ANTI-CXCR4 ANTIBODIES

The present invention relates to monoclonal antibodies against CXCR4 and their use in treating diseases in which pathogenesis is mediated by CXCR4 and SDF-1.

CXCR4, a chemokine receptor, is a G protein-coupled, seven-transmembrane receptor. Like other chemokine receptors, CXCR4 plays an important role in immune and inflammatory responses by mediating the directional migration and activation of leukocytes. CXCR4 is expressed or overexpressed in a variety of cancer cell lines and tissues including breast, prostate, lung, ovarian, colon, pancreatic, kidney, and brain, as well as non-Hodgkin's lymphoma and chronic lymphocytic leukemia. The only known ligand to CXCR4 is stromal cell-derived factor-1 (SDF-1, or CXCL12). The CXCR4 and SDF-1 interaction plays an important role in multiple phases of tumorigenesis, including tumor growth, invasion, angiogenesis, and metastasis.

In view of the involvement of CXCR4 in various serious diseases, CXCR4 has been studied as a therapeutic target. For example, AMD3100, a bicyclam CXCR4 antagonist, is available for patients with multiple myeloma and non-Hodgkins lymphoma. CTCE9908, a peptide CXCR4 antagonist, is currently in Phase Ib/II clinical trials for cancer. In addition, antibodies targeting CXCR4 are disclosed in the art (WO 06/089141, US 07/0059308, and in Carnec et al [Carnec X, Quan L, Olson W, Hazan U, Dragic T. (2005) Anti-CXCR4 Monoclonal Antibodies Recognizing Overlapping Epitopes Differ Significantly in Their Ability To Inhibit Entry of Human Immunodeficiency Virus Type I. Journal of Virology. Feb.2005: 1930-1933]).

Although there are various agents under development that target CXCR4, there still exists a need for additional therapeutic agents targeting CXCR4. The antibodies of the present invention are therapeutically useful CXCR4 antagonists possessing a number of desirable properties. Antibodies of the present invention have increased chemical and physical stability, and solubility. The present invention provides CXCR4 antibodies that bind human CXCR4 with high affinity and inhibit human CXCR4 binding to SDF-1. High potency permits the use of low doses in therapeutic regimens. In addition, these antibodies interfere with the interaction of SDF-1 to CXCR4, and thus reduce tumorigenesis, including tumor growth, invasion, angiogenesis, and metastasis. Furthermore, antibodies of the present invention induce apoptosis of tumor cells.

The present invention includes a human engineered antibody or a binding fragment thereof, that binds human CXCR4, and which:

a. inhibits binding of human SDF-1α (SEQ ID NO:33) to CXCR4 with an IC_{50} for human CXCR4 between 10 nM and 0.05 nM in a human CXCR4/^{125}I-SDF-1α binding inhibition assay as described herein;
b. inhibits migration of cells bearing CXCR4 on their surface with an IC_{50} between 30 nM and 0.3 nM in the chemotaxis assay as described herein; and
c. exhibits an affinity, K_D between 15 nM and 0.05 nM in the Surface Plasmon Reasonance (BIAcore) assay as described herein.

The present invention preferably provides a human engineered antibody or a binding fragment thereof, that binds human CXCR4, and which inhibits binding of human SDF-1α (SEQ ID NO:33) to CXCR4 with an IC_{50} for human CXCR4 between 0.5 nM and 0.05 nM in a human CXCR4/^{125}I-SDF-1α binding inhibition assay as described herein.

The present invention preferably provides a human engineered antibody or a binding fragment thereof, that binds human CXCR4, and which inhibits migration of cells bearing CXCR4 on their surface with an IC_{50} between 3.0 nM and 0.3 nM in the chemotaxis assay as described herein.

The present invention preferably provides a human engineered antibody or a binding fragment thereof, that binds human CXCR4, and which exhibits an affinity, K_D between 1.0 nM and 0.05 nM in the Surface Plasmon Reasonance (BIAcore) assay as described herein.

The present invention preferably provides a human engineered antibody or a binding fragment thereof, that binds human CXCR4, and which exhibits anti-tumorigenesis activity by preventing tumor growth in a tumor xenograft model as described herein when administered at 1 mg/kg.

The present invention preferably provides a human engineered antibody or a binding fragment thereof, that binds human CXCR4, and which induces apoptosis of tumor cells in an apoptosis assay as described herein when administered between 2 μg/mL and 10 μg/mL.

The present invention includes a human engineered antibody or a binding fragment thereof, that comprises a light chain comprising a light chain variable region that comprises framework regions, CDRL1 having the amino acid sequence of SEQ ID NO:8, CDRL2 having the amino acid sequence of SEQ ID NO:9, and CDRL3 having the amino acid sequence of SEQ ID NO:10, and a heavy chain comprising a heavy chain variable region that comprises framework regions, CDRH1 having the amino acid sequence of SEQ ID NO:1, CDRH3 having the amino acid sequence of SEQ ID NO:3, and CDRH2 having an amino sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, wherein the antibody binds to human CXCR4.

Further, the present invention includes an antibody that binds human CXCR4, wherein a light chain comprises an amino acid sequence of SEQ ID NO:16, and a heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15.

In addition, the present invention includes an antibody that binds human CXCR4, wherein a light chain comprises an amino acid sequence of SEQ ID NO:22, and a heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.

The present invention also includes an antibody that binds human CXCR4, wherein an antibody is selected from the group consisting of an antibody comprising SEQ ID NO:17 and SEQ ID NO:22, an antibody comprising SEQ ID NO:18 and SEQ ID NO:22, an antibody comprising SEQ ID NO:19 and SEQ ID NO:22, an antibody comprising SEQ ID NO:20 and SEQ ID NO:22, and an antibody comprising SEQ ID NO:21 and SEQ ID NO:22.

The antibodies of the present invention as defined herein are characterized by having an IC_{50} of 10 nM or less in a human CXCR4/^{125}I-SDF-1α binding inhibition assay as described herein. Preferred antibodies of the invention have a binding affinity for human CXCR4 of 5.0 nM or less. Most preferred antibodies of the invention have a binding affinity for human CXCR4 of 0.5 nM or less. Further preferred, antibodies of the present invention have an IC_{50} for human CXCR4 between 10 nM and 0.05 nM in a human CXCR4/^{125}I-SDF-1α binding inhibition assay as described herein. Further preferred, antibodies of the present invention have an IC_{50} for human CXCR4 between

0.5 nM and 0.05 nM in a human CXCR4/^{125}I-SDF-1α binding inhibition assay as described herein.

The antibodies of the present invention as defined herein are characterized by having an IC_{50} of 30 nM or less in a chemotaxis assay as described herein. Preferred antibodies of the invention have an IC_{50} of 15 nM or less in the chemotaxis assay. More preferred antibodies of the invention have an IC_{50} of 3.0 nM or less in the chemotaxis assay. Further preferred, antibodies of the present invention have an IC_{50} between 30 nM and 0.3 nM in the chemotaxis assay as described herein. Further preferred, antibodies of the present invention have an IC_{50} between 3.0 nM and 0.3 nM in the chemotaxis assay as described herein.

The antibodies of the present invention as defined herein are characterized by having a K_D of 15 nM or less in an assay that evaluates the binding activities of the antibodies by Surface Plasmon Reasonance (BIAcore) as described herein. More preferred antibodies of the invention K_D of 10 nM or less in the BIAcore assay. Most preferred antibodies of the invention have K_D of 1.0 nM or less in the BIAcore assay. Further preferred, antibodies of the present invention have K_D between 15 nM and 0.05 nM in the BIAcore assay as described herein. Further preferred, antibodies of the present invention have K_D between 1.0 nM and 0.05 nM in the BIAcore assay as described herein.

The antibodies of the present invention as defined herein are characterized by having anti-tumorigenesis activity by preventing tumor growth in a tumor xenograft model using NOD/SCID mice and human non-Hodgkin's lymphoma Namalwa cells as described herein when administered at 10 mg/kg. More preferred antibodies of the invention have having anti-tumorigenesis activity by preventing tumor growth when administered at 1 mg/kg.

The antibodies of the present invention as defined herein are characterized by inducing apoptosis of tumor cells in an apoptosis assay as described herein. More preferred antibodies of the invention induce nuclear fragmentation and activation of caspase 3, hallmarks of apoptosis, in multiple tumor cells including Namalwa and CEM cells when administered between 2 μg/mL and 10 μg/mL.

The present invention includes a pharmaceutical composition comprising an antibody as described herein in combination with one or more pharmaceutically

acceptable carriers, diluents, or excipients. In addition, the present invention includes a pharmaceutical composition for the treatment of tumorigenesis, including tumor growth, invasion, angiogenesis, or metastasis, comprising an antibody as variously described herein, in combination with one or more pharmaceutically acceptable carriers, diluents, or excipients. Further, the present invention includes a pharmaceutical composition for the treatment of a cancer selected from the group consisting of breast cancer, pancreatic cancer, melanoma, prostate cancer, kidney cancer, neuroblastoma, non-Hodgkin's lymphoma, lung cancer, ovarian cancer, colorectal cancer, multiple myeloma, glioblastoma multiforme, and leukemia comprising an antibody as variously described herein, in combination with one or more pharmaceutically acceptable carriers, diluents, or excipients.

The present invention includes the use of an antibody as described herein for the preparation of a medicament for the treatment of tumorigenesis, including tumor growth, invasion, angiogenesis, or metastasis. In addition, the present invention includes the use of an antibody as described herein for the preparation of a medicament for the treatment of a cancer selected from the group consisting of breast cancer, pancreatic cancer, melanoma, prostate cancer, kidney cancer, neuroblastoma, non-Hodgkin's lymphoma, lung cancer, ovarian cancer, colorectal cancer, multiple myeloma, glioblastoma multiforme, and leukemia.

The present invention includes a method of treating tumorigenesis, including tumor growth, invasion, angiogenesis, or metastasis, comprising administering to a patient in need of an antibody as described herein. Further, the present invention includes a method of treating a cancer selected from the group consisting of breast cancer, pancreatic cancer, melanoma, prostate cancer, kidney cancer, neuroblastoma, non-Hodgkin's lymphoma, lung cancer, ovarian cancer, colorectal cancer, multiple myeloma, glioblastoma multiforme, and leukemia, comprising administering to a patient in need of an antibody as described herein.

The general structure of an "antibody," is very well-known in the art. For an antibody of the IgG type, there are four amino acid chains (two "heavy" chains and two "light" chains) that are cross-linked via intra- and inter-chain disulfide bonds. When expressed in certain biological systems, antibodies having unmodified human Fc sequences are glycosylated in the Fc region. Antibodies may be glycosylated at other

positions as well. The subunit structures and three-dimensional configurations of antibodies are well known in the art. Each heavy chain is comprised of an N-terminal heavy chain variable region ("HCVR") and a heavy chain constant region ("HCCR"). The heavy chain constant region is comprised of three domains (CHI, CH2, and CH3) for IgG, IgD, and IgA; and 4 domains (CH1, CH2, CH3, and CH4) for IgM and IgE. Each light chain is comprised of a light chain variable region (herein "LCVR") and a light chain constant region ("LCCR").

The variable regions of each light/heavy chain pair form the antibody binding site. The HCVR and LCVR regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDRs), interspersed with regions that are more conserved, termed framework regions (FR). Each HCVR and LCVR are composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. Herein, the 3 CDRs of the heavy chain are referred to as "CDRH1, CDRH2, and CDRH3" and the 3 CDRs of the light chain are referred to as "CDRL1, CDRL2 and CDRL3." The CDRs contain most of the residues which form specific interactions with the antigen. The assignment of amino acids to each domain is in accordance with well-known conventions [e.g., Kabat, "Sequences of Proteins of Immunological Interest," National Institutes of Health, Bethesda, Md. (1991)].

Antibodies of the present invention may have a heavy chain constant region selected from any of the immunoglobulin classes (IgA, IgD, IgG, IgM, and IgE). Furthermore, antibodies of the present invention contain an Fc portion which is derived from human IgG4 Fc region because of its reduced ability to bind complement factors as compared to other IgG sub-types.

An antibody may be derived from a single copy or clone, including e.g., any eukaryotic, prokaryotic, or phage clone. Preferably an antibody of the invention exists in a homogeneous or substantially homogeneous population. An antibody can be intact, comprising complete or full length constant regions, including the Fc region, or a portion or fragment of such an antibody provided that any shortened form comprises the antigen-binding portion and retains antigen-binding capability. Such shortened forms include, e.g., a Fab fragment, Fab' fragment or F(ab') 2 fragment that includes the CDRs or the variable regions of the anti-CXCR4 antibodies disclosed. Furthermore, such shortened

antibody forms can be a single chain Fv fragment that may be produced by joining the DNA encoding the LCVR and HCVR with a linker sequence. (See, Pluckthun, The Pharmacology of Monoclonal Antibodies, vol. 113, Rosenburg and Moore eds., Springer-Verlag, New York, pp 269-315, 1994). Regardless of whether fragments or portions are specified, the term "antibody" as used herein includes such fragments or portions as well as single chain forms unless otherwise indicated. As long as the protein retains the ability to specifically or preferentially bind CXCR4 and includes a sequence or sequences disclosed herein, it is included within the term "antibody." Antibodies of the invention can be produced using techniques well known in the art, e.g., recombinant technologies, phage display technologies, synthetic technologies or combinations of such technologies or other technologies readily known in the art.

The term “human engineered antibody” refers to an antibody having frameworks, hinge regions, and constant regions of human origin that are identical with or substantially identical (substantially human) with frameworks and constant regions derived from human genomic sequences. Fully human frameworks, hinge regions, and constant regions are those human germline sequences as well as sequences with naturally-occurring somatic mutations. A human engineered antibody may comprise framework, hinge, or constant regions derived from a fully human framework, hinge, or constant region containing one or more amino acid substitutions, deletions, or additions therein. Often, a human engineered antibody is preferably substantially non-immunogenic in humans.

A variety of different human framework sequences may be used singly or in combination as a basis for the human engineered antibodies of the present invention. Preferably, the framework regions of the antibodies of the invention are of human origin or substantially human (at least 95%, 97% or 99% of human origin.) The sequences of framework regions of human origin may be obtained from The Immunoglobulin Factsbook, by Marie-Paule Lafranc, Gerard Lefranc, Academic Press 2001, ISBN 012441351.

The framework sequence for the human engineered antibodies of the present invention serves as the “donor” variable framework region and can be used to create additional human engineered antibodies with the same CDRs specified herein using methodology known in the art. Furthermore, the framework sequence for the human

engineered antibodies of the present invention can be compared to other known human framework sequences to generate additional human engineered antibodies. Thus, this information can be used to "back-mutate" another selected homologous human framework region to the human engineered donor amino acid residue at these positions. Further, any "rare" amino acids can be detected in additional human frameworks such that the concensus or donor human engineered amino acid residue can be used at the relevant position.

The term "inhibit" means the ability to substantially antagonize, prohibit, prevent, restrain, slow, disrupt, eliminate, stop, reduce or reverse the biological effects of binding to the CXCR4 receptor.

"CXCR4" or "human CXCR4" refers to any human CXCR4, as well as functionally active, mutated forms thereof. Examples include, but are not limited to, SEQ ID NO:30, SEQ ID NO:31, and SEQ ID NO:32.

A "patient" is a mammal, preferably a human.

The term "treating" (or "treat" or "treatment") means slowing, stopping, reducing, or reversing the progression or severity of a symptom, disorder, condition, or disease.

The term "preventing" (or "prevent" or "prevention") means prohibiting, restraining, or inhibiting the incidence or occurrence of a symptom, disorder, condition, or disease. Acute events and chronic conditions may be treated and prevented. In an acute event, antibody is administered at the onset of a symptom, disorder, condition, or disease and discontinued when the acute event ends, whereas a chronic symptom, disorder, condition, or disease is treated over a more protracted time frame.

The term "therapeutically effective amount" refers to the amount or dose of an antibody of this invention which, upon single or multiple dose administration to a patient, provides the desired treatment or prevention. The therapeutically effective amount can comprise an amount of about 0.001 to 20 mg/kg per single (e.g., bolus), multiple or continuous administration.

Particular antibodies of this invention include: an antibody comprising amino acid sequences of SEQ ID NOs: 1, 2, 3, 8, 9, and 10; an antibody comprising amino acid sequences of SEQ ID NOs: 1, 4, 3, 8, 9, and 10; an antibody comprising amino acid sequences of SEQ ID NOs: 1, 5, 3, 8, 9, and 10; an antibody comprising amino acid sequences of SEQ ID NOs: 1, 6, 3, 8, 9, and 10; an antibody comprising amino acid

sequences of SEQ ID NOs: 1, 7, 3, 8, 9, and 10. The listed sequences represent CDRH1, CDRH2, CDRH3, CDRL1, CDRL2, and CDRL3, respectively.

Particular antibodies of this invention include: an antibody comprising a LCVR having an amino acid sequence of SEQ ID NO: 16 and a HCVR having an amino acid sequence of SEQ ID NO: 11; an antibody comprising a LCVR having an amino acid sequence of SEQ ID NO: 16 and a HCVR having an amino acid sequence of SEQ ID NO:12; an antibody comprising a LCVR having an amino acid sequence of SEQ ID NO: 16 and a HCVR having an amino acid sequence of SEQ ID NO:13; an antibody comprising a LCVR having an amino acid sequence of SEQ ID NO: 16 and a HCVR having an amino acid sequence of SEQ ID NO:14; an antibody comprising a LCVR having an amino acid sequence of SEQ ID NO: 16 and a HCVR having an amino acid sequence of SEQ ID NO:15.

The present invention includes five antibodies that bind and inhibit CXCR4 activity. In particular, the present invention includes: an antibody comprising a light chain having an amino acid sequence of SEQ ID NO: 22 and a heavy chain having an amino acid sequence of SEQ ID NO: 17; an antibody comprising a light chain having an amino acid sequence of SEQ ID NO: 22 and a heavy chain having an amino acid sequence of SEQ ID NO: 18; an antibody comprising a light chain having an amino acid sequence of SEQ ID NO: 22 and a heavy chain having an amino acid sequence of SEQ ID NO: 19; an antibody comprising a light chain having an amino acid sequence of SEQ ID NO: 22 and a heavy chain having an amino acid sequence of SEQ ID NO: 20; an antibody comprising a light chain having an amino acid sequence of SEQ ID NO: 22 and a heavy chain having an amino acid sequence of SEQ ID NO: 21.

Preferably, an antibody of the present invention wherein all six CDRs, the HCVR, the LCVR, the HCVR and the LCVR, the entire heavy chain, the entire light chain, or the entire heavy chain and light chain are limited by a particular sequence as shown by a SEQ ID NO: herein is further characterized by having an IC_{50} in a human CXCR4/^{125}I-SDF-1α binding inhibition assay as described herein of about of 10 nM or less, more preferably about 5.0 nM or less, and most preferably of 0.5 nM or less. Further preferred, an antibody of the present invention wherein all six CDRs, the HCVR, the LCVR, the HCVR and the LCVR, the entire heavy chain, the entire light chain, or the entire heavy chain and light chain are limited by a particular sequence as shown by a SEQ ID NO:

herein is further characterized by having an IC_{50} for human CXCR4 between 10 nM and 0.05 nM in a human CXCR4/^{125}I-SDF-1α binding inhibition assay as described herein. Further preferred, an antibody of the present invention wherein all six CDRs, the HCVR, the LCVR, the HCVR and the LCVR, the entire heavy chain, the entire light chain, or the entire heavy chain and light chain are limited by a particular sequence as shown by a SEQ ID NO: herein is further characterized by having an IC_{50} for human CXCR4 between 0.5 nM and 0.05 nM in a human CXCR4/^{125}I-SDF-1α binding inhibition assay as described herein.

More preferably, an antibody of the present invention wherein all six CDRs, the HCVR, the LCVR, the HCVR and the LCVR, the entire heavy chain, the entire light chain, or the entire heavy chain and light chain are limited by a particular sequence as shown by a SEQ ID NO: herein is further characterized by having an IC_{50} in a chemotaxis assay as described herein of about 30 nM or less, more preferably about 15 nM or less, and even more preferably 3.0 nM or less. Further preferred, an antibody of the present invention wherein all six CDRs, the HCVR, the LCVR, the HCVR and the LCVR, the entire heavy chain, the entire light chain, or the entire heavy chain and light chain are limited by a particular sequence as shown by a SEQ ID NO: herein is further characterized by having an IC_{50} between 30 nM and 0.3 nM in the chemotaxis assay as described herein. Further preferred, an antibody of the present invention wherein all six CDRs, the HCVR, the LCVR, the HCVR and the LCVR, the entire heavy chain, the entire light chain, or the entire heavy chain and light chain are limited by a particular sequence as shown by a SEQ ID NO: herein is further characterized by having an IC_{50} between 3.0 nM and 0.3 nM in the chemotaxis assay as described herein.

Even more preferably, an antibody of the present invention wherein all six CDRs, the HCVR, the LCVR, the HCVR and the LCVR, the entire heavy chain, the entire light chain, or the entire heavy chain and light chain are limited by a particular sequence as shown by a SEQ ID NO: herein is further characterized by having a K_D in an assay that evaluates the binding activities of the antibodies by Surface Plasmon Reasonance (BIAcore) as described herein of about 15 nM or less, more preferably about 10 nM or less, and most preferably 1.0 nM or less. Further preferred, an antibody of the present invention wherein all six CDRs, the HCVR, the LCVR, the HCVR and the LCVR, the entire heavy chain, the entire light chain, or the entire heavy chain and light chain are

limited by a particular sequence as shown by a SEQ ID NO: herein is further characterized by having a K_D in an assay that evaluates the binding activities of the antibodies by Surface Plasmon Reasonance (BIAcore) as described herein between 15 nM and 0.05 nM in the BIAcore assay as described herein. Further preferred, an antibody of the present invention wherein all six CDRs, the HCVR, the LCVR, the HCVR and the LCVR, the entire heavy chain, the entire light chain, or the entire heavy chain and light chain are limited by a particular sequence as shown by a SEQ ID NO: herein is further characterized by having a K_D in an assay that evaluates the binding activities of the antibodies by Surface Plasmon Reasonance (BIAcore) as described herein between 1.0 nM and 0.05 nM in the BIAcore assay as described herein.

More preferably, an antibody of the present invention wherein all six CDRs, the HCVR, the LCVR, the HCVR and the LCVR, the entire heavy chain, the entire light chain, or the entire heavy chain and light chain are limited by a particular sequence as shown by a SEQ ID NO: herein is further characterized by having anti-tumorigenesis activity by preventing tumor growth in a tumor xenograft model using NOD/SCID mice and human non-Hodgkin's lymphoma Namalwa cells as described herein when administered at 10 mg/kg, and even more preferably when administered at 1 mg/kg.

Most preferably, an antibody of the present invention wherein all six CDRs, the HCVR, the LCVR, the HCVR and the LCVR, the entire heavy chain, the entire light chain, or the entire heavy chain and light chain are limited by a particular sequence as shown by a SEQ ID NO: herein is further characterized by inducing apoptosis of tumor cells in an apoptosis assay as described herein. More preferred, an antibody of the present invention wherein all six CDRs, the HCVR, the LCVR, the HCVR and the LCVR, the entire heavy chain, the entire light chain, or the entire heavy chain and light chain are limited by a particular sequence as shown by a SEQ ID NO: herein is further characterized by inducing nuclear fragmentation and activation of caspase 3, hallmarks of apoptosis, in multiple tumor cells including Namalwa and CEM cells when administered between 2 μg/mL and 10 μg/mL.

EXAMPLES

Antibodies I, II, III, IV, and V can be made and purified as follows. An appropriate host cell, such as HEK 293 EBNA or CHO, is either transiently or stably

transfected with an expression system for secreting antibodies using an optimal predetermined HC:LC vector ratio or a single vector system encoding both HC, such as SEQ ID NO: 23, SEQ ID NO: 24, SEQ ID NO: 25, SEQ ID NO: 26, or SEQ ID NO: 27 and LC, such as SEQ ID NO: 28. Clarified media, into which the antibody has been secreted, is purified using any of many commonly-used techniques. For example, the medium may be conveniently applied to a Protein A or G Sepharose FF column that has been equilibrated with a compatible buffer, such as phosphate buffered saline (pH 7.4). The column is washed to remove nonspecific binding components. The bound antibody is eluted, for example, by pH gradient (such as 0.1 M sodium phosphate buffer pH 6.8 to 0.1 M sodium citrate buffer pH 2.5). Antibody fractions are detected, such as by SDS-PAGE, and then are pooled. Further purification is optional, depending on the intended use. The antibody may be concentrated and/or sterile filtered using common techniques. Soluble aggregate and multimers may be effectively removed by common techniques, including size exclusion, hydrophobic interaction, ion exchange, or hydroxyapatite chromatography. The purity of the antibody after these chromatography steps is greater than 99%. The product may be immediately frozen at -70°C or may be lyophilized. The amino acid sequences for these antibodies are provided below.

SEQ ID NOs

Antibody	Heavy Chain	Light Chain	HCVR	LCVR
I	17	22	11	16
II	18	22	12	16
III	19	22	13	16
IV	20	22	14	16
V	21	22	15	16

Antibody	HCDR1	HCDR2	HCDR3	LCDR1	LCDR2	LCDR3
I	1	2	3	8	9	10

II	1	4	3	8	9	10
III	1	5	3	8	9	10
IV	1	6	3	8	9	10
V	1	7	3	8	9	10

Human CXCR4/^{125}I-SDF-1α Binding Inhibition Assay

SDF-1 binding to CXCR4 is the first step in activating the CXCR4 intracellular signaling pathway. To determine if an antibody can block the interaction of SDF-1 and CXCR4, human leukemia CCRF-CEM cells expressing endogenous CXCR4 are used in an ^{125}I-labeled SDF-1α binding assay. The assay is performed in a 96-well U-bottom, non-treated polystyrene plate. The binding assay buffer is prepared with RPMI 1640 medium containing 10 mM N-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (HEPES), pH 7.5, and 0.2% bovine serum albumin (BSA). Briefly, 200 μL reaction mixtures containing 300 pM ligand (60 pM ^{125}I-SDF-1α and 240 pM cold SDF-1α), different concentrations of the test antibody in assay buffer, 100,000 human CCRF-CEM cells, and 0.5 mg scintillation proximity assay (SPA) beads are incubated at room temperature for 2 hours. Plates are then counted in a liquid scintillation and luminescence counter in SPA mode. CXCR4 antagonists decrease the bound radioactivity in this assay in a dose-dependent manner. The inhibitory potency (IC_{50}) of a test antibody is calculated using GraphPad Prism software, based on the dose-dependent decrease of bound radioactivity.

Antibodies exemplified herein exhibit an IC_{50} value of 10 nM or less in this assay. For example, the antibody III exhibits an average IC50 of 0.45 nM in this assay. The data demonstrate that antibodies exemplified herein bind to human CXCR4 with high affinity and inhibit human CXCR4 binding to SDF-1.

Chemotaxis Assay

CXCR4/SDF-1 interaction regulates migration (chemotaxis) of cells bearing CXCR4 on their surface. To determine the antagonist and cellular activities of a test antibody, a chemotaxis assay using human histiocytic lymphoma U937 cells that express endogenous CXCR4 is employed. Briefly, U937 cells, grown in Dulbecco's Modified

Eagle Medium (D-MEM) containing 10% fetal bovine serum, 1% Minimum Essential Medium (MEM) sodium pyruvate solution, 1% MEM nonessential amino acids, and 1% L-glutamine, are harvested and washed once with chemotaxis assay buffer (1x RPMI medium containing 10 mM HEPES, pH 7.5, and 0.3% BSA.) After washing, cells are resuspended in assay buffer at a concentration of 5 x 10^6 cells/mL. The assay is performed in a 96-well cell migration plate. Generally, 50 µL of cell mixture with or without test antibody, ranging from 0.5 µg/mL to 50 µg/mL, is plated on the upper chamber, and 30 µL of SDF-1α (10 ng/mL) prepared in 1x chemotaxis assay buffer is added to the lower chamber. After assembly, the plate is incubated for 2.5 hours at 37°C under 5% carbon dioxide. Following the incubation, 5 µL of cell proliferation solution is added into the lower chamber. The plate is then incubated for 60 minutes at 37°C, and the migrated cells are detected by measuring the absorbance at 492 nm with a microplate reader. CXCR4 antagonists inhibit cell migration, reducing the absorbance reading. The inhibitory potency (IC_{50}) of a test antibody in this assay is calculated using GraphPad Prism software, based on the dose-dependent decrease of absorbance at 492 nm.

Antibodies exemplified herein exhibit an average IC50 value of 30 nM or less in this assay. The antibody III exhibits an average IC50 value of 5.90 nM in this assay. The data demonstrate that antibodies exemplified herein bind to human CXCR4 with high affinity and inhibit human CXCR4 binding to SDF-1.

Evaluation of binding activities of anti-CXCR4 antibodies by Surface Plasmon Reasonance (BIAcore)

A Biacore® 2000 instrument is used to measure binding kinetics and affinity. The Biacore® utilizes the optical properties of surface plasmon resonance to detect alteration in protein concentration of interacting molecules within a dextran biosensor matrix. Except as noted, all reagents and materials are purchased from Biacore® AB (Upsala, Sweden). All measurements are performed at 4°C. The binding experiment is performed essentially as described in Stenlund et al (Stenlund P, Babcock GJ, Sodroski J, Myszka DG. (2003) Capture and reconstitution of G protein-coupled receptors on a biosensor surface. Anal Biochem. 316(2):243-50) and Navratilo et al (Navratilova I, Sodroski J, Myszka DG. (2005) Solubilization, stabilization, and purification of

chemokine receptors using biosensor technology. Anal Biochem. 339(2):271-81). Running buffer is 50 mM HEPES, 5 mM magnesium chloride, 1 mM calcium chloride, 150 mM sodium chloride, 2 mg/mL BSA, pH 7.5. The human CXCR4 receptor with a C-terminal linear C9 peptide tag (SEQ ID NO: 29), is cloned and overexpressed in canine thymocyte Cf2Th cells in the same manner as described previously by Mirzabekov et al (Mirzabekov, N. Bannert, M. Farzan, W. Hofmann, P. Kolchinsky, L. Wu, R. Wyatt and J. Sodroski- Enhanced expression, native purification, and characterization of CCR5, a principal HIV-1 coreceptor. J. Biol. Chem. 274 (1999), pp. 28745–28750). The C-terminal linear C9 peptide tag is recognized by the 1D4 monoclonal antibody (D.D. Oprian, R.S. Molday, R.J. Kaufman and H.G. Khorana, Expression of a synthetic bovine rhodopsin gene in monkey kidney cells. Proc. Natl. Acad. Sci. USA 84 (1987), pp. 8874–8878).

Binding is evaluated using multiple analytical cycles as follows. 1D4 Mab (Monoclonal clone 1D4, University of British Columbia) is immobilized to a CM5 chip via amine coupling (about 10,000-20,000 Resonance Units antibody). Cells are resuspended in 20 mM tris(hydroxymethyl)aminomethane (pH 7.0), 0.1 M ammonium sulphate, 10% glycerol, 5 mM magnesium chloride, 1 mM calcium chloride, plus complete ethylenediaminetetraacetic acid-free protease inhibitor tablet. $4x10^6$ cells/mL final for injection onto the chip (i.e. $2.0x10^6$ cells, 0.5 mL final volume) are used. Transfected cells and running buffer with detergent (2% cholesteryl hemisuccinate ester, 10% dodecyl maltoside, 10% 3-[(3-Cholamidopropyl)dimethylammonio]-1-propanesulfonate) at a ratio of 5:1 (cells to buffer volume ratio) are transferred into an auto-mixer. This mixture is incubated for 10 minutes. After incubation 150 µL solubilized receptor is injected over 1D4 surface at a flow rate of 20 µL/minute. The sample loop is then washed with running buffer. This is followed by an injection of 20 µL of antibody at flow rate of 100 µL /minute. The chip is then regenerated with two 10 second pulses of 10 mM sodium hydroxide + 1% n-octyl-β-D-glucopyranoside at 100 µL/minute. Association rate constants ("k_{on}") and dissociation rate constants ("k_{off}") for each cycle are evaluated using a "1:1 with mass transfer" binding model in the BIAevaluation software. "K_D" is the dissociation constant and it is calculated by the formula: $k_{off}/k_{on} = K_D$. The binding parameters are summarized below. The data

demonstrate that antibodies exemplified herein bind to human CXCR4 with high affinity and inhibit human CXCR4 binding to SDF-1.

Antibody	K_{on} ($M^{-1}s^{-1}$)	K_{off} (s^{-1})	K_D (nM)
I	2.36×10^6	1.61×10^{-3}	0.68
II	2.50×10^6	1.81×10^{-3}	0.72
III	1.12×10^6	1.72×10^{-3}	1.54
IV	7.74×10^5	3.98×10^{-3}	5.14
V	1.91×10^6	1.68×10^{-3}	0.88

Anti-Tumor Activity in a SCID/Namalwa Xenograft Model

SDF-1/CXCR4 interaction appears to play an important role in multiple stages of tumorigenesis, including tumor growth, invasion, angiogenesis, and metastasis. To evaluate *in vivo* anti-tumor activity of a test antibody in cancer, a tumor xenograft model using NOD/SCID mice and human non-Hodgkin's lymphoma Namalwa cells is employed. Briefly, 200,000 Namalwa cells mixed with matrigel (1:1) are implanted subcutaneously into the rear flank of the animals. The implanted tumor cells grow as solid tumors, the dimensions of which can be continuously monitored and measured using a caliper. To determine the *in vivo* efficacy of a test antibody in this model, animals (10/group) are treated with different doses of test antibodies dissolved in saline or phosphate buffered saline, 48 hours post tumor cell implantation. Antibodies are dosed subcutaneously in the range of 1 μg/mouse, 10 μg/mouse, and 100 μg/mouse, and tumor volume and body weight are determined every 2 or 3 days. The studies last generally 3-4 weeks, depending on the tumor growth. The anti-tumor growth activity of a test antibody is determined by the percent reduction in tumor volume in treatment groups compared to tumor volume in control groups treated with vehicle alone.

Antibody I inhibits tumor growth in this assay when administered at 10μg/mouse, which is approximately 0.4 mg/kg. The data demonstrate that Antibody I has tumorigenesis activity by preventing tumor growth.

SCID/Namalwa Hematological Lymphoma Model

To further investigate anti-tumor activity of CXCR4 antibody in lymphoma, a hematological lymphoma model is established by injecting 200,000 Namalwa cells into SCID mice via tail vein. Generally, the mice injected with tumor cells die in 5-6 weeks. To test efficacy of the antibody in this model, animals (10 each group) are treated with 30 μg/mouse or 100 μg/mouse of test antibody 24 hours post tumor cell injection. The antibody is dosed subcutaneously once every 4 days for 6 weeks, and the animal survival is recorded on daily basis.

Antibody I treatment groups have shown statistically significant survival benefit when compared with vehicle and isotype IgG control groups in this hematological lymphoma model.

Anti-Tumor Activity in a SCID/CEM Xenograft Model

To evaluate *in vivo* anti-tumor activity of a test antibody in cancer, a tumor xenograft model using NOD/SCID mice and CEM cells is employed. Briefly, $5.0x10^6$ CEM cells mixed with matrigel (1:1) are implanted subcutaneously into the rear flank of the animals. The implanted tumor cells grow as solid tumors which can be continuously monitored and measured by caliber. To determine the efficacy of a test antibody in this model, animals (10 each group) are treated with 10 μg/mouse, 30 μg/mouse, or 100 μg/mouse of test antibody 24 hours post tumor cell implantation. The antibody is dosed subcutaneously once every 4 days, and the tumor volume and body weight are measured every 2 or 3 days.

A dose-dependent tumor growth inhibition is observed among Antibody I treatment groups compared with vehicle and isotype IgG control groups. Antibody I at all three doses inhibits tumor growth significantly.

Apoptosis Assay

To investigate if CXCR4 antibodies induce apoptosis, multiple tumor cell lines expressing high levels of CXCR4 are treated with test antibody. The cells are treated with different concentrations of test antibody for 2-4 days in their growth medium with 1% or 10 % FBS. After treatment, cells are fixed with 3.7% formaldehyde and washed in D-PBS. Cells are permeabilized with 0.1% Triton X-100 in D-PBS, washed and blocked in

D-PBS containing 1% BSA. Cells are then incubated for 1 hour with rabbit anti-activated Caspase3 polyclonal antibody (Cat# 557135 BD Biosciences, NC) diluted in D-PBS with 1% BSA. Cells are washed 2 times with D-PBS then incubated for 1 hour with Alexa Fluor 488 goat anti Rabbit IgG (Invitrogen, Carlsbad, CA) and 200ng/mL Hoechst 33342 (Invitrogen, Carlsbad, CA) diluted in D-PBS with 1% BSA. Stained plates are scanned using ArrayScan Vti (Cellomics, Pittsburgh, Pennsylvania) and the Target Activation bioapplication is used for quantitation of fluorescent signal.

The results demonstrate that Antibody I induces nuclear fragmentation and activation of caspase 3 in multiple tumor cells including Namalwa and CEM cells. Nuclear fragmentation and caspase 3 activation are hallmarks of apoptosis. Therefore, the data demonstrate that Antibody I induces apoptosis of tumor cells when administered between 2 μg/mL and 10 μg/mL.

To further confirm that Antibody I induces apoptosis, annexin V changes are investigated by flow cytometry in Namalwa cells after treatment with test antibody or isotype IgG control. Antibody I induces a dose-dependent increase of annexin V, while isotype IgG has no effect. Furthermore, Antibody I induces apoptosis which is also observed in CEM xenograft tumors by TUNEL staining.

SEQ ID Listing

Heavy Chain CDRs

SEQ ID NO:1	GFTSTDYYFS
SEQ ID NO:2	FIRTKSKGYTTEYSGSVKG
SEQ ID NO:3	EPITTDPRDY
SEQ ID NO:4	FIRSKSKGYTTEYSGSVKG
SEQ ID NO:5	FIRYKSKGYTTEYSGSVKG
SEQ ID NO:6	FIRNKRKGYTTEYSGSVKG
SEQ ID NO:7	FIRHKSKGYTTEYSGSVKG

Light Chain CDRs

SEQ ID NO:8	KSSQSLFNSRTRKKYLA
SEQ ID NO:9	WASKRKS
SEQ ID NO:10	KQSRFLRA

Heavy Chain Variable Regions

SEQ ID NO:11
EVQLVESGGGLVQPGGSLRLSCAASGFTSTDYYFSWVRQAPGKGLEWVGFIRTK
SKGYTTEYSGSVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCAREPITTDPRD
YWGQGTLVTVSS

SEQ ID NO:12
EVQLVESGGGLVQPGGSLRLSCAASGFTSTDYYFSWVRQAPGKGLEWVGFIRSK
SKGYTTEYSGSVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCAREPITTDPRD
YWGQGTLVTVSS

SEQ ID NO:13
EVQLVESGGGLVQPGGSLRLSCAASGFTSTDYYFSWVRQAPGKGLEWVGFIRYK
SKGYTTEYSGSVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCAREPITTDPRD
YWGQGTLVTVSS

SEQ ID NO:14
EVQLVESGGGLVQPGGSLRLSCAASGFTSTDYYFSWVRQAPGKGLEWVGFIRNK
RKGYTTEYSGSVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCAREPITTDPRD
YWGQGTLVTVSS

SEQ ID NO:15
EVQLVESGGGLVQPGGSLRLSCAASGFTSTDYYFSWVRQAPGKGLEWVGFIRHK
SKGYTTEYSGSVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCAREPITTDPRD
YWGQGTLVTVSS

Light Chain Variable Regions

SEQ ID NO:16
DIVMTQSPDSLAVSLGERATINCKSSQSLFNSRTRKKYLAWYQQKPGQPPKLLIY
WASKRKSGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCKQSRFLRAFGQGTKLE
IK

Complete Heavy Chains

SEQ ID NO:17
EVQLVESGGGLVQPGGSLRLSCAASGFTSTDYYFSWVRQAPGKGLEWVGFIRTK
SKGYTTEYSGSVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCAREPITTDPRD
YWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRV
ESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY
TQKSLSLSLG

SEQ ID NO:18
EVQLVESGGGLVQPGGSLRLSCAASGFTSTDYYFSWVRQAPGKGLEWVGFIRSK
SKGYTTEYSGSVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCAREPITTDPRD
YWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRV
ESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY
TQKSLSLSLG

SEQ ID NO:19
EVQLVESGGGLVQPGGSLRLSCAASGFTSTDYYFSWVRQAPGKGLEWVGFIRYK
SKGYTTEYSGSVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCAREPITTDPRD
YWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRV
ESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY
TQKSLSLSLG

SEQ ID NO:20
EVQLVESGGGLVQPGGSLRLSCAASGFTSTDYYFSWVRQAPGKGLEWVGFIRNK
RKGYTTEYSGSVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCAREPITTDPRD
YWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS

GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRV
ESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY
TQKSLSLSLG

SEQ ID NO:21
EVQLVESGGGLVQPGGSLRLSCAASGFTSTDYYFSWVRQAPGKGLEWVGFIRHK
SKGYTTEYSGSVKGRFTISRDDSKNSLYLQMNSLKTEDTAVYYCAREPITTDPRD
YWGQGTLVTVSSASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNS
GALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRV
ESKYGPPCPPCPAPEAAGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQ
FNWYVDGVEVHNAKTKPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNK
GLPSSIEKTISKAKGQPREPQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWE
SNGQPENNYKTTPPVLDSDGSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHY
TQKSLSLSLG

Complete Light Chain

SEQ ID NO:22
DIVMTQSPDSLAVSLGERATINCKSSQSLFNSRTRKKYLAWYQQKPGQPPKLLIY
WASKRKSGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCKQSRFLRAFGQGTKLE
IKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQ
ESVTEQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Nucleotide Sequences - Heavy Chain Variable Region

SEQ ID NO:23
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGAGGGTCCC
TGAGACTCTCCTGTGCAGCCTCTGGCTTCACCAGTACCGACTACTACTTTAGC
TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTGGCTTCATCCGGA
CGAAGTCGAAGGGCTACACCACCGAGTACAGCGGCAGCGTGAAGGGCAGAT
TCACCATCTCAAGAGATGATTCAAAGAACTCACTGTACCTGCAGATGAACAG
CCTGAAAACCGAGGACACGGCCGTGTATTACTGTGCTAGAGAGCCCATCACC
ACCGACCCTCGGGACTACTGGGGCCAAGGGACCCTGGTCACCGTCTCCTCA

SEQ ID NO:24
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGAGGGTCCC
TGAGACTCTCCTGTGCAGCCTCTGGCTTCACCAGTACCGACTACTACTTTAGC
TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTGGCTTCATCCGGT
CTAAGTCGAAGGGCTACACCACCGAGTACAGCGGCAGCGTGAAGGGCAGATT
CACCATCTCAAGAGATGATTCAAAGAACTCACTGTACCTGCAGATGAACAGC
CTGAAAACCGAGGACACGGCCGTGTATTACTGTGCTAGAGAGCCCATCACCA
CCGACCCTCGGGACTACTGGGGCCAAGGGACCCTGGTCACCGTCTCCTCA

SEQ ID NO:25
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGAGGGTCCC
TGAGACTCTCCTGTGCAGCCTCTGGCTTCACCAGTACCGACTACTACTTTAGC
TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTGGCTTCATCCGGT
ATAAGTCGAAGGGCTACACCACCGAGTACAGCGGCAGCGTGAAGGGCAGATT
CACCATCTCAAGAGATGATTCAAAGAACTCACTGTACCTGCAGATGAACAGC
CTGAAAACCGAGGACACGGCCGTGTATTACTGTGCTAGAGAGCCCATCACCA
CCGACCCTCGGGACTACTGGGGCCAAGGGACCCTGGTCACCGTCTCCTCA

SEQ ID NO:26
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGAGGGTCCC
TGAGACTCTCCTGTGCAGCCTCTGGCTTCACCAGTACCGACTACTACTTTAGC
TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTGGCTTCATCCGGA
ACAAGCGGAAGGGCTACACCACCGAGTACAGCGGCAGCGTGAAGGGCAGAT
TCACCATCTCAAGAGATGATTCAAAGAACTCACTGTACCTGCAGATGAACAG
CCTGAAAACCGAGGACACGGCCGTGTATTACTGTGCTAGAGAGCCCATCACC
ACCGACCCTCGGGACTACTGGGGCCAAGGGACCCTGGTCACCGTCTCCTCA

SEQ ID NO:27
GAGGTGCAGCTGGTGGAGTCTGGGGGAGGCTTGGTCCAGCCTGGAGGGTCCC
TGAGACTCTCCTGTGCAGCCTCTGGCTTCACCAGTACCGACTACTACTTTAGC
TGGGTCCGCCAGGCTCCAGGGAAGGGGCTGGAGTGGGTTGGCTTCATCCGGC
ACAAGTCGAAGGGCTACACCACCGAGTACAGCGGCAGCGTGAAGGGCAGAT
TCACCATCTCAAGAGATGATTCAAAGAACTCACTGTACCTGCAGATGAACAG
CCTGAAAACCGAGGACACGGCCGTGTATTACTGTGCTAGAGAGCCCATCACC
ACCGACCCTCGGGACTACTGGGGCCAAGGGACCCTGGTCACCGTCTCCTCA

Nucleotide Sequences - Light Chain Variable Region

SEQ ID NO:28
GACATCGTGATGACCCAGTCTCCAGACTCCCTGGCTGTGTCTCTGGGCGAGAG
GGCCACCATCAACTGCAAGAGCAGCCAGAGCCTGTTCAACAGCCGGACCCGG
AAGAAGTACCTGGCCTGGTACCAGCAGAAACCAGGACAGCCTCCTAAGCTGC
TCATTTACTGGGCCAGCAAGAGAAAGAGCGGGGTCCCTGACCGATTCAGTGG
CAGCGGGTCTGGGACAGATTTCACTCTCACCATCAGCAGCCTGCAGGCTGAA
GATGTGGCAGTTTATTACTGTAAGCAGAGCCGTTTTCTGAGAGCCTTTGGCCA
AGGGACCAAGCTGGAGATCAAA

C-Terminal Linear C9 Peptide Tag for Human CXCR4 Receptor

SEQ ID NO:29 TETSQVAPA

Human CXCR4

SEQ ID NO:30
MEGISSIPLPLLQIYTSDNYTEEMGSGDYDSMKEPCFREENANFNKIFLPTIYSIIFL
TGIVGNGLVILVMGYQKKLRSMTDKYRLHLSVADLLFVITLPFWAVDAVANWY
FGNFLCKAVHVIYTVNLYSSVLILAFISLDRYLAIVHATNSQRPRKLLAEKVVYVG
VWIPALLLTIPDFIFANVSEADDRYICDRFYPNDLWVVVFQFQHIMVGLILPGIVIL
SCYCIIISKLSHSKGHQKRKALKTTVILILAFFACWLPYYIGISIDSFILLEIIKQGCEF
ENTVHKWISITEALAFFHCCLNPILYAFLGAKFKTSAQHALTSVSRGSSLKILSKG
KRGGHSSVSTESESSSFHSS

Human CXCR4 isoform A

SEQ ID NO:31
MSIPLPLLQIYTSDNYTEEMGSGDYDSMKEPCFREENANFNKIFLPTIYSIIFLTGIV
GNGLVILVMGYQKKLRSMTDKYRLHLSVADLLFVITLPFWAVDAVANWYFGNF
LCKAVHVIYTVNLYSSVLILAFISLDRYLAIVHATNSQRPRKLLAEKVVYVGVWIP
ALLLTIPDFIFANVSEADDRYICDRFYPNDLWVVVFQFQHIMVGLILPGIVILSCYC
IIISKLSHSKGHQKRKALKTTVILILAFFACWLPYYIGISIDSFILLEIIKQGCEFENTV
HKWISITEALAFFHCCLNPILYAFLGAKFKTSAQHALTSVSRGSSLKILSKGKRGG
HSSVSTESESSSFHSS

Human CXCR4 isoform B

SEQ ID NO:32
MEGISIYTSDNYTEEMGSGDYDSMKEPCFREENANFNKIFLPTIYSIIFLTGIVGNG
LVILVMGYQKKLRSMTDKYRLHLSVADLLFVITLPFWAVDAVANWYFGNFLCK
AVHVIYTVNLYSSVLILAFISLDRYLAIVHATNSQRPRKLLAEKVVYVGVWIPALL
LTIPDFIFANVSEADDRYICDRFYPNDLWVVVFQFQHIMVGLILPGIVILSCYCIIIS
KLSHSKGHQKRKALKTTVILILAFFACWLPYYIGISIDSFILLEIIKQGCEFENTVHK
WISITEALAFFHCCLNPILYAFLGAKFKTSAQHALTSVSRGSSLKILSKGKRGGHSS
VSTESESSSFHSS

Human SDF-1 α

SEQ ID NO:33
KPVSLSYRCPCRFFESHVARANVKHLKILNTPNCALQIVARLKNNNRQVCIDPKL
KWIQEYLEKALNK

WE CLAIM:

1. A human engineered antibody or a binding fragment thereof, that binds human CXCR4, and which:
   a. inhibits binding of human SDF-1α (SEQ ID NO:33) to CXCR4 with an IC_{50} for human CXCR4 between 10 nM and 0.05 nM in a human CXCR4/^{125}I-SDF-1α binding inhibition assay as described herein;
   b. inhibits migration of cells bearing CXCR4 on their surface with an IC_{50} between 30 nM and 0.3 nM in the chemotaxis assay as described herein; and
   c. exhibits an affinity, K_D between 15 nM and 0.05 nM in the Surface Plasmon Reasonance (BIAcore) assay as described herein.
2. The antibody of Claim 1, which inhibits binding of human SDF-1α (SEQ ID NO:33) to CXCR4 with an IC_{50} for human CXCR4 between 0.5 nM and 0.05 nM.
3. The antibody of either Claim 1 or Claim 2, which inhibits migration of cells bearing CXCR4 on their surface with an IC_{50} between 3.0 nM and 0.3 nM.
4. The antibody of any one of Claims 1 to 3, which exhibits an affinity, K_D between 1.0 nM and 0.05 nM.
5. The antibody of any one of Claims 1 to 4, which exhibits anti-tumorigenesis activity by preventing tumor growth in a tumor xenograft model as described herein when administered at 1 mg/kg.
6. The antibody of any one of Claims 1 to 5, which induces apoptosis of tumor cells in an apoptosis assay as described herein when administered between 2 μg/mL and 10 μg/mL.
7. A human engineered antibody or a binding fragment thereof, that comprises a light chain comprising a light chain variable region that comprises framework regions, CDRL1 having the amino acid sequence of SEQ ID NO:8, CDRL2 having the amino acid sequence of SEQ ID NO:9, and CDRL3 having the amino acid sequence of SEQ ID NO:10, and a heavy chain comprising a heavy chain variable region that comprises framework regions, CDRH1 having the amino acid sequence of SEQ ID NO:1, CDRH3 having the amino acid

sequence of SEQ ID NO:3, and CDRH2 having an amino sequence selected from the group consisting of SEQ ID NO:2, SEQ ID NO:4, SEQ ID NO:5, SEQ ID NO:6, and SEQ ID NO:7, wherein the antibody binds to human CXCR4.

8. The antibody of Claim 7, wherein a light chain comprises the amino acid sequence of SEQ ID NO:16.

9. The antibody of either Claim 7 or Claim 8, wherein a heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NO:11, SEQ ID NO:12, SEQ ID NO:13, SEQ ID NO:14, and SEQ ID NO:15.

10. The antibody of any one of Claims 7 to 9, wherein a light chain comprises the amino acid sequence of SEQ ID NO:22.

11. The antibody of any one of Claims 7 to 10, wherein a heavy chain comprises an amino acid sequence selected from the group consisting of SEQ ID NO:17, SEQ ID NO:18, SEQ ID NO:19, SEQ ID NO:20, and SEQ ID NO:21.

12. The antibody of any one of Claims 7 to 11, which is selected from the group consisting of an antibody comprising SEQ ID NO:17 and SEQ ID NO:22, an antibody comprising SEQ ID NO:18 and SEQ ID NO:22, an antibody comprising SEQ ID NO:19 and SEQ ID NO:22, an antibody comprising SEQ ID NO:20 and SEQ ID NO:22, and an antibody comprising SEQ ID NO:21 and SEQ ID NO:22.

13. The antibody of any one of Claims 7 to 12 that comprises two light chains of SEQ ID NO: 22, and two heavy chains of SEQ ID NO: 17.

14. A pharmaceutical composition comprising an antibody of any one of Claims 1 to 13 in combination with one or more pharmaceutically acceptable carriers, diluents, or excipients.

15. An antibody of any one of Claims 1 to 13 for use in therapy.

16. An antibody of any one of Claims 1 to 13 for use in the treatment of tumorigenesis, including tumor growth, invasion, angiogenesis, or metastasis.

17. The use of an antibody of any one of Claims 1 to 13 in the manufacture of a medicament for the treatment of tumorigenesis, including tumor growth, invasion, angiogenesis, or metastasis.

18. A method of treating tumorigenesis, including tumor growth, invasion, angiogenesis, or metastasis comprising administering to a patient in need thereof a therapeutically effective amount of an antibody of any one of Claims 1 to 13.

19. An antibody of any one of Claims 1 to 13 for use in the treatment of a cancer selected from the group consisting of breast cancer, pancreatic cancer, melanoma, prostate cancer, kidney cancer, neuroblastoma, non-Hodgkin's lymphoma, lung cancer, ovarian cancer, colorectal cancer, multiple myeloma, glioblastoma multiforme, and leukemia.

20. The use of an antibody of any one of Claims 1 to 13 in the manufacture of a medicament for the treatment of a cancer selected from the group consisting of breast cancer, pancreatic cancer, melanoma, prostate cancer, kidney cancer, neuroblastoma, non-Hodgkin's lymphoma, lung cancer, ovarian cancer, colorectal cancer, multiple myeloma, glioblastoma multiforme, and leukemia.

21. A method of treating a cancer selected from the group consisting of breast cancer, pancreatic cancer, melanoma, prostate cancer, kidney cancer, neuroblastoma, non-Hodgkin's lymphoma, lung cancer, ovarian cancer, colorectal cancer, multiple myeloma, glioblastoma multiforme, and leukemia, comprising administering to a patient in need thereof a therapeutically effective amount of an antibody of any one of Claims 1 to 13.