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Mobilization of Hematopoietic Stem and Progenitor Cells Using Inhibitors of CXCR4 and VLA-4

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Abstract

Successful hematopoietic stem cell transplant (HSCT) requires the infusion of a sufficient number of hematopoietic stem/progenitor cells (HSPCs) that are capable of homing to the bone marrow cavity and regenerating durable trilineage hematopoiesis in a timely fashion. Stem cells harvested from peripheral blood are the most commonly used graft source in HSCT. While granulocyte colony-stimulating factor (G-CSF) is the most frequently used agent for stem cell mobilization, the use of G-CSF alone results in suboptimal stem cell yields in a significant proportion of patients. Both the chemokine receptor CXCR4 and the integrin $\alpha_4\beta_1$ (VLA-4) play important roles in the homing and retention of HSPCs within the bone marrow microenvironment. Preclinical and/or clinical studies have shown that targeted disruption of the interaction of CXCR4 or VLA-4 with their ligands results in the rapid and reversible mobilization of hematopoietic stem cells into the peripheral circulation and is synergistic when combined with G-CSF. In this review we discuss the development of small molecule CXCR4 and VLA-4 inhibitors and how they may improve the utility and convenience of peripheral blood stem cell transplantation.

Keywords

Hematopoietic stem cell transplantation; hematopoietic stem cell mobilization; CXCR4; VLA-4; plerixafor

Introduction

The majority of hematopoietic stem and progenitor cells (HSPCs) reside in the bone marrow in a highly organized microenvironment consisting of marrow stromal cells, osteoblasts, osteoclasts and other extracellular matrix proteins (e.g., collagens, fibronectins, proteoglycans).[1–5] HSPCs express a number of cell surface molecules such as very late antigen 4 (VLA-4), CXCR4, CXCR2, CD44, CD62L, lymphocyte function-associated antigen-1 (LFA-1), CD117 (c-kit), and Robo4 that mediate their adherence in the BM microenvironment.[3, 4, 6, 7] These interactions play important roles in regulating HSPC trafficking, as well as self-renewal, proliferation and differentiation processes.[4, 8]

Mobilized HSPCs collected from peripheral blood have essentially replaced bone marrow as a source of stem cells for autologous and allogeneic transplantation. There was initial concern regarding the use of mobilized peripheral blood stem cells (PBSCs) as a source of graft for allogeneic stem cell transplantation. This concern was based on the presence of a

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10- to 50-fold increase in the T cell content of the mobilized peripheral blood products, which could potentially lead to higher rates of acute and chronic GVHD. However, transplantation with mobilized PBSCs was associated with faster engraftment, reduced infectious complications, enhanced immune reconstitution, shorter hospitalization, and reduced costs.[9–12]

Currently, granulocyte colony-stimulating factor (G-CSF) alone or G-CSF plus chemotherapy are the most commonly used methods for stem cell mobilization. Unfortunately, 5–30% of patients do not respond to these agents.[13] New strategies are needed to manage patients who fail initial mobilization, decrease the number of leukaphereses required to collect adequate number of HSPCs, improve immune reconstitution and decrease total cost. This article will first briefly review the standard approaches to HSPC mobilization using G-CSF. We will then discuss new strategies to mobilize stem cells through the use of CXCR4 and VLA-4 small molecule antagonists.

Standard approaches to hematopoietic stem and progenitor cell mobilization

Cytokines, especially G-CSF, can be used alone or in combination with chemotherapy (disease-specific chemotherapy or cyclophosphamide) to increase the number of circulating $CD34^+$ cells, the surrogate marker of HSPCs in humans.[13–15] Studies have shown that although mobilization with G-CSF plus chemotherapy generates higher HSPC yield when compared to G-CSF alone, failure rates are not different between the two mobilization groups (5–30%).[16–18] Factors that may limit the collection of an adequate amount of $CD34^+$ HSPC include age, sex, extent and type of chemotherapy, previous use of immunomodulatory drugs, previous exposure to radiation, previous attempts at mobilization, depressed peripheral blood $CD34^+$ HSPC counts before mobilization, disease status, and involvement of bone marrow in the disease process.[19,20] The optimal time for collection of stem cells after mobilization with chemotherapy plus G-CSF is 10–14 days as opposed to 4–5 days when G-CSF is used as single agent.[13] Although mobilization with chemotherapy plus G-CSF generally requires fewer leukaphereses to collect an adequate number of stem cells, individual differences in response to chemotherapy, and the potential for complications result in more unpredictable collections that can delay the transplant and result in increased morbidity and cost. The use of chemotherapy as a part of mobilization is associated with significantly higher toxicities including increased risk for secondary malignances, impairment of fertility, cardiac toxicity, hemorrhagic cystitis, anaphylactic reactions, and higher cost.

G-CSF is well tolerated, with skeletal pain, fatigue and nausea being most frequent side effects. Rare episodes of spontaneous splenic rupture have been reported.[21–24] Strategies to manage patients who fail their initial mobilization include dose escalation of G-CSF (12.5–50 μg/kg/day), addition of another cytokine such as GM-CSF, addition of chemotherapy or harvesting the bone marrow; however, no standard approach exists. In general, patients who fail initial mobilization are more likely to fail remobilization regardless of the remobilization regimen.[17, 25] Furthermore, additional mobilization efforts often result in poorer patient outcomes and increased resource utilization. For these reasons, novel mobilization agents that are less toxic, more rapid and increase the yield of collected $CD34^+$ cells for transplantation are needed. Plerixafor (AMD3100, Mozobil; Genzyme, Massachusetts, USA), a novel small molecule antagonist of CXCR4, was approved by the United States Food and Drug Administration (FDA) for use with G-CSF in December of 2008 to mobilize HSPC to the peripheral blood for collection and subsequent autologous transplantation in patients with non-Hodgkin's lymphoma (NHL) and multiple myeloma (MM). Below we will briefly discuss CXCR4/CXCL12 biology and clinical results obtained using plerixafor as a mobilizing agent in autologous and allogeneic transplantation. We will then discuss

some of the limitations with the use of plerixafor for HSPC mobilization and new agents in clinical development that target the CXCR4/CXCL12 axis.

HSPC mobilizing agents that target the CXCL12/CXCR4 axis

CXCL12/CXCR4 axis

CXCL12 or stromal cell derived factor 1α (SDF-1α) is a chemokine that is constitutively produced at high levels in the bone marrow by stromal cells such as osteoblasts, endothelial cells and a subset of reticular cells.[26–30] It is a potent chemoattractant for HSPCs and has been shown to regulate cell adhesion, survival, and cell-cycle status.[31–33] CXCL12 requires the amino terminus of CXCR4 for binding and activates downstream signaling pathways via the second extracellular loop.[34] Further structure-function studies indicate that the third intracellular loop (IL3) of CXCR4 is necessary for G_i-dependent signaling, and intracellular loops 2 and 3 as well as the C-terminus part of CXCR4 are required for chemotaxis.[35] Interestingly, CXCL12 gene polymorphism has been proposed as a conditional factor for human $CD34^+$ stem cell mobilization, with the presence of the SDF1-3′A allele as a predictive factor of good $CD34^+$ cell mobilization.[36, 37] More recently, a second receptor, CXCR7, was identified that binds CXCL12 with an affinity that is approximately tenfold higher than the affinity for CXCR4.[38, 39] Though the role of CXCR7 in CXCL12-dependent chemotaxis is not fully understood, there is evidence that CXCR7 lacks intrinsic chemotactic activity towards CXCL12 and rather functions by sequestering CXCL12 and modifying CXCR4 signaling.[40–43]

CXCR4 is a member of the large family of seven transmembrane domain receptors coupled to heterotrimeric G_i proteins and functions as a coreceptor for human immunodeficiency virus type 1 (HIV-1) cell entry.[44–48] CXCR4 exists in different isoforms as the result of differential splicing, which affect the length of its N-terminus.[49] Post-translational modifications of CXCR4 include N-glycosylation, tyrosine sulfation, and modification of tyrosine-21 by chondroitin sulfate.[49] The binding of CXCR4 to CXCL12 results in activation of multiple signal transduction pathways ultimately triggering chemotaxis.[49, 50] More recently, both trefoil factor family 2 (TFF2)[51] and macrophage migrating inhibiting factor (MIF)[52] were described as additional ligands of CXCR4. Finally, the expression of CXCR4 on human $CD34^+$ stem cells is dynamic and Flt3-ligand,[53] SCF,[54] IL-3,[55] IL-8,[56] hepatocyte growth factor,[57] andG-CSF[58] are all known to modulate the SDF/CXCR4 pathway.

Genetic alteration of CXCR4

Mice deficient for CXCL12 or CXCR4 die perinatally and exhibit similar defects in neuron migration, organ vascularization, and hematopoiesis. $CXCR4^{-/-}$ and $CXCL12^{-/-}$embryos have severely reduced B cell lymphopoiesis, reduced myelopoiesis in fetal liver, and nearly complete loss of myelopoiesis in the bone marrow.[59–62] This data, along with the observation of increased numbers of circulating myeloid cells in the fetal blood of CXCR4-deficient embryos,[63] demonstrated the pivotal role for the CXCR4/CXCL12 axis in bone marrow colonization.

Since the lethality caused by deficiency in CXCR4 prevents analysis of its role in adult hematopoiesis, investigators generated hematopoietic chimeras by transferring CXCR4-deficient cells into lethally irradiated wild type recipients. Unexpectedly, $CXCR4^{-/-}$ fetal liver cells engrafted in the marrow of the irradiated recipients when high cell numbers were transplanted.[63–65] These results imply that early HSPC homing to the BM can occur in the absence of CXCR4. However, mice reconstituted with CXCR4-deficient fetal liver cells exhibited significantly reduced numbers of donor derived $B220^+$ B cells, $Mac\text{-}1^+$ granulocytic-monocytic cells, $Gr\text{-}1^+$ granulocytic cells, and $CD61^+$ megakaryocytic cells in

the bone marrow.[63–65] Furthermore, there was a marked increase in the number of circulating immature granulocytes, CFU-Cs (30-fold increase), and $Lin^{-}Sca\text{-}1^{+}c\text{-}kit^{+}$ (LSK) cells in mice stably engrafted with CXCR4-deficient fetal liver cells.[63–65] In separate studies, $CXCR4^{-/-}$ and wild-type BM cells were competitively transplanted into lethally irradiated congenic recipients, and HSPC engraftment was evaluated by their multi-lineage contribution to the peripheral blood at 8 weeks post-injection.[66] These experiments also revealed that $CXCR4^{-/-}$ BM cells exhibited impaired engrafting capacity, with 5-fold more $CXCR4^{-/-}$ BM cells being required to achieve chimerism levels similar to the wild-type BM cells. Taken together, these data suggest that the impaired hematopoietic reconstitution potential of adoptively transferred $CXCR4^{-/-}$ cells is related to their altered homing and retention within the bone marrow microenvironment.

To further characterize the role of CXCR4 in hematopoietic stem cells, two groups have independently used the Cre-loxP recombination system to selectively delete CXCR4 in the hematopoietic system. In these studies, CXCR4-floxed mice were crossed to either MxCre[33] or tamoxifen-inducible Cre[66] transgenic mice and Cre was activated by injecting poly(I)-poly(C) or tamoxifen, respectively. Consistent with the phenotype of mice reconstituted with CXCR4-deficient fetal liver cells, conditional ablation of CXCR4 in mice caused a severe defect in B cell lymphopoiesis and a significant increase in the number of circulating LSK stem cells. In both studies, primitive hematopoietic stem cells (LSK cells) were retained in the bone marrow and became hyperproliferative following CXCR4 inactivation.33, 66 In the study by Nie et al.[66], elevated numbers of bone marrow and circulating LSK cell numbers as well as sustained hematopoiesis were observed for at least 8 months after tamoxifen induction of Cre and consequent excision of CXCR4. In contrast, Sugiyama et al.[33] reported a drastically reduced number of LSK cells in the bone marrow and impaired hematopoiesis 4 months after poly(I)-poly(C)-mediated induction of Cre and deletion of CXCR4. Nie et al.[66] suggested that these discrepancies in the maintenance of LSK cells and hematopoiesis following CXCR4 ablation might be related to the toxicity of poly(I)-poly(C) on LSK cells. Whatever the explanation, the data indicate that HSPCs can be retained in the BM microenvironment through a CXCR4-independent mechanism. Furthermore, the hyperproliferative state of LSK cells following CXCR4 ablation indicates that CXCR4 acts intrinsically in primitive HSPCs to enforce quiescence.[66]

Additional evidence for the critical role that CXCR4 plays in leukocyte trafficking has been obtained from patients with the genetic immunodeficiency syndrome WHIM (warts, hypogammaglobulinemia, infections, myelokathexis). Most cases of WHIM syndrome have been linked to autosomal dominant mutations in CXCR4, all of which truncate the C-terminal tail of CXCR4.[67–69] Multiple studies have demonstrated that loss of the intracellular tail of CXCR4 prevents its internalization and desensitization in response to CXCL12.[67, 68, 70] This loss of homologous desensitization leads to long-lasting activation of G-proteins and sustained functional activity of the chemokine receptor as evidenced by increased chemotaxis to CXCL12, F-actin polymerization, intracellular calcium release, and endothelial adhesion.[67, 68, 71] Some functional consequences of dysregulated CXCR4 signaling in WHIM syndrome is the failure of mature neutrophils to exit the bone marrow (myelokathexis) resulting in peripheral neutropenia, defects in B-cell development, reduced immune function, and other similarities to murine CXCL12 and CXCR4 deficient embryos.[72, 73]

The data obtained from WHIM patients and studies using genetically modified mice supporta model in which CXCL12 signaling through CXCR4 provides a keyretention signal for HSPCs in the bone marrow. Furthermore, multiple preclinical and clinical studies have shown that pharmacologic disruption of the CXCL12/CXCR4 axis using various CXCR4 modulators, including small molecule antagonists, peptide agonists, and anti-CXCR4

antibodies stimulate HSPC mobilization in a target-dependent manner.[74, 75] Below we will discuss some of these different types of CXCR inhibitors in greater detail.

Plerixafor

Plerixafor is a bicyclam derivative that reversibly competes with and inhibits CXCL12 binding to CXCR4. This compound was originally tested clinically as an agent for treatment of human immunodeficiency virus (HIV) infection by blocking the HIV entry into $CD4^+$ T cells.[76, 77] Initial studies in healthy volunteers showed good tolerance with minimal and reversible adverse effects.[78, 79] During those clinical trials, leukocytosis was noted after a single intravenous dose of plerixafor. Further investigation demonstrated that $CD34^+$ cells were one component of this generalized leukocytosis. Bioavailability after subcutaneous (sc) injection was 87% with a dose dependent increase in $CD34^+$ cells in the peripheral blood. No drug was detected after oral administration.

A single subcutaneous dose of plerixafor at 160–240 μg/kg resulted in a 6- to 10-fold increase in $CD34^+$ cell count starting 1 hour, peaking at 9 hours after injection and declining to baseline within 24 hours.[80] Pharmacokinetic studies of plerixafor have identified no differences between older and younger patients. Thus, no dose adjustment beyond that based on renal function is recommended in elderly patients. The use of plerixafor has not been studied in individuals aged <18 years. Most frequently noted adverse effects were transient pain and injection site erythema, headache, paresthesias, diarrhea, bloating and nausea. An additional study showed plerixafor could be combined with G-CSF to further increase the yield of $CD34^+$ cells. On the basis of these results plerixafor was further pursued for HSPC mobilization in the clinical setting.[81] Testing of plerixafor as a HIV drug was abandoned due to a lack of antiviral effect and the occurrence of asymptomatic premature ventricular contractions in two patients.

Phase I studies with plerixafor

Devine et al.[82] assessed safety and clinical effects of plerixafor in patients with NHL and multiple myeloma. Plerixafor caused a rapid and statistically significant increase in the total WBC and peripheral blood $CD34^+$ cell counts at both 4 and 6 hours following a single injection. The absolute number of circulating $CD34^+$ cells at 4 and 6 hours after plerixafor administration were higher in the 240 μg/kg group compared with the 160 μg/kg group with a maximum 6-fold increase in circulating $CD34^+$ cells.

Phase II studies with plerixafor

The initial clinical trial of plerixafor in human HSPC mobilization by Flomenberg et al.[83] were based on the hypothesis that the combination of plerixafor plus G-CSF would be superior to G-CSF alone and that the plerixafor plus G-CSF-mobilized cells would engraft at least as well as their G-CSF-mobilized counterparts. Patients with MM or NHL in first or second complete or partial remission were eligible for enrollment. Initially, patients were randomly assigned to receive plerixafor plus G-CSF or G-CSF alone as their mobilizing regimen, followed by a 2-week washout period and remobilization with the alternate regimen. Subsequently, randomization was discontinued and all patients received G-CSF alone as the initial mobilizing regimen. G-CSF mobilization consisted of the daily subcutaneous administration of the drug at a dose of 10 μg/kg and pheresis was begun on day 4 or 5 of administration. During plerixafor plus G-CSF mobilization, plerixafor was administered subcutaneously at a dose of 160–240 μg/kg beginning on day 4 or 5 followed 6 hours later by apheresis. The apheresis procedure was limited to 3 blood volumes per day. Patients treated with plerixafor plus G-CSF mobilized more $CD34^+$ cells per leukapheresis, required fewer leukaphereses to reach the target $CD34^+$ cell count, and mobilized higher total $CD34^+$ cell yield. Fifty-six percent of patients mobilized with plerixafor plus G-CSF

yielded at least 2×10^6 $CD34^+$ cells/kg recipient body weight after one leukapheresis and 100% after two leukaphereses (compared to 64% with G-CSF alone). In addition, all 9 of 25 patients who failed to yield the minimum $CD34^+$ cells/kg after four leukaphereses with G-CSF alone were successfully remobilized with plerixafor plus G-CSF.

Plerixafor given with G-CSF has been shown to mobilize $CD34^+$ cells in NHL, MM, and Hodgkin's disease (HD) patients who could not collect sufficient cells for autologous transplant following other mobilization regimens. These poor mobilizers were excluded from company-sponsored trials, but have been included in a plerixafor Single Patient Use protocol, referred to as a Compassionate Use Protocol (CUP). A cohort of 115 data-audited poor mobilizers in CUP were assessed, with the objective being to collect 2×10^6 $CD34^+$ cells per kg following plerixafor plus G-CSF mobilization. The rates of successful $CD34^+$ cell collection were similar for patients who previously failed chemotherapy mobilization or cytokine-only mobilization: NHL—60.3%, MM—71.4% and HD—76.5%. Following transplant, median times to neutrophil and PLT engraftment were 11 days and 18 days, respectively. Engraftment was durable.[84]

Several additional studies in heavily pretreated patients with NHL, Hodgkin's lymphoma and multiple myeloma had similar results, confirming that plerixafor is a well-tolerated and effective mobilizing agent.[85–87] In addition, these studies support the use of plerixafor plus G-CSF in those patients who have failed initial attempts at mobilization with G-CSF or chemotherapy plus G-CSF. Pharmacokinetic and pharmacodynamic studies of plerixafor in patients with lymphoma and multiple myeloma were comparable to those in healthy volunteers, supporting the current recommended dosing of plerixafor (240 mg/kg subcutaneous).[88] Plerixafor was also tested in combination with chemotherapy and G-CSF for HSPC mobilization in patients with multiple myeloma and NHL. Addition of plerixafor to chemo-mobilization accelerated the rate of increase in $CD34^+$ cells.[89] However, the use of chemotherapy in plerixafor-based mobilization regimens is unlikely to be justified since adequate yield of HSPCs can be collected without subjecting the patient to the toxicities of chemotherapy. A retrospective study comparing plerixafor plus G-CSF to a historical cohort mobilized with chemotherapy plus G-CSF showed similar cost but more predictable days of leukapheresis and less hospitalization in the plerixafor plus G-CSF group.[90]

Phase III studies with plerixafor

Two phase III, multicenter, randomized, double-blind, placebo-controlled studies compared plerixafor plus G-CSF to G-CSF alone for mobilization of stem cells in patients with MM and NHL.[91, 92] Patients received G-CSF at 10 μg/kg/day for 4 days and on the evening of the fourth day they received either plerixafor at 240 μg/kg sc or placebo. The leukaphereses were started on day 5, after the morning dose of G-CSF, and continued until $CD34^+$ cell yield was $\geq 5 \times 10^6$/kg (NHL) or $\geq 6 \times 10^6$/kg (MM) or a total of 4 leukaphereses. Patients continued receiving their morning dose of G-CSF and evening dose of study drug until collection was completed. Patients who failed to collect $\geq 2 \times 10^6$ $CD34^+$ cells/kg with G-CSF alone were eligible for rescue with plerixafor plus G-CSF.

In the NHL trial (N=298), 59% of patients in the plerixafor arm reached the primary endpoint of 5×10^6 $CD34^+$ cells/kg compared to 20% in the placebo arm ($P < 0.001$). Importantly, 130/150 (87%) of patients in the plerixafor arm and only 70/148 (47%) in the placebo arm reached the secondary endpoint of at least 2×10^6 $CD34^+$ cells/kg ($P < 0.001$). Patients failing to yield at least 2×10^6 $CD34^+$ cells/kg were eligible for 'rescue' mobilization with plerixafor plus G-CSF. After rescue therapy, 33/52 patients from the placebo arm, and 4/10 patients from the plerixafor arm had successful remobilization.[93] A total of 35% of patients in the placebo arm failed the mobilization process versus 7% of patients in the plerixafor arm. In the multiple myeloma trial (N=302), the primary endpoint

of 6×10^6 $CD34^+$ cells/kg was met in 72% of patients from the plerixafor group and only 34% from the placebo group ($P < 0.001$).

In both the MM and NHL studies plerixafor was well tolerated with minimal side-effects. Patients receiving transplants had rapid hematopoietic recovery and durable grafts across all treatment groups.[91, 92] On the basis of the results of these two phase III randomized placebo controlled trials, plerixafor was FDA-approved, in combination with G-CSF, for HSPC mobilization in patients with NHL and multiple myeloma in December 2008.

Use of plerixafor in allogeneic transplantation

Plerixafor was tested for HSPC mobilization in allogeneic transplantation.[94] Normal sibling donors were mobilized with plerixafor 240 μg/kg subcutaneously and underwent leukapheresis 4 hours later. The FDA mandated for the first 8 patients that we also collect, after a 10-day washout period, a G-CSF mobilized backup product. Two-thirds of the donors mobilized with plerixafor alone yielded the minimum goal of 2×10^6 $CD34^+$ cells/kg recipient body weight after a single leukapheresis (100% after two collections; 20L/apheresis). Allografts mobilized with plerixafor contained less $CD34^+$ cells and higher numbers of T, B and NK cells compared to G-CSF mobilized allografts (Table 1). With a median follow-up of 277 days after allo-transplantation, engraftment was prompt, acute GVHD (grades 2–4) occurred in 35% of patients, and no unexpected adverse events were observed. It is possible that the allografts would have contained higher yields of $CD34^+$ cells if leukapheresis were started 6–10 hours after plerixafor, which is considered the peak of mobilization in both patients and normal allogeneic donors. Several ongoing studies are testing different routes of administration (intravenous vs. subcutaneous), doses, and schedules of plerixafor alone or in combination with G-CSF for HSPC mobilization (Table 2).

The pharmacokinetics of subcutaneous plerixafor requires that it be administered the night before leukapheresis so that the morning collection would correspond to the peak of the circulating HSPCs. Such administration is associated with inconvenience and additional cost. To improve the kinetics of mobilization, intravenous plerixafor is being tested in both autologous and allogeneic HSPC transplant clinical trials (Table 1). In our Phase I allogeneic transplant trial, twenty-one healthy donors were initially mobilized with increasing doses of intravenous plerixafor (80, 160, 240, 320, 400 or 480 μg/kg).[95] After 4 days of drug clearance, the same donors were then mobilized with a single subcutaneous dose of 240 μg/kg plerixafor followed 4 hours later by leukapheresis.

Peak numbers of circulating $CD34^+$ cells were observed 4–6 hours after intravenous dosing (vs. 6–9 hours after subcutaneous dosing) and donors given 240 μg/kg intravenous plerixafor, had higher peak levels of $CD34^+$ cells/μL compared to the same donors who received 240 μg/kg subcutaneous plerixafor. There was a clear dose-response relationship of intravenous plerixafor on mobilization of $CD34^+$ HSPCs, with the 320 μg/kg dose yielding a maximum 8-fold increase in circulating $CD34^+$ cells at 4 hours after injection. We also noted that intravenous dosing (especially doses > 240 μg/kg) resulted in prolonged mobilization of $CD34^+$ cells such that levels approached 20 $CD34^+$ cells/μL at 24 hours after intravenous dosing.

In our Phase II study, twenty-eight sibling donors were treated with plerixafor at a dose of 320 μg/kg by intravenous injection, followed 4 hours later by leukapheresis. Successful mobilization was defined as a minimum leukapheresis yield of $\geq 2.0 \times 10^6$/kg $CD34^+$ cell/kg actual recipient body weight. Six of twenty-three evaluable donors (26%) did not achieve the minimum cell dose of 2×10^6 $CD34^+$ cells/kg in a single 20 liter leukapheresis procedure.[96] This mobilization failure rate of 26% (6/23) with 320 μg/kg intravenous

plerixafor is similar to the failure rate of 33% (15/45) we observe following administration of 240 μg/kg subcutaneous plerixafor. Four of the six patients who failed to collect ≥ 2.0 × 10^6 $CD34^+$ cells/kg recipient body weight after their first leukapheresis procedure met goal after a second mobilization and collection procedure. In both subcutaneous and intravenous plerixafor studies only 3/68 normal allogeneic donors (4.4%) failed to mobilize > 2 × 10^6 $CD34^+$ cells/kg after two 20 liter apheresis procedures.

Plerixafor and G-CSF mobilize phenotypically different $CD34^+$ cell subsets

While evaluating the $CD34^+$ cells obtained from the 8 healthy donors mobilized sequentially with plerixafor and G-CSF in our allogeneic transplant trial, we found that plerixafor mobilized a population of $CD34^{dim}$ cells which were present on average in nearly 5-fold higher numbers compared to the G-CSF-mobilized $CD34^+$ cells.[97] Previous studies by others have demonstrated that human $CD34^+$ cells can be divided into at least three distinct subsets based on their cell surface expression of CD45RA and CD123 (IL-3Rα): (i.) $CD34^+CD45RA^-CD123^{+/-}$ cells containing common myeloid progenitors (CMPs), megakaryocyte/erythrocyte progenitors (MEPs) and more primitive HSPCs, (ii.) $CD34^+CD45RA^+CD123^{+/-}$ cells containing more committed granulocyte/macrophage (GMP) and lymphoid progenitors, and (iii.) $CD34^{dim}CD45RA^+CD123^{hi}$ cells.[98, 99] Strikingly, we observed that plerixafor preferentially mobilized $CD45RA^+CD123^{hi}$ cells, with the relative frequency of this $CD34^{dim}$ population increasing from 2% before treatment to 18% at 4 hours after the administration of the drug. In contrast, G-CSF mobilized grafts were enriched for $CD34^+CD45RA^-CD123^{+/-}$ cells (86% of the $CD34^+$ cells) and contained less than 1% of the $CD34^{dim}CD45RA^+CD123^{hi}$ cells. Further analyses by flow cytometry determined that the $CD34^{dim}$ population represents a plasmacytoid pro-DC2 (for progenitor of pre-dendritic cell type 2) progenitor compartment as indicated by their $CD45RA^+CD123^{hi}BDCA\text{-}2^+BDCA\text{-}4^+CD36^+CD4^{dim}CD25^-CD13^-$ phenotype. Of interest, two of the key molecules responsible for stem cell homing, retention and trafficking, CXCR4 and VLA-4, were significantly over-expressed in the $CD34^{dim}CD45RA^+$ subset compared to the $CD34^+CD45RA^-$ and $CD34^+CD45RA^+$ cells.[100]

A summary of the major differences between G-CSF and plerixafor-mobilized stem cell grafts is shown in in Table 1. These differences were recently reviewed by Fruehauf et al.[101] and clearly indicate that the composition of G-CSF and plerixafor-mobilized grafts are significantly different. What impact these different types of grafts have on the engraftment and function of HSPCs after hematopoietic stem cell transplantation remains largely unknown.

Alternative drugs in development targeting the CXCL12/CXCR4 axis

The success of plerixafor significantly addresses a number of the limitations involved with the use of G-CSF in hematopoietic stem cell mobilization. However, nearly one-third of healthy donors mobilized with plerixafor alone require more than one apheresis to obtain the minimal number of $CD34^+$ cells (≥2.0 × 10^6 $CD34^+$ cells/kg) necessary for transplantation. Furthermore, plerixafor was withdrawn from trials involving HIV patients due to its lack of oral bioavailability and cardiotoxicity. Importantly, the cardiac-related side effect of plerixafor was not a result of the compound's ability to block CXCR4 function, but rather due to its presumed structural capacity for encapsulating metals. Because of these limitations, efforts to discover and develop potent and orally available inhibitors of the CXCR4/CXCL12 axis continue. Below we will discuss the status of some lead drug candidates that target the CXCR4/CXCL12 axis.

POL6326 (Polyphor) is a selective and reversible CXCR4 inhibitor belonging to a novel drug class based on Polyphor's proprietary β-hairpin Protein Epitope Mimetics (PEM)

Technology.[102] The parent compound to POL6326, POL3026, was designed and optimized starting from the naturally occurring CXCR4 inhibitor polyphemusin-II.[103, 104] The design involved incorporating residues from a truncated polyphemusin-II analogue (TC14011) into a macrocyclic template-bound β-hairpin mimetic. POL3026 binds at least 50–100-fold better to CXCR4 than does plerixafor. POL6326 was synthesized from POL3026 to improve its pharmacological properties while maintaining or improving potency and selectivity for CXCR4. A single injection of POL6326 to mice produces an 11–12-fold increase in circulating progenitor cells with a peak at 2–4 hours post-dosing.[102] A Phase I clinical trial involving administration of POL6326 to 74 healthy volunteers was successfully completed in the UK in July 2008. A Phase IIa, proof of concept study to determine the degree of mobilization of $CD34^+$ cells following intravenous administration of POL6326 in patients with multiple myeloma is ongoing (ClinicalTrials.gov Identifier NCT01105403).[105]

BKT-140 (4F-benzoyl-TN14003; Biokine Therapeutics) is a 14-residue polypeptide antagonist of CXCR4 that was also downsized from polyphemusin-II.[106] BKT-140 binds CXCR4 with an affinity of approximately 1 nM and a single injection of the drug alone results in mobilization of murine CFU-Cs (> 6-fold increase over baseline) that exhibit long-term hematopoietic reconstituting activity.[107, 108] Peripheral blood CFU-C levels peaked 2 hours after BKT-140 administration and returned to baseline by 24 hours. BKT-140 and G-CSF act synergistically to mobilize WBC and HSPCs. Comparative studies between BKT-140 and plerixafor suggest that the two compounds bind and inhibit CXCR4 via different mechanisms.[108–110] BKT-140 functions as an inverse agonist and binds CXCR4 residues in extracellular domains and regions of the hydrophobic core proximal to the cell surface. In contrast, plerixafor functions as an antagonist[111] or weak partial agonist[109, 110] and binds amino acids in the central hydrophobic core of CXCR4. Interestingly BKT140, but not plerixafor, selectively, specifically and rapidly stimulates human leukemia and myeloma cell death in vitro and in vivo.[112] A Phase I/IIA, non-randomized, open label, single dose, dose-escalation, and safety study of BKT140 in patients with MM is now open (ClinicalTrials.gov Identifier NCT01010880).[113]

NOX-A12 (NOXXON Pharma) is a L-enantiomeric RNA oligonucleotide (Spiegelmer) that binds and neutralizes CXCL12.[114] Spiegelmers are mirror-image nuclease-resistant ribonucleotides that bind and inhibit target molecules in a manner conceptually similar to antibodies.[115, 116] The 45 nucleotide long NOX-A12 binds CXCL12 with an affinity of less than 1 nM and has an IC_{50} value of 300 pM in a Jurkat cell migration assay.[114] Preclinical studies in the mouse and monkey indicate that NOX-A12 induces a dose-dependent mobilization of murine CFU-Cs and a >3-fold reversible mobilization of monkey WBCs with peak numbers of circulating cells being maintained between 3 and 8 hours post-injection. A first-in-man clinical trial with NOX-A12 was successfully completed in May 2010 (ClinicalTrials.gov Identifier NCT00976378) and a multiple-dose Phase I trial started in August 2010 (ClinicalTrials.gov Identifier NCT01194934). This second clinical study aims to determine the safety, tolerability, pharmacokinetics, and pharmacodynamics of repeated intravenous doses of NOX-A12 alone and in combination with G-CSF in healthy subjects.

TG-0054 (Taigen Biotechnology) is a CXCR4 antagonist of undisclosed structure. The compound blocks CXCL12 binding to CXCR4 with an IC_{50} value of 10 nM.[117] In Phase I study, the drug exhibited a favorable safety and pharmacokinetic profile in healthy subjects.[118] A single dose of TG-0054 at 1.12–4.40 mg/kg resulted in a 3- to 14-fold increase in $CD34^+$ cell count starting at 2 hours, peaking at 4–6 hours after injection and declining to baseline within 24 hours. The mean number of circulating $CD34^+$ cells averaged approximately 30 cells/μL at peak mobilization.[118] A phase II study to evaluate the safety, pharmacokinetics, and hematopoietic stem cell mobilization of TG-0054 in

patients with multiple myeloma, non-Hodgkin lymphoma or Hodgkin disease is currently open (ClinicalTrials.gov Identifier NCT01018979).

MDX-1338 (BMS-936564; Medarex/Bristol-Myers Squibb) is a fully human antibody that targets CXCR4.[119, 120] Since MDX-1338 is an IgG_4 antibody, it lacks complement dependent cytotoxicity (CDC) activity and antibody dependent cell mediated cytotoxicity (ADCC) activity. Preclinical studies have shown that MDX-1338 binds to CXCR4-expressing cells with low nanomolar affinity, blocks CXCL12 ligand binding to CXCR4 expressing cells and inhibits CXCL12 induced migration and calcium flux with low nanomolar EC_{50} values.[120] In addition, MDX-1338 also reduced tumor growth in acute myelogenous leukemia and lymphoma xenograft models.[119] A Phase I, dose escalation study of MDX-1338 as a monotherapy with chemotherapy is expected to enroll up to 34 patients with relapsed/refractory AML (ClinicalTrials.gov Identifier NCT01120457). Moreover, this trial is designed to establish and evaluate the safety, tolerability and maximum tolerated dose, as well as preliminary pharmacodynamics and efficacy of MDX-1338.

CXCR4 pepducins (Anchor Therapeutics) are highly stable synthetic lipopeptide pharmacophores that modulate CXCR4 activity from inside the cell membrane.[121] The CXCR4 receptor compounds are derived from the intracellular loops of CXCR4 and act as antagonists of CXCR4 G-protein signaling.[121] The pepducins PZ-218 and PZ-305 are based on the first intracellular loop of CXCR4, while PZ-210 targets the third intracellular loop.[122] Preclinical studies have shown that all three of these CXCR4 pepducins inhibit CXCL12-mediated calcium flux and chemotaxis of human neutrophils.[122] Furthermore, CXCR4 pepducins mobilized murine HSPCs that exhibit long-term hematopoietic reconstituting activity with efficacy similar to plerixafor.

ALX-0651 (Ablynx) is an anti-CXCR4 Nanobody® which is being developed for hematopoietic stem cell mobilization.[123] Nanobodies® are a novel class of therapeutic single-domain proteins derived from the smallest antibody-binding fragment of camelid heavy chain only antibodies.[124] Their discovery dates back to the early 1990s when Hamers-Casterman et al.[125] found that sera of camels, dromedaries and llamas contain fully functional antibodies that lack light chains. These heavy-chain antibodies contain a single variable domain (VHH) and two constant domains (C_H2 and C_H3). The VHH domain is a perfectly stable polypeptide harboring the full antigen-binding capacity of the original heavy-chain antibody and forms the basis of the Nanobody technology. Ablynx reported the successful generation of two types of Nanobodies against CXCR4: highly potent antagonistic Nanobodies, as well as anti-CXCR4 Nanobodies with inverse agonist function.[123] Furthermore, they report that a single, intravenous administration of anti-CXCR4 Nanobody resulted in rapid mobilization of stem cells in a pre-clinical animal model. ALX-0651 is reportedly a biparatopic Nanobody directed against two different epitopes of CXCR4.

AMD070 (Genzyme Corp.) is an orally bioavailable non-cyclam CXCR4 antagonist derived from plerixafor.[126] Administration of AMD070 to HIV-1 infected patients suppressed the replication of X4 and dual/mixed strains of viruses.127, 128 Leukocytosis followed AMD070 administration in all subjects, ranging from 1.3-fold (50-mg single dose subject) to 2.9-fold (400-mg single dose subject) above baseline, with a peak between 2 and 4 hours after injection.[128] Similar kinetics and magnitudes of mobilization where observed across al leukocyte subsets tested, which included neutrophils, lymphocytes, monocytes, basophils, eosinophils, $CD4^+$ T cells, and $CD34^+$ stem cells. AMD070 was slowly eliminated, with a terminal elimination half-life of 11 to 16 hours.[128] Unfortunately, the FDA had to place

AMD070 on clinical hold due to liver histology changes in long-term preclinical toxicity studies.

GSK812397 (GlaxoSmithKline) is a novel, orally bioavailable noncompetitive CXCR4 antagonist.[129] The compound was developed from a structure-avidity study aimed at improving the antiviral properties of AMD070 through iterative structural modifications. Preclinical studies have shown that GSK812397 binds to CXCR4-expressing cells with low nanomolar affinity, blocks infection of host cells by X4-utilizing HIV-1 viruses and inhibits CXCL12 induced migration and calcium flux with low nanomolar EC_{50} values.[129] The bioavailability of GSK812397 was similar across several species, including rat (21%), dog (21%), and monkey (17%). Furthermore, the half-life was greater than 12 h in both dog and monkey. No data has been published on the effects of GSK812397 on WBC and HSPC mobilization.

KRH-3955 (Kureha Chemical Industries) is an orally bioavailable, non-cyclam, non-peptide small molecule CXCR4 antagonist designed from KRH-1636.[130] KRH-1636 was discovered through screening more than 1000 compounds from the Kureha Corp chemical library.[131] Preclinical studies have shown that KRH-3955 binds to CXCR4-expressing cells with low nanomolar affinity, blocks infection of host cells by X4-utilizing HIV-1 viruses and inhibits CXCL12 induced calcium flux.[130] The compound shows an oral bioavailability of 26% in rats and an intravenous half-life of 99 hours. The authors suggest that the long half-life of KRH-3955 is likely caused by its accumulation in tissues which may be disadvantageous in terms of toxicity.[130] No data has been published on the effects of KRH-3955 on WBC and HSPC mobilization.

FC131 is a cyclopentapeptide [c(Gly_1-D-Tyr_2-Arg_3-Arg_4-Nal_5); Nal is 2-naphthylalanine] developed by molecular size reduction of the 14-residue T140 CXCR4 antagonist.[132] The size reduction (from T140 to FC131) was done based on the identification of the four bioactive amino acid residues Arg2, Nal3, Tyr5, and Arg14 of the T140 molecule. Fuji et al[132] reported that FC131 exerts anti-HIV activity as well as CXCR4 antagonist activity equipotent to T140. Several analogues of FC131 have been reported, but all show lower potency. No data has been published on the effects of KRH-3955 on WBC and HSPC mobilization.

WZ811 (N1,N4-di-2-pyridinyl-1,4-benzenedimethanamine) is the lead candidate drug in a new class of CXCR4 antagonists that contain two aromatic amine moieties connected by a para-xylylene group.[133] WZ811 is a potent inhibitor of binding of an SDF-1 peptide mimic to CXCR4 (EC_{50} = 0.3 nM), prevents CXCL12-mediated modulation of cAMP (EC_{50} = 1.2 nM) in cells and blocks CXCL12-induced Matrigel invasion by MDA-MB-231 human breast adenocarcinoma cells (EC_{50} = 5.2 nM).[133] Studies to improve the pharmacokinetic profile of WZ811 led to the discovery of MSX-122 (WZ40). MSX-122 is an orally bioavailable small molecule that was produced by Metastatix, Inc. There is a report that MSX-122 induces leukocytosis in monkeys beginning 4–6 hours after administration with a peak total white blood cell count at 12–18 hours that was 1.5–2 fold higher than baseline.[134] A phase I trial to determine the safety and pharmacokinetics of oral MSX-122 in patients with refractory metastatic or locally advanced solid tumors was suspended in June 2008 (ClinicalTrials.gov Identifier NCT00591682). No data has been published on the effects of WZ811 or MSX-122 on HSPC mobilization.

HSPC mobilizing agents that target VLA-4

Integrins

Integrins are a large family of transmembrane glycoproteins that mediate cell-cell and cell-matrix interaction and communication.[135–137] All integrins are non-covalently linked, heterodimeric molecules containing a α and a β subunit. Each subunit contains a small cytoplasmic domain, a single transmembrane spanning region, and a large extracellular domain.138, 139 In vertebrates, 18 α and 8 β subunit genes have been identified and can combine to form 24 different integrin receptors, which vary in their ligand specificity, expression and signaling.[140] Integrin β1 (CD29) associates with at least 12 different α subunits (α1- α11 and αV) and forms the largest integrin subfamily. The integrin chains α4, α5, α6 and α9 are expressed with β1 on HSPCs and play important roles in hematopoietic stem cell migration, homing and engraftment.[141, 142]

Integrins mediate a wide variety of physiological processes including adhesion, migration, survival, and differentiation of cells. These functions are largely controlled by the strength of the ligand-binding affinity of the integrin. Integrin affinity for extracellular ligands is regulated through receptor clustering by multivalent ligands and/or conformational changes in the integrins.[143, 144] Extensive research has shown that integrins can adopt at least three major conformational states: (i.) inactive (low affinity), (ii.) primed or activated (high affinity) and (iii.) ligand occupied.[144] Integrin activity is regulated through both the interactions of intracellular proteins with integrin cytoplasmic domains (inside-out activation) or by interactions with extracellular ligands (outside-in activation).[144–146]

The α_4 integrin family

The α_4 integrin family consists of two integrins, $\alpha_4\beta_1$ (VLA-4)[147] and $\alpha_4\beta_7$[148]. Like most integrins, $\alpha_4\beta_1$ normally exists in the inactive (low affinity) state. Although the precise mechanism of activation in vivo is not clear, VLA-4 can be activated in vitro in a variety of ways, including by ligand[149], divalent cations[149–151], monoclonal antibodies[149–151], CXCL12[152–155], IL-3[156], c-kit ligand[156, 157], GM-CSF[156], stem cell factor[158] and phorbol esters. Once activated, the primary counterligands for VLA-4 are vascular cell adhesion molecule-1 (VCAM-1; CD106)[159, 160] and the alternatively spliced connecting segment 1 (CS-1) domain of fibronectin[161, 162] found in the extracellular matrix.[163] VLA-4 also binds to osteopontin, Mucosal Addressin Cell Adhesion Molecule 1 (MadCAM-1), thrombospondin, ICAM-4, and ADAM family members; however, the biological significance of these interactions is less clear.[140] The primary ligand for $\alpha_4\beta_7$ integrin is the immunoglobulin adhesion ligand MAdCAM-1.[164, 165] Both VCAM-1 and MadCAM are normally expressed in the gastrointestinal tract, however VCAM expression extends into peripheral organs[166, 167], while MadCAM expression is confined to organs of the gut.[166, 168, 169]

VLA-4 is constitutively expressed on most leukocytes, including monocytes, lymphocytes, eosinophils, basophils, and $CD34^+$ hematopoietic precursorcells. The expression of VLA-4 on $CD34^+$ HSPCs is known to be upregulated by IL-3 and SCF[170] and down-regulated by G-CSF[170–175]. Studies by Lichterfeld et al.[175] showed that the affinity and avidity of VLA-4 for a soluble VCAM1-Ig fusion protein was significantly reduced on circulating $CD34^+$ after treatment with G-CSF in comparison to $CD34^+$ cells from steady-state BM. Furthermore, the authors found that the number of circulating $CD34^+$ cells following G-CSF administration was inversely related to the number of $CD34^+$/VCAM1-Ig^+ cells in the periphery, indicating that a low activation state of VLA-4 on $CD34^+$ cells during G-CSF mobilization is associated with a higher number of circulating HSPCs.[175] These

observations suggest that both reduced VLA-4-expression and reduced VLA-4 avidity are associated with mobilization of $CD34^+$ cells by G-CSF.

Genetic ablation of VLA-4 in mice

Both the α_4 and β1 subunits of VLA-4 have been inactivated in mice. Table 3 summarizes the phenotypes of mice following genetic ablation of the α_4 or β1 integrin subunits, with a particular emphasis on the impact each deletion has on HSPC formation, maintenance, distribution and migration. Since lack of α_4 or β1 integrin is embryonic lethal due to nonhematological defects[176–178], somatic chimeric mice were generated by injecting α4 or β1 integrin-deficient embryonic stem (ES) cells into wild-type (wt) or recombination activating gene (RAG)-deficient blastocysts. Although $\beta_1^{-/-}$ HSPCs formed and were capable of differentiating into multiple cell lineages in vitro, they failed to migrate to the fetal liver (as well as adult sites of hematopoiesis) and establish hematopoiesis in the $\beta_1^{-/-}$/wt chimeras.[178–180] In contrast, α4 integrins were not essential for HSPC colonization of the fetal liver in the somatic chimeric mice.[181, 182] These data suggest that α subunits other than α4 integrin mediate the migration of integrin $\beta1^+$ HSPCs to sites of hematopoiesis during fetal development. However, α4 integrin was absolutely required for hematopoietic maintenance and development, as the $\alpha4^{-/-}$/wt chimeras were incapable of completing erythroid, myeloid and lymphoid (B and T cell) differentiation in adult mice.[181, 182]

The Cre/loxP system of conditional gene ablation, where one or more critical exons is flanked by loxP (floxed) sites to allow cell-specific knockout, has been used to examine the role of α_4 and β1 integrins on adult BM-derived HSPCs. In contrast to the striking inability of α_4 or β1 integrin-deficient HSPCs to be maintained and differentiate in somatic chimeric mice, conditional deletion of α_4 or β1 integrin in adult mice caused much less severe perturbations in hematopoiesis. Genetic ablation of floxed α_4 integrin in both hematopoietic and nonhematopoietic compartments using Mx-Cre induced a 8-fold increase in the number of circulating HSPCs (CFU-Cs) compared with $\alpha_4^{+/+}$ controls at 2 weeks after deletion.[183] Interestingly, the circulating levels of CFU-Cs remained significantly elevated for at least 50 weeks after α_4 integrin ablation and were accompanied by sequestration of CFU-Cs in the spleen. Similar long-term release of CFU-Cs into the peripheral blood was reported following deletion of floxed α_4 integrin in hematopoietic and endothelial cells using Tie2Cre.[184]

To more clearly determine whether the alterations in progenitor biodistrubution were solely caused by the loss of α_4 integrin on hematopoietic cells, BM cells from wild type ($\alpha_4^{+/+}$) and α_4-deficient animals were injected into lethally irradiated recipients and their homing, distribution and hematopoietic reconstitution potential were evaluated. Cells lacking α_4 integrins displayed impaired homing to the BM, increased numbers of circulating CFU-Cs and a competitive disadvantage in both short-term and long-term hematopoietic reconstitution compared to normal competitors.[183–185] These data suggest that expression of α_4 integrin on HSPCs plays an important role in their retention within the BM microenvironment. Of note, different results were obtained following transplantation of wild-type or α_4-deficient cells isolated from the tissues of embryonic day 12.5 (E12.5) embryos into lethally irradiated adult recipients.[186] Here, α_4 integrin-deficient cells displayedsimilar hematopoietic engraftment, multilineage differentiation, and competitive repopulation capacity compared to $\alpha_4^{+/+}$ cells. The reason for this discrepancy between the two transplant models remains unclear.

Somewhat conflicting results have also been reported following genetic ablation of β1 integrin in adult BM-derived HSPCs. Initial experiments generated β1 integrin deficient HSPCs in vitro by transducing BM cells of mice homozygous for a floxed β_1 integrin gene with a Cre recombinase retrovirus. Transplantation of these ex-vivo-generated $\beta_1^{-/-}$ HSPCs

into lethally irradiated recipients failed to provide radioprotection and BM engraftment.[179] In contrast, no significant alterations were reported in the number, distribution, or function of β_1 integrin-deficient HSPCs when they were generated in vivo using polyIC and the MxCre/loxP system.[187] A similar lack of overt phenotype was reported when both β_1 and $\beta_{7,}$ which are the only known partners of α4 integrin, were conditionally ablated in the hematopoietic system.[188] However, a 8-fold increase in the number of circulating HSPCs was reported following genetic ablation of β_1 in both the hematopoietic and non-hematopoietic systems of β_7-deficient mice.[188] This increase in circulating HSPCs is similar to the 8-fold increase in circulating CFU-Cs observed following genetic ablation of α_4,[183] and suggests that loss of β_1 integrin function on nonhematopoietic cells might contribute to the release of HSPCs from the bone marrow to the peripheral blood.

Mobilization of HSPCs by anti-α_4 integrin antibodies

Studies with antibodies have also suggested that the VLA-4 receptor plays an important role during the migration of HSPCs to the BM and their retention in the BM. Treatment of donor BM cells with an α_4 integrin antibody before injection into lethally irradiated recipients inhibits their homing to the femurs of recipient mice.[189, 190] This inhibition of BM homing was accompanied by an increase in the number of HSPCs in the peripheral blood and spleen. Since similar results were obtained by pre-treatment of recipients with an antibody to VCAM-1,[189] a primary ligand for VLA-4, the data indicate that the VLA-4/VCAM-1 axis plays an important role in the initial stages of HSPC homing to the BM.

Additional studies have shown that in vivo administration of antibodies to VLA-4 increases the number of circulating HSPCs in mice, primates, and humans. The blockade of α_4 integrin in mice[189–194] and nonhuman primates[191, 193, 195] results in rapid (<8 hours) and prolonged (> 10 days) mobilization of HSPCs. Both CFU-C assays and transplantation experiments definitively demonstrated that the anti-α4 integrin antibody mobilized committed progenitors and long-term repopulating cells. A functional kit receptor is required for HSPC mobilization by anti-α4 integrin antibody[194] and either additive or synergistic mobilization of HSPCs was observed when the antibody was combined with G-CSF[193, 195], plerixafor[191], kit ligand[193] and/or Flt3-ligand[193]. This enhanced mobilization of HSPCs following addition of G-CSF, plerixafor and/or Flt3-ligand to the anti-α4 integrin antibody therapy is likely mediated via their disruption of CXCR4/CXCL12 signaling in the bone marrow.[196] Finally, although no significant mobilization is observed following blockade of β2 integrin alone, synergistic mobilization of murine and primate HSPCs occurs following concurrent inhibition of β2 integrin and blockade of VLA-4 by administration of an anti-α_4 integrin antibody.[192]

Elevated circulating levels of HSPCs are also observed following treatment of multiple sclerosis(MS) patients with an anti-VLA4 antibody. Natalizumab (Tysabri;Biogen/Idec, Cambridge, MA) is a recombinant humanized neutralizing IgG4 monoclonal antibody that binds to the α_4 subunit of the α_4 β_1 (VLA-4) and α_4 β_7 integrins. Natalizumab is approved by the FDA for the treatment of Crohn's disease and relapsing MS and is postulated to function in these conditions by inhibiting the transmigration of leukocytes through the blood-brain barrier (MS) and intestines (Crohn's). Similar to mice and nonhuman primates following anti-α_4 integrin antibody administration, natalizumab-treated MS patients display a rapid and sustained increase in circulating HSPCs.[197–199] Both $CD34^+$ cell counts and CFU-Cs were significantly increased within 24 hours after a single injection of natalizumab to previously untreated MS patients. Overall, the number of circulating $CD34^+$ cells/μL blood increased 3- to 5-fold during the first 72 hours after treatment and remained elevated at these levels (8–10 CD34+ cells/μL) for at least a month after natalizumab injection.[197–199] Somewhat surprisingly, repeated administration of the antibody failed to mobilize additional $CD34^+$ cells. To put the magnitude of $CD34^+$ cell mobilization by

natalizumab in context with other mobilizing regimens, the number of circulating $CD34^+$ cells in natalizumab-treated MS patients are approximately one-sixth and one-third of those observed in G-CSF- and plerixafor-treated healthy donors, respectively.[13, 94, 197–199] Finally, limited phenotyping studies on the $CD34^+$ cells mobilized by natalizumab indicated that they were primarily quiescent (>90% in G_0), failed to transmigrate towards SDF-1, and belonged to the subset of more committed progenitors co-expressing CD38 and enriched for erythroid BFU-E.[197–199]

Although natalizumab has shown clinical efficacy and provided validation for the involvement of the α_4 integrin pathway in autoimmune and inflammatory conditions, its use as a clinical stem cell mobilizing agent in normal healthy donors is not justifiable because of its prolonged immune-modulating effects. More specifically, in addition to the prolonged mobilization of $CD34^+$ HSPCs, natalizumab also produces a lymphocytosis that can be sustained for at least a month after antibody administration.[200–204] This prolonged disruption of normal lymphocyte trafficking poses unacceptable risks to a healthy individual. Indeed, MS patients undergoing natalizumab monotherapy are at risk of developing progressive multifocal leukoencephalopathy (PML).[205, 206] PML is a rare and often fatal demyelinating disease of the central nervous system caused by infection of oligodendrocytes and astrocytes by the John Cunningham polyomavirus. As of May 2010, a total of 49 cases of PML were reported among approximately 67,700 natalizumab-treated patients worldwide.[205]

Clinical trials with small molecule α_4 integrin antagonists

Because natalizumab has shown clinical efficacy in the treatment of MS and Crohn's disease, the development of small molecule VLA-4 antagonists is seen as a promising pharmaceutical approach to a novel class of therapeutic agents.[207–210] Small molecule antagonist research in the α_4 integrin area has been ongoing for nearly 20 years and over 250 patents describing α_4 integrin antagonists as potential anti-inflammatory agents have been published.[207] The small molecule antagonists of α_4 integrin that have progressed into clinical trials are summarized in Table 4 and can be classified into two major structures: (1) the tripeptide motif of leucine–aspartic acid–valine (LDV) mimics, whose sequence is responsible for VLA-4 interaction with fibronectin or (2) N-acylphenylalanine-based compounds.[208–210] In general, the LDV-based antagonists show high potency and selectivity for $\alpha_4\beta_1$ (VLA-4) whereas the N-acylphenylalanine derivatives exhibit dual inhibitory activity for both $\alpha_4\beta_1$ and $\alpha_4\beta_7$. Early studies with the α_4 integrin antagonists suffered from poor bioavailability and a short half-life. More recent trials with orally active new generation antagonists like SB-683699/Firategrast[208, 211], AJM-300[212, 213], and Compound 14e[214] suggest that these compounds exhibit more favorable pharmacological properties. Finally, although many of the clinical trials listed in Table 4 have reported a transient lymphocytosis following antagonist administration, there is no published information on the effects these different α_4 integrin antagonists have on $CD34^+$ HSPC mobilization.

Mobilization of murine HSPCs by small molecule antagonists of VLA-4

The mobilization of murine, primate and human HSPCs by α_4 integrin antibodies led to the hypothesis that small molecule antagonists of α_4 integrins would represent a novel approach to mobilizing HSPCs. BIO5192 [2(*S*)-{[1-3,5-dichloro-benzenesulfonyl)-pyrrolidine-2(*S*)-carbonyl]-amino}-4-[4-methyl-2(*S*)-(methyl-{2-[4-(3-o-tolyl-ureido)-phenyl]acetyl}-amino)-pentanoylamino]-butyricacid] is a potent (K_d of < 10 pM) and highlyselective small molecule inhibitor of both the unactivated and activated forms of human, mouse, and rat $\alpha_4\beta_1$ integrin.[215] BIO5192 is an LDV-based α_4 integrin antagonist and exhibits high affinity for $\alpha_4\beta_1$ integrin because of an extremely slow dissociation rate (dissociation half-life >12h) of the inhibitor from both the unactivated and activated states of $\alpha_4\beta_1$ integrin.

The affinity of BIO5192 for $\alpha_4\beta_1$ integrin is at least 250-fold higher than for $\alpha_4\beta_7$ integrin.[215]

Similar to the clinical trials involving α_4 integrin antagonists discussed above (Table 4), initial preclinical studies using murine experimental models of autoimmune encephalomyelitis and other inflammatory diseases showed induction of leukocytosis and lymphocytosis following BIO5192 administration.[215] To determine whether HSPCs can be mobilized into the peripheral circulation by BIO5192, we treated mice with BIO5192 alone or in combination with plerixafor and/or G-CSF. We reported that blockade of $\alpha_4\beta_1$ integrin with a single injection of BIO5192 alone results in a 30-fold increase in mobilization of murine CFU-Cs over basal levels.[216] Peripheral blood CFU-GM levels peaked 0.5 to 1 hour after BIO5192 administration and returned to baseline by 6 hours. A similar magnitude of CFU-C mobilization was observed following treatment with plerixafor alone. Interestingly, an additive affect on HSPC mobilization was observed when BIO5192 was combined with plerixafor (3-fold compared to plerixafor alone), G-CSF (5-fold compared to G-CSF alone) or their combination (17-fold compared to G-CSF alone).[216] Importantly, BIO5192 mobilized long-term repopulating cells that successfully engraft and expand in a multi-lineage fashion in secondary transplant experiments. Since BIO5192 will not be clinically developed, we have performed identical studies with similar results using the small molecule VLA-4 antagonist firategrast, which is currently in clinical development for the treatment of multiple sclerosis by GlaxoSmithKline (data not shown). These data provided the first evidence for the utility of small molecule inhibitors of VLA-4 either alone or in combination with G-CSF or plerixafor for mobilization of HSPCs.

To better understand the relationship between CXCR4 and VLA-4 in HSPC mobilization, Christopher et al.[196] generated $CXCR4^{-/-}$ bone marrow chimeras by transplanting $CXCR4^{-/-}$ fetal liver cells into lethally irradiated wild type recipient mice. Following hematopoietic reconstitution, the $CXCR4^{-/-}$ chimeras were used to investigate the function of different mobilization agents in the absence of CXCR4/CXCL12 signaling. Surprisingly, mobilization by G-CSF or Groβ was completely abrogated in $CXCR4^{-/-}$ bone marrow chimeras.[196] In contrast, HSPC mobilization by BIO5192 was robust; exhibiting a nearly 3000-fold increase in the number of circulating CFU-Cs in the $CXCR4^{-/-}$ chimeras compared to untreated control mice. These observations suggest that α_4 integrin antagonist-induced mobilization of HSPCs occurs independently of CXCR4/CXCL12 signaling and further support the notion that CXCR4 and VLA-4 are the dominant receptors regulating HSPC migration from and retention within the bone marrow.

Summary

Plerixafor represents a significant advance in stem cell mobilization. Mobilization with G-CSF plus plerixafor in autologous stem cell mobilization decreases the number of patients who fail to collect the minimum number of $CD34^+$ stem cells necessary for transplantation.[91, 92] Even in patients who are unlikely to fail mobilization with G-CSF alone, plerixafor provides the benefits of a higher stem cell yield and fewer apheresis procedures. Consequently, fewer patients will be transplanted with suboptimal numbers of HSPCs, which can lead to delayed hematopoietic recovery and the associated increases in blood transfusions, rates of infection and length of hospitalization.

Plerixafor is also effective as a single agent in the allogeneic transplant setting. We have demonstrated that hematopoietic stem cells mobilized by plerixafor alone are functional and provide prompt and durable hematopoietic engraftment following transplantation into HLA-identical siblings with advanced hematological malignancies.[94] These preliminary data demonstrate that the length of time required to mobilize and procure a functional

hematopoietic allograft can be reduced from a 5-day to a 1-day process by directly targeting the CXCR4/CXCL12 axis. Unfortunately, nearly one-third of healthy donors mobilized with plerixafor alone fail to collect the minimal number of $CD34^+$ cells ($\geq 2.0 \times 10^6$ $CD34^+$ cells/kg recipient body weight) necessary for transplantation in a single leukapheresis. One strategy being explored to reduce this mobilization failure rate is to design and develop a more potent CXCR4 inhibitor. Several novel CXCR4 inhibitors in various stages of clinical development were discussed in this review. Alternatively, since an additive affect on murine HSPC mobilization is observed when plerixafor is combined with the VLA-4 antagonist BIO5192[216], a more successful mobilization regimen might involve the co-administration of small molecule inhibitors to CXCR4 and VLA-4. In general, the development of a G-CSF-free mobilization regimen is attractive both to avoid potential toxicities of G-CSF and to save time and resources during a 4–5 day G-CSF-based mobilization.

Recent research has shown that G-CSF induces HSPC mobilization by phagocyte depletion and modulation of the sympathetic nervous system (Figure 1).[217–224] *Cxcl12* down-regulation is critical in both of these processes[217–224], and no G-CSF-mediated mobilization is observed following neutralization of CXCR4 with monoclonal antibodies[225] or in $CXCR4^{-/-}$ BM chimeras[196]. These data indicate that disruption of the CXCR4/CXCL12 axis plays a dominant role in HSPC mobilization by G-CSF. However, the observation that a single injection of plerixafor can synergize with multiple injections of G-CSF indicate that the mechanisms involved in G-CSF and plerixafor HSPC mobilization are not completely overlapping.[91, 92, 226] Combining the effects of G-CSF (phagocyte signaling and depletion, loss of osteoblasts, down-regulation of HSPC retention genes like *Cxcl12* in $Nestin^+$ niche cells), with pharmacologic inhibition of CXCR4 by plerixafor more effectively inhibits the CXCR4/CXCL12 axis and results in increased HSPC mobilization *in vivo*. Indeed, Chow *et al.*[217] recently showed that mimicking one effect of G-CSF administration, in vivo phagocyte depletion, prior to plerixafor treatment, induced a two-fold increase in the magnitude of HSPC mobilization by the drug. Further, others and we have shown that plerixafor mobilizes HSPCs expressing higher levels of CXCR4 both before and during G-CSF administration.[7, 100, 227–229] Although not tested experimentally, it is tempting to speculate that these high CXCR4 expressing HSPCs are tethered to the BM microenvironment in a predominantly CXCR4-dependent manner and are less reliant on other adhesive interactions for the their retention in the bone marrow niche. Alternatively, G-CSF and plerixafor may mobilize HSPCs from different niches within the bone marrow environment. In this regard, putative HSPCs have been found near the endosteum lined by osteoblasts (endosteal niche) and in association with sinusoidal endothelium (perivascular niche).[4, 8]

Numerous reports have documented the importance of the VCAM-1/VLA-4 axis in modulating the homing and retention of HSPCs within the BM microenvironment. The observation that HSPCs are rapidly released into the peripheral circulation following targeted disruption of the VCAM-1/VLA-4 axis indicate that this interaction provides an alternative pathway for HSPC mobilization that is independent of the CXCR4/CXCL12 axis.[189–194, 196–199, 216] This concept is further supported by the additive or synergistic mobilization of HSPCs that is observed following the addition of a VLA-4 inhibitor to a plerixafor and/or G-CSF-based mobilization regimen.[191, 193, 216] Interestingly, although disruption of the CXCR4/CXCL12 axis via *Cxcl12* down-regulation appears to play a dominant role in HSPC mobilization by G-CSF[196], it is important to note that the growth factor also down-regulates the expression of transcripts encoding other HSPC retention genes, including SCF and VCAM-1, in cells that comprise the BM niche.[222] Downregulation of these alternative genes involved in HSPC retention within the BM microenvironment may be an additional mechanism whereby G-CSF induces greater mobilization of HSPCs relative to a specific inhibitor of CXCR4 like plerixafor. It is also

important to note that the binding of CXCL12 to CXCR4 enhances the adhesive properties of HSCs by inside-out signaling leading to activation of the integrins VLA-4, VLA-5, and LFA-1.[153, 230–233] Since the CXCR4/CXCL12 and VCAM-1/VLA-4 axes interact in regulating HSPC trafficking and adhesion to the BM, others and we have sought to increase HSPC mobilization by co-administering inhibitors to both CXCR4 and VLA-4.[191, 216] This dual inhibitor approach may ultimately provide a more efficient method to collect a functional hematopoietic graft in a single day.

Following disruption of the adhesive interactions mediating stem cell retention in the bone marrow niche, HSPCs must transit from the bone marrow parenchyma through the endothelium and into the sinusoids, where they are temporarily retained until their release into the general circulation. Relatively little is known about the physiologic mechanisms that govern the egress of HSPCs into the blood. Recent investigations have unveiled a role for the sphingosine-1-phosphate receptor 1 (SIP_1) in mobilizing HSPCs from the BM under steady state conditions and following CXCR4 antagonist mediated mobilization.[234–236] Receptors for sphingosine-1-phosphate (S1P) are present on human $CD34^+$ HSPCs and mediate their chemotactic response towards S1P as well as S1P-induced upregulation of integrins.[236–240] Although controversial, a shifted balance between intra- and extra-BM S1P might therefore play a role on HSPC mobilization into the peripheral blood. Interestingly, administration of a $S1P_1$ agonist, SEW2871, enhanced plerixafor-mediated mobilization.[235] These data imply that S1P receptors, particularly $S1P_1$, regulate the egress of HSPCs from the bone marrow.

Finally, CXCR4 and VLA-4 inhibitors are likely to find important uses beyond hematopoietic stem cell mobilization. CXCR4 inhibitors may function as therapeutic agents in cancer therapy as anti-metastatic or chemosensitization agents[241–245], as well as in inflammation and tissue repair.[246–253] Furthermore, several studies have indicated an important role of VLA-4 in cell adhesion-mediated inflammatory pathologies including asthma, multiple sclerosis (MS), rheumatoid arthritis (RA), atherosclerosis, and inflammatory bowel disease (IBD).[208–210] Specific orally available small molecule inhibitors of the VLA-4 interaction with its ligands would thus be expected to be of therapeutic benefit.

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References

1. Xie T, Spradling AC. A niche maintaining germ line stem cells in the Drosophila ovary. Science. 2000; 290:328–330. [PubMed: 11030649]
2. Kiger AA, White-Cooper H, Fuller MT. Somatic support cells restrict germline stem cell self-renewal and promote differentiation. Nature. 2000; 407:750–754. [PubMed: 11048722]
3. Adams GB, Scadden DT. The hematopoietic stem cell in its place. Nat Immunol. 2006; 7:333–337. [PubMed: 16550195]
4. Wilson A, Trumpp A. Bone-marrow haematopoietic-stem-cell niches. Nat Rev Immunol. 2006; 6:93–106. [PubMed: 16491134]
5. Kollet O, Dar A, Lapidot T. Themultiple roles of osteoclasts in host defense: bone remodeling and hematopoietic stem cell mobilization. Annu Rev Immunol. 2007; 25:51–69. [PubMed: 17042735]

6. Lapidot T, Dar A, Kollet O. How do stem cells find their way home? Blood. 2005; 106:1901–1910. [PubMed: 15890683]
7. Smith-Berdan S, Nguyen A, Hassanein D, Zimmer M, Ugarte F, Ciriza J, et al. Robo4 cooperates with CXCR4 to specify hematopoietic stem cell localization to bone marrow niches. Cell Stem Cell. 2011; 8:72–83. [PubMed: 21211783]
8. Kiel MJ, Morrison SJ. Uncertainty in the niches that maintain haematopoietic stem cells. Nat Rev Immunol. 2008; 8:290–301. [PubMed: 18323850]
9. Bensinger WI, Martin PJ, Storer B, Clift R, Forman SJ, Negrin R, et al. Transplantation of bone marrow as compared with peripheral-blood cells from HLA-identical relatives in patients withhematologic cancers. N Engl J Med. 2001; 344:175–181. [PubMed: 11172139]
10. Beyer J, Schwella N, Zingsem J, Strohscheer I, Schwaner I, Oettle H, et al. Hematopoietic rescue after high-dose chemotherapy using autologous peripheral-blood progenitor cells or bone marrow: a randomized comparison. J Clin Oncol. 1995; 13:1328–1335. [PubMed: 7538556]
11. Hartmann O, Le Corroller AG, Blaise D, Michon J, Philip I, Norol F, et al. Peripheral blood stem cell and bone marrow transplantation for solid tumors and lymphomas: hematologic recovery and costs. A randomized, controlled trial. Ann Intern Med. 1997; 126:600–607. [PubMed: 9103126]
12. Schmitz N, Linch DC, Dreger P, Goldstone AH, Boogaerts MA, Ferrant A, et al. Randomised trial of filgrastim-mobilised peripheral blood progenitor cell transplantation versus autologous bone-marrow transplantation in lymphoma patients. Lancet. 1996; 347:353–357. [PubMed: 8598700]
13. Bensinger W, Dipersio JF, McCarty JM. Improving stem cell mobilization strategies: future directions. Bone Marrow Transplant. 2009
14. Kessinger A, Armitage JO, Landmark JD, Weisenburger DD. Reconstitution of human hematopoietic function with autologous cryopreserved circulating stem cells. Exp Hematol. 1986; 14:192–196. [PubMed: 2868909]
15. Korbling M, Dorken B, Ho AD, Pezzutto A, Hunstein W, Fliedner TM. Autologous transplantation of blood-derived hemopoietic stem cells after myeloablative therapy in a patient with Burkitt's lymphoma. Blood. 1986; 67:529–532. [PubMed: 2867797]
16. Dreger P, Kloss M, Petersen B, Haferlach T, Loffler H, Loeffler M, et al. Autologous progenitor cell transplantation: prior exposure to stem cell-toxic drugs determines yield and engraftment of peripheral blood progenitor cell but not of bone marrow grafts. Blood. 1995; 86:3970–3978. [PubMed: 7579368]
17. Pusic I, Jiang SY, Landua S, Uy GL, Rettig MP, Cashen AF, et al. Impact of mobilization and remobilization strategies on achieving sufficient stem cell yields for autologous transplantation. Biol Blood Marrow Transplant. 2008; 14:1045–1056. [PubMed: 18721768]
18. Weaver CH, Hazelton B, Birch R, Palmer P, Allen C, Schwartzberg L, et al. An analysis of engraftment kinetics as a function of the CD34 content of peripheral blood progenitor cell collections in 692 patients after the administration of myeloablative chemotherapy. Blood. 1995; 86:3961–3969. [PubMed: 7579367]
19. Haas R, Mohle R, Fruhauf S, Goldschmidt H, Witt B, Flentje M, et al. Patient characteristics associated with successful mobilizing and autografting of peripheral blood progenitor cells in malignant lymphoma. Blood. 1994; 83:3787–3794. [PubMed: 7515721]
20. Sugrue MW, Williams K, Pollock BH, Khan S, Peracha S, Wingard JR, et al. Characterization and outcome of "hard to mobilize"' lymphoma patients undergoing autologous stem cell transplantation. Leuk Lymphoma. 2000; 39:509–519. [PubMed: 11342334]
21. Anderlini P, Przepiorka D, Seong D, Miller P, Sundberg J, Lichtiger B, et al. Clinical toxicity and laboratory effects of granulocyte-colony-stimulating factor (filgrastim) mobilization and blood stem cell apheresis from normal donors, and analysis of charges for the procedures. Transfusion. 1996; 36:590–595. [PubMed: 8701453]
22. Becker PS, Wagle M, Matous S, Swanson RS, Pihan G, Lowry PA, et al. Spontaneous splenic rupture following administration of granulocyte colony-stimulating factor (G-CSF): occurrence in an allogeneic donor of peripheral blood stem cells. Biol Blood Marrow Transplant. 1997; 3:45–49. [PubMed: 9209740]
23. Falzetti F, Aversa F, Minelli O, Tabilio A. Spontaneous rupture of spleen during peripheral blood stem-cell mobilisation in a healthy donor. Lancet. 1999; 353:555. [PubMed: 10028986]

24. Fortanier C, Kuentz M, Sutton L, Milpied N, Michalet M, Macquart-Moulin G, et al. Healthy sibling donor anxiety and pain during bone marrow or peripheral blood stem cell harvesting for allogeneic transplantation: results of a randomised study. Bone Marrow Transplant. 2002; 29:145–149. [PubMed: 11850709]
25. Goterris R, Hernandez-Boluda JC, Teruel A, Gomez C, Lis MJ, Terol MJ, et al. Impact of different strategies of second-line stem cell harvest on the outcome of autologous transplantation in poor peripheral blood stem cell mobilizers. Bone Marrow Transplant. 2005; 36:847–853. [PubMed: 16113660]
26. Dar A, Goichberg P, Shinder V, Kalinkovich A, Kollet O, Netzer N, et al. Chemokine receptor CXCR4-dependent internalization and resecretion of functional chemokine SDF-1 by bone marrow endothelial and stromal cells. Nat Immunol. 2005; 6:1038–1046. [PubMed: 16170318]
27. Imai K, Kobayashi M, Wang J, Shinobu N, Yoshida H, Hamada J, et al. Selective secretion of chemoattractants for haemopoietic progenitor cells by bone marrow endothelial cells: a possible role in homing of haemopoietic progenitor cells to bone marrow. Br J Haematol. 1999; 106:905–911. [PubMed: 10519991]
28. Jung Y, Wang J, Schneider A, Sun YX, Koh-Paige AJ, Osman NI, et al. Regulation of SDF-1 (CXCL12) production by osteoblasts; a possible mechanism for stem cell homing. Bone. 2006; 38:497–508. [PubMed: 16337237]
29. Ponomaryov T, Peled A, Petit I, Taichman RS, Habler L, Sandbank J, et al. Induction of the chemokine stromal-derived factor-1 following DNA damage improves human stem cell function. J Clin Invest. 2000; 106:1331–1339. [PubMed: 11104786]
30. Calvi LM, Adams GB, Weibrecht KW, Weber JM, Olson DP, Knight MC, et al. Osteoblastic cells regulate the haematopoietic stem cell niche. Nature. 2003; 425:841–846. [PubMed: 14574413]
31. Watt SM, Forde SP. The central role of the chemokine receptor, CXCR4, in haemopoietic stem cell transplantation: will CXCR4 antagonists contribute to the treatment of blood disorders? Vox Sang. 2008; 94:18–32. [PubMed: 18042197]
32. Peled A, Petit I, Kollet O, Magid M, Ponomaryov T, Byk T, et al. Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. Science. 1999; 283:845–848. [PubMed: 9933168]
33. Sugiyama T, Kohara H, Noda M, Nagasawa T. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. Immunity. 2006; 25:977–988. [PubMed: 17174120]
34. Doranz BJ, Orsini MJ, Turner JD, Hoffman TL, Berson JF, Hoxie JA, et al. Identification of CXCR4 domains that support coreceptor and chemokine receptor functions. J Virol. 1999; 73:2752–2761. [PubMed: 10074122]
35. Roland J, Murphy BJ, Ahr B, Robert-Hebmann V, Delauzun V, Nye KE, et al. Role of the intracellular domains of CXCR4 in SDF-1-mediated signaling. Blood. 2003; 101:399–406. [PubMed: 12393663]
36. Benboubker L, Watier H, Carion A, Georget MT, Desbois I, Colombat P, et al. Association between the SDF1-3′A allele and high levels of CD34(+) progenitor cells mobilized into peripheral blood in humans. Br J Haematol. 2001; 113:247–250. [PubMed: 11328308]
37. Bogunia-Kubik K, Gieryng A, Dlubek D, Lange A. The CXCL12-3′A allele is associated with a higher mobilization yield of CD34 progenitors to the peripheral blood of healthy donors for allogeneic transplantation. Bone Marrow Transplant. 2009; 44:273–278. [PubMed: 19252530]
38. Burns JM, Summers BC, Wang Y, Melikian A, Berahovich R, Miao Z, et al. A novel chemokine receptor for SDF-1 and I-TAC involved in cell survival, cell adhesion, and tumor development. J Exp Med. 2006; 203:2201–2213. [PubMed: 16940167]
39. Balabanian K, Lagane B, Infantino S, Chow KY, Harriague J, Moepps B, et al. The chemokine SDF-1/CXCL12 binds to and signals through the orphan receptor RDC1 in T lymphocytes. J Biol Chem. 2005; 280:35760–35766. [PubMed: 16107333]
40. Sierro F, Biben C, Martinez-Munoz L, Mellado M, Ransohoff RM, Li M, et al. Disrupted cardiac development but normal hematopoiesis in mice deficient in the second CXCL12/SDF-1 receptor, CXCR7. Proc Natl Acad Sci U S A. 2007; 104:14759–14764. [PubMed: 17804806]

41. Boldajipour B, Mahabaleshwar H, Kardash E, Reichman-Fried M, Blaser H, Minina S, et al. Control of chemokine-guided cell migration by ligand sequestration. Cell. 2008; 132:463–473. [PubMed: 18267076]
42. Hartmann TN, Grabovsky V, Pasvolsky R, Shulman Z, Buss EC, Spiegel A, et al. A crosstalk between intracellular CXCR7 and CXCR4 involved in rapid CXCL12-triggered integrin activation but not in chemokine-triggered motility of human T lymphocytes and CD34+ cells. J Leukoc Biol. 2008; 84:1130–1140. [PubMed: 18653785]
43. Naumann U, Cameroni E, Pruenster M, Mahabaleshwar H, Raz E, Zerwes HG, et al. CXCR7 functions as a scavenger for CXCL12 and CXCL11. PLoS One. 2010; 5:e9175. [PubMed: 20161793]
44. Loetscher M, Geiser T, O'Reilly T, Zwahlen R, Baggiolini M, Moser B. Cloning of a human seven-transmembrane domain receptor, LESTR, that is highly expressed in leukocytes. J Biol Chem. 1994; 269:232–237. [PubMed: 8276799]
45. Bleul CC, Farzan M, Choe H, Parolin C, Clark-Lewis I, Sodroski J, et al. The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. Nature. 1996; 382:829–833. [PubMed: 8752280]
46. Oberlin E, Amara A, Bachelerie F, Bessia C, Virelizier JL, Arenzana-Seisdedos F, et al. 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. [PubMed: 8752281]
47. Feng Y, Broder CC, Kennedy PE, Berger EA. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. Science. 1996; 272:872–877. [PubMed: 8629022]
48. Fredriksson R, Lagerstrom MC, Lundin LG, Schioth HB. The G-protein-coupled receptors in the human genome form five main families. Phylogenetic analysis, paralogon groups, and fingerprints. Mol Pharmacol. 2003; 63:1256–1272. [PubMed: 12761335]
49. Busillo JM, Benovic JL. Regulation of CXCR4 signaling. Biochim Biophys Acta. 2007; 1768:952–963. [PubMed: 17169327]
50. Kucia M, Jankowski K, Reca R, Wysoczynski M, Bandura L, Allendorf DJ, et al. CXCR4-SDF-1 signalling, locomotion, chemotaxis and adhesion. J Mol Histol. 2004; 35:233–245. [PubMed: 15339043]
51. Dubeykovskaya Z, Dubeykovskiy A, Solal-Cohen J, Wang TC. Secreted trefoil factor 2 activates the CXCR4 receptor in epithelial and lymphocytic cancer cell lines. J Biol Chem. 2009; 284:3650–3662. [PubMed: 19064997]
52. Bernhagen J, Krohn R, Lue H, Gregory JL, Zernecke A, Koenen RR, et al. MIF is a noncognate ligand of CXC chemokine receptors in inflammatory and atherogenic cell recruitment. Nat Med. 2007; 13:587–596. [PubMed: 17435771]
53. Fukuda S, Broxmeyer HE, Pelus LM. Flt3 ligand and the Flt3 receptor regulate hematopoietic cell migration by modulating the SDF-1alpha(CXCL12)/CXCR4 axis. Blood. 2005; 105:3117–3126. [PubMed: 15618475]
54. Dutt P, Wang JF, Groopman JE. Stromal cell-derived factor-1 alpha and stem cell factor/kit ligand share signaling pathways in hemopoietic progenitors: a potential mechanism for cooperative induction of chemotaxis. J Immunol. 1998; 161:3652–3658. [PubMed: 9759889]
55. Aiuti A, Tavian M, Cipponi A, Ficara F, Zappone E, Hoxie J, et al. Expression of CXCR4, the receptor for stromal cell-derived factor-1 on fetal and adult human lympho-hematopoietic progenitors. Eur J Immunol. 1999; 29:1823–1831. [PubMed: 10382744]
56. Pruijt JF, Fibbe WE, Laterveer L, Pieters RA, Lindley IJ, Paemen L, et al. Prevention of interleukin-8-induced mobilization of hematopoietic progenitor cells in rhesus monkeys by inhibitory antibodies against the metalloproteinase gelatinase B (MMP-9). Proc Natl Acad Sci U S A. 1999; 96:10863–10868. [PubMed: 10485917]
57. Kollet O, Shivtiel S, Chen YQ, Suriawinata J, Thung SN, Dabeva MD, et al. HGF, SDF-1, and MMP-9 are involved in stress-induced human CD34+ stem cell recruitment to the liver. J Clin Invest. 2003; 112:160–169. [PubMed: 12865405]

58. Levesque JP, Hendy J, Takamatsu Y, Simmons PJ, Bendall LJ. Disruption of the CXCR4/CXCL12 chemotactic interaction during hematopoietic stem cell mobilization induced by GCSF or cyclophosphamide. J Clin Invest. 2003; 111:187–196. [PubMed: 12531874]
59. Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa S, Kitamura Y, et al. Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. Nature. 1996; 382:635–638. [PubMed: 8757135]
60. Tachibana K, Hirota S, Iizasa H, Yoshida H, Kawabata K, Kataoka Y, et al. The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract. Nature. 1998; 393:591–594. [PubMed: 9634237]
61. Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development. Nature. 1998; 393:595–599. [PubMed: 9634238]
62. Ma Q, Jones D, Borghesani PR, Segal RA, Nagasawa T, Kishimoto T, et al. Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4-and SDF-1-deficient mice. Proc Natl Acad Sci US A. 1998; 95:9448–9453.
63. Ma Q, Jones D, Springer TA. The chemokine receptor CXCR4 is required for the retention of B lineage and granulocytic precursors within the bone marrow microenvironment. Immunity. 1999; 10:463–471. [PubMed: 10229189]
64. Foudi A, Jarrier P, Zhang Y, Wittner M, Geay JF, Lecluse Y, et al. Reduced retention of radioprotective hematopoietic cells within the bone marrow microenvironment in CXCR4−/− chimeric mice. Blood. 2006; 107:2243–2251. [PubMed: 16291599]
65. Kawabata K, Ujikawa M, Egawa T, Kawamoto H, Tachibana K, Iizasa H, et al. A cell-autonomous requirement for CXCR4 in long-term lymphoid and myeloid reconstitution. Proc Natl Acad Sci U S A. 1999; 96:5663–5667. [PubMed: 10318941]
66. Nie Y, Han YC, Zou YR. CXCR4 is required for the quiescence of primitive hematopoietic cells. J Exp Med. 2008; 205:777–783. [PubMed: 18378795]
67. Balabanian K, Lagane B, Pablos JL, Laurent L, Planchenault T, Verola O, et al. WHIM syndromes with different genetic anomalies are accounted for by impaired CXCR4 desensitization to CXCL12. Blood. 2005; 105:2449–2457. [PubMed: 15536153]
68. Gulino AV, Moratto D, Sozzani S, Cavadini P, Otero K, Tassone L, et al. Altered leukocyte response to CXCL12 in patients with warts hypogammaglobulinemia, infections, myelokathexis (WHIM) syndrome. Blood. 2004; 104:444–452. [PubMed: 15026312]
69. Hernandez PA, Gorlin RJ, Lukens JN, Taniuchi S, Bohinjec J, Francois F, et al. Mutations in the chemokine receptor gene CXCR4 are associated with WHIM syndrome, a combined immunodeficiency disease. Nat Genet. 2003; 34:70–74. [PubMed: 12692554]
70. Kawai T, Choi U, Whiting-Theobald NL, Linton GF, Brenner S, Sechler JM, et al. Enhanced function with decreased internalization of carboxy-terminus truncated CXCR4 responsible for WHIM syndrome. Exp Hematol. 2005; 33:460–468. [PubMed: 15781337]
71. Balabanian K, Levoye A, Klemm L, Lagane B, Hermine O, Harriague J, et al. Leukocyte analysis from WHIM syndrome patients reveals a pivotal role for GRK3 in CXCR4 signaling. J Clin Invest. 2008; 118:1074–1084. [PubMed: 18274673]
72. Gorlin RJ, Gelb B, Diaz GA, Lofsness KG, Pittelkow MR, Fenyk JR Jr. WHIM syndrome, an autosomal dominant disorder: clinical, hematological, and molecular studies. Am J Med Genet. 2000; 91:368–376. [PubMed: 10767001]
73. Gulino AV. WHIM syndrome: a genetic disorder of leukocyte trafficking. Curr Opin Allergy Clin Immunol. 2003; 3:443–450. [PubMed: 14612668]
74. Pelus LM. Peripheral blood stem cell mobilization: new regimens, new cells, where do we stand. Curr Opin Hematol. 2008; 15:285–292. [PubMed: 18536564]
75. Nervi B, Link DC, DiPersio JF. Cytokines and hematopoietic stem cell mobilization. J Cell Biochem. 2006; 99:690–705. [PubMed: 16888804]
76. De Clercq E. The bicyclam AMD3100 story. Nat Rev Drug Discov. 2003; 2:581–587. [PubMed: 12815382]

77. Donzella GA, Schols D, Lin SW, Este JA, Nagashima KA, Maddon PJ, et al. AMD3100, a small molecule inhibitor of HIV-1 entry via the CXCR4 co-receptor. Nat Med. 1998; 4:72–77. [PubMed: 9427609]

78. Hendrix CW, Flexner C, MacFarland RT, Giandomenico C, Fuchs EJ, Redpath E, et al. 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. [PubMed: 10817726]

79. Lack NA, Green B, Dale DC, Calandra GB, Lee H, MacFarland RT, et al. A pharmacokinetic-pharmacodynamic model for the mobilization of CD34+ hematopoietic progenitor cells by AMD3100. Clin Pharmacol Ther. 2005; 77:427–436. [PubMed: 15900288]

80. Liles WC, Broxmeyer HE, Rodger E, Wood B, Hubel K, Cooper S, et al. Mobilization of hematopoietic progenitor cells in healthy volunteers by AMD3100, a CXCR4 antagonist. Blood. 2003; 102:2728–2730. [PubMed: 12855591]

81. Liles WC, Rodger E, Broxmeyer HE, Dehner C, Badel K, Calandra G, et al. Augmented mobilization and collection of CD34+ hematopoietic cells from normal human volunteers stimulated with granulocyte-colony-stimulating factor by single-dose administration of AMD3100, a CXCR4 antagonist. Transfusion. 2005; 45:295–300. [PubMed: 15752146]

82. Devine SM, Flomenberg N, Vesole DH, Liesveld J, Weisdorf D, Badel K, et al. Rapid mobilization of CD34+ cells following administration of the CXCR4 antagonist AMD3100 to patients with multiple myeloma and non-Hodgkin's lymphoma. J Clin Oncol. 2004; 22:1095–1102. [PubMed: 15020611]

83. Flomenberg N, Devine SM, Dipersio JF, Liesveld JL, McCarty JM, Rowley SD, et al. The use of AMD3100 plus G-CSF for autologous hematopoietic progenitor cell mobilization is superior to G-CSF alone. Blood. 2005; 106:1867–1874. [PubMed: 15890685]

84. Calandra G, McCarty J, McGuirk J, Tricot G, Crocker SA, Badel K, et al. AMD3100 plus G-CSF can successfully mobilize CD34+ cells from non-Hodgkin's lymphoma, Hodgkin's disease and multiple myeloma patients previously failing mobilization with chemotherapy and/or cytokine treatment: compassionate use data. Bone Marrow Transplant. 2008; 41:331–338. [PubMed: 17994119]

85. Cashen A, Lopez S, Gao F, Calandra G, MacFarland R, Badel K, et al. A phase II study of plerixafor (AMD3100) plus G-CSF for autologous hematopoietic progenitor cell mobilization in patients with Hodgkin lymphoma. Biol Blood Marrow Transplant. 2008; 14:1253–1261. [PubMed: 18940680]

86. Fowler CJ, Dunn A, Hayes-Lattin B, Hansen K, Hansen L, Lanier K, et al. Rescue from failed growth factor and/or chemotherapy HSC mobilization with G-CSF and plerixafor(AMD3100): an institutional experience. Bone Marrow Transplant. 2009; 43:909–917. [PubMed: 19182831]

87. Stiff P, Micallef I, McCarthy P, Magalhaes-Silverman M, Weisdorf D, Territo M, et al. Treatment with plerixafor in non-Hodgkin's lymphoma and multiple myeloma patients to increase the number of peripheral blood stem cells when given a mobilizing regimen of G-CSF: implications for the heavily pretreated patient. Biol Blood Marrow Transplant. 2009; 15:249–256. [PubMed: 19167685]

88. Stewart DA, Smith C, MacFarland R, Calandra G. Pharmacokinetics and pharmacodynamics of plerixafor in patients with non-Hodgkin lymphoma and multiple myeloma. Biol Blood Marrow Transplant. 2009; 15:39–46. [PubMed: 19135941]

89. Dugan MJ, Maziarz RT, Bensinger WI, Nademanee A, Liesveld J, Badel K, et al. Safety and preliminary efficacy of plerixafor (Mozobil) in combination with chemotherapy and G-CSF: an open-label, multicenter, exploratory trial in patients with multiple myeloma and non-Hodgkin's lymphoma undergoing stem cell mobilization. Bone Marrow Transplant. 2010; 45:39–47. [PubMed: 19483760]

90. Shaughnessy P, Islas-Ohlmayer M, Murphy J, Hougham M, MacPherson J, Winkler K, et al. Plerixafor Plus G-CSF Compared to Chemotherapy Plus G-CSF for Mobilization of Autologous CD34+ Cells Resulted in Similar Cost but More Predictable Days of Apheresis and Less Hospitalization. Blood (ASH Annual Meeting Abstracts). 2009; 114:abstract [2277].

91. DiPersio JF, Micallef IN, Stiff PJ, Bolwell BJ, Maziarz RT, Jacobsen E, et al. Phase III prospective randomized double-blind placebo-controlled trial of plerixafor plus granulocyte colony-stimulating

factor compared with placebo plus granulocyte colony-stimulating factor for autologous stem-cell mobilization and transplantation for patients with non-Hodgkin's lymphoma. J Clin Oncol. 2009; 27:4767–4773. [PubMed: 19720922]

92. DiPersio JF, Stadtmauer EA, Nademanee A, Micallef IN, Stiff PJ, Kaufman JL, et al. Plerixafor and G-CSF versus placebo and G-CSF to mobilize hematopoietic stem cells for autologous stem cell transplantation in patients with multiple myeloma. Blood. 2009; 113:5720–5726. [PubMed: 19363221]
93. Micallef IN, Stiff PJ, DiPersio JF, Maziarz RT, McCarty JM, Bridger G, et al. Successful stem cell remobilization using plerixafor (mozobil) plus granulocyte colony-stimulating factor in patients with non-hodgkin lymphoma: results from the plerixafor NHL phase 3 study rescue protocol. Biol Blood Marrow Transplant. 2009; 15:1578–1586. [PubMed: 19896082]
94. Devine SM, Vij R, Rettig M, Todt L, McGlauchlen K, Fisher N, et al. Rapid mobilization of functional donor hematopoietic cells without G-CSF using AMD3100, an antagonist of the CXCR4/SDF-1 interaction. Blood. 2008; 112:990–998. [PubMed: 18426988]
95. Rettig MP, Shannon WD, Ritchey J, Holt M, McFarland K, Lopez S, et al. Characterization of human CD34+ hematopoietic stem cells following administration of G-CSF or plerixafor. Blood (ASH Annual Meeting Abstracts). 2008; 112:3476.
96. Rettig M, Lopez S, McFarland K, DiPersio JF. Rapid and Prolonged Mobilization of Human CD34+ Hematopoietic Stem Cells Following Intravenous (IV) Administration of Plerixafor. Blood (ASH Annual Meeting Abstracts). 2010; 116:2261.
97. Rettig M, McFarland K, Ritchey J, Holt M, Deych E, Lopez S, et al. Preferential Mobilization of CD34+ Plasmacytoid Dendritic Cell Precursors by Plerixafor. Blood (ASH Annual Meeting Abstracts). 2009; 114:32.
98. Manz MG, Miyamoto T, Akashi K, Weissman IL. Prospective isolation of human clonogenic common myeloid progenitors. Proc Natl Acad Sci U SA. 2002; 99:11872–11877.
99. Weissman IL, Shizuru JA. The origins of the identification and isolation of hematopoietic stem cells, and their capability to induce donor-specific transplantation tolerance and treat autoimmune diseases. Blood. 2008; 112:3543–3553. [PubMed: 18948588]
100. Rettig MP, Ramirez P, Nervi B, DiPersio JF. CXCR4 and mobilization of hematopoietic precursors. Methods Enzymol. 2009; 460:57–90. [PubMed: 19446720]
101. Fruehauf S, Tricot G. Comparison of unmobilized and mobilized graft characteristics and the implications of cell subsets on autologous and allogeneic transplantation outcomes. Biol Blood Marrow Transplant. 2010; 16:1629–1648. [PubMed: 20144908]
102. Robinson JA, Demarco S, Gombert F, Moehle K, Obrecht D. The design, structures and therapeutic potential of protein epitope mimetics. Drug Discov Today. 2008; 13:944–951. [PubMed: 18725320]
103. DeMarco SJ, Henze H, Lederer A, Moehle K, Mukherjee R, Romagnoli B, et al. Discovery of novel, highly potent and selective beta-hairpin mimetic CXCR4 inhibitors with excellent anti-HIV activity and pharmacokinetic profiles. Bioorg Med Chem. 2006; 14:8396–8404. [PubMed: 17010618]
104. Moncunill G, Armand-Ugon M, Clotet-Codina I, Pauls E, Ballana E, Llano A, et al. Anti-HIV activity and resistance profile of the CXC chemokine receptor 4 antagonist POL3026. Mol Pharmacol. 2008; 73:1264–1273. [PubMed: 18182480]
105. Schmitt S, Weinhold N, Dembowsky K, Neben K, Witzens-Harig M, Braun M, et al. First Results of a Phase-II Study with the New CXCR4 Antagonist POL6326 to Mobilize Hematopoietic Stem Cells (HSC) In Multiple Myeloma (MM). Blood (ASH Annual Meeting Abstracts). 2010; 116:824.
106. Juarez J, Bradstock KF, Gottlieb DJ, Bendall LJ. Effects of inhibitors of the chemokine receptor CXCR4 on acute lymphoblastic leukemia cells in vitro. Leukemia. 2003; 17:1294–1300. [PubMed: 12835717]
107. Abraham M, Beider K, Wald H, Weiss ID, Zipori D, Galun E, et al. The CXCR4 antagonist 4F-benzoyl-TN14003 stimulates the recovery of the bone marrow after transplantation. Leukemia. 2009; 23:1378–1388. [PubMed: 19322207]

108. Abraham M, Biyder K, Begin M, Wald H, Weiss ID, Galun E, et al. Enhanced unique pattern of hematopoietic cell mobilization induced by the CXCR4 antagonist 4F-benzoyl-TN14003. Stem Cells. 2007; 25:2158–2166. [PubMed: 17525235]

109. Trent JO, Wang ZX, Murray JL, Shao W, Tamamura H, Fujii N, et al. Lipid bilayer simulations of CXCR4 with inverse agonists and weak partial agonists. J Biol Chem. 2003; 278:47136–47144. [PubMed: 12958314]

110. Zhang WB, Navenot JM, Haribabu B, Tamamura H, Hiramatu K, Omagari A, et al. 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. [PubMed: 11923301]

111. 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. [PubMed: 9334378]

112. Beider K, Begin M, Abraham M, Wald H, Weiss I, Wald O, et al. Anti-Leukemia and Multiple Myeloma Selective Activity of CXCR4 Antagonist 4F-Benzoyl-TN14003 Involves Apoptotic Death Pathway. Blood (ASH Annual Meeting Abstracts). 2009; 114:3857.

113. Nagler A, Shimoni A, Avivi I, Rowe JM, Beider K, Hardan I, et al. BKT140 Is a Novel CXCR4 Antagonist with Stem Cell Mobilization and Antimyeloma Effects: An Open-Label First Human Trial In Patients with Multiple Myeloma Undergoing Stem Cell Mobilization for Autologous Transplantation. Blood (ASH Annual Meeting Abstracts). 2010; 116:2260.

114. Sayyed SG, Hagele H, Kulkarni OP, Endlich K, Segerer S, Eulberg D, et al. Podocytes produce homeostatic chemokine stromal cell-derived factor-1/CXCL12, which contributes to glomerulosclerosis, podocyte loss and albuminuria in a mouse model of type 2 diabetes. Diabetologia. 2009; 52:2445–2454. [PubMed: 19707743]

115. Eulberg D, Klussmann S. Spiegelmers: biostable aptamers. Chembiochem. 2003; 4:979–983. [PubMed: 14523914]

116. Klussmann S, Nolte A, Bald R, Erdmann VA, Furste JP. Mirror-image RNA that binds D-adenosine. Nat Biotechnol. 1996; 14:1112–1115. [PubMed: 9631061]

117. Huang Y, Liu Y, Yen C, Chen H, Chen S, King CR, et al. Rapid Mobilization of Murine Hematopoietic Stem and Progenitor Cells with TG-0054, a Novel CXCR4 Antagonist. Blood (ASH Annual Meeting Abstracts). 2009; 114:3542.

118. Chung DT, Chang L, Huang Y, Tsai C, Hsu C, King CR, et al. TG-0054, a Novel and Potent Stem Cell Mobilizer, Displays Excellent PK/PD and Safety Profile in Phase I Trial. Blood (ASH Annual Meeting Abstracts). 2009; 114:866.

119. Garber K. First results for agents targeting cancer-related inflammation. J Natl Cancer Inst. 2009; 101:1110–1112. [PubMed: 19671776]

120. Kuhne, MR.; Mulvey, T.; Belanger, B.; Chen, S.; Pan, C.; Chong, C., et al. A fully human anti-CXCR4 antibody induces apoptosis in vitro and shows anti tumor activity iin vivo. Proceedings of the 100th Annual Meeting of the American Association for Cancer Research; 2009. p. abstract[LB-150]

121. Kaneider NC, Agarwal A, Leger AJ, Kuliopulos A. Reversing systemic inflammatory response syndrome with chemokine receptor pepducins. Nat Med. 2005; 11:661–665. [PubMed: 15880119]

122. O'Callaghan KM, Hsieh M, VanEtten RA, Covic L, Kuliopulos A. CXCR4 Pepducins in Stem Cell Mobilization. Blood (ASH Annual Meeting Abstracts). 2009; 114:2440.

123. Moses, E.; Law, D. Ablynx's R&D Investor Day. 2010. http://wwwablynxcom/investorrelations/english/documents/RDDayUSA_12Feb_2010_FINALVERSIONpdf

124. Kolkman JA, Law DA. Nanobodies–from llamas to therapeutic proteins. dRUG Discov Today: tECHNOL. 2010 doi: 10.1016/.

125. Hamers-Casterman C, Atarhouch T, Muyldermans S, Robinson G, Hamers C, Songa EB, et al. Naturally occurring antibodies devoid of light chains. Nature. 1993; 363:446–448. [PubMed: 8502296]

126. Skerlj RT, Bridger GJ, Kaller A, McEachern EJ, Crawford JB, Zhou Y, et al. Discovery of novel small molecule orally bioavailable C-X-C chemokine receptor 4 antagonists that are potent

inhibitors of T-tropic (X4) HIV-1 replication. J Med Chem. 2010; 53:3376–3388. [PubMed: 20297846]

127. Moyle G, DeJesus E, Boffito M, Wong RS, Gibney C, Badel K, et al. Proof of activity with AMD11070, an orally bioavailable inhibitor of CXCR4-tropic HIV type 1. Clin Infect Dis. 2009; 48:798–805. [PubMed: 19193109]
128. Stone ND, Dunaway SB, Flexner C, Tierney C, Calandra GB, Becker S, et al. Multiple-dose escalation study of the safety, pharmacokinetics, and biologic activity of oral AMD070, a selectiveCXCR4 receptor inhibitor, in human subjects. Antimicrob Agents Chemother. 2007; 51:2351–2358. [PubMed: 17452489]
129. Jenkinson S, Thomson M, McCoy D, Edelstein M, Danehower S, Lawrence W, et al. Blockade of X4-tropic HIV-1 cellular entry by GSK812397, a potent noncompetitive CXCR4 receptor antagonist. Antimicrob Agents Chemother. 2010; 54:817–824. [PubMed: 19949058]
130. Murakami T, Kumakura S, Yamazaki T, Tanaka R, Hamatake M, Okuma K, et al. The novel CXCR4 antagonist KRH-3955 is an orally bioavailable and extremely potent inhibitor of human immunodeficiency virus type 1 infection: comparative studies with AMD3100. Antimicrob Agents Chemother. 2009; 53:2940–2948. [PubMed: 19451305]
131. Ichiyama K, Yokoyama-Kumakura S, Tanaka Y, Tanaka R, Hirose K, Bannai K, et al. A duodenally absorbable CXC chemokine receptor 4 antagonist, KRH-1636, exhibits a potent and selective anti-HIV-1 activity. Proc Natl Acad Sci U S A. 2003; 100:4185–4190. [PubMed: 12642669]
132. Fujii N, Oishi S, Hiramatsu K, Araki T, Ueda S, Tamamura H, et al. Molecular-size reduction of a potent CXCR4-chemokine antagonist using orthogonal combination of conformation-and sequence-based libraries. Angew Chem Int Ed Engl. 2003; 42:3251–3253. [PubMed: 12876735]
133. Zhan W, Liang Z, Zhu A, Kurtkaya S, Shim H, Snyder JP, et al. Discovery of small molecule CXCR4 antagonists. J Med Chem. 2007; 50:5655–5664. [PubMed: 17958344]
134. Natchus, M.; Arrendale, R.; Donald, L. MSX-122, an orally available small molecule CXCR4 antagonist, promotes leukocytosis in monkeys at doses that were well tolerated in a 28 day toxicology study. AACR Annual Meeting 2009 Proceedings; 2008 Apr. p. 49p. abstract [1189]
135. Humphries MJ. Integrin structure. Biochem Soc Trans. 2000; 28:311–339. [PubMed: 10961914]
136. Hynes RO. Integrins: bidirectional, allosteric signaling machines. Cell. 2002; 110:673–687. [PubMed: 12297042]
137. Johnson MS, Lu N, Denessiouk K, Heino J, Gullberg D. Integrins during evolution: evolutionary trees and model organisms. Biochim Biophys Acta. 2009; 1788:779–789. [PubMed: 19161977]
138. Springer TA, Wang JH. The three-dimensional structure of integrins and their ligands, and conformational regulation of cell adhesion. Adv Protein Chem. 2004; 68:29–63. [PubMed: 15500858]
139. Arnaout MA, Mahalingam B, Xiong JP. Integrin structure, allostery, and bidirectional signaling. Annu Rev Cell Dev Biol. 2005; 21:381–410. [PubMed: 16212500]
140. Humphries JD, Byron A, Humphries MJ. Integrin ligands at a glance. J Cell Sci. 2006; 119:3901–3903. [PubMed: 16988024]
141. Barczyk M, Carracedo S, Gullberg D. Integrins. Cell Tissue Res. 2010; 339:269–280. [PubMed: 19693543]
142. Imai Y, Shimaoka M, Kurokawa M. Essential roles of VLA-4 in the hematopoietic system. Int J Hematol. 2010; 91:569–575. [PubMed: 20352381]
143. Carman CV, Springer TA. Integrin avidity regulation: are changes in affinity and conformation underemphasized? Curr Opin Cell Biol. 2003; 15:547–556. [PubMed: 14519389]
144. Askari JA, Buckley PA, Mould AP, Humphries MJ. Linking integrin conformation to function. J Cell Sci. 2009; 122:165–170. [PubMed: 19118208]
145. Liddington RC, Ginsberg MH. Integrin activation takes shape. J Cell Biol. 2002; 158:833–839. [PubMed: 12213832]
146. Chigaev A, Zwartz G, Graves SW, Dwyer DC, Tsuji H, Foutz TD, et al. Alpha4beta1 integrin affinity changes govern cell adhesion. J Biol Chem. 2003; 278:38174–38182. [PubMed: 12844491]

147. Hemler ME, Elices MJ, Parker C, Takada Y. Structure of the integrin VLA-4 and its cell-cell and cell-matrix adhesion functions. Immunol Rev. 1990; 114:45–65. [PubMed: 2142475]

148. Berlin C, Berg EL, Briskin MJ, Andrew DP, Kilshaw PJ, Holzmann B, et al. Alpha 4 beta 7 integrin mediates lymphocyte binding to the mucosal vascular addressin MAdCAM-1. Cell. 1993; 74:185–195. [PubMed: 7687523]

149. Masumoto A, Hemler ME. Multiple activation states of VLA-4. Mechanistic differences between adhesion to CS1/fibronectin and to vascular cell adhesion molecule-1. J Biol Chem. 1993; 268:228–234. [PubMed: 7677996]

150. Lobb RR, Antognetti G, Pepinsky RB, Burkly LC, Leone DR, Whitty A. A direct binding assay for the vascular cell adhesion molecule-1 (VCAM1) interaction with alpha 4 integrins. Cell Adhes Commun. 1995; 3:385–397. [PubMed: 8640376]

151. Jakubowski A, Rosa MD, Bixler S, Lobb R, Burkly LC. Vascular cell adhesion molecule (VCAM)-Ig fusion protein defines distinct affinity states of the very late antigen-4 (VLA-4) receptor. Cell Adhes Commun. 1995; 3:131–142. [PubMed: 7583006]

152. Petty JM, Lenox CC, Weiss DJ, Poynter ME, Suratt BT. Crosstalk between CXCR4/stromal derived factor-1 and VLA-4/VCAM-1 pathways regulates neutrophil retention in the bone marrow. J Immunol. 2009; 182:604–612. [PubMed: 19109194]

153. Peled A, Grabovsky V, Habler L, Sandbank J, Arenzana-Seisdedos F, Petit I, et al. The chemokine SDF-1 stimulates integrin-mediated arrest of CD34(+) cells on vascular endothelium under shear flow. J Clin Invest. 1999; 104:1199–1211. [PubMed: 10545519]

154. DiVietro JA, Brown DC, Sklar LA, Larson RS, Lawrence MB. Immobilized stromal cell-derived factor-1alpha triggers rapid VLA-4 affinity increases to stabilize lymphocyte tethers on VCAM-1 and subsequently initiate firm adhesion. J Immunol. 2007; 178:3903–3911. [PubMed: 17339490]

155. Sanz-Rodriguez F, Hidalgo A, Teixido J. Chemokine stromal cell-derived factor-1alpha modulates VLA-4 integrin-mediated multiple myeloma cell adhesion to CS-1/fibronectin and VCAM-1. Blood. 2001; 97:346–351. [PubMed: 11154207]

156. Levesque JP, Leavesley DI, Niutta S, Vadas M, Simmons PJ. Cytokines increase human hemopoietic cell adhesiveness by activation of very late antigen (VLA)-4 and VLA-5 integrins. J Exp Med. 1995; 181:1805–1815. [PubMed: 7536795]

157. Kodama H, Nose M, Niida S, Nishikawa S. Involvement of the c-kit receptor in the adhesion of hematopoietic stem cells to stromal cells. Exp Hematol. 1994; 22:979–984. [PubMed: 7522185]

158. Kovach NL, Lin N, Yednock T, Harlan JM, Broudy VC. Stem cell factor modulates avidity of alpha 4 beta 1 and alpha 5 beta 1 integrins expressed on hematopoietic cell lines. Blood. 1995; 85:159–167. [PubMed: 7528564]

159. Makarem R, Newham P, Askari JA, Green LJ, Clements J, Edwards M, et al. Competitive binding of vascular cell adhesion molecule-1 and the HepII/IIICS domain of fibronectin to the integrin alpha 4 beta 1. J Biol Chem. 1994; 269:4005–4011. [PubMed: 7508437]

160. Elices MJ, Osborn L, Takada Y, Crouse C, Luhowskyj S, Hemler ME, et al. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. Cell. 1990; 60:577–584. [PubMed: 1689216]

161. Guan JL, Hynes RO. Lymphoid cells recognize an alternatively spliced segment of fibronectin via the integrin receptor alpha 4 beta 1. Cell. 1990; 60:53–61. [PubMed: 2295088]

162. Wayner EA, Garcia-Pardo A, Humphries MJ, McDonald JA, Carter WG. Identification and characterization of the T lymphocyte adhesion receptor for an alternative cell attachment domain (CS-1) in plasma fibronectin. J Cell Biol. 1989; 109:1321–1330. [PubMed: 2527858]

163. Williams DA, Rios M, Stephens C, Patel VP. Fibronectin and VLA-4 in haematopoietic stem cell-microenvironment interactions. Nature. 1991; 352:438–441. [PubMed: 1861722]

164. Fong S, Jones S, Renz ME, Chiu HH, Ryan AM, Presta LG, et al. Mucosal addressin cell adhesion molecule-1(MAdCAM-1). Its binding motif for alpha 4 beta 7 and role in experimental colitis. Immunol Res. 1997; 16:299–311. [PubMed: 9379078]

165. Viney JL, Jones S, Chiu HH, Lagrimas B, Renz ME, Presta LG, et al. Mucosal addressin cell adhesion molecule-1: a structural and functional analysis demarcates the integrin binding motif. J Immunol. 1996; 157:2488–2497. [PubMed: 8805649]

166. Connor EM, Eppihimer MJ, Morise Z, Granger DN, Grisham MB. Expression of mucosal addressin cell adhesion molecule-1 (MAdCAM-1) in acute and chronic inflammation. J Leukoc Biol. 1999; 65:349–355. [PubMed: 10080539]

167. Quinlan KL, Song IS, Naik SM, Letran EL, Olerud JE, Bunnett NW, et al. VCAM-1 expression on human dermal microvascular endothelial cells is directly and specifically up-regulated by substance P. J Immunol. 1999; 162:1656–1661. [PubMed: 9973426]

168. Shyjan AM, Bertagnolli M, Kenney CJ, Briskin MJ. Human mucosal addressin cell adhesion molecule-1 (MAdCAM-1) demonstrates structural and functional similarities to the alpha 4 beta 7-integrin binding domains of murine MAdCAM-1, but extreme divergence of mucin-like sequences. J Immunol. 1996; 156:2851–2857. [PubMed: 8609404]

169. Erle DJ, Briskin MJ, Butcher EC, Garcia-Pardo A, Lazarovits AI, Tidswell M. Expression and function of the MAdCAM-1 receptor, integrin alpha 4 beta 7, on human leukocytes. J Immunol. 1994; 153:517–528. [PubMed: 7517418]

170. Bellucci R, De Propris MS, Buccisano F, Lisci A, Leone G, Tabilio A, et al. Modulation of VLA-4 and L-selectin expression on normal CD34+ cells during mobilization with G-CSF. Bone Marrow Transplant. 1999; 23:1–8. [PubMed: 10037043]

171. Prosper F, Stroncek D, McCarthy JB, Verfaillie CM. Mobilization and homing of peripheral blood progenitors is related to reversible downregulation of alpha4 beta1 integrin expression and function. J Clin Invest. 1998; 101:2456–2467. [PubMed: 9616217]

172. Mohle R, Murea S, Kirsch M, Haas R. Differential expression of L-selectin, VLA-4, and LFA-1 on CD34+ progenitor cells from bone marrow and peripheral blood during G-CSF-enhanced recovery. Exp Hematol. 1995; 23:1535–1542. [PubMed: 8542944]

173. Leavesley DI, Oliver JM, Swart BW, Berndt MC, Haylock DN, Simmons PJ. Signals from platelet/endothelial cell adhesion molecule enhance the adhesive activity of the very late antigen-4 integrin of human CD34+ hemopoietic progenitor cells. J Immunol. 1994; 153:4673–4683. [PubMed: 7525710]

174. Yamaguchi M, Ikebuchi K, Hirayama F, Sato N, Mogi Y, Ohkawara J, et al. Different adhesive characteristics and VLA-4 expression of CD34(+) progenitors in G0/G1 versus S+G2/M phases of the cell cycle. Blood. 1998; 92:842–848. [PubMed: 9680352]

175. Lichterfeld M, Martin S, Burkly L, Haas R, Kronenwett R. Mobilization of CD34+ haematopoietic stem cells is associated with a functional inactivation of the integrin very late antigen 4. Br J Haematol. 2000; 110:71–81. [PubMed: 10930981]

176. Stephens LE, Sutherland AE, Klimanskaya IV, Andrieux A, Meneses J, Pedersen RA, et al. Deletion of beta 1 integrins in mice results in inner cell mass failure and peri-implantation lethality. Genes Dev. 1995; 9:1883–1895. [PubMed: 7544312]

177. Yang JT, Rayburn H, Hynes RO. Cell adhesion events mediated by alpha 4 integrins are essential in placental and cardiac development. Development. 1995; 121:549–560. [PubMed: 7539359]

178. Fassler R, Meyer M. Consequences of lack of beta 1 integrin gene expression in mice. Genes Dev. 1995; 9:1896–1908. [PubMed: 7544313]

179. Potocnik AJ, Brakebusch C, Fassler R. Fetal and adult hematopoietic stem cells require beta1 integrin function for colonizing fetal liver, spleen, and bone marrow. Immunity. 2000; 12:653–663. [PubMed: 10894165]

180. Hirsch E, Iglesias A, Potocnik AJ, Hartmann U, Fassler R. Impaired migration but not differentiation of haematopoietic stem cells in the absence of beta1 integrins. Nature. 1996; 380:171–175. [PubMed: 8600394]

181. Arroyo AG, Yang JT, Rayburn H, Hynes RO. Alpha4 integrins regulate the proliferation/differentiation balance of multilineage hematopoietic progenitors in vivo. Immunity. 1999; 11:555–566. [PubMed: 10591181]

182. Arroyo AG, Yang JT, Rayburn H, Hynes RO. Differential requirements for alpha4 integrins during fetal and adult hematopoiesis. Cell. 1996; 85:997–1008. [PubMed: 8674127]

183. Scott LM, Priestley GV, Papayannopoulou T. Deletion of alpha4 integrins from adult hematopoietic cells reveals roles in homeostasis, regeneration, and homing. Mol Cell Biol. 2003; 23:9349–9360. [PubMed: 14645544]

184. Priestley GV, Ulyanova T, Papayannopoulou T. Sustained alterations in biodistribution of stem/progenitor cells in Tie2Cre+ alpha4(f/f) mice are hematopoietic cell autonomous. Blood. 2007; 109:109–111. [PubMed: 16931623]

185. Priestley GV, Scott LM, Ulyanova T, Papayannopoulou T. Lack of alpha4 integrin expression in stem cells restricts competitive function and self-renewal activity. Blood. 2006; 107:2959–2967. [PubMed: 16357327]

186. Gribi R, Hook L, Ure J, Medvinsky A. The differentiation program of embryonic definitive hematopoietic stem cells is largely alpha4 integrin independent. Blood. 2006; 108:501–509. [PubMed: 16551970]

187. Brakebusch C, Fillatreau S, Potocnik AJ, Bungartz G, Wilhelm P, Svensson M, et al. Beta1 integrin is not essential for hematopoiesis but is necessary for the T cell-dependent IgM antibody response. Immunity. 2002; 16:465–477. [PubMed: 11911830]

188. Bungartz G, Stiller S, Bauer M, Muller W, Schippers A, Wagner N, et al. Adult murine hematopoiesis can proceed without beta1 and beta7 integrins. Blood. 2006; 108:1857–1864. [PubMed: 16735603]

189. Papayannopoulou T, Craddock C, Nakamoto B, Priestley GV, Wolf NS. The VLA4/VCAM-1 adhesion pathway defines contrasting mechanisms of lodgement of transplanted murine hemopoietic progenitors between bone marrow and spleen. Proc Natl Acad Sci U S A. 1995; 92:9647–9651. [PubMed: 7568190]

190. Vermeulen M, Le Pesteur F, Gagnerault MC, Mary JY, Sainteny F, Lepault F. Role of adhesion molecules in the homing and mobilization of murine hematopoietic stem and progenitor cells. Blood. 1998; 92:894–900. [PubMed: 9680357]

191. Bonig H, Watts KL, Chang KH, Kiem HP, Papayannopoulou T. Concurrent blockade of alpha4-integrin and CXCR4 in hematopoietic stem/progenitor cell mobilization. Stem Cells. 2009; 27:836–837. [PubMed: 19350684]

192. Papayannopoulou T, Priestley GV, Nakamoto B, Zafiropoulos V, Scott LM, Harlan JM. Synergistic mobilization of hemopoietic progenitor cells using concurrent beta1 and beta2 integrin blockade or beta2-deficient mice. Blood. 2001; 97:1282–1288. [PubMed: 11222371]

193. Craddock CF, Nakamoto B, Andrews RG, Priestley GV, Papayannopoulou T. Antibodies to VLA4 integrin mobilize long-term repopulating cells and augment cytokine-induced mobilization in primates and mice. Blood. 1997; 90:4779–4788. [PubMed: 9389694]

194. Papayannopoulou T, Priestley GV, Nakamoto B. Anti-VLA4/VCAM-1-induced mobilization requires cooperative signaling through the kit/mkit ligand pathway. Blood. 1998; 91:2231–2239. [PubMed: 9516120]

195. Papayannopoulou T, Nakamoto B. Peripheralization of hemopoietic progenitors in primates treated with anti-VLA4 integrin. Proc Natl Acad Sci U S A. 1993; 90:9374–9378. [PubMed: 7692447]

196. Christopher MJ, Liu F, Hilton MJ, Long F, Link DC. Suppression of CXCL12 production by bone marrow osteoblasts is a common and critical pathway for cytokine-induced mobilization. Blood. 2009

197. Bonig H, Wundes A, Chang KH, Lucas S, Papayannopoulou T. Increased numbers of circulating hematopoietic stem/progenitor cells are chronically maintained in patients treated with the CD49d blocking antibody natalizumab. Blood. 2008; 111:3439–3441. [PubMed: 18195093]

198. Jing D, Oelschlaegel U, Ordemann R, Holig K, Ehninger G, Reichmann H, et al. CD49d blockade by natalizumab in patients with multiple sclerosis affects steady-state hematopoiesis and mobilizes progenitors with a distinct phenotype and function. Bone Marrow Transplant. 2010; 45:1489–1496. [PubMed: 20098455]

199. Zohren F, Toutzaris D, Klarner V, Hartung HP, Kieseier B, Haas R. The monoclonal anti-VLA-4 antibody natalizumab mobilizes CD34+ hematopoietic progenitor cells in humans. Blood. 2008; 111:3893–3895. [PubMed: 18235044]

200. Polman CH, O'Connor PW, Havrdova E, Hutchinson M, Kappos L, Miller DH, et al. A randomized, placebo-controlled trial of natalizumab for relapsing multiple sclerosis. N Engl J Med. 2006; 354:899–910. [PubMed: 16510744]

201. Rudick RA, Stuart WH, Calabresi PA, Confavreux C, Galetta SL, Radue EW, et al. Natalizumab plus interferon beta-1a for relapsing multiple sclerosis. N Engl J Med. 2006; 354:911–923. [PubMed: 16510745]
202. Hauser SL, Weiner HL. Natalizumab: immune effects and implications for therapy. Ann Neurol. 2006; 59:731–732. [PubMed: 16634007]
203. Stuve O, Marra CM, Jerome KR, Cook L, Cravens PD, Cepok S, et al. Immune surveillance in multiple sclerosis patients treated with natalizumab. Ann Neurol. 2006; 59:743–747. [PubMed: 16634029]
204. Rice GP, Hartung HP, Calabresi PA. Anti-alpha4 integrin therapy for multiple sclerosis: mechanisms and rationale. Neurology. 2005; 64:1336–1342. [PubMed: 15851719]
205. Foley J. Recommendations for the selection, treatment, and management of patients utilizing natalizumab therapy for multiple sclerosis. Am J Manag Care. 2010; 16:S178–183. [PubMed: 20615054]
206. Ransohoff RM. Natalizumab for multiple sclerosis. N Engl J Med. 2007; 356:2622–2629. [PubMed: 17582072]
207. Davenport RJ, Munday JR. Blocking alpha4-integrins -A small molecule approach to treatment of multiple sclerosis. J Neurol Sci. 2008; 274:27–30. [PubMed: 18603264]
208. Davenport RJ, Munday JR. Alpha4-integrin antagonism--an effective approach for the treatment of inflammatory diseases? Drug Discov Today. 2007; 12:569–576. [PubMed: 17631252]
209. Jackson DY. Alpha 4 integrin antagonists. Curr Pharm Des. 2002; 8:1229–1253. [PubMed: 12052218]
210. Yang GX, Hagmann WK. VLA-4 antagonists: potent inhibitors of lymphocyte migration. Med Res Rev. 2003; 23:369–392. [PubMed: 12647315]
211. Sagi, K.; Izawa, H.; Chiba, A.; Okuzumi, T.; Yoshimura, T.; Tanaka, Y., et al. Novel phenylalanine derivative. Patent. WO2003070709. 2003.
212. Ghosh S, Panaccione R. Anti-adhesion molecule therapy for inflammatory bowel disease. Therapeutic Advances in Gastroenterology. 2010; 3:239–258. [PubMed: 21180606]
213. Takazoe M, Watanabe M, Kawaguchi T, Matsumoto T, Oshitani N, Matsui T, et al. Oral Alpha-4 Integrin Inhibitor (AJM300) in Patients with Active Crohn's Disease — A Randomized, Double-Blind, Placebo-Controlled Trial. Gastroenterology. 2009; 136:abstract [S1066].
214. Muro F, Iimura S, Sugimoto Y, Yoneda Y, Chiba J, Watanabe T, et al. Discovery of trans-4-[1-[[2,5-Dichloro-4-(1-methyl-3-indolylcarboxamido)phenyl]acetyl]-(4S)-methoxy-(2S)-pyrrolidinylmethoxy]cyclohexanecarboxylic acid: an orally active, selective very late antigen-4 antagonist. J Med Chem. 2009; 52:7974–7992. [PubMed: 19891440]
215. Leone DR, Giza K, Gill A, Dolinski BM, Yang W, Perper S, et al. An assessment of the mechanistic differences between two integrin alpha 4 beta 1 inhibitors, the monoclonal antibody TA-2 and the small molecule BIO5192, in rat experimental autoimmune encephalomyelitis. J Pharmacol Exp Ther. 2003; 305:1150–1162. [PubMed: 12626659]
216. Ramirez P, Rettig MP, Uy GL, Deych E, Holt MS, Ritchey JK, et al. BIO5192, a small molecule inhibitor of VLA-4, mobilizes hematopoietic stem and progenitor cells. Blood. 2009; 114:1340–1343. [PubMed: 19571319]
217. Chow A, Lucas D, Hidalgo A, Mendez-Ferrer S, Hashimoto D, Scheiermann C, et al. Bone marrow CD169+ macrophages promote the retention of hematopoietic stem and progenitor cells in the mesenchymal stem cell niche. J Exp Med. 2011; 208:261–271. [PubMed: 21282381]
218. Ehninger A, Trumpp A. The bone marrow stem cell niche grows up: mesenchymal stem cells and macrophages move in. J Exp Med. 2011; 208:421–428. [PubMed: 21402747]
219. Winkler IG, Sims NA, Pettit AR, Barbier V, Nowlan B, Helwani F, et al. Bone marrow macrophages maintain hematopoietic stem cell (HSC) niches and their depletion mobilizes HSCs. Blood. 2010; 116:4815–4828. [PubMed: 20713966]
220. Christopher MJ, Rao M, Liu F, Woloszynek JR, Link DC. Expression of the G-CSF receptor in monocytic cells is sufficient to mediate hematopoietic progenitor mobilization by G-CSF in mice. J Exp Med. 2011; 208:251–260. [PubMed: 21282380]
221. Lymperi S, Ersek A, Ferraro F, Dazzi F, Horwood NJ. Inhibition of osteoclast function reduces hematopoietic stem cell numbers in vivo. Blood. 2011; 117:1540–1549. [PubMed: 21131587]

222. Mendez-Ferrer S, Michurina TV, Ferraro F, Mazloom AR, Macarthur BD, Lira SA, et al. Mesenchymal and haematopoietic stem cells form a unique bone marrow niche. Nature. 2010; 466:829–834. [PubMed: 20703299]

223. Mendez-Ferrer S, Lucas D, Battista M, Frenette PS. Haematopoietic stem cell release is regulated by circadian oscillations. Nature. 2008; 452:442–447. [PubMed: 18256599]

224. Mendez-Ferrer S, Battista M, Frenette PS. Cooperation of beta(2)-and beta(3)-adrenergic receptors in hematopoietic progenitor cell mobilization. Ann N Y Acad Sci. 2010; 1192:139–144. [PubMed: 20392229]

225. Petit I, Szyper-Kravitz M, Nagler A, Lahav M, Peled A, Habler L, et al. G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. Nat Immunol. 2002; 3:687–694. [PubMed: 12068293]

226. Broxmeyer HE, Orschell CM, Clapp DW, Hangoc G, Cooper S, Plett PA, et al. Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. J Exp Med. 2005; 201:1307–1318. [PubMed: 15837815]

227. Fruehauf S, Seeger T, Maier P, Li L, Weinhardt S, Laufs S, et al. 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. [PubMed: 16863911]

228. Fruehauf S, Veldwijk MR, Seeger T, Schubert M, Laufs S, Topaly J, et al. 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. [PubMed: 19929463]

229. Larochelle A, Krouse A, Metzger M, Orlic D, Donahue RE, Fricker S, et al. AMD3100 mobilizes hematopoietic stem cells with long-term repopulating capacity in nonhuman primates. Blood. 2006; 107:3772–3778. [PubMed: 16439684]

230. Hidalgo A, Sanz-Rodriguez F, Rodriguez-Fernandez JL, Albella B, Blaya C, Wright N, et al. Chemokine stromal cell-derived factor-1alpha modulates VLA-4 integrin-dependent adhesion to fibronectin and VCAM-1 on bone marrow hematopoietic progenitor cells. Exp Hematol. 2001; 29:345–355. [PubMed: 11274763]

231. Kucia M, Reca R, Miekus K, Wanzeck J, Wojakowski W, Janowska-Wieczorek A, et al. Trafficking of normal stem cells and metastasis of cancer stem cells involve similar mechanisms: pivotal role of the SDF-1-CXCR4 axis. Stem Cells. 2005; 23:879–894. [PubMed: 15888687]

232. Peled A, Kollet O, Ponomaryov T, Petit I, Franitza S, Grabovsky V, et al. The chemokine SDF-1 activates the integrins LFA-1, VLA-4, and VLA-5 on immature human CD34(+) cells: role in transendothelial/stromal migration and engraftment of NOD/SCID mice. Blood. 2000; 95:3289–3296. [PubMed: 10828007]

233. Wysoczynski M, Reca R, Ratajczak J, Kucia M, Shirvaikar N, Honczarenko M, et al. Incorporation of CXCR4 into membrane lipid rafts primes homing-related responses of hematopoietic stem/progenitor cells to an SDF-1 gradient. Blood. 2005; 105:40–48. [PubMed: 15328152]

234. Golan K, Vagima Y, Ludin A, Itkin T, Kalinkovich A, Cohen-Gur S, et al. The Chemotactic Lipid S1P Regulates Hematopoietic Progenitor Cell Egress and Mobilization Via Its Major Receptor S1P1 and by SDF-1 Inhibition In a p38/Akt/mTOR Dependent Manner. Blood (ASH Annual Meeting Abstracts). 2010; 116:553.

235. Harun N, Thien M, Juarez JG, Bradstock KF, Bendall LJ. S1P1 Agonists for Use as Adjunct Mobilizing Agents. Blood (ASH Annual Meeting Abstracts). 2010; 116:826.

236. Ratajczak MZ, Lee H, Wysoczynski M, Wan W, Marlicz W, Laughlin MJ, et al. Novel insight into stem cell mobilization-plasma sphingosine-1-phosphate is a major chemoattractant that directs the egress of hematopoietic stem progenitor cells from the bone marrow and its level in peripheral blood increases during mobilization due to activation of complement cascade/membrane attack complex. Leukemia. 2010; 24:976–985. [PubMed: 20357827]

237. Yanai N, Matsui N, Furusawa T, Okubo T, Obinata M. Sphingosine-1-phosphate and lysophosphatidic acid trigger invasion of primitive hematopoietic cells into stromal cell layers. Blood. 2000; 96:139–144. [PubMed: 10891442]

238. Kimura T, Boehmler AM, Seitz G, Kuci S, Wiesner T, Brinkmann V, et al. The sphingosine 1-phosphate receptor agonist FTY720 supports CXCR4-dependent migration and bone marrow homing of human CD34+ progenitor cells. Blood. 2004; 103:4478–4486. [PubMed: 14988150]

239. Xue X, Cai Z, Seitz G, Kanz L, Weisel KC, Mohle R. Differential effects of G protein coupled receptors on hematopoietic progenitor cell growth depend on their signaling capacities. Ann N Y Acad Sci. 2007; 1106:180–189. [PubMed: 17360805]

240. Allende ML, Tuymetova G, Lee BG, Bonifacino E, Wu YP, Proia RL. S1P1 receptor directs the release of immature B cells from bone marrow into blood. J Exp Med. 2010; 207:1113–1124. [PubMed: 20404103]

241. Burger JA, Peled A. CXCR4 antagonists: targeting the microenvironment in leukemia and other cancers. Leukemia. 2009; 23:43–52. [PubMed: 18987663]

242. Lane SW, Scadden DT, Gilliland DG. The leukemic stem cell niche: current concepts and therapeutic opportunities. Blood. 2009; 114:1150–1157. [PubMed: 19401558]

243. Nervi B, Ramirez P, Rettig MP, Uy GL, Holt MS, Ritchey JK, et al. Chemosensitization of AML following mobilization by the CXCR4 antagonist AMD3100. Blood. 2008

244. Tavor S, Petit I. Can inhibition of the SDF-1/CXCR4 axis eradicate acute leukemia? Semin Cancer Biol. 2010; 20:178–185. [PubMed: 20637871]

245. Zeng Z, Shi YX, Samudio IJ, Wang RY, Ling X, Frolova O, et al. Targeting the leukemia microenvironment by CXCR4 inhibition overcomes resistance to kinase inhibitors and chemotherapy in AML. Blood. 2009; 113:6215–6224. [PubMed: 18955566]

246. 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. [PubMed: 16735597]

247. Jujo K, Hamada H, Iwakura A, Thorne T, Sekiguchi H, Clarke T, et al. 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. [PubMed: 20534467]

248. Khan A, Greenman J, Archibald SJ. Small molecule CXCR4 chemokine receptor antagonists: developing drug candidates. Curr Med Chem. 2007; 14:2257–2277. [PubMed: 17896975]

249. Kirton JP, Xu Q. Endothelial precursors in vascular repair. Microvasc Res. 2010; 79:193–199. [PubMed: 20184904]

250. Petit I, Jin D, Rafii S. The SDF-1-CXCR4 signaling pathway: a molecular hub modulating neo-angiogenesis. Trends Immunol. 2007; 28:299–307. [PubMed: 17560169]

251. Pitchford SC, Furze RC, Jones CP, Wengner AM, Rankin SM. Differential mobilization of subsets of progenitor cells from the bone marrow. Cell Stem Cell. 2009; 4:62–72. [PubMed: 19128793]

252. Ratajczak MZ, Zuba-Surma EK, Wysoczynski M, Ratajczak J, Kucia M. Very small embryonic-like stem cells: characterization, developmental origin, and biological significance. Exp Hematol. 2008; 36:742–751. [PubMed: 18474305]

253. Shepherd RM, Capoccia BJ, Devine SM, Dipersio J, Trinkaus KM, Ingram D, et al. Angiogenic cells can be rapidly mobilized and efficiently harvested from the blood following treatment with AMD3100. Blood. 2006; 108:3662–3667. [PubMed: 16912220]

254. Dreger P, Haferlach T, Eckstein V, Jacobs S, Suttorp M, Loffler H, et al. G-CSF-mobilized peripheral blood progenitor cells for allogeneic transplantation: safety, kinetics of mobilization, and composition of the graft. Br J Haematol. 1994; 87:609–613. [PubMed: 7527648]

255. Korbling M, Huh YO, Durett A, Mirza N, Miller P, Engel H, et al. Allogeneic blood stem cell transplantation: peripheralization and yield of donor-derived primitive hematopoietic progenitor cells (CD34+ Thy-1dim) and lymphoid subsets, and possible predictors of engraftment and graft-versus-host disease. Blood. 1995; 86:2842–2848. [PubMed: 7545476]

256. Stroncek DF, Clay ME, Herr G, Smith J, Jaszcz WB, Ilstrup S, et al. The kinetics of G-CSF mobilization of CD34+ cells in healthy people. Transfus Med. 1997; 7:19–24. [PubMed: 9089980]

257. Gazitt Y, Freytes CO, Akay C, Badel K, Calandra G. Improved mobilization of peripheral blood CD34+ cells and dendritic cells by AMD3100 plus granulocyte-colony-stimulating factor in non-Hodgkin's lymphoma patients. Stem Cells Dev. 2007; 16:657–666. [PubMed: 17784839]

258. Hess DA, Bonde J, Craft TP, Wirthlin L, Hohm S, Lahey R, et al. 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. [PubMed: 17382247]

259. Donahue RE, Jin P, Bonifacino AC, Metzger ME, Ren J, Wang E, et al. 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. [PubMed: 19602709]

260. Jin P, Wang E, Ren J, Childs R, Shin JW, Khuu H, et al. Differentiation of two types of mobilized peripheral blood stem cells by microRNA and cDNA expression analysis. J Transl Med. 2008; 6:39. [PubMed: 18647411]

261. Lin K, Ateeq HS, Hsiung SH, Chong LT, Zimmerman CN, Castro A, et al. Selective, tight-binding inhibitors of integrin alpha4beta1 that inhibit allergic airway responses. J Med Chem. 1999; 42:920–934. [PubMed: 10072689]

262. Norris V, Choong L, Tran D, Corden Z, Boyce M, Arshad H, et al. Effect of IVL745, a VLA-4 antagonist, on allergen-induced bronchoconstriction in patients with asthma. J Allergy Clin Immunol. 2005; 116:761–767. [PubMed: 16210048]

263. Baldwin, JJ.; McDonald, E.; Moriarity, K.; Sarko, C.; Machinaga, N.; Nakayama, A., et al. VLA-4 Inhibitor compounds. Patent WO2001000206. 2000.

264. Hijazi Y, Welker H, Dorr AE, Tang JP, Blain R, Renzetti LM, et al. Pharmacokinetics, safety, and tolerability of R411, a dual alpha4beta1-alpha4beta7 integrin antagonist after oral administration at single and multiple once-daily ascending doses in healthy volunteers. J Clin Pharmacol. 2004; 44:1368–1378. [PubMed: 15545307]

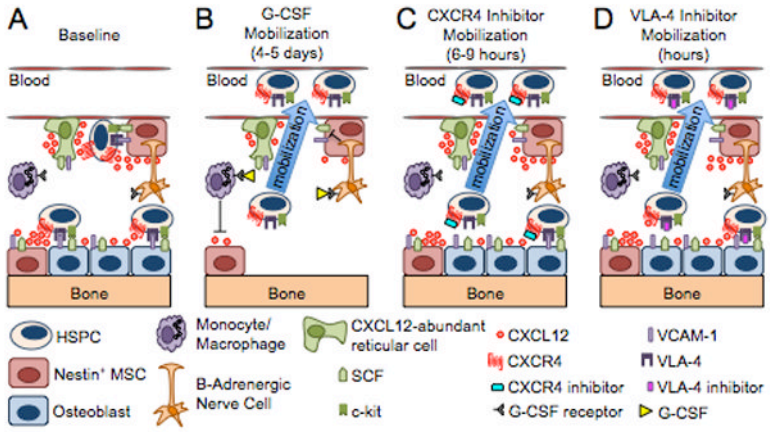

Figure 1. Models of HSPC mobilization by G-CSF and inhibitors of CXCR4 and VLA-4

(**A**) Bone marrow environment at baseline. HSPCs in the endosteal niche are likely in close contact with osteoblasts and nestin$^+$ mesenchymal stem cells (MSCs), both of which express numerous HSPC retention factors, including CXCL12, VCAM-1 and SCF. The perivascular niche is more distant from the endosteum and includes both nestin$^+$ MSCs and CXCL12 abundant reticular cells. Cells of the monocytic lineage support the maintenance of osteoblasts and MSCs. β-adrenergic nerve cells of the sympathetic nervous system regulate MSC proliferation and induce circadian oscillations of CXCL12 expression. (**B**) Model of G-CSF-induced HSPC mobilization. Cells of the monocytic lineage express the receptor for G-CSF and provide factors that support the survival of MSCs and osteoblasts. Upon 4 to 5 days of stimulation with G-CSF, the monocytes/macrophages disappear, leading to the loss of osteoblast lineage cells and reduced expression of CXCL12, VCAM-1, and SCF on MSCs. Reduced expression of these key HSPC retention factors is also observed following G-CSF signaling through β-adrenergic nerve cells. The net effect of these signaling cascades is the disruption of HSPC retention interactions and mobilization of HSPCs into the peripheral blood. (**C–D**) Model of HSPC mobilization by inhibitors of CXCR4 or VLA-4. Targeted disruption of the interaction of CXCR4 or VLA-4 with their ligands results in the rapid (within hours) and reversible mobilization of HSPCs into the peripheral circulation. An additive or synergistic affect on HSPC mobilization is observed when a CXCR4 inhibitor is combined with a VLA-4 antagonist, G-CSF or their combination. When used alone, inhibitors of CXCR4 and VLA-4 mobilize fewer HSPCs than G-CSF.

Table 1

Comparison of HSPC mobilization by plerixafor and/or G-CSF

Characteristic	Species	Comparison of Plerixafor to G-CSF	Reference
Time of peak $CD34^+$ cell mobilization	Human	Plerixafor induces peak mobilization of $CD34^+$ cells within 4–9 hours while G-CSF requires 4–5 days	80, 94, 226, 254–256
Magnitude of $CD34^+$ cell mobilization	Human	Plerixafor alone mobilizes fewer $CD34^+$ cells/μL blood than G-CSF and plerixafor-mobilized grafts contain fewer $CD34^+$ cells/kg recipient body weight compared with G-CSF.	94
	Human	Plerixafor significantly increases both G-CSF-stimulated mobilization of $CD34^+$ cells and leukapheresis yields of $CD34^+$ cells.	83, 84, 86, 87, 91–93, 228
$CD34^+$ stem cell phenotype	Human	Plerixafor preferentially mobilizes a $CD34^{dim}CD45RA^+CD123^{hi}CXCR4^{hi}$ plasmacytoid DC precursor	97
Primitive $CD34^+CD38^-$ HSC mobilization	Human	Plerixafor and G-CSF-mobilized grafts have a higher proportion of primitive 34^+38^- cells than grafts mobilized with G-CSF alone	228
CFU content	Human	Plerixafor mobilizes decreased numbers of BFU-E & CFU-GEMM compared to G-CSF	226
LTC-IC content	Human	Plerixafor mobilizes a higher frequency of LTC-IC compared to G-CSF	228
DC content	Human	Plerixafor and G-CSF-mobilized grafts have 2-fold more DCs (DC1 & DC2) than grafts mobilized with G-CSF alone	257
Frequency of SRCs	Human	Plerixafor mobilizes increased numbers of SRCs compared to G-CSF	226, 258
Cell cycle status	Rhesus macaque	Plerixafor-mobilized grafts have a higher proportion of $CD34^+$ cells in G_1 phase of cell cycle	229
CXCR4 expression	Rhesus macaque	Plerixafor-mobilized grafts have a higher percentage of $CD34^+$ cells expressing CXCR4 and VLA-4	229
	Human	Plerixafor and G-CSF-mobilized $CD34^+$ cells express more CXCR4 than $CD34^+$ cells mobilized with G-CSF alone	227, 228
VLA-4 expression	Rhesus macaque	Plerixafor-mobilized grafts have a higher percentage of $CD34^+$ cells expressing VLA-4	229
microRNA and cDNA expression	Rhesus macaque and Human	Plerixafor-mobilized HSPCs have unique microRNA and cDNA expression profiles compared to $CD34^+$ cells mobilized with G-CSF alone	227, 229, 259, 260
Lymphocyte Content	Human	Grafts mobilized with plerixafor contain more T, B and NK cells	94

Abbreviations: HSPC, hematopoietic stem and progenitor cell; G-CSF, granulocyte colony-stimulating factor; BFU-E, burst-forming unit-erythroid; CFU, colony forming unit; GEMM, granulocyte erythroid macrophage, megakaryocyte; LTC-IC, long-term culture-initiating cell; DC, dendritic cell; DC1, type 1 DC; DC2, type 2 DC; SRC, SCID repopulating cell

Table 2

Ongoing clinical trials using CXCR4 inhibitors for HSPC mobilization

Indication	Institution	Study Title	ClinicalTrials. gov Identifier
Autologous transplantation	Washington University School of Medicine	A phase I/II study of intravenous AMD3100 added to a mobilization regimen of G-CSF to increase the number of autologous peripheral blood stem cells collected from patients with lymphoma	NCT00733824
Autologous transplantation	Cincinnati Children's Hospital	AMD3100 in combination with G-CSF to mobilize peripheral blood stem cells in patients with Fanconi anemia: a phase I/II study	NCT00479115
Autologous transplantation	Duke University	An observational study of plerixafor mobilization rescue for autologous stem cell transplant patients with inadequate response to G-CSF	NCT00901225
Autologous transplantation	Mayo Clinic	Phase II trial of intravenously administered AMD3100 for stem cell mobilization in patients with multiple myeloma undergoing autologous stem cell transplantation following a lenalidomide-based initial therapy	NCT00998049
Autologous transplantation	CancerCare Manitoba	Open Label Phase II Trial of an Augmented Mobilization Strategy With Plerixafor in a Population at Risk for Poor Stem Cell Mobilization	NCT01037517
Autologous transplantation	City of Hope Medical Center	A Phase I/Pilot Study of Intravenous Plerixafor Following Cyclophosphamide Mobilization in Patients With Multiple Myeloma	NCT01074060
Autologous transplantation	Emory University	Evaluation of Plerixafor in Combination With Chemotherapy and G-CSF for CD34+ Cell Mobilization	NCT01095757
Autologous transplantation	Emory University	WCI1680-09: Evaluation of Alterations in Time of Administration of Plerixafor in Combination With G-CSF on Safety and CD34+ Cell Mobilization	NCT01149863
Autologous transplantation	Fred Hutchinson Cancer Research Center	Mobilization of Autologous Peripheral Blood Stem Cells in CD20+ Lymphoma Patients Using RICE, G-CSF, and Plerixafor	NCT01097057
Autologous transplantation	Sheba Medical Center	Plerixafor + Recombinant Human G-CSF for Autologous Peripheral Blood Stem Cell Transplantation in Hard to Mobilize Patients: a Phase IIB Study	NCT01164345
Autologous transplantation	University of Liverpool	A Comparison of Plerixafor/G-CSF With Chemotherapy/G-CSF for Stem Cell Mobilisation	NCT01186224
Autologous transplantation	University of Heidelberg	A Phase IIa, Proof of Concept Study is to Determine the Degree of Mobilisation of CD34+ Cells Following Administration of POL6326 in Patients With Multiple Myeloma	NCT01105403
Autologous transplantation	Chaim Sheba Medical Center	A Phase I/IIA, Non-Randomized, Open Label, Single Dose, Dose-Escalation, Safety Study of BKT140, a CXCR4 Antagonist in Patients With Multiple Myeloma	NCT01010880
Autologous transplantation	Multiple	A Phase II, Randomized, Open-Label, Multi-Center Study to Evaluate the Safety,	NCT01018979

Indication	Institution	Study Title	ClinicalTrials. gov Identifier
		Pharmacokinetics, and Hematopoietic Stem Cell Mobilization of TG-0054 in Patients With Multiple Myeloma, Non-Hodgkin Lymphoma or Hodgkin Disease	
Allogeneic transplantation	Washington University School of Medicine	A phase II study evaluating the safety and efficacy of intravenous AMD3100 for the mobilization and transplantation of HLA-matched sibling donor hematopoietic stem cells in patients with advanced hematological malignancies	NCT00914849
Healthy donors	Charite Research Organisation GmbH	A Single Center, Open-label, Repeated Dose, Phase I Study in Healthy Subjects to Assess the Safety, Tolerability, Pharmacokinetics and the Effect on Mobilization of Hematopoietic Stem Cells of NOX-A12 Alone and in Combination With Filgrastim	NCT01194934
Allogeneic transplantation	MD Anderson Cancer Center	G-CSF and plerixafor with busulfan and fludarabine for allogeneic stem cell transplantation for myeloid leukemias	NCT00822770
Allogeneic transplantation	Fred Hutchinson Cancer Research Center	A Pilot Study of Combined Plerixafor + Filgrastim for Mobilization of Peripheral Blood Stem Cells From Normal Donors	NCT01076270
Allogeneic transplantation	St. Jude Children's Research Hospital	A Pediatric Study of a Plerixafor Containing Regimen In Second Allogeneic Stem Cell Transplantation	NCT01068301

Abbreviations: G-CSF, granulocyte colony stimulating factor.

Table 3

Phenotype of mice following genetic ablation of α_4 or β1 integrin subunits

Integrin	Model	Phenotype	Reference
β_1	$\beta_1^{-/-}$ ES cells injected into wt or Rag-2$^{-/-}$ blastocysts	$\beta_1^{-/-}$ HSPCs develop and can differentiate into different lineages in vitro, but fail to migrate to fetal liver	178–180
α_4	$\alpha_4^{-/-}$ ES cells injected into wt or Rag-2$^{-/-}$ blastocysts	$\alpha_4^{-/-}$ HSPCs develop and migrate to fetal liver, spleen and BM, but exhibit decreased erythroid, myeloid and lymphoid hematopoiesis	181, 182
α_4	MxCre-mediated ablation of floxed α_4 in hematopoietic and nonhematopoietic cells	Eight-fold increase in circulating $\alpha_4^{-/-}$ CFU-Cs and increased sequestration of CFU-Cs in spleen.	183
α_4	Tie2Cre-mediated ablation of floxed α_4 in hematopoietic and endothelial cells	Increased numbers of circulating CFU-Cs	184
α_4	Transplantation of wt and/or $\alpha_4^{-/-}$ BM cells into adult irradiated recipients	$\alpha_4^{-/-}$ BM cells exhibit impaired homing to the BM, increased numbers of circulating CFU-Cs and decreased competitive repopulating activity	183–185
α_4	E12.5 cells from wt or $\alpha_4^{-/-}$ mice cotransplanted with wt BM cells into adult irradiated recipients	$\alpha_4^{-/-}$ HSPCs displayedsimilar engraftment, multilineage differentiation, and competitive repopulation capacity compared to wt cells	186
β_1	$\beta_1^{-/-}$ cells generated ex vivo were injected into lethally irradiated recipients	$\beta_1^{-/-}$ HSPCs were not radioprotective and failed to migrate to the spleen and BM	179
β_1	MxCre-mediated ablation of floxed β_1 in hematopoietic cells	No significant alterations in hematopoiesis or HSPC biodistribution	187
β_1 & β_7	MxCre-mediated ablation of floxed β_1 in $\beta_7^{-/-}$ hematopoietic cells	Transient increase of CFU-Cs in the BM and peripheral blood at 2 mos after β_1 deletion with return to baseline at 10 mos.	188
β_1 & β_7	MxCre-mediated ablation of floxed β_1 in $\beta_7^{-/-}$ hematopoietic and nonhematopoietic cells	Eight-fold increase in circulating HSPCs (CFU-C).	188

Abbreviations: ES, embryonic stem; Rag, recombination activating gene; E12.5; HSPC, hematopoietic stem and progenitor cell; BM, bone marrow; CFU-C, colony-forming unit-cells; wt, wild type; Embryonic day 12.5.

Table 4

Summary of α_4 integrin antagonists tested in human clinical trials

Drug	Company	Class	Disease	Clinical trial status	Reference
Bio-1211	Biogen/Merck	LDV	Asthma	Phase II	261
IVL-745	Aventis	LDV	Asthma	Phase II	262
TBC-4746	Encysive/Schering Plough	LDV	Asthma/MS	Phase I	208
DW-908e	Daiichi-Sankyp/Pharmacopeia	LDV	Asthma	Phase I	263
R-411/Valetegrast	Roche	N-acyl phenylalanine	Asthma	Phase II	264
AJM-300	Ajinomoto	N-acyl phenylalanine	IBD	Phase II	212, 213
SB-683699/Firategrast	GlaxoSmithKliine	N-acyl phenylalanine	MS	Phase II	208, 211
CDP323	UCB/Biogen	N-acyl phenylalanine	MS	Phase II	207
Compound 14e	Daiichi Sankyo	N-acyl phenylalanine	NA	Phase I	214

Abbreviations: LDV, leucine–aspartic acid–valine; MS, multiple sclerosis; IBD, inflammatory bowel disease.