Leukemia (2009) 23, 43–52

www.nature.com/leu

REVIEW

CXCR4 antagonists: targeting the microenvironment in leukemia and other cancers

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Hematopoietic and epithelial cancer cells express CXCR4, a seven-transmembrane G-protein-coupled chemokine receptor. Stromal cells within the bone marrow microenvironment constitutively secrete stromal cell-derived factor-1 (SDF-1/CXCL12), the ligand for CXCR4. Activation of CXCR4 induces leukemia cell trafficking and homing to the marrow microenvironment, where CXCL12 retains leukemia cells in close contact with marrow stromal cells that provide growth and drug resistance signals. CXCR4 antagonists, such as Plerixafor (AMD3100) and T140 analogs, can disrupt adhesive tumor–stroma interactions and mobilize leukemia cells from their protective stromal microenvironment, making them more accessible to conventional drugs. Therefore, targeting the CXCR4-CXCL12 axis is a novel, attractive therapeutic approach that is explored in ongoing clinical trials in leukemia patients. Initially, CXCR4 antagonists were developed for the treatment of HIV, where CXCR4 functions as a co-receptor for virus entry into T cells. Subsequently, CXCR4 antagonists were noticed to induce leukocytosis, and are currently used clinically for mobilization of hematopoietic stem cells. However, because CXCR4 plays a key role in cross-talk between leukemia cells (and a variety of other tumor cells) and their microenvironment, cancer treatment may become the ultimate application of CXCR4 antagonists. Here, we summarize the development of CXCR4 antagonists and their preclinical and clinical activities, focusing on leukemia and other cancers.
Leukemia (2009) **23,** 43–52; doi:10.1038/leu.2008.299; published online 6 November 2008
Keywords: CXCR4; CXCR4 antagonists; leukemia microenvironment; leukemia stem cells; CXCL12

CXCR4: a unique chemokine receptor

Chemokines are defined by their ability to induce directional migration of cells toward a gradient of the chemokine (chemotaxis) through binding to a subset of seven-transmembrane, G-protein-coupled (chemokine) receptors. Chemokines are small (~8–14 kDa) secreted proteins that are divided into the two main chemokine subfamilies on the basis of the arrangement of two N-terminal cysteine residues. These cysteine residues either have an amino acid between, or they are adjacent, accounting for CXC or CC chemokines.[1] In a more functional sense, chemokines can also be classified as inflammatory or homeostatic chemokines that are induced during inflammation to attract inflammatory cells,[2] or constitutively secreted by stromal cells (homeostatic chemokines). Homeostatic chemokines, such as CXCL12, coordinate cell trafficking and homing, which is essential during development and for homeostasis and function of the immune system.[3,4]

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Received 25 August 2008; revised 27 September 2008; accepted 1 October 2008; published online 6 November 2008

Stromal cell-derived factor-1 (SDF-1), now designated CXCL12,[1] signals through the CXCR4 chemokine receptor,[5,6] and was initially described as a pre-B-cell growth factor in 1994.[7] In 1996, the co-receptor function of CXCR4 for the entry of T-tropic (X4) human immunodeficiency virus (HIV)-1 strains into CD4-positive T cells was discovered.[5,6,8] Subsequently, CXCL12 and CXCR4 gene-deleted mice were described with an identical, lethal phenotype, suggesting a monogamous relationship between this chemokine and its receptor. The phenotype of these mice is characterized by deficient hematopoiesis with defects in B-cell development and myelopoiesis, and abnormal neuronal and cardiovascular development.[9–12] More recently, CXCR7 has been described as an alternate receptor for CXCL12, which appears to function by sequestering CXCL12[13] and modifying CXCR4 signaling rather than displaying autonomous signaling in response to CXCL12.[14] Mesenchymal stromal cells (MSCs) are considered a major source for CXCL12 in the adult organism. CXCL12-secreting stromal cells can be found in various tissues, such as the liver, lungs, lymphatic tissues and the marrow.[15] Constitutive high-level CXCL12 secretion by reticular stromal cells in the marrow is essential for homing[16] and maintenance of hematopoietic stem cells (HSCs) in distinct vascular and endosteal niches for their development and growth.[17] Through CXCL12, these stromal cells also attract circulating hematopoietic progenitor cells[16] or leukemia cells[18] for homing to the marrow (Figure 1). Actually, CXCR4 is the only functional chemokine receptor on hematopoietic progenitor cells,[19] emphasizing the predominant role of this chemokine receptor for homing and maintenance of HSC in the marrow niches. More recently, the architecture of niches for hematopoietic and other tissue stem cells (that is, germline stem cells, follicle stem cell, intestinal stem cell, central nervous system stem cell, and others) and the mechanism that govern stem cell homeostasis within these niches are emerging.[20,21] Regulated migration and homing of stem cells to tissue niches are critical steps not only during embryonic development or tissue repair but also in cancer (stem) cell dissemination.[22] In this context, the CXCR4-CXCL12 axis functions as a migration mechanism broadly conserved across species that is essential for stem cell migration in multiple tissues in both the embryo and adult. The responsiveness to CXCL12 significantly changes during differentiation of hematopoietic cells, as demonstrated for lymphoid[23] and myeloid[24] cells. These maturation-dependent changes in CXCL12 responsiveness, which are not necessarily accompanied by changes in CXCR4 expression levels,[23] are thought to regulate trafficking and homing to distinct tissue microenvironments. For example, high CXCL12 responsiveness allows for the retention and homing of immature and mature B cells (pre- and pro-B cells, and plasma cells) to the marrow.[25–27] This maturation-dependent CXCL12 responsiveness appears to be retained in malignancies that correspond to respective maturation stages of their normal counterparts; that is, pre- and

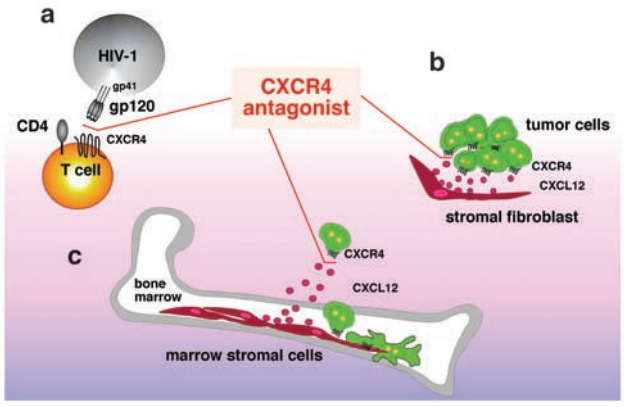

Figure 1 CXCR4 antagonists in human immunodeficiency (HIV-1) and cancer. (**a**) CXCR4 is the co-receptor used along with CD4 by T cell-tropic (X4) HIV-1 strains for cellular entry into T cells. A trimeric unit of viral envelope glycoproteins (gp120) that are anchored by gp41 binds to CD4 on the surface of T cells, inducing a conformational change of gp120, allowing it to interact with CXCR4 through the V3 loop of gp120. CXCR4 antagonists block the CXCR4-binding site for X4 HIV-1, and thereby prevent fusion of HIV-1 with T cells. (**b**) Stromal fibroblasts within the tumor microenvironment secrete CXCL12 and thereby attract and retain tumor cells in contact with the stroma. Adhesion of tumor cells to stromal cells confers survival, growth and drug resistance signals (cell adhesion-mediated drug resistance (CAM-DR)) that are, at least in part, mediated by activation of CXCR4 on the tumor cells. Stromal cell-mediated activation of CXCR4 is also called a 'paracrine' activation of tumor cells through CXCL12.[48] CXCR4 antagonists can disrupt the adhesive interactions between tumor cells and tumoral fibroblasts, mobilizing them from the tumor microenvironment, and making the tumor cells more accessible to cytotoxic drugs. (**c**) Tumor cells (hematopoietic and non-hematopoietic) also utilize the CXCR4-CXCL12 axis to migrate and home to target organs, such as the marrow. CXCL12 is constitutively secreted by marrow stromal cells retains leukemia cells in protective marrow niches and attracts circulating tumor cells for directional homing/metastasis. CXCR4 antagonists can inhibit this mechanism of tumor cell homing by blocking CXCR4 receptors responsible for migration to CXCL12-secreting stromal cells, thereby mobilizing tumor cells from tissue sites, such as the marrow.

pro-B-cell acute lymphoblastic leukemia (ALL) cells and multiple myeloma cells utilize the CXCR4/CXCL12 axis for bone marrow homing.[18,28]

There is also growing evidence suggesting that leukemia progression is driven by a sub-population of cells referred to as leukemia stem cells (LSCs) that are more leukemogenic than other cells of the same clone. LSCs share phenotypic and functional characteristics with their normal counterparts, and the hierarchical organization of the neoplastic clone mimics differentiation and cell turnover as part of homeostasis or tissue repair, nurtured by infrequent stem cells.[29–31] Normal and malignant stem cells apparently have a particular requirement for distinct niches: HSCs localize to CXCL12$^+$ stromal cells that are in close proximity to the marrow vasculature (vascular niche) or the endosteum.[17] Interestingly, brain tumor stem cells also reside in vascular niches adjacent to blood vessels,[32] suggesting that normal stem cells, LSCs and other cancer stem cells (CSCs) have fundamentally similar requirements toward their niches.[21,33] This concept implies that LSCs would preferentially localize through CXCR4 to vascular and endosteal niches that are normally restricted to HSC. Consequently, CXCR4 antagonists could mobilize LSCs and/or CSCs that are normally protected in tissue niches and make them accessible to conventional anticancer drugs.

Current clinical trials with AMD3100 utilized this mechanism of CXCR4-mediated homing to the marrow to mobilize HSC to the peripheral blood for HSC collection for autologous stem cell transplantation.[34] In phase II trials, mobilization with the combination of AMD3100 and granulocyte colony-stimulating factor (G-CSF) results in the collection of more progenitor cells than G-CSF alone.[35] The peptide CXCR4 antagonist TN14003 is also a potent mobilizer of HSC alone and in combination with G-CSF; and apparently TN14003 displays a pattern of hematopoietic cells and overall potency for HSC mobilization that is different from AMD3100.[36]

The CXCR4-CXCL12 axis is also related to tissue hypoxia and repair of hypoxic damage.[37] The transcription factor hypoxia-inducible factor-1 (HIF-1), which gets upregulated in hypoxic states, induces the local expression of CXCL12,[38] which in turn can attract circulating progenitor cells for tissue repair. This function of the CXCR4-CXCL12 axis plays a prominent role in the recruitment of marrow-derived progenitors to the heart after myocardial infarction.[39,40] Currently, there are research efforts to utilize this mechanism by local intramyocardial delivery of a protease-resistant CXCL12 to attract progenitor cells to sites of hypoxic damage after myocardial infarction.[41] Moreover, in hypoxic tumors or in tumors that display mutations in the von Hippel–Lindau tumor suppressor protein pVHL (a negative regulator of HIF-1), HIF-1 upregulates CXCR4 expression,[42,43] providing a survival benefit for tumor cells with high CXCR4 expression.

Blocking CXCR4 in the treatment of leukemia and other cancers

CXCR4 chemokine receptors are expressed by leukemia cells from patients with acute and chronic leukemias and also various solid tumors, such as breast cancer, lung cancer, prostate cancer and others.[37,44] In general, CXCR4 expression by the tumor cells allows for tumor cell migration, and homing of the neoplastic cells to sites where non-malignant stromal cells express CXCL12, the ligand for CXCR4.[45] This concept implies that tumor cell metastasis is not random, but guided by the expression of chemokine receptors and adhesion molecules on the neoplastic cells, and respective ligands in the target organs.[15,46] Tumor cells apparently utilize this mechanism to access microenvironments, such as the marrow, that provide factors that favor their growth. For example, the importance of CXCR4 for breast cancer metastasis to different target organs was demonstrated *in vitro* and *in vivo*.[15] Leukemia cells from patients with chronic lymphocytic leukemia (CLL) and ALL utilize CXCR4 for homing to marrow stromal cells (MSCs) *in vitro*[47] and *in vivo*.[18] Moreover, CXCL12 has a direct growth- and survival-promoting effect for various cancer cells, such as breast cancer cells[48] or CLL cells.[49] CXCL12 also promotes tumor progression by recruiting endothelial progenitor cells to tumors for tumor angiogenesis.[48] Stromal fibroblasts, also called carcinoma-associated fibroblasts or mesenchymal stromal cells (MSC), are part of the tumor microenvironment. These stromal fibroblasts constitutively secrete CXCL12, and contact between tumor cells and stromal cells is largely dependent on the CXCR4-CXCL12 axis. For example, co-culture between CLL cells[47] or lung cancer cells[50] and CXCL12-secreting stromal cells induces strong adhesion and spontaneous migration of the neoplastic cells beneath the stromal cells (pseudoemperipolesis) in a CXCR4-dependent manner. Nonspecific and specific

CXCR4 antagonists, such as pertussis toxin and CXCR4 antagonists (T140 and AMD3100) can block this adhesion and migration.[51] Adhesion to stromal cells confers resistance to spontaneous and drug-induced cell death of tumor cells, and therefore is also termed cell adhesion-mediated drug resistance.[52,53] Tumor cells that adhere to stromal cells through CXCR4 are therefore, at least partially, protected from the effects of cytotoxic chemotherapy and represent a reservoir for minimal residual disease (MRD) and relapses commonly seen in the treatment of patients with various cancers. However, the overall role of MSC in the tumor microenvironment remains controversial. Although on the one hand, MSC can provide survival, growth and drug resistance signals,[48,54] MSC can also induce cell cycle and growth arrest in epithelial cancer[55–57] and leukemia cells.[58] On the basis of these findings, the therapeutic use of MSC to decelerate tumor growth has been proposed.[59,60] These controversial findings regarding the capacity of MSCs to induce tumor progression (*in vivo*) and also to induce tumor cell growth arrest (*in vitro*) could, at least in part, be due to the ability of MSC to provide niches for CSCs *in vivo*. In these tissue niches, MSCs are thought to maintain and support a sub-population of growth-arrested tumor cells/CSCs that are resistant to cytotoxic treatments and function as a reservoir of the disease with the potential to proliferate and sustain the malignant process.[22]

In summary, the rationale for targeting CXCR4 with CXCR4 antagonists in leukemia and other cancers is as follows:

1. disrupting the adhesive stromal interactions that confer survival and drug resistance signals to leukemia and other cancer cells;
2. mobilizing tumor cells from tissue sites, such as the marrow, and thereby making them better accessible to conventional therapy;
3. blocking of migration and dissemination of tumor cells in the process of tumor cell metastasis;
4. blocking of paracrine growth and survival signals through activation of the CXCR4-CXCL12 axis and
5. blocking pro-angiogenesis effects of CXCL12.

These different mechanisms through which CXCR4 antagonists may display activity in treatment of neoplastic diseases raise questions about when during the course of the disease and for how long they should be administered, and whether CXCR4 antagonists should be given alone or in combination with conventional anticancer drugs. This question will be addressed by clinical trials that are currently designed or ongoing.

CXCR4 antagonists

CXCR4 antagonists were initially developed as new drugs for the treatment of HIV-1 infection. At the time of their discovery in the early 1990s, the mechanism of anti-HIV activity of the most prominent CXCR4 antagonists, T140 and its analogs,[61,62] AMD3100[63,64] and ALX-4C,[65] was unknown. After the discovery of the co-receptor function of CXCR4 for T tropic HIV-1, the specific CXCR4-blocking function of the different CXCR4 antagonists was rapidly demonstrated.[66–68] With the rapid increase in our knowledge of other, non-HIV-related functions of CXCR4 over the past 11 years, other potential applications such as HSC mobilization and treatment of cancer and autoimmune disease are emerging and have gradually replaced the original intent to use CXCR4 antagonists as anti-HIV drugs.

In general, four major classes of CXCR4 antagonists and agonists can be distinguished: (a) small peptide CXCR4 antagonists, such as T140 and its analogs (TN14003 and others), (b) non-peptide CXCR4 antagonists, such as the bicyclam AMD3100, (c) antibodies to CXCR4 and (d) modified agonists and antagonists for SDF-1. Below, we will summarize the current status of preclinical and clinical development for CXCR4 antagonists.

Small peptide antagonist of CXCR4

Initially, this group of small peptide CXCR4 antagonists was discovered screening naturally occurring peptides for anti-HIV activity. In that process, self-defense peptides from horseshoe crabs, called tachyplesin (from the Japanese horseshoe crab *Tachypleus tridentatus*) and polyphemusin (from the American horseshoe crab *Limulus polyphemus*), were identified and chemically modified, leading to the synthesis of the anti-HIV peptides T22,[62] T134 and T140.[69] Initially, these compounds were thought to function by inhibiting HIV-1–T-cell fusion or the viral uncoating.[61] However, the precise mechanism of anti-HIV activity remained unclear until the discovery that T tropic HIV-1 (X4-HIV-1) utilizes CXCR4 as a co-receptor for cellular entry into CD4-positive T cells. Soon after this, it was demonstrated the T22 specifically binds to CXCR4 and blocks CXCR4 receptor regions that are critical for HIV-1 viral entry and for activation by its natural ligand, CXCL12.[68] T140 is considered the most active CXCR4 peptide antagonist among the initially synthesized peptides, but lacks serum stability due to cleavage of the C-terminal Arg. Therefore, C-terminally amidated T140 analogs were developed to overcome serum instability,[70] leading to the synthesis of TN14003 and TC14012. Further work revealed the binding regions for T140 within the extracellular domains and regions of the hydrophobic core of CXCR4, which are distinct from the binding region for AMD3100.[71] Also, in a series of experiments to elucidate the mechanism of CXCR4 signaling, it was noticed that T140 decreased autonomous CXCR4 signaling in CXCR4 wild-type or constitutively active CXCR4 mutants, characterizing T140 as an inverse CXCR4 agonist, whereas AMD3100 and ALX40-4C displayed partial agonist activity in this study.[72] Clinical trials will help to determine whether this characteristic of CXCR4 peptide antagonists correlates with a profile of activities and/or side effects that is distinct from AMD3100.

The efficacy of T140 and its analogs for blocking CXCR4 *in vitro* and *in vivo* has been documented in numerous preclinical studies, including *in vivo* models for breast cancer and melanoma,[73,74] rheumatoid arthritis[75] and stem cell mobilization.[36] Other studies explored the activity of these agents in acute[76,77] and chronic leukemias,[54] multiple myeloma,[78] small cell lung cancer (SCLC),[50] malignant melanoma[74] and pancreatic cancer.[79] Besides these disease-oriented studies, T140 and its analogs have been used in basic studies exploring the function of CXCR4 in dendritic cell development[80] and migration,[81] B-cell homing and germinal center positioning within lymphatic tissues,[82] and HSC homing.[83] Currently, the T140 analog TN14003 is under clinical development by Biokine Therapeutics Ltd, Rehovot, Israel.

ALX40-4C is a polypeptide of nine Arg residues that is stabilized by terminal protection and inclusion of D-amino acids. ALX40-4C is a specific CXCR4 antagonist,[66] and was the first CXCR4 antagonist clinically used in phase I and phase I/II trials in HIV patients conducted by the Canadian company Allelix Biopharmaceuticals, Mississauga, ON, Canada.[65] This peptide is no longer under development, particularly because of formulation difficulties and lack of efficacy, and also because it is unlikely that an oral formulation of this complex peptide can be produced.

Non-peptide CXCR4 antagonists

AMD3100 is a bicyclam, in which two cyclam rings are connected through an aromatic bridge. AMD3100 possesses the highest anti-HIV activity among a series of bicyclams that were synthesized in the early 1990s.[64,84] AMD3100 is a specific antagonist of CXCL12 binding to CXCR4, inhibiting CXCL12-mediated calcium mobilization, chemotaxis and GTP binding, and does not cross-react with other chemokine receptors.[85] AMD3100 was initially considered to interfere with HIV-1 fusion or uncoating.[63] Initially developed at Johnson Matthey in collaboration with the Rega Institute for Medical Research (Leuven University, Leuven, Belgium), this compound was first called JM3100, which changed to AMD3100 after a new company (AnorMED, Langley, BC, Canada) took over the development. The anti-HIV-1 activity of AMD3100, restricted to X4-HIV-1 strains, and the blocking function of AMD3100 on gp120 interaction with CXCR4 during viral entry[86] were the initial focus during the early development of this drug. However, an unexpected rapid, transient leukocytosis was noticed during phase I/II clinical trials of AMD3100 in volunteers and HIV-infected patients, caused by the mobilization of various hematopoietic cells, including CD34-positive HSC, to the blood.[87,88] In the second trial in HIV patients, one patient receiving the highest dose of AMD3100 (160 μg/kg/h) had a significant drop in his viral load, but overall the efficacy of AMD3100 in affecting disease activity in HIV-1 patients was considered low and therefore this application was not further pursued for AMD3100. Instead, AnorMED explored AMD3100 as a mobilizing agent for HSC,[88] and a subsequent series of preclinical and clinical trials demonstrated that AMD3100 alone and in combination with G-CSF mobilizes HSC.[34,89,90] AMD3100 (recently re-named as Plerixafor or Mozobil) is now owned by Genzyme Corporation (Cambridge, MA, USA) after a recent takeover of AnorMED by Genzyme in late 2006. Plerixafor is currently used in phase III trials in lymphoma and multiple myeloma patients undergoing autologous stem cell mobilization, and current plans are to file for US and European approval of the drug in 2008. The activity of Plerixafor to inhibit CXCR4 activation in various *in vitro* and *in vivo* tumor models, such as inhibition of CXCL12-induced tumor cell migration and downstream signaling and activity in murine tumor models has been reported. These studies are summarized in Table 1.

AMD070 is another orally bioavailable small molecule CXCR4 antagonist with anti-HIV activity that Genzyme is currently developing in phase II trials for HIV-1 treatment.[91]

KRH-1636 is an orally available, non-peptide CXCR4 antagonist that inhibits infection by X4-HIV-1 virus and blocks responses to stimulation with CXCL12, such as calcium mobilization.[92]

Development antibodies to CXCR4

Neutralizing the interaction between CXCL12, the ligand for CXCR4, and CXCR4 by using anti-CXCR4 antibodies significantly inhibit HIV infection and tumor cell migration *in vitro*.[47,93,94] Furthermore, anti-human CXCR4 or CXCL12 antibodies also significantly impair metastasis and progression of non-Hodgkin's lymphoma, breast, lung and prostate tumors in animal models.[95–98] The unique properties of monoclonal antibody (mAb) therapies, including their high affinity and specificity, and the differential expression of target antigen in tumor cells versus normal cells make them attractive agents for cancer immunotherapy. Development of therapeutic mAbs to CXCR4 is challenging due to the fact that CXCR4 can exhibit conformational heterogeneity. Using a panel of mAbs to CXCR4, it was found that CXCR4 on both primary and transformed T, B and myeloid cells exhibited considerable conformational heterogeneity.[99] This conformational heterogeneity of CXCR4 explains the cell-type-dependent ability of CXCR4 antibodies to block chemotaxis to its ligand CXCL12. In addition, the mAb most commonly used to study CXCR4 expression, 12G5, recognizes only a sub-population of CXCR4 molecules on all primary cell types analyzed. As a result, CXCR4 concentrations on these important cell types have been underestimated to date. The factors responsible for altering CXCR4 conformation are not known. However, CXCR4 can be post-translationally modified by sulfation of its N-terminal tyrosines, and by a chondroitinsulfate chain at serine 18. This phenmenon may explain, in part, the difference in confirmation, antibody specificity and function of CXCR4.[100] Altered glycosylation patterns, neo-expression and underexpression or overexpression of glycans are hallmarks of cancer and may significantly affect the activity of various CXCR4 antagonists in development.

Modified CXCL12

CTCE-9908 and CTCE-0214 are peptide analogs of CXCL12 with inhibitory and agonist activity, respectively. CTCE-9908 that has received orphan drug status by the Food and Drug Administration for the treatment of osteogenic sarcoma. CTCE-9908 decreases growth and adhesion of osteosarcoma cells and the metastatic dissemination of cancer cells in two murine models.[101] CTCE-9908 is developed by Chemokine Therapeutics Corp., Vancouver, BC, Canada.

CXCR4 antagonists in selected cancers

CLL

B-cell CLL is a leukemia of mature, antigen-experienced B cells. CLL cells accumulate in the blood, marrow and secondary lymphoid tissues. Despite their apparent longevity *in vivo*, isolated CLL cells generally undergo spontaneous apoptosis *in vitro* when cultured under conditions that support the growth of human B-cell lines.[49] CLL cells express high levels of CXCR4,[47,102] and CLL cells spontaneously migrate beneath MSCs that secrete CXCL12 in a CXCR4-dependent manner.[47] Stromal cells and nurse-like cells,

Table 1 CXCR4 antagonists that are currently in preclinical and clinical development

Product name	*Company*	*Structure*	*Administration*	*Indication*	*Study phase*
Plerixafor (AMD3100)	Genzyme	Bicyclam	s.c.	Stem cell mobilization	Phase III
AMD070	Genzyme	Bicyclam derived	Oral	HIV	Phase I/II
CTCE-9908 antagonist	Chemokine Therapeutics Corp.	Modified SDF-1	s.c./i.v.	Solid tumors	Phase I/II
CTCE-0214-agonist	Chemokine Therapeutics Corp.	Modified SDF-1	s.c./i.v.	Mobilization BM recovery	Phase I/II
No name	Northwest Biotherapeutics, Bethesda, MD, USA	Antibody	s.c./i.v.	Cancer	Preclinical
TG-0054	TaiGen Biotechnology Co., Taipei, Taiwan	?	?	Stem mobilization for regeneration	Phase I/II
BKT140	Biokine Therapeutics	Modified peptide	s.c./oral	MM and leukemia	Phase I

Table 2 *In vitro* and *in vivo* efficacy of CXCR4 antagonists in solid tumors and leukemia/lymphoma

Cancer type	*In vitro studies*	*In vivo studies*
Solid tumors		
Breast cancer	AMD3100: blocks CXCL12-induced HER2-neu activation[133]	T140: reduced metastasis in murine model[73]; AMD3100: prolongs survival in murine model[128]
Small cell lung cancer (SCLC)	T140 and its analogs block adhesion and survival pathways[50,130]	
Pancreatic cancer	AMD3100 inhibits tumor cell migration and growth[134]	
Cholangiocarcinoma	AMD3100 inhibits tumor cell migration[135]	
Gastric cancer		AMD3100 reduced tumor growth in a murine model[136]
Colorectal cancer	AMD3100 inhibits tumor cell growth[137]	
Malignant melanoma	AMD3100 inhibits tumor cell activation and proliferation[138]	T140 analog inhibits metastatic melanoma,[74] T22 increases efficacy of immunotherapy in metastatic melanoma[139]
Glioma	AMD3100 inhibits tumor cell invasion[140]	
Other CNS tumors		AMD3100 inhibits glioblastoma and medulloblastoma growth in xenograft model[141]
Ovarian cancer	AMD3100 inhibits cancer cell migration and activation[142]	
Rhabdomyosarcoma	T140 blocked *in vitro* responses to CXCL12 [143]	
Prostate cancer	T140 blocks tumor cell invasion and signaling[144]	
Leukemia/lymphoma		
Chronic lymphocytic leukemia (CLL)	T140, TC14012 and TN14003 block migration, adhesion and stromal protection[54]; AMD3100 blocks actin polymerization in CLL cells[54]	
Acute myelogenous leukemia (AML)	RCP168 and AMD3465 block migration and CXCR4 signaling;[145] AMD3100 reduced AML cell survival[111]	
Acute lymphoblastic leukemia (ALL)	T140 and its analogs and AMD3100 inhibit ALL cell migration and adhesion[76]	T140 analogs, AMD3100 and AMD3465 mobilize ALL cells[77]
Multiple myeloma	T140 analogs block CXCL12-induced osteoclast activity[78]	AMD3100 inhibits *in vivo* homing of myeloma cells[28]
Non-Hodgkin's lymphoma		CXCR4 neutralization inhibited lymphoma growth[95]

Abbreviation: CNS, central nervous system.
The diseases in which CXCR4 antagonists showed activity, along with the respective references, are listed.

another stromal cell type derived from monocytes, protect CLL cells from spontaneous or drug-induced apoptosis in a contact-dependent manner. These observations support a model proposing that expression of CXCR4 by CLL cells allows for their recirculation between the blood and the marrow or lymphoid tissues, where they receive protective survival signals. We demonstrated earlier that CXCR4 antagonists effectively block CXCL12-induced activation, migration and signaling of CLL cells.[54] Also, CXCR4 antagonists reversed stromal cell-mediated protection from spontaneous or fludarabine-induced apoptosis of CLL cells, suggesting a potential role of CXCR4 antagonists in combination with a B-cell-targeted therapy in the treatment of CLL. Because of the high-level CXCR4 expression, and the particular requirement of stromal cell support for CLL cell survival, it appears that CLL patients would particularly respond to CXCR4 antagonists. The expected response to CXCR4 antagonists would be a mobilization of the CLL cells from the tissues (marrow and lymphoid tissues) to the blood, where CLL cells then could be targeted by mAbs, such as anti-CD20 or anti-CD52 mAbs, or cytotoxic agents.

Acute myeloid leukemia

Despite a general sensitivity to chemotherapy, long-term disease-free survival in patients with acute myelogenous leukemia (AML) is low because a majority of patients relapse from MRD. The marrow is considered the primary site for MRD where adhesion to stromal elements may protect AML cells from cytotoxic drugs. Several studies indicated that adhesion to MSCs affects the survival and proliferation of AML cells[58,103,104] and protects AML cells from chemotherapy *in vitro*[105] and *in vivo*.[106] Adhesion molecules, in particular the very late antigen-4 (VLA-4) integrins, along with CXCR4 chemokine receptors, are essential for AML cell adhesion to respective ligands (fibronectin and VCAM-1) on stromal cells[107] and for protection of AML cells from spontaneous or drug-induced apoptosis.[106,108] CXCR4 receptors are functional in AML,[58,109] and surface CXCR4 expression, which is generally low when compared with lymphoid cells, correlates with functional responses, such as chemotaxis.[110] CXCR4-dependent engraftment of AML cells in non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice was demonstrated by Tavor *et al.*,[111] and this group also reported that the proteolytic enzyme elastase is involved in regulating SDF-1-dependent migration and proliferation of AML cells *in vitro* and *in vivo*.[112] CXCR4, in cooperation with VLA-4 integrins, mediates spontaneous migration of AML cells beneath MSCs, along with a decreased proliferation of migrated AML cells within stromal layers.[58] This suggests that CXCR4 expression by AML cells

favors the enrichment of a non-cycling sub-population of AML cells within the stromal layer. These cells may be less susceptible to cytotoxic treatments, and they may represent dormant leukemia progenitors serving as a reservoir for MRD.[113] This function of CXCR4 could explain why CXCR4 surface expression on AML cells has such a profound negative prognostic impact in AML.[114–116] Plerixafor is currently used in an ongoing clinical trial for mobilization of AML cells from the protective marrow microenvironment to the blood, where the AML cells are then targeted by conventional cytotoxic drugs. The feasibility of using Plerixafor for AML cell mobilization to the blood in an animal model and in AML patients on this trial was recently reported.[117,118]

CXCR4 in ALL

Precursor B-cell ALL, the most common childhood malignancy and the second most common adult acute leukemia, is characterized by a high motility of the leukemia cells, resulting in leukemic infiltrates into extramedullary sites. In particular, ALL cells have a high affinity for the central nervous system, which is the most common location for extramedullary relapses, but also for the liver, spleen and the lymph nodes. The presumed normal counterparts of B-ALL cells are precursor B cells that are highly dependent on the stromal microenvironment during their maturation in the marrow.[119] Contact between precursor B cell and CXCL12-secreting stromal cells is maintained by CXCR4 expression on the B cells, and gene deletion of CXCR4 or CXCL12 in mouse models results in premature release of B-cell precursors into the circulation.[25] We reported that B-ALL cells express functional CXCR4 receptors that induce leukemia cell chemotaxis to CXCL12 and spontaneous migration beneath CXCL12-secreting stromal cells in a CXCR4- and VLA4 integrin-dependent manner, using the B-cell precursor lines NALM-6 and REH,[47,120] findings that were subsequently confirmed with primary ALL cells.[121,122] CXCR4 receptors on ALL cells participate in homing of leukemia cells to the marrow in NOD/SCID mice.[123,124] A recent study by Sipkins *et al.*[18] provided *in vivo* evidence that CXCR4 is necessary for homing of ALL cells to the marrow. Blocking CXCR4 on ALL cells with specific CXCR4 receptor antagonists blocked the migration of ALL cells to CXCL12 and MSCs, and partially disrupted the protection of ALL cells from cytotoxic agents by MSCs.[76] One of the mechanisms by which MSCs protect ALL cells from chemotherapeutic agents is related to asparagine synthetase, an enzyme that is critical to the biosynthesis of asparagine. MSCs constitutively secrete asparagine synthetase,[125] which interferes with asparaginase, a drug that has a critical impact in the treatment of ALL patients. Through this mechanism, ALL cells may survive in marrow stromal niches and thus represent the seed for residual disease and relapses.[126]

Collectively, these studies suggest that ALL cells should be particularly responsive to therapeutic attempts for mobilization with CXCR4 antagonists. ALL cells mobilized in such a way from marrow or other tissue niches then could be better targeted by conventional ALL treatments.

CXCR4 in breast cancer

CXCR4 was the first chemokine receptor that was described to be functionally expressed by breast cancer cells.[15] Initially, the main function of CXCR4 in breast cancer was considered to be the directed, organ-specific metastasis of circulating CXCR4-positive cancer cells to CXCL12-expressing target organs, such as the lungs, liver, lymphatic tissues and the marrow.[15] Treatment with anti-CXCR4 antibodies reduced local and systemic metastasis of breast cancer in an animal model.[15] Subsequently, additional functions of the CXCR4-CXCL12 axis in breast cancer have been described: first, CXCR4 activation on breast cancer cells induces a growth response in the tumor cells (paracrine function of CXCL12), and second, CXCL12 recruits endothelial progenitor cells to the tumor for tumor angiogenesis (endocrine function of CXCL12).[48] Stromal fibroblasts, also termed carcinoma-associated fibroblasts, constitutively secrete CXCL12 into the tumor microenvironment.[45,48] High-level expression of CXCR4 on neoplastic cells is associated with relatively poor overall survival in patients with breast cancer.[127] The multiple tumor-promoting effects of CXCL12 in breast cancer suggest that CXCR4 antagonists alone or in combination with cytotoxic drugs could decrease the rate of recurrence in the adjuvant setting where patients are likely to have MRD, and/or increase the response rates to conventional therapy in advanced stages of the disease. Animal breast cancer models using T140[73] and Plerixafor[128] have shown promising results, suggesting that CXCR4 antagonists should be explored in this cancer.

CXCR4 in lung cancer

SCLC is an aggressive, rapidly metastasizing cancer. Even with combination chemotherapy and radiotherapy treatments, the 5-year survival is only 5% due to rapid development of drug resistance. CXCR4 is the major chemokine receptor expressed by primary SCLC cells or SCLC cell lines.[50,129] CXCR4 activation induces migratory and invasive responses in SCLC cells, and adhesion to CXCL12-secreting stromal cells in a CXCR4- and integrin-dependent manner.[50] Moreover, signaling through CXCR4 on SCLC cells induces activation and signaling of integrin adhesion molecules expressed on the SCLC cells.[130] Integrin-mediated adhesion of SCLC cells to stroma and extracellular matrix in turn protects SCLC cells from chemotherapy-induced apoptosis.[130,131] Collectively, CXCR4 cooperates with integrins in SCLC cells, mediating tumor cell adhesion to stromal cells, which in turn confers drug resistance and tumor cell growth. Moreover, CXCR4 may direct the distinct metastatic pattern observed in patients with SCLC with a high propensity for bone marrow involvement. The neoplastic cells in NSCLC also express CXCR4, but at levels that are lower than in SCLC.[50] Because of the very transient responses to cytotoxic chemotherapy in lung cancer patients, particularly in SCLC, mobilization of tumor cells from their protective microenvironment using CXCR4 antagonists could be an attractive new approach and combination partner for conventional chemotherapy of lung cancer patients.

Potential side effects of CXCR4 antagonists

Permanent or long-term inhibition of the CXCR4-CXCL12 axis would potentially expose patients to risks of immune system and hematopoietic dysfunctions. T and B lymphocytes utilize CXCR4 for trafficking and homing to distinct microenvironments within lymphoid tissues and the thymus during development and immune surveillance.[82,132] Another general concern regarding the use of CXCR4 antagonists in cancer patients is the mobilization of normal progenitor cells, such as HSC from their microenvironments to the blood. Mobilized HSCs that are normally protected in marrow niches would be exposed to the effects of cytotoxic drugs in trials where CXCR4 antagonists are administered along with cytotoxic drugs, which could result in prolonged cytopenias. More specific side effects are Plerixafor-

related cardiac complications (premature ventricular contractions) in two patients treated with 40 and 160 μg/kg/h, who had a history of cardiac problems.[88] No cardiac side effects were reported for the subsequent clinical trials of Plerixafor for stem cell mobilization.

Collectively, CXCR4 antagonists provide a new, targeted tool to mobilize leukemia cells from their protective marrow microenvironment. Mobilized leukemia cells then become more accessible to conventional drugs, and therefore this strategy may help to overcome MRD and relapses commonly seen in the treatment of leukemia patients. Currently, CXCR4 antagonists are explored in proof-of-principle studies in leukemia patients in whom leukemia cell mobilization can be easily assessed and monitored. However, given the expression of functional CXCR4 receptors by a variety of other hematopoietic and epithelial cancers,[37] a broader use of CXCR4 antagonists in other cancers is expected provided that the ongoing studies are successful (Table 2).

Acknowledgements

We apologize that due to space limitation we were not able to discuss and cite a number of additional studies of other investigators that are related to CXCR4 antagonists in leukemia and other neoplastic diseases. This study was supported by an ASCO Career Development Award (to JAB), a Kimmel Scholar Award by the Sidney Kimmel Foundation for Cancer Research (to JAB) and a CLL Global Research Foundation grant (to JAB).

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