Translating an Antagonist of Chemokine Receptor CXCR4: From Bench to Bedside

Donald Wong and Walter Korz

**Abstract**

The majority of current cancer therapies focus on a primary tumor approach. However, it is metastases that cause the majority of cancer deaths. The metastatic process has been shown repeatedly to be greatly influenced by chemokines such as CXCL12 [stromal cell derived factor-1 (SDF-1)] and its receptor CXCR4. The activation of this pathway has been reported to modulate cell migration, survival, proliferation, and gene transcription through G proteins, phosphoinositide-3 kinase, Akt, extracellular signal-regulated kinase, arrestin, and Janus-activated kinase/signal transducers and activators of transcription. A wide variety of strategies, such as peptides, small molecules, antibodies, and small interfering RNA, have been used to target this pathway. Treatments in combination with current therapies seem to be especially promising in preclinical studies. A few compounds are advancing into early stages of clinical development. In this article, we will review the development of CXCR4 antagonists in oncology.

**Background**

Metastasis is a complex process that can be driven by hypoxia and insulins to the tumor, such as chemotherapy. It is the metastatic process that accounts for >90% of cancer-related deaths. To date, most therapeutic approaches focus on techniques that affect the primary tumor. There is currently no drug approved that specifically targets the metastatic process. It has been long known that different types of cancer preferentially metastasize to specific organs. Recent reports show that pivotal to this phenomenon is the production of chemokines by the organs to which the tumor cells metastasize. One of the major chemokine receptors that is expressed by cancer is CXCR4, the receptor for CXCL12 [stromal cell derived factor-1 (SDF-1)].

The CXCL12/CXCR4 pathway has been widely studied. CXCR4 is a 352-amino-acid, seven-transmembrane G-protein coupled receptor. CXCL12 is its sole ligand (1). The two most investigated isoforms of CXCL12 are CXCL12α and CXCL12β, the latter having four additional amino acids at the COOH terminus compared with the former. Four additional isoforms (CXCL12γ, CXCL12δ, CXCL12ζ, and CXCL12ε) were recently described, each with different number of amino acid extensions compared with CXCL12α (2). There is remarkable homology between CXCL12α expressed by human and mice with only a single conservative change in amino acids (3). This is perhaps a fortuitous circumstance for translational research. This pathway is critical especially during early development, in the processes of hematopoiesis, organogenesis, and vascularization (4–6). Gene knockout is lethal in mice. In humans, heterozygous mutations in the CXCR4 gene lead to the development of the warts, hypogammaglobulinemia, infections, and myelokathexis syndrome (7). The CXCL12/CXCR4 pathway has also been co-opted by cancer cells in the processes of metastasis, growth, and survival.

**The CXCL12/CXCR4 Signal Transduction Pathway**

CXCL12 is the sole ligand for the chemokine receptor CXCR4. This receptor has been described as undergoing dimerization after binding to CXCL12 or alternately in its unbound configuration. The dimer state seems to be important to activate signal transduction pathways (8, 9). Upon binding of CXCL12 to CXCR4, the receptor is stabilized into a conformation that activates the heterotrimeric G-protein, Gi being a major component (refs. 9, 10; Fig. 1). However, other G-protein subtypes and non–G-protein-mediated pathways are also used. Gia and Gip both dissociate from the receptor and regulate a wide variety of downstream pathways, including activation of phospholipase C, phosphoinositide-3 kinase, and inactivation of adenyl cyclase. Signaling through the phosphoinositide-3 kinase pathway leads to the activation of PAK and cell polarization, the first step in migration. Phosphoinositide-3 kinase and various tyrosine kinases that activate Akt and Cdc42 are involved in actin polymerization. Phospholipase C–mediated events, such as calcium release and protein kinase C activation, as well as focal adhesion kinase, pyk2, paxillin, and extracellular signal-regulated kinase are important in the adhesion process, leading to cell migration (refs. 11–14; Fig. 1).

In parallel, activation of Akt, extracellular signal-regulated kinase, and tyrosine kinases such as Src leads to transcription of genes involved in migration (13, 14). Whether CXCR4 is involved in the survival and proliferation of tumor cells remains controversial, perhaps because this may be tumor
dependent. The activation of extracellular signal-regulated kinase and Akt can both potentially contribute to the survival and growth of tumor cells (refs. 15, 16; Fig. 1). Migration, survival, and growth are all components of the metastatic process.

Traditionally, it is believed that the Janus-activated kinase/signal transducers and activators of transcription (JAK/STAT) pathway is activated through CXCR4, partially independent of G-protein. The association of JAK with CXCR4 activates STAT proteins, which translocate to the nucleus and regulate transcription (9). However, a recent report that used genetically modified cells rather than inhibitors raised questions about this activity (17). It remains unclear whether CXCL12 binding to CXCR4 in patients with tumors activates the JAK/STAT pathway. This perhaps reflects the differences among cell lines, normal cells, and primary tumor cells as Moriguchi et al. suggested (17).

Down-regulation of the CXCR4 receptor is initiated by phosphorylation of its cytoplasmic tail. Subsequent to the binding of arrestin to the COOH terminus of CXCR4, the receptor is internalized through endocytosis (Fig. 1). Degradation of CXCR4 occurs in the lysosome. However, binding of arrestin also elicits signals through various mitogen-activated protein kinases and modulates various signal pathways (18). Stimulation of other G-protein coupled receptors may also down-regulate CXCR4 signaling through a process called heterologous desensitization (19).

A recent report described a number of CXCR4 isoforms and mutant CXCR4 receptors. They vary in levels of glycosylation and functionality (20). Thus, although CXCR4 is expressed, it may differ structurally and functionally in different cells. It is not completely surprising that CXCR4+ neuroblastoma cells that do not undergo chemotaxis toward bone marrow–derived CXCL12 have been isolated (21). However, it remains a possibility that the primary tumor from which these cells were derived remains responsive to CXCL12 and these cells subsequently became desensitized. Similarly, a panel of breast cancer cells that all express CXCR4 uniformly exhibit different metastatic potentials in mice (10). Only the highly invasive cells show signal transduction, actin polymerization, and chemotaxis subsequent to CXCL12 exposure. The reason is partly related to the inability of the G-protein subunits to form an αβγ heterotrimeric complex with CXCR4 in nonmetastatic lines. This also suggests that screening potential patients for

![Signal Transduction Pathway](image)

**Fig. 1.** Signal transduction pathway induced by CXCL12 binding to receptor CXCR4. Downstream actions mainly related to migration and chemotaxis. Other effects include survival, proliferation, and transcription. Current inhibitors of this pathway are peptides, small molecules, and antibodies that inhibit ligand/receptor binding.
clinical trials by simply assessing CXCR4 expression may be misleading.

**Clinical-Translational Advances**

It was reported in 2004 that 23 types of cancers express CXCR4 (22). This number continues to grow. Prominent CXCR4+ tumors include breast cancer (23), ovarian cancer (24), prostate cancer (25), hepatocellular carcinoma (26), and hematologic malignancies (27). The expression of CXCR4 is regulated in many tumors by hypoxia and cytokines such as VEGF and TNF (28–30). The classic article linking CXCR4/CXCL12 to metastasis *in vivo* is the article by Müller et al. that described the impairment of metastasis in murine breast cancer models by a neutralizing antibody against CXCR4 (31). Since then, a large body of supportive work has been done in a variety of *in vivo* cancer models using various blockade strategies. The CXCR4 antagonist peptide designated CTCE-9908 has been used to show that blocking CXCR4 reduces metastasis consistently in eight different murine cancer models (32–38). These include i.v. and intracardiac injection of human breast cancer (34), mouse osteosarcoma, and mouse melanoma cells (32) as models of metastasis; s.c. implantation and metastasis from the subcutaneous site of Lewis Lung carcinoma (35); orthotopic xenografts of hepatocellular carcinoma (37), prostate cancer (33), and breast cancer (34); and transgenic model of metastatic breast cancer (38).

Similar *in vivo* observations have been made with CXCR4 blocking small molecules, such as AMD3100 (39, 40); peptides, such as T22 (41) and TN14003 (42); antibodies (43); and small interfering RNA (22, 40). Administration of AMD3100 following injection of ovarian tumor cells resulted in reduced peritoneal metastasis but did not affect survival (39). In a xenograft model with i.v. injected breast cancer cells, both AMD3100 and RNA inhibition by small interfering RNA reduced lung metastases (40). However, overall survival was unaffected (40). When CXCR4 knockdown breast cancer cells (by RNA inhibition) were orthotopically implanted, all mice survived without metastatic lesions in contrast to control animals that all died from metastatic disease (40). I.v. injection of melanoma cells generates tumor masses in the lungs that were inhibited by T22 (41). The analogue to T22 designated TN14003 suppressed primary tumor growth by inhibiting tumor angiogenesis and prevented lung metastasis in an orthotopic head and neck cancer model (42). By using the same tumor cells in an i.v. model, TN14003 treatment reduced the number of lung metastases (42). Injection of prostate cancer cells into the left cardiac ventricle resulted in osseous metastases (42). Injection of prostate cancer cells in an i.v. model, TN14003 treatment reduced the number of lung metastases (42). Injection of prostate cancer cells into the left cardiac ventricle resulted in osseous metastases (42). Injection of prostate cancer cells in an i.v. model, TN14003 treatment reduced the number of lung metastases (42). Injection of prostate cancer cells in an i.v. model, TN14003 treatment reduced the number of lung metastases (42). Injection of prostate cancer cells in an i.v. model, TN14003 treatment reduced the number of lung metastases (42).

CXCR4 activation not only induces a panel of cytoskeletal changes leading to cell migration (as discussed in the previous section) but also induces the production of matrix metalloproteinases at the primary tumor site (14). This is important for detachment from the tumor and migration through the extracellular matrix into the circulation. Upon arrival in an organ high in CXCL12, interaction of CXCR4 with CXCL12 activates integrins on the tumor cells, allowing them to adhere to endothelial cells and subsequently migrate across the endothelium into the organ to form metastases (22, 44). The regulation of these processes may affect their role in metastasis of individual tumors.

Other less studied CXCR4-related effects are survival, proliferation, angiogenesis, and vasculogenesis. CXCL12 produced by stromal cells may induce a survival or antiapoptotic signal in tumors that reduce their susceptibility to current treatments such as chemotherapy (45, 46). This is similar to usurping the role CXCL12 usually plays in hematopoiesis in the bone marrow when tumor cells metastasize to the bone (14). CXCR4 antagonists also reduced tumor growth in several murine models (47, 48). It has been shown in some studies that angiogenesis, the growth of new blood vessels from preexisting blood vessels, can be induced by CXCL12 and reduced by CXCR4 antagonists (49, 50). However, there are also reports that show there is no change in microvessel density with CXCR4 inhibitor treatment. Striet et al. proposed that some tumor cells have such high levels of CXCR4 expression that all the CXCR4 inhibitors would be bound and none may be left to affect the endothelium which may express much lower levels of CXCR4 (51). This phenomenon may be dependent on the tumor and its stage of development when angiogenesis is required (angiogenic switch). Similarly, some reports show that some tumors, under normal circumstances or when insulted by current therapies, recruit bone marrow cells such as endothelial progenitor cells (EPC), hematangiocytes/ hematopoietic progenitor cells, and mesenchymal stem cells (52–56). The main function of EPC and hematangiocytes would be in vasculogenesis, bringing fresh nourishment to repair injured tumor tissue. Also, these cells may secrete growth factors and cytokines important in the growth of new vasculature and survival of the tumor. Circulating EPC have been found to be increased in breast cancer patients by a number of groups, one of which also found an increase in hematopoietic progenitor cells/hematangiocytes (53, 54). In murine glioma models, CXCR4 blockade by AMD3100 reduced recruitment of EPC to vasculogenic gliomas (57).

Treatment of mice and cancer patients with taxanes, such as paclitaxel, but not other chemotherapies, such as gemcitabine, mobilized EPC at least in part by secretion of CXCL12 (58). Pretreatment with a VEGFR-2 blocking antibody prevented the mobilization. Blockage of recruitment of EPC by a CXCR4 antagonist may also be effective and may lead to reduced tumor viability. The CXCR4 antagonist CTCE-9908 has been successfully used in combination with docetaxel in murine prostate cancer and breast cancer models. In the prostate cancer model, a proportion of the mice became free of primary tumor and metastases, although it continued to grow in cases in which the primary was not eradicated (36). In the breast cancer model, an additive effect was observed both in reducing the growth of the primary tumor and in inhibiting lung metastases (38).

Approximately 40% of hepatocellular carcinoma patients are routinely treated with transarterial chemoembolization as first-line therapy in which chemotherapy is administered directly to the tumor followed by vessel occlusion. It was observed that the tumor responds by recruiting EPC and hemangiocytes for detachment from the tumor and vessel occlusion. It was observed that the tumor responds by recruiting EPC and hemangiocytes for detachment from the tumor and vessel occlusion. It was observed that the tumor responds by recruiting EPC and hemangiocytes for detachment from the tumor and vessel occlusion. It was observed that the tumor responds by recruiting EPC and hemangiocytes for detachment from the tumor and vessel occlusion. It was observed that the tumor responds by recruiting EPC and hemangiocytes for detachment from the tumor and vessel occlusion.
bone marrow cells, decreasing blood vessel density in the tumor, and causing the tumor to become necrotic. The overall survival of rats was significantly increased (56).

In glioblastoma patients, the escape of the tumor from anti-VEGF therapy correlated with an increase in plasma CXCL12 (59). This represents a promising opportunity to use a CXCR4 antagonist in combination with an anti-VEGF agent. CXCL12 and VEGF also work synergistically in ovarian cancer (60). In the case of breast cancer, HER2-positive tumor cells exhibit increased expression of CXCR4. This is related to inhibition of receptor degradation through the ubiquitin pathway (61). Moreover, CXCL12/CXCR4 seems to transactivate HER2 in breast cancer and prostate cancer (62, 63). It may be postulated that a CXCR4 antagonist can be used in combination with therapies targeting VEGF and HER2. Indeed, CXCR4 antagonist CTCE-9908 has been recently used in combination with the VEGFR2 blocking antibody DC101 in a breast cancer model. The results showed a further inhibition of the primary tumor and lung metastases comparing the combination therapy with either therapy alone (38).

It is clear that there is more to CXCR4 antagonists than just antimetastasis. The best strategy for their use may be in combination with other targeted therapies or standards of care that induce CXCL12 secretion or migration of CXCR4+ cells, whether bone marrow–derived cells or tumor cells. The design of a clinical trial to examine strictly antimetastatic effects may involve surgery and chemotherapy to treat the primary tumor and current metastases. A CXCR4 antagonist given in a consolidation setting may inhibit any future metastases (64). However, this would probably require a protracted clinical trial to prove sufficient evidence of efficacy. Moreover, a regulatory approvable end point for inhibition of metastasis has not been recognized. Time to progression, tumor response, and similar end points are not appropriate to measure changes in the amount or rate of metastasis. Further work with regulatory agencies is needed to identify end points suitable to quantify disease burden in the context of metastasis and its ultimate effect on overall survival.

Translational Approaches toward Clinical Studies

A leading CXCR4 antagonist for solid tumors is CTCE-9908 (Chemokine Therapeutics Corp.). This compound is derived from the amino acid sequence of the NH2 terminus of human CXCL12. Preclinical efficacy studies were done in eight metastatic models as discussed in the previous section (32–38). Throughout, CTCE-9908 showed a consistent anti-metastatic effect whether tumor cells were injected (i.v., intracardiac), implanted (s.c., orthotopic), or spontaneously developed in a transgenic model. In the breast cancer models, it was observed to reduce the growth of the primary tumor by 40% to 80% (34, 38). Recently, reduction of primary tumor growth or shrinkage of primary tumor has been reported in combination with various treatments, such as chemotherapy, anti-VEGF therapy, and hepatic artery ligation in mouse and rat models (36, 38, 56). A phase I/II clinical trial is completed and final results have been reported (65). Adverse events such as phlebitis are mild. More importantly, early signs of efficacy have been observed in the form of patients with stable disease. A phase II clinical trial in hepatocellular carcinoma with the use of CTCE-9908 in combination with transarterial chemoembolization is being initiated. The hypothesis is that CTCE-9908 would block the recruitment of bone marrow–derived support cells by the tumor after the transarterial chemoembolization procedure in addition to blocking the metastatic process. Primary tumor response, effects on disease progression, and survival are anticipated.

On the hematologic malignancy side, AMD3100 (Genzyme Corp.) is currently being tested in a phase I/II trial in combination with mitoxantrone, etoposide, and cytarabine as listed in clinicaltrials.gov. The investigators hypothesized that the bone marrow stroma interacts with AML blasts through CXCL12/CXCR4 to produce chemoresistance. Disruption of this interaction would sensitize the acute myelogenous leukemia blasts to the cytotoxic effect of chemotherapy. A number of preclinical animal studies have shown this effect in hematologic malignancies (66). A similar observation was reported with the CXCR4 antagonist peptide RCP168, an analogue of the viral macrophage inflammatory protein II, although no clinical studies are reported (45). Another CXCR4 antagonist has recently entered clinical trials. MSX-122 is an orally available small molecule produced by Metastatix, Inc. A phase I trial was reported as suspended on clinicaltrials.gov on June 30, 2008. There is a report that this compound inhibits the growth of primary tumor and synergizes with paclitaxel in an orthotopic model with human lung adenocarcinoma (67). A number of other compounds show activity in the preclinical stage, some showing efficacy in mouse cancer models whereas others are targeting other diseases and disorders. These include CXCR4 antagonist AMD3465 and AMD070 (Genzyme Corp.), as well as T22, T140, FC131, and other derivatives (Biokine Therapeutics, Ltd., and Kyoto University).

Conclusion

It is clear that CXCR4 blockade for oncology is moving from preclinical research into the clinical. A number of challenges remain, including a method to screen for patients and tumor types likely to respond, biomarkers as indicators of efficacy, the appropriate stage of disease to treat, regulatory approvable end points, and appropriate combinations with current and future therapies. Marketing applications for AMD3100 in the setting of stem cell mobilization and transplantation have been submitted to the Food and Drug Administration and European Union. The future is bright for CXCR4 antagonists as a therapeutic approach for the management of cancer.

Disclosure of Potential Conflicts of Interest

D. Wong, W. Korz: employees and ownership interest: Chemokine Therapeutics Corporation.

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