Potential of CXCR4 antagonists for the treatment of metastatic lung cancer


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Despite advances in surgery, chemotherapy and radiotherapy over the last decades, the death rate from lung cancer has remained largely unchanged, which is mainly due to metastatic disease. Because of the overall poor prognosis, new treatment strategies for lung cancer patients are urgently needed, and targeting CXCR4 constitutes such a novel, attractive strategy. Tumor cell migration and metastasis share many similarities with leukocyte trafficking, which is critically regulated by chemokine receptors and adhesion molecules. Lung cancer cells express CXCR4 (CD184), a seven-transmembrane G-protein-coupled chemokine receptor. Stromal cells within the tumor microenvironment constitutively secrete stromal cell-derived factor-1 (SDF-1/CXCL12), the ligand for CXCR4. Activation of CXCR4 induces lung cancer cell migration and adhesion to stromal cells, which in turn provides growth- and drug-resistance signals to the tumor cells. CXCR4 antagonists, such as Plerixafor (AMD3100) and T140 analogues (TN14003/BKT140), can disrupt CXCR4-mediated tumor cell adhesion to stromal cells and sensitize lung cancer cells to cytotoxic drugs. Therefore, targeting the CXCR4–CXCL12 axis is a novel, attractive therapeutic approach in small-cell lung cancer and non-small-cell lung cancer. In this article, we summarize data about the cellular and molecular microenvironment in small-cell lung cancer and non-small-cell lung cancer, as well as the role of CXCR4 in tumor–stroma crosstalk. In addition, we review the current status of the preclinical and clinical development of CXCR4 antagonists.

Keywords: CXCL12 • CXCR4 • CXCR4 antagonists • NSCLC • SCLC • tumor microenvironment • tumor stem cells

The CXCR4 chemokine receptor & its ligand, CXCL12

Chemokines induce the directional migration of cells towards a gradient of the chemokine (chemotaxis) by binding to seven-transmembrane G-protein-coupled (chemokine) receptors. Chemokines are small (~8–14 kDa) secreted proteins that are divided into 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 them, or they are adjacent, defining 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 or are 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 and stem cell systems.
are associated with a rare disease known as warts, hypogamma-globulinemia, immunodeficiency and myelokathexis (WHIM) syndrome [9].

Bone marrow stromal cells and other mesenchymal stromal cells (MSCs) are considered the major source for CXCL12 in adult individuals. CXCL12-secreting stromal cells can be found in various tissues, such as the liver, lungs, lymphatic tissues and the marrow [10]. Constitutive high-level CXCL12 secretion by reticular stromal cells and endothelial cells in the marrow is essential for homing, retention [11] and maintenance of hematopoietic stem cells (HSCs) in distinct vascular and endosteal niches for their development and growth [12]. Via CXCL12, these stromal cells also attract circulating hematopoietic progenitor cells [11] or leukemia cells [13] for homing to the marrow. CXCR4 is the only functional chemokine receptor on hematopoietic progenitor cells [14], emphasizing the predominant role of this chemokine receptor for homing and maintenance of HSCs in marrow niches. Current clinical trials with AMD3100 utilized this mechanism of CXCR4-mediated homing to the marrow in order to mobilize HSCs to the peripheral blood for HSC collection for autologous stem cell transplantation. In Phase II trials, mobilization with the combination of AMD3100 and granulocyte–colony-stimulating factor (G-CSF) results in the collection of higher numbers of progenitor cells than with G-CSF alone [15]. The peptide CXCR4 antagonist 4F-benzoyl-TN14003 is also a potent mobilizing agent of HSC alone and in combination with G-CSF [16].

CXCR4 has also been described to function as a key migration factor for targeted cancer cell metastasis to organs that secrete CXCL12, the ligand for CXCR4. In the tumor microenvironment, the CXCR4–CXCL12 axis furthermore supports cancer cell survival and growth, and participates in tumor angiogenesis [17].

**CXCR4 & the tumor microenvironment**

Compelling evidence has emerged in recent years indicating that accessory cells, collectively referred to as ‘stromal cells’, play a critical role in disease progression in various cancers of both the hematopoietic and nonhematopoietic system. The importance of the microenvironment, defined as tissue niches where progenitor cells reside and are maintained by accessory ‘feeder’ cells, was initially recognized for hematopoietic stem cells, based upon the observation that transplanted hematopoietic progenitor cells specifically home to the marrow microenvironment where they find conditions necessary for their maintenance and propagation. More recently, the architecture of distinct niches for hematopoietic and various tissue stem cells, and the mechanisms that govern stem cell homeostasis within these niches, are emerging [18,19].

Regulated migration and homing of stem cells to tissue niches is a critical step during embryonic development or tissue repair, but also in cancer (stem) cell dissemination [20].

The interactions of cancer cells with components of their tumor microenvironment are bidirectional and crucial for cancer progression [21–23]. In order to organize their microenvironment, tumor cells communicate with their surrounding microenvironment via a network of secreted growth factors, cytokines and chemokines [24,25].

Among the various cells that compose reactive infiltrates into tumors, MSCs [26,27], monocyte/macrophage lineage cells and T lymphocytes [28], along with the extracellular matrix (ECM) and blood vessels, are key cellular players in tumor–microenvironment crosstalk. When associated with epithelial cancers, MSCs are oftentimes called carcinoma-associated fibroblasts (CAFs) [17] or myofibroblasts, and can be identified by staining with α-smooth muscle actin antibodies.

Different types of cancers express different chemokines and chemokine receptors [25,29,30]. However, the chemokine receptor CXCR4 is the only one that is expressed by the majority of cancer types. Tumor cells from at least 23 different types of cancers express CXCR4 [25]. Furthermore, MSCs/CAFs constitutively secrete the chemokine CXCL12 (SDF-1), which in turn promotes tumor progression via direct and indirect mechanisms (Figure 1) [30]. Cells of monocyte/macrophage lineage are another highly important cell type in the tumor microenvironment [31].

When associated with tumor, lymphoma or chronic lymphocytic leukemia cells, monocytes/macrophages are referred to as tumor-associated macrophages, lymphoma-associated macrophages or nurse-like cells, respectively. These cells support cancer progression by inducing cancer cell motility and metastasis, and angiogenesis. Among many others, CXCL12 is a prominent mediator accounting for tumor-associated macrophage/lymphoma-associated macrophage accumulation in tumors [32]. Increased numbers of regulatory T cells in the tumor microenvironment can be detected by forkhead box P3 staining, and are thought to suppress immune recognition and response to the neoplastic cells [28]. Among the many molecular pathways of tumor–stroma crosstalk, the CXCL12–CXCR4 axis plays a critical role, as demonstrated in solid and hematologic tumors [17,33]. MSCs/CAFs are well known for constitutive secretion of CXCL12. CXCL12 promotes carcinoma cell growth directly through the CXCR4 receptor expressed on tumor cells, and indirectly by recruitment of endothelial progenitor cells into tumors, thereby causing neangiogenesis [17].

Vascular endothelial growth factor is one of the most potent and specific regulators of angiogenesis, and is required for viability and growth of various solid tumors. VEGF can induce the perivascular expression of CXCL12 by myofibroblasts, which, in turn, positions monocyte–macrophages in the target tissue, where they promote proliferation of tumor cells [34]. Recently, the VEGFR antagonist Avastin® was approved in combination with carboplatin and paclitaxel for the initial systemic treatment of patients with unresectable, locally advanced, recurrent or metastatic, non-squamous non-small-cell lung cancer (NSCLC) [35].

In addition, abnormal expression or function of EGF family members may stimulate tumor progression and can affect CXCR4 expression and function. The EGF receptor (EGFR) HER2 increases CXCR4 expression and inhibits CXCR4 degradation, which could explain increased invasion and metastasis of HER2-positive breast cancer cells [36]. A substantial percentage of lung cancers express cell surface EGFRs, and similar interactions between EGFRs and CXCR4 may also exist in lung cancer.
CXCR4 in NSCLC

In all major subtypes of NSCLC, the tumor cells expressed CXCR4 by immunohistochemistry (Figure 2), and NSCLC patients with higher CXCR4 surface-expressing tumors are more likely to have metastatic disease [37]. Spano and colleagues reported that strong CXCR4-positive nuclear staining was associated with a better outcome in early-stage NSCLC [38], which was confirmed by Wagner et al. [39]. Based on a large sample size, these authors proposed high CXCR4 surface expression as an independent high-risk factor in NSCLC, whereas nuclear staining conferred a survival benefit [39]. The primary sites of NSCLC metastasis (the lymph nodes, bone, liver, adrenal glands and brain) are all sites of high-level CXCL12 expression [10], and therefore CXCR4 is considered a key guidance mechanism for NSCLC metastasis. Phillips and colleagues demonstrated that anti-CXCL12 antibodies abrogated metastasis in a murine NSCLC model [40]. Oonakahara et al. demonstrated a correlation between CXCR4 expression and NSCLC dissemination into the pleural space [41]. Chen et al. recently reported that high-level CXCR4 expression correlates with brain-specific metastasis of NSCLC [42].

Some studies suggested a link between high-level CXCL12 expression and a more advanced disease stage [39,43]. We have previously shown that CXCL12 is expressed in the majority of NSCLC tissue sections (up to 80%) obtained from stage IA–IIB disease patients [43]. Both neoplastic cells and stromal cells (CAFs and vascular endothelial cells) stained positive for CXCL12. CXCL12-expressing CAFs and other stromal cells were mostly detected in proximity to CXCL12-negative tumor tissues, and vice versa. In vitro stimulation of NSCLC cells with CXCL12 increased their colony-forming capacity and induced ERK phosphorylation. These findings indicate that CXCL12–CXCR4 interactions in NSCLC occur in situ and act locally to support tumor growth. CXCL12 levels in NSCLC tumors were elevated compared with peripheral blood, and high CXCL12 expression correlated with increased tumor infiltration by accessory cells, including regulatory T-cell

Figure 1. Importance of the CXCR4 chemokine receptor and its ligand, CXCL12, in the tumor microenvironment and for targeted metastasis. Within hypoxic areas of tumors, both CXCL12 expression by fibroblasts and CXCR4 expression on tumor cells, such as SCLCs and NSCLCs, increases, which stimulates tumor cell motility and invasiveness. These relationships are indicated by the triangles in the top part of the figure. CAFs stimulate tumor progression by CXCL12 secretion. Two major mechanisms by which fibroblast-derived CXCL12 promotes tumor progression have been identified. First, CXCL12 promote tumor cell growth in a paracrine fashion by directly stimulating tumor cell growth via CXCR4. Second, CXCL12 from CAFs induces recruitment of endothelial progenitors, which allow for tumor angiogenesis (endocrine effect of CXCL12). Targeted metastasis to the marrow or other sites of high CXCL12 expression involves CXCR4 activation on circulating tumor cells that ‘hijack’ the CXCR4–CXCL12 axis for homing to microenvironments that are normally restricted to HPCs. CXCL12 gradients attract CXCR4-positive tumor cells to the marrow and other sites where stromal cells secrete high levels of CXCL12. As a consequence, tumor cells can displace HPCs from their protective microenvironment, resulting in hematopoietic dysfunction. Moreover, tumor cells may invade adjacent tissues, resulting in bone destruction.

CAFs: Carcinoma-associated fibroblast; HPC: Hematopoietic progenitor cell; NSCLC: Non-small-cell lung cancer; pO$_2$: Partial pressure of oxygen; SCLC: Small-cell lung cancer.

Adapted from [33].
lymphocytes [43]. Overall, the CXCL12–CXCR4 axis appears to be a key player in NSCLC pathogenesis, acting in an autocrine and paracrine fashion in the tumor microenvironment, and as a chemotactant and growth-promoting factor at primary and metastatic sites.

**CXCR4 in small-cell lung cancer**
Extensive-stage small-cell lung cancer (SCLC), which accounts for approximately 70% of all SCLC cases, is primarily treated with chemotherapy with a high initial response rate of 60–70%.

Despite this high initial response rate, the median survival of extensive SCLC is only 10 months, because the vast majority of patients relapse from minimal residual disease [44].

There is growing evidence suggesting that interactions with accessory stromal cells, which are dispersed with SCLC cells in primary tumors and at metastatic sites, promote SCLC growth, drug resistance and overall disease progression [45,46]. Like NSCLC, SCLC cells are engaged in a complex crosstalk with accessory cells (mesenchymal fibroblasts, inflammatory cells, vasculature and others), soluble factors and ECM components. SCLC immunohistochemistry reveals an infiltration by stromal cells, consisting of either fine fibrous septa or a desmoplastic response [47]. This stroma in SCLC also reveals high levels of the ECM, including fibronectin, collagen IV and tenasin [45,48]. Adhesive interactions between SCLC cells and the ECM confer survival- and drug-resistance signals that are mediated through adhesion molecules, in particularly β1 integrins [45]. This SCLC cell adhesion-mediated drug resistance is also termed cell adhesion-mediated drug resistance (CAM-DR) [49], and can be targeted, for example by β1 integrin-targeting antibodies [48].

The marrow microenvironment, an example of a microenvironment into which SCLC cells preferentially metastasize, includes ECM-diffusible growth factors, chemokines and other cytokines, and a heterogeneous population of adherent cells, including mesenchymal fibroblasts (also called MSCs), osteoblasts, endothelial cells and others, and they create distinct narrow niches that normally support hematopoiesis [50]. CXCR4 is highly expressed on SCLC cells [46,51], and its activation induces firm adhesion of SCLC cells to marrow stromal cells [46], which in turn confers drug resistance and integrin activation [52], and enhances the invasiveness of lung cancer cells by increasing matrix metalloproteinase-9 expression [53]. CXCL12-induced integrin activation resulted in an increased adhesion of SCLC cells to fibronectin and collagen [52]. This CXCR4-induced adhesion was mediated by α2, α4, α5 and β1 integrins, and could be inhibited by CXCR4 antagonists. Stromal cells protected SCLC cells from chemotherapy-induced apoptosis, and this protection/CAM-DR could also be antagonized by CXCR4 inhibitors. Therefore, we concluded that the activation of integrins and CXCR4 chemokine receptors co-operate in mediating adhesion and survival signals in SCLC.

**Lung cancer stem cells**
There is evidence suggesting that cancer growth is driven by a subpopulation of cells referred to as cancer stem cells (CSCs) that are more tumorigenic than other tumor cells of the same clone. CSCs oftentimes 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 [54,55]. This concept was initially developed in acute myelogenous leukemia where leukemia-initiating (stem) cells account for approximately 1 in 250,000 leukemia cells in the peripheral blood [56]. Normal and malignant stem cells apparently have a particular affinity for homing to distinct niches: HSCs localize to CXCL12+ stromal cells that are in close proximity to the
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Review

Bertolini et al. recently provided robust evidence for a connection between lung cancer progenitor cells and CXCR4. Cisplatin treatment of lung cancer cells in vitro resulted in enrichment of the CD133+ fraction, and a consistent enrichment of a CD133+/CXCR4+ population after cisplatin treatment of tumor xenografts [60], providing the first in vivo evidence for a highly tumorigenic and metastatic subpopulation in lung cancer, characterized by expression of CD133 together with CXCR4.

Blocking CXCR4 in lung cancer

CXCR4 chemokine receptors are expressed by various solid and hematologic tumors, such as breast cancer, lung cancer, prostate cancer, and acute and chronic leukemias. In general, CXCR4 expression by the tumor cells allows for tumor cell migration and homing to sites where nonmalignant stromal cells express CXCL12. This concept implies that tumor cell metastasis is guided by the expression of chemokine receptors and adhesion molecules on the neoplastic cells, and expression of respective ligands in target tissues [10,69]. Tumor cells exploit this mechanism to access microenvironments, such as the marrow, that provide factors that favor their growth and protect them from conventional cytotoxic agents. Lung cancer cells, both primary tumors and cell lines, express high levels of CXCR4 [40,51], and rapidly adhere to MSCs in a CXCR4-dependent fashion [46]. Furthermore, CXCR4 activation induces activation of signaling pathways in lung cancer cells that are linked to cell survival and growth, such as the p44/42 MAPK (ERK-1/2) [46] and the PI3K pathway with phosphorylation of Akt and p70 S6 kinase [51]. MSCs protected SCLC cells from etoposide-induced cell death, and this protective effect was antagonized by the CXCR4 antagonist TN14003 [52]. CXCL12 also promotes tumor progression by recruiting endothelial progenitor cells to tumors for angiogenesis [17]. As such, the rationale for CXCR4 antagonists is based on the presence of CAFs/MSCs in the tumor microenvironment, both at primary and metastatic sites. Constitutive secretion of CXCL12 by the stromal cells induces migration and adhesion of SCLC cells to the stromal cells via CXCR4 activation and CXCR4-dependent integrin activation [46,52]. Specific CXCR4 antagonists, such as TN14003, can block stromal cell adhesion, antagonize CAM-DR and thereby sensitize SCLC to drugs such as etoposide [52]. In vivo, we assume that tumor cells that are adherent to stromal cells via CXCR4 could, at least partially, be sensitized to chemotherapy, and therefore CXCR4 antagonists may help to reduce minimal residual disease and relapses in SCLC. The enrichment of CXCR4+ cells in chemo-resistant CSC populations in vivo [60] provides further strong rationale to target CXCR4 in lung cancer.

CXCR4 antagonists

CXCR4 antagonists were initially developed for treatment of HIV-1 infection. At the time of their discovery in the early 1990s, the mechanism of anti-HIV activity of the CXCR4 antagonists T140 and its analogues [66,67], AMD3100 [68,69] and ALX-4C [70], was unknown. However, rapidly following the discovery of the coreceptor function of CXCR4 for T-tropic HIV-1, the activity of the different CXCR4 antagonists was demonstrated to be due to specific binding of these drugs to CXCR4 [70–72]. In general, four major classes of CXCR4 antagonists and agonists can be distinguished:

- Nonpeptide CXCR4 antagonists, such as the bicyclam AMD3100;
- Small-peptide CXCR4 antagonists, such as T140 and its analogues (TN14003 and others);
- Antibodies to CXCR4;
- Modified agonists and antagonists for SDF-1/CXCL12, which will be discussed later.

Nonpeptide CXCR4 antagonists

AMD3100 is a bicyclam, in which two cyclam rings are linked through an aromatic bridge. AMD3100 was among a series of bicyclams that were synthesized in the early 1990s and found to have high anti-HIV activity [73]. AMD3100, a specific antagonist of CXCR4, binds to CXCR4, inhibits CXCL12-induced chemotaxis and GTP-binding, and does not crossreact with other chemokine receptors [74,75]. Initially, AMD3100 was developed at Johnson Matthey (NJ, USA) in collaboration with the Rega Institute for Medical Research (Leuven University, Brussels, Belgium), and therefore this compound was first called JM3100. The name then changed to AMD3100 after a new company, AnorMED (BC, Canada), took over the development. The anti-HIV-1 activity of AMD3100 and the blocking function of AMD3100 on gp120 interaction with CXCR4 during viral entry was the initial focus during the early development of this drug [76]. However, a 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+ HSCs) to the blood [77,78]. Overall, safety and pharmacokinetic issues, and a relatively low effect on the viral load, precluded further development of AMD3100 in HIV. Instead, AnorMED explored AMD3100 as a mobilizing agent for HSCs [79], and a subsequent series of preclinical and clinical trials demonstrated that AMD3100 alone and in combination with G-CSF is an effective mobilizing agent for HSCs [79–82]. The precise mechanism through which AMD3100 mobilizes HSCs is still under investigation. AMD3100 (recently renamed as Plerixafor or Mozobil™) is now owned by Genzyme (MA, USA) after acquisition of AnorMED by Genzyme in 2006. Plerixafor was approved by the US FDA.
for subcutaneous injection for use in combination with G-CSF to mobilize hematopoietic stem cells to the peripheral blood for collection and subsequent autologous transplantation in patients with non-Hodgkin’s lymphoma and multiple myeloma, based upon the data of two randomized Phase III trials [83,84]. Currently, Plerixafor is also used in leukemia patients for leukemia cell mobilization and subsequent targeting by conventional drugs. The activity of Plerixafor to inhibit CXCR4 activation in various tumor models, such as inhibition of CXCL12-induced tumor cell migration and downstream signaling, and activity in animal models has been reported. These studies demonstrate activity of Plerixafor in vitro and in animal models of breast cancer, NSCLC, pancreatic cancer, cholangiocarcinoma, colorectal cancer, malignant melanoma, glioma, ovarian cancer, glioblastoma and medulloblastoma (reviewed in [85]). AMD070 is another orally bioavailable small-molecule CXCR4 antagonist with anti-HIV activity [86]. KRH-1636 is an orally available, nonpeptide CXCR4 antagonist that inhibits infection by X4 HIV-1 virus and blocks responses to stimulation with CXCL12, such as calcium mobilization.

Small-peptide antagonist of CXCR4
Initially, this group of small-peptide CXCR4 antagonists was discovered by screening naturally occurring peptides for anti-HIV activity. In that process, the self-defense peptides tachyplesin (from the Japanese horseshoe crab Tachypleus tridentatus) and polyphemusin (from the American horseshoe crab Limulus polyphemus) were discovered and chemically modified, leading to the synthesis of the anti-HIV peptides T22 [67], T134 and T140 [87]. Initially, these compounds were thought to function by inhibiting HIV-1-T cell fusion or viral uncoating. T22 specifically binds to CXCR4 and blocks CXCR4 receptor regions that are critical for HIV-1 entry and for activation by CXCL12 [72]. 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 arginine (Arg). Therefore, C-terminally amidated T140 analogues were developed to overcome serum instability [88], 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 [89]. In addition, 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 [90]. This is, however, a controversial issue, because other investigators have not found any (partial) agonistic activity of AMD3100 [74,75].

The efficacy of T140 and its analogues for blocking CXCR4 in vitro and in vivo has been documented in numerous preclinical studies, including in vitro and in vivo models for SCLC, breast cancer and melanoma, rheumatoid arthritis, and stem cell mobilization [85]. Other studies explored the activity of these agents in acute and chronic leukemias, multiple myeloma, malignant melanoma, and pancreatic cancer [85]. Besides these disease-oriented studies, T140 and its analogues have been used in basic studies exploring the function of CXCR4 in dendritic cell development [91] and migration [92], B-cell homing and germinal center positioning within lymphatic tissues [93], and hematopoietic stem cell homing [94]. Currently, the T140 analogue TN14003 (BKT140) is under clinical development for patients with multiple myeloma by Biokine Therapeutics Ltd (Rehovot, Israel).

ALX40-4C is a polypeptide of nine Arg residues that is stabilized by terminal protection and the inclusion of d-amino acids. ALX40-4C is a specific CXCR4 antagonist [70], 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 (ON, Canada) [95]. This peptide is no longer under development, particularly because of formulation difficulties, lack of efficacy, and because it is unlikely that an oral formulation of this complex peptide can be produced.

Development antibodies to CXCR4
Neutralizing the interaction between CXCL12 (the ligand for CXCR4) and CXCR4 by using the anti-CXCR4 antibody 12G5 significantly inhibits HIV infection and tumor cell migration in vitro [96]. 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 [90,97-98]. Development of therapeutic monoclonal antibodies (mAbs) to CXCR4 is complicated because of the conformational heterogeneity of CXCR4. 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 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 subgroup 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 largely unknown. However, CXCR4 can be post-translationally modified by sulfation of its N-terminal tyrosines, and by a chondroitinsulfate chain at serine 18 [100]. This phenomenon may in part explain the difference in confirmation, antibody specificity and function of CXCR4. Altered glycosylation patterns, neoexpression, and underexpression or overexpression of glycans are a hallmark of cancer and may significantly affect the activity of various CXCR4 antagonists in development.

Other CXCR4-targeting agents
CTCE-9908 [101] and CTCE-0214 [102] are peptide analogues of CXCL12 with inhibitory and agonist activity, respectively. CTCE-9908 has received orphan drug status by the FDA 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 [103]. CTCE-9908 is developed by Chemokine Therapeutics (BC, Canada). Compounds from the family of chalcones [104] and Spiegelmer technology are under development for neutralization of CXCL12.
Another potential approach is RNA-mediated CXCR4 silencing by RNAi. This typically involves siRNA that directs the cleavage and degradation of complementary mRNA target molecules [105]. In breast cancer cells, siRNA targeting CXCR4 inhibited breast cancer cell migration in vitro [106]. Finally, Jähnichen et al. recently characterized immunoglobulin single variable domains against CXCR4 called nanobodies. Two highly selective monovalent nanobodies, 238D2 and 238D4, displayed strong antiretroviral activity against T-cell-tropic and dual-tropic HIV-1 strains, blocked CXCR4 activation by CXCL12, and induced CD34+ stem cell mobilization in Cynomolgus monkeys [107].

Conclusion
CXCR4 is a chemokine receptor expressed on lung cancer cells and lung CSCs. CXCR4 mediates lung cancer cell migration and adhesion to stromal cells (CAFs) that are present within the tumor microenvironment. CAFs constitutively secrete the ligand for CXCR4 – CXCL12 – and thereby attract and activate lung cancer cells. Adhesion to stromal cells provides lung cancer cells with growth- and drug-resistance signals that could be responsible for residual disease after conventional chemotherapies for SCLC. Persistence of CD133+/CXCR4+ CSCs after conventional chemotherapy in vivo further emphasizes that CXCR4 is a valid new therapeutic target in lung cancer. Targeting CXCR4 in lung cancer could help to overcome primary drug resistance (CAM-DR) and eradicate residual disease, and potentially improve response rates to current treatments.

Expert commentary
CXCR4 antagonists provide a new, targeted tool to inhibit lung cancer cell adhesion to stromal cells (CAFs) that secrete CXCL12. Thereby, CXCR4 antagonists can reverse CAM-DR, making lung cancer cells more accessible to conventional drugs. Moreover, this approach can reverse direct CXCR4-derived growth and survival signals and indirect, proangiogenic circuits between CAFs and endothelial progenitors. As such, this strategy may help to overcome residual disease and relapses that account for the high mortality in lung cancer. Because of the relatively high expression of CXCR4, its high propensity for metastasis to target organs that express CXCL12, and the limited therapeutic options for lung cancer patients, we propose that lung cancer represents a very suitable disease to start investigating the therapeutic activity of CXCR4 antagonists in solid cancers. Currently, CXCR4 antagonists are explored in proof-of-principle studies in leukemia patients in which leukemia cell mobilization can be easily assessed and monitored. However, given the expression and function of CXCR4 receptors in lung cancer, and particularly in lung CSCs, the clinical efficacy of CXCR4 antagonists in lung cancer should be explored.

Five-year view
Increasing emphasis will be placed over the coming years towards targeting the microenvironment, and not only the tumor cells. CXCR4 and its ligand CXCL12 represent a key receptor–ligand pair involved in the complex crosstalk between tumor cells and their microenvironment in various cancers, including lung cancer (SCLC and NSCLC). Significant further benefit for lung cancer patients from new chemotherapeutic strategies or chemotherapeutic combinations is not expected. Instead, combinations of conventional chemotherapeutic drugs with drugs that target the tumor microenvironment, for example CXCR4 antagonists, could improve response rates and outcome, and should be pursued further in the treatment of lung cancer. Additional preclinical studies in lung cancer, both in vivo and in vitro studies, will accelerate the development of these new therapeutic concepts and will help define the best drug combinations.

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First paper to describe the CXCL4–CXCR12 axis as a key player in metastasis of solid tumors (breast cancer).


• Defined the tumor-promoting role of cancer-associated fibroblasts (CAFs) and the key role of the CXCL4–CXCR12 axis in this crosstalk. In addition, it defined that CAF-derived CXCL12 plays multiple roles in tumor progression through direct and indirect mechanisms.


Extensive review of the function of CXCR4 in cancer biology and its potential role as a therapeutic target.


• Introduces the concept of stromal-mediated drug resistance in lung cancer.


• Highlights expression and function of CXCR4 on small-cell lung cancer (SCLC) cells and explores the activity of CXCR4 antagonists for disrupting SCLC–stroma interactions.


discussed the use of CXCR4 antagonists in various contexts, including cancer therapy, stem cell mobilization, and HIV-1 infection. The role of CXCR4 in stem cell mobilization was highlighted, with studies showing the importance of CXCL12-CXCR4 engagement in hematopoietic stem cell mobilization, particularly in the context of bone marrow stromal cell niches. The use of AMD3100, a CXCR4 antagonist, in stem cell transplantation was also discussed, demonstrating the potential of such agents in enhancing stem cell mobilization.

In the context of HIV-1 infection, the CXCR4 co-receptor plays a crucial role in the viral entry process. The use of CXCR4 antagonists, such as AMD3100, was shown to inhibit HIV-1 entry and infection, particularly in cells infected with HIV-1 strains that utilize CXCR4 as a co-receptor. The role of CXCR4 antagonists in HIV-1 entry was also discussed, emphasizing the potential of these agents as novel therapeutic approaches for the treatment of HIV-1 infection.

Several studies highlighted the potential of CXCR4 antagonists in the treatment of cancer, including prostate cancer. The use of AMD3100 in prostate cancer models was shown to inhibit chemotaxis and HIV-1 infection, demonstrating the potential of CXCR4 antagonists in the treatment of prostate cancer.

In summary, the use of CXCR4 antagonists in various contexts, including cancer therapy, stem cell mobilization, and HIV-1 infection, was discussed. The potential of these agents in enhancing stem cell mobilization, inhibiting HIV-1 entry, and treating cancer was highlighted, providing a strong foundation for further research and development in this field.