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(54) Title: HUMAN CXCR3 RECEPTOR VARIANTS AND METHODS OF USE

(57) Abstract: The present invention provides alternatively spliced CXCR3 variants, as well as related nucleic acid molecules and screening methods.



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HUMAN CXCR3 RECEPTOR VARIANTS AND METHODS OF USE

This patent application claims priority pursuant to 35 U.S.C. §119(e) to provisional patent application 5 Serial No. 60/572459 filed May 18, 2004, which is hereby incorporated by reference in its entirety.

Chemokines are small secreted molecules that attract and activate specific leukocyte subpopulations. By regulating trafficking of leukocytes in animals, chemokines 10 play key roles in initiating and maintaining normal inflammatory responses. In some cases, the selective accumulation and activation of leukocytes in inflamed tissues contributes to the pathogenesis of inflammatory and autoimmune diseases such as infection, rheumatoid 15 arthritis, allergic asthma, atopic dermatitis, and multiple sclerosis.

Chemokines are classified into subgroups based on the presence or absence of an amino acid between the first two cysteine residues. Generally, CXC chemokines that 20 contain a proximal "ELR" motif attract neutrophils; CXC chemokines that lack a proximal "ELR" motif attract lymphocytes; and CC chemokines attract mononuclear cells. Each of the various types of chemokines functions by binding to at least one respective chemokine receptor.

25 Chemokine receptors belong to the superfamily of G protein-coupled seven-transmembrane domain receptors (GPCRs). Based on ligand binding specificity, chemokine receptors have been classified into C-; CC-; CXC-, and

CX3C-chemokine receptors, as well as orphan chemokine receptors. In particular, CXC receptor 3 (CXCR3), which binds to CXCL10, I-TAC and Mig, is involved in regulating T cell chemotaxis. The involvement of CXCR3 in immunological diseases has been documented in several preclinical studies using CXCR3 deficient mice, anti-CXCR3 antibodies and CXCR3 antagonists, suggesting that CXCR3 is an attractive therapeutic target for a variety of diseases. In addition, there is evidence that a CXCR3 isoform termed "CXCR3-B" can mediate additional activities of the CXCR3 ligands, including angiostatic activity.

CXCR3 splice variants thus can encode protein isoforms that have physiological activities that differ in degree or type from related isoforms. A CXCR3 isoform arising from a splice variant can differ, for example, in stability, clearance rate, tissue or cellular localization, tissue expression pattern, temporal pattern of expression, regulation, or response to agonists or antagonists, in comparison to another isoform.

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The presence or level of a specific isoform of a protein can contribute to, or protects against, a pathological condition. As such, a protein isoform such as an isoform encoded by a newly identified splice variant, can represent a new drug target or diagnostic marker. Because a drug can have differential activity on one isoform compared to another, knowledge of isoforms that represent drug targets can contribute to improved understanding of drug effectiveness, as well as improved drug screening strategies and drug design. For example,

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drug screening can be performed using more than one CXCR3 isoform in order to improve specificity for the target isoform.

5 Thus, there exists a need for identification of CXCR3 polypeptide isoforms that can be used, for example, to design more specific drugs with fewer side effects. The present invention satisfies this need and provides related advantages as well.

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SUMMARY OF THE INVENTION

The invention provides an isolated CXCR3 variant polypeptide containing the amino acid sequence referenced as SEQ ID NO:11, the polypeptide having at least 50%
15 identity with SEQ ID NO:8 or 10. The amino acid sequence of the CXCR3 variant polypeptide can contain, for example, one of SEQ ID NOS:2 or 4, or a conservative variant thereof. Further provided by the invention is an isolated CXCR3C polypeptide comprising an amino acid sequence having
20 at least 80% identity with SEQ ID NO:6.

Also provided by the invention is a CXCR3 variant binding agent that binds specifically to SEQ ID NO:11, or a portion thereof. In addition, a CXCR3 variant binding agent that binds specifically to SEQ ID NO:12 is provided.
25 In one embodiment, the binding agent can be an antibody or antigen binding fragment thereof.

Further provided by the invention is a cell that exogenously expresses a polypeptide containing the amino

acid sequence referenced as SEQ ID NO:11, the polypeptide having at least 50% identity with SEQ ID NO:8 or 10, and a cell that exogenously expresses polypeptide containing an amino acid sequence having at least 80% identity with SEQ
5 ID NO:6.

The invention provides methods for identifying an agonist of a CXCR3 variant. In one aspect, the method involves a) contacting a CXCR3 variant polypeptide with a candidate compound, the polypeptide containing SEQ ID NO:11
10 or a conservative variant thereof, and having at least 50% identity with SEQ ID NO:8 or 10, and b) identifying a compound that selectively promotes production of a CXCR3 signal, the compound being characterized as an agonist of a CXCR3 variant. In an embodiment, the CXCR3 variant
15 polypeptide contains an amino acid sequence selected from SEQ ID NO:2 or 4, or a conservative variant thereof. In another aspect, the method involves a) contacting a CXCR3C polypeptide with a candidate compound, the polypeptide comprising an amino acid sequence having at least 80%
20 identity with SEQ ID NO:6, and b) identifying a compound that selectively promotes production of a CXCR3 signal, the compound being characterized as an agonist of a CXCR3C. In embodiments of the invention, the CXCR3 variant polypeptide can be isolated or expressed in a genetically engineered
25 cell. In further embodiments of the invention, the CXCR3 signal can be, for example, calcium mobilization, cAMP upregulation, and ligand binding. A compound tested using a method of the invention can be, for example, a polypeptide or small molecule.

The invention also provides methods for identifying an antagonist of a CXCR3 variant. In one aspect, the method involves a) contacting a CXCR3 variant polypeptide with a candidate compound in the presence of a
5 CXCR3 ligand, the polypeptide containing SEQ ID NO:11 or a conservative variant thereof and having at least 50% identity with SEQ ID NO:8 or 10, and b) identifying a compound that selectively inhibits production of a CXCR3 signal, the compound being characterized as an antagonist
10 of the CXCR3 variant. In one embodiment, the CXCR3 variant polypeptide contains an amino acid sequence selected from SEQ ID NO:2 or 4, or a conservative variant thereof. In another aspect, the method involves a) contacting a CXCR3C polypeptide with a candidate compound in the presence of a
15 CXCR3 ligand, the polypeptide comprising an amino acid sequence having at least 80% identity with SEQ ID NO:6, and b) identifying a compound that selectively inhibits production of a CXCR3 signal, the compound being characterized as an antagonist of the CXCR3. In
20 embodiments of the invention, the CXCR3 variant polypeptide can be isolated or expressed in a genetically engineered cell. In further embodiments of the invention, the CXCR3 signal can be, for example, calcium mobilization, cAMP upregulation, and ligand binding. A compound tested using
25 a method of the invention can be, for example, a polypeptide or small molecule.

The invention provides a methods for identifying a compound that specifically binds to a CXCR3 variant. In one aspect, the method involves a) contacting a CXCR3
30 variant polypeptide with a candidate compound, the

polypeptide containing SEQ ID NO:11 or a conservative variant thereof and having at least 50% identity with SEQ ID NO:8 or 10, and b) identifying a compound that specifically binds to the CXCR3 variant polypeptide. In one
5 embodiment, the CXCR3 variant polypeptide contains an amino acid sequence selected from SEQ ID NO:2 or 4, or a conservative variant thereof. In another aspect, the method involves a) contacting a CXCR3 polypeptide with a candidate compound, the polypeptide comprising an amino
10 acid sequence having at least 80% identity with SEQ ID NO:6, and b) identifying a compound that specifically binds to the CXCR3 variant polypeptide. In embodiments of the invention, the CXCR3 variant polypeptide can be isolated or expressed in a genetically engineered cell. A compound
15 tested using a method of the invention can be, for example, a polypeptide or small molecule.

Further provided by the invention is a method for identifying a compound that differentially modulates a
20 CXCR3 isoform. In one aspect, the method involves a) contacting a CXCR3 variant polypeptide with a candidate compound, the polypeptide containing SEQ ID NO:11 or a conservative variant thereof and having at least 50% identity with SEQ ID NO:8 or 10, and b) determining a CXCR3
25 signal for the CXCR3 variant polypeptide; c) contacting a distinct CXCR3 isoform polypeptide with the candidate compound; d) determining a CXCR3 signal for the distinct CXCR3 isoform polypeptide; and e) comparing the CXCR3 signals determined for the CXCR3 variant polypeptide and
30 the distinct CXCR3 isoform polypeptide, wherein a

difference between the CXCR3 signals indicates that the candidate compound is a compound that differentially modulates a CXCR3 isoform. In an embodiment, the distinct CXCR3 isoform polypeptide contains SEQ ID NO:11 or a conservative variant thereof. For example, a CXCR3 variant polypeptide can contain an amino acid sequence selected from SEQ ID NO:2 or 4, or a conservative variant thereof. In another aspect, the method involves a) contacting a CXCR3C polypeptide with a candidate compound, the polypeptide comprising an amino acid sequence having at least 80% identity with SEQ ID NO:6, and b) determining an a CXCR3 signal for the CXCR3C polypeptide; c) contacting a distinct CXCR3 isoform polypeptide with the candidate compound; d) determining a corresponding CXCR3 signal for the distinct CXCR3 isoform polypeptide; and e) comparing CXCR3 signals determined for the CXCR3C polypeptide and the distinct CXCR3 isoform polypeptide, wherein a difference between the signals indicates that the candidate compound is a compound that differentially modulates a CXCR3 isoform. In embodiments of the invention, the CXCR3 signal can be, for example, calcium mobilization, cAMP upregulation or IP3 release. A candidate compound used in the method can be, for example, a polypeptide or small molecule.

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The invention also provides a method for identifying a compound that differentially binds to a CXCR3 variant. In one aspect, the method involves a) contacting the CXCR3 variant polypeptide with a candidate compound, wherein the polypeptide contains SEQ ID NO:11 or a

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conservative variant thereof and having at least 50% identity with SEQ ID NO:8 or 10; b) determining specific binding of the candidate compound to the CXCR3 variant polypeptide; c) contacting a distinct CXCR3 isoform polypeptide with the candidate compound; d) determining specific binding of the candidate compound to the distinct CXCR3 isoform polypeptide, and e) comparing specific binding determined for the CXCR3 variant polypeptide with specific binding determined for the distinct CXCR3 isoform polypeptide, wherein a difference between the specific binding indicates that the candidate compound is a compound that differentially binds to a CXCR3 isoform. In an embodiment, the distinct CXCR3 isoform polypeptide contains SEQ ID NO:11 or a conservative variant. For example, a CXCR3 variant contains an amino acid sequence selected from SEQ ID NO:2 or 4, or a conservative variant thereof. In another aspect, the method involves a) contacting a CXCR3C polypeptide with a candidate compound, the polypeptide comprising an amino acid sequence having at least 80% identity with SEQ ID NO:6; b) determining specific binding of the candidate compound to the CXCR3C polypeptide; c) contacting a distinct CXCR3 isoform polypeptide with the candidate compound; d) determining specific binding of the candidate compound to the distinct CXCR3 isoform polypeptide, and e) comparing specific binding determined for the CXCR3C polypeptide with specific binding determined for the distinct CXCR3 isoform polypeptide, wherein a difference between the specific binding indicates that the candidate compound is a compound that differentially binds to a CXCR3 isoform.

In embodiments of the invention, the CXCR3 signal can be, for example, calcium mobilization, cAMP upregulation or IP3 release. A candidate compound used in the method can be, for example, a polypeptide or small molecule.

Also provided by the invention is an isolated CXCR3 variant nucleic acid molecule, containing a nucleotide sequence that encodes a polypeptide containing the amino acid sequence referenced as SEQ ID NO:11, the polypeptide having at least 50% identity with SEQ ID NO:8 or 10. The amino acid sequence of the CXCR3 variant polypeptide can contain, for example, SEQ ID NOS:2 or 4, or a conservative variant thereof. In one embodiment, the nucleotide sequence can be selected from SEQ ID NOS:1, 3, and 5. Further provided is an isolated CXCR3C nucleic acid molecule containing a nucleotide sequence that encodes a polypeptide that comprises an amino acid sequence having at least 80% identity with SEQ ID NO:6. In addition, the invention provides a vector containing the CXCR3 variant nucleic acid molecule, as well as a host cell containing the vector.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1A shows the nucleotide sequence of human CXCR3 variant CXCR3A-2 (SEQ ID NO:1). The underlined sequence indicates nucleotides that differ between the originally identified human CXCR3 receptor isoform, CXCR3A (SEQ ID NO:7), and newly identified CXCR3 variant CXCR3A-2 (SEQ ID NO:1). Figure 1B shows the amino acid sequence of

CXCR3A-2 (SEQ ID NO:2). Figure 1C shows a comparison of the amino acid sequences of human CXCR3A (SEQ ID NO:8) and CXCR3A-2 (SEQ ID NO:2), which indicates that CXCR3A-2 contains 256 amino acids while CXCR3A contains 368 amino acids, and that amino acids 210-256 of CXCR3A-2 differ from the corresponding amino acids in CXCR3A.

Figure 2A shows the nucleotide sequence of CXCR3 variant CXCR3B-2 (SEQ ID NO:3). The underlined sequence indicates nucleotides that differ between the CXCR3 receptor variant identified as CXCR3B (SEQ ID NO:9) and newly identified CXCR3 variant CXCR3B-2 (SEQ ID NO:3). Figure 2B shows the amino acid sequence of CXCR3B-2 (SEQ ID NO:4). Figure 2C shows a comparison of the amino acid sequences of CXCR3B and CXCR3B-2, which indicates that CXCR3A-2 contains 303 amino acids while CXCR3B contains 415 amino acids, and that amino acids 257-303 of CXCR3B-2 differ from the corresponding amino acids in CXCR3B.

Figure 3A shows the nucleotide sequence of CXCR3 variant CXCR3C (SEQ ID NO:5). Figure 3B shows the amino acid sequence of CXCR3C (SEQ ID NO:6). Figure 3C shows a comparison of the amino acid sequences of CXCR3A and CXCR3C, with the portions of CXCR3C that differ from CXCR3A shown in bold.

Figure 4 shows the intron/exon structure of human CXCR3A genomic DNA clone NM_001504, human CXCR3B genomic DNA clone AF429635, human CXCR3C genomic clone NT_011669.14 and newly identified CXCR3 variants CXCR3A-2, CXCR3B-2 and CXCR3C. Exons 1 and 2 of human CXCR3A and CXCR3B genomic

DNAs are shown, with the start codon indicated by an arrow. Alternatively spliced exons 2A and 2B of human CXCR3A-2 and CXCR3B-2 and alternatively spliced exons 3A and 3B of human CXCR3C are shown, with the start codon indicated by an arrow. Intron sequence is shown as a thin line, while exon sequence is shown as a thick line.

Figure 5A shows distribution of CXCR3 variant CXCR3A and CXCR3A-2 mRNA in various tissues using multiple tissue RT-PCR analysis. The location of PCR products of the correct size for CXCR3A and CXCR3A-2 are indicated by arrows. Figure 5B shows distribution of CXCR3 variant CXCR3B and CXCR3B-2 mRNA in various tissues using multiple tissue RT-PCR analysis. The location of PCR products of the correct size for CXCR3B and CXCR3B-2 are indicated by arrows.

Figure 6A shows a hydropathic profile of the amino acid sequence of human CXCR3A-2. Figure 6B shows a hydropathic profile of the amino acid sequence of human CXCR3B-2

DETAILED DESCRIPTION OF THE INVENTION

The present invention is directed to the discovery of novel CXC-Chemokine receptor R3 (CXCR3R) variants. The CXCR3 variants can be used to determine and refine the specificity of binding of compounds that bind to the originally identified isoforms of CXCR3. The variants also can be used to identify compounds that differentially modulate or bind to a specific CXCR3 isoform. Such a

compound can be, for example, a ligand that specifically binds to a novel CXCR3 variant disclosed herein.

As disclosed herein in Example I, several novel CXCR3 variants have been identified using the reverse transcription polymerase chain reaction (RT-PCR). In particular, three novel alternatively spliced human CXCR3 variants, referred to herein as CXCR3A-2, CXCR3B-2 and CXCR3C, were identified as distinct from the originally identified CXCR3 isoforms CXCR3A and CXCR3B (see Figures 1 to 4).

As further disclosed herein, sequence analysis of nucleic acid molecules encoding the newly identified CXCR3 variant isoforms revealed novel carboxy-terminal amino acid sequences. The intron/exon structures of the originally identified CXCR3A and the alternatively spliced CXCR3A-2; the intron/exon structures of the originally identified CXCR3B and the alternatively spliced CXCR3B-2, and the intron/exon structures of alternatively spliced CXCR3C are shown in Figure 4. As is shown, exon 1 is conserved between originally identified CXCR3A and CXCR3B and the CXCR3 variant isoforms CXCR3A-2, CXCR3B-2 and CXCR3C. Also shown is that CXCR3A-2 and CXCR3B-2 contain two alternatively spliced exons (exons 2A and 2B) not present in the originally identified CXCR3 isoforms, and that CXCR3C contain two alternatively spliced exons (exons 3A and 3B) not present in the originally identified CXCR3 isoforms.

Comparison of originally identified CXCR3A amino acid sequence (SEQ ID NO:8) to CXCR3 variant isoform CXCR3A-2 (SEQ ID NO:2) revealed that CXCR3A-2 contains 209 amino acids at the N-terminus derived from conserved exon 1 and exon 2A, while the C-terminal 47 amino acids are derived from exon 2B. As is shown in Figure 4, exons 2A and 2B of CXCR3A-2 correspond to alternatively spliced portions of exon 2 from originally identified CXCR3A that contains a 335 bp insert. The unique C-terminal 47 amino acids of CXCR3A-2 not present in CXCR3A are

GSSSGSGCGCCSCAWAAPTREGSRGSHRLPAGIHPGLRPQRPPTAC (SEQ ID NO:11). Similarly, comparison of originally identified CXCR3B amino acid sequence (SEQ ID NO:10) to the CXCR3 variant isoform CXCR3B-2 (SEQ ID NO:4) revealed that

CXCR3B-2 contains 256 amino acids at the N-terminus derived from conserved exon 1 and exon 2A while the C-terminal 47 amino acids are derived from exon 2B. As is shown in Figure 4, exons 2A and 2B of CXCR3B-2 correspond to a variant of exon 2 from originally identified CXCR3B that contains a 335 bp insert. The unique C-terminal 47 amino acids of CXCR3B-2 not present in CXCR3B are

GSSSGSGCGCCSCAWAAPTREGSRGSHRLPAGIHPGLRPQRPPTAC (SEQ ID NO:11).

As is disclosed in Example III, expression of CXCR3 variant mRNAs for CXCR3A-2 and CXCR3B-2 was observed in a variety of tissues including liver, kidney, brain, small intestine, spleen, lung, skeletal muscle, and heart (see Figures 5A and 5B).

As is further disclosed herein, a new CXCR3 isoform distinct from previously identified CXCR3A and CXCR3B has now been discovered by the inventors and termed "CXCR3C." CXCR3C (SEQ ID NO:6) contains an N-terminal region identical to CXCR3A within amino acids 1 to 93 and a C-terminal region different from CXCR3A within amino acids 94 to 280 (see Figure 3C). As is shown in Figure 4, exons 3A and 3B of CXCR3C are separated by a 259 bp insert. The portion of CXCR3C that differs from the previously identified CXCR3A isoform therefore corresponds to

FALPDFIFLSAHHDERLNATHCQYNFPQVGRTALRVLQLVAGFLLPLLVA
YCYAHILAVLLVSRGQRRLRAMRLVVVVVAFALCWTPYHLVVLVDILMDLGALARNCG
RESRVDVAKSVTSGLGVMHCCLNPLLYAFVGVKFRERMWMLLLRLGCPNQRLQRPSS
SRDSSWSETSEASYSGL (SEQ ID NO:12).

Based on the discoveries described above, the present invention provides CXCR3 variant polypeptides CXCR3A-2 (SEQ ID NO:2) and CXCR3B-2 (SEQ ID NO:4) that arise from alternative splicing; encoding nucleic acid molecules; CXCR3 variant binding agents; and screening methods in which the CXCR3 variant polypeptides are employed. In particular, the invention provides an isolated polypeptide containing SEQ ID NO:11, which is the amino acid sequence corresponding to the unique carboxy-terminal portions of newly identified CXCR3 variants CXCR3A-2 and CXCR3B-2, and having at least 50% amino acid identity with SEQ ID NO:8 or 10, which are the originally identified human CXCR3A and CXCR3B amino acid sequences. Also provided herein is an isolated polypeptide containing the amino acid sequence of SEQ ID NO:2 (CXCR3A-2), 4 (CXCR3B-2) or 6 (CXCR3C), or a conservative variant thereof.

The invention further provides an isolated polypeptide consisting of the amino acid sequence of SEQ ID NO:2(CXCR3A-2), 4 (CXCR3B-2) or 6 (CXCR3C).

CXCR3 is a G-protein coupled receptor (GPCR) with 5 selectivity for three chemokines, termed IP10 (interferon gamma-inducible 10 kDa protein), Mig (monokine induced by interferon gamma), and I-TAC (interferon-inducible T cell a-chemoattractant). IP10, Mig and I-TAC belong to the structural family of CXC chemokines, in which a single 10 amino acid residue separates the first two of four conserved cysteine residues. CXCR3 has also been referred to as the IP-10 receptor, Mig receptor, CKR-L2, GPR-9 and CD183. See Murphy et al. Pharmacol Rev. 52:145-176 (2000) for a more complete discussion of CXCR3 nomenclature. The 15 human CXCR3 cDNA encodes a 368 amino acid polypeptide. Expression of CXCR3 polypeptide has been observed in T cells (Loetscher et al. Eur. J. Immunol. 28:3696-3705 (1998)), and minor subsets of B and NK cells (Qin et al. J Clin. Invest. 101:746-754 (1998)). Although a major role 20 of ligands for CXCR3 is recruitment of activated T cells (Rollins, Blood 90:909-928 (1997)), IP-10 has also exhibited monocyte chemoattraction (Taub et al. J. Exp. Med. 177:1809-1814 (1993)). In addition, IP-10 is involved in a variety of cellular activities, including vascular 25 smooth muscle cell migration and proliferation (Wang et al. J. Biol. Chem. 271:24286-24293 (1996)) and astrocyte chemoattraction (Wang et al. J. Neurochem 71:1194-1204 (1998)).

CXCR3 variant polypeptides

The invention provides isolated CXCR3 variant polypeptides. In one embodiment, the CXCR3 variant polypeptide contains the amino acid sequence referenced as
5 SEQ ID NO:11, the polypeptide having at least 50% identity with SEQ ID NO:8 or 10. As used herein, the terms "CXCR3 variant" and "CXCR3 variant polypeptide" means a polypeptide containing an amino acid sequence that has at least 30% amino acid identity with the corresponding
10 originally identified (wild-type) CXCR3 receptor and further containing amino acid sequence SEQ ID NO:11 or SEQ ID NO:12, or a conservative variant thereof. For example, a CXCR3A variant means a polypeptide containing an amino acid sequence that has at least 30%
15 amino acid identity with human CXCR3A SEQ ID NO:8, such as SEQ ID NO:2; a CXCR3B variant means a polypeptide containing an amino acid sequence that has at least 30% amino acid identity with human CXCR3B SEQ ID NO:10, such as SEQ ID NO:6. A CXCR3 variant can contain an amino acid
20 sequence having, for example, at least 30% amino acid identity, at least 40% amino acid identity, at least 50% amino acid identity, at least 60% amino acid identity, at least 70% amino acid identity, at least 80% amino acid identity, at least 90% amino acid identity, at least 95%
25 amino acid identity, or at least 99% amino acid identity with the corresponding originally identified CXCR3 receptor. As non-limiting examples, a CXCR3A variant can contain an amino acid sequence having at least 50% amino acid identity with SEQ ID NO:8 and containing the amino
30 acid sequence SEQ ID NO:11, or a conservative variant

thereof, such as SEQ ID NO:2; and a CXCR3B variant can contain an amino acid sequence having at least 50% amino acid identity with SEQ ID NO:10 and containing the amino acid sequence SEQ ID NO:11, or a conservative variant
5 thereof, such as SEQ ID NO:4. Another type of CXCR3 variant is a CXCR3C that contains an amino acid sequence having at least 50% amino acid identity with SEQ ID NO:6 and containing the amino acid sequence SEQ ID NO:12, or a conservative variant thereof. Based on the above, it is
10 understood that species orthologs of CXCR3 variants that contain amino acid sequence SEQ ID NO:11 or SEQ ID NO:12, or a conservative variant thereof, are encompassed by the definition of a CXCR3 variant.

The invention also provides an isolated CXCR3C
15 polypeptide containing the amino acid sequence containing an amino acid sequence having at least 80% identity with SEQ ID NO:6. The amino acid sequence of a CXCR3C polypeptide can have at least 85% identity with SEQ ID NO:6, at least 90% identity, at least 95 % identity, at
20 least 98% identity or at least 99% identity with SEQ ID NO:6. The amino acids of a CXCR3C polypeptide that differ from SEQ ID NO:6 can be, for example, one or more conservative substitutions.

As used herein in reference to a specified amino
25 acid sequence such as SEQ ID NO:6, SEQ ID NO:11 or SEQ ID NO:12, the term "conservative variant" or "conservative substitution" means a sequence in which a selected amino acid is replaced by another amino acid or amino acid analog having at least one biochemical property similar to that of

the selected amino acid; similar properties include, yet are not limited to, similar size, charge, hydrophobicity or hydrogen-bonding capacity.

A CXCR3 variant of the invention differs from the 5 originally identified (wild-type) human CXCR3 polypeptides, CXCR3A and CXCR3B, because it contains amino acid sequence of SEQ ID NO:11, or a conservative variant of SEQ ID NO:11, or amino acid sequence SEQ ID NO:12, or a conservative variant of SEQ ID NO:12. As an example, a conservative 10 variant can be a sequence containing one or more conservative substitutions, such as a sequence in which a first uncharged polar amino acid is conservatively substituted with a second (non-identical) uncharged polar amino acid such as cysteine, serine, threonine, tyrosine, 15 glycine, glutamine or asparagine or an analog thereof. A conservative variant also can be a sequence in which a first basic amino acid is conservatively substituted with a second basic amino acid such as arginine, lysine, histidine, 5-hydroxylysine, N-methyllysine or an analog 20 thereof. Similarly, a conservative variant can be a sequence in which a first hydrophobic amino acid is conservatively substituted with a second hydrophobic amino acid such as alanine, valine, leucine, isoleucine, proline, methionine, phenylalanine or tryptophan or an analog 25 thereof. In the same way, a conservative variant can be a sequence in which a first acidic amino acid is conservatively substituted with a second acidic amino acid such as aspartic acid or glutamic acid or an analog thereof; a sequence in which an aromatic amino acid such as 30 phenylalanine is conservatively substituted with a second

aromatic amino acid or amino acid analog, for example, tyrosine; or a sequence in which a first relatively small amino acid such as alanine is substituted with a second relatively small amino acid or amino acid analog such as
5 glycine or valine or an analog thereof. It is understood that a conservative variant of SEQ ID NO:11, 2, 4, 6 or X can have one, two, three, four, five, six, ten, or more amino acid substitutions relative to the specified sequence and that such a conservative variant can include naturally
10 and non-naturally occurring amino acid analogs.

In addition, a CXCR3 variant of the invention can have a minor modification. Such a minor modification can be a chemical or enzymatic modification to the polypeptide, such as replacement of hydrogen by an alkyl, acyl, or amino
15 group; esterification of a carboxyl group with a suitable alkyl or aryl moiety; alkylation of a hydroxyl group to form an ether derivative; phosphorylation or dephosphorylation of a serine, threonine or tyrosine residue; or N - or O-linked glycosylation.

20 It is understood that minor modifications in primary amino acid sequence can result in a polypeptide that has a substantially equivalent function as compared to a polypeptide of the invention. These modifications can be deliberate, as through site-directed mutagenesis, or may be
25 accidental such as through spontaneous mutation. For example, it is understood that only a portion of the entire primary structure of CXCR3 variant can be required in order to bind to a ligand such as CXCL10, Mig-1 or I-TAC.

Those skilled in the art can determine whether minor modifications to a CXCR3 variant sequence are advantageous. Such modifications can be made, for example, to enhance the stability, bioavailability or bioactivity of the CXCR3 variant. A modified CXCR3 variant can be prepared, for example, by recombinant methods, by synthetic methods, by post-synthesis chemical or enzymatic methods, or by a combination of these methods, and tested for ability to bind to a CXCR3 ligand or signal through a G-protein coupled signal transduction pathway.

Those skilled in the art also can determine regions in a CXCR3 variant amino acid sequence that can be modified without abolishing ligand binding or signaling through a G-protein coupled signal transduction pathway. Structural and sequence information can be used to determine the amino acid residues important for CXCR3 variant activity. For example, comparisons of amino acid sequences of CXCR3 variant sequences from different species can provide guidance in determining amino acid residues that can be altered without abolishing activity.

Further, a large number of published GPCR structure-function studies have indicated regions of GPCRs involved in ligand interaction, G-protein coupling and in forming transmembrane regions, and indicate regions of GPCRs tolerant to modification (see, for example, Burstein et al., J. Biol. Chem., 273(38):24322-7 (1998) and Burstein et al., Biochemistry, 37(12):4052-8 (1998)). In addition, computer programs known in the art can be used to determine which amino acid residues of a GPCR, such as a CXCR3

variant, can be modified as described above without abolishing activity (see, for example, Eroshkin et al., Comput. Appl. Biosci. 9:491-497 (1993)).

The invention further provides functional
5 fragments of a CXCR3 variant containing the amino acid sequence SEQ ID NO:11 or SEQ ID NO:12. A functional fragment of a CXCR3 variant can be, for example, a ligand-binding fragment or a fragment of a CXCR3 variant that is involved in signal transduction. Such a functional
10 fragment can be useful in a method of the invention in place of the corresponding full-length CXCR3 variant. As non-limiting examples, a functional fragment can include the seven transmembrane helices and can further include, for example, the second extracellular loop or the carboxy
15 terminus (C-terminal 156 residues), as described in Stillman et al., Eur. J. Pharm. 357:73-82 (1998), and Bastepe and Ashby, Mol. Pharm. 51:343-349 (1997).

As further understood by one skilled in the art, a CXCR3 variant can optionally include a heterologous amino
20 acid sequence. A heterologous amino acid sequence is derived from a nucleotide sequence other than a gene encoding a CXCR3 or CXCR3 variant. Non-limiting examples of heterologous amino acid sequences that can be fused to a polypeptide of the invention include purification and
25 detection tags, such as GST, Myc tags, Flag tags, polyhistidine tags and the like.

Further provided herein is an isolated polypeptide containing or consisting of substantially the

same amino acid sequence as SEQ ID NO:2, 4 or 6. As used herein, the term "isolated" indicates that the molecule is altered by the hand of man from how it is found in its natural environment. An "isolated" CXCR3 variant
5 polypeptide can be a "substantially purified" molecule, that is at least 60%, 70%, 80%, 90 or 95% free from cellular components with which it is naturally associated. An isolated polypeptide can be in any form, such as in a buffered solution, a suspension, a lyophilized powder,
10 recombinantly expressed in a heterologous cell, bound to a receptor or attached to a solid support.

Such an isolated polypeptide can contain or consist of a polypeptide having a similar, non-identical sequence that is considered by those skilled in the art to
15 be a functionally equivalent amino acid sequence. An amino acid sequence that is substantially the same as a reference amino acid sequence can have at least 70%, at least 80%, at least 90%, or at least 95% or more identity to the reference sequence. The term substantially the same amino
20 acid sequence also includes sequences encompassing, for example, modified forms of naturally occurring amino acids such as D-stereoisomers, non-naturally occurring amino acids, amino acid analogs and mimetics, so long as the polypeptide containing such a sequence retains a functional
25 activity of the reference CXCR3 variant. A functional activity of a CXCR3 variant of the invention can be, for example, the ability to bind to CXCL10, Mig or I-TAC, or the ability to initiate a particular intracellular signal transduction pathway, such as modulating calcium
30 mobilization or cAMP regulation.

CXCR3 variant binding agents

The invention further provides a CXCR3 variant binding agent that binds a polypeptide of the invention, such as amino acid sequence SEQ ID NO:11 or SEQ ID NO:12, 5 or an epitope thereof. As discussed above, SEQ ID NO:11 represents the unique amino acid sequence of alternatively spliced CXCR3 variants CXCR3A-2 and CXCR3B-2 disclosed herein, with respect to originally identified CXCR3 polypeptide isoforms, while SEQ ID NO:12 represents the 10 unique amino acid sequence of alternatively spliced variant CXCR3C disclosed herein, with respect to the originally identified CXCR3A isoform. A CXCR3 variant binding agent of the invention can be, without limitation, an antibody or antigen binding fragment thereof that binds selectively to 15 amino acid SEQ ID NO:11 or SEQ ID NO:12, or an epitope thereof.

As used herein, the term "CXCR3 variant binding agent" means a molecule that specifically binds the unique sequence of a CXCR3 variant amino acid sequence disclosed 20 herein without substantial cross-reactivity with other polypeptides. A binding agent of the invention can be, for example, a simple or complex organic molecule, carbohydrate, peptide, peptidomimetic, protein, glycoprotein, lipoprotein, lipid, nucleic acid molecule, 25 antibody, aptamer or the like. In one embodiment, the CXCR3 variant binding agent specifically binds to amino acid sequence SEQ ID NO:11 or SEQ ID NO:12, or an epitope thereof.

The affinity of a CXCR3 variant binding agent of the invention generally is greater than about 10^{-4} M and can be greater than about 10^{-6} M, typically being in the range of 10^{-4} M to 10^{-10} M. A CXCR3 variant binding agent of the
5 invention can bind, for example, with high affinity such as an affinity of 10^{-7} M to 10^{-10} M. Specific examples of binding agents of the invention include, but are not limited to, polyclonal and monoclonal antibodies that specifically bind an epitope of SEQ ID NO:11 or SEQ ID
10 NO:12; and nucleic acid molecules, nucleic acid analogs, and small organic molecules, identified, for example, by affinity screening of a nucleic acid or small molecule library against SEQ ID NO:11 or SEQ ID NO:12. For certain applications, a CXCR3 variant binding agent can
15 preferentially recognize a particular conformational or post-translationally modified state of SEQ ID NO:11 or SEQ ID NO:12. It is understood that a CXCR3 variant binding agent of the invention can be labeled with a detectable moiety, if desired, or rendered detectable by specific
20 binding to a detectable secondary agent.

In one embodiment, a CXCR3 variant binding agent of the invention is an antibody or antigen-binding fragment thereof. As used herein, the term "antibody" is used in
25 its broadest sense to mean a polyclonal or monoclonal antibody or an antigen binding fragment of such an antibody. Such an antibody of the invention is characterized by having specific binding activity for SEQ ID NO:11 or SEQ ID NO:12, or an epitope thereof, of at
30 least about 1×10^5 M⁻¹. Thus, Fab, F(ab')₂, Fd and Fv

fragments of an antibody, which retain specific binding activity for SEQ ID NO:11 or SEQ ID NO:12, or an epitope thereof, are included within the definition of antibody as used herein. Specific binding activity can be readily
5 determined by one skilled in the art, for example, by comparing the binding activity of the antibody to SEQ ID NO:11, versus a control sequence. Methods of preparing polyclonal or monoclonal antibodies are well known to those skilled in the art. See, for example, Harlow and Lane,
10 Antibodies: A Laboratory Manual Cold Spring Harbor Laboratory Press (1988).

It is understood that the term antibody includes naturally occurring antibodies as well as non-naturally occurring antibodies such as, without limitation, single
15 chain antibodies, chimeric, bi-functional and humanized antibodies, and antigen-binding fragments thereof. Such non-naturally occurring antibodies can be constructed using solid phase peptide synthesis, produced recombinantly or obtained, for example, by screening combinatorial libraries
20 consisting of variable heavy chains and variable light chains as described in Huse et al., Science 246:1275-1281 (1989). These and other methods of making, for example, chimeric, humanized, CDR-grafted, single chain, and bi-functional antibodies are well known to those skilled in
25 the art (Winter and Harris, Immunol. Today 14:243-246 (1993); Ward et al., Nature 341:544-546 (1989); Harlow and Lane, *supra*, 1988; Hilyard et al., Protein Engineering: A practical approach (IRL Press 1992); and Borrabeck, Antibody Engineering 2d ed. (Oxford University Press
30 1995)).

An antibody of the invention can be prepared using as an antigen a polypeptide or peptide containing SEQ ID NO:11 or SEQ ID NO:12, or an epitope thereof, which can be prepared, for example, from natural sources, 5 produced recombinantly, or chemically synthesized. Such a polypeptide or peptide is a functional antigen if the polypeptide or peptide can be used to generate an antibody that specifically binds SEQ ID NO:11 or SEQ ID NO:12, or an epitope thereof. As is well known in the art, a 10 non-antigenic or weakly antigenic polypeptide or peptide can be made antigenic by coupling the polypeptide or peptide to a carrier molecule such as bovine serum albumin (BSA) or keyhole limpet hemocyanin (KLH). Various other carrier molecules and methods for coupling a polypeptide or 15 peptide to a carrier molecule are well known in the art (see, for example, Harlow and Lane, *supra*, 1988). An antigenic polypeptide or peptide can also be generated by expressing the polypeptide or peptide as a fusion protein, for example, fused to glutathione S transferase, polyHis or 20 the like. Methods for expressing polypeptide fusions are well known to those skilled in the art as described, for example, in Ausubel et al., Current Protocols in Molecular Biology (Supplement 47), John Wiley & Sons, New York (1999).

25 Screening methods

The invention provides methods for identifying compounds that modulate a CXCR3 variant. Compounds so identified can be used, for example, to modulate a CXCR3 variant in an individual for therapeutic benefit. A

compound identified to modulate a particular CXCR3 variant can also be used to modulate an alternative CXCR3 isoform, such as a different CXCR3 variant or originally identified (wild-type) CXCR3. Using screening assays such as those
5 described below, a compound identified to modulate a particular CXCR3 variant can be tested for its ability to modulate a corresponding wild-type CXCR3 or related CXCR3 variant, if desired.

A compound that modulates a CXCR3 variant has the
10 ability to alter a characteristic of the CXCR3 variant. A characteristic of a CXCR3 variant that can be altered can be, without limitation, an activity or physical conformation of the CXCR3 variant. As a non-limiting example, a compound that modulates a CXCR3 variant can
15 increase or decrease the binding of the CXCR3 variant to a ligand such as Mig, IP-10 or I-TAC, or can increase or decrease the binding of the CXCR3 variant to an intracellular molecule that transduces a signaling pathway within a cell. It is understood that compounds that
20 modulate a CXCR3 variant include compounds that specifically bind to the CXCR3 variant as well as compounds that do not specifically bind to the CXCR3 variant. Exemplary types of compounds that modulate a CXCR3 variant include agonists and antagonists.

25 Accordingly, the invention provides a method for identifying an agonist of a CXCR3 variant. The method involves a) contacting a CXCR3 variant with a candidate compound, the CXCR3 variant containing SEQ ID NO:11 or a conservative variant thereof, and having at least 50%

identity with SEQ ID NO:8 or 10 , and b) identifying a compound that selectively promotes production of a CXCR3 signal, the compound being characterized as an agonist of the CXCR3 variant. In one embodiment, the CXCR3 variant
5 polypeptide contains an amino acid sequence selected from SEQ ID NO:2 or 4, or a conservative variant thereof. In embodiments of the invention, the CXCR3 variant polypeptide can be isolated or expressed in a genetically engineered cell. In further embodiments of the invention, the CXCR3
10 signal can be calcium mobilization, cAMP upregulation, and ligand binding. A compound tested using a method of the invention can be, for example, a polypeptide or small molecule.

The invention also provides a method for
15 identifying an antagonist of a CXCR3 variant. The method involves a) contacting a CXCR3 variant with a candidate compound in the presence of a CXCR3 ligand, the CXCR3 variant containing SEQ ID NO:11 or a conservative variant thereof, and having at least 50% identity with SEQ ID NO:8
20 or 10, and b) identifying a compound that selectively inhibits production of a CXCR3 signal, the compound being characterized as an antagonist of the CXCR3 variant. In one embodiment, the CXCR3 variant polypeptide contains an amino acid sequence selected from SEQ ID NO:2 or 4, or a
25 conservative variant thereof. In embodiments of the invention, the CXCR3 variant polypeptide can be isolated or expressed in a genetically engineered cell. In further embodiments of the invention, the CXCR3 signal can be calcium mobilization, cAMP upregulation, and IP3 release.

A compound tested using a method of the invention can be, for example, a polypeptide or small molecule.

As used herein, the terms "agonist of a CXCR3 variant" and "CXCR3 variant agonist" mean a compound that
5 selectively promotes or enhances normal signal transduction through a CXCR3 variant. A CXCR3 variant agonist can act by any agonistic mechanism, such as by binding a CXCR3 variant at the normal ligand binding site, thereby promoting CXCR3 variant signaling. A CXCR3 variant agonist
10 can also act, for example, by potentiating the binding activity of a ligand, such as Mig, IP-10 and I-TAC.

As used herein, the terms "antagonist of a CXCR3 variant" and "CXCR3 variant antagonist" mean a compound
15 that selectively inhibits or decreases normal signal transduction through a CXCR3 variant. A CXCR3 variant antagonist can act by any antagonistic mechanism, such as by binding a CXCR3 variant or CXCR3 ligand, thereby inhibiting binding between CXCR3 variant and the ligand. A
20 CXCR3 variant antagonist can also inhibit binding between a specific or non-specific CXCR3 variant agonist and CXCR3 variant. A CXCR3 variant antagonist can also act, for example, by inhibiting the binding activity of a CXCR3 ligand to the CXCR3 variant, or signaling activity of CXCR3
25 variant. For example, a CXCR3 variant antagonist can act by altering the state of phosphorylation or glycosylation of a CXCR3 variant. A CXCR3 variant antagonist can also be an inverse agonist, which decreases CXCR3 variant signaling from a baseline amount of constitutive CXCR3 variant
30 signaling activity.

It is understood that a CXCR3 variant agonist or antagonist identified using a method of the invention can also function as an agonist or antagonist of a CXCR3
5 isoform other than the one selected for use in the screening method. For example, a CXCR3 variant agonist or antagonist can function as an agonist or antagonist of an originally identified CXCR3, such as human CXCR3A or CXCR3B, or a different CXCR3 variant.

10

A screening assay used in a method of the invention for identifying a CXCR3 variant agonist or antagonist can involve detecting a CXCR3 signal produced by a CXCR3 variant. As used herein, the term "CXCR3 signal"
15 is intended to mean a readout, detectable by any analytical means, that is a qualitative or quantitative indication of activation of G-protein-dependent signal transduction through a CXCR3 or CXCR3 variant. Assays used to determine such qualitative or quantitative activation of G-protein-
20 dependent signal transduction through the CXCR3 or CXCR3 variant, are referred to below as "signaling assays." G-proteins, or heterotrimeric GTP binding proteins, are signal transducing polypeptides having subunits designated $G\alpha$, $G\beta$ and $G\gamma$, that couple to seven-transmembrane cell
25 surface receptors. G-proteins couple to such receptors to transduce a variety of extracellular stimuli, including light, neurotransmitters, hormones and odorants to various intracellular effector proteins. G-proteins are present in
30 mammals, other vertebrates, flies and yeast.

A signaling assay can be performed to determine whether a candidate compound is a CXCR3 variant agonist or antagonist. In such an assay, a CXCR3 variant, such as CXCR3A-2 or CXCR3B-2, is contacted with one or more candidate compounds under conditions wherein the CXCR3 variant produces a CXCR3 signal in response to a CXCR3 variant agonist, such as Mig, IP-10 or I-TAC. In response to CXCR3 variant activation, a CXCR3 signal can increase or a decrease from an unstimulated CXCR3 variant baseline signal. A CXCR3 signal is an increasing signal, for example, when the amount of detected second messenger molecule is increased in response to CXCR3 variant activation. A CXCR3 signal is a decreasing signal, for example, when the detected second messenger molecule is destroyed, for example, by hydrolysis, in response to CXCR3 variant activation. Accordingly, a CXCR3 variant signaling assay can be used to identify a CXCR3 variant agonist that promotes production of a CXCR3 signal, whether the agonist promotes an increase in a CXCR3 signal that positively correlates with CXCR3 variant activity, or a decrease in a CXCR3 signal that negatively correlates with CXCR3 variant activity. Similarly, a signaling assay can be performed to determine whether a candidate compound is a CXCR3 variant antagonist. In such a signaling assay, a CXCR3 variant is contacted with one or more candidate compounds under conditions wherein the CXCR3 variant produces a CXCR3 signal in response to a CXCR3 variant agonist, such as Mig, IP-10 or I-TAC, and a compound is identified that reduces production of the CXCR3 signal.

Signaling through G proteins can lead to increased or decreased production or liberation of second messengers, including, for example, arachidonic acid, acetylcholine, diacylglycerol, cGMP, cAMP, inositol phosphate, such as inositol-1,4,5-trisphosphate, and ions, including Ca^{++} ions; altered cell membrane potential; GTP hydrolysis; influx or efflux of amino acids; increased or decreased phosphorylation of intracellular proteins; or activation of transcription. The specificity of a G-protein for cell-surface receptors is determined by the C-terminal five amino acids of the $\text{G}\alpha$ subunit. The nucleotide sequences and signal transduction pathways of different classes and subclasses of $\text{G}\alpha$ subunits in a variety of eukaryotic and prokaryotic organisms are well known in the art. Thus, any convenient G-protein mediated signal transduction pathway can be assayed by preparing a chimeric $\text{G}\alpha$ containing the C-terminal residues of a $\text{G}\alpha$ that couples to an isoform of CXCR3, such as $\text{G}\alpha_q$, with the remainder of the protein corresponding to a $\text{G}\alpha$ that couples to the signal transduction pathway it is desired to assay. Methods of recombinantly expressing chimeric $\text{G}\alpha$ proteins are known in the art and are described, for example, in Conklin et al., Nature 363:274-276 (1993), Komatsuzaki et al., FEBS Letters 406:165-170 (1995), and Saito et al., Nature 400:265-269 (1999). Additionally, chimeric $\text{G}\alpha$ proteins can be prepared by synthetic methods.

Various assays, including high throughput automated screening assays, to identify alterations in G-protein coupled signal transduction pathways are well known in the art. Various screening assay that measure Ca^{++} ,

cAMP, voltage changes and gene expression are reviewed, for example, in Gonzalez et al., Curr. Opin. Biotech. 9:624-631 (1998); Jayawickreme et al., Curr. Opin. Biotech. 8:629-634 (1997); and Coward et al., Anal. Biochem. 270:2424-248
5 (1999). Yeast cell-based bioassays for high-throughput screening of drug targets for G-protein coupled receptors are described, for example, in Pausch, Trends in Biotech. 15:487-494 (1997). A variety of cell-based expression systems, including bacterial, yeast, baculovirus/insect
10 systems and mammalian cells, useful for detecting G-protein coupled receptor agonists and antagonists are reviewed, for example, in Tate et al., Trends in Biotech. 14:426-430 (1996).

Assays to detect and measure G-protein-coupled
15 signal transduction can involve first contacting a sample containing a CXCR3 variant, such as an isolated cell, membrane or artificial membrane, such as a liposome or micelle, with a detectable indicator. A detectable indicator can be any molecule that exhibits a detectable
20 difference in a physical or chemical property in the presence of the substance being measured, such as a color change. Calcium indicators, pH indicators, and metal ion indicators, and assays for using these indicators to detect and measure selected signal transduction pathways are
25 described, for example, in Haugland, Molecular Probes Handbook of Fluorescent Probes and Research Chemicals, Sets 20-23 and 25 (1992-94). For example, calcium indicators and their use are well known in the art, and include compounds like Fluo-3 AM, Fura-2, Indo-1, FURA RED, CALCIUM
30 GREEN, CALCIUM ORANGE, CALCIUM CRIMSON, BTC, OREGON GREEN

BAPTA, which are available from Molecular Probes, Inc., Eugene OR, and described, for example, in U.S. Patent Nos. 5,453,517, 5,501,980 and 4,849,362.

Another type of signaling assay involves
5 determining changes in gene expression in response to a CXCR3 variant agonist or antagonist. A variety of signal transduction pathways contribute to the regulation of transcription in animal cells by stimulating the interaction of transcription factors with genetic sequences
10 termed response elements in the promoter regions of responsive genes. Assays for determining the interaction of transcription factors with promoter regions to stimulate gene expression are well known to those skilled in the art and are commercially available.

15 An exemplary assay that measures activation of a CXCR3 signal transduction pathway is based on melanophores, which are skin cells that provide pigmentation to an organism (Lerner, Trends Neurosci. 17:142-146 (1994)). In numerous animals, including fish, lizards and amphibians,
20 melanophores are used, for example, for camouflage. The color of the melanophore is dependent on the intracellular position of melanin-containing organelles, termed melanosomes. Melanosomes move along a microtubule network and are clustered to give a light color or dispersed to
25 give a dark color. The distribution of melanosomes is regulated by G protein coupled receptors and cellular signaling events, where increased concentrations of second messengers such as cyclic AMP and diacylglycerol result in melanosome dispersion and darkening of melanophores.

Conversely, decreased concentrations of cyclic AMP and diacylglycerol result in melanosome aggregation and lightening of melanophores.

A melanophore-based assay can be advantageously
5 used to identify a compound that modulates or specifically binds to a CXCR3 variant, due to the regulation of melanosome distribution by a CXCR3 variant-stimulated intracellular signaling. For example, a CXCR3 variant can be over-expressed in genetically engineered melanophore
10 cells, for example, frog melanophore cells. Compounds that modulate or specifically bind to the CXCR3 variant can stimulate or inhibit G protein coupled receptor signaling. Both stimulation or inhibition of signaling can be determined since the system can be used to detect both
15 aggregation of melanosomes and lightening of cells, and dispersion of melanosomes and darkening of cells. Thus, the color of the cells, determined by the level of melanin in the cells, is an indicator that can be used to identify a compound that modulates or specifically binds to a CXCR3
20 variant in a method of the invention.

A binding assay can be performed to identify compounds that are CXCR3 variant agonists or antagonists. In such an assay, the CXCR3 variant can be contacted with one or more candidate compounds under conditions in which
25 ligand binds to the selected receptor and a compound that binds to the selected receptor or that reduces binding of an agonist to selected receptor can be identified. Contemplated binding assays can involve detectably labeling a candidate compound, or competing an unlabeled candidate

compound with a detectably labeled CXCR3 ligand, such as Mig, IP-10 and I-TAC. A detectable label can be, for example, a radioisotope, fluorochrome, ferromagnetic substance, or luminescent substance. Exemplary radiolabels
5 useful for labeling compounds include ^{125}I , ^{14}C and ^3H . Methods of detectably labeling organic molecules, either by incorporating labeled amino acids into the compound during synthesis, or by derivatizing the compound after synthesis, are known in the art.

10 In order to determine whether a candidate compound decreases binding of detectably labeled ligand to a CXCR3 variant, the amount of binding of a given amount of the detectably labeled ligand is determined in the absence of the candidate compound. Generally the amount of
15 detectably labeled ligand will be less than its K_d , for example, 1/10 of its K_d . Under the same conditions, the amount of binding of the detectably labeled ligand in the presence of the candidate compound is determined. A decrease in binding due to a candidate compound
20 characterized as a CXCR3 variant ligand is evidenced by at least 2-fold less, such as at least 10-fold to at least 100-fold less, such as at least 1000-fold less, binding of detectably labeled ligand to CXCR3 variant in the presence of the candidate compound than in the absence of the
25 candidate compound. An exemplary assay for determining binding of detectably labeled ligand to a GPCR is the radioligand filter binding assay described in Clark-Lewis et al. J Biol Chem. 278(1):289-95 (2003), which is incorporated herein by reference

Additional assays suitable for determining specific binding of a compound to a CXCR3 variant in a screening method of the invention include, without limitation, UV and chemical cross-linking assays (Fancy, 5 Curr. Opin. Chem. Biol. 4:28-33 (2000)) and biomolecular interaction analyses (Weinberger et al., Pharmacogenomics 1:395-416 (2000)). Specific binding of a compound a CXCR3 variant can be determined by cross-linking these two components, if they are in contact with each other, using 10 UV or a chemical cross-linking agent. In addition, a biomolecular interaction analysis (BIA) can detect whether two components are in contact with each other. In such an assay, one component, such as a CXCR3 variant (for example, a membrane preparation containing a CXCR3 variant) is bound 15 to a BIA chip, and a second component such as a compound is passed over the chip. If the two components specifically bind, the contact results in an electrical signal, which is readily detected.

In addition, virtual computational methods and 20 the like can be used to identify compounds that modulate or specifically bind to a CXCR3 variant in a screening method of the invention. Exemplary virtual computational methodology involves virtual docking of small-molecule compounds on a virtual representation of a CXCR3 variant 25 structure in order to determine or predict specific binding. See, for example, Lengauer et al., Current Opinions in Structural Biology 6:402-406 (1996); Choichet et al., Journal of Molecular Biology 221:327-346 (1991); Cherfils et al., Proteins 11:271-280 (1991); Palma et al., 30 Proteins 39:372-384 (2000); Eckert et al., Cell 99:103-115

(1999); Loo et al., Med. Res. Rev. 19:307-319 (1999); and Kramer et al., J. Biol. Chem. (2000).

Additional assays suitable for detecting selective binding interactions between a receptor and a
5 ligand include, for example, fluorescence correlation spectroscopy (FCS) and scintillation proximity assays (SPA) reviewed in Major, J. Receptor and Signal Transduction Res. 15:595-607 (1995); and in Sterrer et al., J. Receptor and Signal Transduction Res. 17:511-520 (1997)). Scintillation
10 proximity assays involve the use of a fluomicrosphere coated with an acceptor molecule, such as an antibody, to which an antigen will bind selectively in a reversible manner. For example, a compound can be bound to a fluomicrosphere using an antibody that specifically binds
15 to the compound, and contacted with a labeled CXCR3 variant. If the labeled CXCR3 variant specifically binds to the compound, the radiation energy from the labeled CXCR3 variant is absorbed by the fluomicrosphere, thereby producing light which is easily measured. Variations of
20 such assays can also be performed where the CXCR3 variant is bound to the fluomicrosphere, and the compound is labeled.

Other assays suitable to identifying a CXCR3 variant agonist or antagonist include cell-based high
25 throughput screening systems such as melanophore assays, yeast-based assay systems, and mammalian cell expression systems (Jayawickreme and Kost, Curr. Opin. Biotechnol. 8:629-634 (1997)). Automated and miniaturized high throughput screening assays are also useful in the methods

of the invention (Houston and Banks, Curr. Opin. Biotechnol. 8:734-740 (1997)). High throughput screening assays are designed to identify "hits" or "lead compounds" having the desired modulating or specific binding activity, 5 from which modified compounds can be prepared to improve a property of the initial lead compound. Chemical modification of the "hit" or "lead compound" can be based on an identifiable structure/activity relationship (SAR) between the "hit" and a CXCR3 variant of the invention.

10 The methods of the invention can utilize high throughput screening (HTS) techniques to identify compounds that modulate or specifically bind to a CXCR3 variant. HTS assays permit screening of large numbers of compounds in an efficient manner. A variety of other assays well known in 15 the art can be used to determine specific binding of a compound to a CXCR3 variant in a method of the invention. Such assays include, without limitation, detecting specific binding of a labeled compound to a CXCR3 variant which is immobilized. For example, a compound can be conjugated to 20 a radiolabel, fluorescent label or enzyme label such as alkaline phosphatase, horse radish peroxidase or luciferase. Labeled compound can then bind to a CXCR3 variant, for example a CXCR3 variant membrane preparation, which is immobilized, for example, on a solid support such 25 as a latex bead. Unbound compound can be washed away, and the amount of specifically bound compound can be detected based on its label. Fluorescently labeled compound can also be bound to a CXCR3 variant in solution and bound complexes detected, for example, using a fluorescence 30 polarization assay (Degterev et al., Nature Cell Biology

3:173-182 (2001)). Such assays also can be performed where the CXCR3 variant is labeled and the compound is immobilized or in solution. One skilled in the art understands that a variety of additional means can be used
5 to determine specific binding to a CXCR3 variant; as non-limiting examples, binding of a compound to a ¹⁵N-labeled CXCR3 variant can be detected using nuclear magnetic resonance (NMR), or specific binding can be determined using an antibody that specifically recognizes a
10 ligand-bound CXCR3 variant.

An assay to identify compounds that function as CXCR3 variant agonists or antagonists are generally performed under conditions in which contacting the receptor with a known receptor agonist would produce a predetermined
15 signal. If desired, the assay can be performed in the presence of a known CXCR3 variant agonist, such as a Mig, IP-10 or I-TAC. The agonist concentration can be within 10-fold of the EC50. Thus, an agonist that competes with ligand, for signaling through the CXCR3 variant, or
20 indirectly potentiates the signaling activity of ligand, can be readily identified. Similarly, an agonist that competes with ligand for signaling through the CXCR3 variant can be readily identified.

Likewise, an antagonist that prevents ligand from
25 binding the CXCR3 variant, or indirectly decreases the signaling activity of CXCR3 variant, also can be identified. The candidate compound can be tested at a range of concentrations to establish the concentration where half-maximal signaling occurs; such a concentration

is generally similar to the dissociation constant (K_d) for CXCR3 variant binding.

Assay methods for identifying compounds that selectively bind to or modulate signaling through a CXCR3 variant generally involve comparison to a control. One type of a "control" is a preparation that is treated identically to the test preparation, except the control is not exposed to the candidate compound. Another type of "control" is a preparation that is similar to the test preparation, except that the control preparation does not express the receptor, or has been modified so as not to respond selectively to ligand. In this situation, the response of the test preparation to a candidate compound is compared to the response (or lack of response) of the control preparation to the same compound under substantially the same reaction conditions.

A compound identified to be an agonist or antagonist of one or more CXCR3 variants can be tested for its ability to modulate one or more effects on the function of a cell or animal. For example, a CXCR3 variant agonist or antagonist can be tested for an ability to modulate an inflammatory response.

General assay formats

In the methods of the invention for identifying a compound that modulates, or specifically binds to, a CXCR3 variant, an isolated CXCR3 variant or CXCR3 variant over-expressed in a genetically engineered cell can be contacted with a compound in a solution under conditions

suitable for interaction between the CXCR3 variant and compound. Such contact can occur in an isolated cell in cell culture, in a whole or partially purified cell extract, or with an isolated polypeptide. As used herein, 5 the term "*in vitro*" means in an artificial environment outside of a living organism or cell. Assays performed in a test tube, microcentrifuge tube, 96 well plate, 384 well plate, 1536 well plate or other assay format outside of an organism or living cell are *in vitro* assays. Experiments 10 performed in cells or tissues that have been fixed and are therefore dead (sometimes referred to as *in situ* experiments) or using cell-free extracts from cells are *in vitro*. Contact can also occur *in vivo* using, for example, living primary or tissue culture cells, isolated tissues or 15 organs, or whole animals.

Conditions suitable for contacting an isolated CXCR3 variant expressed in a genetically engineered cell with a compound are dependent on the characteristics of the CXCR3 variant and the compound. For example, the overall 20 charge of the CXCR3 variant and the compound can be considered when adjusting the salt concentration or pH of a buffering solution to optimize the specific binding or modulation of the CXCR3 variant by the compound. Usually a salt concentration and pH in the physiological range, for 25 example, about 100 mM KCl and pH 7.0 are reasonable starting points. In addition, other components such as glycerol or protease inhibitors can be added to the solution, for example, to inhibit polypeptide degradation. It is understood that the stability of the contact between 30 the CXCR3 variant and the compound can be effected by the

temperature at which such contact occurs and that the optimal temperature for contact can be routinely determined by those skilled in the art. For example, reactions can be performed on ice (4°C), at room temperature (about 25°C) or 5 at body temperature (37°C). Suitable conditions can be similar or identical to conditions used for binding of a compound to the wild-type human CXCR3. Such conditions are known in the art and include, for example, contact in a binding buffer containing 10mM MES/KOH (pH 6.0), 1 mM EDTA, 10 and 10mM MnCl₂, and incubation at 30°C for 45 minutes, as described in Bastien et al., J. Biol. Chem. 269:11873-11877 (1994).

Comparative screening assays

In addition to the methods described above for 15 identifying a compound that modulates or specifically binds a CXCR3 variant, the invention also provides related methods for identifying a compound that differentially modulates or differentially binds to a CXCR3 variant. It is understood that the CXCR3 variant, cells, compounds, 20 indicators, conditions for contacting, and assays described above also can be applied to methods for identifying a compound that differentially modulates or differentially binds to a CXCR3 variant.

Further provided by the invention is a method for 25 identifying a compound that differentially modulates a CXCR3 isoform. The method involves a) contacting a CXCR3 variant polypeptide with a candidate compound, the polypeptide containing SEQ ID NO:11 or a conservative

variant thereof and having at least 50% identity with SEQ ID NO:8 or 10, and b) determining a CXCR3 signal for the CXCR3 variant polypeptide; c) contacting a distinct CXCR3 isoform polypeptide with the candidate compound; d) 5 determining a CXCR3 signal for the distinct CXCR3 isoform polypeptide; and e) comparing the CXCR3 signals determined for the CXCR3 variant polypeptide and the distinct CXCR3 isoform polypeptide, wherein a difference between the CXCR3 signals indicates that the candidate compound is a compound 10 that differentially modulates a CXCR3 isoform. In an embodiment, the distinct CXCR3 isoform polypeptide contains SEQ ID NO:11 or a conservative variant thereof. For example, a CXCR3 variant polypeptide can contain an amino acid sequence selected from SEQ ID NO:2 or 4, or a 15 conservative variant thereof. In embodiments of the invention, the CXCR3 signal can be, for example, calcium mobilization, cAMP upregulation or IP3 release. A candidate compound used in the method can be, for example, a polypeptide or small molecule.

20 The invention also provides a method for identifying a compound that differentially binds to a CXCR3 variant. The method involves a) contacting the CXCR3 variant polypeptide with a candidate compound, wherein the polypeptide contains SEQ ID NO:11 or a conservative variant 25 thereof and having at least 50% identity with SEQ ID NO:8 or 10; b) determining specific binding of the candidate compound to the CXCR3 variant polypeptide; c) contacting a distinct CXCR3 isoform polypeptide with the candidate compound; d) determining specific binding of the candidate 30 compound to the distinct CXCR3 isoform polypeptide, and e)

comparing specific binding determined for the CXCR3 variant polypeptide with specific binding determined for the distinct CXCR3 isoform polypeptide, wherein a difference between the specific binding indicates that the candidate
5 compound is a compound that differentially binds to a CXCR3 isoform. In an embodiment, the distinct CXCR3 isoform polypeptide contains SEQ ID NO:11 or a conservative variant. For example, a CXCR3 variant contains an amino acid sequence selected from SEQ ID NO:2 or 4, or a conservative
10 variant thereof. In embodiments of the invention, the CXCR3 signal can be, for example, calcium mobilization, cAMP upregulation or IP3 release. A candidate compound used in the method can be, for example, a polypeptide or small molecule.

15 As set forth above with respect to methods for identifying a compound that modulates or specifically binds a CXCR3 variant, the CXCR3 variant can be any of a variety of CXCR3 variants such as an isolated polypeptide containing amino acid sequence SEQ ID NO:11, or a
20 conservative variant thereof, and having at least 50% amino acid identity with SEQ ID NO:8 or 10 and or a conservative variant thereof; or an isolated polypeptide containing amino acid sequence SEQ ID NO:2, 4 or 6, or a conservative variant thereof. In one embodiment, the CXCR3 variant is
25 expressed in a genetically engineered cell. In another embodiment, the CXCR3 variant is exogenously expressed in a genetically engineered cell.

 In the methods of the invention for identifying a compound that differentially modulates or differentially

binds CXCR3 variant, the second receptor can be any receptor of interest. For example, the second receptor can be a G-protein coupled receptor such as, without limitation, any other CXCR3 such as a different CXCR3 variant or an originally identified CXCR3. In particular embodiments, the second receptor is an originally identified CXCR3 containing the amino acid sequence SEQ ID NO: 2 or 4, or a functional fragment thereof.

It is understood that the methods of the invention can be practiced using a CXCR3 variant and second receptor expressed, for example, in different cells. In addition, the methods of the invention can be practiced using a CXCR3 variant and second receptor expressed in the same cell, for example, where the CXCR3 variant has distinct binding specificity or signal transduction effects as compared to the co-expressed second receptor.

Compounds

As used herein, the term "candidate compound" refers to any biological or chemical compound. For example, a candidate compound can be a naturally occurring macromolecule, such as a polypeptide, nucleic acid, carbohydrate, lipid, or any combination thereof. A candidate compound also can be a partially or completely synthetic derivative, analog or mimetic of such a macromolecule, or a small organic molecule prepared by combinatorial chemistry methods. If desired in a particular assay format, a candidate compound can be detectably labeled or attached to a solid support.

Methods for preparing large libraries of compounds, including simple or complex organic molecules, metal-containing compounds, carbohydrates, peptides, proteins, peptidomimetics, glycoproteins, lipoproteins, 5 nucleic acids, antibodies, and the like, are well known in the art and are described, for example, in Huse, U.S. Patent No. 5,264,563; Francis et al., Curr. Opin. Chem. Biol. 2:422-428 (1998); Tietze et al., Curr. Biol., 2:363-371 (1998); Sofia, Mol. Divers. 3:75-94 (1998); Eichler et 10 al., Med. Res. Rev. 15:481-496 (1995); and the like. Libraries containing large numbers of natural and synthetic compounds also can be obtained from commercial sources.

The number of different candidate compounds to test in the methods of the invention will depend on the 15 application of the method. For example, one or a small number of candidate compounds can be advantageous in manual screening procedures, or when it is desired to compare efficacy among several predicted ligands, agonists or antagonists. However, it will be appreciated that the 20 larger the number of candidate compounds, the greater the likelihood of identifying a compound having the desired activity in a screening assay. Additionally, large numbers of compounds can be processed in high-throughput automated screening assays.

25 In a screening method of the invention, the members of a library of compounds can be assayed for activity individually, in pools, or *en masse*. An example of *en masse* screening to identify a compound that modulates or specifically binds to a CXCR3 variant is as follows: a

library of compounds is assayed in pools for the ability to modulate or specifically bind a CXCR3 variant; the sub-population which modulates or specifically binds the CXCR3 variant is subdivided; and the assay is repeated as
5 needed in order to isolate an individual compound or compounds from the library that modulate or specifically bind the CXCR3 variant.

Screening Compositions

10 The screening methods of the invention can be practiced using, for example, using a CXCR3 variant expressed or over-expressed in a genetically engineered cell. As used herein, the term "genetically engineered cell" means a cell having genetic material which is altered
15 by the hand of man. Such a cell can contain a transient or permanent alteration of its genetic material including, for example, alteration in genomic or episomal genetic material. The genetic material in a genetically engineered cell can be altered using, without limitation, an
20 exogenously expressed nucleic acid molecule, chemical mutagen or transposable element. It is understood that a genetically engineered cell can contain one or more man-made alterations; for example, a cell can be co-transfected with more than one expression vector. As used herein in
25 relation to a CXCR3 variant in a genetically engineered cell, the term "over-expressed" means having a protein level of a CXCR3 variant greater than the level seen in a corresponding non-genetically engineered cell.

As understood by one skilled in the art, a CXCR3 variant can be over-expressed in a genetically engineered cell, for example, by exogenously expressing a nucleic acid molecule encoding the CXCR3 variant in a cell as described 5 herein above. It is further understood that a CXCR3 variant can be over-expressed in a cell that does not normally express the CXCR3 variant, or in a cell that naturally expresses the endogenous CXCR3 variant. As a non-limiting example, a CXCR3 variant can be over-expressed 10 in a cell that expresses endogenous CXCR3 variant at a low level. In addition, a CXCR3 variant can be over-expressed in a genetically engineered cell, for example, by expressing a regulatory molecule in the cell to increase expression of the endogenous CXCR3 variant. Another 15 example of a method whereby a CXCR3 variant can be over-expressed in a genetically engineered cell is recombination of a heterologous regulatory region such as, without limitation, a promoter, enhancer or 3' regulator, in the cell such that the heterologous regulatory region results 20 in over-expression of endogenous CXCR3 variant. As understood by one skilled in the art, over-expression of a CXCR3 variant in a genetically engineered cell includes, without limitation, over-expression of the variant on the surface of the cell, within a cell membrane or in the 25 cytosolic portion of the cell.

A CXCR3 variant also can be over-expressed in a cell using a chemical agent. Thus, the invention provides a method for identifying a compound that modulates a CXCR3 variant by contacting the CXCR3 variant with a compound, 30 where the CXCR3 variant is over-expressed in a cell using a

chemical agent, and determining the level of an indicator which correlates with modulation of the CXCR3 variant, where an alteration in the level of the indicator as compared to a control level indicates that the compound is
5 a compound that modulates the CXCR3 variant. The invention also provides a method for identifying a compound that specifically binds to a CXCR3 variant by contacting the CXCR3 variant with a compound, where the CXCR3 variant is over-expressed in a cell using a chemical agent, and
10 determining specific binding of the compound to the CXCR3 variant. Chemical agents that can result in over-expression of a CXCR3 variant can include, without limitation, chemicals that induce the level or activity of a regulatory factor, such as a transcription factor, that
15 promotes CXCR3 variant expression; and chemicals that suppress the level or activity of a regulatory factor, such as a transcription factor, that inhibits CXCR3 variant expression.

As described above, the methods of the invention
20 can be practiced with a cell that over-expresses a CXCR3 variant. In addition, it is understood that cellular extracts prepared from cells that over-express a CXCR3 variant such as genetically engineered cells that over-express a CXCR3 variant can be useful in the methods of the
25 invention. Methods for generating different types of cellular extracts including, without limitation, whole cell extracts, membrane extracts, cytosolic extracts and nuclear extracts are well known in the art. As a non-limiting example, receptor enriched plasma membrane fractions can be
30 obtained by continuous or discontinuous gradients of, for

example, sucrose, as described in Woodward and Lawrence, Biochemical Pharmacology 47:1567-1674 (1994).

Isolated CXCR3 also can be useful in the screening methods of the invention. For example, an
5 isolated CXCR3 variant derived from a cell can be substantially purified away from other polypeptides in the cell. An isolated CXCR3 variant can contain non-polypeptide components, for example, an isolated CXCR3 variant can be associated with a natural or artificial
10 lipid containing membrane. In addition, it is understood that cell extracts prepared from cells that over-express a CXCR3 variant such as genetically engineered cells that over-express a CXCR3 variant can be useful in the methods of the invention. Methods for generating different types
15 of cell extracts including, without limitation, whole cell extracts, membrane extracts, cytosolic extracts and nuclear extracts are well known in the art. As a non-limiting example, receptor enriched plasma membrane fractions can be obtained by continuous or discontinuous gradients of, for
20 example, sucrose, as described in Woodward and Lawrence, Biochemical Pharmacology 47:1567-1674 (1994).

A screening composition containing a CXCR3 variant, such as CXCR3A-2 or CXCR3B-2, in the presence of a CXCR3 ligand, such as Mig, IP-10 or I-TAC, can be useful in
25 a screening method of the invention. Either the CXCR3 variant or ligand, or both, can be detectably labeled for use in a screening method.

Preparation of CXCR3 variants

A CXCR3 variant of the invention can be prepared in isolated form using conventional biochemical purification methods, starting either from a tissue
5 containing the desired CXCR3 variant or from a recombinant source. A CXCR3 variant can be isolated by any of a variety of methods well-known in the art, including, but not limited to, precipitation, gel filtration, ion-exchange, reverse-phase and affinity chromatography,
10 and combinations thereof. Other well-known methods for protein isolation are described in Deutscher et al., Guide to Protein Purification: Methods in Enzymology Vol. 182, (Academic Press, (1990)). Methods suitable for isolating a CXCR3 variant using biochemical purification are known in
15 the art as described for example, in Venter and Harrison, (eds), Receptor Purification Procedures (Liss, (1984)); Litwack, Receptor Purification: Receptors for CNS Agents, Growth Factors, Hormones, & Related Substances (Humana Press, (1990)); or Litwack, Receptor Purification:
20 Receptors for Steroid Hormones, Thyroid Hormones, Water Balancing Hormone, & Others (Humana Press, (1990)). Purification of the receptor variant can be routinely monitored, for example, by an immunological assay, functional assay or ligand binding assay.

25 An isolated CXCR3 variant of the invention also can be produced by chemical synthesis. As a non-limiting example, synthetic isolated CXCR3 variants, including fragments thereof, can be produced using an Applied Biosystems, Inc., Model 430A or 431A automatic peptide

synthesizer employing the chemistry provided by the manufacturer (Applied BioSystems, Inc.; Foster City, CA). Methods for synthesizing isolated polypeptides are well known in the art. See, for example, Bodanzsky, Principles of Peptide Synthesis (1st ed. & 2d rev. ed.), Springer-Verlag, New York, New York (1984 & 1993), Chapter 7; and Stewart and Young, Solid Phase Peptide Synthesis (2d ed.), Pierce Chemical Co., Rockford, Illinois (1984).

If desired, such as to optimize their functional activity, selectivity, stability or bioavailability, chemically synthesized polypeptides can be modified to include D-stereoisomers, non-naturally occurring amino acids, and amino acid analogs and mimetics. Examples of modified amino acids and their uses are presented in Sawyer, Peptide Based Drug Design, ACS, Washington (1995) and Gross and Meienhofer, The Peptides: Analysis, Synthesis, Biology, Academic Press, Inc., New York (1983). For certain applications, it can also be useful to incorporate one or more detectably labeled amino acids into a chemically synthesized polypeptide or peptide, such as radiolabeled or fluorescently labeled amino acids.

An isolated polypeptide can be in any form, such as in a buffered solution, a suspension, a lyophilized powder, recombinantly expressed in a heterologous cell, bound to a receptor or attached to a solid support.

Nucleic acid molecules

The invention provides an isolated nucleic acid molecule that encodes a CXCR3 variant. The nucleic acid

molecule includes a nucleotide sequence that encodes the amino acid sequence referenced as SEQ ID NO:11, and the encoded polypeptide has at least 50% identity with SEQ ID NO:8 or 10. In one embodiment, the isolated nucleic acid molecule comprises a nucleotide sequence that encodes an amino acid sequence selected from SEQ ID NOS:2 or 4, or a conservative variant thereof. In another embodiment, the isolated nucleic acid molecule consists of a nucleotide sequence that encodes an amino acid sequence selected from SEQ ID NOS:2 or 4. In a further embodiment, the isolated nucleic acid molecule is selected from SEQ ID NOS:1, 3, and 5.

Isolated nucleic acid molecules include DNA and RNA molecules as well as both sense or complementary anti-sense strands. It is understood that an isolated nucleic acid molecule of the invention can be a double-stranded or single-stranded molecule, an RNA or DNA molecule, and can optionally include non-coding sequence. Nucleic acid molecules of the invention further include molecules which are wholly or partially chemically synthesized.

The nucleic acid molecules of the invention optionally include heterologous nucleic acid sequences that are not part of the CXCR3 variant-encoding sequences in nature. Such a heterologous nucleic acid sequence can be optionally separated from the CXCR3 variant-encoding sequence by an encoded cleavage site that facilitates removal of non-CXCR3 variant sequences from the expressed fusion protein. Heterologous nucleic acid sequences

include, without limitation, restriction sites, promoter sequences, sequences encoding poly-histidine sequences, FLAG tags and other epitopes, and glutathione-S-transferase, thioredoxin, and maltose binding protein domains or other domains or sequences that facilitate purification or detection of the fusion protein containing a CXCR3 variant of the invention.

An isolated nucleic acid molecule of the invention contains nucleotide sequence that is distinct from the genomic sequence encoding the originally identified human CXCR3-A and CXCR3-B polypeptides. For example, the nucleotide sequences encoding CXCR3A-2 (SEQ ID NO:2) and CXCR3B-2 (SEQ ID NO:4) contain additional sequence with respect to exon 2 of the CXCR3A and CXCR3B genomic sequences. The intron/exon structure of the human CXCR3-A and CXCR3-B genomic clones is shown in Figure 4.

The portions of exons of human CXCR3A and CXCR3B genomic clones that are present in alternatively spliced CXCR3A-2 and CXCR3B-2 genomic clones are as follows: CXCR3A-2 alternatively spliced sequence corresponds to exon 2 of human genomic sequence at ranges from +8783055 to +8782438 and +8782103 to +8781510.

A nucleic acid molecule of the invention can be used in a variety of recombinant cloning methods and methods for detecting CXCR3 nucleic acid molecules. As non-limiting examples, nucleic acid molecules of the invention can be used in hybridization reactions such as Southern and Northern blots, to encode polypeptide sequence

in recombinant cloning methods, and as primers in polymerase chain reactions.

Vectors

Further provided by the invention are vectors
5 that contain a nucleic acid molecule of the invention, and
isolated host cells containing the vector. Such a vector
contains an isolated nucleic acid molecule that encodes a
CXCR3 variant. The nucleic acid molecule contained in the
vector includes a nucleotide sequence that encodes a
10 polypeptide including the amino acid sequence referenced as
SEQ ID NO:11, or a conservative variant thereof, the
polypeptide having at least 50% identity with SEQ ID NO:8
or 10. In one embodiment, the isolated nucleic acid
molecule comprises a nucleotide sequence that encodes an
15 amino acid sequence selected from SEQ ID NOS:2 or 4, or a
conservative variant thereof. In another embodiment, the
isolated nucleic acid molecule consists of a nucleotide
sequence that encodes an amino acid sequence selected from
SEQ ID NOS:2 or 4. In a further embodiment, the isolated
20 nucleic acid molecule is selected from SEQ ID NOS:1, 3, and
5.

Exemplary vectors include vectors derived from a
virus, such as a bacteriophage, a baculovirus or a
retrovirus, and vectors derived from bacteria or a
25 combination of bacterial sequences and sequences from other
organisms, such as a cosmid or a plasmid. The vectors of
the invention will generally contain elements such as an
origin of replication compatible with the intended host

cells; transcription termination and RNA processing signals; one or more selectable markers compatible with the intended host cells; and one or more multiple cloning sites. Optionally, the vector will further contain
5 sequences encoding tag sequences, such as GST tags, and/or a protease cleavage site, such as a Factor Xa site, which facilitate expression and purification of the encoded polypeptide.

The choice of particular elements to include in a
10 vector will depend on factors such as the intended host cells; the insert size; whether expression of the inserted sequence is desired; the desired copy number of the vector; the desired selection system, and the like. The factors involved in ensuring compatibility between a host cell and
15 a vector for different applications are well known in the art.

In applications in which the vectors are to be used for recombinant expression of the encoded polypeptide, the isolated nucleic acid molecules will generally be
20 operatively linked to a promoter of gene expression, which may be present in the vector or in the inserted nucleic acid molecule. An isolated nucleic acid molecule encoding a CXCR3 variant can be operatively linked to a promoter of gene expression. As used herein, the term "operatively
25 linked" means that the nucleic acid molecule is positioned with respect to either the endogenous promoter, or a heterologous promoter, in such a manner that the promoter will direct the transcription of RNA using the nucleic acid molecule as a template.

Methods for operatively linking a nucleic acid to a heterologous promoter are well known in the art and include, for example, cloning the nucleic acid into a vector containing the desired promoter, or appending the promoter to a nucleic acid sequence using PCR. A nucleic acid molecule operatively linked to a promoter of RNA transcription can be used to express CXCR3 variant transcripts and polypeptides in a desired host cell or in vitro transcription-translation system.

10 The choice of promoter to operatively link to an invention nucleic acid molecule will depend on the intended application, and can be determined by those skilled in the art. For example, if a particular gene product may be detrimental to a particular host cell, it may be desirable
15 to link the invention nucleic acid molecule to a regulated promoter, such that gene expression can be turned on or off. Alternatively, it may be desirable to have expression driven by either a weak or strong constitutive promoter. Exemplary promoters suitable for mammalian cell systems
20 include, for example, the SV40 early promoter, the cytomegalovirus (CMV) promoter, the mouse mammary tumor virus (MMTV) steroid-inducible promoter, and the Moloney murine leukemia virus (MMLV) promoter. Exemplary promoters suitable for bacterial cell systems include, for example,
25 T7, T3, SP6, lac and trp promoters. An exemplary vector suitable for fusion protein expression in bacterial cells is the pGEX-3X vector (Amersham Pharmacia Biotech, Piscataway, NJ).

Cells containing vectors

Also provided are cells containing an isolated nucleic acid molecule encoding a CXCR3 variant. The
5 isolated nucleic acid molecule will generally be contained within a vector, and can be maintained episomally, or incorporated into the host cell genome.

The cells of the invention can be used, for example, for molecular biology applications such as
10 expansion, subcloning or modification of the isolated nucleic acid molecule. For such applications, bacterial cells, such as laboratory strains of *E. coli*, are useful, and expression of the encoded polypeptide is not required.

The invention also provides methods for preparing
15 an isolated polypeptide corresponding to a CXCR3 variant, by culturing host cells so as to express a recombinant CXCR3 variant. A variety of well-known methods can be used to introduce a vector into a host cell for expression of a recombinant polypeptide (see, for example, Sambrook et al.,
20 Molecular Cloning: A Laboratory Manual, Cold Spring Harbor Laboratory, New York (1992) and Ansubel et al., Current Protocols in Molecular Biology, John Wiley and Sons, Baltimore, MD (1998)). The selected method will depend, for example, on the selected host cells. A cell can be
25 generated that transiently or stably expresses an exogenously expressed polypeptide of the invention. Expression vectors for transient or stable expression of a polypeptide of the invention can be introduced into cells

using transfection methods well known to one skilled in the art. Such methods include, without limitation, infection using viral vectors, lipofection, electroporation, particle bombardment and transfection such as calcium-phosphate mediated transfection. Detailed procedures for these methods can be found in Sambrook et al., Molecular Cloning: A Laboratory Manual Cold Spring Harbor Laboratory Press (1989), and the references cited therein. Useful mammalian expression vectors and methods of introducing such vectors into mammalian cells either *ex vivo* or *in vivo* are well known in the art. As non-limiting examples, a plasmid expression vector can be introduced into a cell by calcium-phosphate mediated transfection, DEAE dextran-mediated transfection, lipofection, polybrene- or polylysine-mediated transfection, electroporation, or by conjugation to an antibody, gramicidin S, artificial viral envelope or other intracellular carrier. A viral expression vector can be introduced into a cell by infection or transduction, for example, or by encapsulation in a liposome. It further is understood that polypeptides can be delivered directly into cells using a lipid-mediated delivery system (Zelphati et al., J. Biol. Chem. 276:35103-35110 (2001)) to produce a cell that contains an exogenously expressed polypeptide of the invention.

Exemplary host cells that can be used to exogenously express a polypeptide of the invention include, yet are not limited to, primary mammalian cells; established mammalian cell lines such as COS, CHO, HeLa, NIH3T3, HEK 293, and HEK 293/EBNA cells; amphibian cells such as *Xenopus* embryos and oocytes; and other vertebrate

cells. Exemplary host cells further include, without limitation, insect cells such as *Drosophila*, *Spodoptera frugiperda* and other cells compatible with baculovirus expression systems (Murakimi et al., Cytokine 13:18-24
5 (2001); yeast cells such as *Saccharomyces cerevisiae*, *Saccharomyces pombe*, or *Pichia pastoris*; and prokaryotic cells such as *Escherichia coli*. Following transfection, cells exogenously expressing a polypeptide of the invention can be selected, for example, using drug resistance. A
10 quantitative assay such as, for example, immunoblot analysis, immunoprecipitation or ELISA can determine the amount of a polypeptide of the invention expressed in a transfected cell. Such methods are known to one skilled in the art and can be found, for example, in Ausubel et al.,
15 *supra*, 1989, or in Harlow et al., *supra*, 1988.

Use of CXCR3 variant agonists and antagonists

The invention provides screening assays for identifying compounds that modulate CXCR3 variant activity, such as agonists and antagonists of CXCR3 variant. The
20 agonists and antagonists identified using the methods of the invention can be used to beneficially modulate CXCR3 variant activity to treat an individual having condition associated with CXCR3 or a CXCR3 variant. For example, because CXCR3 ligands mediate inflammation, CXCR3 variant
25 agonists and antagonists can be used to treat inflammatory disorders, including autoimmune disorders. As used herein, a "condition associated with CXCR3 or a CXCR3 variant" means any disease or condition in which modulation of the activity of CXCR3 or a CXCR3 variant can be beneficial. It

is understood that the underlying cause of the disease may or may not be due to an abnormality in expression or activity of CXCR3 or a CXCR3 variant.

5 A condition associated with CXCR3 or a CXCR3 variant can be, without limitation, a renal disorder, such as glomerulonephritis; an autoimmune disorder, such as lupus; a thyroid disorder, such as Grave's Disease; a respiratory system disorder, such as chronic obstructive
10 pulmonary diseases (COPD) and asthma; cancer, such as leukemias including chronic lymphocytic leukemia; a metabolic disorder, such as diabetes; a vascular disorder, such as stroke; alloimmune response to transplanted organs or tissues; and other inflammatory disorders, such as
15 inflammatory bowel disease, rheumatoid arthritis, psoriasis and the like.

 A compound identified according to a method of the invention also can be used to prevent or reduce the
20 severity of a cancer, which, as used herein, is a term that means any neoplastic disease including both solid tumors and hematopoietic cancers. Exemplary cancers to be prevented or reduced in severity according to a method of the invention include, without limitation, leukemias; B
25 cell malignancies; cervical cancers; melanomas, adenocarcinomas and other carcinomas; osteosarcomas; epithelial tumors such as breast and ovarian carcinomas; endometrial cancers; glioblastomas; renal cancers; bladder cancers; gastric cancers; pancreatic cancers; colorectal
30 cancers; prostate cancers; lung cancers, neuroblastomas;

glioblastomas; leukemias and lymphomas; and vascular cell tumors such as hemangiomas, Kaposi's sarcomas, lymphangiomas, angiosarcomas and hemangioendotheliomas. It is understood that the methods of the invention can be used
5 to prevent or reduce the severity of any of the above or other cancers known in the art, including cancers of various severities and stages.

The present invention also provides methods of preventing or reducing the severity of an ocular condition
10 by administering a compound that modulates or differentially modulates a CXCR3 or a CXCR3 variant. Ocular conditions that can be prevented or reduced in severity with a compound that modulates or differentially modulates a CXCR3 or a CXCR3 variant include, without
15 limitation, maculopathies and retinal degeneration, such as Non-Exudative Age Related Macular Degeneration (ARMD), Exudative Age Related Macular Degeneration (ARMD), Choroidal Neovascularization, Diabetic Retinopathy, Central Serous Chorioretinopathy, Cystoid Macular Edema, Diabetic
20 Macular Edema, Myopic Retinal Degeneration; inflammatory diseases, such as Acute Multifocal Placoid Pigment Epitheliopathy, Behcet's Disease, Birdshot Retinochoroidopathy, Infectious (Syphilis, Lyme, Tuberculosis, Toxoplasmosis), Intermediate Uveitis (Pars
25 Planitis), Multifocal Choroiditis, Multiple Evanescent White Dot Syndrome (MEWDS), Ocular Sarcoidosis, Posterior Scleritis, Serpiginous Choroiditis, Subretinal Fibrosis and Uveitis Syndrome, Vogt-Koyanagi-Harada Syndrome, Punctate Inner Choroidopathy, Acute Posterior Multifocal Placoid
30 Pigment Epitheliopathy, Acute Retinal Pigment

Epitheliitis, Acute Macular Neuroretinopathy; vascular and exudative diseases, such as Diabetic retinopathy, Central Retinal Arterial Occlusive Disease, Central Retinal Vein Occlusion, Disseminated Intravascular Coagulopathy, Branch
5 Retinal Vein Occlusion, Hypertensive Fundus Changes, Ocular Ischemic Syndrome, Retinal Arterial Microaneurysms, Coat's Disease, Parafoveal Telangiectasis, Hemi-Retinal Vein Occlusion, Papillophlebitis, Central Retinal Artery Occlusion, Branch Retinal Artery Occlusion, Carotid Artery
10 Disease (CAD), Frosted Branch Angiitis, Sickle Cell Retinopathy and other Hemoglobinopathies, Angioid Streaks, Familial Exudative Vitreoretinopathy; Eales Disease; traumatic, surgical and environmental disorders, such as Sympathetic Ophthalmia, Uveitic Retinal Disease, Retinal
15 Detachment, Trauma, Retinal Laser, Photodynamic therapy, Photocoagulation, Hypoperfusion During Surgery, Radiation Retinopathy, Bone Marrow Transplant Retinopathy; proliferative disorders, such as Proliferative Vitreal Retinopathy and Epiretinal Membranes; infectious disorders,
20 such as Ocular Histoplasmosis, Ocular Toxocariasis, Presumed Ocular Histoplasmosis Syndrome (POHS), Endophthalmitis, Toxoplasmosis, Retinal Diseases Associated with HIV Infection, Choroidal Disease Associate with HIV Infection, Uveitic Disease Associate with HIV Infection,
25 Viral Retinitis, Acute Retinal Necrosis, Progressive Outer Retinal Necrosis, Fungal Retinal Diseases, Ocular Syphilis, Ocular Tuberculosis, Diffuse Unilateral Subacute Neuroretinitis, Myiasis; genetic disorders, such as Retinitis Pigmentosa, Systemic Disorders with Associated
30 Retinal Dystrophies, Congenital Stationary Night Blindness,

Cone Dystrophies, Stargardt's Disease And Fundus
Flavimaculatus, Best's Disease, Pattern Dystrophy of the
Retinal Pigmented Epithelium, X-Linked Retinoschisis,
Sorsby's Fundus Dystrophy, Benign Concentric Maculopathy,
5 Bietti's Crystalline Dystrophy, pseudoxanthoma elasticum;
retinal injuries, such as Macular Hole, Giant Retinal Tear;
retinal tumors, such as Retinal Disease Associated With
Tumors, Congenital Hypertrophy Of The RPE, Posterior Uveal
Melanoma, Choroidal Hemangioma, Choroidal Osteoma,
10 Choroidal Metastasis, Combined Hamartoma of the Retina and
Retinal Pigmented Epithelium, Retinoblastoma,
Vasoproliferative Tumors of the Ocular Fundus, Retinal
Astrocytoma, and Intraocular Lymphoid Tumors.

For treating a condition associated with CXCR3 or
15 a CXCR3 variant, more than one therapeutic approach or
compound can be provided to an individual for maximal
symptom control. Thus, for use in treating or preventing
an inflammatory disorder, a CXCR3 variant antagonist or
agonist can advantageously be administered concurrently or
20 sequentially with another therapeutic mode or formulated
with a second compound that controls the same or related
symptoms. For example, in treating inflammatory disorders,
a CXCR3 variant antagonist or agonist can be administered
while an individual is receiving another anti-inflammatory
25 or pro-inflammatory treatment. A compound identified using
a method of the invention therefore can be administered
alone, in combination with, or in sequence with, other
compounds or modalities. The skilled clinician will be
able to determine concurrent or sequential therapies

appropriate for use with a CXCR3 or CXCR3 variant antagonist or agonist.

Formulation of compounds

5 In the methods of the invention for preventing or reducing the severity of a condition associated with CXCR3 or a CXCR3 variant, a compound that modulates or differentially modulates a CXCR3 variant can be formulated together with a pharmaceutically acceptable carrier.

10 Suitable pharmaceutically acceptable carriers are well known in the art and include, for example, aqueous or organic solvents such as physiologically buffered saline, glycols, glycerol, oils or injectable organic esters. A pharmaceutically acceptable carrier can also contain a

15 physiologically acceptable agent that acts, for example, to stabilize or increase solubility of a pharmaceutical composition. Such a physiologically acceptable agent can be, for example, a carbohydrate such as glucose, sucrose or dextrans; an antioxidant such as ascorbic acid or

20 glutathione; a chelating agent; a low molecular weight polypeptide; or another stabilizer or excipient. Pharmaceutically acceptable carriers including solvents, stabilizers, solubilizers and preservatives, are well known in the art as described, for example, in Martin,

25 Remington's Pharm. Sci. 15th Ed. (Mack Publ. Co., Easton, 1975).

Ophthalmic compositions can contain an ophthalmically acceptable carrier, which is any carrier

that has substantially no long term or permanent detrimental effect on the eye to which it is administered. Examples of ophthalmically acceptable carriers include, without limitation, water, such as distilled or deionized 5 water; saline; and other aqueous media. Topical ophthalmic compositions can include, without limitation, ocular drops, ocular ointments, ocular gels and ocular creams. Such ophthalmic compositions are easy to apply and deliver the active compound effectively. A preservative can be 10 included, if desired, in an ophthalmic composition. Such a preservative can be, without limitation, benzalkonium chloride, chlorobutanol, purite, thimerosal, phenylmercuric acetate, or phenylmercuric nitrate. Vehicles useful in a topical ophthalmic composition include, yet are not limited 15 to, polyvinyl alcohol, povidone, hydroxypropyl methyl cellulose, poloxamers, carboxymethyl cellulose, hydroxyethyl cellulose and purified water. A tonicity adjustor also can be included, if desired, in an ophthalmic composition. Such a tonicity adjustor can be, without 20 limitation, a salt such as sodium chloride, potassium chloride, mannitol or glycerin, or another pharmaceutically or ophthalmically acceptable tonicity adjustor.

Various buffers and means for adjusting pH can be used to prepare an ophthalmic composition, provided that 25 the resulting preparation is ophthalmically acceptable. Such buffers include, but are not limited to, acetate buffers, citrate buffers, phosphate buffers and borate buffers. It is understood that acids or bases can be used to adjust the pH of the composition as needed. 30 Ophthalmically acceptable antioxidants useful in preparing

an ophthalmic composition include, yet are not limited to, sodium metabisulfite, sodium thiosulfate, acetylcysteine, butylated hydroxyanisole and butylated hydroxytoluene.

Those skilled in the art can formulate a compound
5 that modulates, differentially modulates, specifically binds, or differentially binds a CXCR3 variant to ensure proper compound distribution and bioavailability in vivo. For example, some regions of the eye can be inaccessible to some systemically administered drugs, and as a result
10 topical drug delivery can be used. Polymers can be added to ophthalmic solutions to increase bioavailability (Ludwig and Ootenhgm, S.T.P. Pharm. Sci. 2:81-87 (1992)). In addition, colloidal systems such as, without limitation, liposomes, microparticles or nanoparticles can be used to
15 increase penetration of a compound into the eye. Ocular drug absorption also can be enhanced using, for example, iontophoresis, prodrugs, and cyclodextrins.

Methods of ensuring appropriate distribution in vivo also can be provided by rechargeable or biodegradable
20 devices, particularly where concentration gradients or continuous delivery is desired. Various slow release polymeric devices are known in the art for the controlled delivery of drugs, and include both biodegradable and non-degradable polymers and hydrogels. Polymeric device
25 inserts can allow for accurate dosing, reduced systemic absorption and in some cases, better patient compliance resulting from a reduced frequency of administration. Those skilled in the art understand that the choice of the pharmaceutical formulation and the appropriate preparation

of the compound will depend on the intended use and mode of administration.

A compound identified using a method of the invention can be administered to a individual by any
5 effective route. Suitable routes of administration include, but are not limited to, oral, topical, sublingual, intraocular, intradermal, parenteral, intranasal, intravenous, intramuscular, intraspinal, intracerebral and subcutaneous routes. The present invention also provides
10 compounds containing an acceptable carrier such as any of the standard pharmaceutical carriers, including phosphate buffered saline solution, water and emulsions such as an oil and water emulsion, and various types of wetting agents.

15 A compound identified by a method of the invention can be peripherally administered to a individual an effective amount. As used herein, the term "peripherally administering" or "peripheral administration" means introducing the compound into a individual outside of
20 the central nervous system. Thus, peripheral administration encompasses any route of administration other than direct administration to the spine or brain.

An effective amount of a compound identified using a method of the invention can be administered to a
25 individual by any of a variety of means depending, for example, on the type of condition to be treated, the pharmaceutical formulation, and the history, risk factors and symptoms of the individual. Routes of peripheral

administration suitable for the methods of the invention include both systemic and local administration. As non-limiting examples, an effective amount of a compound of the invention can be administered orally; sublingually; 5 parenterally; by subcutaneous pump; by dermal patch; by intravenous, intra-articular, subcutaneous or intramuscular injection; by topical drops, creams, gels or ointments; as an implanted or injected extended release formulation; or by subcutaneous minipump or other implanted device, and by 10 inhalation by aerosol and similar devices.

One skilled in the art understands that peripheral administration can be local or systemic. Local administration results in significantly more of a compound 15 of the invention being delivered to and about the site of local administration than to regions distal to the site of administration. Systemic administration results in delivery of a compound of the invention essentially throughout at least the entire peripheral system of the 20 individual.

Routes of peripheral administration useful in the methods of the invention encompass, without limitation, oral administration, sublingual administration, topical administration, intravenous or other injection, and 25 implanted minipumps or other extended release devices or formulations. A compound of the invention can be peripherally administered, without limitation, orally in any acceptable form such as in a tablet, pill, capsule, powder, liquid, suspension, emulsion or the like; as an 30 aerosol; as a suppository; by intravenous, intraperitoneal,

intramuscular, subcutaneous or parenteral injection; by transdermal diffusion or electrophoresis; topically in any acceptable form such as in drops, creams, gels or ointments; and by minipump or other implanted extended
5 release device or formulation. A compound of the invention optionally can be packaged in unit dosage form suitable for single administration of precise dosages, or in sustained release dosage form for continuous controlled administration.

10 It is understood that slow-release formulations can be useful in the methods of the invention. It is further understood that the frequency and duration of dosing will be dependent, in part, on the effect desired and the half-life of the modulating compound and that a
15 variety of routes of administration are useful for delivering slow-release formulations, as detailed herein above.

 An effective dose of a compound identified using a method of the invention can be determined, for example,
20 by extrapolation from the concentration required in a CXCR3 polypeptide binding or activity assay such as one of the assays disclosed herein above. An effective dose of a compound for the treatment of a CXCR3-associated disorder also can be determined from appropriate animal models, such
25 as transgenic animal models. As non-limiting examples, animal models for pathologies such as cardiovascular disease and ocular disease are well-known in the art. An effective dose for preventing or reducing the severity of a disease is a dose that results in either partial or

complete alleviation of at least one symptom of the disease. The appropriate dose of a compound for treatment of a human individual can be determined by those skilled in the art; and is dependent, for example, on the particular
5 disease being treated and its severity, the nature and bioactivity of the particular compound, the desired route of administration, the gender, age and general health of the individual, and the number of doses and duration of treatment.

10 Diagnostic applications

Because isoforms of CXCR3 variant can be correlated with disease, the presence of such isoforms can be used as a diagnostic or prognostication indicator. Analysis of CXCR3 variant mRNA or polypeptide can be used
15 in such diagnostic methods to identify the presence of an isoform of the CXCR3 variant that correlates with a disease or condition. Direct sequencing, binding, or hybridization assays including PCR, RT-PCR, Northern blot, Southern blot, and RNase protection can be used to detect a CXCR3 isoform.
20 For example, PCR amplification or RT-PCR amplification of a region of a known difference between the originally identified receptor (or particular isoform) and a diagnostic isoform disclosed herein, such as SEQ ID NOS: 2, 4 or 6, or SEQ ID NO:11, can be used. Similarly, an
25 antibody that binds to a region of known difference between the originally identified receptor (or particular isoform) and a diagnostic isoform can be used. Similarly, reverse transcription reactions coupled with PCR amplification can be used to identify a CXCR3 variant, such as SEQ ID NOS:2

or 4, or 6. Any of these methods can be used to detect disease, monitor disease progression and/or regression, and to evaluate the effects of treatments based on the presence or absence of a CXCR3 variant.

5 All journal article, reference and patent citations provided herein, including referenced sequence accession numbers of nucleotide and amino acid sequences contained in various databases, in parentheses or otherwise, whether previously stated or not, are
10 incorporated herein by reference in their entirety.

It is understood that modifications which do not substantially affect the activity of the various embodiments of this invention are also included within the definition of the invention provided herein. Accordingly,
15 the following examples are intended to illustrate but not limit the present invention.

EXAMPLE I

Identification of Alternatively Spliced CXCR3 receptor Variants

20 This example describes the discovery of two alternatively spliced CXCR3 receptor variants.

Using two sets of CXCR3 gene specific primers, RT-PCT was performed to assess CXCR3 gene expression in eight different human tissues (see Figure 5). The primers
25 used for amplification of CXCR3A-2 and CXCR3C were:
catttggaacacgatgtgc (SEQ ID NO:13) and
gatggctaggacccagatga (SEQ ID NO:14). The primers used for

amplification of CXCR3B-2 were: agttcctgccaggccttta (SEQ ID NO:15) and acatccgctcccgggaact (SEQ ID NO:16). As is shown in Figure 5, short PCR products corresponding to newly identified CXCR3A-2 and CXCR3B-2 were observed. Sequence analysis of the short PCR products was performed using standard procedures, and revealed the sequences of alternatively spliced CXCR3 isoforms CXCR3A-2 and CXCR3B-2, which are shown in Figures 1 and 2.

10

EXAMPLE II**Tissue Distribution of Alternatively Spliced
CXCR3 receptor Variants**

This example demonstrates the tissue distribution of alternatively spliced CXCR3 receptor variants CXCR3A-2 and CXCR3B-2 using RT-PCR.

Human multiple tissue RNA samples were purchased from BD Biosciences (Clontech). Using 5 mg of human total RNA, first strand cDNA was synthesized by SuperScript II RNase H reverse transcriptase (Life Technologies). Reactions (20 ml) containing 5 ml of RNA, 250 ng of oligo (dT), and 100 units of reverse transcriptase were incubated at 42°C for 1 hour and terminated by 100°C for 3 minutes.

PCR reactions contained the following: PCR buffer (10 mM Tris-HCl, pH 8.3, 50 mM KCl, 2 mM MgCl), 2.5 units AmpliTaq DNA polymerase, 0.2 mM forward and reverse primers, in a final volume of 50 ml. After an initial incubation for 5 minutes at 94°C, samples were individually

processed to 30 cycles of 30 seconds at 95°C, 30 seconds at 58°C, and 30 seconds at 72°C in a PE 9700 thermal cycler.

Multiple tissue RT-PCR analysis was performed to detect the alternatively spliced CXCR3 receptor variant 5 mRNA.

EXAMPLE III

Screening Assays using Alternatively Spliced CXCR3 Receptor Variants

This example describes assays for identifying 10 compounds that selectively interact with alternatively spliced CXCR3 receptor variants, such as agonists and antagonists of a CXCR3A-2, CXCR3B-2 or CXCR3C receptor variant.

HEK 293/EBNA cells transiently or stably 15 expressing CXCR3A-2 or CXCR3B-2/pcDNA3.1 plasmids are seeded at a density of 5×10^3 cells per well in Biocoat® Poly-D-lysine-coated black-wall, clear-bottom 96-well plates (Becton-Dickinson; Franklin Lakes, New Jersey) and allowed to attach overnight. At 48 hours after 20 transfection, the cells are washed two times with HBSS-HEPES buffer (Hanks Balanced Salt Solution without bicarbonate and phenol red, 20 mM HEPES, pH 7.4) using a Lab Systems Cellwash plate washer. After 45 minutes of dye-loading in the dark, using the calcium-sensitive dye 25 Fluo-4 AM at a final concentration of 2 mM, the plates are washed four times with HBSS-HEPES buffer to remove excess dye leaving 100 µl in each well. Plates are re-

equilibrated to 37°C for a few minutes. The cells are excited with an Argon laser at 488 nm, and emission is measured through a 510-570 nm bandwidth emission filter (FLIPR™; Molecular Devices; Sunnyvale, CA). Compound 5 solution is added in a 50 µl volume to each well to give the desired final concentration. The peak increase in fluorescence intensity is recorded for each well. To generate concentration-response curves, compounds are tested in duplicate in a concentration range between 10⁻¹¹ 10 and 10⁻⁵ M. The duplicate values are averaged.

All journal article, reference and patent citations provided herein, including referenced sequence accession numbers of nucleotide and amino acid sequences contained in various databases, in parentheses or 15 otherwise, whether previously stated or not, are incorporated herein by reference in their entirety.

Although the invention has been described with reference to the disclosed embodiments, those skilled in 20 the art will readily appreciate that the specific experiments detailed are only illustrative of the invention. It should be understood that various modifications can be made without departing from the spirit of the invention.

We claim:

1. An isolated CXCR3 variant polypeptide comprising the amino acid sequence referenced as SEQ ID NO:11, said polypeptide having at least 50% identity
5 with SEQ ID NO:8 or SEQ ID NO:10.
2. An isolated CXCR3 variant polypeptide, comprising an amino acid sequence selected from SEQ ID NOS:2 or 4, or a conservative variant thereof.
3. The isolated CXCR3 variant polypeptide of
10 claim 2, comprising an amino acid sequence selected from SEQ ID NOS:2 or 4.
4. The isolated CXCR3 variant polypeptide of claim 3, consisting of an amino acid sequence selected from SEQ ID NOS:2 or 4.
- 15 5. A CXCR3 variant binding agent that binds specifically to SEQ ID NO:11 or SEQ ID NO:12, or a portion thereof.
6. The binding agent of claim 5, which is an antibody or antigen binding fragment thereof.
- 20 7. A cell that exogenously expresses a polypeptide comprising the amino acid sequence referenced as SEQ ID NO:11, or a conservative variant thereof, said polypeptide having at least 50% identity with SEQ ID NO:8 or SEQ ID NO:10.

8. A method for identifying an agonist of a CXCR3 variant, comprising:

a) contacting a CXCR3 variant polypeptide
5 with a candidate compound, said polypeptide comprising SEQ ID NO:11, or a conservative variant thereof, and having at least 50% identity with SEQ ID NO:8 or SEQ ID NO 10, and

b) identifying a compound that selectively promotes production of a CXCR3 signal, said compound being
10 characterized as an agonist of a CXCR3 variant.

9. The method of claim 8, wherein said CXCR3 variant polypeptide comprises an amino acid sequence selected from SEQ ID NO:2 and 4, or a conservative variant thereof.

15 10. The method of claim 8, wherein said CXCR3 variant polypeptide is isolated.

11. The method of claim 8, wherein said CXCR3 variant polypeptide is expressed in a genetically engineered cell.

20 12. The method of claim 8, wherein said contacting occurs *in vitro*.

13. The method of claim 8, wherein said CXCR3 signal is calcium mobilization.

25 14. The method of claim 8, wherein said CXCR3 signal is cAMP upregulation.

15. The method of claim 8, wherein said CXCR3 signal is ligand binding.

16. The method of claim 8, wherein said compound is a polypeptide.

5 17. The method of claim 8, wherein said compound is a small molecule.

18. A method for identifying an antagonist of a CXCR3 variant, comprising:

a) contacting a CXCR3 variant polypeptide
10 with a candidate compound in the presence of a CXCR3 ligand, said polypeptide comprising SEQ ID NO:11, or a conservative variant thereof, and having at least 50% identity with SEQ ID NO:8 or SEQ ID NO:10, and

b) identifying a compound that selectively
15 inhibits production of a CXCR3 signal, said compound being characterized as an antagonist of a CXCR3 variant.

19. The method of claim 18, wherein said CXCR3 variant polypeptide comprises an amino acid sequence selected from SEQ ID NO:2 or 4, or a conservative variant
20 thereof.

20. The method of claim 18, wherein said CXCR3 variant polypeptide is isolated.

21. The method of claim 18, wherein said CXCR3 variant polypeptide is expressed in a genetically
25 engineered cell.

22. The method of claim 18, wherein said contacting occurs *in vitro*.

23. The method of claim 18, wherein said CXCR3 signal is calcium mobilization.

5 24. The method of claim 18, wherein said CXCR3 signal is cAMP upregulation.

25. The method of claim 18, wherein said CXCR3 signal is ligand binding.

26. The method of claim 18, wherein said
10 candidate compound is a polypeptide.

27. The method of claim 18, wherein said candidate compound is a small molecule.

28. A method for identifying a compound that specifically binds to a CXCR3 variant polypeptide,
15 comprising:

- a) contacting a CXCR3 variant polypeptide with a candidate compound, said polypeptide comprising SEQ ID NO:11, or a conservative variant thereof, and having at least 50% identity with SEQ ID NO:8 or SEQ ID NO:10, and
- 20 b) identifying a compound that specifically binds to said CXCR3 variant polypeptide.

29. The method of claim 28, wherein said CXCR3 variant polypeptide comprises an amino acid sequence selected from SEQ ID NO:2 or 4, or a conservative variant thereof.

5 30. The method of claim 28, wherein said CXCR3 variant polypeptide is isolated.

31. The method of claim 28, wherein said CXCR3 variant polypeptide is expressed in a genetically engineered cell.

10 32. The method of claim 28, wherein said contacting occurs *in vitro*.

33. The method of claim 28, wherein said candidate compound is a polypeptide.

15 34. The method of claim 28, wherein said candidate compound is a small molecule.

35. A method for identifying a compound that differentially modulates a CXCR3 isoform, comprising:

a) contacting a CXCR3 variant polypeptide with a candidate compound, said polypeptide comprising
20 SEQ ID NO:11, or a conservative variant thereof, and having at least 50% identity with SEQ ID NO:8 or SEQ ID NO:10 and

b) determining an a CXCR3 signal for said CXCR3 variant polypeptide;

c) contacting a distinct CXCR3 isoform polypeptide with said candidate compound;

d) determining a corresponding CXCR3 signal for said distinct CXCR3 isoform polypeptide; and

5 e) comparing CXCR3 signals determined for said CXCR3 variant polypeptide and said distinct CXCR3 isoform polypeptide, wherein a difference between said signals indicates that said candidate compound is a compound that differentially modulates a CXCR3 isoform.

10 36. The method of claim 35, wherein said distinct CXCR3 isoform polypeptide comprises SEQ ID NO:11, or a conservative variant thereof

37. The method of claim 35, wherein said CXCR3 variant polypeptide comprises an amino acid sequence
15 selected from SEQ ID NO:2 or 4, or a conservative variant thereof.

38. The method of claim 35, wherein said distinct CXCR3 isoform polypeptide comprises an amino acid sequence selected from SEQ ID NO:2 or 4, or a conservative
20 variant thereof.

39. The method of claim 35, wherein said CXCR3 variant polypeptide is isolated.

40. The method of claim 35, wherein said CXCR3 variant polypeptide is expressed in a genetically
25 engineered cell.

41. The method of claim 35, wherein said distinct CXCR3 isoform polypeptide is isolated.

42. The method of claim 35, wherein said distinct CXCR3 isoform polypeptide is expressed in a 5 genetically engineered cell.

43. The method of claim 35, wherein said contacting occurs *in vitro*.

44. The method of claim 35, wherein said CXCR3 signal is calcium mobilization.

10 45. The method of claim 35, wherein said CXCR3 signal is cAMP upregulation.

46. The method of claim 35, wherein said CXCR3 signal is IP3 release.

47. A method for identifying a compound that 15 differentially binds to a CXCR3 variant polypeptide, comprising:

a) contacting said CXCR3 variant polypeptide with a candidate compound, wherein said polypeptide comprises SEQ ID NO:11, or a conservative variant thereof, 20 and having at least 50% identity with SEQ ID NO:8 or SEQ ID NO:10;

b) determining specific binding of said candidate compound to said CXCR3 variant polypeptide;

c) contacting a distinct CXCR3 isoform 25 polypeptide with said candidate compound;

d) determining specific binding of said candidate compound to said distinct CXCR3 isoform polypeptide, and

e) comparing specific binding determined for
5 said CXCR3 variant polypeptide with specific binding determined for said distinct CXCR3 isoform polypeptide, wherein a difference between said specific binding indicates that said candidate compound is a compound that differentially binds to a CXCR3 isoform.

10 48. The method of claim 47, wherein said distinct CXCR3 isoform polypeptide comprises SEQ ID NO:11, or a conservative variant thereof

 49. The method of claim 47, wherein said CXCR3 variant polypeptide comprises an amino acid sequence
15 selected from SEQ ID NO:2 or 4, or a conservative variant thereof.

 50. The method of claim 47, wherein said distinct CXCR3 isoform polypeptide comprises an amino acid sequence selected from SEQ ID NO:2 or 4, or a conservative
20 variant thereof.

 51. The method of claim 47, wherein said CXCR3 variant polypeptide is isolated.

 52. The method of claim 47, wherein said CXCR3 variant polypeptide is expressed in a genetically
25 engineered cell.

53. The method of claim 47, wherein said distinct CXCR3 isoform polypeptide is isolated.

54. The method of claim 47, wherein said distinct CXCR3 isoform polypeptide is expressed in a
5 genetically engineered cell.

55. The method of claim 47, wherein said contacting occurs *in vitro*.

56. An isolated nucleic acid molecule,
comprising a nucleotide sequence that encodes a polypeptide
10 comprising the amino acid sequence referenced as SEQ ID
NO:11, said polypeptide having at least 50% identity with
SEQ ID NO:8 or 10.

57. An isolated nucleic acid molecule,
comprising a nucleotide sequence that encodes an amino acid
15 sequence selected from SEQ ID NOS:2 or 4, or a conservative
variant thereof.

58. The isolated nucleic acid molecule of claim
57, comprising a nucleotide sequence that encodes an amino
acid sequence selected from SEQ ID NOS:2 or 4.

20 59. The isolated nucleic acid molecule of claim
58, consisting of a nucleotide sequence that encodes an
amino acid sequence selected from SEQ ID NOS:2 or 4.

60. The isolated nucleic acid molecule of claim
59, wherein said nucleotide sequence is selected from SEQ
25 ID NOS:1 and 3.

61. A vector, comprising the isolated nucleic acid molecule of claim 56.

62. A host cell, comprising the vector of claim 61.

5

63. An isolated CXCR3C polypeptide comprising an amino acid sequence having at least 80% identity with SEQ ID NO:6.

10

64. A method for identifying an agonist of a CXCR3C, comprising:

a) contacting the CXCR3C polypeptide with a candidate compound, said polypeptide comprising an amino acid sequence having at least 80% identity with SEQ ID
15 NO:6, and

b) identifying a compound that selectively promotes production of a CXCR3 signal, said compound being characterized as an agonist of a CXCR3C.

65. A method for identifying an antagonist of a
20 CXCR3C, comprising:

a) contacting the CXCR3C polypeptide with a candidate compound in the presence of a CXCR3 ligand, said polypeptide comprising an amino acid sequence having at least 80% identity with SEQ ID NO:6, and

25

b) identifying a compound that selectively inhibits production of a CXCR3 signal, said compound being characterized as an antagonist of the CXCR3.

66. A method for identifying a compound that specifically binds to a CXCR3C, comprising:

a) contacting the CXCR3 polypeptide with a candidate compound, said polypeptide comprising an amino acid sequence having at least 80% identity with SEQ ID NO:6, and

b) identifying a compound that specifically binds to said CXCR3 variant polypeptide.

67. A method for identifying a compound that differentially modulates a CXCR3 isoform, comprising:

a) contacting a CXCR3C polypeptide with a candidate compound, said polypeptide comprising an amino acid sequence having at least 80% identity with SEQ ID NO:6, and

b) determining an a CXCR3 signal for said CXCR3C polypeptide;

c) contacting a distinct CXCR3 isoform polypeptide with said candidate compound;

d) determining a corresponding CXCR3 signal for said distinct CXCR3 isoform polypeptide; and

e) comparing CXCR3 signals determined for said CXCR3C polypeptide and said distinct CXCR3 isoform polypeptide, wherein a difference between said signals indicates that said candidate compound is a compound that differentially modulates a CXCR3 isoform.

68. A method for identifying a compound that differentially binds to a CXCR3C, comprising:

- a) contacting the CXCR3C polypeptide with a candidate compound, said polypeptide comprising an amino acid sequence having at least 80% identity with SEQ ID NO:6;
- b) determining specific binding of said candidate compound to said CXCR3C polypeptide;
- c) contacting a distinct CXCR3 isoform polypeptide with said candidate compound;
- d) determining specific binding of said candidate compound to said distinct CXCR3 isoform polypeptide, and
- e) comparing specific binding determined for said CXCR3C polypeptide with specific binding determined for said distinct CXCR3 isoform polypeptide, wherein a difference between said specific binding indicates that said candidate compound is a compound that differentially binds to a CXCR3 isoform.

69. An isolated nucleic acid molecule, comprising a nucleotide sequence that encodes a polypeptide that comprises an amino acid sequence having at least 80% identity with SEQ ID NO:6.

A

SEQ ID NO:1 CXCR3A-2 nucleotide sequence

```

atggtccttg aggtgagtga ccaccaagtg ctaaatagacg ccgaggttgc cgccctcctg 60
gagaacttca gctcttccta tgactatgga gaaaacgaga gtgactcgtg ctgtacctcc 120
ccgcccctgcc cacaggactt cagcctgaac ttcgaccggg ccttcctgcc agccctctac 180
agcctcctct ttctgctggg gctgctgggc aacggcgcgg tggcagccgt gctgctgagc 240
cggcggacag ccctgagcag caccgacacc ttcctgctcc acctagctgt agcagacacg 300
ctgctggtgc tgacactgcc gctctgggca gtggacgctg ccgtccagtg ggtctttggc 360
tctggcctct gcaaagtggc aggtgccctc ttcaacatca acttctacgc aggagccctc 420
ctgctggcct gcatcagctt tgaccgctac ctgaacatag ttcatgccac ccagctctac 480
cgccgggggc ccccgggccc cgtgaccctc acctgcctgg ctgtctgggg gctctgcctg 540
cttttcgccc tcccagactt catcttcctg tcggcccacc acgacgagcg cctcaacgcc 600
accactgcc aatacaactt cccacagggg tcaagttccg ggagcggatg tggatgctgc 660
tcttgcgctt gggctgcccc aaccagagag ggctccagag gcagccatcg tcttcccgcg 720
gggattcatc ctgggtctgag acctcagagg cctcctactc gggcttgtga 780

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B

SEQ ID NO:2 CXCR3A-2 amino acid sequence

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MVLEVSDHQV LNDAEVAALL ENFSSSYDYG ENESDSCCTS PPCPQDFSLN FDRAFLPALY
SLLFLLGLLG NGAAVAVLLS RRTALSSTDT FLLHLAVADT LLVLTPLWA VDAAVQWVFG
SGLCKVAGAL FNINFYAGAL LLACISFDY LNIVHATQLY RRGPPARVTL TCLAVWGLCL
LFALPDFIFL SAHDERLNA THCQYNFPQG SSSGSGCGCC SCAWAAPTRE GSRGSHRLPA
GIHPGLRPQR PPTRAC

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C

Comparison of amino acid sequences of human CXCR3A (top; SEQ ID NO:8) and human CXCR3A-2 (bottom)

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MVLEVSDHQV LNDAEVAALL ENFSSSYDYG ENESDSCCTS PPCPQDFSLN FDRAFLPALY
MVLEVSDHQV LNDAEVAALL ENFSSSYDYG ENESDSCCTS PPCPQDFSLN FDRAFLPALY

SLLFLLGLLG NGAAVAVLLS RRTALSSTDT FLLHLAVADT LLVLTPLWA VDAAVQWVFG
SLLFLLGLLG NGAAVAVLLS RRTALSSTDT FLLHLAVADT LLVLTPLWA VDAAVQWVFG

SGLCKVAGAL FNINFYAGAL LLACISFDY LNIVHATQLY RRGPPARVTL TCLAVWGLCL
SGLCKVAGAL FNINFYAGAL LLACISFDY LNIVHATQLY RRGPPARVTL TCLAVWGLCL

LFALPDFIFL SAHDERLNA THCQYNFPQV GRTALRVLQL VAGFLLPLLV MAYCYAHILA
LFALPDFIFL SAHDERLNA THCQYNFPQG SSSGSGCGCC SCAWAAPTRE GSRGSHRLPA

VLLVSRGQRR LRAMRLVVVV VVAFALCWTP YHLVVLVDIL MDLGALARNC GRESRVDVAK
GIHPGLRPQR PPTRAC

SVTSGLYMH CCLNPLLYAF VGVKFRERMW MLLLRLGCPN QRGLQRQPSS SRRDSSWSET
SEASYSGL

```

Figure 1

A

SEQ ID NO:3 CXCR3B-2 nucleotide sequence

```

atggagttga ggaagtacgg ccctggaaga ctggcgggga cagttatagg aggagctgct 60
cagagtaaat cacagactaa atcagactca atcacaaaag agttcctgcc aggcctttac 120
acagcccctt cctccccgtt cccgccctca cagggtgagt accaccaagt gctaaatgac 180
gccgaggttg ccgccctcct ggagaacttc agctcttcct atgactatgg agaaaacgag 240
agtgactcgt gctgtacctc cccgccctgc ccacaggact tcagcctgaa cttcgaccgg 300
gccttcctgc cagccctcta cagcctcctc tttctgctgg ggctgctggg caacggcgcg 360
gtggcagccg tgctgctgag ccggcggaca gccctgagca gcaccgacac cttcctgctc 420
cacttagctg tagcagacac gctgctggtg ctgacactgc cgctctgggc agtggacgct 480
gccgtccagt gggctcttgg ctctggcctc tgcaaagtgg cagggtgcct cttcaacatc 540
aacttctacg caggagccct cctgctggcc tgcacagct ttgaccgcta cctgaacata 600
gttcatgcca ccagctcta ccgccggggg cccccggccc gcgtgaccct cacctgcctg 660
gctgtctggg ggctctgcct gcttttcgcc ctcccagact tcattcttct gtcggccac 720
cacgacgagc gcctcaacgc caccactgc caatacaact tcccacagg gtcaagtcc 780
gggagcggat gtggatgctg ctcttgcgcc tgggctgccc caaccagaga gggctccaga 840
ggcagccatc gtcttccgcg cgggattcat cctggcttga gacctcagag gcctcctact 900
cgggcttgta a 960

```

B

SEQ ID NO:4 CXCR3B-2 amino acid sequence

```

MELRKYPGR LAGTVIGGAA QSKSQTKSDS ITKEFLPGLY TAPSSPFPPS QVSDHQVLND AEVAALLENF
SSSYDYGENE SDSCCTSPPC PQDFSLNFDR AFLPALYSLL FLLGLLGNGA VAAVLLSRRT ALSSTDFTLL
HLAVADTLLV LTLPLWAVDA AVQWVFGSGL CKVAGALFNI NFYAGALLLA CISFDRLNI VHATQLYRRG
PPARVTLTCL AVWGLCLLFA LPDFIFLSAH HDERLNATHC QYNFPQGSSS GSGCGCCSCA WAAPTREGSR
GSHRLPAGIH PGLRPQRPPT RAC

```

C

Comparison of amino acid sequences of human CXCR3B (top; SEQ ID NO:10) with human CXCR3B-2 (bottom)

```

MELRKYPGR LAGTVIGGAA QSKSQTKSDS ITKEFLPGLY TAPSSPFPPS QVSDHQVLND AEVAALLENF
MELRKYPGR LAGTVIGGAA QSKSQTKSDS ITKEFLPGLY TAPSSPFPPS QVSDHQVLND AEVAALLENF

SSSYDYGENE SDSCCTSPPC PQDFSLNFDR AFLPALYSLL FLLGLLGNGA VAAVLLSRRT ALSSTDFTLL
SSSYDYGENE SDSCCTSPPC PQDFSLNFDR AFLPALYSLL FLLGLLGNGA VAAVLLSRRT ALSSTDFTLL

HLAVADTLLV LTLPLWAVDA AVQWVFGSGL CKVAGALFNI NFYAGALLLA CISFDRLNI VHATQLYRRG
HLAVADTLLV LTLPLWAVDA AVQWVFGSGL CKVAGALFNI NFYAGALLLA CISFDRLNI VHATQLYRRG

PPARVTLTCL AVWGLCLLFA LPDFIFLSAH HDERLNATHC QYNFPQVGRT ALRVLQLVAG FLLPLLVMAY
PPARVTLTCL AVWGLCLLFA LPDFIFLSAH HDERLNATHC QYNFPQGSSS GSGCGCCSCA WAAPTREGSR

CYAHILAVLL VSRGQRLRA MRLVVVVVA FALCWTPYHL VVLVDILMDL GALARNCGRE SRVDVAKSVT
GSHRLPAGIH PGLRPQRPPT RAC

SGLGYMHCC L NPLLYAFVGV KFRERMWMLL LRLGCPNQRG LQRQPSSSR DSSWSETSEA SYSG L

```

Figure 2

A

SEQ ID NO:5 CXCR3C nucleotide sequence

```

1  ccaaccacaa gcaccaaagc agagggggcag gcagcacacc acccagcagc cagagcacca
61  gcccagccat ggtccttgag gtgagtgacc accaagtgct aaatgacgcc gaggttgccg
121 cctcctcgga gaacttcagc tcttcctatg actatggaga aaacgagagt gactcgtgct
181 gtacctcccc gccctgcccc caggacttca gcctgaactt cgaccgggccc ttctctgccag
241 ccctctacag cctcctcttt ctgctggggc tgctgggcaa cggcgcggtg gcagccgtgc
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B

SEQ ID NO:6 CXCR3C amino acid sequence

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MVLEVSDHQV LNDAEVAALL ENFSSSYDYG ENESDSCCTS PPCPQDFSLN FDRAFLPALY
SLLFLLGLLG NGAVAAVLLS RRTALSSTDT FLLFALPDFI FLSAHHDERL NATHCQYNFP
QVGR TALRVL QLVAGFLLPL LVMAYCYAHI LAVLLVSRGQ RRLRAMRLVV VVVVAFALCW
TPYHLVVLVD ILMDLGALAR NCGRESRVDV AKSVTSG LGY MHCCLNPLLY AFVGVKFRER
MWMLLLRLGC PNQRGLQRQP SSSRRDSSWS ETSEASYSGL

```

Figure 3

C

Comparison of amino acid sequences of human CXCR3A (top) with human CXCR3C (bottom)

```

3A MVLEVSDHQV LNDAEVAALL ENFSSSYDYG ENESDSCCTS PPCPQDFSLN FDRAFLPALY
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3A SLLFLLGLLG NGAVAAVLLS RRTALSSTDT FLLHLAVADT LLVLTPLPLWA VDAAVQWVFG
3C SLLFLLGLLG NGAVAAVLLS RRTALSSTDT FLLFALPDFI FLSAHDERL NATHCQYNFP

3A SGLCKVAGAL FNINFYAGAL LLACISFDYR LNIVHATQLY RRGPPARVTL TCLAVWGLCL
3C QVGRTALRVL QLVAGFLLPL LVMAYCYAHI LAVLLVSRGQ RRLRAMRLVV VVVVAFALCW

3A LFALPDFIFL SAHDERLNA THCQYNFPQV GRTALRVLQL VAGFLLPLLVM MAYCYAHILA
3C TPYHLVVLVD ILMDLGALAR NCGRESRVDV AKSVTSGLGY MHCCLNPLLY AFVGVKFRER

3A VLLVSRGQRR LRAMRLVVVV VVAFALCWTP YHLVVLVDIL MDLGALARNC GRESRVDVAK
3C MWMLLLRLGC PNQRLQRP SSSRDSSWS ETSEASYSGL -----

3A SVTSGLGVMH CCLNPLLYAF VGVKFRERMW MLLRLGCPN QRLQRPSS SRDSSWSET
3C SEASYSGL

3A FALPDFIFLS AHDERLNAT HCQYNFPQVG RTALRVLQLV AGFLLPLLVM AYCYAHILAV

3A LLVSRGQRR LRAMRLVVVV VVAFALCWTP YHLVVLVDIL MDLGALARNCGR ESRVDVAKSV

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3A ASYSGL

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Figure 3, cont.

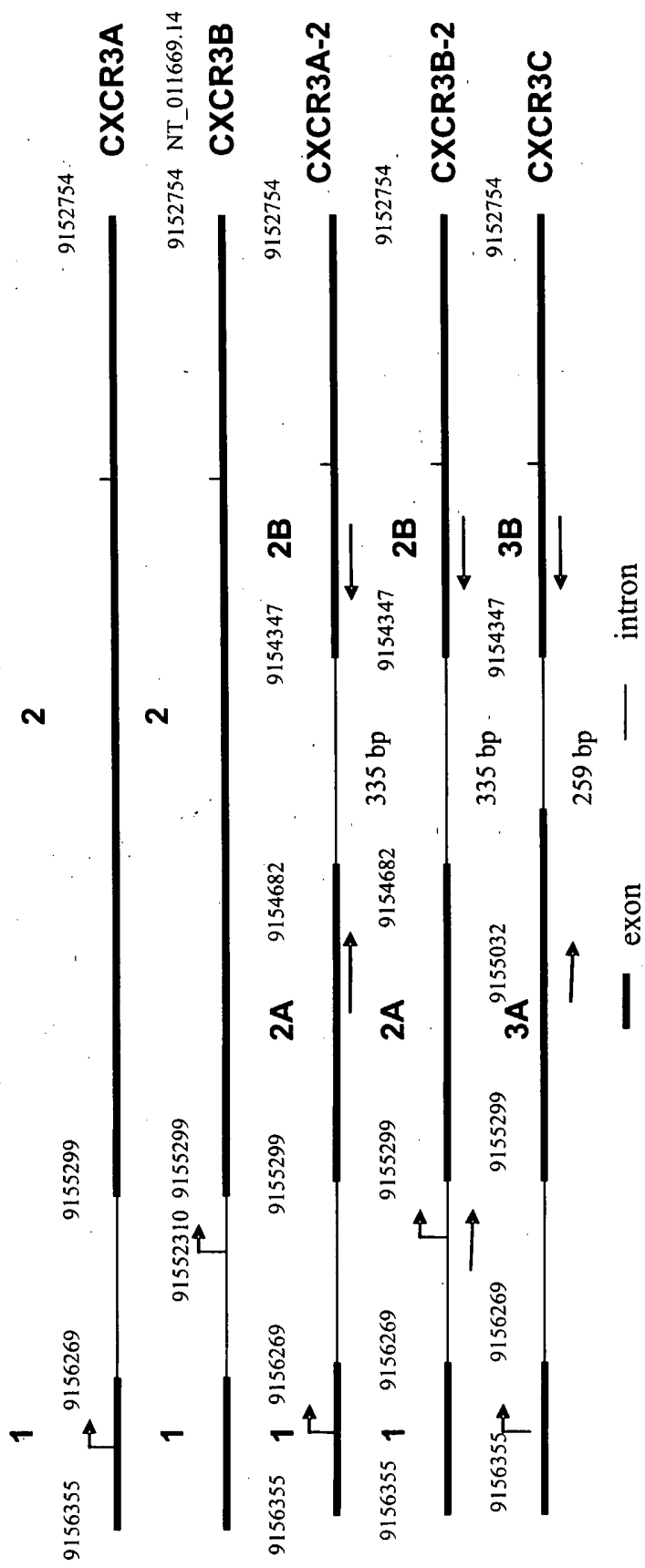


Figure 4

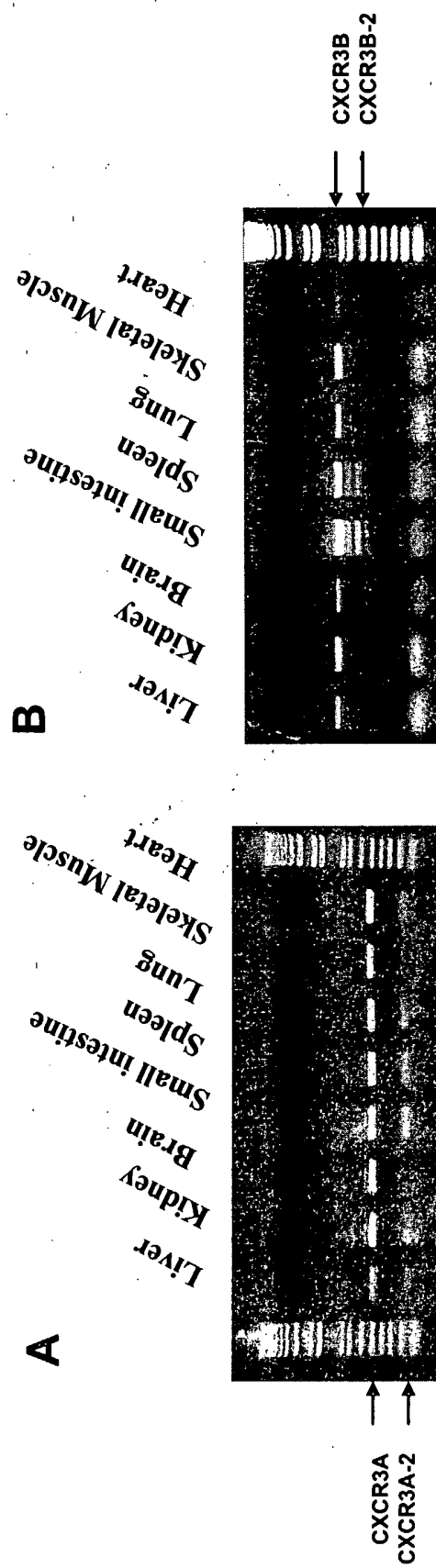


Figure 5

SEQUENCE LISTING

<110> Liang, Yanbin
Woodward, Davis F.

<120> Human CXCR3 Receptor Variants and
Methods of Use

<130> 17713 (AP)

<160> 12

<170> FastSEQ for Windows Version 4.0

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<212> DNA

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ccgccttgcc cacaggactt cagcctgaac ttcgaccggg ccttcctgcc agccctctac 180
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Glu Ser Asp Ser Cys Cys Thr Ser Pro Pro Cys Pro Gln Asp Phe Ser
 35           40           45
Leu Asn Phe Asp Arg Ala Phe Leu Pro Ala Leu Tyr Ser Leu Leu Phe
 50           55           60
Leu Leu Gly Leu Leu Gly Asn Gly Ala Val Ala Val Leu Leu Ser
 65           70           75           80
Arg Arg Thr Ala Leu Ser Ser Thr Asp Thr Phe Leu Leu His Leu Ala
 85           90           95
Val Ala Asp Thr Leu Leu Val Leu Thr Leu Pro Leu Trp Ala Val Asp
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			20					25					30		
Lys	Glu	Phe	Leu	Pro	Gly	Leu	Tyr	Thr	Ala	Pro	Ser	Ser	Pro	Phe	Pro
		35					40					45			
Pro	Ser	Gln	Val	Ser	Asp	His	Gln	Val	Leu	Asn	Asp	Ala	Glu	Val	Ala

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Ala Leu Leu Glu Asn Phe	Ser Ser Ser Tyr Asp	Tyr Gly Glu Asn Glu
65	70	75
Ser Asp Ser Cys Cys Thr	Ser Pro Pro Cys Pro	Gln Asp Phe Ser Leu
85	90	95
Asn Phe Asp Arg Ala Phe	Leu Pro Ala Leu Tyr	Ser Leu Leu Phe Leu
100	105	110
Leu Gly Leu Leu Gly Asn	Gly Ala Val Ala Ala	Val Leu Leu Ser Arg
115	120	125
Arg Thr Ala Leu Ser Ser	Thr Asp Thr Phe Leu	Leu His Leu Ala Val
130	135	140
Ala Asp Thr Leu Leu Val	Leu Thr Leu Pro Leu	Trp Ala Val Asp Ala
145	150	155
Ala Val Gln Trp Val Phe	Gly Ser Gly Leu Cys	Lys Val Ala Gly Ala
165	170	175
Leu Phe Asn Ile Asn Phe	Tyr Ala Gly Ala Leu	Leu Leu Ala Cys Ile
180	185	190
Ser Phe Asp Arg Tyr Leu	Asn Ile Val His Ala	Thr Gln Leu Tyr Arg
195	200	205
Arg Gly Pro Pro Ala Arg	Val Thr Leu Thr Cys	Leu Ala Val Trp Gly
210	215	220
Leu Cys Leu Leu Phe Ala	Leu Pro Asp Phe Ile	Phe Leu Ser Ala His
225	230	235
His Asp Glu Arg Leu Asn	Ala Thr His Cys Gln	Tyr Asn Phe Pro Gln
245	250	255
Gly Ser Ser Ser Gly Ser	Gly Cys Gly Cys Cys	Ser Cys Ala Trp Ala
260	265	270
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          20          25          30
Glu Ser Asp Ser Cys Cys Thr Ser Pro Pro Cys Pro Gln Asp Phe Ser
          35          40          45
Leu Asn Phe Asp Arg Ala Phe Leu Pro Ala Leu Tyr Ser Leu Leu Phe
          50          55          60
Leu Leu Gly Leu Leu Gly Asn Gly Ala Val Ala Ala Val Leu Leu Ser
65          70          75          80
Arg Arg Thr Ala Leu Ser Ser Thr Asp Thr Phe Leu Leu Phe Ala Leu
          85          90          95
Pro Asp Phe Ile Phe Leu Ser Ala His His Asp Glu Arg Leu Asn Ala
          100          105          110
Thr His Cys Gln Tyr Asn Phe Pro Gln Val Gly Arg Thr Ala Leu Arg
          115          120          125
Val Leu Gln Leu Val Ala Gly Phe Leu Leu Pro Leu Leu Val Met Ala
          130          135          140
Tyr Cys Tyr Ala His Ile Leu Ala Val Leu Leu Val Ser Arg Gly Gln
145          150          155          160
Arg Arg Leu Arg Ala Met Arg Leu Val Val Val Val Val Ala Phe
          165          170          175
Ala Leu Cys Trp Thr Pro Tyr His Leu Val Val Leu Val Asp Ile Leu
          180          185          190
Met Asp Leu Gly Ala Leu Ala Arg Asn Cys Gly Arg Glu Ser Arg Val
          195          200          205
Asp Val Ala Lys Ser Val Thr Ser Gly Leu Gly Tyr Met His Cys Cys
          210          215          220
Leu Asn Pro Leu Leu Tyr Ala Phe Val Gly Val Lys Phe Arg Glu Arg
225          230          235          240
Met Trp Met Leu Leu Leu Arg Leu Gly Cys Pro Asn Gln Arg Gly Leu
          245          250          255
Gln Arg Gln Pro Ser Ser Ser Arg Arg Asp Ser Ser Trp Ser Glu Thr
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Ser Glu Ala Ser Tyr Ser Gly Leu
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<213> CXCR3A-1

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Glu Ser Asp Ser Cys Cys Thr Ser Pro Pro Cys Pro Gln Asp Phe Ser
35      40      45
Leu Asn Phe Asp Arg Ala Phe Leu Pro Ala Leu Tyr Ser Leu Leu Phe
50      55      60
Leu Leu Gly Leu Leu Gly Asn Gly Ala Val Ala Val Leu Leu Ser
65      70      75      80
Arg Arg Thr Ala Leu Ser Ser Thr Asp Thr Phe Leu Leu His Leu Ala
85      90      95
Val Ala Asp Thr Leu Leu Val Leu Thr Leu Pro Leu Trp Ala Val Asp
100      105      110
Ala Ala Val Gln Trp Val Phe Gly Ser Gly Leu Cys Lys Val Ala Gly
115      120      125
Ala Leu Phe Asn Ile Asn Phe Tyr Ala Gly Ala Leu Leu Leu Ala Cys
130      135      140
Ile Ser Phe Asp Arg Tyr Leu Asn Ile Val His Ala Thr Gln Leu Tyr
145      150      155      160
Arg Arg Gly Pro Pro Ala Arg Val Thr Leu Thr Cys Leu Ala Val Trp
165      170      175
Gly Leu Cys Leu Leu Phe Ala Leu Pro Asp Phe Ile Phe Leu Ser Ala
180      185      190
His His Asp Glu Arg Leu Asn Ala Thr His Cys Gln Tyr Asn Phe Pro
195      200      205

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Val	Leu	Leu	Val	Ser	Arg	Gly	Gln	Arg	Arg	Leu	Arg	Ala	Met	Arg	Leu
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Val	Val	Val	Val	Val	Val	Ala	Phe	Ala	Leu	Cys	Trp	Thr	Pro	Tyr	His
			260					265					270		
Leu	Val	Val	Leu	Val	Asp	Ile	Leu	Met	Asp	Leu	Gly	Ala	Leu	Ala	Arg
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 35 40 45
 Pro Ser Gln Val Ser Asp His Gln Val Leu Asn Asp Ala Glu Val Ala
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 Ala Leu Leu Glu Asn Phe Ser Ser Ser Tyr Asp Tyr Gly Glu Asn Glu
 65 70 75 80
 Ser Asp Ser Cys Cys Thr Ser Pro Pro Cys Pro Gln Asp Phe Ser Leu
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 Asn Phe Asp Arg Ala Phe Leu Pro Ala Leu Tyr Ser Leu Leu Phe Leu
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 Leu Gly Leu Leu Gly Asn Gly Ala Val Arg Ala Val Leu Leu Ser Arg
 115 120 125
 Arg Thr Ala Leu Ser Ser Thr Asp Thr Phe Leu Leu His Leu Ala Val
 130 135 140
 Ala Asp Thr Leu Leu Val Leu Thr Leu Pro Leu Trp Ala Val Asp Ala
 145 150 155 160
 Ala Val Gln Trp Val Phe Gly Ser Gly Leu Cys Lys Val Ala Gly Ala
 165 170 175
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 Ser Phe Asp Arg Tyr Leu Asn Ile Val His Ala Thr Gln Leu Tyr Arg
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 Arg Gly Pro Pro Ala Arg Val Thr Leu Thr Cys Leu Ala Val Trp Gly
 210 215 220
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 225 230 235 240
 His Asp Glu Arg Leu Asn Ala Thr His Cys Gln Tyr Asn Phe Pro Gln
 245 250 255
 Val Gly Arg Thr Ala Leu Arg Val Leu Gln Leu Val Ala Gly Phe Leu
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 275 280 285
 Leu Leu Val Ser Arg Gly Gln Arg Arg Leu Arg Ala Met Arg Leu Val
 290 295 300
 Val Val Val Val Val Ala Phe Ala Leu Cys Trp Thr Pro Tyr His Leu
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 Val Val Leu Val Asp Ile Leu Met Asp Leu Gly Ala Leu Ala Arg Asn
 325 330 335
 Cys Gly Arg Glu Ser Arg Val Asp Val Ala Lys Ser Val Thr Ser Gly
 340 345 350
 Leu Gly Tyr Met His Cys Cys Leu Asn Pro Leu Leu Tyr Ala Phe Val
 355 360 365

Gly Val Lys Phe Arg Glu Arg Met Trp Met Leu Leu Leu Arg Leu Gly
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 Ile His Pro Gly Leu Arg Pro Gln Arg Pro Pro Thr Arg Ala Cys
 35 40 45

<210> 12
 <211> 187
 <212> PRT
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<400> 12
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 1 5 10 15
 Leu Asn Ala Thr His Cys Gln Tyr Asn Phe Pro Gln Val Gly Arg Thr
 20 25 30
 Ala Leu Arg Val Leu Gln Leu Val Ala Gly Phe Leu Leu Pro Leu Leu
 35 40 45
 Val Met Ala Tyr Cys Tyr Ala His Ile Leu Ala Val Leu Leu Val Ser
 50 55 60
 Arg Gly Gln Arg Arg Leu Arg Ala Met Arg Leu Val Val Val Val
 65 70 75 80
 Val Ala Phe Ala Leu Cys Trp Thr Pro Tyr His Leu Val Val Leu Val
 85 90 95
 Asp Ile Leu Met Asp Leu Gly Ala Leu Ala Arg Asn Cys Gly Arg Glu
 100 105 110
 Ser Arg Val Asp Val Ala Lys Ser Val Thr Ser Gly Leu Gly Tyr Met
 115 120 125
 His Cys Cys Leu Asn Pro Leu Leu Tyr Ala Phe Val Gly Val Lys Phe
 130 135 140
 Arg Glu Arg Met Trp Met Leu Leu Leu Arg Leu Gly Cys Pro Asn Gln
 145 150 155 160
 Arg Gly Leu Gln Arg Gln Pro Ser Ser Ser Arg Arg Asp Ser Ser Trp
 165 170 175
 Ser Glu Thr Ser Glu Ala Ser Tyr Ser Gly Leu
 180 185

INTERNATIONAL SEARCH REPORT

Int. Application No.
PCT/US2005/017636

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C07K14/715 C12N5/10 G01N33/50 C12N15/19

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

IPC 7 C07K C12N G01N

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

EPO-Internal, BIOSIS, EMBASE, WPI Data, PAJ, Sequence Search

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	LASAGNI LAURA ET AL: "An alternatively spliced variant of CXCR3 mediates the inhibition of endothelial cell growth induced by IP-10, Mig, and I-TAC, and acts as functional receptor for platelet factor 4" JOURNAL OF EXPERIMENTAL MEDICINE, TOKYO, JP, vol. 197, no. 11, 2 June 2003 (2003-06-02), pages 1537-1549, XP002246737 ISSN: 0022-1007 page 1539, column 2 - page 1546, column 1 -----	1-4,6-69
Y	WO 99/50299 A (ASTRA PHARMACEUTICALS LTD; ASTRA AKTIEBOLAG; DELANEY, STEPHEN) 7 October 1999 (1999-10-07) figure 2 ----- -/--	1-4,6-69



Further documents are listed in the continuation of box C.



Patent family members are listed in annex.

* Special categories of cited documents:

- *A* document defining the general state of the art which is not considered to be of particular relevance
- *E* earlier document but published on or after the international filing date
- *L* document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)
- *O* document referring to an oral disclosure, use, exhibition or other means
- *P* document published prior to the international filing date but later than the priority date claimed

- *T* later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
- *X* document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
- *Y* document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.
- * & * document member of the same patent family

Date of the actual completion of the international search

9 September 2005

Date of mailing of the international search report

06/10/2005

Name and mailing address of the ISA

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Mabit, H.

INTERNATIONAL SEARCH REPORT

onal Application No
PCT/US2005/017636

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
A	US 6 140 064 A (LOETSCHER ET AL) 31 October 2000 (2000-10-31) claims 1-88 -----	
P,A	EHLERT JAN ERIK ET AL: "Identification and partial characterization of a variant of human CXCR3 generated by posttranscriptional exon skipping" JOURNAL OF IMMUNOLOGY, vol. 173, no. 10, 15 November 2004 (2004-11-15), pages 6234-6240, XP002344010 ISSN: 0022-1767 -----	

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US2005/017636

Box II Observations where certain claims were found unsearchable (Continuation of item 2 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☒ Claims Nos.: **claim 5**
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:
see FURTHER INFORMATION sheet PCT/ISA/210
3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box III Observations where unity of invention is lacking (Continuation of item 3 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. ☐ As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; It is covered by claims Nos.:

Remark on Protest

- ☐ The additional search fees were accompanied by the applicant's protest.
- ☐ No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box II.2

Claims Nos.: claim 5

Claim 5 relates to a CXCR3 variant binding agent without giving a true technical characterization of the claimed agent (except when related to the embodiment disclosed in claim 6, i.e antibody). Consequently, the scope of said claims is ambiguous and vague, and its subject-matter is not sufficiently disclosed and supported (Art. 5 and 6 PCT). The non-compliance with the substantive provisions is such to an extent that no meaningful search of claim 5 (when not related to the embodiment of claim 6) could be carried out (Article 17(2) PCT and PCT Guidelines 9.26).

The applicant's attention is drawn to the fact that claims relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure. If the application proceeds into the regional phase before the EPO, the applicant is reminded that a search may be carried out during examination before the EPO (see EPO Guideline C-VI, 8.5), should the problems which led to the Article 17(2) declaration be overcome.

INTERNATIONAL SEARCH REPORT

Int al Application No
PCT/US2005/017636

Patent document cited in search report		Publication date	Patent family member(s)	Publication date
WO 9950299	A	07-10-1999	AU 3543599 A	18-10-1999
			WO 9950299 A1	07-10-1999
US 6140064	A	31-10-2000	AU 734090 B2	07-06-2001
			AU 4260897 A	02-04-1998
			CA 2265915 A1	19-03-1998
			EP 0925358 A1	30-06-1999
			JP 2002513388 T	08-05-2002
			WO 9811218 A1	19-03-1998
			US 2003158392 A1	21-08-2003
			US 6833439 B1	21-12-2004
			US 6686175 B1	03-02-2004
			US 6184358 B1	06-02-2001