic nature of the hematopoietic bone marrow niche may itself regulate the CXCR4/SDF-1α signaling axis, perhaps further identifying this axis as a unifying mobilization mechanism. The importance of CXCR4 signaling in HSPC retention and mobilization is certainly
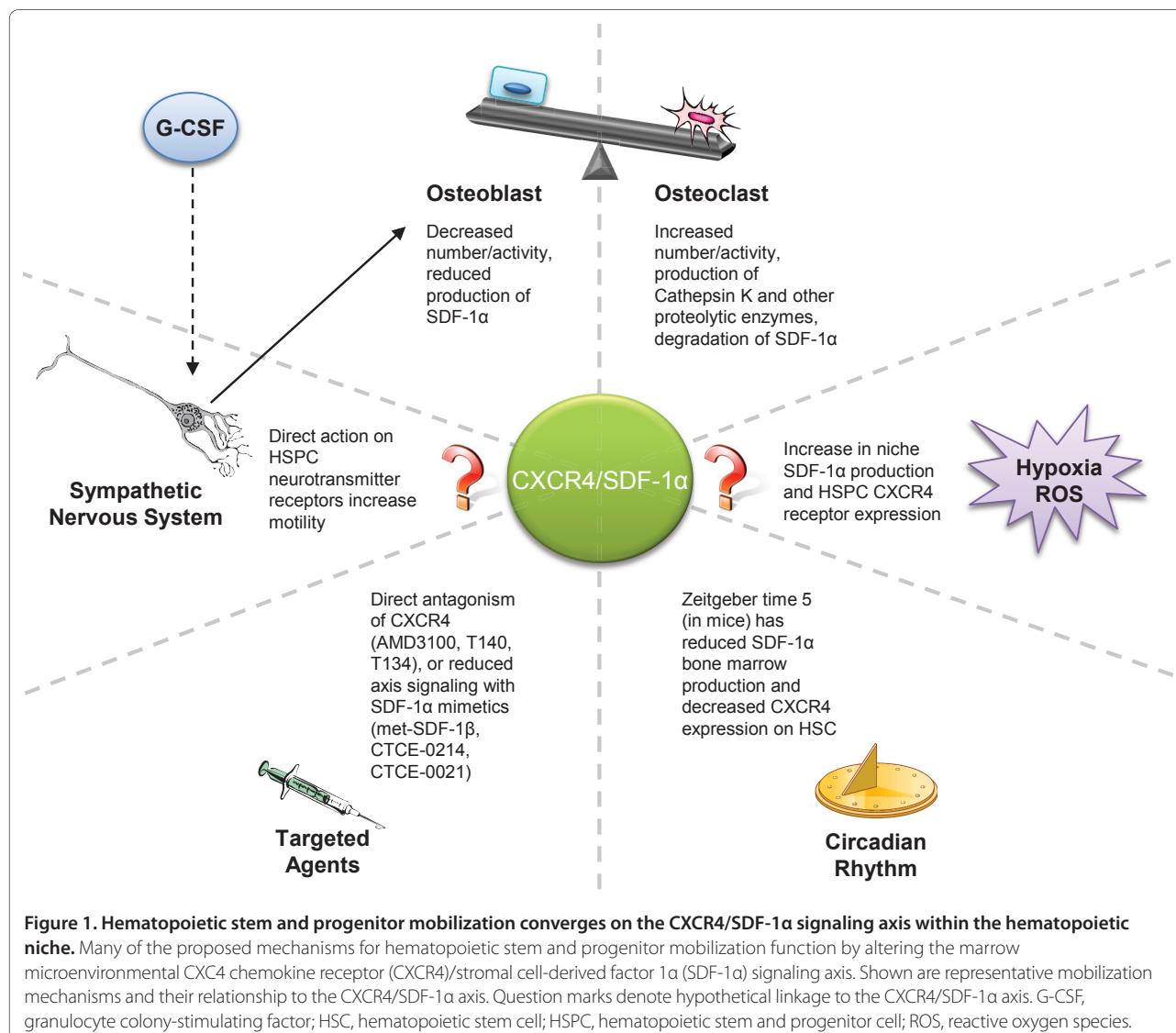
Figure 1. Hematopoietic stem and progenitor mobilization converges on the CXCR4/SDF-1α signaling axis within the hematopoietic niche. Many of the proposed mechanisms for hematopoietic stem and progenitor mobilization function by altering the marrow microenvironmental CXC4 chemokine receptor (CXCR4)/stromal cell-derived factor 1α (SDF-1α) signaling axis. Shown are representative mobilization mechanisms and their relationship to the CXCR4/SDF-1α axis. Question marks denote hypothetical linkage to the CXCR4/SDF-1α axis. G-CSF, granulocyte colony-stimulating factor; HSC, hematopoietic stem cell; HSPC, hematopoietic stem and progenitor cell; ROS, reactive oxygen species.

supported by the abundance of agents that directly antagonize, or compete with SDF-1α and partially agonize, the CXCR4 receptor and result in HSPC mobilization. Even a rapid mobilizing agent such as GROβ (CXCR2 agonist) may function by increasing proteolytic cleavage of SDF-1α [86,87], or altering a homeostatic balance between the CXCR4 and CXCR2 signaling pathways [88].

While perhaps connecting many of the proposed mechanistic pathways for HSPC mobilization, however, the CXCR4/SDF-1α pathway does not appear to be an exclusive target for HSPC mobilization. Continued investigation of the molecular mechanism(s) for action of

G-CSF and other HSPC mobilizers is warranted and may define new molecular targets that can be used to enhance the magnitude and/or ease of HSPC collection for hematopoietic transplant.

Abbreviations

CXCR4, CXC4 chemokine receptor; G-CSF, granulocyte colony-stimulating factor; HIF-1α, hypoxia inducible factor 1α; HPC, hematopoietic progenitor cell; HSC, hematopoietic stem cell; HSPC, hematopoietic stem and progenitor cell; mTOR, mammalian target of rapamycin; RANK, receptor activator NF-κB; SDF-1α, stromal cell-derived factor 1α; VCAM-1, vascular cell adhesion molecule 1; VLA-4, late antigen 4.

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