

## REVIEW

# CXCR4 antagonists: targeting the microenvironment in leukemia and other cancers

JA Burger<sup>1</sup> and A Peled<sup>2</sup>

<sup>1</sup>Department of Leukemia, The University of Texas MD Anderson Cancer Center, Houston, TX, USA and <sup>2</sup>Goldyne Savad Institute of Gene Therapy, Hadassah Hebrew University Hospital, Jerusalem, Israel

**Hematopoietic and epithelial cancer cells express CXCR4, a seven-transmembrane G-protein-coupled chemokine receptor. Stromal cells within the bone marrow microenvironment constitutively secrete stromal cell-derived factor-1 (SDF-1/CXCL12), the ligand for CXCR4. Activation of CXCR4 induces leukemia cell trafficking and homing to the marrow microenvironment, where CXCL12 retains leukemia cells in close contact with marrow stromal cells that provide growth and drug resistance signals. CXCR4 antagonists, such as Plerixafor (AMD3100) and T140 analogs, can disrupt adhesive tumor-stroma interactions and mobilize leukemia cells from their protective stromal microenvironment, making them more accessible to conventional drugs. Therefore, targeting the CXCR4-CXCL12 axis is a novel, attractive therapeutic approach that is explored in ongoing clinical trials in leukemia patients. Initially, CXCR4 antagonists were developed for the treatment of HIV, where CXCR4 functions as a co-receptor for virus entry into T cells. Subsequently, CXCR4 antagonists were noticed to induce leukocytosis, and are currently used clinically for mobilization of hematopoietic stem cells. However, because CXCR4 plays a key role in cross-talk between leukemia cells (and a variety of other tumor cells) and their microenvironment, cancer treatment may become the ultimate application of CXCR4 antagonists. Here, we summarize the development of CXCR4 antagonists and their preclinical and clinical activities, focusing on leukemia and other cancers.**

*Leukemia* (2009) 23, 43–52; doi:10.1038/leu.2008.299; published online 6 November 2008

**Keywords:** CXCR4; CXCR4 antagonists; leukemia microenvironment; leukemia stem cells; CXCL12

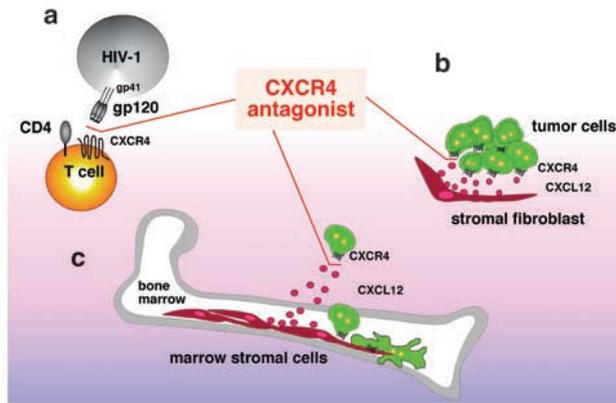
## CXCR4: a unique chemokine receptor

Chemokines are defined by their ability to induce directional migration of cells toward a gradient of the chemokine (chemotaxis) through binding to a subset of seven-transmembrane, G-protein-coupled (chemokine) receptors. Chemokines are small (~8–14 kDa) secreted proteins that are divided into the 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, or they are adjacent, accounting for CXC or CC chemokines.<sup>1</sup> In a more functional sense, chemokines can also be classified as inflammatory or homeostatic chemokines that are induced during inflammation to attract inflammatory cells,<sup>2</sup> or 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 system.<sup>3,4</sup>

Stromal cell-derived factor-1 (SDF-1), now designated CXCL12,<sup>1</sup> signals through the CXCR4 chemokine receptor,<sup>5,6</sup> and was initially described as a pre-B-cell growth factor in 1994.<sup>7</sup> In 1996, the co-receptor function of CXCR4 for the entry of T-tropic (X4) human immunodeficiency virus (HIV)-1 strains into CD4-positive T cells was discovered.<sup>5,6,8</sup> Subsequently, CXCL12 and CXCR4 gene-deleted mice were described with an identical, lethal phenotype, suggesting a monogamous relationship between this chemokine and its receptor. The phenotype of these mice is characterized by deficient hematopoiesis with defects in B-cell development and myelopoiesis, and abnormal neuronal and cardiovascular development.<sup>9–12</sup> More recently, CXCR7 has been described as an alternate receptor for CXCL12, which appears to function by sequestering CXCL12<sup>13</sup> and modifying CXCR4 signaling rather than displaying autonomous signaling in response to CXCL12.<sup>14</sup> Mesenchymal stromal cells (MSCs) are considered a major source for CXCL12 in the adult organism. CXCL12-secreting stromal cells can be found in various tissues, such as the liver, lungs, lymphatic tissues and the marrow.<sup>15</sup> Constitutive high-level CXCL12 secretion by reticular stromal cells in the marrow is essential for homing<sup>16</sup> and maintenance of hematopoietic stem cells (HSCs) in distinct vascular and endosteal niches for their development and growth.<sup>17</sup> Through CXCL12, these stromal cells also attract circulating hematopoietic progenitor cells<sup>16</sup> or leukemia cells<sup>18</sup> for homing to the marrow (Figure 1). Actually, CXCR4 is the only functional chemokine receptor on hematopoietic progenitor cells,<sup>19</sup> emphasizing the predominant role of this chemokine receptor for homing and maintenance of HSC in the marrow niches. More recently, the architecture of niches for hematopoietic and other tissue stem cells (that is, germline stem cells, follicle stem cell, intestinal stem cell, central nervous system stem cell, and others) and the mechanism that govern stem cell homeostasis within these niches are emerging.<sup>20,21</sup> Regulated migration and homing of stem cells to tissue niches are critical steps not only during embryonic development or tissue repair but also in cancer (stem) cell dissemination.<sup>22</sup> In this context, the CXCR4-CXCL12 axis functions as a migration mechanism broadly conserved across species that is essential for stem cell migration in multiple tissues in both the embryo and adult. The responsiveness to CXCL12 significantly changes during differentiation of hematopoietic cells, as demonstrated for lymphoid<sup>23</sup> and myeloid<sup>24</sup> cells. These maturation-dependent changes in CXCL12 responsiveness, which are not necessarily accompanied by changes in CXCR4 expression levels,<sup>23</sup> are thought to regulate trafficking and homing to distinct tissue microenvironments. For example, high CXCL12 responsiveness allows for the retention and homing of immature and mature B cells (pre- and pro-B cells, and plasma cells) to the marrow.<sup>25–27</sup> This maturation-dependent CXCL12 responsiveness appears to be retained in malignancies that correspond to respective maturation stages of their normal counterparts; that is, pre- and

Correspondence: Dr JA Burger, Department of Leukemia, The University of Texas MD Anderson Cancer Center, Unit 428, PO Box 301402, Houston, TX 77230-1402, USA.  
E-mail: jaburger@mdanderson.org

Received 25 August 2008; revised 27 September 2008; accepted 1 October 2008; published online 6 November 2008



**Figure 1** CXCR4 antagonists in human immunodeficiency (HIV-1) and cancer. (a) CXCR4 is the co-receptor used along with CD4 by T cell-tropic (X4) HIV-1 strains for cellular entry into T cells. A trimeric unit of viral envelope glycoproteins (gp120) that are anchored by gp41 binds to CD4 on the surface of T cells, inducing a conformational change of gp120, allowing it to interact with CXCR4 through the V3 loop of gp120. CXCR4 antagonists block the CXCR4-binding site for X4 HIV-1, and thereby prevent fusion of HIV-1 with T cells. (b) Stromal fibroblasts within the tumor microenvironment secrete CXCL12 and thereby attract and retain tumor cells in contact with the stroma. Adhesion of tumor cells to stromal cells confers survival, growth and drug resistance signals (cell adhesion-mediated drug resistance (CAM-DR)) that are, at least in part, mediated by activation of CXCR4 on the tumor cells. Stromal cell-mediated activation of CXCR4 is also called a ‘paracrine’ activation of tumor cells through CXCL12.<sup>48</sup> CXCR4 antagonists can disrupt the adhesive interactions between tumor cells and tumoral fibroblasts, mobilizing them from the tumor microenvironment, and making the tumor cells more accessible to cytotoxic drugs. (c) Tumor cells (hematopoietic and non-hematopoietic) also utilize the CXCR4-CXCL12 axis to migrate and home to target organs, such as the marrow. CXCL12 is constitutively secreted by marrow stromal cells retains leukemia cells in protective marrow niches and attracts circulating tumor cells for directional homing/metastasis. CXCR4 antagonists can inhibit this mechanism of tumor cell homing by blocking CXCR4 receptors responsible for migration to CXCL12-secreting stromal cells, thereby mobilizing tumor cells from tissue sites, such as the marrow.

pro-B-cell acute lymphoblastic leukemia (ALL) cells and multiple myeloma cells utilize the CXCR4/CXCL12 axis for bone marrow homing.<sup>18,28</sup>

There is also growing evidence suggesting that leukemia progression is driven by a sub-population of cells referred to as leukemia stem cells (LSCs) that are more leukemogenic than other cells of the same clone. LSCs 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.<sup>29–31</sup> Normal and malignant stem cells apparently have a particular requirement for distinct niches: HSCs localize to CXCL12<sup>+</sup> stromal cells that are in close proximity to the marrow vasculature (vascular niche) or the endosteum.<sup>17</sup> Interestingly, brain tumor stem cells also reside in vascular niches adjacent to blood vessels,<sup>32</sup> suggesting that normal stem cells, LSCs and other cancer stem cells (CSCs) have fundamentally similar requirements toward their niches.<sup>21,33</sup> This concept implies that LSCs would preferentially localize through CXCR4 to vascular and endosteal niches that are normally restricted to HSC. Consequently, CXCR4 antagonists could mobilize LSCs and/or CSCs that are

normally protected in tissue niches and make them accessible to conventional anticancer drugs.

Current clinical trials with AMD3100 utilized this mechanism of CXCR4-mediated homing to the marrow to mobilize HSC to the peripheral blood for HSC collection for autologous stem cell transplantation.<sup>34</sup> In phase II trials, mobilization with the combination of AMD3100 and granulocyte colony-stimulating factor (G-CSF) results in the collection of more progenitor cells than G-CSF alone.<sup>35</sup> The peptide CXCR4 antagonist TN14003 is also a potent mobilizer of HSC alone and in combination with G-CSF; and apparently TN14003 displays a pattern of hematopoietic cells and overall potency for HSC mobilization that is different from AMD3100.<sup>36</sup>

The CXCR4-CXCL12 axis is also related to tissue hypoxia and repair of hypoxic damage.<sup>37</sup> The transcription factor hypoxia-inducible factor-1 (HIF-1), which gets upregulated in hypoxic states, induces the local expression of CXCL12,<sup>38</sup> which in turn can attract circulating progenitor cells for tissue repair. This function of the CXCR4-CXCL12 axis plays a prominent role in the recruitment of marrow-derived progenitors to the heart after myocardial infarction.<sup>39,40</sup> Currently, there are research efforts to utilize this mechanism by local intramyocardial delivery of a protease-resistant CXCL12 to attract progenitor cells to sites of hypoxic damage after myocardial infarction.<sup>41</sup> Moreover, in hypoxic tumors or in tumors that display mutations in the von Hippel-Lindau tumor suppressor protein pVHL (a negative regulator of HIF-1), HIF-1 upregulates CXCR4 expression,<sup>42,43</sup> providing a survival benefit for tumor cells with high CXCR4 expression.

### Blocking CXCR4 in the treatment of leukemia and other cancers

CXCR4 chemokine receptors are expressed by leukemia cells from patients with acute and chronic leukemias and also various solid tumors, such as breast cancer, lung cancer, prostate cancer and others.<sup>37,44</sup> In general, CXCR4 expression by the tumor cells allows for tumor cell migration, and homing of the neoplastic cells to sites where non-malignant stromal cells express CXCL12, the ligand for CXCR4.<sup>45</sup> This concept implies that tumor cell metastasis is not random, but guided by the expression of chemokine receptors and adhesion molecules on the neoplastic cells, and respective ligands in the target organs.<sup>15,46</sup> Tumor cells apparently utilize this mechanism to access microenvironments, such as the marrow, that provide factors that favor their growth. For example, the importance of CXCR4 for breast cancer metastasis to different target organs was demonstrated *in vitro* and *in vivo*.<sup>15</sup> Leukemia cells from patients with chronic lymphocytic leukemia (CLL) and ALL utilize CXCR4 for homing to marrow stromal cells (MSCs) *in vitro*<sup>47</sup> and *in vivo*.<sup>18</sup> Moreover, CXCL12 has a direct growth- and survival-promoting effect for various cancer cells, such as breast cancer cells<sup>48</sup> or CLL cells.<sup>49</sup> CXCL12 also promotes tumor progression by recruiting endothelial progenitor cells to tumors for tumor angiogenesis.<sup>48</sup> Stromal fibroblasts, also called carcinoma-associated fibroblasts or mesenchymal stromal cells (MSC), are part of the tumor microenvironment. These stromal fibroblasts constitutively secrete CXCL12, and contact between tumor cells and stromal cells is largely dependent on the CXCR4-CXCL12 axis. For example, co-culture between CLL cells<sup>47</sup> or lung cancer cells<sup>50</sup> and CXCL12-secreting stromal cells induces strong adhesion and spontaneous migration of the neoplastic cells beneath the stromal cells (pseudoemperipolesis) in a CXCR4-dependent manner. Nonspecific and specific

CXCR4 antagonists, such as pertussis toxin and CXCR4 antagonists (T140 and AMD3100) can block this adhesion and migration.<sup>51</sup> Adhesion to stromal cells confers resistance to spontaneous and drug-induced cell death of tumor cells, and therefore is also termed cell adhesion-mediated drug resistance.<sup>52,53</sup> Tumor cells that adhere to stromal cells through CXCR4 are therefore, at least partially, protected from the effects of cytotoxic chemotherapy and represent a reservoir for minimal residual disease (MRD) and relapses commonly seen in the treatment of patients with various cancers. However, the overall role of MSC in the tumor microenvironment remains controversial. Although on the one hand, MSC can provide survival, growth and drug resistance signals,<sup>48,54</sup> MSC can also induce cell cycle and growth arrest in epithelial cancer<sup>55–57</sup> and leukemia cells.<sup>58</sup> On the basis of these findings, the therapeutic use of MSC to decelerate tumor growth has been proposed.<sup>59,60</sup> These controversial findings regarding the capacity of MSCs to induce tumor progression (*in vivo*) and also to induce tumor cell growth arrest (*in vitro*) could, at least in part, be due to the ability of MSC to provide niches for CSCs *in vivo*. In these tissue niches, MSCs are thought to maintain and support a sub-population of growth-arrested tumor cells/CSCs that are resistant to cytotoxic treatments and function as a reservoir of the disease with the potential to proliferate and sustain the malignant process.<sup>22</sup>

In summary, the rationale for targeting CXCR4 with CXCR4 antagonists in leukemia and other cancers is as follows:

1. disrupting the adhesive stromal interactions that confer survival and drug resistance signals to leukemia and other cancer cells;
2. mobilizing tumor cells from tissue sites, such as the marrow, and thereby making them better accessible to conventional therapy;
3. blocking of migration and dissemination of tumor cells in the process of tumor cell metastasis;
4. blocking of paracrine growth and survival signals through activation of the CXCR4-CXCL12 axis and
5. blocking pro-angiogenesis effects of CXCL12.

These different mechanisms through which CXCR4 antagonists may display activity in treatment of neoplastic diseases raise questions about when during the course of the disease and for how long they should be administered, and whether CXCR4 antagonists should be given alone or in combination with conventional anticancer drugs. This question will be addressed by clinical trials that are currently designed or ongoing.

### CXCR4 antagonists

CXCR4 antagonists were initially developed as new drugs for the treatment of HIV-1 infection. At the time of their discovery in the early 1990s, the mechanism of anti-HIV activity of the most prominent CXCR4 antagonists, T140 and its analogs,<sup>61,62</sup> AMD3100<sup>63,64</sup> and ALX-4C,<sup>65</sup> was unknown. After the discovery of the co-receptor function of CXCR4 for T tropic HIV-1, the specific CXCR4-blocking function of the different CXCR4 antagonists was rapidly demonstrated.<sup>66–68</sup> With the rapid increase in our knowledge of other, non-HIV-related functions of CXCR4 over the past 11 years, other potential applications such as HSC mobilization and treatment of cancer and autoimmune disease are emerging and have gradually replaced the original intent to use CXCR4 antagonists as anti-HIV drugs.

In general, four major classes of CXCR4 antagonists and agonists can be distinguished: (a) small peptide CXCR4 antagonists, such as

T140 and its analogs (TN14003 and others), (b) non-peptide CXCR4 antagonists, such as the bicyclam AMD3100, (c) antibodies to CXCR4 and (d) modified agonists and antagonists for SDF-1. Below, we will summarize the current status of preclinical and clinical development for CXCR4 antagonists.

### Small peptide antagonist of CXCR4

Initially, this group of small peptide CXCR4 antagonists was discovered screening naturally occurring peptides for anti-HIV activity. In that process, self-defense peptides from horseshoe crabs, called tachyplesin (from the Japanese horseshoe crab *Tachyplesus tridentatus*) and polyphemusin (from the American horseshoe crab *Limulus polyphemus*), were identified and chemically modified, leading to the synthesis of the anti-HIV peptides T22,<sup>62</sup> T134 and T140.<sup>69</sup> Initially, these compounds were thought to function by inhibiting HIV-1–T-cell fusion or the viral uncoating.<sup>61</sup> However, the precise mechanism of anti-HIV activity remained unclear until the discovery that T tropic HIV-1 (X4-HIV-1) utilizes CXCR4 as a co-receptor for cellular entry into CD4-positive T cells. Soon after this, it was demonstrated the T22 specifically binds to CXCR4 and blocks CXCR4 receptor regions that are critical for HIV-1 viral entry and for activation by its natural ligand, CXCL12.<sup>68</sup> 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 Arg. Therefore, C-terminally amidated T140 analogs were developed to overcome serum instability,<sup>70</sup> 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.<sup>71</sup> Also, 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.<sup>72</sup> Clinical trials will help to determine whether this characteristic of CXCR4 peptide antagonists correlates with a profile of activities and/or side effects that is distinct from AMD3100.

The efficacy of T140 and its analogs for blocking CXCR4 *in vitro* and *in vivo* has been documented in numerous preclinical studies, including *in vivo* models for breast cancer and melanoma,<sup>73,74</sup> rheumatoid arthritis<sup>75</sup> and stem cell mobilization.<sup>36</sup> Other studies explored the activity of these agents in acute<sup>76,77</sup> and chronic leukemias,<sup>54</sup> multiple myeloma,<sup>78</sup> small cell lung cancer (SCLC),<sup>50</sup> malignant melanoma<sup>74</sup> and pancreatic cancer.<sup>79</sup> Besides these disease-oriented studies, T140 and its analogs have been used in basic studies exploring the function of CXCR4 in dendritic cell development<sup>80</sup> and migration,<sup>81</sup> B-cell homing and germinal center positioning within lymphatic tissues,<sup>82</sup> and HSC homing.<sup>83</sup> Currently, the T140 analog TN14003 is under clinical development by Biokine Therapeutics Ltd, Rehovot, Israel.

ALX40-4C is a polypeptide of nine Arg residues that is stabilized by terminal protection and inclusion of D-amino acids. ALX40-4C is a specific CXCR4 antagonist,<sup>66</sup> 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, Mississauga, ON, Canada.<sup>65</sup> This peptide is no longer under development, particularly because of formulation difficulties and lack of efficacy, and also because it is unlikely that an oral formulation of this complex peptide can be produced.

### Non-peptide CXCR4 antagonists

AMD3100 is a bicyclam, in which two cyclam rings are connected through an aromatic bridge. AMD3100 possesses the highest anti-HIV activity among a series of bicyclams that were synthesized in the early 1990s.<sup>64,84</sup> AMD3100 is a specific antagonist of CXCL12 binding to CXCR4, inhibiting CXCL12-mediated calcium mobilization, chemotaxis and GTP binding, and does not cross-react with other chemokine receptors.<sup>85</sup> AMD3100 was initially considered to interfere with HIV-1 fusion or uncoating.<sup>63</sup> Initially developed at Johnson Matthey in collaboration with the Rega Institute for Medical Research (Leuven University, Leuven, Belgium), this compound was first called JM3100, which changed to AMD3100 after a new company (AnorMED, Langley, BC, Canada) took over the development. The anti-HIV-1 activity of AMD3100, restricted to X4-HIV-1 strains, and the blocking function of AMD3100 on gp120 interaction with CXCR4 during viral entry<sup>86</sup> were the initial focus during the early development of this drug. However, an unexpected 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-positive HSC, to the blood.<sup>87,88</sup> In the second trial in HIV patients, one patient receiving the highest dose of AMD3100 (160 µg/kg/h) had a significant drop in his viral load, but overall the efficacy of AMD3100 in affecting disease activity in HIV-1 patients was considered low and therefore this application was not further pursued for AMD3100. Instead, AnorMED explored AMD3100 as a mobilizing agent for HSC,<sup>88</sup> and a subsequent series of preclinical and clinical trials demonstrated that AMD3100 alone and in combination with G-CSF mobilizes HSC.<sup>34,89,90</sup> AMD3100 (recently re-named as Plerixafor or Mozobil) is now owned by Genzyme Corporation (Cambridge, MA, USA) after a recent takeover of AnorMED by Genzyme in late 2006. Plerixafor is currently used in phase III trials in lymphoma and multiple myeloma patients undergoing autologous stem cell mobilization, and current plans are to file for US and European approval of the drug in 2008. The activity of Plerixafor to inhibit CXCR4 activation in various *in vitro* and *in vivo* tumor models, such as inhibition of CXCL12-induced tumor cell migration and downstream signaling and activity in murine tumor models has been reported. These studies are summarized in Table 1.

AMD070 is another orally bioavailable small molecule CXCR4 antagonist with anti-HIV activity that Genzyme is currently developing in phase II trials for HIV-1 treatment.<sup>91</sup>

KRH-1636 is an orally available, non-peptide CXCR4 antagonist that inhibits infection by X4-HIV-1 virus and blocks responses to stimulation with CXCL12, such as calcium mobilization.<sup>92</sup>

### Development antibodies to CXCR4

Neutralizing the interaction between CXCL12, the ligand for CXCR4, and CXCR4 by using anti-CXCR4 antibodies

significantly inhibit HIV infection and tumor cell migration *in vitro*.<sup>47,93,94</sup> 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.<sup>95-98</sup> The unique properties of monoclonal antibody (mAb) therapies, including their high affinity and specificity, and the differential expression of target antigen in tumor cells versus normal cells make them attractive agents for cancer immunotherapy. Development of therapeutic mAbs to CXCR4 is challenging due to the fact that CXCR4 can exhibit conformational heterogeneity. 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.<sup>99</sup> This conformational 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 sub-population 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 not known. However, CXCR4 can be post-translationally modified by sulfation of its N-terminal tyrosines, and by a chondroitinsulfate chain at serine 18. This phenomenon may explain, in part, the difference in confirmation, antibody specificity and function of CXCR4.<sup>100</sup> Altered glycosylation patterns, neo-expression and underexpression or overexpression of glycans are hallmarks of cancer and may significantly affect the activity of various CXCR4 antagonists in development.

### Modified CXCL12

CTCE-9908 and CTCE-0214 are peptide analogs of CXCL12 with inhibitory and agonist activity, respectively. CTCE-9908 that has received orphan drug status by the Food and Drug Administration 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.<sup>101</sup> CTCE-9908 is developed by Chemokine Therapeutics Corp., Vancouver, BC, Canada.

## CXCR4 antagonists in selected cancers

### CLL

B-cell CLL is a leukemia of mature, antigen-experienced B cells. CLL cells accumulate in the blood, marrow and secondary lymphoid tissues. Despite their apparent longevity *in vivo*, isolated CLL cells generally undergo spontaneous apoptosis *in vitro* when cultured under conditions that support the growth of human B-cell lines.<sup>49</sup> CLL cells express high levels of CXCR4,<sup>47,102</sup> and CLL cells spontaneously migrate beneath MSCs that secrete CXCL12 in a CXCR4-dependent manner.<sup>47</sup> Stromal cells and nurse-like cells,

**Table 1** CXCR4 antagonists that are currently in preclinical and clinical development

Product name	Company	Structure	Administration	Indication	Study phase
Plerixafor (AMD3100)	Genzyme	Bicyclam	s.c.	Stem cell mobilization	Phase III
AMD070	Genzyme	Bicyclam derived	Oral	HIV	Phase I/II
CTCE-9908 antagonist	Chemokine Therapeutics Corp.	Modified SDF-1	s.c./i.v.	Solid tumors	Phase I/II
CTCE-0214-agonist	Chemokine Therapeutics Corp.	Modified SDF-1	s.c./i.v.	Mobilization BM recovery	Phase I/II
No name	Northwest Biotherapeutics, Bethesda, MD, USA	Antibody	s.c./i.v.	Cancer	Preclinical
TG-0054	TaiGen Biotechnology Co., Taipei, Taiwan	?	?	Stem mobilization for regeneration	Phase I/II
BKT140	Biokine Therapeutics	Modified peptide	s.c./oral	MM and leukemia	Phase I

**Table 2** *In vitro* and *in vivo* efficacy of CXCR4 antagonists in solid tumors and leukemia/lymphoma

Cancer type	<i>In vitro</i> studies	<i>In vivo</i> studies
<i>Solid tumors</i>		
Breast cancer	AMD3100: blocks CXCL12-induced HER2-neu activation <sup>133</sup>	T140: reduced metastasis in murine model <sup>73</sup> ; AMD3100: prolongs survival in murine model <sup>128</sup>
Small cell lung cancer (SCLC)	T140 and its analogs block adhesion and survival pathways <sup>50,130</sup>	
Pancreatic cancer	AMD3100 inhibits tumor cell migration and growth <sup>134</sup>	
Cholangiocarcinoma	AMD3100 inhibits tumor cell migration <sup>135</sup>	
Gastric cancer		AMD3100 reduced tumor growth in a murine model <sup>136</sup>
Colorectal cancer	AMD3100 inhibits tumor cell growth <sup>137</sup>	
Malignant melanoma	AMD3100 inhibits tumor cell activation and proliferation <sup>138</sup>	T140 analog inhibits metastatic melanoma, <sup>74</sup> T22 increases efficacy of immunotherapy in metastatic melanoma <sup>139</sup>
Glioma	AMD3100 inhibits tumor cell invasion <sup>140</sup>	
Other CNS tumors		AMD3100 inhibits glioblastoma and medulloblastoma growth in xenograft model <sup>141</sup>
Ovarian cancer	AMD3100 inhibits cancer cell migration and activation <sup>142</sup>	
Rhabdomyosarcoma	T140 blocked <i>in vitro</i> responses to CXCL12 <sup>143</sup>	
Prostate cancer	T140 blocks tumor cell invasion and signaling <sup>144</sup>	
<i>Leukemia/lymphoma</i>		
Chronic lymphocytic leukemia (CLL)	T140, TC14012 and TN14003 block migration, adhesion and stromal protection <sup>54</sup> ; AMD3100 blocks actin polymerization in CLL cells <sup>54</sup>	
Acute myelogenous leukemia (AML)	RCP168 and AMD3465 block migration and CXCR4 signaling; <sup>145</sup> AMD3100 reduced AML cell survival <sup>111</sup>	
Acute lymphoblastic leukemia (ALL)	T140 and its analogs and AMD3100 inhibit ALL cell migration and adhesion <sup>76</sup>	T140 analogs, AMD3100 and AMD3465 mobilize ALL cells <sup>77</sup>
Multiple myeloma	T140 analogs block CXCL12-induced osteoclast activity <sup>78</sup>	AMD3100 inhibits <i>in vivo</i> homing of myeloma cells <sup>28</sup>
Non-Hodgkin's lymphoma		CXCR4 neutralization inhibited lymphoma growth <sup>95</sup>

Abbreviation: CNS, central nervous system.

The diseases in which CXCR4 antagonists showed activity, along with the respective references, are listed.

another stromal cell type derived from monocytes, protect CLL cells from spontaneous or drug-induced apoptosis in a contact-dependent manner. These observations support a model proposing that expression of CXCR4 by CLL cells allows for their recirculation between the blood and the marrow or lymphoid tissues, where they receive protective survival signals. We demonstrated earlier that CXCR4 antagonists effectively block CXCL12-induced activation, migration and signaling of CLL cells.<sup>54</sup> Also, CXCR4 antagonists reversed stromal cell-mediated protection from spontaneous or fludarabine-induced apoptosis of CLL cells, suggesting a potential role of CXCR4 antagonists in combination with a B-cell-targeted therapy in the treatment of CLL. Because of the high-level CXCR4 expression, and the particular requirement of stromal cell support for CLL cell survival, it appears that CLL patients would particularly respond to CXCR4 antagonists. The expected response to CXCR4 antagonists would be a mobilization of the CLL cells from the tissues (marrow and lymphoid tissues) to the blood, where CLL cells then could be targeted by mAbs, such as anti-CD20 or anti-CD52 mAbs, or cytotoxic agents.

#### Acute myeloid leukemia

Despite a general sensitivity to chemotherapy, long-term disease-free survival in patients with acute myelogenous

leukemia (AML) is low because a majority of patients relapse from MRD. The marrow is considered the primary site for MRD where adhesion to stromal elements may protect AML cells from cytotoxic drugs. Several studies indicated that adhesion to MSCs affects the survival and proliferation of AML cells<sup>58,103,104</sup> and protects AML cells from chemotherapy *in vitro*<sup>105</sup> and *in vivo*.<sup>106</sup> Adhesion molecules, in particular the very late antigen-4 (VLA-4) integrins, along with CXCR4 chemokine receptors, are essential for AML cell adhesion to respective ligands (fibronectin and VCAM-1) on stromal cells<sup>107</sup> and for protection of AML cells from spontaneous or drug-induced apoptosis.<sup>106,108</sup> CXCR4 receptors are functional in AML,<sup>58,109</sup> and surface CXCR4 expression, which is generally low when compared with lymphoid cells, correlates with functional responses, such as chemotaxis.<sup>110</sup> CXCR4-dependent engraftment of AML cells in non-obese diabetic/severe combined immunodeficient (NOD/SCID) mice was demonstrated by Tavor *et al.*,<sup>111</sup> and this group also reported that the proteolytic enzyme elastase is involved in regulating SDF-1-dependent migration and proliferation of AML cells *in vitro* and *in vivo*.<sup>112</sup> CXCR4, in cooperation with VLA-4 integrins, mediates spontaneous migration of AML cells beneath MSCs, along with a decreased proliferation of migrated AML cells within stromal layers.<sup>58</sup> This suggests that CXCR4 expression by AML cells

favors the enrichment of a non-cycling sub-population of AML cells within the stromal layer. These cells may be less susceptible to cytotoxic treatments, and they may represent dormant leukemia progenitors serving as a reservoir for MRD.<sup>113</sup> This function of CXCR4 could explain why CXCR4 surface expression on AML cells has such a profound negative prognostic impact in AML.<sup>114–116</sup> Plerixafor is currently used in an ongoing clinical trial for mobilization of AML cells from the protective marrow microenvironment to the blood, where the AML cells are then targeted by conventional cytotoxic drugs. The feasibility of using Plerixafor for AML cell mobilization to the blood in an animal model and in AML patients on this trial was recently reported.<sup>117,118</sup>

### *CXCR4 in ALL*

Precursor B-cell ALL, the most common childhood malignancy and the second most common adult acute leukemia, is characterized by a high motility of the leukemia cells, resulting in leukemic infiltrates into extramedullary sites. In particular, ALL cells have a high affinity for the central nervous system, which is the most common location for extramedullary relapses, but also for the liver, spleen and the lymph nodes. The presumed normal counterparts of B-ALL cells are precursor B cells that are highly dependent on the stromal microenvironment during their maturation in the marrow.<sup>119</sup> Contact between precursor B cell and CXCL12-secreting stromal cells is maintained by CXCR4 expression on the B cells, and gene deletion of CXCR4 or CXCL12 in mouse models results in premature release of B-cell precursors into the circulation.<sup>25</sup> We reported that B-ALL cells express functional CXCR4 receptors that induce leukemia cell chemotaxis to CXCL12 and spontaneous migration beneath CXCL12-secreting stromal cells in a CXCR4- and VLA4 integrin-dependent manner, using the B-cell precursor lines NALM-6 and REH,<sup>47,120</sup> findings that were subsequently confirmed with primary ALL cells.<sup>121,122</sup> CXCR4 receptors on ALL cells participate in homing of leukemia cells to the marrow in NOD/SCID mice.<sup>123,124</sup> A recent study by Sipkins *et al.*<sup>18</sup> provided *in vivo* evidence that CXCR4 is necessary for homing of ALL cells to the marrow. Blocking CXCR4 on ALL cells with specific CXCR4 receptor antagonists blocked the migration of ALL cells to CXCL12 and MSCs, and partially disrupted the protection of ALL cells from cytotoxic agents by MSCs.<sup>76</sup> One of the mechanisms by which MSCs protect ALL cells from chemotherapeutic agents is related to asparagine synthetase, an enzyme that is critical to the biosynthesis of asparagine. MSCs constitutively secrete asparagine synthetase,<sup>125</sup> which interferes with asparaginase, a drug that has a critical impact in the treatment of ALL patients. Through this mechanism, ALL cells may survive in marrow stromal niches and thus represent the seed for residual disease and relapses.<sup>126</sup>

Collectively, these studies suggest that ALL cells should be particularly responsive to therapeutic attempts for mobilization with CXCR4 antagonists. ALL cells mobilized in such a way from marrow or other tissue niches then could be better targeted by conventional ALL treatments.

### *CXCR4 in breast cancer*

CXCR4 was the first chemokine receptor that was described to be functionally expressed by breast cancer cells.<sup>15</sup> Initially, the main function of CXCR4 in breast cancer was considered to be the directed, organ-specific metastasis of circulating CXCR4-positive cancer cells to CXCL12-expressing target organs, such as the lungs, liver, lymphatic tissues and the marrow.<sup>15</sup>

Treatment with anti-CXCR4 antibodies reduced local and systemic metastasis of breast cancer in an animal model.<sup>15</sup> Subsequently, additional functions of the CXCR4-CXCL12 axis in breast cancer have been described: first, CXCR4 activation on breast cancer cells induces a growth response in the tumor cells (paracrine function of CXCL12), and second, CXCL12 recruits endothelial progenitor cells to the tumor for tumor angiogenesis (endocrine function of CXCL12).<sup>48</sup> Stromal fibroblasts, also termed carcinoma-associated fibroblasts, constitutively secrete CXCL12 into the tumor microenvironment.<sup>45,48</sup> High-level expression of CXCR4 on neoplastic cells is associated with relatively poor overall survival in patients with breast cancer.<sup>127</sup> The multiple tumor-promoting effects of CXCL12 in breast cancer suggest that CXCR4 antagonists alone or in combination with cytotoxic drugs could decrease the rate of recurrence in the adjuvant setting where patients are likely to have MRD, and/or increase the response rates to conventional therapy in advanced stages of the disease. Animal breast cancer models using T140<sup>73</sup> and Plerixafor<sup>128</sup> have shown promising results, suggesting that CXCR4 antagonists should be explored in this cancer.

### *CXCR4 in lung cancer*

SCLC is an aggressive, rapidly metastasizing cancer. Even with combination chemotherapy and radiotherapy treatments, the 5-year survival is only 5% due to rapid development of drug resistance. CXCR4 is the major chemokine receptor expressed by primary SCLC cells or SCLC cell lines.<sup>50,129</sup> CXCR4 activation induces migratory and invasive responses in SCLC cells, and adhesion to CXCL12-secreting stromal cells in a CXCR4- and integrin-dependent manner.<sup>50</sup> Moreover, signaling through CXCR4 on SCLC cells induces activation and signaling of integrin adhesion molecules expressed on the SCLC cells.<sup>130</sup> Integrin-mediated adhesion of SCLC cells to stroma and extracellular matrix in turn protects SCLC cells from chemotherapy-induced apoptosis.<sup>130,131</sup> Collectively, CXCR4 cooperates with integrins in SCLC cells, mediating tumor cell adhesion to stromal cells, which in turn confers drug resistance and tumor cell growth. Moreover, CXCR4 may direct the distinct metastatic pattern observed in patients with SCLC with a high propensity for bone marrow involvement. The neoplastic cells in NSCLC also express CXCR4, but at levels that are lower than in SCLC.<sup>50</sup> Because of the very transient responses to cytotoxic chemotherapy in lung cancer patients, particularly in SCLC, mobilization of tumor cells from their protective microenvironment using CXCR4 antagonists could be an attractive new approach and combination partner for conventional chemotherapy of lung cancer patients.

### **Potential side effects of CXCR4 antagonists**

Permanent or long-term inhibition of the CXCR4-CXCL12 axis would potentially expose patients to risks of immune system and hematopoietic dysfunctions. T and B lymphocytes utilize CXCR4 for trafficking and homing to distinct microenvironments within lymphoid tissues and the thymus during development and immune surveillance.<sup>82,132</sup> Another general concern regarding the use of CXCR4 antagonists in cancer patients is the mobilization of normal progenitor cells, such as HSC from their microenvironments to the blood. Mobilized HSCs that are normally protected in marrow niches would be exposed to the effects of cytotoxic drugs in trials where CXCR4 antagonists are administered along with cytotoxic drugs, which could result in prolonged cytopenias. More specific side effects are Plerixafor-

related cardiac complications (premature ventricular contractions) in two patients treated with 40 and 160 µg/kg/h, who had a history of cardiac problems.<sup>88</sup> No cardiac side effects were reported for the subsequent clinical trials of Plerixafor for stem cell mobilization.

Collectively, CXCR4 antagonists provide a new, targeted tool to mobilize leukemia cells from their protective marrow microenvironment. Mobilized leukemia cells then become more accessible to conventional drugs, and therefore this strategy may help to overcome MRD and relapses commonly seen in the treatment of leukemia patients. Currently, CXCR4 antagonists are explored in proof-of-principle studies in leukemia patients in whom leukemia cell mobilization can be easily assessed and monitored. However, given the expression of functional CXCR4 receptors by a variety of other hematopoietic and epithelial cancers,<sup>37</sup> a broader use of CXCR4 antagonists in other cancers is expected provided that the ongoing studies are successful (Table 2).

### Acknowledgements

We apologize that due to space limitation we were not able to discuss and cite a number of additional studies of other investigators that are related to CXCR4 antagonists in leukemia and other neoplastic diseases. This study was supported by an ASCO Career Development Award (to JAB), a Kimmel Scholar Award by the Sidney Kimmel Foundation for Cancer Research (to JAB) and a CLL Global Research Foundation grant (to JAB).

### References

- Zlotnik A, Yoshie O. Chemokines: a new classification system and their role in immunity. *Immunity* 2000; **12**: 121–127.
- Loetscher P, Moser B, Baggiolini M. Chemokines and their receptors in lymphocyte traffic and HIV infection. *Adv Immunol* 2000; **74**: 127–180.
- Baggiolini M. Chemokines and leukocyte traffic. *Nature* 1998; **392**: 565–568.
- Moser B, Loetscher P. Lymphocyte traffic control by chemokines. *Nat Immunol* 2001; **2**: 123–128.
- Bleul CC, Farzan M, Choe H, Parolin C, Clark-Lewis I, Soderroski J *et al.* The lymphocyte chemoattractant SDF-1 is a ligand for LESTR/fusin and blocks HIV-1 entry. *Nature* 1996; **382**: 829–833.
- Oberlin E, Amara A, Bachelier F, Bessia C, Virelizier JL, Arenzana-Seisdedos F *et al.* The CXC chemokine SDF-1 is the ligand for LESTR/fusin and prevents infection by T-cell-line-adapted HIV-1 [published erratum appears in *Nature* 1996; **384**: 288]. *Nature* 1996; **382**: 833–835.
- Nagasawa T, Kikutani H, Kishimoto T. Molecular cloning and structure of a pre-B-cell growth-stimulating factor. *Proc Natl Acad Sci USA* 1994; **91**: 2305–2309.
- Feng Y, Broder CC, Kennedy PE, Berger EA. HIV-1 entry cofactor: functional cDNA cloning of a seven-transmembrane, G protein-coupled receptor [see comments]. *Science* 1996; **272**: 872–877.
- Nagasawa T, Hirota S, Tachibana K, Takakura N, Nishikawa S, Kitamura Y *et al.* Defects of B-cell lymphopoiesis and bone-marrow myelopoiesis in mice lacking the CXC chemokine PBSF/SDF-1. *Nature* 1996; **382**: 635–638.
- Tachibana K, Hirota S, Iizasa H, Yoshida H, Kawabata K, Kataoka Y *et al.* The chemokine receptor CXCR4 is essential for vascularization of the gastrointestinal tract [see comments]. *Nature* 1998; **393**: 591–594.
- Zou YR, Kottmann AH, Kuroda M, Taniuchi I, Littman DR. Function of the chemokine receptor CXCR4 in haematopoiesis and in cerebellar development [see comments]. *Nature* 1998; **393**: 595–599.
- Ma Q, Jones D, Borghesani PR, Segal RA, Nagasawa T, Kishimoto T *et al.* Impaired B-lymphopoiesis, myelopoiesis, and derailed cerebellar neuron migration in CXCR4- and SDF-1-deficient mice. *Proc Natl Acad Sci USA* 1998; **95**: 9448–9453.
- Boldajipour B, Mahabaleswar H, Kardash E, Reichman-Fried M, Blaser H, Minina S *et al.* Control of chemokine-guided cell migration by ligand sequestration. *Cell* 2008; **132**: 463–473.
- Sierro F, Biben C, Martinez-Munoz L, Mellado M, Ransohoff RM, Li M *et al.* Disrupted cardiac development but normal hematopoiesis in mice deficient in the second CXCL12/SDF-1 receptor, CXCR7. *Proc Natl Acad Sci USA* 2007; **104**: 14759–14764.
- Muller A, Homey B, Soto H, Ge N, Catron D, Buchanan ME *et al.* Involvement of chemokine receptors in breast cancer metastasis. *Nature* 2001; **410**: 50–56.
- Peled A, Petit I, Kollet O, Magid M, Ponomaryov T, Byk T *et al.* Dependence of human stem cell engraftment and repopulation of NOD/SCID mice on CXCR4. *Science* 1999; **283**: 845–848.
- Sugiyama T, Kohara H, Noda M, Nagasawa T. Maintenance of the hematopoietic stem cell pool by CXCL12-CXCR4 chemokine signaling in bone marrow stromal cell niches. *Immunity* 2006; **25**: 977–988.
- Sipkins DA, Wei X, Wu JW, Runnels JM, Cote D, Means TK *et al.* *In vivo* imaging of specialized bone marrow endothelial microdomains for tumour engraftment. *Nature* 2005; **435**: 969–973.
- Wright DE, Bowman EP, Wagers AJ, Butcher EC, Weissman IL. Hematopoietic stem cells are uniquely selective in their migratory response to chemokines. *J Exp Med* 2002; **195**: 1145–1154.
- Moore KA, Lemischka IR. Stem cells and their niches. *Science* 2006; **311**: 1880–1885.
- Morrison SJ, Spradling AC. Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. *Cell* 2008; **132**: 598–611.
- Laird DJ, von Andrian UH, Wagers AJ. Stem cell trafficking in tissue development, growth, and disease. *Cell* 2008; **132**: 612–630.
- Honczarenko M, Douglas RS, Mathias C, Lee B, Ratajczak MZ, Silberstein LE. SDF-1 responsiveness does not correlate with CXCR4 expression levels of developing human bone marrow B cells. *Blood* 1999; **94**: 2990–2998.
- Martin C, Burdon PC, Bridger G, Gutierrez-Ramos JC, Williams TJ, Rankin SM. Chemokines acting via CXCR2 and CXCR4 control the release of neutrophils from the bone marrow and their return following senescence. *Immunity* 2003; **19**: 583–593.
- Ma Q, Jones D, Springer TA. The chemokine receptor CXCR4 is required for the retention of B lineage and granulocytic precursors within the bone marrow microenvironment. *Immunity* 1999; **10**: 463–471.
- Hargreaves DC, Hyman PL, Lu TT, Ngo VN, Bidgol A, Suzuki G *et al.* A coordinated change in chemokine responsiveness guides plasma cell movements. *J Exp Med* 2001; **194**: 45–56.
- Cyster JG. Homing of antibody secreting cells. *Immunol Rev* 2003; **194**: 48–60.
- Alsayed Y, Ngo H, Runnels J, Leleu X, Singha UK, Pitsillides CM *et al.* Mechanisms of regulation of CXCR4/SDF-1 (CXCL12)-dependent migration and homing in multiple myeloma. *Blood* 2007; **109**: 2708–2717.
- Lapidot T, Sirard C, Vormoor J, Murdoch B, Hoang T, Caceres-Cortes J *et al.* A cell initiating human acute myeloid leukaemia after transplantation into SCID mice. *Nature* 1994; **367**: 645–648.
- Bonnet D, Dick JE. Human acute myeloid leukemia is organized as a hierarchy that originates from a primitive hematopoietic cell. *Nat Med* 1997; **3**: 730–737.
- Lessard J, Sauvageau G. Bmi-1 determines the proliferative capacity of normal and leukaemic stem cells. *Nature* 2003; **423**: 255–260.
- Calabrese C, Poppleton H, Kocak M, Hogg TL, Fuller C, Hamner B *et al.* A perivascular niche for brain tumor stem cells. *Cancer Cell* 2007; **11**: 69–82.
- Yang ZJ, Wechsler-Reya RJ. Hit 'em where they live: targeting the cancer stem cell niche. *Cancer Cell* 2007; **11**: 3–5.
- Broxmeyer HE, Orschell CM, Clapp DW, Hangoc G, Cooper S, Plett PA *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.

- 35 Cashen AF, Nervi B, DiPersio J. AMD3100: CXCR4 antagonist and rapid stem cell-mobilizing agent. *Future Oncol* 2007; **3**: 19–27.
- 36 Abraham M, Biyder K, Begin M, Wald H, Weiss ID, Galun E *et al.* Enhanced unique pattern of hematopoietic cell mobilization induced by the CXCR4 antagonist 4F-benzoyl-TN14003. *Stem Cells* 2007; **25**: 2158–2166.
- 37 Burger JA, Kipps TJ. CXCR4: a key receptor in the crosstalk between tumor cells and their microenvironment. *Blood* 2006; **107**: 1761–1767.
- 38 Ceradini DJ, Kulkarni AR, Callaghan MJ, Tepper OM, Bastidas N, Kleinman ME *et al.* Progenitor cell trafficking is regulated by hypoxic gradients through HIF-1 induction of SDF-1. *Nat Med* 2004; **10**: 858–864.
- 39 Abbott JD, Huang Y, Liu D, Hickey R, Krause DS, Giordano FJ. Stromal cell-derived factor-1 $\alpha$  plays a critical role in stem cell recruitment to the heart after myocardial infarction but is not sufficient to induce homing in the absence of injury. *Circulation* 2004; **110**: 3300–3305.
- 40 Hu X, Dai S, Wu WJ, Tan W, Zhu X, Mu J *et al.* Stromal cell derived factor-1  $\alpha$  confers protection against myocardial ischemia/reperfusion injury: role of the cardiac stromal cell derived factor-1  $\alpha$  CXCR4 axis. *Circulation* 2007; **116**: 654–663.
- 41 Segers VF, Tokounou T, Higgins LJ, MacGillivray C, Gannon J, Lee RT. Local delivery of protease-resistant stromal cell derived factor-1 for stem cell recruitment after myocardial infarction. *Circulation* 2007; **116**: 1683–1692.
- 42 Staller P, Sulitkova J, Lisztwan J, Moch H, Oakeley EJ, Krek W. Chemokine receptor CXCR4 downregulated by von Hippel-Lindau tumour suppressor pVHL. *Nature* 2003; **425**: 307–311.
- 43 Zagzag D, Krishnamachary B, Yee H, Okuyama H, Chiriboga L, Ali MA *et al.* Stromal cell-derived factor-1 $\alpha$  and CXCR4 expression in hemangioblastoma and clear cell-renal cell carcinoma: von Hippel-Lindau loss-of-function induces expression of a ligand and its receptor. *Cancer Res* 2005; **65**: 6178–6188.
- 44 Burger JA, Burkle A. The CXCR4 chemokine receptor in acute and chronic leukaemia: a marrow homing receptor and potential therapeutic target. *Br J Haematol* 2007; **137**: 288–296.
- 45 Orimo A, Weinberg RA. Stromal fibroblasts in cancer: a novel tumor-promoting cell type. *Cell Cycle* 2006; **5**: 1597–1601.
- 46 Liotta LA. An attractive force in metastasis. *Nature* 2001; **410**: 24–25.
- 47 Burger JA, Burger M, Kipps TJ. Chronic lymphocytic leukemia B cells express functional CXCR4 chemokine receptors that mediate spontaneous migration beneath bone marrow stromal cells. *Blood* 1999; **94**: 3658–3667.
- 48 Orimo A, Gupta PB, SgROI DC, Arenzana-Seisdedos F, Delaunay T, Naeem R *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.
- 49 Burger JA, Tsukada N, Burger M, Zvaifler NJ, Dell'Aquila M, Kipps TJ. Blood-derived nurse-like cells protect chronic lymphocytic leukemia B cells from spontaneous apoptosis through stromal cell-derived factor-1. *Blood* 2000; **96**: 2655–2663.
- 50 Burger M, Glodek A, Hartmann T, Schmitt-Graff A, Silberstein LE, Fujii N *et al.* Functional expression of CXCR4 (CD184) on small-cell lung cancer cells mediates migration, integrin activation, and adhesion to stromal cells. *Oncogene* 2003; **22**: 8093–8101.
- 51 Burger M, Hartmann T, Krome M, Rawluk J, Tamamura H, Fujii N *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.
- 52 Damiano JS, Cress AE, Hazlehurst LA, Shtil AA, Dalton WS. Cell adhesion mediated drug resistance (CAM-DR): role of integrins and resistance to apoptosis in human myeloma cell lines. *Blood* 1999; **93**: 1658–1667.
- 53 Li ZW, Dalton WS. Tumor microenvironment and drug resistance in hematologic malignancies. *Blood Rev* 2006; **20**: 333–342.
- 54 Burger M, Hartmann T, Krome M, Rawluk J, Tamamura H, Fujii N *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.
- 55 Ramasamy R, Lam EW, Soeiro I, Tisato V, Bonnet D, Dazzi F. Mesenchymal stem cells inhibit proliferation and apoptosis of tumor cells: impact on *in vivo* tumor growth. *Leukemia* 2007; **21**: 304–310.
- 56 Maestroni GJ, Hertens E, Galli P. Factor(s) from nonmacrophage bone marrow stromal cells inhibit Lewis lung carcinoma and B16 melanoma growth in mice. *Cell Mol Life Sci* 1999; **55**: 663–667.
- 57 Ohlsson LB, Varas L, Kjellman C, Edvardsen K, Lindvall M. Mesenchymal progenitor cell-mediated inhibition of tumor growth *in vivo* and *in vitro* in gelatin matrix. *Exp Mol Pathol* 2003; **75**: 248–255.
- 58 Burger JA, Spoo A, Dwenger A, Burger M, Behringer D. CXCR4 chemokine receptors (CD184) and  $\alpha$ 4 $\beta$ 1 integrins mediate spontaneous migration of human CD34 $^{+}$  progenitors and acute myeloid leukaemia cells beneath marrow stromal cells (pseudoemperipolesis). *Br J Haematol* 2003; **122**: 579–589.
- 59 Pisati F, Belicchi M, Acerbi F, Marchesi C, Giussani C, Gavina M *et al.* Effect of human skin-derived stem cells on vessel architecture, tumor growth, and tumor invasion in brain tumor animal models. *Cancer Res* 2007; **67**: 3054–3063.
- 60 Khakoo AY, Pati S, Anderson SA, Reid W, Elshal MF, Rovira II *et al.* Human mesenchymal stem cells exert potent antitumorigenic effects in a model of Kaposi's sarcoma. *J Exp Med* 2006; **203**: 1235–1247.
- 61 Nakashima H, Masuda M, Murakami T, Koyanagi Y, Matsumoto A, Fujii N *et al.* Anti-human immunodeficiency virus activity of a novel synthetic peptide, T22 ([Tyr-5,12, Lys-7]polyphemusin II): a possible inhibitor of virus-cell fusion. *Antimicrob Agents Chemother* 1992; **36**: 1249–1255.
- 62 Masuda M, Nakashima H, Ueda T, Naba H, Ikoma R, Otaka A *et al.* A novel anti-HIV synthetic peptide, T-22 ([Tyr5,12, Lys7]-polyphemusin II). *Biochem Biophys Res Commun* 1992; **189**: 845–850.
- 63 De Clercq E, Yamamoto N, Pauwels R, Baba M, Schols D, Nakashima H *et al.* Potent and selective inhibition of human immunodeficiency virus (HIV)-1 and HIV-2 replication by a class of bicyclams interacting with a viral uncoating event. *Proc Natl Acad Sci USA* 1992; **89**: 5286–5290.
- 64 De Clercq E, Yamamoto N, Pauwels R, Balzarini J, Witvrouw M, De Vreese K *et al.* Highly potent and selective inhibition of human immunodeficiency virus by the bicyclam derivative JM3100. *Antimicrob Agents Chemother* 1994; **38**: 668–674.
- 65 Doranz BJ, Filion LG, Diaz-Mitoma F, Sitar DS, Sahai J, Baribaud F *et al.* Safe use of the CXCR4 inhibitor ALX40-4C in humans. *AIDS Res Hum Retroviruses* 2001; **17**: 475–486.
- 66 Doranz BJ, Grovit-Ferbas K, Sharron MP, Mao SH, Goetz MB, Daar ES *et al.* A small-molecule inhibitor directed against the chemokine receptor CXCR4 prevents its use as an HIV-1 coreceptor. *J Exp Med* 1997; **186**: 1395–1400.
- 67 Schols D, Struyf S, Van Damme J, Este JA, Henson G, De Clercq E. Inhibition of T-tropic HIV strains by selective antagonization of the chemokine receptor CXCR4. *J Exp Med* 1997; **186**: 1383–1388.
- 68 Murakami T, Nakajima T, Koyanagi Y, Tachibana K, Fujii N, Tamamura H *et al.* A small molecule CXCR4 inhibitor that blocks T cell line-tropic HIV-1 infection. *J Exp Med* 1997; **186**: 1389–1393.
- 69 Tamamura H, Xu Y, Hattori T, Zhang X, Arakaki R, Kanbara K *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.
- 70 Tamamura H, Omagari A, Hiramatsu K, Gotoh K, Kanamoto T, Xu Y *et al.* Development of specific CXCR4 inhibitors possessing high selectivity indexes as well as complete stability in serum based on an anti-HIV peptide T140. *Bioorg Med Chem Lett* 2001; **11**: 1897–1902.
- 71 Trent JO, Wang ZX, Murray JL, Shao W, Tamamura H, Fujii N *et al.* Lipid bilayer simulations of CXCR4 with inverse agonists and weak partial agonists. *J Biol Chem* 2003; **278**: 47136–47144.
- 72 Zhang WB, Navenot JM, Haribabu B, Tamamura H, Hiramatsu K, Omagari A *et al.* A point mutation that confers constitutive activity to CXCR4 reveals that T140 is an inverse agonist and that

- AMD3100 and ALX40-4C are weak partial agonists. *J Biol Chem* 2002; **277**: 24515–24521.
- 73 Tamamura H, Hori A, Kanzaki N, Hiramatsu K, Mizumoto M, Nakashima H *et al*. T140 analogs as CXCR4 antagonists identified as anti-metastatic agents in the treatment of breast cancer. *FEBS Lett* 2003; **550**: 79–83.
- 74 Takenaga M, Tamamura H, Hiramatsu K, Nakamura N, Yamaguchi Y, Kitagawa A *et al*. A single treatment with microcapsules containing a CXCR4 antagonist suppresses pulmonary metastasis of murine melanoma. *Biochem Biophys Res Commun* 2004; **320**: 226–232.
- 75 Tamamura H, Fujisawa M, Hiramatsu K, Mizumoto M, Nakashima H, Yamamoto N *et al*. Identification of a CXCR4 antagonist, a T140 analog, as an anti-rheumatoid arthritis agent. *FEBS Lett* 2004; **569**: 99–104.
- 76 Juarez J, Bradstock KF, Gottlieb DJ, Bendall LJ. Effects of inhibitors of the chemokine receptor CXCR4 on acute lymphoblastic leukemia cells *in vitro*. *Leukemia* 2003; **17**: 1294–1300.
- 77 Juarez J, Dela Pena A, Baraz R, Hewson J, Khoo M, Cisterne A *et al*. CXCR4 antagonists mobilize childhood acute lymphoblastic leukemia cells into the peripheral blood and inhibit engraftment. *Leukemia* 2007; **21**: 1249–1257.
- 78 Zannettino AC, Farrugia AN, Kortessidis A, Manavis J, To LB, Martin SK *et al*. Elevated serum levels of stromal-derived factor-1alpha are associated with increased osteoclast activity and osteolytic bone disease in multiple myeloma patients. *Cancer Res* 2005; **65**: 1700–1709.
- 79 Mori T, Doi R, Koizumi M, Toyoda E, Ito D, Kami K *et al*. CXCR4 antagonist inhibits stromal cell-derived factor 1-induced migration and invasion of human pancreatic cancer. *Mol Cancer Ther* 2004; **3**: 29–37.
- 80 Kohara H, Omatsu Y, Sugiyama T, Noda M, Fujii N, Nagasawa T. Development of plasmacytoid dendritic cells in bone marrow stromal cell niches requires CXCL12-CXCR4 chemokine signaling. *Blood* 2007; **110**: 4153–4160.
- 81 Kabashima K, Shiraishi N, Sugita K, Mori T, Onoue A, Kobayashi M *et al*. CXCL12-CXCR4 engagement is required for migration of cutaneous dendritic cells. *Am J Pathol* 2007; **171**: 1249–1257.
- 82 Allen CD, Ansel KM, Low C, Lesley R, Tamamura H, Fujii N *et al*. Germinal center dark and light zone organization is mediated by CXCR4 and CXCR5. *Nat Immunol* 2004; **5**: 943–952.
- 83 Petit I, Szyper-Kravitz M, Nagler A, Lahav M, Peled A, Habler L *et al*. G-CSF induces stem cell mobilization by decreasing bone marrow SDF-1 and up-regulating CXCR4. *Nat Immunol* 2002; **3**: 687–694.
- 84 De Clercq E. The bicyclam AMD3100 story. *Nat Rev Drug Discov* 2003; **2**: 581–587.
- 85 Fricker SP, Anastassov V, Cox J, Darkes MC, Grujic O, Idzan SR *et al*. Characterization of the molecular pharmacology of AMD3100: a specific antagonist of the G-protein coupled chemokine receptor, CXCR4. *Biochem Pharmacol* 2006; **72**: 588–596.
- 86 Donzella GA, Schols D, Lin SW, Este JA, Nagashima KA, Maddon PJ *et al*. AMD3100, a small molecule inhibitor of HIV-1 entry via the CXCR4 co-receptor. *Nat Med* 1998; **4**: 72–77.
- 87 Hendrix CW, Flexner C, MacFarland RT, Giandomenico C, Fuchs EJ, Redpath E *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.
- 88 Hendrix CW, Collier AC, Lederman MM, Schols D, Pollard RB, Brown S *et al*. Safety, pharmacokinetics, and antiviral activity of AMD3100, a selective CXCR4 receptor inhibitor, in HIV-1 infection. *J Acquir Immune Defic Syndr* 2004; **37**: 1253–1262.
- 89 Liles WC, Broxmeyer HE, Rodger E, Wood B, Hubel K, Cooper S *et al*. Mobilization of hematopoietic progenitor cells in healthy volunteers by AMD3100, a CXCR4 antagonist. *Blood* 2003; **102**: 2728–2730.
- 90 Devine SM, Flomenberg N, Vesole DH, Liesveld J, Weisdorf D, Badel K *et al*. Rapid mobilization of CD34+ cells following administration of the CXCR4 antagonist AMD3100 to patients with multiple myeloma and non-Hodgkin's lymphoma. *J Clin Oncol* 2004; **22**: 1095–1102.
- 91 Stone ND, Dunaway SB, Flexner C, Tierney C, Calandra GB, Becker S *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.
- 92 Ichiyama K, Yokoyama-Kumakura S, Tanaka Y, Tanaka R, Hirose K, Bannai K *et al*. A duodenally absorbable CXC chemokine receptor 4 antagonist, KRH-1636, exhibits a potent and selective anti-HIV-1 activity. *Proc Natl Acad Sci USA* 2003; **100**: 4185–4190.
- 93 Endres MJ, Clapham PR, Marsh M, Ahuja M, Turner JD, McKnight A *et al*. CD4-independent infection by HIV-2 is mediated by fusin/CXCR4. *Cell* 1996; **87**: 745–756.
- 94 D'Apuzzo M, Rolink A, Loetscher M, Hoxie JA, Clark-Lewis I, Melchers F *et al*. The chemokine SDF-1, stromal cell-derived factor 1, attracts early stage B cell precursors via the chemokine receptor CXCR4. *Eur J Immunol* 1997; **27**: 1788–1793.
- 95 Bertolini F, Dell'Agnola C, Mancuso P, Rabascio C, Burlini A, Monestiroli S *et al*. CXCR4 neutralization, a novel therapeutic approach for non-Hodgkin's lymphoma. *Cancer Res* 2002; **62**: 3106–3112.
- 96 Chen GS, Yu HS, Lan CC, Chow KC, Lin TY, Kok LF *et al*. CXCR4 chemokine receptor expression enhances tumorigenesis and angiogenesis of basal cell carcinoma. *Br J Dermatol* 2006; **154**: 910–918.
- 97 Phillips RJ, Burdick MD, Lutz M, Belperio JA, Keane MP, Strieter RM. The stromal derived factor-1/CXCL12-CXC chemokine receptor 4 biological axis in non-small cell lung cancer metastases. *Am J Respir Crit Care Med* 2003; **167**: 1676–1686.
- 98 Engl T, Relja B, Marian D, Blumenberg C, Muller I, Beecken WD *et al*. CXCR4 chemokine receptor mediates prostate tumor cell adhesion through alpha5 and beta3 integrins. *Neoplasia* 2006; **8**: 290–301.
- 99 Baribaud F, Edwards TG, Sharron M, Brelot A, Heveker N, Price K *et al*. Antigenically distinct conformations of CXCR4. *J Virol* 2001; **75**: 8957–8967.
- 100 Farzan M, Babcock GJ, Vasilieva N, Wright PL, Kiprilov E, Mirzabekov T *et al*. The role of post-translational modifications of the CXCR4 amino terminus in stromal-derived factor 1 alpha association and HIV-1 entry. *J Biol Chem* 2002; **277**: 29484–29489.
- 101 Kim SY, Lee CH, Midura BV, Yeung C, Mendoza A, Hong SH *et al*. Inhibition of the CXCR4/CXCL12 chemokine pathway reduces the development of murine pulmonary metastases. *Clin Exp Metastasis* 2008; **25**: 201–211.
- 102 Mohle R, Failenschmid C, Bautz F, Kanz L. Overexpression of the chemokine receptor CXCR4 in B cell chronic lymphocytic leukemia is associated with increased functional response to stromal cell-derived factor-1 (SDF-1). *Leukemia* 1999; **13**: 1954–1959.
- 103 Scholzel C, Lowenberg B. Stimulation of proliferation and differentiation of acute myeloid leukemia cells on a bone marrow stroma in culture. *Exp Hematol* 1985; **13**: 664–669.
- 104 Bendall LJ, Daniel A, Kortlepel K, Gottlieb DJ. Bone marrow adherent layers inhibit apoptosis of acute myeloid leukemia cells. *Exp Hematol* 1994; **22**: 1252–1260.
- 105 Konopleva M, Konoplev S, Hu W, Zaritsky AY, Afanasiev BV, Andreeff M. Stromal cells prevent apoptosis of AML cells by up-regulation of anti-apoptotic proteins. *Leukemia* 2002; **16**: 1713–1724.
- 106 Matsunaga T, Takemoto N, Sato T, Takimoto R, Tanaka I, Fujimi A *et al*. Interaction between leukemic-cell VLA-4 and stromal fibronectin is a decisive factor for minimal residual disease of acute myelogenous leukemia. *Nat Med* 2003; **9**: 1158–1165.
- 107 Bendall LJ, Kortlepel K, Gottlieb DJ. Human acute myeloid leukemia cells bind to bone marrow stroma via a combination of beta-1 and beta-2 integrin mechanisms. *Blood* 1993; **82**: 3125–3132.
- 108 Delforge M, Raets V, Van Duppen V, Vandenberghe P, Boogaerts M. CD34+ marrow progenitors from MDS patients with high levels of intramedullary apoptosis have reduced expression of alpha4beta1 and alpha5beta1 integrins. *Leukemia* 2005; **19**: 57–63.
- 109 Hamada T, Mohle R, Hesselgesser J, Hoxie J, Nachman RL, Moore MA *et al*. Transendothelial migration of megakaryocytes in response to stromal cell-derived factor 1 (SDF-1) enhances platelet formation. *J Exp Med* 1998; **188**: 539–548.

- 110 Mohle R, Schittenhelm M, Failenschmid C, Bautz F, Kratz-Albers K, Serve H *et al.* Functional response of leukaemic blasts to stromal cell-derived factor-1 correlates with preferential expression of the chemokine receptor CXCR4 in acute myelomonocytic and lymphoblastic leukaemia. *Br J Haematol* 2000; **110**: 563–572.
- 111 Tavor S, Petit I, Porozov S, Avigdor A, Dar A, Leider-Trejo L *et al.* CXCR4 regulates migration and development of human acute myelogenous leukemia stem cells in transplanted NOD/SCID mice. *Cancer Res* 2004; **64**: 2817–2824.
- 112 Tavor S, Petit I, Porozov S, Goichberg P, Avigdor A, Sagiv S *et al.* Motility, proliferation, and egress to the circulation of human AML cells are elastase dependent in NOD/SCID chimeric mice. *Blood* 2005; **106**: 2120–2127.
- 113 Hope KJ, Jin L, Dick JE. Human acute myeloid leukemia stem cells. *Arch Med Res* 2003; **34**: 507–514.
- 114 Spoo AC, Lubbert M, Wierda WG, Burger JA. CXCR4 is a prognostic marker in acute myelogenous leukemia. *Blood* 2007; **109**: 786–791.
- 115 Rombouts EJ, Pavic B, Lowenberg B, Ploemacher RE. Relation between CXCR-4 expression, Flt3 mutations, and unfavorable prognosis of adult acute myeloid leukemia. *Blood* 2004; **104**: 550–557.
- 116 Konoplev S, Rassidakis GZ, Estey E, Kantarjian H, Liakou CI, Huang X *et al.* Overexpression of CXCR4 predicts adverse overall and event-free survival in patients with unmutated FLT3 acute myeloid leukemia with normal karyotype. *Cancer* 2007; **109**: 1152–1156.
- 117 Uy GL, Rettig MP, Ramirez P, Nervi B, Abboud CN, DiPersio JF. Kinetics of human and murine mobilization of acute myelogenous leukemia in response to AMD3100. *Blood* 2007; **110**: 265a.
- 118 Andreeff M, Konoplev S, Wang RY, Zeng Z, McQueen T, Shi YX *et al.* Massive mobilization of AML cells into circulation by disruption of leukemia/stroma cell interactions using CXCR4 antagonist AMD3100: first evidence in patients and potential for abolishing bone marrow microenvironment-mediated resistance. *Blood* 2006; **108** (ASH Annual Meeting Abstracts): 176a (Abstract 568).
- 119 Nagasawa T. Microenvironmental niches in the bone marrow required for B-cell development. *Nat Rev Immunol* 2006; **6**: 107–116.
- 120 Burger JA, Zvaifler NJ, Tsukada N, Firestein GS, Kipps TJ. Fibroblast-like synoviocytes support B-cell pseudoemperipolesis via a stromal cell-derived factor-1- and CD106 (VCAM-1)-dependent mechanism. *J Clin Invest* 2001; **107**: 305–315.
- 121 Bradstock KF, Makrynikola V, Bianchi A, Shen W, Hewson J, Gottlieb DJ. Effects of the chemokine stromal cell-derived factor-1 on the migration and localization of precursor-B acute lymphoblastic leukemia cells within bone marrow stromal layers [in process citation]. *Leukemia* 2000; **14**: 882–888.
- 122 Corcione A, Arduino N, Ferretti E, Pistorio A, Spinelli M, Ottonello L *et al.* Chemokine receptor expression and function in childhood acute lymphoblastic leukemia of B-lineage. *Leuk Res* 2006; **30**: 365–372.
- 123 Shen W, Bendall LJ, Gottlieb DJ, Bradstock KF. The chemokine receptor CXCR4 enhances integrin-mediated *in vitro* adhesion and facilitates engraftment of leukemic precursor-B cells in the bone marrow. *Exp Hematol* 2001; **29**: 1439–1447.
- 124 Spiegel A, Kollet O, Peled A, Abel L, Nagler A, Bieleorai B *et al.* Unique SDF-1-induced activation of human precursor-B ALL cells as a result of altered CXCR4 expression and signaling. *Blood* 2004; **103**: 2900–2907.
- 125 Iwamoto S, Mihara K, Downing JR, Pui CH, Campana D. Mesenchymal cells regulate the response of acute lymphoblastic leukemia cells to asparaginase. *J Clin Invest* 2007; **117**: 1049–1057.
- 126 Williams DA. A new mechanism of leukemia drug resistance? *N Engl J Med* 2007; **357**: 77–78.
- 127 Li YM, Pan Y, Wei Y, Cheng X, Zhou BP, Tan M *et al.* Upregulation of CXCR4 is essential for HER2-mediated tumor metastasis. *Cancer Cell* 2004; **6**: 459–469.
- 128 Smith MC, Luker KE, Garbow JR, Prior JL, Jackson E, Piwnicka-Worms D *et al.* CXCR4 regulates growth of both primary and metastatic breast cancer. *Cancer Res* 2004; **64**: 8604–8612.
- 129 Kijima T, Maulik G, Ma PC, Tibaldi EV, Turner RE, Rollins B *et al.* Regulation of cellular proliferation, cytoskeletal function, and signal transduction through CXCR4 and c-Kit in small cell lung cancer cells. *Cancer Res* 2002; **62**: 6304–6311.
- 130 Hartmann TN, Burger JA, Glodek A, Fujii N, Burger M. CXCR4 chemokine receptor and integrin signaling co-operate in mediating adhesion and chemoresistance in small cell lung cancer (SCLC) cells. *Oncogene* 2005; **24**: 4462–4471.
- 131 Sethi T, Rintoul RC, Moore SM, MacKinnon AC, Salter D, Choo C *et al.* Extracellular matrix proteins protect small cell lung cancer cells against apoptosis: a mechanism for small cell lung cancer growth and drug resistance *in vivo*. *Nat Med* 1999; **5**: 662–668.
- 132 Ara T, Itoi M, Kawabata K, Egawa T, Tokoyoda K, Sugiyama T *et al.* A role of CXC chemokine ligand 12/stromal cell-derived factor-1/pre-B cell growth stimulating factor and its receptor CXCR4 in fetal and adult T cell development *in vivo*. *J Immunol* 2003; **170**: 4649–4655.
- 133 Cabioglu N, Summy J, Miller C, Parikh NU, Sahin AA, Tuzlali S *et al.* CXCL-12/stromal cell-derived factor-1alpha transactivates HER2-neu in breast cancer cells by a novel pathway involving Src kinase activation. *Cancer Res* 2005; **65**: 6493–6497.
- 134 Marchesi F, Monti P, Leone BE, Zerbi A, Vecchi A, Piemonti L *et al.* Increased survival, proliferation, and migration in metastatic human pancreatic tumor cells expressing functional CXCR4. *Cancer Res* 2004; **64**: 8420–8427.
- 135 Ohira S, Sasaki M, Harada K, Sato Y, Zen Y, Isse K *et al.* Possible regulation of migration of intrahepatic cholangiocarcinoma cells by interaction of CXCR4 expressed in carcinoma cells with tumor necrosis factor-alpha and stromal-derived factor-1 released in stroma. *Am J Pathol* 2006; **168**: 1155–1168.
- 136 Yasumoto K, Koizumi K, Kawashima A, Saitoh Y, Arita Y, Shinohara K *et al.* Role of the CXCL12/CXCR4 axis in peritoneal carcinomatosis of gastric cancer. *Cancer Res* 2006; **66**: 2181–2187.
- 137 Ottaiano A, Franco R, Aiello Talamanca A, Liguori G, Tatangelo F, Delrio P *et al.* Overexpression of both CXC chemokine receptor 4 and vascular endothelial growth factor proteins predicts early distant relapse in stage II–III colorectal cancer patients. *Clin Cancer Res* 2006; **12**: 2795–2803.
- 138 Scala S, Giuliano P, Ascierto PA, Ierano C, Franco R, Napolitano M *et al.* Human melanoma metastases express functional CXCR4. *Clin Cancer Res* 2006; **12**: 2427–2433.
- 139 Lee CH, Kakinuma T, Wang J, Zhang H, Palmer DC, Restifo NP *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.
- 140 Hong X, Jiang F, Kalkanis SN, Zhang ZG, Zhang XP, DeCarvalho AC *et al.* SDF-1 and CXCR4 are up-regulated by VEGF and contribute to glioma cell invasion. *Cancer Lett* 2006; **236**: 39–45.
- 141 Rubin JB, Kung AL, Klein RS, Chan JA, Sun Y, Schmidt K *et al.* A small-molecule antagonist of CXCR4 inhibits intracranial growth of primary brain tumors. *Proc Natl Acad Sci USA* 2003; **100**: 13513–13518.
- 142 Scotton CJ, Wilson JL, Scott K, Stamp G, Wilbanks GD, Fricker S *et al.* Multiple actions of the chemokine CXCL12 on epithelial tumor cells in human ovarian cancer. *Cancer Res* 2002; **62**: 5930–5938.
- 143 Libura J, Drukala J, Majka M, Tomescu O, Navenot JM, Kucia M *et al.* CXCR4-SDF-1 signaling is active in rhabdomyosarcoma cells and regulates locomotion, chemotaxis, and adhesion. *Blood* 2002; **100**: 2597–2606.
- 144 Hart CA, Brown M, Bagley S, Sharrard M, Clarke NW. Invasive characteristics of human prostatic epithelial cells: understanding the metastatic process. *Br J Cancer* 2005; **92**: 503–512.
- 145 Zeng Z, Samudio IJ, Munsell M, An J, Huang Z, Estey E *et al.* Inhibition of CXCR4 with the novel RCP168 peptide overcomes stroma-mediated chemoresistance in chronic and acute leukemias. *Mol Cancer Ther* 2006; **5**: 3113–3121.