

CXCR4 ligands: the next big hit?

Annemiek M. E. Walenkamp, Constantin Lapa, Ken Herrmann, Hans-Jürgen Wester

Angaben zur Veröffentlichung / Publication details:

Walenkamp, Annemiek M. E., Constantin Lapa, Ken Herrmann, and Hans-Jürgen Wester. 2017. "CXCR4 ligands: the next big hit?" *Journal of Nuclear Medicine* 58 (Supplement 2): 77–82. <https://doi.org/10.2967/jnumed.116.186874>.



CXCR4 Ligands: The Next Big Hit?

Annemiek M.E. Walenkamp¹, Constantin Lapa², Ken Herrmann^{3,4}, and Hans-Jürgen Wester^{5,6}

¹Department of Medical Oncology, University Medical Center Groningen, University of Groningen, Groningen, The Netherlands;

²Department of Nuclear Medicine, University Hospital Würzburg, Würzburg, Germany; ³Department of Nuclear Medicine, University Hospital Essen, Essen, Germany; ⁴Department of Molecular and Medical Pharmacology, David Geffen School of Medicine, UCLA, Los Angeles, California; ⁵Pharmaceutical Radiochemistry, Technische Universität München, Munich, Germany; and ⁶Scintomics GmbH, Fuerstenfeldbruck, Germany

Chemokine receptors form a large family of proteins that mediate the chemotaxis of cells toward a gradient of chemokines. C-X-C chemokine receptor 4 (CXCR4) is a G protein-coupled chemokine receptor encoded on chromosome 2 (1). The receptor has a 7-transmembrane structure with 7 helical regions connected by 6 extramembrane loops (2). CXCR4 exerts its biologic effect by binding its ligand, C-X-C chemokine ligand 12 (CXCL12) (3,4), and activating the downstream signaling pathway; these

actions lead to an alteration of gene expression, actin polymerization, cell skeleton rearrangement, and cell migration (Fig. 1) (5).

CXCR4 expression plays a key role during embryonic development, as CXCR4 expression on progenitor cells allows migration from their place of origin to their destination, where they will differentiate into organs and tissues. The CXCR4 pathway is important in neoangiogenesis, immunity, and infections (6,7). In the late 1990s, CXCR4 expressed on cluster of differentiation 4-positive (CD4⁺) T cells was discovered to serve as a coentry receptor for HIV type 1 (8). The role of CXCR4 in numerous physiologic and pathologic circumstances, such as systemic lupus erythematosus, rheumatoid arthritis, and multiple sclerosis, has been elucidated (5). CXCR4 and CXCL12 play decisive roles in tumorigenesis, including the enhancement of cell proliferation, migration, and invasion; cancer cell-tumor micro-environment interactions; and angiogenesis (9–11).

TARGETING OF CXCR4–CXCL12 AXIS

Multiple agents with different modes of antagonistic activity against CXCR4–CXCL12 have been developed. In general, 4 major classes of CXCR4 antagonists and agonists can be distinguished: nonpeptide CXCR4 antagonists, such as the bicyclam derivative AMD3100; small-peptide CXCR4 antagonists, such as T140 and even smaller cyclic peptides; antibodies to CXCR4; and modified agonists and antagonists for CXCL12 (12).

The bicyclam derivative AMD3100 (plerixafor injection; Mozobil [Sanofi]), previously called JM3100, acts as a specific antagonist by blocking the binding pocket of CXCR4 (13,14). The first clinical trials with AMD3100 were designed for the treatment of HIV. Interestingly, an increased amount of white blood cells was observed in healthy volunteers in phase 1 clinical trials (15). This finding led to the discovery that AMD3100 mobilizes CD34⁺ human hematopoietic stem and progenitor cells from the bone marrow to peripheral blood (16). Finally, AMD3100 was approved by the U.S. Food and Drug Administration as a mobilizer of hematopoietic CD34⁺ cells from the bone marrow to the circulation (17,18). Orally available CXCR4 antagonists include AMD070 (19,20).

The earliest efforts to find CXCR4 antagonists focused mainly on peptide derivatives (21), including [Tyr^{5,12},Lys⁷]polypheumisin II, which was designated T22 (22); the highly potent CXCR4 antagonist T140, a 14-mer peptide with a disulfide bridge; and its smaller derivative, the cyclic pentapeptide FC131 (23,24). These efforts were followed by the development of antibodies specifically directed against CXCR4. Müller et al. described decreased metastasis in murine breast cancer models when a neutralizing antibody against CXCR4 was used (10). In addition to inhibition of metastasis, the discovery of a direct apoptotic effect

For correspondence contact: Annemiek M.E. Walenkamp, University Medical Center Groningen, University of Groningen, P.O. Box 30.001, 9700 RB Groningen, The Netherlands.
E-mail: a.walenkamp@umcg.nl

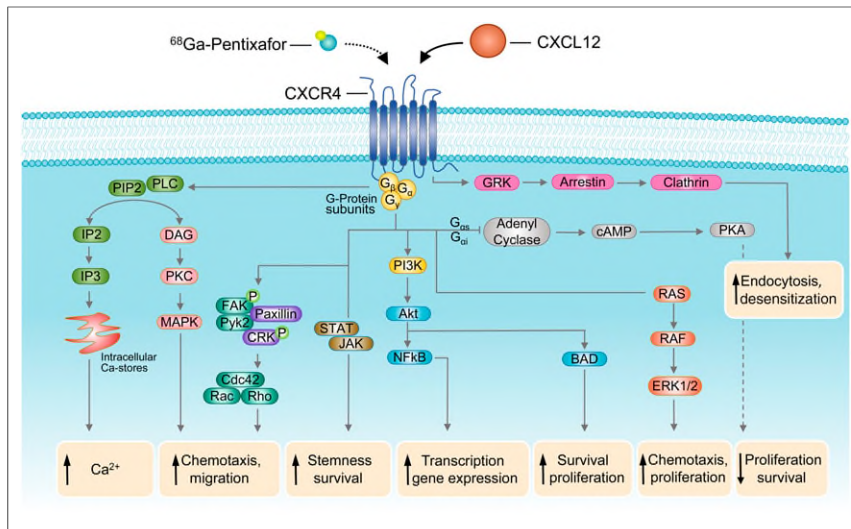


FIGURE 1. Some key signaling pathways thought to be involved in CXCR4–CXCL12 signaling. On agonistic binding to CXCR4, CXCL12 is internalized and finally subjected to lysosomal degradation. Activation of CXCR4 induces β -arrestin-mediated signaling. On the basis of their sequence similarity, G α subunits are divided into 4 families (G α s, G α i, G α q, and G α 12) that regulate G protein-coupled receptor signals via different routes.

of antibodies suggested that direct killing may be a mechanism for tumor growth inhibition.

BMS-936564/MDX-1338 is a fully human anti-human CXCR4 IgG4 monoclonal antibody that is currently in phase I trials for the treatment of relapsed or refractory acute myeloid leukemia, chronic lymphatic leukemia, non-Hodgkin lymphoma, and multiple myeloma (25). Neutralization of the interaction between CXCL12 and CXCR4 by use of the anti-CXCR4 antibody 12G5 significantly inhibited HIV infection and tumor cell migration in vitro (26). Recently, the development of a fully human single-domain antibody-like scaffold (termed an *i-body*) with activity against human CXCR4 was reported (27). Another CXCR4-targeting agent is CTCE-9908, a 17-amino-acid-sequence peptide analog of CXCL12 with inhibitory capacity and approved for clinical use in patients with osteosarcoma (28,29). Other agents or strategies for interfering with the CXCR4–CXCL12 axis include the anti-CXCL12 aptamer NOX-A12 (Noxxon Pharma AG), CCX2066, and RNA interference (30,31).

ROLE OF CXCR4–CXCL12 IN TUMOR CELL–MICROENVIRONMENT INTERACTIONS

Classic chemotherapeutic anticancer treatments induce cell death through DNA damage by taking advantage of the proliferative behavior of cancer cells. An alternative approach strikes at the critical driving forces of cancer cells. The importance of the microenvironment in the tumorigenic potential of epithelial cells is well established (32,33). The tumor microenvironment contains numerous cell types in addition to cancer cells—including stromal fibroblasts, endothelial cells, immune cells, connective tissue, and extracellular matrix—that support tumor structure, angiogenesis, and growth (33).

High levels of CXCL12 expressed by cancer cells and tumor-associated stromal cells directly stimulated the proliferation and invasiveness of breast cancer cells in autocrine and paracrine manners (9,33). Moreover, in mouse models of human breast

cancer (9) and prostate cancer (33), high CXCL12 levels in the tumors attracted CXCR4-positive inflammatory, vascular, and stromal cells to the tumor mass, where they would eventually support tumor growth by secreting growth factors, cytokines, chemokines, and proangiogenic factors.

In the bone marrow, constitutive CXCL12 secretion by stromal cells is crucial for homing and sustaining CXCR4-expressing hematopoietic stem and progenitor cells in their niches (34,35). As shown in acute myeloid leukemia human xenotransplant mouse models, leukemic cells also localize in CXCL12-rich niches of bone marrow, where the protective microenvironment favors their growth and survival during cytotoxic treatment (36). As shown in murine models of chronic myelogenous leukemia (37), acute myeloid leukemia (38), and chronic lymphocytic leukemia (39), CXCR4 antagonists can disrupt tumor–stroma interactions and mobilize leukemic cells to the peripheral blood, making them more sensitive to conventional

anticancer drugs (40–46).

CXCR4 antagonists have also been evaluated for inhibition of the cross talk between tumor and stromal cells and for mobilization of cancer cells from the protective microenvironment of solid tumors, making them more sensitive to conventional chemotherapy or radiotherapy and antiangiogenic therapy (3,34,47–49).

In a glioblastoma mouse model, combined vascular endothelial growth factor and CXCR4 antagonism targeting the glioblastoma stem cell population resulted in improved survival (50). Glioblastoma recurrence involves the persistence of a subpopulation of cells that have enhanced tumor-initiating capacity and that reside within the perivascular space, or niche. Antiangiogenic therapies may prevent the formation of new perivascular space but have not prevented recurrence in clinical trials (51,52), suggesting that they cannot abrogate tumor-initiating capacity. Anti-vascular endothelial growth factor and CXCR4 treatment indicates that targeting the structure and function of the perivascular space, or niche, has a superior antitumor effect. This concept was set to be evaluated in a clinical trial aiming to explore the efficacy of plerixafor and bevacizumab (ClinicalTrials.gov identifier: NCT01339039); however, the study was terminated because of a low accrual rate.

Another CXCR4 inhibitor, USL311, is currently under investigation alone and in combination with lomustine in a phase 1/2, dose escalation and dose expansion study in subjects with advanced solid tumors (phase 1) and subjects with relapsing or recurrent glioblastoma (phase 2). The study is designed to explore the safety, tolerability, pharmacokinetics, and preliminary efficacy of USL311 alone and in combination with lomustine (ClinicalTrials.gov identifier: NCT02765165).

ROLE OF CXCR4–CXCL12 IN IMMUNITY IN TUMOR MICROENVIRONMENT

Immune checkpoints consisting of inhibitory pathways are a part of the immune system that is crucial for maintaining self-tolerance and modulating the duration and amplitude of physiologic immune

responses in peripheral tissues to minimize collateral tissue damage. Tumors coopt certain immune checkpoint pathways as a major mechanism of immune resistance, particularly against T cells that are specific for tumor antigens (53). Cytotoxic T-lymphocyte–associated antigen 4 antibodies, programmed cell death protein 1 (PD-1), and programmed cell death ligand 1 antibodies promote the killing of cancer cells by cytotoxic T cells and have been successful in subsets of patients with melanoma, non–small cell lung cancer, urothelial bladder cancer, renal cell cancer, and head and neck cancer (54–60). Nevertheless, it has become apparent that even if these T-cell checkpoint antagonists overcome some of the immunosuppressive effects of the tumor microenvironment, other, more fundamental inhibitory reactions in the tumor microenvironment may explain why most patients—especially those with microsatellite stable colorectal cancer, ovarian cancer, prostate cancer, and pancreatic ductal adenocarcinoma—rarely exhibit objective responses to these therapies (34,55,61).

Effective immunotherapy requires that these T cells physically contact cancer cells. Cancer-associated fibroblasts were shown to exclude T cells by mediating the extracellular matrix (60,62). Live-cell imaging of lung tumor tissue slices from patients revealed active T-cell motility in regions of loose fibronectin and collagen, whereas T cells migrated poorly in dense matrix areas surrounding tumor nests (63). When collagenase was added to reduce matrix rigidity or when the chemokine CCL5 was experimentally produced by tumor cells, increased T-cell movement out of the stromal regions and into contact with cancer cells was observed. Also, cancer-associated fibroblasts excluded T cells by biosynthesis of CXCL12. The administration of AMD3100 in an autochthonous pancreatic ductal adenocarcinoma mouse model induced rapid T-cell accumulation among cancer cells and acted synergistically with anti-programmed cell death ligand 1 to diminish cancer cells (64).

The stromal cell–derived factor 1 α /CXCR4 pathway mediated stroma polarization toward an immunosuppressive microenvironment and contributed to systemic disease progression after anti-angiogenic treatment in a hepatocellular mouse model (65). AMD3100 prevented this immunosuppressive microenvironment after sorafenib treatment, inhibited tumor growth, reduced lung metastasis, and improved survival (66). In treatment-naïve tumors in mice with orthotopic hepatocellular cancer, CXCR4 inhibition in the tumor microenvironment was demonstrated to facilitate anti-PD-1 immunotherapy. In an in vivo lung metastatic mouse

model of human melanoma, the CXCR4 antagonist T22 sensitized melanoma cells for immunity–augmenting low-dose cyclophosphamide and anticytotoxic T-lymphocyte–associated antigen 4 monoclonal antibody therapy, resulting in 70% and 50% fewer lung metastases, respectively, than cyclophosphamide and anticytotoxic T-lymphocyte–associated antigen 4 monoclonal antibody therapy alone (67).

The concept of inhibition of the CXCL12–CXCR4 axis in combination with treatment with an immune checkpoint inhibitor is currently being tested in a phase 1a/b trial evaluating the safety and tolerability of CXCR4 peptide antagonist LY2510924 in combination with a monoclonal antibody targeting programmed cell death ligand 1 durvalumab in patients with advanced refractory solid tumors.

CXCR4 RECEPTOR IMAGING AND CXCR4-DIRECTED RADIONUCLIDE THERAPY

Given the paramount importance of the CXCL12–CXCR4 axis in a multitude of tumors, the chemokine receptor represents a promising target for imaging and therapy. Recently, noninvasive molecular imaging of CXCR4 expression was made feasible by the introduction of radiolabeled receptor ligands that allow for whole-body SPECT or PET (68–73). For example, labeling of AMD3100 with ^{99m}Tc resulted in specific binding in organs with high levels of CXCR4 expression and CXCR4-positive tumors (71). The development of ^{68}Ga -pentixafor can be regarded as a milestone for clinical PET imaging of CXCR4 expression (69,70). Proof-of-concept visualization with this tracer could be demonstrated not only for several different hematologic and other neoplasms—including leukemia, lymphoma, multiple myeloma, adrenocortical carcinoma, and small cell lung cancer (Fig. 2) (72–77)—but also for other solid tumors and disease conditions—such as splenosis, stroke, atherosclerosis, and myocardial infarction (78–84).

Interestingly, patients can present with striking inter- and intraindividual receptor expression heterogeneity. Solid tumors, in particular, have demonstrated lower levels of in vivo CXCR4 expression, as is generally described for in vitro expression profiles (78). As a potential explanation, differences between either transcript or whole-protein level analysis of CXCR4 expression and membrane-associated tracer binding in the primary tumor and metastases have been considered (85). Additionally, chemokine receptor expression on the cell surface

has been shown to be a dynamic process that is responsive to concomitant or intermittent therapy (Constantin Lapa, unpublished data, 2017). Future studies should investigate the underlying mechanisms and biologic implications (76). So far, the main value of CXCR4 imaging in oncology is not staging of disease but identification of suitable candidates for chemokine receptor–targeted treatment, including radiolabeled and nonradiolabeled options. Most available data concern multiple myeloma, with about two thirds of patients having CXCR4-positive disease (76).

Recently, a peptide ligand that can be labeled with α - or β -emitters (pentixafor) and that represents a therapeutic counterpart

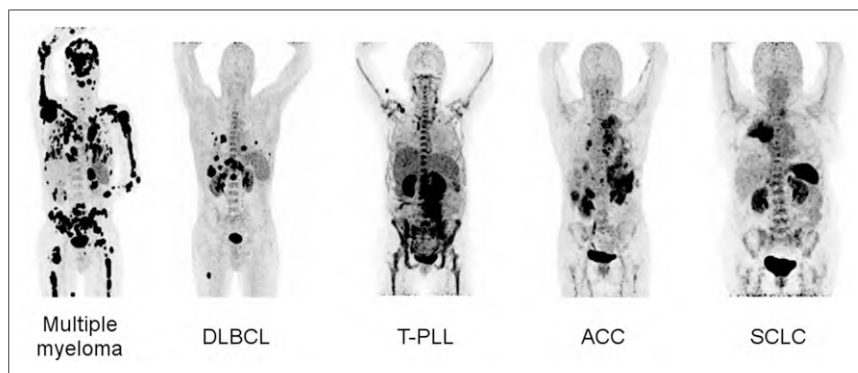


FIGURE 2. Maximum-intensity projections of different tumor entities undergoing ^{68}Ga -pentixafor PET/CT: multiple myeloma, diffuse large B-cell lymphoma (DLBCL), T-cell prolymphocytic leukemia (T-PLL), adrenocortical carcinoma (ACC), and small cell lung cancer (SCLC).

to the diagnostic PET/SPECT agents was developed (86). Thus, the so-called theranostic concept known from neuroendocrine tumors or even thyroid carcinoma can be applied to various diseases, offering new, exciting options for individualized medicine in terms of endoradiotherapy. The first encouraging results were obtained from small pilot studies with end-stage multiple myeloma patients, in whom CXCR4-directed endoradiotherapy resulted in high initial response rates (87,88). However, given the ultra-high-risk patient population, the duration of the response was relatively short, with median progression-free and overall survival times of 54 and 223 d, respectively (88).

Experience and promising data have also been gained for other hematologic malignancies, such as (relapsed or refractory) acute myeloid leukemia and diffuse large B-cell lymphoma, which could also be effectively targeted by radionuclide therapy (Constantin Lapa, unpublished data, 2015–2017). Up to now, more than 30 therapies have been safely performed and have been well tolerated, without any significant adverse reaction in most cases. However, tumor lysis syndrome was observed in a single case with a high tumor burden and highly dedifferentiated myeloma (88). In all cases, endoradiotherapy was added to standard high-dose chemotherapy as part of the conditioning regimen before stem cell transplantation to augment tumor cell killing and to mediate (expected) CXCR4-directed therapy-induced myeloablation due to receptor expression on the tumor and on physiologic bone marrow progenitor cells. Therefore, endoradiotherapy is especially suitable for hematologic disease, in which tumor and bone marrow ablation is highly desirable and stem cell rescue is not a concern.

Whereas side effects in bone marrow have prevented the use of radionuclide therapy for solid cancers so far, endoradiotherapy could be considered an option for malignancies such as adrenocortical cancer or small cell lung cancer, given the intense receptor expression in relapsed stages and an otherwise dismal prognosis. Future trials will investigate the benefit of endoradiotherapy in patients with multiple myeloma or lymphoma at earlier disease stages (COLPRIT trial; European Union Drug Regulating Authorities Clinical Trials number 2015-001817-28). Future research will also focus on combinations of endoradiotherapy and “conventional” therapies, which could lead to synergistic effects. Both preclinical studies in myeloma cell lines and clinical observations in patients with various diseases (such as myeloma, diffuse large B-cell lymphoma, and acute myeloid leukemia) have suggested the possibility of up- or downregulating CXCR4 on the cell surface. ⁶⁸Ga-pentixafor could prove to be a useful tool for studying the spatial and temporal orchestration of CXCR4 biology in the process of metastasis, response to and acquisition of resistance to treatment, or disease relapse.

CONCLUSION

CXCR4, a surface receptor that is overexpressed in multiple cancer types, plays a role in various stages of tumor progression and other diseases. CXCR4 inhibitors are in clinical use as bone marrow stem cell or progenitor cell mobilizers and are being investigated either alone or in combination with other systemic treatments in numerous clinical trials. The next major development may be CXCR4-directed endoradiotherapy, for which the results of clinical trials are eagerly awaited.

DISCLOSURE

No potential conflict of interest relevant to this article was reported.

REFERENCES

- Caruz A, Samsom M, Alonso JM, et al. Genomic organization and promoter characterization of human CXCR4 gene. *FEBS Lett.* 1998;426:271–278.
- Hamm HE. The many faces of G protein signaling. *J Biol Chem.* 1998;273:669–672.
- Burger JA, Kipps TJ. CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. *Blood.* 2006;107:1761–1767.
- Wojcechowskyj JA, Lee JY, Seeholzer SH, et al. Quantitative phosphoproteomics of CXCL12 (SDF-1) signaling. *PLoS One.* 2011;6:e24918.
- Domanska UM, Kruizinga RC, Nagengast WB, et al. A review on CXCR4/CXCL12 axis in oncology: no place to hide. *Eur J Cancer.* 2013;49:219–230.
- Loetscher P, Moser B, Baggiolini M. Chemokines and their receptors in lymphocyte traffic and HIV infection. *Adv Immunol.* 2000;74:127–180.
- Kruizinga RC, Bestebroer J, Berghuis P, et al. Role of chemokines and their receptors in cancer. *Curr Pharm Des.* 2009;15:3396–3416.
- Feng Y, Broder CC, Kennedy PE, et al. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor. *Science.* 1996;272:872–877.
- Orimo A, Gupta PB, Sgroi DC, et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. *Cell.* 2005;121:335–348.
- Müller A, Homey B, Soto H, et al. Involvement of chemokine receptors in breast cancer metastasis. *Nature.* 2001;410:50–56.
- Guo F, Wang Y, Liu J, et al. CXCL12/CXCR4: a symbiotic bridge linking cancer cells and their stromal neighbors in oncogenic communication networks. *Oncogene.* 2016;35:816–826.
- Burger JA, Stewart DJ, Wald O, et al. Potential of CXCR4 antagonists for the treatment of metastatic lung cancer. *Expert Rev Anticancer Ther.* 2011;11:621–630.
- De Clercq E, Yamamoto N, Pauwels R, et al. Highly potent and selective inhibition of human immunodeficiency virus by the bicyclam derivative JM3100. *Antimicrob Agents Chemother.* 1994;38:668–674.
- Donzella GA, Schols D, Lin SW, et al. AMD3100, a small molecule inhibitor of HIV-1 entry via the CXCR4 co-receptor. *Nat Med.* 1998;4:72–77.
- Hendrix CW, Flexner C, MacFarland RT, et al. Pharmacokinetics and safety of AMD-3100, a novel antagonist of the CXCR-4 chemokine receptor, in human volunteers. *Antimicrob Agents Chemother.* 2000;44:1667–1673.
- Broxmeyer HE, Orschell CM, Clapp DW, et al. Rapid mobilization of murine and human hematopoietic stem and progenitor cells with AMD3100, a CXCR4 antagonist. *J Exp Med.* 2005;201:1307–1318.
- DiPersio JF, Micallef IN, Stiff PJ, et al. Phase III prospective randomized double-blind placebo-controlled trial of plerixafor plus granulocyte colony-stimulating factor compared with placebo plus granulocyte colony-stimulating factor for autologous stem-cell mobilization and transplantation for patients with non-Hodgkin's lymphoma. *J Clin Oncol.* 2009;27:4767–4773.
- Devine SM, Vij R, Rettig M, et al. Rapid mobilization of functional donor hematopoietic cells without G-CSF using AMD3100, an antagonist of the CXCR4/SDF-1 interaction. *Blood.* 2008;112:990–998.
- Stone ND, Dunaway SB, Flexner C, et al. Multiple-dose escalation study of the safety, pharmacokinetics, and biologic activity of oral AMD070, a selective CXCR4 receptor inhibitor, in human subjects. *Antimicrob Agents Chemother.* 2007;51:2351–2358.
- Ichiyama K, Yokoyama-Kumakura S, Tanaka Y, et al. A duodenally absorbable CXCR4 PI3K chemokine receptor 4 antagonist, KRH-1636, exhibits a potent and selective anti-HIV-1 activity. *Proc Natl Acad Sci USA.* 2003;100:4185–4190.
- Debnath B, Xu S, Grande F, et al. Small molecule inhibitors of CXCR4. *Theranostics.* 2013;3:47–75.
- Masuda M, Nakashima H, Ueda T, et al. A novel anti-HIV synthetic peptide, T-22 ([Tyr^{5,12}, Lys⁷]-polyphemusin II). *Biochem Biophys Res Commun.* 1992;189:845–850.
- Tamamura H, Xu Y, Hattori T, et al. A low-molecular-weight inhibitor against the chemokine receptor CXCR4: a strong anti-HIV peptide T140. *Biochem Biophys Res Commun.* 1998;253:877–882.
- Narumi T, Aikawa H, Tanaka T, et al. Low-molecular-weight CXCR4 ligands with variable spacers. *ChemMedChem.* 2013;8:118–124.
- Kuhne MR, Mulvey T, Belanger B, et al. BMS-936564/MDX-1338: a fully human anti-CXCR4 antibody induces apoptosis in vitro and shows antitumor activity in vivo in hematologic malignancies. *Clin Cancer Res.* 2013;19:357–366.

26. Endres MJ, Clapham PR, Marsh M, et al. CD4-independent infection by HIV-2 is mediated by fusin/CXCR4. *Cell*. 1996;87:745–756.
27. Griffiths K, Dolezal O, Cao B, et al. i-bodies, human single domain antibodies that antagonize chemokine receptor CXCR4. *J Biol Chem*. 2016;291:12641–12657.
28. Porvasnik S, Sakamoto N, Kusmartsev S, et al. Effects of CXCR4 antagonist CTCE-9908 on prostate tumor growth. *Prostate*. 2009;69:1460–1469.
29. Kim SY, Lee CH, Midura BV, et al. Inhibition of the CXCR4/CXCL12 chemokine pathway reduces the development of murine pulmonary metastases. *Clin Exp Metastasis*. 2008;25:201–211.
30. Duda DG, Kozin SV, Kirkpatrick ND, et al. CXCL12 (SDF1alpha)-CXCR4/CXCR7 pathway inhibition: an emerging sensitizer for anticancer therapies? *Clin Cancer Res*. 2011;17:2074–2080.
31. Chen Y, Jacamo R, Konopleva M, et al. CXCR4 downregulation of let-7a drives chemoresistance in acute myeloid leukemia. *J Clin Invest*. 2013;123:2395–2407.
32. Wong D, Korz W. Translating an antagonist of chemokine receptor CXCR4: from bench to bedside. *Clin Cancer Res*. 2008;14:7975–7980.
33. Olumi AF, Grossfeld GD, Hayward SW, et al. Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. *Cancer Res*. 1999;59:5002–5011.
34. Burger JA, Peled A. CXCR4 antagonists: targeting the microenvironment in leukemia and other cancers. *Leukemia*. 2009;23:43–52.
35. Kucia M, Reza R, Miekus K, et al. Trafficking of normal stem cells and metastasis of cancer stem cells involve similar mechanisms: pivotal role of the SDF-1-CXCR4 axis. *Stem Cells*. 2005;23:879–894.
36. Ishikawa F, Yoshida S, Saito Y, et al. Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. *Nat Biotechnol*. 2007;25:1315–1321.
37. Dillmann F, Veldwijk MR, Laufs S, et al. Plerixafor inhibits chemotaxis toward SDF-1 and CXCR4-mediated stroma contact in a dose-dependent manner resulting in increased susceptibility of BCR-ABL⁺ cell to imatinib and nilotinib. *Leuk Lymphoma*. 2009;50:1676–1686.
38. Nervi B, Ramirez P, Rettig MP, et al. Chemosensitization of acute myeloid leukemia (AML) following mobilization by the CXCR4 antagonist AMD3100. *Blood*. 2009;113:6206–6214.
39. Burger M, Hartmann T, Krome M, et al. Small peptide inhibitors of the CXCR4 chemokine receptor (CD184) antagonize the activation, migration, and antiapoptotic responses of CXCL12 in chronic lymphocytic leukemia B cells. *Blood*. 2005;106:1824–1830.
40. Rashidi A, Uy GL. Targeting the microenvironment in acute myeloid leukemia. *Curr Hematol Malig Rep*. 2015;10:126–131.
41. Uy GL, Rettig MP, Motabi IH, et al. A phase I/II study of chemosensitization with the CXCR4 antagonist plerixafor in relapsed or refractory acute myeloid leukemia. *Blood*. 2012;119:3917–3924.
42. Andritsos L, Byrd JC, Jones JA, et al. Preliminary results from a phase I dose escalation study to determine the maximum tolerated dose of plerixafor in combination with rituximab in patients with relapsed chronic lymphocytic leukemia [abstract]. *Blood*. 2010;116:2450.
43. Uy GL, Avigan D, Cortes JE, et al. Safety and tolerability of plerixafor in combination with cytarabine and daunorubicin in patients with newly diagnosed acute myeloid leukemia: preliminary results from a phase I study [abstract]. *Blood*. 2011;118:82.
44. Roboz GJ, Scandura JM, Ritchie E, et al. Combining decitabine with plerixafor yields a high response rate in newly diagnosed older patients with AML [abstract]. *Blood*. 2013;122:621.
45. Andreeff M, Zeng Z, Kelly MA, et al. Mobilization and elimination of FLT3-ITD⁺ acute myelogenous leukemia (AML) stem/progenitor cells by plerixafor/G-CSF/sorafenib: results from a phase I trial in relapsed/refractory AML patients [abstract]. *Blood*. 2012;120:142.
46. Becker PS, Foran JM, Altman JK, et al. Targeting the CXCR4 pathway: safety, tolerability and clinical activity of ulocuplumab (BMS-936564), an anti-CXCR4 antibody, in relapsed/refractory acute myeloid leukemia [abstract]. *Blood*. 2014;124:386.
47. Liu T, Li X, You S, Bhuyan SS, Dong L. Effectiveness of AMD3100 in treatment of leukemia and solid tumors: from original discovery to use in current clinical practice. *Exp Hematol Oncol*. 2016;5:19.
48. Tamas K, Domanska UM, van Dijk TH, et al. CXCR4 and CXCL12 expression in rectal tumors of stage IV patients before and after local radiotherapy and systemic neoadjuvant treatment. *Curr Pharm Des*. 2015;21:2276–2283.
49. Domanska UM, Timmer-Bosscha H, Nagengast WB, et al. CXCR4 inhibition with AMD3100 sensitizes prostate cancer to docetaxel chemotherapy. *Neoplasia*. 2012;14:709–718.
50. Barone A, Sengupta R, Warrington NM, et al. Combined VEGF and CXCR4 antagonism targets the GBM stem cell population and synergistically improves survival in an intracranial mouse model of glioblastoma. *Oncotarget*. 2014;5:9811–9822.
51. Taal W, Oosterkamp HM, Walenkamp AM, et al. Single-agent bevacizumab or lomustine versus a combination of bevacizumab plus lomustine in patients with recurrent glioblastoma (BELOB trial): a randomised controlled phase 2 trial. *Lancet Oncol*. 2014;15:943–953.
52. Wick W, Brandes AA, Gorlia T, et al. EORTC 26101 phase III trial exploring the combination of bevacizumab and lomustine in patients with first progression of a glioblastoma. *J Clin Oncol*. 2016;34(suppl):2001.
53. Pardoll DM. The blockade of immune checkpoints in cancer immunotherapy. *Nat Rev Cancer*. 2012;12:252–264.
54. Hodi FS, O'Day SJ, McDermott DF, et al. Improved survival with ipilimumab in patients with metastatic melanoma. *N Engl J Med*. 2010;363:711–723.
55. Brahmer JR, Tykodi SS, Chow LQ, et al. Safety and activity of anti-PD-L1 antibody in patients with advanced cancer. *N Engl J Med*. 2012;366:2455–2465.
56. Topalian SL, Hodi FS, Brahmer JR, et al. Safety, activity, and immune correlates of anti-PD-1 antibody in cancer. *N Engl J Med*. 2012;366:2443–2454.
57. Powles T, Eder JP, Fine GD, et al. MPDL3280A (anti-PD-L1) treatment leads to clinical activity in metastatic bladder cancer. *Nature*. 2014;515:558–562.
58. Herbst RS, Soria JC, Kowanetz M, et al. Predictive correlates of response to the anti-PD-L1 antibody MPDL3280A in cancer patients. *Nature*. 2014;515:563–567.
59. Tumeh PC, Harview CL, Yearley JH, et al. PD-1 blockade induces responses by inhibiting adaptive immune resistance. *Nature*. 2014;515:568–571.
60. Joyce JA, Fearon DT. T cell exclusion, immune privilege, and the tumor microenvironment. *Science*. 2015;348:74–80.
61. Royal RE, Levy C, Turner K, et al. Phase 2 trial of single agent ipilimumab (anti-CTLA-4) for locally advanced or metastatic pancreatic adenocarcinoma. *J Immunother*. 2010;33:828–833.
62. Scotton CJ, Wilson JL, Scott K, et al. Multiple actions of the chemokine CXCL12 on epithelial tumor cells in human ovarian cancer. *Cancer Res*. 2002;62:5930–5938.
63. Salmon H, Franciszkiewicz K, Damotte D, et al. Matrix architecture defines the preferential localization and migration of T cells into the stroma of human lung tumors. *J Clin Invest*. 2012;122:899–910.
64. Feig C, Jones JO, Kraman M, et al. Targeting CXCL12 from FAP-expressing carcinoma-associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. *Proc Natl Acad Sci USA*. 2013;110:20212–20217.
65. Chen Y, Huang Y, Reiberger T, et al. Differential effects of sorafenib on liver versus tumor fibrosis mediated by stromal-derived factor 1 alpha/C-X-C receptor type 4 axis and myeloid differentiation antigen-positive myeloid cell infiltration in mice. *Hepatology*. 2014;59:1435–1447.
66. Chen Y, Ramjiawan RR, Reiberger T, et al. CXCR4 inhibition in tumor microenvironment facilitates anti-programmed death receptor-1 immunotherapy in sorafenib-treated hepatocellular carcinoma in mice. *Hepatology*. 2015;61:1591–1602.
67. Lee CH, Kakinuma T, Wang J, et al. Sensitization of B16 tumor cells with a CXCR4 antagonist increases the efficacy of immunotherapy for established lung metastases. *Mol Cancer Ther*. 2006;5:2592–2599.
68. Jacobson O, Weiss ID, Kiesewetter DO, Farber JM, Chen X. PET of tumor CXCR4 expression with 4-¹⁸F-T140. *J Nucl Med*. 2010;51:1796–1804.
69. Demmer O, Gourni E, Schumacher U, Kessler H, Wester HJ. PET imaging of CXCR4 receptors in cancer by a new optimized ligand. *ChemMedChem*. 2011;6:1789–1791.
70. Gourni E, Demmer O, Schottelius M, et al. PET of CXCR4 expression by a ⁶⁸Ga-labeled highly specific targeted contrast agent. *J Nucl Med*. 2011;52:1803–1810.
71. Hartmath SV, Domanska UM, Walenkamp AM, et al. [^{99m}Tc]O₂-AMD3100 as a SPECT tracer for CXCR4 receptor imaging. *Nucl Med Biol*. 2013;40:507–517.
72. Wester HJ, Keller U, Schottelius M, et al. Disclosing the CXCR4 expression in lymphoproliferative diseases by targeted molecular imaging. *Theranostics*. 2015;5:618–630.
73. Herrmann K, Lapa C, Wester HJ, et al. Biodistribution and radiation dosimetry for the chemokine receptor CXCR4-targeting probe ⁶⁸Ga-pentixafor. *J Nucl Med*. 2015;56:410–416.
74. Lapa C, Luckertath K, Kleinlein I, et al. ⁶⁸Ga-pentixafor-PET/CT for imaging of chemokine receptor 4 expression in glioblastoma. *Theranostics*. 2016;6:428–434.
75. Lapa C, Luckertath K, Rudelius M, et al. [⁶⁸Ga]pentixafor-PET/CT for imaging of chemokine receptor 4 expression in small cell lung cancer: initial experience. *Oncotarget*. 2016;7:9288–9295.

76. Lapa C, Schreder M, Schirbel A, et al. [⁶⁸Ga]pentixafor-PET/CT for imaging of chemokine receptor CXCR4 expression in multiple myeloma: comparison to [¹⁸F]FDG and laboratory values. *Theranostics*. 2017;7:205–212.
77. Herhaus P, Habringer S, Philipp-Abbrederis K, et al. Targeted positron emission tomography imaging of CXCR4 expression in patients with acute myeloid leukemia. *Haematologica*. 2016;101:932–940.
78. Vag T, Gemgross C, Herhaus P, et al. First experience with chemokine receptor CXCR4-targeted PET imaging of patients with solid cancers. *J Nucl Med*. 2016;57:741–746.
79. Derlin T, Wester HJ, Bengel FM, Hueper K. Visualization of posttraumatic splenosis on chemokine receptor CXCR4-targeted PET/CT. *Clin Nucl Med*. 2017;6:e317–e318.
80. Schmid JS, Schirbel A, Buck AK, Kropf S, Wester HJ, Lapa C. [⁶⁸Ga]pentixafor-positron emission tomography/computed tomography detects chemokine receptor CXCR4 expression after ischemic stroke. *Circ Cardiovasc Imaging*. 2016;9:e005217.
81. Hyafil F, Pelisek J, Laitinen I, et al. Imaging the cytokine receptor CXCR4 in atherosclerotic plaques with the radiotracer ⁶⁸Ga-pentixafor for PET. *J Nucl Med*. 2017;58:499–506.
82. Lapa C, Reiter T, Werner RA, et al. [⁶⁸Ga]pentixafor-PET/CT for imaging of chemokine receptor 4 expression after myocardial infarction. *JACC Cardiovasc Imaging*. 2015;8:1466–1468.
83. Rischpler C, Nekolla SG, Kossmann H, et al. Upregulated myocardial CXCR4-expression after myocardial infarction assessed by simultaneous GA-68 pentixafor PET/MRI. *J Nucl Cardiol*. 2016;23:131–133.
84. Thackeray JT, Derlin T, Haghighi A, et al. Molecular imaging of the chemokine receptor CXCR4 after acute myocardial infarction. *JACC Cardiovasc Imaging*. 2015;8:1417–1426.
85. Shim H, Lau SK, Devi S, Yoon Y, Cho HT, Liang Z. Lower expression of CXCR4 in lymph node metastases than in primary breast cancers: potential regulation by ligand-dependent degradation and HIF-1α. *Biochem Biophys Res Commun*. 2006;346:252–258.
86. Schottelius M, Osl T, Poschenrieder A, et al. [¹⁷⁷Lu]pentixather: comprehensive preclinical characterization of a first CXCR4-directed endoradiotherapeutic agent. *Theranostics*. 2017;7:2350–2362.
87. Herrmann K, Schottelius M, Lapa C, et al. First-in-human experience of CXCR4-directed endoradiotherapy with ¹⁷⁷Lu- and ⁹⁰Y-labeled pentixather in advanced-stage multiple myeloma with extensive intra- and extramedullary disease. *J Nucl Med*. 2016;57:248–251.
88. Lapa C, Herrmann K, Schirbel A, et al. CXCR4-directed endoradiotherapy induces high response rates in extramedullary relapsed multiple myeloma. *Theranostics*. 2017;7:1589–1597.