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The role of chemokine receptor CXCR4 in lung cancer

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Lung cancer is the leading cause of cancer deaths worldwide. Small cell lung cancer (SCLC), which comprises 15% of all lung cancers, is almost exclusively due to smoking and is highly aggressive due to early widespread metastasis. While combination chemotherapy has lead to modest improvements in outcome, the five-year overall survival for SCLC remains at 5%. Identifying distinct biochemical pathways of metastasis and chemotherapy resistance in SCLC may lead to novel therapeutic approaches and improve survival in SCLC patients. The chemokine receptor CXCR4 is emerging as an important target in cancer growth, metastasis, relapse and resistance to therapy. In this article, we review the structure and function of CXCR4 and its ligand, CXCL12, as well as mechanisms of CXCR4/CXCL12 signal transduction in lung cancer. We review the current preclinical and translational research involving this pathway in lung cancer and the clinical development of several novel agents targeting the CXCR4/CXCL12 pathway. Further understanding of the CXCR4/CXCL12 pathway in SCLC and NSCLC may provide a rationale for innovative research on the CXCR4 receptor as a potential novel therapeutic target in lung cancer.

Background

Lung cancer is the leading cause of cancer deaths worldwide with approximately 220,000 new cases and 160,000 deaths estimated for 2009 in the United States alone.[1] Small cell lung cancer (SCLC), which comprises 15% of all lung cancers, is almost exclusively due to smoking and is highly aggressive due to early widespread metastasis. While combination chemotherapy has lead to modest improvements in outcome, the 5 y overall survival for SCLC remains at 5%. As in most cancers, early stage lung cancer can often be controlled with locally directed therapy including radiation and surgery; it is the almost inevitable development of metastatic disease that leads to a high mortality rate in SCLC.

Many genetic abnormalities involved in the pathogenesis of SCLC are distinct from those in non small cell lung cancer (NSCLC). While the tumor suppressor gene, *p53*, is mutated in more than 90% of SCLC patients, it is mutated in about 50% of patients with NSCLC. The oncogene *K-RAS* is mutated in 30% of NSCLC patients; however, no *K-RAS* mutations have been detected in SCLC. *EGFR* mutations are often identified in NSCLC but rarely occur in SCLC.[2] The identification of several tyrosine kinase receptors important in the pathogenesis of lung cancer, including epidermal growth factor receptor (EGFR), has lead to the development of targeted therapies, including erlotinib and cetuximab. However, while these EGFR targeted agents have improved survival in patients with NSCLC, therapy for advanced stage SCLC relies largely on response to cytotoxic chemotherapy. Therefore identifying distinct biochemical pathways of metastasis and chemotherapy resistance in SCLC may lead to novel therapeutic approaches and improve survival in SCLC patients.

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Chemokines and Chemokine Receptors

Chemokines, or chemotactic cytokines, are a group of related small soluble peptides that were originally noted to direct leukocyte movement to inflammatory tissues.[3,4] They are classified into four subfamilies according to the number and spacing of their N-terminal cysteine residues: C, CC, CXC and CX_3C.[5] Chemokines designated CXC, for example, have an amino acid between these cysteine residues whereas CC chemokines do not. The CXC family has been further subdivided in two classes based on whether or not there is an 'ELR' motif (glutamic acid-leucine-arginine) preceding the first cysteine residue.[6] Chemokines effect signal transduction through cells by binding with 7-transmembrane domain G-protein coupled receptors (7TM-GPCR) which are designated according to the names of their corresponding ligands.[3,4]

In normal physiology, chemokines play a role in pro-inflammatory as well as non-inflammatory cell homing.[6] Chemokines mediate the migration of leukocytes to inflammatory sites and also play roles in the regulation of hematopoietic stem cells, angiogenesis and the extracellular matrix. The growing field of chemokine research has lead to a greater understanding of their additional roles in diverse fields including development, immunology and cancer. About 50 chemokines and 20 chemokine receptors have been identified to date. Many chemokine receptors interact with more than one chemokine. Likewise, several chemokines may activate the same chemokine receptor.

Chemokine ligand 12 (CXCL12), also known as stromal cell-derived factor 1α (SDF-1α), is the only known ligand for chemokine receptor 4 (CXCR4), a well-characterized 7TM-GPCR. Although the CXCR4/CXCL12 receptor/ligand pair is unique in that the interaction has long been viewed as exclusive, more recent evidence suggests CXCL12 signaling through CXCR7 as well,[7] which has also been hypothesized to play a role in cancer pathogenesis.[8-10] Unlike the majority of human chemokines, the genes of which cluster together on chromosomes 4 and 17, the gene for CXCL12 is located on chromosome 10q11.21, suggesting a more highly conserved specialized function.[11] The CXCR4/CXCL12 receptor/ligand pair controls the chemotaxis of human and mouse hematopoietic progenitor cells, regulating hematopoietic stem cell homing to the bone marrow.[12] CXCL12 knockout mice die perinatally (embryonic day 18.5) with significantly reduced progenitor B-cells as well as cardiovascular defects, indicating a critical role in lymphopoiesis, myelopoiesis and development.[13] Mice lacking CXCR4 exhibit identical defects, as well as abnormal neuronal cell migration during development.[14] In humans, CXCL12 acts as a chemoattractant for $CD34^+$ progenitor cells in peripheral blood.[12]

CXCL12 was first cloned by Tashiro et al. in 1993.[15] A few years later, CXCR4, a previously unknown cofactor required for HIV-1 virion entry into $CD4^+$ cells, was cloned.[16] Sequence analysis revealed that the cofactor belonged to the seven transmembrane G-protein coupled receptor family. Since CXCR4 had not yet been identified as the receptor for CXCL12, it was functionally identified only as a co-receptor, along with the CD4 cell surface receptor, for HIV-1 *env* mediated cell fusion and entry. The ligand for CXCR4 was later identified as CXCL12.[17]

The gene that encodes CXCR4 is located on chromosome 2 band q22.1, clustered with genes for other CXC chemokine receptors. Alternative splicing results in two isoforms which differ slightly in the N-terminus region: one isoform consists of 356 amino acids beginning with the residues MSIPLPLLQ while the predominantly expressed isoform contains 352 amino acids, beginning with the amino acid residues MEGIS (**Fig. 1**). At least four single nucleotide polymorphisms (SNP) have been identified in the exonic portions of the CXCR4 gene, including non-synonymous SNPs.[18] The synonymous variation rs2228014 has been studied in a cohort of CLL patients; while no clinical association was identified, three mutations were identified in that study.[19] A well established mutation in CXCR4 causes the WHIM syndrome (warts-hypogammaglobulinemia-infections-myelokathexis), a primary immunodeficiency disorder that can be caused by either a nonsense or a frameshift truncation mutation affecting the receptor's serine and threonine rich C-terminal cytoplasmic tail domain.[20]

The protein structure of CXCR4 is characterized by the classical 7-transmembrane domains with an extracellular N-terminus and three extracellular loops between the transmembrane domains. The C-terminal cytoplasmic tail is serine and threonine rich. Ligand binding requires the proximal amino terminus near the transmembrane region. Signal transduction requires specific residues in the second intracellular loop, a conserved 'DRY' motif as well as the second extracellular loop, which contains a sequence of negatively charged amino acids.[21] Mutant receptors with alterations in these regions do not demonstrate an increase in intracellular calcium ions in the presence of CXCL12, suggesting the involvement of these regions in ligand activated signal transduction. Interestingly, truncation of the serine and threonine rich region of the distal C terminus does not effect signal transduction, though this region contains potential phosphorylation sites.[21] Post-translational modifications in chemokine receptors are known to affect ligand binding properties. In CXCR4, post-translational sulfation at tyrosine 21 contributes to CXCR4 binding to CXCL12.[22] CXCR4 exists as a constitutive homodimer. CXCL12 ligand binding induces conformational changes within these preformed receptor dimers, without promoting the formation or dissociation of dimers.[23] CXCR4 dimerization is specific, constitutive and likely occurs soon after receptor synthesis and folding.[24]

CXCR4 in Cancer

Since Paget first proposed the "seed and soil" concept in 1889,[25] several authors have explored this theory of cancer metastasis. Clinicians have long recognized that certain cancers metastasize to distant sites in a predictable pattern. This clinical concept forms the basis of many well-developed hypotheses regarding the nature of cancer metastasis. The idea that certain organs provide the optimal "soil" or environment to allow for distant growth of the tumor "seed" leads to the question of which factors comprise the ideal microenvironment for tumor growth. Mueller was the first to describe increased CXCL12 levels in organs of breast cancer metastasis, proposing a role for a chemotactic gradient and the CXCR4/CXCL12 pathway in tumor cells homing to distant sites.[26]

Based on the well-characterized roles of CXCL12 and CXCR4 in chemotaxis and the similarities between chemotactic cell migration and cancer cell movement to distant sites, this receptor-ligand pair has been hypothesized to play a role in cancer pathogenesis and metastasis. While GPCRs have been well described in the past, recently there is growing evidence supporting an important role for GPCRs in cancer biology, with implications in several tumor types.[27] CXCR4 has previously been shown to be involved in abnormal homing in chronic myeloid leukemia.[28] In breast cancer, CXCR4 has been noted to be undetectable in normal mammary epithelium with significant upregulation in breast cancer cells. The receptor mediates actin polymerization, pseudopod formation and invasive responses. In addition, high levels of CXCL12 expression were observed in organs to which breast cancer metastasizes. Experimental neutralization of CXCR4 with an anti-human CXCR4 monoclonal antibody inhibited lung metastasis in vivo in a mouse model.[26] CXCR4 inhibition blocked CXCL12 induced migration and invasion in pancreatic cancer cell lines[29] and has been shown to synergize with cytotoxic chemotherapy in glioblastoma cells in vitro and in vivo.[30] Prostate cancer cells show increased migration and invasion in response to stimulation by CXCL12, which was inhibited by antibody to CXCR4.[31] In hepatocellular carcinoma,

Table 1. Clinical trials involving CXCR4 inhibition currently active in the United States

Drug name	Patient population	Primary endpoint	Dosing	Trial stage/center
Plerixafor	MM, NHL, HD patients with <20 $CD34^+$ cells/ul after 5 d of mobilization with G-CSF alone	Percent who achieve greater than or equal to 2 x 10^6 $CD34^+$ cells/kg within 3 d of apheresis	240 ug/kg subcutaneous following 5 d of G-CSF mobilization	Phase II Duke
Plerixafor	De novo AML	DLTs of Plerixafor in combination with cytarabine and daunorubicin	Escalating subcutaneous dose levels to determine the MTD	Phase I Multiple Centers
Plerixafor	Relapsed/refractory MM	Safety, MTD and response rate of plerixafor and bortezomib	Escalating subcutaneous dose levels days 1–6 of each 21 d cycle	Phase I and II Dana-Farber Cancer Institute
Plerixafor	Allogeneic stem cell transplantation for myeloid leukemias	PFS, biological effects and safetly in combination with busulfan, fludarabine and allogeneic hematopoietic transplantation	Escalating subcutaneous dose levels up to 240 μg/kg daily for 4 d with G-CSF, busulfan and fludarabine	Phase I and II MD Anderson Cancer Center
Plerixafor	Front line mobilization, transplantation in NHL, HD, MM	Safety in stem cell mobilization	240 μg/kg subcutaneous daily up to 5 consecutive d	Phase III Multiple sites
Plerixafor	WHIMS	Safety	Escalating subcutaneous dose levels over 5 d	Phase I NIH
Plerixafor	CLL/SLL	MTD and response when used with rituximab	Escalating subcutaneous dose levels to 320 μg/kg, x3 per w for 3 w	Phase I and II Multiple sites
Plerixafor	HNL, HD	Determine the minimum effective dose of intravenous AMD3100 with G-CSF	Escalating intravenious dose levels from 160–400 μg/kg on day 0 and daily until apheresis is complete after subcutaneous test dose or 240 μg/kg	Phase I and II Washington University
Plerixafor	Relapsed or refractory AML	Determine the optimal dose and schedule	Escalating subcutaneous dose levels to 240 μg/kg, days 0–5 with mitoxantrone, etoposide and cytarabine	Phase I and II Washington University
Plerixafor	Donors, hematologic neoplasms	Number of donors needing a second collection to obtain the minimum $CD34^+$ cells necessary for allogeneic stem cell transplant	320 μg/kg intravenous for 1–2 d	Phase II Washington University
BKT140 (Biokine)	MM	White blood cell count	Escalating subcutaneous dose levels. One dose of 0.03, 0.1, 0.3 or 0.9 mg/kg	Phase I and II Israel

increased immunohistochemical expression of CXCR4 was significantly associated with local tumor progression as well as lymph node and distant metastases.[31] In addition, the CXCR4 pathway may play a role in several other malignancies including pancreatic adenocarcinoma,[32] colon cancer,[33] esophageal cancer,[34] sarcomas[35-37] and other tumors.

Implications for Lung Cancer

CXCR4 and CXCL12 expression in lung cancer. Several studies have demonstrated a correlation between CXCR4 expression and clinical outcomes in lung cancer, with increased expression in tumor tissue over normal lung tissue, and increased expression in tumors of patients with metastatic disease versus those without clinical metastasis.[38] However, analysis of the subcellular localization of CXCR4 yields conflicting results. While one study of 46 NSCLC samples noted a significant association in five NSCLC samples with nuclear CXCR4 immunohistochemical expression and increasing lymph node metastasis ($p = 0.008$),[39] another study demonstrated strong CXCR4 nuclear staining in 17 of 61 samples from patients with stage I NSCLC was associated with a significantly better outcome in compared to patients with no CXCR4 nuclear staining ($p = 0.039$).[40] In a similar study of 154 primary NSCLC samples, nuclear CXCR4 staining was associated with improved disease-free survival and a lower T stage in patients with adenocarcinoma, whereas cytomembranous staining was associated with distant metastasis and decreased disease-free survival.[41] In a multivariate analysis, cytomembranous CXCR4 staining was associated with a significantly worse disease free survival ($p = 0.004$).[41] The same study found that intense CXCL12 staining was associated with nodal metastasis but not with survival.[41] In another study of 16 NSCLC patients, high levels of CXCR4 expression on pan-cytokeratin positive circulating peripheral blood mononuclear cells was associated with a significant decrease in overall survival compared with low CXCR4 expression ($p = 0.03$). Combined pan-cytokeratin and CXCR4 expression was higher in the 16 patients with NSCLC compared with ten normal healthy donors; however, the difference was not statistically significant ($p = 0.11$).[42] Aberrant CXCL12 methylation in NSCLC cell lines corresponds to decreased

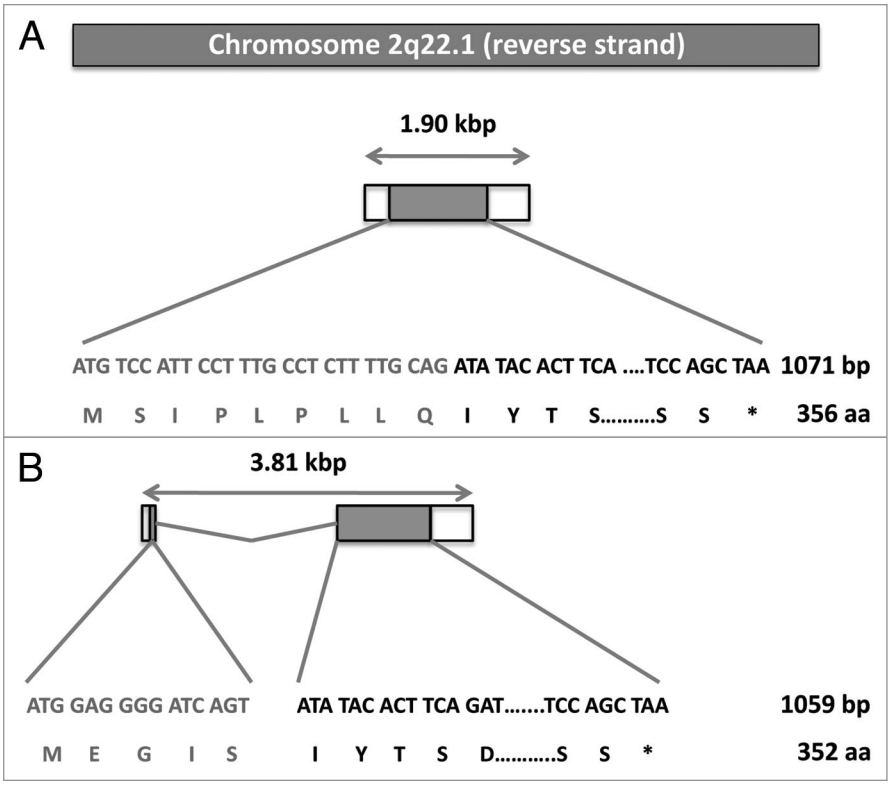

Figure 1. The gene that encodes CXCR4 is located on chromosome 2 band q22.1. Alternative splicing results in two isoforms, which differ slightly in the distal N-terminus region. (A) Variant 1 consists of 356 amino acids beginning with the residues MSIPLPLLQ. (B) Variant 2, the predominantly expressed sequence, contains 352 amino acids, beginning with the amino acid residues MEGIS.

CXCL12 mRNA expression, which is restored after treatment with a demethylating agent.[43] Aberrant CXCL12 methylation was present in 85 of 236 patients with stage I NSCLC, compared with only 11 out of 163 samples of corresponding non-malignant lung tissue. CXCL12 methylation was independently associated with a poor prognosis in all patients ($p = 0.15$) and in stage I patients ($p = 0.17$) compared with non-methylation. However, there was no relationship between CXCL12 methylation and protein expression of CXCL12 and CXCR4, which may be due to the fact that immunohistochemistry cannot distinguish between endogenous and exogenous CXCL12 present in tumor samples. In this study, CXCL12 expression was present in significantly more cases with nodal metastasis and with advanced stages and also correlated with a poor prognosis in adenocarcinomas. CXCR4 expression was also present in significantly more cases with advanced stages (II, III or IV) compared to stage I ($p = 0.0121$). High CXCR4 expression was associated with a poor progression in NSCLC ($p = 0.023$) and high CXCL12 expression was associated with a poor prognosis in adenocarcinoma ($p = 0.015$).[43]

Pathways of CXCR4 Signal Transduction in Lung Cancer

In small cell lung cancer (SCLC), a malignancy with highly aggressive metastatic behavior, there has been limited research on the CXCR4/CXCL12 axis. CXCL12 is highly expressed by human bone marrow osteoblasts and endothelial cells[44] making this chemokine and its receptor valuable candidates for investigation in SCLC, which has a high rate of early metastasis to the hematopoietic compartment of bone. Ubiquitous expression of CXCR4 has previously been demonstrated in ten SCLC cell lines. Specifically, all ten SCLC cell lines tested expressed CXCR4 and responded to its ligand CXCL12 with an increase in cell proliferation, adhesion and motility that can be attributed, in part, to increased PI3K signaling.[45] In vivo studies demonstrate that SCLC tissues are surrounded by an extensive stroma of Extracellular Matrix (ECM) at both primary and metastatic sites.[46] In vitro analysis showed that SCLC cells have an increased expression of β1 integrin as well as α3, α6 and αv integrins that mediate binding to fibronectin, laminin, collagen IV and tenascin expressed by the ECM. This increased binding leads to an increased tyrosine-kinase activity that blocks caspase activation thereby resulting in decreased chemotherapy-induced cytotoxicity.[46] In a subsequent study, SCLC samples from patients were found to express high levels of CXCR4. In SCLC cells, CXCL12 induced signaling through CXCR4, actin polymerization and activation of MAPK signaling.[47] CXCL12 also induced SCLC migration into the ECM of the stromal cells in the bone marrow, which could be inhibited by either a CXCR4 antagonist or an antibody directed against vascular cell adhesion molecule-1 (VCAM-1) implicating CXCR4 activation and α4β1 integrin induced binding in the interaction between SCLC cells and the tumor microenvironment.[47] These findings were also confirmed by Hartmann and colleagues, who showed that α2, α4, α5 and β1 integrins, as well as CXCR4 activation, play a role in SCLC metastasis.[48] Blocking CXCR4 also decreased the etoposide resistance of SCLC cells in vitro. In addition, this study noted increased phosphorylation of paxillin, a component of the focal adhesion complex, in SCLC cells in response to CXCL12, as well as constitutive activation of FAK and CRK-L, both of which are downstream effectors of paxillin signaling.[48] Since CXCR4 has previously been shown to activate the JAK2/STAT3 pathway,[49] the effect of CXCR4 activation on this pathway was investigated in SCLCs.[50] This study found that STAT3 is constitutively phosphorylated in SCLC cells, with increased phosphorylation in response to CXCL12 activation, as well as increased adhesion to VCAM-1. Inhibitors of either CXCR4 or JAK2 decreased cell growth in soft agar, indicating the role of both CXCR4 and JAK2/STAT3 signaling in anchorage independent cell growth. Increased STAT3 phosphorylation was also seen in primary SCLC tumor samples.[50]

In NSCLC cells, in vitro studies have demonstrated that CXCL12 increases CXCR4 mediated motility and cell surface expression of integrins, mediated by phosphorylation of extracellular signal regulated kinase (ERK) and downstream activation of the IKKαβ/NFκβ/RELA signaling.[51] Related to integrin signaling, it has also been shown that the breast cancer metastasis suppressor 1 (BRMS1) gene, a histone deacetylase, decreases CXCR4 expression thus inhibiting CXCL12 induced chemotaxis, whereas BRMS1 silencing increases CXCL12 induced chemotaxis.[52] In this setting, BRMS1 may act as a transcriptional regulator of CXCR4 expression by modulating NFκB, which has been shown to bind the CXCR4 promoter. In a cohort of 132 NSCLC patient samples, CXCR4 mRNA expression was inversely correlated with BRMS1 mRNA expression. Furthermore, samples with distant metastases had a markedly lower level of BRMS1 mRNA and higher levels of CXCR4 mRNA than the non-distant metastasis group.[52] Another downstream target of MAPK and NFκB upregulation in NSCLC is matrix metalloproteinase-9 (MMP-9). Increased expression of MMP-9 due to NFκB binding to its promoter in response to CXCL12 has been shown to induce MAPK activation.[53]

Other tumor environmental factors can also influence CXCR4 expression in lung cancer. Hypoxia has been shown to promote CXCR4 expression in NSCLC cells by activating hypoxia inducible factor 1α (HIF-1α) and HIF-1β expression, which then mediate CXCR4 transcription by promoter binding.[54] Epidermal growth factor receptor (EGFR) activation by EGF has also been shown to increase CXCR4 expression in NSCLC cells, an effect that is markedly enhanced under hypoxic conditions.[55] This increase in CXCR4 expression is regulated by the PI3K/PTEN/AKT/mTOR signaling pathway that upregulates HIF-1 genes and ultimately increases CXCR4 transcription.[55] Finally, CXCR4 has been described as a marker of highly tumorigenic, stem-like lung cancer cells.[56] In vitro treatment of NSCLC cells with cisplatin results in the enrichment of a population of cells positive for CD133, a marker of cancer initiating cells in several tumor types. Furthermore, a population $CD133^+/CXCR4^+$ cells were spared by in vivo cisplatin treatment of lung cancer xenografts established from primary tumors, suggesting a role for CXCR4 in the mechanisms of resistance to chemotherapy and relapse in lung cancer.[56]

Clearly, there is evidence that the CXCR4/CXCL12 pathway plays an important role in attracting cancer cells to sites of distant metastasis as well as in promoting cell growth and proliferation in a protective environment. Combining CXCR4 antagonists with cytotoxic chemotherapy might provide a means of overcoming chemoresistance and relapse in lung cancer, particularly in SCLC, by mobilizing tumor cells from the protective marrow environments, in addition to inhibiting tumor cell proliferation and metastasis along chemotactic gradients.

Inhibition and Clinical Trials

As demonstrated by the in vitro and in vivo preclinical studies described above, targeting CXCR4 may be active as a monotherapy. In addition, CXCR4 inhibition may enhance cytotoxicity when used in combination with chemotherapeutic regimens. Several inhibitors of the CXCR4/CXCL12 pathway have been shown to be effective in pre-clinical studies involving animal tumor models, the most notable of which is the CXCR4 antagonist, plerixafor. Previously known as AMD3100, the drug was initially identified as a highly potent and selective inhibitor of HIV entry.[57] AMD3100 is a symmetrical bicyclam composed of two macrocyclic 1,4,8,11-tetraazacyclotetradecane moieties connected by phenylenebismethylene linker.[58] The bicyclam binds to aspartate residue 171 in the fourth transmembrane domain of CXCR4 and aspartate residue 262 in the sixth transmembrane domain with each of its cyclam moieties. Each cyclam ring has an overall positive charge at physiological pH and can form a complex with carboxylic acid groups, which are found at the aspartate residues. Binding may be associated with a conformational change in the receptor as a result of the phenylene-bismethylene linker connecting the two cyclam components that bind two distinct regions of the receptor.[58]

AMD 3100 was shown to be well tolerated in a Phase I single dose trial of 12 healthy volunteers.[59] Of note, volunteers were noted to have a transient, dose dependent leukocytosis after administration of AMD3100. Further analysis revealed the mobilized white blood cells were haematopoietic ($CD34^+$) cells. Based on this pharmacodynamic observation, AMD3100 was clinically developed for use in hematopoeitic stem cell mobilization. Its safety, tolerability and efficacy have subsequently been demonstrated in clinical trials[60-66] leading to its approval by the Food and Drug Administration in December 2008 for use in combination with Granulocyte Colony-Stimulating Factor (GCSF) to mobilize stem cells for collection prior to autologous transplantation in patients with non-Hodgkin lymphoma and multiple myeloma. In addition, treatment of stem cell donors with AMD3100 has been shown to allow for rapid mobilization and collection of hematopoietic cells just 4 h after a single treatment dose, followed by successful engraftment in all 20 recipient patients studied.[61] This rapid and effective stem cell mobilization to peripheral blood through direct inhibition of CXCR4/CXCL12 underscores the importance of this pathway in cell homing to the bone marrow. By blocking the interaction, cells are quickly released from the marrow into circulation. In vitro and in vivo studies demonstrate reduced CXCR4 signaling in the presence of AMD3100, as evidenced by decreased Akt phosphorylation. AMD3100 also inhibits the protective effects of the bone marrow microenvironment in myeloma cells, disrupting the interaction between bone marrow stromal cells and myeloma cells thereby releasing myeloma cells into circulation and enhancing their sensitivity to chemotherapy.[67] In a mouse model of epithelial ovarian cancer, intraperitoneal treatment with AMD3100 reduced dissemination, possibly by inhibiting CXCL12 induced attachment between tumor cells and peritoneal cells.[68] AMD3100, as well as PI3K and MAPK inhibitors, decreased this attachment in vitro further implicating the interaction of those two signaling cascades with CXCR4/CXCL12 axis.[68]

These recurrent pathobiological themes of disrupting the tumor microenvironment to overcome resistance to treatment have implications for the treatment of lung cancer, in which relapse

may involve a CXCR4-mediated protective tumor microenvironment in the bone marrow. Plerixafor is currently being tested in a Phase I/II trial in combination with mitoxantrone, etoposide and cytarabine (MEC) to determine the optimal dose and schedule in patients with relapsed or refractory acute myeloid leukemia (AML) with the rationale that disrupting the interaction between AML blast cells and the bone marrow microenvironment will increase the cytotoxic effect of chemotherapy. Another planned study aims to further optimize the dosing and pharmacokinetics of plerixafor by changing from daily subcutaneous to twice daily intravenous dosing in combination with MEC and GCSF, which acts synergistically with plerixafor by downregulating CXCL12. Early phase trials of plerixafor are also ongoing in combination with daunorubicin and cytarabine in AML, with bortezomib in relapsed or refractory myeloma, with filgrastim, busulfan and fludarabine for allogeneic stem cell transplantation in myeloid leukemias and with rituximab in patients with chronic lymphocytic leukemia (CLL) or small lymphocytic lymphoma (SLL), among other trials in hematologic malignancies (**Tab. 1**). The drug has been well tolerated with the major reactions being injection site reactions and mild gastrointestinal symptoms.[65,66]

Recently, a novel CXCR4 antagonist, AMD070 (Genzyme Corporation), was shown to be well tolerated in healthy volunteers[12] and in combination with ritonavir (antiviral and protease inhibitor) in patients with HIV.[69] A dose-dependent elevation in white blood cell count was observed as a pharmacodynamic marker of anti-CXCR4 activity in HIV patients. In addition, other CXCR4 inhibitors are currently being tested in patients with HIV and cancer in Phase I and II clinical trials.[70] BKT140 (Biokine Therapeutics) is a highly selective modified peptide CXCR4 antagonist being tested in a Phase I/IIA trial in myeloma patients. TG-0054 (TaiGen Biotechnology Co., Ltd.) is a CXCR4 antagonist currently being evaluated in a randomized, double-blind, placebo-controlled, sequential ascending single intravenous dose study to assess safety, tolerability, pharmacokinetics and pharmacodynamics in healthy volunteers. The primary endpoint of the trial is to determine the maximum tolerated dose of TG-0054. A secondary endpoint is to assess the plasma pharmacokinetics profile and potential pharmacodynamic effects of TG-0054 on $CD34^+$ stem cells, $CD133^+$ progenitor cells, white blood cells, red blood cells, platelets and differential counts.[18]

CTCE-9908 (Chemokine Therapeutics, Vancouver, BC, CA) is a modified CXCL12 antagonist analog that acts as a receptor antagonist by competitively binding CXCR4 receptors, thereby preventing interaction with CXCL12. CXCR4 inhibition with CTCE-9908 has been shown to decrease metastatic tumor burden in a mouse breast cancer model; however, the incidence of metastasis was not decreased.[71] Additionally, other studies have shown decreased metastasis and primary tumor growth in mouse models of breast cancer[72] and decreased tumor size in a mouse model of prostate cancer.[73] A Phase I study of CTCE-9908 in healthy adults did not reveal any significant toxicity. The primary objective of this Phase I/II clinical trial was to determine the tolerability and safety profile of repeated administration of CTCE-9908. The secondary objective was to evaluate early signs of efficacy such as tumor stabilization and reduction of tumor burden. A total of 25 patients with advanced metastatic disease that had stopped responding to standard treatments or for whom no curative therapy exists were enrolled into the study and received at least one dose of CTCE-9908. Eight patients were treated in the dose escalation portion of the study, while the remaining 17 were treated at the highest dose of 5 mg/kg/day. All patients in the study had at least one previous surgery and had received an average of three previous chemotherapy regimens. CTCE-9908 was well tolerated in patients with only mild irritation at the injection site.[74]

Another CXCR4 antagonist being studied is AMD3465. Both in vivo and in vitro studies of AMD3465 have demonstrated the important role of the CXCR4/CXCL12 pathway in interactions between the tumor and the bone marrow microenvironment.[75] In a mouse model, treatment with this CXCR4 inhibitor resulted in the mobilization of AML cells into circulation and increased the effects of chemotherapy on reducing leukemia burden by disrupting the protective microenvironment of the bone marrow.[75] AMD3465 also significantly blocked growth in mouse xenograft models of medulloblastoma and glioblastoma.[75] In addition, several CXCR4 inhibitors have been evaluated in pre-clinical in vitro and in vivo studies.

Conclusions

To make an impact on patient survival in lung cancer, novel targeted therapies are needed. Several targeted agents have recently been approved for treatment of NSCLC, but in SCLC, a malignancy with highly aggressive and predictable metastatic behavior, only cytotoxic chemotherapy has been effective to date. The CXCR4/CXCL12 pathway plays an important role in SCLC proliferation, metastasis, resistance to treatment and relapse. This pathway may provide a novel therapeutic approach by targeting tumor progression and spread as well as the chemoprotective tumor microenvironment. Innovative research in pathways of metastasis and chemotherapy resistance is essential to understanding the pathogenesis of small cell lung cancer and developing targeted treatment approaches.

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