Physiology and Pharmacology of Plerixafor

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Summary

Autologous hematopoietic stem cell (HSC) transplantation is an important therapeutic option for patients with non-Hodgkin's lymphoma and multiple myeloma. The primary source of HSC is from the peripheral blood which requires mobilization from the bone marrow. Current mobilization regimens include cytokines such as G-CSF and/or chemotherapy. However not all patients mobilize enough HSC to proceed to transplant. The chemokine receptor CXCR4 and its ligand CXCL12 are an integral part of the mechanism of HSC retention in the bone marrow niche. The discovery of plerixafor, a selective inhibitor of CXCR4, has provided a new additional means of mobilizing HSC for autologous transplantation. Plerixafor consists of two cyclam rings with a phenylenebis(methylene) linker. It inhibits CXCL12 binding to CXCR4 and subsequent downstream events including chemotaxis. The molecular interactions of plerixafor have been defined indicating a unique binding mode to CXCR4. Plerixafor rapidly mobilizes HSC within hours compared with the multi-day treatment required by G-CSF in mouse, dog and non-human primate. The mobilized cells once transplanted are capable of timely and enduring engraftment. Additionally CXCR4 has been implicated in the pathology of HIV, inflammatory disease and cancer and the pharmacology of plerixafor in various disease models is described.

Introduction

Plerixafor (Mozobil[®], AMD3100) is a small organic molecule consisting of two cyclam rings connected by a 1,4-phenylenebis(methylene) linker (fig. 1) [1]. The pharma-

cological activity of bicyclam molecules was first identified in the search for new agents to treat HIV [2, 3]. Plerixafor evolved through an intensive medicinal chemistry effort from these early alkyl-linked bicyclams [4, 5].

Mechanistic studies suggested that the bicyclams acted at the early stages of the HIV infection process, but it was not until the discovery that HIV required a chemokine co-receptor, either CCR5 or CXCR4, along with CD4, for host cell entry, that the molecular target for plerixafor was discovered [6–10]. Plerixafor was shown to specifically inhibit infection of CXCR4-using (X4) virus thus pointing to antagonism of the CXCR4 chemokine receptor as the target [11–13]. Plerixafor was subsequently investigated in clinical trials. In a phase I study, a single subcutaneous injection of plerixafor resulted in a marked and rapid increase in circulating white blood cells (WBC) [14]. Initially this was attributed to demargination but subsequently, as described below, this was found to be due to cell mobilization. Though plerixafor was shown to be able to reduce X4-viral load in HIV patients [15], it was the pharmacological action as a mobilizer of hematopoietic stem cells (HSC) that was the subsequent focus for clinical development [16].

HSC are the stem cells from which all blood cells are derived, a process known as hematopoiesis. HSC have the ability to self-renew and are capable of generating every cell lineage of the hematopoietic system including erythrocytes, platelets, lymphoid and myeloid cells. An early step in hematopoiesis is the differentiation of a HSC to a hematopoietic progenitor cell (HPC) that is further committed to differentiation down a particular hematopoietic lineage pathway. As HPCs can be easily quantified by colony-forming assays, they are frequently used as a measure of HSC.

Hematopoietic stem cell transplantation (HSCT) is an important therapeutic strategy for the treatment of hematological malignancies [17]. Allogeneic transplantation, i.e., transplant of HSCs from a HLA-matched donor, is used for leukemia where it is often the only curative option, and autologous HSCT, i.e., use of the patient's own cells, is used for multiple myeloma (MM) and non-Hodgkin's lymphoma (NHL) in sup-

Fig. 1. Structure of plerixafor.

port of myeloablative high-dose chemotherapy and/or total body irradiation. Cells can be obtained by aspiration from the bone marrow but this is a painful procedure requiring general anesthesia and several repeat aspirations and has been replaced in recent years by peripheral blood stem cells as a source of HSC, particularly for autologous transplantation. Though HSC continually exit and return to the bone marrow, the numbers of HSC actually present in the peripheral blood is very small therefore they have to be mobilized from the bone marrow into the circulation before collection by apheresis. The predominant agents for mobilization are either cytokines such as granulocyte-colony stimulating factor (G-CSF) or chemotherapeutic drugs such as cyclophosphamide, or a combination of both [18, 19]. However a proportion of patients (5–40%) fail to mobilize adequate numbers of HSC to allow them to undergo a successful transplant. Plerixafor was approved by the Food and Drug Administration (FDA) in 2008 and by the European Medicines Agency (EMA) in 2009 for the use in combination with G-CSF to mobilize HSC to the peripheral blood for collection and subsequent autologous transplantation in patients with NHL and MM. This review will describe the pharmacology of plerixafor, and discuss the current understanding of its mechanism of action as a small molecule inhibitor of CXCR4.

CXCR4 and Hematopoietic Stem Cells

Chemokine receptors are seven-transmembrane G-protein-coupled receptor (GPCR) proteins. Their cognate ligands, chemokines, are 8–10 kDa proteins [20–22]. Chemokines regulate lymphocyte trafficking, are mediators of hematopoiesis and immune function, and play a role in the inflammatory disease process [21, 23, 24]. The chemokine receptor CXCR4 is ubiquitously expressed on many cell types including WBC, epithelial and endothelial cells. It plays crucial roles in the homing and trafficking of leukocytes and HPCs [25–27]. Moreover, it plays an important role in neonatal development by controlling the migration and positioning of cells during the processes of hematopoiesis, brain development, and vascularization. CXCR4 is expressed on HSC and plays a key role in their retention and maintenance in the bone marrow niche [28–30]. The bone marrow niche has been described as a two compartment system consisting of an endosteal niche provided

by osteoblasts and osteoclasts and a vascular niche comprised of the vascular endothelium [31]. However it is now apparent that this is an oversimplification as these cell types are in close anatomical proximity to one another.

There are two major families of chemokines defined by the number and relative spacing of cysteine residues at the N-terminal end of the protein. These are CC and CXC in which there are two cysteine residues that are either adjacent (CC) or separated by one amino acid residue (CXC) [22]. CXCR4 is a member of the latter family. CXCR4 has one ligand, the chemokine CXCL12 (also known as SDF-1). CXCL12 is produced by bone marrow stromal cells such as osteoblasts, endothelial cells and adventitial reticular cells, also known as CXCL12-abundant reticular cells (CAR) [32]. The expression of CXCL12 and CXCR4 is regulated by hypoxia-inducible factor (HIF-1) and it has been hypothesized that the bone marrow is partially hypoxic resulting in their enhanced expression. However, the hypoxic nature of the bone marrow has been questioned [31]. Other molecules playing a role in HSC homing and retention include c-kit and its ligand stem cell factor (SCF), the adhesion molecules VLA-4, VLA-5 and LFA-1 [33, 34]. CXCL12 can induce expression of VLA-4, and conversely mobilization with cytokines such as G-CSF and SCF result in decreased expression of CXCR4 [32]. What is clear is the importance of the CXCR4/CXCL12 interaction in the homing and retention of HSC within the bone marrow.

Molecular Pharmacology of Plerixafor

As mentioned above the discovery of CXCR4 as a co-receptor for HIV led to the realization that plerixafor was an inhibitor of CXCR4. As chemokine receptors are GPCRs, classical GPCR pharmacological techniques were used to investigate the molecular mechanism of action of plerixafor. Plerixafor was shown to inhibit CXCL12 ligand binding, and CXCL12-mediated G-protein activation, calcium flux, and receptor internalization [35, 36]. Plerixafor was also able to inhibit CXCL12-mediated chemotaxis, an *in vitro* physiological response related to *in vivo* migration. Plerixafor itself was unable to elicit any of these responses, indicating that it is an inhibitor of CXCR4 function without apparent agonist activity [35, 36], though there are reports that at high concentrations it can stimulate activity of a constitutively active mutant of CXCR4 and that it may be a weak agonist against wild type CXCR4 [37]. Plerixafor was shown to be a selective inhibitor of CXCR4 when compared across a panel of other chemokine receptor assays (CXCR1, CXCR2, CXCR3, CXCR7, CCR1, CCR2b, CCR3, CCR4, CCR5, CCR6, CCR7, CCR8, and CCR9) [35, 36]. CXCL12 can also interact with CXCR7 [38] and there is evidence that at high pharmacologically irrelevant concentrations (>10 $\mu\text{mol/l}$) plerixafor may be an allosteric agonist of CXCR7 [39], confirming that plerixafor is selective for CXCR4 over CXCR7. Mechanistic studies further

demonstrated that plerixafor is a tight-binding, slowly reversible inhibitor of CXCR4 [35, 36].

Plerixafor has a unique binding mode to CXCR4 compared with other chemokine receptor inhibitors. A single 1,4,8,11-tetrazacyclotetradecane ring has an overall positive charge of 2+ at physiological pH; therefore, the bicyclam plerixafor has an overall positive charge of 4+, thus allowing charge/charge interactions with amino acid residues on CXCR4. This was tested by site-directed mutagenesis of CXCR4 of selected aspartate and histidine residues and assessing the effect of these mutations on the binding of either radiolabeled Met-SDF-1 α or the CXCR4-specific antibody 12G5 to the receptor [40]. Ligand binding was significantly reduced by the mutations D171N and D262N. These studies highlighted the importance of Asp¹⁷¹ and Asp²⁶² for plerixafor binding to CXCR4. Interaction with a third charged amino acid, Glu²⁸⁸, was confirmed by reconstructing the CXCR4 binding site on CXCR3, which, as noted earlier, is not inhibited by plerixafor. Whereas CXCR4 has a glutamate, Glu²⁸⁸ on transmembrane region VII (TMVII), this is not present in CXCR3 where there is a serine in the equivalent position. In an elegant study the binding site of CXCR4 was reconstructed in CXCR3 by mutating Ser³⁰⁴ on CXCR3 to a glutamate. This resulted in inhibition of CXCR3 by plerixafor [41]. The binding site of plerixafor was therefore concluded to consist of the triad of Asp¹⁷¹ on TMIV, Asp²⁶² on TMVI, and Glu²⁸⁸ on TMVII. These data were used to create a model of plerixafor binding to CXCR4 by creating a CXCR4 homology model based upon the crystal structure of rhodopsin [42] (fig. 2). This binding mode was confirmed in further studies using site-directed mutagenesis together with inhibition of ¹²⁵I-SDF-1 α binding to CXCR4. However in an extensive probing of the binding site other potential amino acids were identified including Tyr⁴⁵, Trp⁹⁴ and Tyr¹¹⁶ suggesting possible alternative binding modes for plerixafor in which one cyclam ring can interact with one of the aspartate residues whilst the other interacts with an aromatic amino acid residue [43].

In vivo Pharmacology of Plerixafor

CXCR4, unlike many other chemokine receptors, is highly conserved across species, and it has been shown that plerixafor can inhibit CXCL12-mediated calcium flux in CXCR4-expressing lymphocytes and lymphocyte cell lines from human, mouse, and dog with similar potency [35, 36, 44]. This makes it possible to investigate plerixafor pharmacology in multiple species; as a result plerixafor has been shown to be capable of mobilizing HPC and HSC in several animal species. Mobilization occurs rapidly within hours of a single injection compared with the repeat injection of G-CSF over a number of days. The first, pivotal, study demonstrated peak mobilization of HPCs, assessed by colony forming unit (CFU) assays, within 1 h after a single subcutaneous injection in C3H/HeJ mice

Fig. 2. The binding mode of plerixafor to CXCR4 showing interactions with AspIV:20, AspVII:23 and GluVII:06 (Asp¹⁷¹, Asp²⁶² and Glu²⁸⁸ on TMIV, TMVI and TMVII respectively).

(fig. 3A) [45]. Mobilization was dose-dependent, with HPCs returning to baseline by 24 h. Importantly for future clinical use, the HPC-mobilizing capacity was not impaired by repeat daily administration. Equally significant was the observation that mobilization could be achieved in several different strains of mice, including those which only mobilized poorly in response to G-CSF. Treatment with plerixafor following G-CSF resulted in synergistic mobilization. HPCs can only provide short-term engraftment, whereas HSCs are capable of true long term engraftment. Whilst HPCs can be assessed by CFU assays, HSCs can only be measured by in vivo transplantation and subsequent reconstitution of the recipient bone marrow. Confirmation that HSC were mobilized was provided by transplant studies in which donor blood cells from CD45.2+ mice were transplanted into lethally irradiated congenic CD45.1+ recipients. Chimerism was assessed by flow cytometry using antibodies to CD45.1 and CD45.2. A >eightfold chimerism was observed (CD45.2:CD45.1) with plerixafor mobilized donor cells compared with control cells, and long-term engraftment was achieved with plerixafor-mobilized cells. Furthermore, the self-renewing capacity of the mobilized HSCs was demonstrated by a secondary transplant of marrow-derived donor cells from the primary recipient mice into secondary recipients. Mobilization of HPC from the bone marrow was confirmed using an in situ perfusion of mouse femoral bone marrow [46], though studies in splenectomized mice have suggested that other tissues may also act as extramedullary sources of the mobilized HPC/HSC under certain conditions [47].

HSCs are identified in the clinical setting by expression of the marker CD34. In addition successful engraftment is moni-

Fig. 3. Plerixafor rapidly mobilizes HSC which are capable of durable and long-term engraftment. **A** Time course of hematopoietic progenitor cell mobilization in response to a single subcutaneous injection of 5mg/kg plerixafor into C3H/HeJ mice. Results are shown as fold change over baseline. (Broxmeyer, H.E. et al. Originally published in *J Exp Med* 2005;201:1307–1318. doi:10.1084/jem.20041385 [45]). CFU-GM = colony forming unit – granulocyte macrophage, BFU-E = burst forming unit – erythroid, CFU-GEMM = colony forming unit – granulocyte erythroid macrophage megakaryocyte. Recovery of peripheral blood cell counts **B** neutrophils and **C** platelets in four dogs after receiving an autologous hematopoietic cell transplant (HCT) with plerixafor mobilized cells. Neutrophils recovered within a median of 9 days and platelets within a median of 25 days typical of normal timely engraftment. (Originally published in Burroughs L, et al.: Durable engraftment of AMD3100-mobilized autologous and allogeneic peripheral-blood mononuclear cells in a canine transplantation model. *Blood* 2005; 06:4002–4008 [44]).

tored by the neutrophil and platelet recovery. A canine model of HSC mobilization and transplantation has been successfully used to study G-CSF mobilization. This model has two advantages over the mouse model; one is that canine HSC express CD34, and secondly successful engraftment can be measured using the clinical parameters of neutrophil and platelet recovery. A single subcutaneous injection of plerixafor resulted in a rapid generalized leukocytosis similar to that observed in humans, together with a mobilization of CD34+ cells with the peak occurring around 8–10 h post-injection [44]. Cells were collected by leukapheresis and an autologous transplantation performed after myeloablative irradiation of the recipient dog. Neutrophil and platelet recoveries occurred at medians of 8 and 26 days, respectively, demonstrating successful bone marrow reconstitution (fig. 3B, C). All recipients had normal marrow function 12 months after transplantation demonstrating long-term durable engraftment of plerixafor-mobilized HSC.

The dog is also a suitable species for investigating allogeneic transplantation. Successful long-term engraftment was obtained after transplant of plerixafor-mobilized cells into dog leukocyte antigen(DLA)-matched littermates after conditioning with total body irradiation. Neutrophil and platelet recovery occurred with a median of 8 and 26 days, respectively [44]. Successful and prompt engraftment without graft-versus-host disease was also obtained in a model of non-myeloablative, MHC-haploidentical HSC transplantation [48].

Rapid mobilization of HSCs was also demonstrated in rhesus macaques. The cells were collected and retrovirally marked prior to transplant. Again rapid neutrophil and platelet recovery was observed. Using the retroviral marker, it was shown that long-term gene-marked myeloid and lymphoid cells were present up to 32 months after transplantation, again indicative of long-term durable engraftment of plerixafor-mobilized cells [49].

Phenotypic analysis showed that the plerixafor-mobilized cells possess intrinsic characteristics different from those of HSCs mobilized with G-CSF. In the mouse studies the plerixafor mobilized HSCs expressed a phenotype characteristic of highly engrafting mouse HSCs [45]. Based upon CXCR4, VLA-4 expression and cell cycle status, the mobilized cells from the rhesus macaque study indicated that the HSCs mobilized by plerixafor possessed a similar phenotype to bone marrow-derived HSCs [49]. In addition, a gene microarray analysis highlighted significant differences in the genes expressed by plerixafor- and G-CSF plus plerixafor-mobilized CD34+ cells compared with G-CSF alone-mobilized cells [50]. This is in line with clinical observations that plerixafor-mobilized human CD34+ cells appear to have a better ability to engraft in SCID mice [45, 51] and have the phenotype typical of a more primitive subset of HSCs than G-CSF [52] and that these cells have a gene profile consistent with the potential to promote engraftment [53].

Fig. 4. Mechanism of HSC mobilization by plerixafor. **A** HSCs are retained in the bone marrow niche by the CXCR4/CXCL12 interaction. **B** Binding of plerixafor to CXCR4 inhibits CXCL12 ligand binding and releases HSC from the bone marrow niche.

Mechanism of Plerixafor HSC Mobilization

The interaction of CXCR4 on HSCs with its cognate ligand produced by bone marrow stromal cells is essential for the retention of HSCs in the bone marrow niches. It is important to note that CXCR4, unlike for example VLA-4, is not an adhesion molecule but a chemokine receptor. Chemokines promote migration of cells towards a chemokine gradient and, in some instances, cause cells to arrest at precise locations [24]. It should also be noted that chemokines are bound to glycosaminoglycans on the cell surface and, in this way, are presented to the chemokine receptor-expressing cells [20]. Additionally chemokine receptors are rapidly internalized upon ligand engagement. It can be therefore envisaged that the retention of HSCs by CXCL12 is a dynamic interaction in which cells are being continually pulled towards the bone marrow niche cells. Disruption of this dynamic interaction will thus allow HSCs to passively move away from and out of the bone marrow niche [31]. Disruption of this interaction can occur by reduced expression of CXCR4 and/or CXCL12 either at the level of mRNA or by proteolytic cleavage [54, 55]. G-CSF-induced mobilization has been associated with increased levels of the serine proteases, neutrophil elastase, and cathepsin G. Proteolysis may also play a regulatory role in chemokine function; CXCL12 is selectively cleaved by CD26 which reduces the chemotactic responsiveness of CD34+ cells [23, 56]. However, non-proteolytic mechanisms seem to play a role as CXCL12 mRNA levels decrease in association with G-CSF treatment [55]. Though the mechanism of G-CSF is still not completely resolved, it is apparent that the CXCR4/CXCL12 interaction plays a central role.

Plerixafor is a selective inhibitor of CXCR4. Plerixafor binds to CXCR4 and blocks the binding of its cognate ligand CXCL12 [35, 36]. It is generally thought that it is the blocking of this interaction that allows HSC mobilization out of the

bone marrow. Further evidence for the disruption of the CXCR4/CXCL12 interaction in HSCs as the mechanism for HSC mobilization by plerixafor interestingly comes from transplant studies examining the effect of plerixafor treatment on HSC engraftment in mice. Mice pre-treated with plerixafor prior to transplantation showed higher engraftment compared with untreated controls [57]. Interestingly, the findings from a different study demonstrated that treatment with plerixafor post-transplantation was also able to improve engraftment [58]. In both cases it was proposed that the effect was mediated by increased marrow niche availability due to emptying of niches by the CXCR4 inhibitory effect of plerixafor.

The homeostatic regulation of the bone marrow niche and egress and ingress of HSCs is coordinated by multiple interacting factors that regulate the interactions of HSCs with their bone marrow niche. These factors include CXCR4 and CXCL12, but also a diverse array of molecules including cytokines and growth factors such as SCF, c-kit ligand and G-CSF [18], parathyroid hormone [59, 60], prostaglandins [61, 62], neuronal signals [63, 64], adhesion molecules such as VLA-4 [65], and chemokines including CXCL12 [23, 28, 30]. An additional mechanism for plerixafor-stimulated HSC mobilization has been proposed in which plerixafor directly stimulates an increase in circulating plasma CXCL12 which in turn drives HSC mobilization [66]. It is conceivable that in such a complex and well regulated system the disruption of the CXCR4/CXCL12 interaction would interfere with other processes in a downstream fashion. However, the simpler explanation seems the most likely; plerixafor-blocked CXCR4 can no longer respond to CXCL12 and hence lose the CXCL12-mediated retention signal resulting in movement away from the bone marrow niche. The accumulated data described above support the inhibition of the CXCR4-mediated retention of HSC as the mechanism of HSC mobilization by plerixafor (fig. 4).

Pharmacology of Plerixafor in Models of Disease

The initial therapeutic interest in CXCR4 came from its role as a co-receptor for HIV [1, 9, 10]. In a phase II clinical trial with HIV patients, treatment with plerixafor validated CXCR4 as a therapeutic target for HIV [15]. However, CXCR4 is widely expressed on many cell types and tissues such as brain, lung, colon, heart, kidney and liver, and on leukocytes, epithelial and endothelial cells [25, 27]. Therefore, it is not surprising that CXCR4 and CXCL12 have been implicated in a variety of disease states such as inflammatory diseases including asthma and rheumatoid arthritis [24], and cancer [67–69]. CXCL12 and CXCR4 have been found to be involved in leukocyte recruitment in autoimmune and inflammatory disease. CXCR4 and CXCL12 expression is elevated on CD4⁺ T cells in the synovium of patients with rheumatoid arthritis, and contributes to T-cell recruitment [70–72]. Plerixafor was tested in two models of collagen-induced rheumatoid arthritis, one using interferon γ (IFN- γ) receptor knock-out (IFN γ R KO) mice, and one in wild-type mice [73, 74]. In both cases plerixafor reduced the severity of the disease, with a concomitant reduction in leukocyte infiltration to the inflamed joint. CXCR4 has also been implicated in leukocyte recruitment in other inflammatory diseases such as uveitis and asthma. Plerixafor was able to decrease airway hyperreactivity and resistance in a cockroach allergen-induced model of asthma in mice [75]. This response was associated with a significant reduction in levels of T_H2 cytokines (IL-4, IL-5) and increases in T_H1 cytokines (IL-12, IFN- γ), indicating a shift of the immune response from T_H2 to T_H1, reflecting the preferential expression of CXCR4 on T_H2 cells over T_H1 cells.

Plerixafor can also mobilize circulating angiogenic cells and endothelial progenitor cells [76–78]. Plerixafor was able to accelerate the restoration of blood flow in a model of hindlimb ischemia in diabetic mice. In addition systemic administration of plerixafor-mobilized CD34⁺ cells accelerated restoration of blood flow [79]. Similar results were obtained in a model of hindlimb ischemia using C57BL/6 mice [76]. Transfer of PBMC mobilized with a combination of G-CSF plus plerixafor also resulted in an improvement in blood flow. Treatment with plerixafor was shown to reduce fibrosis and improve myocardial function and vascularity in a mouse model of myocardial infarction induced by ligation of the left ascending coronary artery [78] and after ischemia/reperfusion injury [80]. This was associated with mobilization of bone marrow-derived endothelial progenitor cells. As yet none of these indications have been translated into the clinical setting.

CXCR4 is also expressed on many types of cancer cells, including those of hematological origin such as leukemia and lymphomas [32, 81, 82] where it is frequently associated with a worsened prognosis [83], and on solid tumors, including breast, ovarian and glioblastoma [84–86]. CXCR4 plays multiple roles in tumor biology depending on the tumor type and location. It can act as a survival factor, promote metastasis, and

can act as a mediator of vasculogenesis [68]. Plerixafor has been shown to have activity against a variety of solid tumors where in vitro it can inhibit migration of cancer cells, and in vivo effect tumor growth and metastasis. Treatment with plerixafor has been shown to decrease growth of glioblastoma xenografts both as a single agent and in combination with BCNU [84, 87]. In another glioblastoma model, tumor vascularization was increased post-irradiation treatment [88]. This was found to be mediated by CD11b-expressing bone marrow-derived cells. These cells are of the monocyte lineage and also express CXCR4. Treatment with plerixafor post-irradiation prevented the infiltration of the CD11b cells, with an associated decrease in vasculogenesis, and abrogation of tumor regrowth [89].

The bone marrow provides a protective environment for leukemia cells. This survival advantage is in part provided by the CXCL12 interaction with CXCR4 on the leukemia cells [90]. Plerixafor has been shown to be able to mobilize B-cell acute lymphocytic leukemia cells (ALL) from the bone marrow and to inhibit their engraftment [91]. Similarly in a model of acute promyelocytic leukemia (APL), plerixafor was able to mobilize APL cells. Furthermore, plerixafor was able to improve survival and decrease the leukemia burden when administered with cytosine arabinoside compared with chemotherapy alone [92]. Plerixafor was also able to enhance the response of the therapeutic antibodies rituximab (anti-CD20) and alemtuzumab (anti-CD52) in a model of disseminated lymphoma [93]. In the latter instance, the therapeutic benefit was in part due to mobilization of neutrophils and enhancement of the antibody-dependent cellular cytotoxicity. These and other studies therefore support the potential use of CXCR4 antagonism as a means to enhance sensitivity to therapeutic agents in hematological malignancies. Preliminary encouraging data has been obtained from a phase II clinical study using plerixafor and chemotherapy with mitoxantrone, etoposide and cytarabine in AML patients [94].

Conclusion

Plerixafor was the first small molecule CXCR4 antagonist to be identified, coming out of a medicinal chemistry program aimed at identifying novel inhibitors of HIV infection [1]. Plerixafor is also only one of two chemokine receptor inhibitors approved for clinical use, the other being the CCR5 inhibitor maraviroc approved for HIV treatment [95, 96]. Plerixafor is a potent, selective inhibitor of CXCR4 with a unique binding mode. The interaction of CXCR4 with its ligand CXCL12 is essential to the retention of HSCs in the bone marrow and inhibition of CXCR4 by plerixafor results in rapid mobilization of HSCs. Non-clinical pharmacology studies in multiple species demonstrated that plerixafor is able to mobilize HSCs with long-term durable engraftment properties. Significantly, plerixafor synergistically enhanced HSC

mobilization with G-CSF. These pharmacological properties successfully translated into the clinical setting. Treatment with plerixafor and G-CSF was shown to significantly increase HSC mobilization compared with G-CSF alone both in healthy volunteers and in patients with NHL and MM [97–99]. Plerixafor was subsequently evaluated in two randomized phase III clinical trials, one in NHL patients [100] and one in MM patients [101]. The success of these two trials led to the approval by the FDA in 2008 for the use of plerixafor in combination with G-CSF to mobilize HSCs to the peripheral blood for collection and subsequent autologous transplantation in patients with NHL and MM. This was followed by approval in Europe by the EMA in 2009. Plerixafor represents a

new treatment option for HSC mobilization for MM and NHL patients.

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References

- De Clercq E: The bicyclam amd3100 story. *Nat Rev Drug Discov* 2003;2:581–587.
- De Clercq E, Yamamoto N, Pauwels R, Baba M, Schols D, Nakashima H, Balzarini J, Debyser Z, Murrer BA, Schwartz D, Thornton D, Bridger G, Fricker S, Henson G, Abrams M, Picker D: Potent and selective inhibition of human immunodeficiency virus (HIV)-1 and HIV-2 replication by a class of bicyclams interacting with a viral uncoating event. *Proc Natl Acad Sci U S A* 1992;89:5286–5290.
- De Clercq E, Yamamoto N, Pauwels R, Balzarini J, Witvrouw M, De Vreese K, Debyser Z, Rosenwirth B, Peichl P, Datema R, Thornton D, Skerlj R, Gaul F, Padmanabhan S, Bridger G, Henson G, Abrams: Highly potent and selective inhibition of human immunodeficiency virus by the bicyclam derivative jm3100. *Antimicrob Agents Chemother* 1994;38:668–674.
- Bridger GJ, Skerlj RT, Padmanabhan S, Martellucci SA, Henson GW, Struyf S, Witvrouw M, Schols D, De Clercq E: Synthesis and structure-activity relationships of phenylenebis(methylene)-linked bis-azamacrocycles that inhibit HIV-1 and HIV-2 replication by antagonism of the chemokine receptor CXCR4. *J Med Chem* 1999;42:3971–3981.
- Bridger GJ, Skerlj RT, Thornton D, Padmanabhan S, Martellucci SA, Henson GW, Abrams MJ, Yamamoto N, De Vreese K, Pauwels R, De Clercq E: Synthesis and structure-activity relationships of phenylenebis(methylene)-linked bis-tetraazamacrocycles that inhibit HIV replication. Effects of macrocyclic ring size and substituents on the aromatic linker. *J Med Chem* 1995;38:366–378.
- Deng H, Liu R, Ellmeier W, Choe S, Unutmaz D, Burkhart M, Di Marzio P, Marmon S, Sutton RE, Hill CM, Davis CB, Peiper SC, Schall TJ, Littman DR, Landau NR: Identification of a major co-receptor for primary isolates of HIV-1. *Nature* 1996;381:661–666.
- Oberlin E, Amara A, Bachelier F, Bessia C, Virelizier JL, Arenzana-Seisdedos F, Schwartz O, Heard JM, Clark-Lewis I, Legler DF, Loetscher M, Baggiolini M, Moser B: The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1. *Nature* 1996;382:833–835.
- Bleul CC, Farzan M, Choe H, Parolin C, Clark-Lewis I, Sodroski J, Springer TA: The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature* 1996;382:829–833.
- Doranz BJ, Berson JF, Rucker J, Doms RW: Chemokine receptors as fusion cofactors for human immunodeficiency virus type 1 (HIV-1). *Immunol Res* 1997;16:15–28.
- Moore JP, Doms RW: The entry of entry inhibitors: a fusion of science and medicine. *Proc Natl Acad Sci U S A* 2003;100:10598–10602.
- Donzella GA, Schols D, Lin SW, Este JA, Nagashima KA, Maddon PJ, Allaway GP, Sakmar TP, Henson G, De Clercq E, Moore JP: Amd3100, a small molecule inhibitor of HIV-1 entry via the CXCR4 co-receptor. *Nat Med* 1998;4:72–77.
- Schols D, Este JA, Henson G, De Clercq E: Bicyclams, a class of potent anti-HIV agents, are targeted at the HIV coreceptor fusin/CXCR-4. *Antiviral Res* 1997;35:147–156.
- Schols D, Struyf S, Van Damme J, Este JA, Henson G, De Clercq E: Inhibition of T-tropic HIV strains by selective antagonization of the chemokine receptor CXCR4. *J Exp Med* 1997;186:1383–1388.
- Hendrix CW, Flexner C, MacFarland RT, Giandomenico C, Fuchs EJ, Redpath E, Bridger G, Henson GW: Pharmacokinetics and safety of AMD-3100, a novel antagonist of the CXCR-4 chemokine receptor, in human volunteers. *Antimicrob Agents Chemother* 2000;44:1667–1673.
- Hendrix CW, Collier AC, Lederman MM, Schols D, Pollard RB, Brown S, Jackson JB, Coombs RW, Glesby MJ, Flexner CW, Bridger GJ, Badel K, MacFarland RT, Henson GW, Calandra G: Safety, pharmacokinetics, and antiviral activity of AMD3100, a selective CXCR4 receptor inhibitor, in HIV-1 infection. *J Acquir Immune Defic Syndr* 2004;37:1253–1262.
- Calandra G, Bridger G, Fricker S: CXCR4 in clinical hematology. *Curr Top Microbiol Immunol* 2010;341:173–191.
- Coplan EA: Hematopoietic stem-cell transplantation. *N Engl J Med* 2006;354:1813–1826.
- Nervi B, Link DC, DiPersio JF: Cytokines and hematopoietic stem cell mobilization. *J Cell Biochem* 2006;99:690–705.
- Pusic I, DiPersio JF: The use of growth factors in hematopoietic stem cell transplantation. *Curr Pharm Des* 2008;14:1950–1961.
- Allen SJ, Crown SE, Handel TM: Chemokine: receptor structure, interactions, and antagonism. *Annu Rev Immunol* 2007;25:787–820.
- Baggiolini M: Chemokines and leukocyte traffic. *Nature* 1998;392:565–568.
- Zlotnik A, Yoshie O: Chemokines: a new classification system and their role in immunity. *Immunity* 2000;12:121–127.
- Broxmeyer HE: Chemokines in hematopoiesis. *Curr Opin Hematol* 2008;15:49–58.
- Viola A, Luster AD: Chemokines and their receptors: drug targets in immunity and inflammation. *Annu Rev Pharmacol Toxicol* 2008;48:171–197.
- Murdoch C: CXCR4: chemokine receptor extraordinaire. *Immunol Rev* 2000;177:175–184.
- Nagasawa T, Kikutani H, Kishimoto T: Molecular cloning and structure of a pre-B-cell growth-stimulating factor. *Proc Natl Acad Sci U S A* 1994;91:2305–2309.
- Nagasawa T, Tachibana K, Kishimoto T: A novel CXC chemokine PBSF/SDF-1 and its receptor CXCR4: Their functions in development, hematopoiesis and HIV infection. *Semin Immunol* 1998;10:179–185.
- Dar A, Kollet O, Lapidot T: Mutual, reciprocal SDF-1/CXCR4 interactions between hematopoietic and bone marrow stromal cells regulate human stem cell migration and development in NOD/SCID chimeric mice. *Exp Hematol* 2006;34:967–975.
- Fruehauf S, Seggewiss R: It's moving day: factors affecting peripheral blood stem cell mobilization and strategies for improvement [corrected]. *Br J Haematol* 2003;122:360–375.
- Lapidot T, Dar A, Kollet O: How do stem cells find their way home? *Blood* 2005;106:1901–1910.
- Bonig H, Papayannopoulou T: Hematopoietic stem cell mobilization: updated conceptual renditions. *Leukemia* 2013;27:24–31.
- Schroeder MA, DiPersio JF: Mobilization of hematopoietic stem and leukemia cells. *J Leukoc Biol* 2012;91:47–57.
- Papayannopoulou T: Mechanisms of stem-/progenitor-cell mobilization: the anti-VLA-4 paradigm. *Semin Hematol* 2000;37:11–18.
- Bonig H, Priestley GV, Papayannopoulou T: Hierarchy of molecular-pathway usage in bone marrow homing and its shift by cytokines. *Blood* 2006;107:79–86.
- Fricker SP, Anastassov V, Cox J, Darkes MC, Grujic O, Idzan SR, Labrecque J, Lau G, Mosi RM, Nelson KL, Qin L, Santucci Z, Wong RS: Characterization of the molecular pharmacology of AMD3100: a specific antagonist of the G-protein coupled chemokine receptor, CXCR4. *Biochem Pharmacol* 2006;72:588–596.

- 36 Hatse S, Princen K, Bridger G, De Clercq E, Schols D: Chemokine receptor inhibition by AMD3100 is strictly confined to CXCR4. *FEBS Letters* 2002; 527:255–262.
- 37 Zhang WB, Navenot JM, Haribabu B, Tamamura H, Hiramatsu K, Omagari A, Pei G, Manfredi JP, Fujii N, Broach JR, Peiper SC: A point mutation that confers constitutive activity to CXCR4 reveals that T140 is an inverse agonist and that AMD3100 and Alx40-4c are weak partial agonists. *J Biol Chem* 2002;277:24515–24521.
- 38 Balabanian K, Lagane B, Infantino S, Chow KY, Harriague J, Moepps B, Arenzana-Seisdedos F, Thelen M, Bachelier F: The chemokine SDF-1/CXCL12 binds to and signals through the orphan receptor RDC1 in T lymphocytes. *J Biol Chem* 2005;280:35760–35766.
- 39 Kalatskaya I, Berchiche YA, Gravel S, Limberg BJ, Rosenbaum JS, Heveker N: AMD3100 is a CXCR7 ligand with allosteric agonist properties. *Mol Pharmacol* 2009;75:1240–1247.
- 40 Gerlach LO, Skerlj RT, Bridger GJ, Schwartz TW: Molecular interactions of cyclam and bicyclam non-peptide antagonists with the CXCR4 chemokine receptor. *J Biol Chem* 2001;276:14153–14160.
- 41 Rosenkilde MM, Gerlach L-O, Jakobsen JS, Skerlj RT, Bridger GJ, Schwartz TW: Molecular mechanism of AMD3100 antagonism in the CXCR4 receptor: transfer of binding site to the CXCR3 receptor. *J Biol Chem* 2004;279:3033–3041.
- 42 Palczewski K, Kumasaka T, Hori T, Behnke CA, Motoshima H, Fox BA, Le Trong I, Teller DC, Okada T, Stenkamp RE, Yamamoto M, Miyano M: Crystal structure of rhodopsin: a G protein-coupled receptor. *Science* 2000;289:739–745.
- 43 Wong RS, Bodart V, Metz M, Labrecque J, Bridger G, Fricker SP: Comparison of the potential multiple binding modes of bicyclam, monocyclam, and nencyclam small-molecule CXCR4 chemokine receptor 4 inhibitors. *Mol Pharmacol* 2008;74:1485–1495.
- 44 Burroughs L, Mielcarek M, Little MT, Bridger G, Macfarland R, Fricker S, Labrecque J, Sandmaier BM, Storb R: Durable engraftment of AMD3100-mobilized autologous and allogeneic peripheral-blood mononuclear cells in a canine transplantation model. *Blood* 2005;106:4002–4008.
- 45 Broxmeyer HE, Orschell CM, Clapp DW, Hangoc G, Cooper S, Plett PA, Liles WC, Li X, Graham-Evans B, Campbell TB, Calandra G, Bridger G, Dale DC, Srouf EF: Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. *J Exp Med* 2005;201:1307–1318.
- 46 Martin C, Bridger GJ, Rankin SM: Structural analogues of AMD3100 mobilise haematopoietic progenitor cells from bone marrow in vivo according to their ability to inhibit CXCL12 binding to CXCR4 in vitro. *Br J Haematol* 2006;134:326–329.
- 47 Ramirez P, Rettig MP, Uy GL, Deych E, Holt MS, Ritchey JK, DiPersio JF: Bio5192, a small molecule inhibitor of VLA-4, mobilizes hematopoietic stem and progenitor cells. *Blood* 2009;114:1340–1343.
- 48 Thakar MS, Santos EB, Fricker S, Bridger G, Storb R, Sandmaier BM: Plerixafor-mobilized stem cells alone are capable of inducing early engraftment across the MHC-haploidentical canine barrier. *Blood* 2010;115:916–917.
- 49 Larochelle A, Krouse A, Metzger M, Orlie D, Donahue RE, Fricker S, Bridger G, Dunbar CE, Hematti P: AMD3100 mobilizes hematopoietic stem cells with long-term repopulating capacity in nonhuman primates. *Blood* 2006;107:3772–3778.
- 50 Donahue RE, Jin P, Bonifacio AC, Metzger ME, Ren J, Wang E, Stroncek DF: Plerixafor (AMD3100) and granulocyte colony-stimulating factor (G-CSF) mobilize different CD34+ cell populations based on global gene and microRNA expression signatures. *Blood* 2009;114:2530–2541.
- 51 Hess DA, Bonde J, Craft TP, Wirthlin L, Hohm S, Lahey R, Todt LM, Dipersio JF, Devine SM, Nolte JA: Human progenitor cells rapidly mobilized by AMD3100 repopulate NOD/SCID mice with increased frequency in comparison to cells from the same donor mobilized by granulocyte colony stimulating factor. *Biol Blood Marrow Transplant* 2007; 13:398–411.
- 52 Fruehauf S, Veldwijk MR, Seeger T, Schubert M, Laufs S, Topaly J, Wuchter P, Dillmann F, Eckstein V, Wenz F, Goldschmidt H, Ho AD, Calandra G: A combination of granulocyte-colony-stimulating factor (G-CSF) and plerixafor mobilizes more primitive peripheral blood progenitor cells than G-CSF alone: results of a European phase II study. *Cytotherapy* 2009;11:992–1001.
- 53 Fruehauf S, Seeger T, Maier P, Li L, Weinhardt S, Laufs S, Wagner W, Eckstein V, Bridger G, Calandra G, Wenz F, Zeller WJ, Goldschmidt H, Ho AD: The CXCR4 antagonist AMD3100 releases a subset of G-CSF-primed peripheral blood progenitor cells with specific gene expression characteristics. *Exp Hematol* 2006;34:1052–1059.
- 54 Lapidot T, Petit I: Current understanding of stem cell mobilization: the roles of chemokines, proteolytic enzymes, adhesion molecules, cytokines, and stromal cells. *Exp Hematol* 2002;30:973–981.
- 55 Motabi IH, DiPersio JF: Advances in stem cell mobilization. *Blood Rev* 2012;26:267–278.
- 56 Christopherson KW 2nd, Cooper S, Broxmeyer HE: Cell surface peptidase CD26/DPP4 mediates G-CSF mobilization of mouse progenitor cells. *Blood* 2003;101:4680–4686.
- 57 Chen J, Larochelle A, Fricker S, Bridger G, Dunbar CE, Abkowitz JL: Mobilization as a preparative regimen for hematopoietic stem cell transplantation. *Blood* 2006;107:3764–3771.
- 58 Kang Y, Chen BJ, Deoliveira D, Mito J, Chao NJ: Selective enhancement of donor hematopoietic cell engraftment by the CXCR4 antagonist AMD3100 in a mouse transplantation model. *PLoS One* 2010; 5:e11316.
- 59 Adams GB, Martin RP, Alley IR, Chabner KT, Cohen KS, Calvi LM, Kronenberg HM, Scadden DT: Therapeutic targeting of a stem cell niche. *Nat Biotechnol* 2007;25:238–243.
- 60 Ballen K: Targeting the stem cell niche: squeezing blood from bones. *Bone Marrow Transplant* 2007; 39:655–660.
- 61 Hoggatt J, Singh P, Sampath J, Pelus LM: Prostaglandin e2 enhances hematopoietic stem cell homing, survival, and proliferation. *Blood* 2009;113: 5444–5455.
- 62 North TE, Goessling W, Walkley CR, Lengerke C, Kopani KR, Lord AM, Weber GJ, Bowman TV, Jang IH, Grosser T, Fitzgerald GA, Daley GQ, Orkin SH, Zon LI: Prostaglandin e2 regulates vertebrate hematopoietic stem cell homeostasis. *Nature* 2007;447:1007–1011.
- 63 Katayama Y, Battista M, Kao WM, Hidalgo A, Peired AJ, Thomas SA, Frenette PS: Signals from the sympathetic nervous system regulate hematopoietic stem cell egress from bone marrow. *Cell* 2006;124:407–421.
- 64 Spiegel A, Kalinkovich A, Shvitiel S, Kollet O, Lapidot T: Stem cell regulation via dynamic interactions of the nervous and immune systems with the microenvironment. *Cell Stem Cell* 2008;3:484–492.
- 65 Papayannopoulou T: Current mechanistic scenarios in hematopoietic stem/progenitor cell mobilization. *Blood* 2004;103:1580–1585.
- 66 Dar A, Schajnovitz A, Lapid K, Kalinkovich A, Itkin T, Ludin A, Kao WM, Battista M, Tesio M, Kollet O, Cohen NN, Margalit R, Buss EC, Baleux F, Oishi S, Fujii N, Larochelle A, Dunbar CE, Broxmeyer HE, Frenette PS, Lapidot T: Rapid mobilization of hematopoietic progenitors by AMD3100 and catecholamines is mediated by CXCR4-dependent SDF-1 release from bone marrow stromal cells. *Leukemia* 2011;25:1286–1296.
- 67 Balkwill F: The significance of cancer cell expression of the chemokine receptor CXCR4. *Semin Cancer Biol* 2004;14:171–179.
- 68 Balkwill F: Cancer and the chemokine network. *Nat Rev Cancer* 2004;4:540–550.
- 69 Teicher BA, Fricker SP: CXCL12 (SDF-1)/CXCR4 pathway in cancer. *Clin Cancer Res* 2010;16:2927–2931.
- 70 Bradfield PF, Amft N, Vernon-Wilson E, Exley AE, Parsonage G, Rainger GE, Nash GB, Thomas AM, Simmons DL, Salmon M, Buckley CD: Rheumatoid fibroblast-like synoviocytes overexpress the chemokine stromal cell-derived factor 1 (CXCL12), which supports distinct patterns and rates of CD4+ and CD8+ T cell migration within synovial tissue. *Arthritis Rheum* 2003;48:2472–2482.
- 71 Buckley CD, Amft N, Bradfield PF, Pilling D, Ross E, Arenzana-Seisdedos F, Amara A, Curnow SJ, Lord JM, Scheel-Toellner D, Salmon M: Persistent induction of the chemokine receptor CXCR4 by TGF-beta 1 on synovial T cells contributes to their accumulation within the rheumatoid synovium. *J Immunol* 2000;165:3423–3429.
- 72 Nanki T, Hayashida K, El-Gabalawy HS, Suson S, Shi K, Girschick HJ, Yavuz S, Lipsky PE: Stromal cell-derived factor-1/CXC chemokine receptor 4 interactions play a central role in CD4+ T cell accumulation in rheumatoid arthritis synovium. *J Immunol* 2000;165:6590–6598.
- 73 De Klerck B, Geboes L, Hatse S, Kelchtermans H, Meyvis Y, Vermeire K, Bridger G, Billiau A, Schols D, Matthys P: Pro-inflammatory properties of stromal cell-derived factor-1 (CXCL12) in collagen-induced arthritis. *Arthritis Res Ther* 2005;7: R1208–1220.
- 74 Matthys P, Hatse S, Vermeire K, Wuyts A, Bridger G, Henson GW, De Clercq E, Billiau A, Schols D: AMD3100, a potent and specific antagonist of the stromal cell-derived factor-1 chemokine receptor CXCR4, inhibits autoimmune joint inflammation in IFN-gamma receptor-deficient mice. *J Immunol* 2001;167:4686–4692.
- 75 Lukacs NW, Berlin A, Schols D, Skerlj RT, Bridger GJ: AMD3100, a CXCR4 antagonist, attenuates allergic lung inflammation and airway hyperreactivity. *Am J Pathol* 2002;160:1353–1360.
- 76 Capoccia BJ, Shepherd RM, Link DC: G-CSF and AMD3100 mobilize monocytes into the blood that stimulate angiogenesis in vivo through a paracrine mechanism. *Blood* 2006;108:2438–2445.
- 77 Shepherd RM, Capoccia BJ, Devine SM, Dipersio J, Trinkaus KM, Ingram D, Link DC: Angiogenic cells can be rapidly mobilized and efficiently harvested from the blood following treatment with AMD3100. *Blood* 2006;108:3662–3667.
- 78 Jujo K, Hamada H, Iwakura A, Thorne T, Sekiguchi H, Clarke T, Ito A, Misener S, Tanaka T, Klyachko E, Kobayashi K, Tongers J, Roncalli J, Tsurumi Y, Hagiwara N, Losordo DW: CXCR4 blockade augments bone marrow progenitor cell recruitment to the neovasculature and reduces mortality after myocardial infarction. *Proc Natl Acad Sci U S A* 2010;107:11008–11013.

- 79 Jiao C, Fricker S, Schatteman GC: The chemokine (C-X-C motif) receptor 4 inhibitor AMD3100 accelerates blood flow restoration in diabetic mice. *Diabetologia* 2006;49:2786–2789.
- 80 Jujo K, Ii M, Sekiguchi H, Klyachko E, Misener S, Tanaka T, Tongers J, Roncalli J, Renault MA, Thorne T, Ito A, Clarke T, Kamide C, Tsurumi Y, Hagiwara N, Qin G, Asahi M, Losordo DW: CXCR4 chemokine receptor 4 antagonist AMD3100 promotes cardiac functional recovery after ischemia/reperfusion injury via endothelial nitric oxide synthase-dependent mechanism. *Circulation* 2013;127:63–73.
- 81 Konopleva M, Tabe Y, Zeng Z, Andreeff M: Therapeutic targeting of microenvironmental interactions in leukemia: mechanisms and approaches. *Drug Resist Updat* 2009;12:103–113.
- 82 Juarez J, Bendall L: SDF-1 and CXCR4 in normal and malignant hematopoiesis. *Histol Histopathol* 2004;19:299–309.
- 83 Spoo AC, Lubbert M, Wierda WG, Burger JA: CXCR4 is a prognostic marker in acute myelogenous leukemia. *Blood* 2007;109:786–791.
- 84 Rubin JB, Kung AL, Klein RS, Chan JA, Sun Y, Schmidt K, Kieran MW, Luster AD, Segal RA: A small-molecule antagonist of CXCR4 inhibits intracranial growth of primary brain tumors. *Proc Natl Acad Sci U S A* 2003;100:13513–13518.
- 85 Scotton CJ, Wilson JL, Scott K, Stamp G, Wilbanks GD, Fricker S, Bridger G, Balkwill FR: Multiple actions of the chemokine CXCL12 on epithelial tumor cells in human ovarian cancer. *Cancer Res* 2002;62:5930–5938.
- 86 Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME, McClanahan T, Murphy E, Yuan W, Wagner SN, Barrera JL, Mohar A, Verastegui E, Zlotnik A: Involvement of chemokine receptors in breast cancer metastasis. *Nature* 2001;410:50–56.
- 87 Redjal N, Chan JA, Segal RA, Kung AL: CXCR4 inhibition synergizes with cytotoxic chemotherapy in gliomas. *Clin Cancer Res* 2006;12:6765–6771.
- 88 Tseng D, Vasquez-Medrano DA, Brown JM: Targeting SDF-1/CXCR4 to inhibit tumour vasculature for treatment of glioblastomas. *Br J Cancer* 2011;104:1805–1809.
- 89 Kioi M, Vogel H, Schultz G, Hoffman RM, Harsh GR, Brown JM: Inhibition of vasculogenesis, but not angiogenesis, prevents the recurrence of glioblastoma after irradiation in mice. *J Clin Invest* 2010;120:694–705.
- 90 Burger JA, Kipps TJ: CXCR4: A key receptor in the crosstalk between tumor cells and their microenvironment. *Blood* 2006;107:1761–1767.
- 91 Juarez J, Dela Pena A, Baraz R, Hewson J, Khoo M, Cisterne A, Fricker S, Fujii N, Bradstock KF, Bendall LJ: CXCR4 antagonists mobilize childhood acute lymphoblastic leukemia cells into the peripheral blood and inhibit engraftment. *Leukemia* 2007;21:1249–1257.
- 92 Nervi B, Ramirez P, Rettig MP, Uy GL, Holt MS, Ritchey JK, Prior JL, Pivnicka-Worms D, Bridger G, Ley TJ, DiPersio JF: Chemosensitization of acute myeloid leukemia (AML) following mobilization by the CXCR4 antagonist AMD3100. *Blood* 2009;113:6206–6214.
- 93 Hu Y, Gale M, Shields J, Garron C, Swistak M, Nguyen TH, Jacques G, Fogle R, Siders W, Kaplan J: Enhancement of the anti-tumor activity of therapeutic monoclonal antibodies by CXCR4 antagonists. *Leuk Lymphoma* 2012;53:130–138.
- 94 Uy GL, Rettig MP, Motabi IH, McFarland K, Trinkaus KM, Hladnik LM, Kulkarni S, Abboud CN, Cashen AF, Stockerl-Goldstein KE, Vij R, Westervelt P, DiPersio JF: A phase 1/2 study of chemosensitization with the CXCR4 antagonist plerixafor in relapsed or refractory acute myeloid leukemia. *Blood* 2012;119:3917–3924.
- 95 Kromdijk W, Huitema AD, Mulder JW: Treatment of HIV infection with the CCR5 antagonist maraviroc. *Expert Opin Pharmacother* 2010;11:1215–1223.
- 96 Perry CM: Maraviroc: A review of its use in the management of CCR5-tropic HIV-1 infection. *Drugs* 2010;70:1189–1213.
- 97 Liles WC, Broxmeyer HE, Rodger E, Wood B, Hubel K, Cooper S, Hangoc G, Bridger GJ, Henson GW, Calandra G, Dale DC: Mobilization of hematopoietic progenitor cells in healthy volunteers by AMD3100, a CXCR4 antagonist. *Blood* 2003;102:2728–2730.
- 98 Liles WC, Rodger E, Broxmeyer HE, Dehner C, Badel K, Calandra G, Christensen J, Wood B, Price TH, Dale DC: 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;45:295–300.
- 99 Flomenberg N, Devine SM, DiPersio JF, Liesveld JL, McCarty JM, Rowley SD, Vesole DH, Badel K, Calandra G: The use of AMD3100 plus G-CSF for autologous hematopoietic progenitor cell mobilization is superior to G-CSF alone. *Blood* 2005;106:1867–1874.
- 100 DiPersio JF, Micallef IN, Stiff PJ, Bolwell BJ, Maziarz RT, Jacobsen E, Nademanee A, McCarty J, Bridger G, Calandra G: 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;27:4767–4773.
- 101 DiPersio JF, Stadtmauer EA, Nademanee A, Micallef IN, Stiff PJ, Kaufman JL, Maziarz RT, Hosking C, Fruehauf S, Horwitz M, Cooper D, Bridger G, Calandra G: Plerixafor and G-CSF versus placebo and G-CSF to mobilize hematopoietic stem cells for autologous stem cell transplantation in patients with multiple myeloma. *Blood* 2009;113:5720–5726.