Emergence of Small-Molecule CXCR4 Antagonists as Novel Immune and Hematopoietic System Regulatory Agents

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ABSTRACT

The chemokine CXCL12 (SDF-1) is a pro-inflammatory regulator of cell trafficking and adhesion that binds to the CXCR4 receptor. This G-protein-coupled receptor and ligand combination leads to a complex series of intracellular signaling pathways that are responsible for a number of important cellular regulatory processes, including chemotaxis, cell survival, proliferation, intracellular calcium stores, and gene transcription. Unlike other chemokine ligand receptor combinations, the CXCL12-CXCR4 axis is fairly homogeneous [Viola and Luster, 2008]. The signaling pathways of CXCR4 are complex and divergent. Downstream of ligand binding, CXCR4 receptor activation is mediated by coupling to an intracellular heterotrimeric G-protein associated with the plasma membrane [Mellado et al., 2001; Goldsmith and Dhanasekaran, 2007]. The intracellular signaling pathways associated with SDF-1 binding to CXCR4 are numerous and include the following: PLC-MAFK and PI3K-FAK controlling calcium flux and chemotaxis; Akt-NFκB/p38, JAK-STAT, and Ras/Raf-ERK controlling transcription, gene expression, survival, and proliferation; and β-arrestin/clathrin-controlling receptor endocytosis and desensitization [Teicher and Fricker, 2010].

INTRODUCTION

The chemokine CXCL12 (SDF-1) is a pro-inflammatory regulator of cell trafficking and adhesion that binds to the CXCR4 receptor. This G-protein-coupled receptor and ligand combination leads to a complex series of intracellular signaling pathways that are responsible for a number of important cellular regulatory processes, including chemotaxis, cell survival, proliferation, intracellular calcium stores, and gene transcription. Unlike other chemokine ligand receptor combinations, the CXCL12-CXCR4 axis is fairly homogeneous [Viola and Luster, 2008]. The signaling pathways of CXCR4 are complex and divergent. Downstream of ligand binding, CXCR4 receptor activation is mediated by coupling to an intracellular heterotrimeric G-protein associated with the plasma membrane [Mellado et al., 2001; Goldsmith and Dhanasekaran, 2007]. The intracellular signaling pathways associated with SDF-1 binding to CXCR4 are numerous and include the following: PLC-MAFK and PI3K-FAK controlling calcium flux and chemotaxis; Akt-NFκB/p38, JAK-STAT, and Ras/Raf-ERK controlling transcription, gene expression, survival, and proliferation; and β-arrestin/clathrin-controlling receptor endocytosis and desensitization [Teicher and Fricker, 2010].

Key words: CXCR4 antagonists; CXCR4 receptor; SDF-1; CXCL12; stem cell mobilization; cancer; HIV; WHIM; lupus; rheumatoid arthritis

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The CXCR4 receptor is expressed in multiple cell types, including T and B lymphocytes, hematopoietic stem cells, endothelial, epithelial, macrophages, monocytes, neutrophils, eosinophils, brain, lung, colon, heart, kidney, liver, microglia, astrocytes, and neuronal cells [Murdoch, 2000]. These cells express functional CXCR4 and migrate and/or invade along CXCL12 gradients. CXCR4 is a lethal knock-out in embryonic mice. CXCL12 is constitutively expressed in several organs, including the heart, lungs, kidney, liver, brain, skin, and bone marrow. CXCL12 (SDF-1) secretion is also associated with tissue damage such as heart infarct, limb ischemia, toxic liver damage, excessive bleeding, effects of total body irradiation, and the after-effects of chemotherapy. The CXCR4 receptor is expressed by endothelial cells, pericytes of hypoxic tissues, injured carotid arteries, and atherosclerotic plaques. Historically, CXCR4 was initially discovered as the co-receptor needed for T-tropic human immune deficiency virus (HIV) entry. The virus enters the cells it infects using either the CXCR4 or CCR5 receptor along with the CD4 receptor. Thus, blocking the virus from binding to the CXCR4 receptor prevents entry and infection [Nagasawa et al., 1998].

The CXCL12–CXCR4 axis plays a critical role in cell trafficking, adhesion, and intracellular signaling. The function of SDF-1 is to act as a homing signal to cells that express the CXCR4 receptor, allowing them to migrate toward the increasing gradient. These three functions together determine pathology at the cellular level [Ratajczak et al., 2006]. In leukemias, such as B-cell chronic lymphoid leukemia (CCL), this interaction leads to migration of these cells into lymphoid organs and bone marrow, and rescues them from apoptosis allowing accumulation and survival. Furthermore, SDF-1/CXCR4 signaling and adhesion provides drug resistance and reduces drug effectiveness for many types of cancer cells. For hematopoietic stem cells, which reside in the bone marrow, disruption of the SDF-1/CXCR4 axis allows these cells to migrate from the marrow and go through the differentiation process from progenitor cells to become all components of the blood and immune systems. The CXCR4–CXCL12 axis plays a fundamental role in the accumulation of memory T cells in the rheumatoid arthritis (RA) synovium, resulting in inhibition of apoptosis and persistent release of inflammatory factors leading to disease.

An antagonist of the CXCR4 receptor would prevent SDF-1 signaling resulting in therapeutic outcomes. Antagonist effects include increased calcium flux and chemotaxis, as well as decreased transcription and gene expression [Teicher and Fricker, 2010]. Mechanistically, an antagonist would cause cells that express the CXCR4 receptor to migrate toward an increasing SDF-1 gradient (the homing signal). It would increase in apoptosis, making cells more sensitive to their environment. These include prevention of T-tropic HIV infection, stem cell therapies, cancer anti-metastatic, cancer chemosensitizer, inflammatory conditions such as RA, and autoimmune disorders such as lupus and WHIM, which stands for Warts, Hypogammaglobulinemia (low immunoglobulin levels), Immunodeficiency (susceptibility to infections), Myelokathexis (trapping of white blood cells in the bone marrow) syndrome [Tamamura et al., 2008].

**DISCUSSION**

The discovery of the chemokine CXCR4 receptor and small-molecule CXCR4 antagonists has resulted in the realization of therapeutic effects in humans. The first Food and Drug administration (FDA)-approved CXCR4 antagonist, AMD3100, is for an indication that was not realized until human clinical trials [De Clercq, 2003]. The link of this receptor to observed therapeutic effects in humans has validated CXCR4 as a drug target for immune system modulation. The discovery and development of CXCR4 antagonists has been evolving over the last decade. Several small molecules have advanced into the clinic for different uses (Table 1). AMD3100 (Fig. 1) also known as plerixafo (Genzyme) is administered for stem cell mobilization in the setting of autologous cell transplantation for non-Hodgkin’s lymphoma [De Clercq, 2010]. (Note that Genzyme is selling, marketing, and conducting further clinical trials on prelixafor/AMD3100. See www.clinicaltrials.gov for a complete list and www.genzyme.com for more information.)

AMD3100 is also being investigated for cancer chemosensitization, and prevention of T-tropic HIV infection. Several other CXCR4 antagonist-based injectable treatments are in the clinic, including one small molecule (TG-0054, Taigen; Fig. 1), a cyclic peptide, and numerous biologics. (Note that Taigen Biotech is developing TG-0054. See www.taigenbiotech.com and www.clinicaltrials.gov for more information.) Another small molecule, AMD11070 (Fig. 1), was studied as an oral treatment for prevention of T-tropic HIV infection and found to mobilize leukocytes in a dose dependent manner [Stone et al., 2007]. A summary of possible CXCR4 based indications and therapeutic uses, as well as compound development status is listed in Table 1.

The first report of CXCR4 involved the identification of this receptor via the role it plays in HIV infection. Involved as a co-receptor with CD4, CXCR4 facilitates the entry of T-cell tropic (T-tropic) virus. The discovery of the CXCR4 receptor was coincided with
the discovery of the first CXCR4 antagonist, AMD3100 [De Clercq, 2003] that led to the first human trial on HIV [Hendrix et al., 2004]. AMD3100 reduced viral load in T-tropic infected patients in a Phase I study. Although the trial was halted, it had been established that AMD3100 could mobilize leukocytes in a dose-time dependent manner leading to its study for hematopoietic stem cell mobilization. The only other CXCR4 antagonist studied in humans for T-tropic HIV eradication is AMD11070, the first orally active CXCR4 antagonist. It underwent a Phase II trial in combination with the HIV protease inhibitor, ritonavir, where it reduces viral load [Cao et al., 2008]. However, 3/4 patients with mixed tropic virus (R5/X4) were reported to have switched viral tropism to CCR5-based virus, indicating the need for either limiting this treatment to X4 tropic infections or co-dosing with an R5 antagonist such as maraviroc. AMD11070 increased levels of midazolam and dextromethorphan via effects on cytochrome P-450-related metabolism [Nyunt et al., 2008]. The development of AMD11070 was halted, and to date there are no CXCR4-based HIV therapies in clinical development.

As CXCR4 antagonists could mobilize a variety of hematopoietic and immune cells including stem cells, leukocytes, neutrophils, and others [De Clercq, 2010], they had potential in the setting of autologous engraftment for the treatment of non-Hodgkin’s lymphoma, in which the patient immune system is restored after radiation therapy. The standard treatment for mobilizing hematopoietic stem cells (HPSCs), the precursor to immune system and blood cells, is

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**TABLE 1. Therapeutic Uses and Current Clinical Status of Small-Molecule CXCR4 Antagonists**

<table>
<thead>
<tr>
<th>Therapeutic target</th>
<th>Use(s)</th>
<th>Status/Developments</th>
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<tbody>
<tr>
<td>Hematopoietic stem cell and endothelial progenitor cell mobilization</td>
<td>Autologous stem cell transplant, neutropenia, anemia, vascular pathologies</td>
<td>AMD3100 approved in 12/2008; various trials and treatment combinations TG-0054 completed Phase I studies for autologous engraftment. AMD3100 in, or planned for, trials for Fanconi anemia, neutropenia, and diabetic foot ulcers TG-0054 planned studies for myocardial infarction and macular degeneration AMD11070 reported to mobilize white blood cells during HIV trial</td>
</tr>
<tr>
<td>HIV-T-tropic virus</td>
<td>Prevention and reduction of T-tropic HIV infection</td>
<td>Phase I trial with AMD3100; Phase II trial with AMD11070 Both discontinued in this area</td>
</tr>
<tr>
<td>Cancer: leukemia, myeloma, lymphoma, others</td>
<td>Enhance success rates of chemotherapy; treat and reduce refractory disease</td>
<td>AMD3100 in several Phase II trials in conjunction with chemotherapeutics for leukemia and myeloma None in current development.</td>
</tr>
<tr>
<td>Lupus (SLE)</td>
<td>Reduce flare-ups and symptom severity, reduce organ inflammation</td>
<td>None in development.</td>
</tr>
<tr>
<td>Rheumatoid arthritis</td>
<td>Reduce memory T-cell population in connective tissues</td>
<td>None in development.</td>
</tr>
<tr>
<td>WHIM</td>
<td>Reduce oversignaling of the CXCR4 receptor</td>
<td>Potential orphan drug use for congenital disease None in development.</td>
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**Fig. 1.** Small-molecule CXCR4 antagonists studied in clinical trials.

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*Drug Dev. Res.*
G-CSF, but this requires weeks of treatment and collection and requires bone marrow harvesting via surgery. HPSCs can be mobilized in the bloodstream by CXCR4 antagonists like AMD3100 (Genzyme) and TG-0054 [Taigen, 2011]; they can be collected by apheresis reducing collection times to 1 or 2 days. Thus, it is anticipated that CXCR4 antagonists may replace G-CSF in this setting [Devine et al., 2008]. Other settings for hematopoietic mobilization include neutropenia and Fanconi anemia. Endothelial cells can also be mobilized by CXCR4 antagonists and may aid in revascularization when endothelial progenitor cells mobilize to areas of ischemia as in vascular pathologies including diabetic foot ulcers, myocardial infarction and macular degeneration for which clinical trials are planned (Genzyme, Taigen).

CXCR4 also plays a role in cancer metathesis and cancer chemotherapy-related drug resistance. The receptor for CXCR4 is the most widely expressed in more than 20 different cancers including solid and non-solid tumors ranging from acute lymphoblastic leukemia to breast cancer to neuroblastoma [Shim et al., 2009]. The SDF-1/CXCR4 axis is involved in a wide range of cellular events, including angiogenesis, invasion, locomotion, directional migration, homing, and cell survival.

AMD3100 can mobilize leukemia cancers from the bone marrow (Genzyme), while receptor activation with SDF-1 can stabilize cell migration, conferring resistance to drug treatment with a variety of cytotoxic agents such asetoposide [Hartman et al., 2005], and also leading to estrogen-resistant propagation of breast cancer [Rhodes et al., 2011]. There is growing evidence indicating that proliferation and survival of cancer cells originates from a small subpopulation of cancer stem cells. These observations have led to the hypothesis that CXCR4 antagonism may enhance the action of cytotoxic drugs, making cancer cells more sensitive to apoptotic mechanisms, and overall reducing their survival [Burger and Stewart, 2009]. As an extension of this rationale, AMD3100 is now in clinical trials as a chemosensitizer in the treatment of acute myeloid leukemia (AML) along with etoposide, mitoxantrone, and cytarabine. AMD3100 is also being investigated to assess its effects on mobilization and chemotherapy in combination with Rituximab in the treatment of lymphoma (Genzyme).

In addition to the effects of HIV infection, cell mobilization, and drug resistance, the CXCR4 receptor is also associated with a number of other immune system disorders. In individuals exhibiting the rare congenital immune-compromised condition WHIM syndrome, CXCR4 receptor dysfunction plays a central role. This condition is characterized by recurrent bacterial infections, widespread warts with HPV serotypes, low neutrophil (neutropenia), and B-lymphocyte counts, low immunoglobulin production, and myelokathexis in the bone marrow [Kawai and Malech, 2009]. Although there are no candidates in development for this indication, G-CSF can help normalize neutrophil counts and reverse bone marrow imbalance in WHIM patients. Within this context, a CXCR4 antagonist could serve in a similar role.

CXCR4 also plays a role in other important immune system-related disorders. In RA, inflammatory processes are propagated by memory T cells that migrate toward and into the soft tissue surrounding the joint areas. These cells become resident and express various inflammatory agents in the tissue. This process is partly regulated by the CXCR4 receptor via homing of the cells to the site of inflammation, expression of inflammatory cytokines, and reduction in apoptosis prolonging their residence [Nanki et al., 2000]. Mechanistically, a CXCR4 antagonist could block inflammatory proteins, resulting in T-cell-mediated apoptosis and clearance from the tissue [Tamura et al., 2008]. Systemic lupus erythematosus (SLE) involves both hyperactive B- and T-cell types that increase the production of many immune factors, including antibodies and cytokines. Because of its involvement in B-cell production, myelopoiesis, integrin activation, and chemotaxis, blocking the CXCR4/CXCL12 axis could blunt the heightened immune response and end-organ inflammation in SLE [Chong and Mohan, 2009].

Conclusions

The discovery of the chemokine CXCR4 receptor and small-molecule CXCR4 antagonists has resulted in the realization of therapeutic effects in humans. The first FDA-approved CXCR4 antagonist, AMD3100, is for an indication that was not realized until human clinical trials. The link of this receptor to observed therapeutic effects in humans has validated CXCR4 as a lucrative drug target for immune system modulation. It is also one of the first fields in which a small molecule will take the place for a biologic therapeutic (G-CSF). As AMD3100 and other agents are further investigated in the clinic, new uses and therapeutic interventions are being discovered. Although AMD3100 is injectable, it was investigated for the treatment of HIV infection; it is being explored for other indications, including as a chemotherapy co-treatment. As such, there is currently one other small-molecule CXCR4 antagonist-based injectable treatment in the clinic (TG-0054). Furthermore, another small molecule (AMD11070) was studied in the clinic as oral treatment for prevention of T-tropic HIV infection. In the future, CXCR4
antagonist development will include therapeutic intervention in various cancers as a chemotherapy enhancer, hematologic and vascular disorders, HIV treatment, and other immune system disorders involving CXCR4 receptor modulation, such as RA and lupus. As such, an increasing emphasis will be placed on the development of CXCR4 antagonists with improved profiles including oral activity.

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