Abstract:

Title: NOVEL ANTIBODIES AND USES THEREFOR

A monoclonal antibody or antigen-binding fragment thereof that binds to human CXCR7 or a fragment thereof, the antibody comprising within the light chain variable region at least one complementarity determining region (CDR) comprising a sequence as set forth in any one of SEQ ID NOs: 1 to 3 or a variant thereof; and/or within the heavy chain variable region at least one complementarity determining region (CDR) comprising a sequence as set forth in any one of SEQ ID NOs: 4 to 6 or a variant thereof.
Novel Antibodies and Uses Therefor

Field of the Invention

The present invention relates generally to antibodies that bind to the chemokine receptor CXCR7. More particularly the invention relates to monoclonal antibodies specific for CXCR7 and having antagonistic or inhibitory activity. The invention also relates to uses of such antibodies in the treatment or prevention of CXCR7-associated disorders. The invention further relates to methods for modulating CXCR7-associated disorders by the administration of antibodies of the invention.

Background of the Invention

Chemokines (chemoattractant cytokines) are a family of structurally and functionally related small (8-10 kDa) proteins that play an important role in host defence, controlling leukocyte and lymphocyte migration from the blood to inflammatory sites, as well as controlling other critical biological processes such as embryogenesis, angiogenesis, stem cell trafficking and neuronal migration during development of the central nervous system. Chemokines are early triggers of the inflammatory response, inducing the release of pro-inflammatory mediators and the chemotaxis of inflammatory cells such as neutrophils and eosinophils to inflamed sites.

Chemokines mediate their biological activity via binding to cell surface receptors of the 7-transmembrane G protein-coupled receptor (GPCR) family. The chemokine receptors, about 20 of which have been described to date, comprise the largest subfamily (Class A rhodopsin-like) in the GPCR superfamily.

More than 50 different chemokines are recognised and these are classified into families (CC, CXC, CX3C, XC) based on the positioning of conserved cysteine residues. For example CXC chemokines are characterised by the first two of four conserved cysteines being separated by a single amino acid. Similarly, chemokine receptors are grouped and named (CC, CXC, C3XC, XC) according to nature of their chemokine ligands.

The chemokine receptor CXCR7 is highly conserved in mammals, with more than 91% identity and 95% similarity between human, mouse and dog proteins. First identified as an orphan receptor expressed in the human thyroid, it is now known that CXCR7 is expressed in a wide range of tissues in humans and binds the CXC chemokines CXCL12 (SDF-1) and CXCL11 (I-TAC). In addition to normal developmental roles, CXCR7 is associated with a number of disorders and disease states.
For example, CXCR7 expression is upregulated in some tumours, such as breast, prostate and lung tumours and blockade of CXCR7 inhibits tumour growth in several mouse models. Similar to some other chemokine receptors, CXCR7 may also facilitate angiogenesis. CXCR7 may also facilitate the migration of populations of progenitor cells. Human renal progenitor cells exhibit high expression of both CXCR4 and CXCR7. In SCID mice with acute renal failure, intravenously injected renal stem/progenitor cells engrafted into injured renal tissue decreased the severity of acute renal failure and prevented renal fibrosis. These beneficial effects were abolished by blocking CXCR7, which suggests that inhibition of CXCR7 might be detrimental for the development of renal fibrosis (Mazzinghi et al., 2008, J. Exp. Med. 205:479-490).

Thus, manipulation of CXCR7 through agonism or antagonism represents an attractive and promising target for the development of therapeutic agents. In particular antagonists such as monoclonal antibodies capable of binding to CXCR7 and competing with the normal CXCR7 ligands may affect a number of cellular processes such as tumour cell growth and development, and the migration of progenitor cells.

However the generation of suitable chemokine receptor inhibitors has to date been retarded by the focus on, and difficulties encountered in, developing small molecule inhibitors to certain members of the chemokine receptor family. Antibody based therapies offer important advantages over small molecules including a long half-life in blood (thus requiring less frequent dosing), and a safer and more predictable route to clinical trial. In addition, certain small molecules targeting CXCR7 can act as agonists rather than antagonists. For instance the CXCR4 antagonist AMD3100 paradoxically serves as an allosteric agonist for CXCR7 (Kalatskaya, et al. 2009, Mol. Pharmacol. 75:1240-1247). Therefore the development of small molecule CXCR7 competitive antagonists may prove challenging.

Accordingly, monoclonal antibodies represent a particularly promising new class of chemokine receptor antagonists. Despite this, and whilst antibodies against CXCR7 are known, no blocking anti-CXCR7 antibodies have to date been isolated and developed for therapeutic use. There remains a need for the development of novel monoclonal antibodies having effective CXCR7 inhibitory activity. The ideal properties of such an antibody would also involve recognition of a conserved epitope between mouse and human CXCR7, which would facilitate clinical development of the antibody.

Summary of the Invention
The articles "a" and "an" are used herein to refer to one or to more than one (i.e., to at least one) of the grammatical object of the article. By way of example, "an element" means one element or more than one element.

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" or "comprising", will be understood to imply the inclusion of a stated integer or step or group of integers or steps but not the exclusion of any other integer or step or group of integers or steps.

The present invention relates generally to antibodies that bind, interact or otherwise associate with CXCR7 or a fragment, portion or part thereof and to antibodies that antagonize or neutralize CXCR7 activity or CXCL12 ligand binding. The antibodies are typically monoclonal antibodies or antigen-binding fragments thereof. Typically, the antibodies are in isolated, homogenous or fully or partially purified form. The antibodies may be, for example, murine monoclonal antibodies, humanized antibodies prepared from such murine antibodies or human monoclonal antibodies which may be prepared, for example, using transgenic mice or by phage display.

According to a first aspect of the present invention there is provided a monoclonal antibody or antigen-binding fragment thereof that binds to human CXCR7 or a fragment thereof, the antibody comprising:

(i) within the light chain variable region at least one complementarity determining region (CDR) comprising a sequence as set forth in any one of SEQ ID N0s:1 to 3 or a variant thereof; and/or

(ii) within the heavy chain variable region at least one complementarity determining region (CDR) comprising a sequence as set forth in any one of SEQ ID N0s:4 to 6 or a variant thereof.

In an embodiment, the antibody or antigen-binding fragment thereof comprises within the light chain variable region at least one CDR comprising a sequence as set forth in any one of SEQ ID N0s:1 to 3 or a variant thereof and within the heavy chain variable region at least one CDR comprising a sequence as set forth in any one of SEQ ID N0s:4 to 6 or a variant thereof.

In an embodiment, the antibody or antigen-binding fragment thereof comprises a light chain variable region comprising the sequence as set forth in SEQ ID NO:13 or a fragment or variant thereof.
embodiment, the antibody or antigen-binding fragment thereof comprises a heavy chain variable region comprising the sequence as set forth in SEQ ID NO:14 or a fragment or variant thereof.

The antibody may comprise a light chain variable region comprising the sequence as set forth in SEQ ID NO:13 or a fragment or variant thereof and a heavy chain variable region comprising the sequence as set forth in SEQ ID NO:14 or a fragment or variant thereof.

The antibody may comprise a light chain variable region comprising the sequence as set forth in SEQ ID NO:13 or a fragment or variant thereof and a heavy chain variable region comprising the sequence as set forth in SEQ ID NO:20 or a fragment or variant thereof.

The antibody may comprise a light chain variable region comprising the sequence as set forth in SEQ ID NO:15 or a fragment or variant thereof and a heavy chain variable region comprising the sequence as set forth in SEQ ID NO:18 or a fragment or variant thereof.

The antibody may comprise a light chain variable region comprising the sequence as set forth in SEQ ID NO:16 or a fragment or variant thereof and a heavy chain variable region comprising the sequence as set forth in SEQ ID NO:14 or a fragment or variant thereof.

The antibody may comprise a light chain variable region comprising the sequence as set forth in SEQ ID NO:16 or a fragment or variant thereof and a heavy chain variable region comprising the sequence as set forth in SEQ ID NO:19 or a fragment or variant thereof.

According to a second aspect of the present invention there is provided a monoclonal antibody or antigen-binding fragment thereof that binds to human CXCR7 or a fragment thereof, the antibody comprising:

(iii) within the light chain variable region at least one complementarity determining region (CDR) comprising a sequence as set forth in any one of SEQ ID NOs:7 to 9 or a variant thereof; and/or

(iv) within the heavy chain variable region at least one complementarity determining region (CDR) comprising a sequence as set forth in any one of SEQ ID NOs:10 to 12 or a variant thereof.

In an embodiment, the antibody or antigen-binding fragment thereof comprises within the light chain variable region at least one CDR comprising a sequence as set forth in any one of SEQ ID NOs:7 to
' 9 or a variant thereof and within the heavy chain variable region at least one CDR comprising a sequence as set forth in any one of SEQ ID NOs:10 to 12 or a variant thereof.

The antibody may comprise a light chain variable region comprising the sequence as set forth in SEQ ID NO: 17 or a fragment or variant thereof and/or a heavy chain variable region comprising the sequence as set forth in SEQ ID NO:21 or a fragment or variant thereof.

The invention also provides for monoclonal antibodies comprising an amino acid sequence having at least about 70% sequence identity to an amino acid sequence as set forth in any one of SEQ ID Nos: 1 to 21.

The invention also provides hybridomas producing the monoclonal antibodies of the invention.

According to a third aspect there is provided a method for the treatment or prevention of a CXCR7-associated disorder, the method comprising administering to a subject in need thereof an effective amount of at least one antibody, or an antigen-binding fragment thereof, of the first or second aspect.

By way of example, the disorder may be a fibrotic disorder, cancer or an inflammatory disorder. The cancer may be selected from, for example, breast or lung cancer. Proliferation of one or more cancer cells may be inhibited following administration of the antibody or antigen-binding fragment. Angiogenesis may be inhibited following administration of the antibody or antigen-binding fragment. The fibrotic disorder may be, for example, pulmonary fibrosis, fibrosis of the airways, cardiac fibrosis, liver fibrosis or renal fibrosis. The inflammatory disorder may be associated with airways inflammation. Airways hyperresponsiveness may be reduced following administration of the antibody or antigen-binding fragment.

According to a fourth aspect there is provided the use of an antibody, or an antigen-binding fragment thereof, of the first or second aspect for the manufacture of a medicament for treating or preventing a CXCR7-associated disorder.

According to a fifth aspect there is provided a method for inhibiting angiogenesis in a subject, the method comprising administering to the subject an effective amount of at least one antibody, or an antigen-binding fragment thereof, of the first or second aspect.
According to a sixth aspect there is provided a method for reducing airway hyperresponsiveness in a subject, the method comprising administering to the subject an effective amount of at least one antibody, or an antigen-binding fragment thereof, of the first or second aspect.

According to a seventh aspect there is provided a method of detecting a cell expressing CXCR7 in a biological sample, the method comprising contacting the biological sample with an antibody or antigen-binding fragment of the first or second aspect and detecting the presence of the antibody or antigen-binding fragment.

Typically, the antibody or antigen-binding fragment is linked to a detectable label.

Also provided herein is the use of antibodies, or antigen-binding fragments thereof, as disclosed herein as therapeutic agents. The antibody or antigen-binding fragment may be, for example, an anti-fibrotic, anti-angiogenic or anti-cancer agent.

Also provided herein are pharmaceutical compositions comprising one or more antibodies, or antigen-binding fragments thereof, as disclosed herein optionally together with suitable pharmaceutically acceptable carriers and/or diluents.

**Brief Description of the Drawings**

The present invention will now be described, by way of non-limiting example only, with reference to the accompanying drawings.

**Figure 1.** A. Dot plots illustrating the specific reactivity of three anti-CXCR7 monoclonal antibodies, designated 7A9, 10D1 and 6G2, with L1.2/hCXCR7 transfectants. These antibodies reacted strongly with human CXCR7 L1.2 transfectants, but not with untransfected L1.2 cells or with CXCR2 transfected L1.2 cells. B. Several of the antibodies also reacted with mouse CXCR7 transfectants.

Plot of fluorescence intensity for the monoclonal antibody 10D1 in transfected and untransfected L1.2 cells, illustrating reactivity of the antibody with both human cxcr7-transfected cells and murine cxcr7-transfected cells. Dotted line, L1.2 non-transfected cells; grey line, murine Cxcr7-transfected L1.2 cells; black line, human CXCR7-transfected L1.2 cells.

**Figure 2.** Graphical representation showing percentage inhibition of binding of 125I labeled CXCL12 to L1.2/hCXCR7 transfected cells (Y-axis) by the indicated monoclonal antibodies.
- Figure 3. Alignment of variable light chain sequences of five anti-CXCR7 monoclonal antibodies, designated 6G2, 6G7, 7A9, 8C1 and 10D1. CDRs 1, 2 and 3 are boxed. The variable light chain consensus sequence is also shown.

- Figure 4. Alignment of variable heavy chain sequences of five anti-CXCR7 monoclonal antibodies, designated 6G2, 6G7, 7A9, 8C1 and 10D1. CDRs 1, 2 and 3 are boxed. The variable heavy chain consensus sequence is also shown.

- Figure 5. Schematic representation of the in vivo mouse model of lung fibrosis used as exemplified herein.

- Figure 6. Reduction in airway resistance (% change in saline) in the presence of anti-CXCR7 monoclonal antibody 10D1. Open diamonds, saline/saline; open squares, saline/isotype control; open circles, saline/10D1; filled diamonds, TGFβ/saline; filled squares, TGFβ/isotype control; filled circles, TGFβ/10D1.

- Figure 7. Effect of anti-CXCR7 monoclonal antibody 10D1 on bleomycin pulmonary fibrosis

A Reduction in airway resistance (% change in saline)
- o- Sham
- □. Saline/Iso
- ' ' Bleomycin/Ab
- _ Bleomycin
- • - Bleo/Iso
- _ Saline/Ab

B Tissue sections from bleomycin administered mice with isotype control antibody (top panel) or 10D1 antibody (bottom panel)

Amino acid and polynucleotide sequences are referred to by a sequence identifier number (SEQ ID NO:). The SEQ ID NOs: correspond numerically to the sequence identifiers <400>1 (SEQ ID NO:1), <400>2 (SEQ ID NO:2), etc. A sequence listing is provided at the end of the specification. Specifically, the amino acid sequences of variable light chains and variable heavy chains and CDRs of antibodies in accordance with the invention are set forth in SEQ ID NOs:1 to 21. The variable light chain (Vk) CDRs of monoclonal antibodies 6G2, 6G7, 7A9 and 8C1 are shown in SEQ ID NOs:1 to 3. The variable heavy chain (Vh) CDRs of monoclonal antibodies 6G2, 6G7, 7A9 and 8C1 are shown in SEQ ID NOs:4 to 6. The variable light chain (Vk) CDRs of monoclonal antibody 10D1
are shown in SEQ ID NOs:7 to 9. The variable heavy chain (Vh) CDRs of monoclonal antibody 10D1 are shown in SEQ ID NOs:10 to 12. The Vk and Vh consensus and individual sequences for antibodies 6G2, 6G7, 7A9, 8C1 and 10D1 are detailed below in Example 3.

Detailed Description of the Invention

In the context of this specification, the term "activity" as it relates to CXCR7 means any cellular function, action, effect or influence exerted by the CXCR7, either by the protein or polypeptide itself or any fragment or portion thereof.

In the context of the present specification reference to an "antibody" or "antibodies" includes reference to all the various forms of antibodies, including but not limited to whole antibodies, antibody fragments, including, for example, Fv, Fab, Fab' and F(ab')2 fragments, humanized antibodies, human antibodies and immunoglobulin-derived polypeptides produced through genetic engineering techniques.

As used herein the term "associated with" when used in the context of a disorder "associated with" CXCR7 means that the disorder may result from, result in, be characterised by, or otherwise associated with the expression and/or activity of CXCR7 or the binding of a ligand to CXCR7. Thus, a CXCR7-associated disorder may be a disorder the development or progression of which is, at least in part, mediated via CXCR7, but this is not necessarily the case. Additionally, the association between the disorder and CXCR7 may be direct or indirect and may be temporally and/or spatially separated.

In the context of the present specification reference to "binding" of an antibody means binding, interacting or associating with or to a target antigen such as CXCR7. Reference to "CXCR7" includes fragments or portions thereof which comprise the epitopes to which an antibody binds. Consequently, reference to an antibody binding to CXCR7 includes within its scope the binding, interaction or association of the antibody or an antigen-binding portion thereof to part, fragment or epitope-containing region of CXCR7. Generally, "binding", "interaction" or "association" means or includes the specific binding, interaction or association of the antibody to CXCR7 or a portion thereof.

As used herein "CXCR7" refers to the chemokine receptor of the 7-transmembrane G protein-coupled receptor (GPCR) superfamily that is also known as RDC1, CCX-CKR2 and Cmkori. The gene or polynucleotide sequence encoding CXCR7 is herein designated cxcr7.
As used herein the term "effective amount" includes within its meaning a non-toxic but sufficient amount of an agent to provide the desired effect. The exact amount or dose required will vary from subject to subject depending on factors such as the species being treated, the age and general condition of the subject, the severity of the condition being treated, the particular agent being administered and the mode of administration and so forth. Thus, it is not possible to specify an exact "effective amount". However, for any given case, an appropriate "effective amount" may be determined by one of ordinary skill in the art using only routine experimentation.

The terms "inhibits" and "inhibiting" as used herein as they relate to the activity of CXCR7 does not necessarily mean completely inhibiting activity. Rather, activity may be inhibited to an extent, and/or for a time, sufficient to produce the desired effect. Thus inhibition of CXCR7 activity may be partial or complete attenuation of one or more biological effects of CXCR7 and such inhibition may be temporally and/or spatially limited. By temporally and/or spatially limited is meant that the inhibition may be limited to particular physiological conditions or circumstances and/or to particular regions of the body.

The term "polypeptide" means a polymer made up of amino acids linked together by peptide bonds. The terms "polypeptide" and "protein" are used interchangeably herein, although for the purposes of the present invention a "polypeptide" may constitute a portion of a full length protein.

The term "subject" as used herein typically refers to mammals including humans, primates, livestock animals (eg. sheep, pigs, cattle, horses, donkeys), laboratory test animals (eg. mice, rabbits, rats, guinea pigs), companion animals (eg. dogs, cats) and captive wild animals (eg. foxes, kangaroos, deer). Preferably, the mammal is human or a laboratory test animal. Even more preferably, the mammal is a human.

As used herein the terms "treating", "treatment", "preventing" and "prevention" refer to any and all uses which remedy a condition or symptoms, prevent the establishment of a condition or disease, or otherwise prevent, hinder, retard, or reverse the progression of a condition or disease or other undesirable symptoms in any way whatsoever. Thus the terms "treating" and "preventing" and the like are to be considered in their broadest context. For example, treatment does not necessarily imply that a patient is treated until total recovery.
' Described herein is the generation of a set of novel anti-CXCR7 monoclonal antibodies raised by immunising CXCR7 deficient mice with the receptor in its natural configuration rather than using the traditional antibody generation approach of immunising with peptides from extracellular domains of the receptor. The inventors have used a rigorous approach involving functional screening of numerous candidate antibodies to select those with the most potent blocking and binding characteristics. Described herein, and subject of the present invention, are novel anti-CXCR7 neutralising monoclonal antibodies with desirable characteristics making them candidates for therapeutic and diagnostic applications. The anti-CXCR7 monoclonal antibodies are unusual in that they recognise CXCR7 from both human and mouse, and presumably many other mammalian species, which makes them suited for preclinical studies in animal models, and for clinical development in humans.

The present invention provides antibodies that function as inhibitors of CXCR7 binding its principle ligand CXCL12 (SDF-1) and may be used for treating certain conditions associated with CXCR7 or CXCL12 expression and/or activity. Also provided are methods for treating such conditions, comprising administering an anti-CXCR7 antibody of the invention to a patient afflicted with such a condition. Also provided are compositions for use in such methods, the compositions comprising one or more anti-CXCR7 antibodies.

The antibodies of the present invention bind, interact or otherwise associate with CXCR7 or a portion thereof. The antibodies are typically specific for CXCR7 from a particular species, such as human CXCR7, or, in view of the high level of sequence similarity between CXCR7 from different species, the antibodies may exhibit cross-reactivity with CXCR7 from two or more species, such as mouse CXCR7. In the case of antibodies directed towards human CXCR7, some level of cross-reactivity with other mammalian forms of CXCR7 may be desirable in certain circumstances, such as for example, in testing antibodies in animal models of a particular disease and for conducting toxicology, safety and dosage studies.

Typically, antibodies of the invention are monoclonal antibodies or antigen-binding fragments thereof. Most preferably, the antibodies are humanized or human antibodies suitable for administration to humans. These include humanized antibodies prepared, for example, from murine monoclonal antibodies and human monoclonal antibodies which may be prepared, for example, using transgenic mice or by phage display.

By way of example, one method for producing an antibody of the present invention comprises immunizing a non-human animal, such as a mouse or a transgenic mouse, with a CXCR7 polypeptide, or immunogenic portion or fragment thereof, whereby antibodies directed against the CXCR7 polypeptide are generated in said animal. The CXCR7 polypeptide or immunogenic portion or fragment thereof that may be used to immunize animals may be from any mammalian source. Typically, the CXCR7 polypeptide or immunogenic portion of fragment thereof is CXCR7.

Antigen-binding fragments of antibodies of the present invention may be produced by conventional techniques. Examples of such fragments include, but are not limited to, Fab, Fab', F(ab')2 and Fv fragments, including single chain Fv fragments (termed sFv or scFv). Antibody fragments and derivatives produced by genetic engineering techniques, such as disulphide stabilized Fv fragments (dsFv), single chain variable region domain (Abs) molecules and minibodies are also contemplated for use. Unless otherwise specified, the terms "antibody" and "monoclonal antibody" as used herein encompass both whole antibodies and antigen-binding fragments thereof.

Such derivatives of monoclonal antibodies directed against CXCR7 may be prepared and screened for desired properties, by known techniques. The techniques may involve, for example, isolating DNA encoding a polypeptide chain (or a portion thereof) of a monoclonal antibody of interest, and manipulating the DNA through recombinant DNA technology. The DNA may be used to generate another DNA of interest, or altered (e.g. by mutagenesis or other conventional techniques) to add, delete, or substitute one or more amino acid residues, for example. DNA encoding antibody polypeptides (e.g. heavy or light chain, variable region only or full length) may be isolated from B-cells of mice that have been immunized with CXCR7. The DNA may be isolated by conventional procedures including polymerase chain reaction (PCR).
Phage display is an alternative example of a suitable technique whereby derivatives of antibodies of the invention may be prepared. In one approach, polypeptides that are components of an antibody of interest are expressed in any suitable recombinant expression system, and the expressed polypeptides are allowed to assemble to form antibody molecules.

Single chain antibodies may be formed by linking heavy and light chain variable region (Fv region) fragments via an amino acid bridge (short peptide linker), resulting in a single polypeptide chain. Such single-chain Fvs (scFvs) may be prepared by fusing DNA encoding a peptide linker between DNAs encoding the two variable region polypeptides (VL and VH). The resulting antibody fragments can form dimers or trimers, depending on the length of a flexible linker between the two variable domains (see Kortt et al., Protein Engineering 10: 423, 1997). Techniques developed for the production of single chain antibodies include those described in U.S. Pat. No. 4,946,778; Bird (Science 242: 423, 1988), Huston et al. (Proc. Natl. Acad Sci USA 85: 5879, 1988) and Ward et al. (Nature 334: 544, 1989). The disclosures thereof are incorporated herein by reference in their entirety. Single chain antibodies derived from antibodies provided herein are encompassed by the present invention.

Exemplary anti-CXCR7 monoclonal antibodies disclosed herein comprise variable light chains and variable heavy chains comprising sequences as set forth in SEQ ID NOs:13 and 14 respectively, SEQ ID NOs:13 and 20 respectively, SEQ ID NOs:15 and 18 respectively, SEQ ID NOs:16 and 14 respectively, SEQ ID NOs:16 and 19 respectively, or SEQ ID NOs:17 and 21 respectively.

It will be appreciated that the amino acid sequences of monoclonal antibodies of the invention may include one or more amino acid substitutions such that although the primary sequence of the polypeptide is altered, the ability of the antibody to bind CXCR7 and the activity of the antibody is retained. The substitution may be a conservative substitution. The term "conservative amino acid substitution" as used herein refers to a substitution or replacement of one amino acid for another amino acid with similar properties within a polypeptide chain (primary sequence of a protein). For example, the substitution of the charged amino acid glutamic acid (Glu) for the similarly charged amino acid aspartic acid (Asp) would be a conservative amino acid substitution.

The present invention contemplates variants of the light chain and heavy chain sequences disclosed herein and such variants are encompassed within the scope of the present invention. The term "variant" as used herein refers to substantially similar sequences. Generally, polypeptide sequence variants possess qualitative biological activity in common. A variant polypeptide sequence may be a
derivative of a sequence as disclosed herein, which derivative comprises the addition, deletion, or substitution of one or more amino acids. Variants may differ from the disclosed sequences within framework regions or within CDRs of either the light or heavy chain sequences. For example, monoclonal antibodies or antigen-binding fragments thereof comprising amino acid sequences having at least about 70% sequence identity to the amino acid sequences set forth in SEQ ID Nos: 1 to 21 are contemplated. The monoclonal antibody or antigen-binding fragment thereof may comprise amino acid sequences having at least about 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98% or 99% sequence identity to the amino acid sequences set forth in SEQ ID Nos: 1 to 21. The term "variant" encompasses antibody sequences modified from those disclosed herein by any suitable means. For example, when used in the context of murine sequences, the term "variant" includes within its scope humanized forms of such sequences. When used in the context of humanized sequences disclosed herein the term "variant" includes within its scope modified sequences comprising one or more murine back mutations.

Antibodies derived from non-human animals, for example mice, may be unsuitable for administration to humans as they may cause an immune response and result in the generation of anti-mouse antibodies (the so-called HAMA response). The HAMA response can neutralize the mouse antibodies by rapidly clearing them from the blood, thus preventing the mouse antibody from binding to its target.

To avoid development of a HAMA response one strategy is to "humanize" the mouse antibody by replacing as many "foreign" residues in the non-epitope binding regions with human sequences. The specificity of the interaction between an antibody and an antigen involves the hypervariable or complementarity-determining regions (CDRs) in the variable domain. These residues are generally not changed during the humanization process. The remaining residues in the variable domain, referred to as the framework (FW) and the constant regions of the antibody, on both heavy and light chains are usually replaced with human sequences. To avoid disrupting the structure of the antibody-binding pocket, and the specificity or affinity of the antibody, certain mouse residues in the framework regions may need to be preserved. Suitable humanization processes, such as CDR grafting, are well known to those skilled in the art. A particularly suitable approach is exemplified herein. Procedures for the production of chimeric and humanized monoclonal antibodies also include those described in, for example, Riechmann et al., Nature 332: 323, 1988; Liu et al., Proc. Natl. Acad. Sci. USA 84: 3439, 1987; Larrick et al., Bio/Technology 7: 934, 1989 and Winter and Harris, TIPS 14: 139, 1993. The complementarity determining regions (CDRs) of a given antibody may be identified using the system described by Kabat et al., in Sequences of Proteins of Immunological Interest, 5th Ed., US Dept. of Health and Human Services, PHS, NIH, NIH Publication No. 91-3242, 1991).
Procedures for generating human antibodies in non-human animals have also been developed and are well known to those skilled in the art. The antibodies may be partially human, or completely human. For example, transgenic mice into which genetic material encoding one or more human immunoglobulin chains has been introduced may be used to produce antibodies. The transgenic mice may be such that human immunoglobulin polypeptide chains replacing endogenous immunoglobulin chains are present in at least some antibodies produced by the animal upon immunization.

Another method for generating human antibodies is phage display. Phage display techniques for generating human antibodies are well known to those skilled in the art, and include the methods used by Cambridge Antibody Technology and MorphoSys and which are described in International Patent Publication Nos. WO 92/01047, WO 92/20791, WO 93/06213 and WO 93/11236 (the disclosures of which are incorporated herein by reference).

Antibodies of the present invention or hybridomas comprising such antibodies may be screened and manipulated further to identify monoclonal antibodies with particularly desirable properties, such as increased binding affinity, reduced immunogenicity and/or increased inhibitory activity against CXCR7.

The present invention provides methods for treating or preventing CXCR7-associated disorders and other disorders which may be beneficially treated by inhibiting or neutralizing CXCR7 activity by the administration of antibodies of the present invention. Disorders which may be treated in accordance with the present invention include, but are not limited to, cancer, fibrotic disorders (such as airway fibrosis, pulmonary fibrosis, liver fibrosis, renal fibrosis and cardiac fibrosis), cardiovascular disorders, inflammatory diseases, metabolic diseases, gastrointestinal and liver diseases, respiratory diseases, endocrine system disorders, haematological disorders, neurological disorders and urological disorders. The cancer may be a solid or haematologic cancer of any type. Anti-CXCR7 antibodies as disclosed herein may not only treat or prevent cancer or tumour development and growth but also metastasis.

Other exemplary disorders that may be treated or prevented in accordance with embodiments of the invention include Alzheimer's disease, multiple sclerosis, kidney dysfunction, rheumatoid arthritis, cardiac allograft rejection, atherosclerosis, asthma, glomerulonephritis, contact dermatitis, inflammatory bowel disease, colitis, and psoriasis, reperfusion injury.
Embodiments of the invention also provide for the inhibition of angiogenesis using anti-CXCR7 antibodies as disclosed herein. The angiogenesis may be tumour angiogenesis. However also contemplated herein is the inhibition of angiogenesis in any subject in need thereof, for example in rheumatoid arthritis; psoriasis; ocular angiogenic diseases, for example, diabetic retinopathy, retinopathy of prematurity, macular degeneration, corneal graft rejection, neovascular glaucoma, retrolental fibroplasia, rubeosis; myocardial angiogenesis; plaque neovascularization; telangiectasia; hemophilic joints; angiofibroma; disease of excessive or abnormal stimulation of endothelial cells, including intestinal adhesions, Crohn's disease, skin diseases such as psoriasis, excema, and scleroderma; diabetes; atherosclerosis; endometriosis; obesity; wound granulation and hypertrophic scars. As angiogenic inhibitors the monoclonal antibodies disclosed herein can be used to prevent or inhibit scarring following transplantation, cirrhosis of the liver, pulmonary fibrosis following acute respiratory distress syndrome or other pulmonary fibrosis of the newborn, implantation of temporary prosthetics, and adhesions after surgery between the brain and the dura.

Additional applications, both in vivo and in vitro, of antibodies of the invention are contemplated. For example, antibodies of the invention may be employed in assays designed to detect the presence of CXCR7 bearing cells, and/or to purify CXCR7 bearing cells for diagnostic or therapeutic purposes. Antibodies may also be tested in animal models of particular diseases and for conducting toxicology, safety and dosage studies.

Additionally provided herein are methods for detecting the presence and/or amount of CXCR7 on the surface of a cell or cells. For example, the method may comprise detecting one or more cells expressing CXCR7 in a biological sample by contacting the biological sample with an antibody or antigen-binding fragment as disclosed herein and detecting the presence of the antibody or antigen-binding fragment, wherein, for example, the antibody or antigen-binding fragment is labelled (directly or indirectly) with a suitable label to enable detection. Suitable means of labelling antibodies to enable detection upon binding of the antibody to CXCR7 are well known to those skilled in the art.

For therapeutic and prophylactic applications, antibodies of the invention are administered to a subject in need thereof in an amount effective to obtain the desired therapeutic or prophylactic effect. It will be understood that the specific effective amount or dose for any particular subject will depend upon a variety of factors including, for example, the activity of the specific antibody(ies) employed, the age, body weight, general health and diet of the individual to be treated, the time of administration, rate of excretion, and combination with any other treatment or therapy. Single or
multiple administrations can be carried out with dose levels and pattern being selected by the treating physician.

In treating or preventing autoimmune and inflammatory conditions, the present invention contemplates the administration of multiple antibodies if required or desirable. Whether it is suitable or desirable to administer one or more antibodies can be determined by those skilled in the art on a case-by-case basis.

The invention also contemplates combination therapies, wherein antibodies as described herein are coadministered with other suitable agents which may facilitate the desired therapeutic or prophylactic outcome. By "coadministered" is meant simultaneous administration in the same formulation or in two different formulations via the same or different routes or sequential administration by the same or different routes. By "sequential" administration is meant a time difference of, for example, from seconds, minutes, hours, days, weeks, months or years between the administration of the two agents. The agents may be administered in any order.

Antibodies may be administered in any suitable form. In accordance with the present invention antibodies are typically administered in the form of pharmaceutical compositions, which compositions may comprise one or more pharmaceutically acceptable carriers, excipients or diluents. Such compositions may be administered systemically, regionally or locally and via any suitable route such as by parenteral (including intravenous, intraarterial or intramuscular), oral, nasal, topical and subcutaneous routes.

Examples of pharmaceutically acceptable carriers or diluents are demineralised or distilled water; saline solution; vegetable based oils such as peanut oil, safflower oil, olive oil, cottonseed oil, maize oil, sesame oil, arachis oil or coconut oil; silicone oils, including polysiloxanes, such as methyl polysiloxane, phenyl polysiloxane and methylphenyl polysiloxane; volatile silicones; mineral oils such as liquid paraffin, soft paraffin or squalane; cellulose derivatives such as methyl cellulose, ethyl cellulose, carboxymethyl cellulose, sodium carboxymethylcellulose or hydroxypropylmethylcellulose; lower alkanols, for example ethanol or iso-propanol; lower aralkanols; lower polyalkylene glycols or lower alkylene glycols, for example polyethylene glycol, polypropylene glycol, ethylene glycol, propylene glycol, 1,3-butylene glycol or glycerin; fatty acid esters such as isopropyl palmitate, isopropyl myristate or ethyl oleate; polyvinylpyridone; agar; carrageenan; gum tragacanth or gum acacia, and petroleum jelly. Typically, the carrier or carriers will form from 10% to 99.9% by weight of the compositions.
Some examples of suitable carriers, diluents, excipients and adjuvants for oral use include peanut oil, liquid paraffin, sodium carboxymethylcellulose, methylcellulose, sodium alginate, gum acacia, gum tragacanth, dextrose, sucrose, sorbitol, mannitol, gelatine and lecithin. In addition these oral formulations may contain suitable flavouring and colouring agents. When used in capsule form the capsules may be coated with compounds such as glyceryl monostearate or glyceryl distearate which delay disintegration. Adjuvants typically include emollients, emulsifiers, thickening agents, preservatives, bactericides and buffering agents.

For administration as an injectable solution or suspension, non-toxic parenterally acceptable diluents or carriers can include, Ringer's solution, medium chain triglyceride (MCT), isotonic saline, phosphate buffered saline, ethanol and 1,2 propylene glycol. Methods for preparing parenterally administrable compositions are known to those skilled in the art, and are described in more detail in, for example, Remington's Pharmaceutical Science, 15th ed., Mack Publishing Company, Easton, Pa., hereby incorporated by reference in its entirety.

The reference in this specification to any prior publication (or information derived from it), or to any matter which is known, is not, and should not be taken as an acknowledgment or admission or any form of suggestion that that prior publication (or information derived from it) or known matter forms part of the common general knowledge in any country in the field of endeavour to which this specification relates.

The present invention will now be described with reference to the following specific examples, which should not be construed as in any way limiting the scope of the invention.

**Examples**

**Example 1 - Generation of anti-CXCR7 monoclonal antibodies**

Monoclonal antibodies reactive with human or human/mouse CXCR7 were generated by immunising CXCR7 deficient mice (Sierra et al., 2007, *Proc Natl Acad Sci USA* 104:14759-14764) on a mixed C57BL/6 x 129 background with 2 x 10^7 L1.2/hCXCR7 transfected cells stimulated 20 hours prior to harvest with 5 mM butyric acid and emulsified in Complete Freund's Adjuvant (1st immunization intraperitoneal) or Incomplete Freund's Adjuvant (2nd - 6th immunizations intraperitoneal), for a total five to six times at 2-wk intervals. The final immunisation was injected intravenously in PBS. Four days later, the spleen was removed and cells were fused with the SP2/0 cell line using standard methods. Hybridomas were grown in DMEM (Gibco/Invitrogen) containing 10% Fetalclone
(HyClone), 1x HAT supplement (Sigma Aldrich) plus mouse IL-6. After 10-14 days growth culture supernatant was taken for initial screening.

Monoclonal antibodies reactive with CXCR7 were identified using human CXCR7 transfected L1.2 cells, and untransfected L1.2 cells, or L1.2 cells transfected with unrelated or closely receptors such as hCXCR1, hCXCR2 or hCXCR3 using immunofluorescent staining and analysis using a FACSCalibur (BD Biosciences). Monoclonal antibody staining of cells was performed using standard procedures as described previously (Lee et al., 2006, Nat. Biotech. 24:1279-1284).

Production of antibodies involved growing hybridomas in tissue culture flasks and harvesting the culture medium. For some experiments, the concentration of antibody in the culture supernatant was sufficient to proceed without further purification. Production of selected antibodies was scaled up and monoclonal antibodies were purified by protein G chromatography, concentrated and buffer exchanged into PBS. Monoclonal antibody concentration was determined using a total IgG ELISA.

L1.2 transfectants expressing high levels of hCXCR7 were used to immunize mice, and approximately 40 monoclonal antibodies were initially identified via flow cytometry that reacted with L1.2 cells transfected with hCXCR7, of which approximately 10 reacted specifically with L1.2/hCXCR7 transfectants but not with untransfected L1.2 cells or with L1.2 cells transfected with the closely related receptors hCXCR1, hCXCR2 or hCXCR3 (data not shown).

To ensure clonality, selected hybridomas were subcloned using dilution plating into a 384-well plate. The specificity of cross-reactivity of the subclones was confirmed by flow cytometry with L1.2/hCXCR7 transfectants and untransfected L1.2 cells. Figure 1A provides dot plots showing the selective reactivity of four anti-CXCR7 monoclonal antibody clones with L1.2 cells transfected with human or mouse CXCR7 as opposed to untransfected L1.2 cells. All of the antibodies also reacted with mouse CXCR7 transfectants (Figure 1B), although far fewer cells were stained as transient transfection of mouse CXCR7 resulted in far fewer cells expressing the mouse CXCR7 molecule on the cell surface.

Example 2 - Competitive inhibition of ligand binding to CXCR7 by monoclonal antibodies

For ligand binding analysis, recombinant human CXCL12 (SDF-Ia) ("ligand") was obtained from Peprotech (New Jersey, USA). \textsuperscript{125}I-Bolton-Hunter-labelled SDF-Ia was purchased from Perkin-Elmer (Boston, MA, USA), with a specific activity of 2200 Ci/mM. Cells were washed once in binding buffer (50 mM Hepes, pH 7.5, 1 mM CaCl, 5 mM MgCb, 0.5% BSA) and resuspended in binding
buffer at a concentration of 2.5 x 10^6 cells/ml. Cold Purified monoclonal antibody or diluted hybridoma culture medium (cold competitor) was added to a 96-well plate followed by an equal volume (40 µl) binding buffer containing 1 x 10^5 cells. Cells and competitor were preincubated at room temperature for 15 min. Then radiolabeled ligand (final concentration 0.5 - 2 nM) was added to each well to give a final reaction volume of 120 µl. After a 60-min incubation at room temperature, the cells were washed three times with 1 ml of binding buffer containing 150 mM NaCl. The radioactivity (amount of bound label) in the cell pellets was counted in a TopCount liquid scintillation counter (Packard). Non-specific background binding was calculated by incubating cells without radiolabelled-ligand. Samples were assayed in duplicate.

Initially, a panel of anti-CXCR7 monoclonal antibodies identified as binding to human CXCR7 transfectants (see Example 1) was screened for their ability to competitively inhibit binding of ^{125}I-labelled ligand to hCXCR7/L1.2 transfectants treated with 5 mM butyric acid for 20 hr prior to assay. After incubation and washing the amount of label bound to cells was measured and the percentage inhibition determined by comparison to a control reaction with no added antibody. Results for a panel of 22 monoclonal antibodies is shown in Figure 2.

**Example 3 - Sequencing of anti-CXCR7 monoclonal antibodies**

The sequences of the heavy and light chain variable domains encoding various anti-CXCR7 monoclonal antibodies demonstrating significant inhibitory effect on ligand binding were determined.

Messenger RNA was extracted from the hybridoma subclones using the RNeasy Mini Kit (Qiagen) and converted to double-stranded cDNA using the Smart RACE cDNA Amplification Kit (Clontech) following the manufacturer's instructions. The heavy and light chain genes were amplified by PCR using constant region-specific reverse primers (for mouse g1, g2b or k genes) and a forward primer complementary to an oligonucleotide added to the 5' end of the cDNA coding strand during synthesis. The primers used for cloning and sequencing the anti-CXCR7 monoclonal antibody heavy and light chain genes are listed in Table 1 below.

<table>
<thead>
<tr>
<th>Primer Name</th>
<th>Sequence 5' &gt; 3'</th>
<th>Specificity/Direction</th>
</tr>
</thead>
<tbody>
<tr>
<td>5'F-long</td>
<td>CTAATACGACTCTATAGGGCAAGCA GTGTATCAACGCAGAGT (SEQ ID NO:22)</td>
<td>forward primer binds 5' end of cDNA made with SmartRACE cDNA Amplification Kit (Clontech)</td>
</tr>
</tbody>
</table>

**Table 3: Oligonucleotide Primer Sequences**
PCR was performed using KOD HotStart DNA polymerase (Merck Biosciences). PCR was performed on an iCycler (Biorad) and comprised an initial 5 min denaturation step at 94°C, then 30 cycles of denaturation for 30 sec at 94°C, annealing for 30 sec at 55-65°C and elongation for 3 min at 72°C followed by a final step of 7 min at 72°C. If non-specific bands were present in the PCR reaction, the required PCR fragments (~500 bp) were gel purified by electrophoresis through a 0.5% agarose gel. DNA was extracted from the gel slice using the QIAquick Gel Extraction Kit (Qiagen). If the PCR produced a single product it was purified using the Jetquick PCR Product Purification Spin Kit (Genomed).

PCR products were A-tailed by incubating with 2 µM dATP (Promega) and Taq DNA polymerase (Promega) in 1x Thermophilic Mg-free DNA polymerase buffer (Promega) for 30 minutes at 70°C. The A-tailed fragments were subcloned into pGEM-T Easy using the pGEM-T Easy Vector System I (Promega) following manufacturer’s instructions. Vector and fragment were mixed with 2x rapid ligase buffer (Promega) at room temperature for 1 hour. Ligation mix (2 µl) was transformed into

<table>
<thead>
<tr>
<th>5’F-short</th>
<th>CTAATACGACTCACTATAGGGC (SEQ ID NO:23)</th>
<th>forward primer binds 5’ end of cDNA made with SmartRACE cDNA Amplification Kit (Clontech)</th>
</tr>
</thead>
<tbody>
<tr>
<td>mlgG1con.r1</td>
<td>TAGCCCTGGACCAGGCATCC (SEQ ID NO:24)</td>
<td>mouse IgG1 constant region CH1 reverse primer (Chardes et al., 1999, FEBS Lett 452:386-394)</td>
</tr>
<tr>
<td>mlgG2bcon.r1</td>
<td>CAGGGATCCAGAGTTCAAG (SEQ ID NO:25)</td>
<td>mouse IgG2b constant region CH1 reverse primer (Chardes et al., 1999, FEBS Lett 452:386-394)</td>
</tr>
<tr>
<td>mlgKcon.r</td>
<td>TGGTGGAGAGATGGATACAG (SEQ ID NO:26)</td>
<td>mouse kappa constant region reverse primer (Chardes et al., 1999, FEBS Lett 452:386-394)</td>
</tr>
<tr>
<td>M13f</td>
<td>GTTTTCCCAGTCAGGAC (SEQ ID NO:27)</td>
<td>universal forward primer</td>
</tr>
<tr>
<td>M13r</td>
<td>CAGGAAACAGCTATGAC (SEQ ID NO:28)</td>
<td>universal reverse primer</td>
</tr>
</tbody>
</table>
One Shot Top10 Competent Cells (Invitrogen) following manufacturer’s protocol and plated out onto LB agar containing Carbicillin (100 µg/ml) and X-gal (Promega) and incubated overnight at 37°C. Individual colonies were grown in 2 ml LB medium containing Carbicillin (100 µg/ml) overnight at 37°C. Plasmid DNA was extracted using Wizard Plus SV Minipreps DNA Purification System (Promega). Plasmid DNA (800 ng) and 10 pmole universal M13 sequencing primer were mixed and sequenced by Supamac (Sydney, Australia).

**Light Chain Sequences**

The light chain gene variable region (Vk) was amplified using a mix of 5’ forward primers (SEQ ID NOs:22 and 23) and a reverse primer specific for the mouse kappa constant region (SEQ ID NO:26). A DNA fragment approximately 540 bp was amplified from each hybridoma cDNA. These fragments were subcloned between the EcoR1 sites of pGEM-T Easy and transformed into E coli TOP10F competent cells. Plasmid DNA was extracted from between 1 and 6 colonies resulting from each transformation and each cloned PCR fragment sequenced in both directions. DNA sequences were translated using ATG start to predict the sequence of each antibody’s Vk region. Figure 3 shows an alignment of the following Vk amino acid sequences for the anti-CXCR7 hybridoma subclones 6G2, 6G7, 7A9, 8C1 and 10D1.

**6G2**

6G2
MVSTSQLGLLLFMTSASRCIDIVLTQPSATLSVTPGDRVSLSCRASQIKDYLHWYQQKSHESPRL
IKYASQISGISRFGSGSGSDFTLSINSVEPEDGVYYCQNGHSFPTFGSGTKLEIKRADAPTV
SIFPPSSEQ (SEQ ID NO:15)

**6G7**

6G7
MVSTSQLGLLLLFWTSASRCIDIVMTQPSATLSVTPGDRVSLSCRASQIKDYLHWYQQKSHESPRL
LIKASQISIFGISRFSGSGSDFTLSINSVEPEDGVYYCQNGHSFPTFGSGTKLEIKRADAPTV
SIFPPSSEQ (SEQ ID NO:16)

**7A9**

7A9
MVSTSQLGLLLLFWTSASRCIDIVMTQPSATLSVTPGDRVSLSCRASQIKDYLHWYQQKSHESPRL
LIKASQISIFGISRFSGSGSDFTLSINSVEPEDGVYYCQNGHSFPTFGSGTKLEIKRADAPTV
SIFPPSSEQ (SEQ ID NO:16)

**8C1**

8C1
MVSTSQLGLLLLFWTSASRCIDIVMTQPSATLSVTPGDRVSLSCRASQIKDYLHWYQQKSHESPRL
Heavy Chain Sequences

The heavy chain gene variable region (Vh) was amplified using a mix of 5’ forward primers (SEQ ID NOs:22 and 23) and a reverse primer specific for either the mouse $g_1$ constant region (SEQ ID NO:24) or the mouse $g_2b$ constant region (SEQ ID NO:25). DNA fragments approximately 580 bp were amplified from each hybridoma cDNA. These fragments were subcloned between the EcoR1 sites of pGEM-T Easy and transformed into E. coli TOP10 competent cells. Plasmid DNA was extracted from between 1 and 6 colonies resulting from each transformation and each cloned for sequencing. DNA sequences were translated using ATG start to predict the protein sequence of each antibody’s Vh domain. Figure 4 shows an alignment of the following Vh amino acid sequences determined for each of the anti-CXCR7 hybridoma subclones 6G2, 6G7, 7A9, 8C1 and 10D1.

6G2
MEWPLIFLSSLGAAGVQSQVQLQSGAELVKPGASVKISCKAAGYAFSTFWMNWVKORPKGKLE
WIGQIYPANGASNYGFEFKGKATLTDSSSTAYMQLSSLTSEDASYFCAGGLTYWGGATLTVS
AAKTTPPSVYPL (SEQ ID NO:18)

6G7
MEWPLIFLSSLGAAGVQSQVQLQSGAELVKPGASVKISCKAAGYAFSTFWMNWVKORPKGKLE
WIGQIYPANGATNYGFKGKATLTDSSSTAYMQLSSLTSEDASYFCAGGLTYWGGATLTVS
AAKTTPPSVYPL (SEQ ID NO:14)

7A9
MEWPUFLSSGAAGVQSQVQLQSGAELVKPGASVKISCKAAGYAFSTFWMNWVKORPKGKLE
WIGQIYPANGATNYGFKGKATLTDSSSTAYMQLSSLTSEDASYFCAGGLTYWGGATLTVS
AAKTTPPSVYPL (SEQ ID NO:19)
Example 4 - Biological activity of anti-CXCR7 monoclonal antibodies

The ability of the anti-CXCR7 monoclonal antibody 10D1 to protect from airway fibrosis was demonstrated in vivo in two mouse models. Figure 5 provides a basic schematic representation of the first mouse model, using normal Balb/c mice. Briefly, at day 0, TGFβ (40 µl) or saline (40 µl sterile PBS as control) was administered intra-tracheally (IT) followed by intraperitoneal (IP) administration from day 1 onwards, up to day 42, of either the 10D1 antibody or an isotype control antibody in 200 µl sterile PBS (versus 200 µl sterile PBS as a control). At day 43 mice were euthanized, lungs perfused and inflated for histology and stored in buffered formalin for histological analysis. The procedures are detailed further below.

Mice were divided into six treatment groups (eight mice per group) as follows:

**Saline/Saline**
- 40 µl sterile PBS days 0 and 1- IT
- 200 µl sterile PBS day 1- IP
- 200 µl sterile PBS x2 week days 1-42- IP

**Saline/Isotype Control Ab**
- 40 µl sterile PBS days 0 and 1- IT
- Isotype Control Ab in 200 µl sterile PBS 2x week from day 1-42 (10 mg/kg first dose and 5 mg/kg thereafter) - IP

**Saline/CXCR7 Ab**
- 40 µl sterile PBS days 0 and 1- IT
- CXCR7 Ab in 200 µl sterile PBS 2x week from day 1-42 (10 mg/kg first dose and 5 mg/kg thereafter) - IP
TGFB/Saline
40μl days 0 and 1- IT
200μl sterile PBS day 1- IP
200μl sterile PBS x 2 week days 2-43- IP

TGFB/ Isotype Control Ab
40μl sterile PBS days 0 and 1- IT
Isotype Control Ab in 200μl sterile PBS 2x week from day 1-42 (10mg/kg first dose and 5mg/kg thereafter) - IP

TGFB/ CXCR7 Ab
40μl sterile PBS days 0 and 1- IT
CXCR7 Ab in 200μl sterile PBS 2x week from day 1-42 (10mg/kg first dose and 5mg/kg thereafter) - IP

Recombinant human TGF® (R&D systems) was reconstituted in 4mM HCL with 1mg/mL BSA as per suppliers instructions. 50μl aliquots of 50μg/ml solution were stored at -80°C and diluted to 5μg/ml in sterile PBS directly before use.

Mice were warmed briefly using a heat lamp and then were anesthetised by IV injection with 12.5 μg/kg Alfaxalone (Jurox, NSW, Australia). Mice were then suspended vertically and were orotracheally intubated with a 22-gauge flexible plastic catheter (Terumo Sureflo, Hospital Supplies of Australia). Endotracheal positioning was confirmed by palpation of the tracheal rings with the catheter tip. Mice were administered 200ng TGF® in 40μl PBS or 40μl of PBS alone on day 0 and day 1. Animals remained vertical for 1-2 min after administration to ensure the inoculums remained in the lungs.

Airways hyperresponsiveness was assessed in vivo by measuring changes in transpulmonary resistance and dynamic compliance using a Buxco (Buxco, Sharon, USA) mouse plethysmograph. Mice were anaesthetised with an intraperitoneal injection of ketamine/xylazine (80-100 mg/kg and 10 mg/kg, respectively) and cannulated via the trachea with an 18G metal tube. Mice were then mechanically ventilated (150 strokes/min, 175μl stroke volume). Volume changes due to thoracic expansion with ventilation were measured by a transducer connected to the plethysmograph flow chamber. Once stabilized, mice were challenged with saline, followed by increasing concentrations of
methacholine (1.25, 2.5, 5, 10 and 20 mg/ml), aerosolized by an ultrasonic nebuliser and administered (10 |μl) directly to the lungs via the inspiratory line. Each aerosol was delivered for a period of 5 min, during which pressure and flow data were continuously recorded, and a computer program (BioSystemXA; Buxco Electronics) was used to calculate pulmonary resistance and compliance. Peak values were taken as the maximum response to the concentration of methacholine being tested, and were expressed as the percentage change over the saline control. Statistical analysis was performed by a two-way ANOVA using Graphpad Prism 5 software.

Mice were then euthanized by sodium pentobarbital overdose and tissue collected as described below. The lungs were perfused with saline to remove blood from the pulmonary capillary bed; the trachea was then cannulated, and lungs were inflated and fixed in 10% buffered formalin.

As shown in Figure 6, the 10D1 anti-CXCR7 monoclonal antibody induced a significant reduction in airway resistance in vivo, demonstrating the protective effect of this antibody against airway fibrosis.

These results were supported by a second mouse model involving the induction of pulmonary fibrosis by bleomycin administration. Briefly, day 0 mice (female Balb/c 6-8 weeks old) were anesthetised with a tail vein injection of Alfaxan (2mg/ml- 100ul volume). 0.3 Units of bleomycin (Sigma) per mouse instilled intracheally in 30ul of saline. Control mice received 30ul of saline only. Day 0 mice then received an intra-peritoneal (IP) injection of anti-CXCR7 10D1, isotype control or saline control (200ug in 100ul saline). Mice then received IP injection of anti-CXCR7, isotype control or saline control x2 per week (100ug in 100ul saline). Day 21 lung function was assessed (Buxco) and tissues collected for histology.

Lung function was assessed in terms of resistance and compliance by invasive plethysmography (Buxco Research System, Wilmington, NC, USA). Mice were anesthetized by intraperitoneal injection of ketamine-xylazine (80mg/kg and 10mg/kg, respectively) the trachea was cannulated and the mouse mechanically ventilated. Once stabilised, mice were challenges with saline, followed by increasing doses of methacholine (1.25, 2.5, 5, 10, 20 mg/ml). Pressure and flow data were continuously recorded and BiosystemXA software used to calculate pulmonary resistance and compliance. Peak values were taken from each methacholine dose, and were expressed as a percentage change from saline control values. As shown in Figure 7 the administration of antibody 10D1 to mice in which fibrosis had been induced with bleomycin led to a significant reduction in resistance.
Claims

1. A monoclonal antibody or antigen-binding fragment thereof that binds to human CXCR7 or a fragment thereof, the antibody comprising:

(i) within the light chain variable region at least one complementarity determining region (CDR) comprising a sequence as set forth in any one of SEQ ID NOs:1 to 3 or a variant thereof; and/or

(ii) within the heavy chain variable region at least one complementarity determining region (CDR) comprising a sequence as set forth in any one of SEQ ID NOs:4 to 6 or a variant thereof.

2. The antibody or antigen-binding fragment thereof according to claim 1, comprising within the light chain variable region at least one CDR comprising a sequence as set forth in any one of SEQ ID NOs:1 to 3 or a variant thereof and within the heavy chain variable region at least one CDR comprising a sequence as set forth in any one of SEQ ID NOs:4 to 6 or a variant thereof.

3. The antibody or antigen-binding fragment thereof according to claim 1 or 2, comprising a light chain variable region comprising the sequence as set forth in SEQ ID NO:13 or a fragment or variant thereof.

4. The antibody or antigen-binding fragment thereof according to any one of claims 1 to 3, comprising a heavy chain variable region comprising the sequence as set forth in SEQ ID NO:14 or a fragment or variant thereof.

5. The antibody or antigen-binding fragment thereof according to any one of claims 1 to 4, comprising a light chain variable region comprising the sequence as set forth in SEQ ID NO:13 or a fragment or variant thereof and a heavy chain variable region comprising the sequence as set forth in SEQ ID NO:14 or a fragment or variant thereof.

6. The antibody or antigen-binding fragment thereof according to any one of claims 1 to 5, comprising a light chain variable region comprising the sequence as set forth in SEQ ID NO:13 or a fragment or variant thereof and a heavy chain variable region comprising the sequence as set forth in SEQ ID NO:20 or a fragment or variant thereof.
7. The antibody or antigen-binding fragment thereof according to any one of claims 1 to 5, comprising a light chain variable region comprising the sequence as set forth in SEQ ID NO:15 or a fragment or variant thereof and a heavy chain variable region comprising the sequence as set forth in SEQ ID NO:18 or a fragment or variant thereof.

8. The antibody or antigen-binding fragment thereof according to any one of claims 1 to 5, comprising a light chain variable region comprising the sequence as set forth in SEQ ID NO:16 or a fragment or variant thereof and a heavy chain variable region comprising the sequence as set forth in SEQ ID NO:14 or a fragment or variant thereof.

9. The antibody or antigen-binding fragment thereof according to any one of claims 1 to 5, comprising a light chain variable region comprising the sequence as set forth in SEQ ID NO:16 or a fragment or variant thereof and a heavy chain variable region comprising the sequence as set forth in SEQ ID NO:19 or a fragment or variant thereof.

10. A monoclonal antibody or antigen-binding fragment thereof that binds to human CXCR7 or a fragment thereof, the antibody comprising:

(i) within the light chain variable region at least one complementarity determining region (CDR) comprising a sequence as set forth in any one of SEQ ID NOs:7 to 9 or a variant thereof; and/or

(ii) within the heavy chain variable region at least one complementarity determining region (CDR) comprising a sequence as set forth in any one of SEQ ID NOs:10 to 12 or a variant thereof.

11. The antibody or antigen-binding fragment thereof according to claim 10, comprising within the light chain variable region at least one CDR comprising a sequence as set forth in any one of SEQ ID NOs:7 to 9 or a variant thereof and within the heavy chain variable region at least one CDR comprising a sequence as set forth in any one of SEQ ID NOs:10 to 12 or a variant thereof.

12. The antibody or antigen-binding fragment thereof according to claim 10 or 11, comprising a light chain variable region comprising the sequence as set forth in SEQ ID NO:17 or a fragment or variant thereof and/or a heavy chain variable region comprising the sequence as set forth in SEQ ID NO:21 or a fragment or variant thereof.
13. A monoclonal antibody comprising a amino acid sequence having at least about 70% sequence identity to an amino acid sequence as set forth in any one of SEQ ID Nos: 1 to 21.

14. A hybridoma producing a monoclonal antibody as defined in any one of claims 1 to 13.

15. A method for the treatment or prevention of a CXCR7-associated disorder, the method comprising administering to a subject in need thereof an effective amount of at least one antibody, or an antigen-binding fragment thereof, of the first or second aspect.

16. The method according to claim 15 wherein the disorder is a fibrotic disorder, cancer or an inflammatory disorder.

17. The method according to claim 16 wherein the cancer is selected from breast, prostate or lung cancer.

18. The method according to any one of claims 15 to 17 wherein proliferation of one or more cancer cells is inhibited following administration of the antibody or antigen-binding fragment.

19. The method according to any one of claims 15 to 18 wherein angiogenesis is inhibited following administration of the antibody or antigen-binding fragment.

20. The method according to claim 16 wherein the fibrotic disorder is pulmonary fibrosis, fibrosis of the airways, cardiac fibrosis, liver fibrosis or renal fibrosis.

21. The method according to claim 16 wherein the inflammatory disorder is associated with airways inflammation.

22. The method according to any one of claims 15 to 21 wherein airways hyperresponsiveness is reduced following administration of the antibody or antigen-binding fragment.

23. Use of an antibody, or an antigen-binding fragment thereof, according to any one of claims 1 to 13 for the manufacture of a medicament for treating or preventing a CXCR7-associated disorder.
24. A method for inhibiting angiogenesis in a subject, the method comprising administering to the subject an effective amount of at least one antibody, or an antigen-binding fragment thereof, according to any one of claims 1 to 13.

25. A method for reducing airway hyperresponsiveness in a subject, the method comprising administering to the subject an effective amount of at least one antibody, or an antigen-binding fragment thereof, according to any one of claims 1 to 13.

26. A method of detecting a cell expressing CXCR7 in a biological sample, the method comprising contacting the biological sample with an antibody or antigen-binding fragment according to any one of claims 1 to 13 and detecting the presence of the antibody or antigen-binding fragment.

27. The method according to claim 26 wherein the antibody or antigen-binding fragment is linked to a detectable label.

28. Use of an antibody, or an antigen-binding fragment thereof, according to any one of claims 1 to 13 as a therapeutic agent.

29. The use according to claim 28 wherein the antibody or antigen-binding fragment is an anti-fibrotic, anti-angiogenic or anti-cancer agent.

30. A pharmaceutical composition comprising one or more antibodies, or antigen-binding fragments thereof according to any one of claims 1 to 13, optionally together with suitable pharmaceutically acceptable carriers and/or diluents.
FIGURE 1

B

mAb 10D1

% of max

Fluorescence intensity
FIGURE 5

TGFβ model of lung fibrosis

<table>
<thead>
<tr>
<th>IT (TGFβ or Saline)</th>
<th>IP (CXCR7 Ab, Isotype Control Ab or Saline)</th>
<th>Endpoint (AHR, Histology)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Day 0</td>
<td>1</td>
<td>43</td>
</tr>
</tbody>
</table>
FIGURE 6

Airway resistance

Control Ab

Anti-CXCR7 Ab

% change in saline

Saline  1.25  2.5  5  10  20

Methacholine (mg/ml)
FIGURE 7

Effect of anti-CXCR7 mAb 10D1 on bleomycin pulmonary fibrosis

A

Lung Resistance

- Sham
- Saline/Iso
- Bleomycin/Ab
- Bleomycin
- Bleo/Iso
- Saline/Ab

Change from Saline (%)

Saline 1.25 2.5 5 10 20
Methacholine (mg/ml)

B

Isotype cont. mAb

Anti-CXCR7 mAb 10D1
INTERNATIONAL SEARCH REPORT

A. CLASSIFICATION OF SUBJECT MATTER

Int. Cl.
C07K 16/28 (2006.01) A61P 11/00(2006.01)
A61K 59/595(2006.01) A61P 35/00(2006.01)

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)

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 practicable, search terms used)
GenomeQuest (SEQ ID NOS 1, 13, 17)
WPI, EPODOC, Medline and keywords (CXCR7, RDC1, antibody and related terms)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

<table>
<thead>
<tr>
<th>Category*</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
</table>

Further documents are listed in the continuation of Box C

See patent family annex

Date of the actual completion of the international search
06 September 2010

Date of mailing of the international search report
17 SEP 2010

Name and mailing address of the ISA/AU
AUSTRALIAN PATENT OFFICE
PO BOX 200, WODEN ACT 2606, AUSTRALIA
E-mail address: pct@ipaustralia.gov.au
Facsimile No. +61 2 6283 7999

Authorized officer
MARGARET CHANG
AUSTRALIAN PATENT OFFICE
(ISO 9001 Quality Certified Service)
Telephone No.: +61 2 6283 2631

Form PCT/ISA/210 (second sheet) (July 2009)
<table>
<thead>
<tr>
<th>Category</th>
<th>Citation of document, with indication, where appropriate, of the relevant passages</th>
<th>Relevant to claim No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>WO 2006/078780 A2 (GENZYME CORPORATION) 27 July 2006</td>
<td></td>
</tr>
<tr>
<td>A</td>
<td>WO 2004/099781 A2 (BAYER HEALTHCARE AG) 18 November 2004</td>
<td></td>
</tr>
</tbody>
</table>
**INTERNATIONAL SEARCH REPORT**

**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.:
   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:

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)

**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

See Supplemental Box I

1. ☐ 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.:

2. ☐ As all searchable claims could be searched without effort justifying additional fees, this Authority did not invite payment of additional fees

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.:

   1, 3 (in parts relating to SEQ ID NOS: 1 and 13), 10, 12 (in parts relating to SEQ ID NOS: 7 and 17), and claims 13-30 (in parts relating to any one of SEQ ID NOS: 1, 13, 7, and 17)

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 and, where applicable, the payment of a protest fee.

☐ The additional search fees were accompanied by the applicant's protest but the applicable protest fee was not paid within the time limit specified in the invitation.

☒ No protest accompanied the payment of additional search fees.

Form PCT/ISA/210 (continuation of first sheet (2)) (July 2009)
Continuation of Box No: III.

This International Application does not comply with the requirements of unity of invention because it does not relate to one invention or to a group of inventions so linked as to form a single general inventive concept.

In assessing whether there is more than one invention claimed, I have given consideration to those features which can be considered to potentially distinguish the claimed combination of features from the prior art. Where different claims have different distinguishing features they define different inventions.

This International Searching Authority has found that there are different inventions as follows:

- Claims 1-9 (in full) and claims 13-30 (in part). It is considered that monoclonal antibody that binds to human CXCR7 comprising any one of SEQ ID NO: 1-6, 13-16, 18-20, as well as hybridoma producing thereof, pharmaceutical composition comprising thereof, or methods of use thereof, comprise the first to thirteenth distinguishing features, wherein each distinguishing feature is defined by each of SEO ID NO: 1-6, 13-16 and 18-20.

- Claims 10-12 (in full) and claims 13-30 (in part). It is considered that monoclonal antibody that binds to human CXCR7 comprising any one of SEQ ID NO: 7-12, 17, 21, as well as hybridoma producing thereof, pharmaceutical composition comprising thereof, or methods of use thereof, comprise the fourteenth to twenty-first distinguishing features, wherein each distinguishing feature is defined by each of SEO ID NO: 7-12, 17 and 21.

PCT Rule 13.2, first sentence, states that unity of invention is only fulfilled when there is a technical relationship among the claimed inventions involving one or more of the same or corresponding special technical features. PCT Rule 13.2, second sentence, defines a special technical feature as a feature which makes a contribution over the prior art.

The only feature common to all of the claims is "monoclonal antibody that binds to human CXCR7". However this concept is not novel in the light of the following prior art document (cited in the specification on page 2):


Mazzinghi et al discloses the use of blocking monoclonal antibodies to CXCR7 to evaluate the migration of ex vivo human renal multipotent progenitor cells grafted into SCID mouse recipients (see Abstract; page 488 "Materials and Methods").

This means that the common feature can not constitute a special technical feature within the meaning of PCT Rule 13.2, second sentence, since it makes no contribution over the prior art.

Because the common feature does not satisfy the requirement for being a special technical feature it follows that it cannot provide the necessary technical relationship between the identified inventions. Therefore the claims do not satisfy the requirement of unity of invention 'a posteriori.'

(continued in Supplemental Box II)
<table>
<thead>
<tr>
<th>Supplemental Box II</th>
</tr>
</thead>
<tbody>
<tr>
<td>(To be used when the space in any of Boxes I to VIII is not sufficient)</td>
</tr>
<tr>
<td><strong>Continuation of Box No: Supplemental Box I</strong></td>
</tr>
</tbody>
</table>

In response to the invitation to pay additional fees, the applicant has elected for an antibody comprising SEQ ID NO: 17 to be searched.

Therefore, this authority has searched the application defined by the first, seventh, fourteenth, and twentieth distinguishing features, i.e. claims 1, 3 (in parts relating to SEQ ID NOS: 1 and 13, the latter of which is a light chain variable fragment comprising a complementarity determining region as defined in SEQ ID NO:1), 10, 12 (in parts relating to SEQ ID NOS: 7 and 17, the latter of which is a light chain variable fragment comprising a complementarity determining region as defined in SEQ ID NO:7), and claims 13-30 (in parts relating to any one of SEQ ID NOS: 1, 13, 7, and 17).
This Annex lists the known "A" publication level patent family members relating to the patent documents cited in the above-mentioned international search report. The Australian Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

<table>
<thead>
<tr>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>US 2009022717 AU 2007313274 CA 2665534</td>
<td>CN 101528257</td>
</tr>
<tr>
<td>EP 2086584</td>
<td>KR 20090088868</td>
</tr>
<tr>
<td>WO 2008048519</td>
<td>MX 2009004167</td>
</tr>
<tr>
<td>US 2004121413 AU 2003303379 US 2010196883</td>
<td>WO 2004058051</td>
</tr>
<tr>
<td>WO 2006078780 NONE</td>
<td></td>
</tr>
<tr>
<td>WO 2004099781 NONE</td>
<td></td>
</tr>
</tbody>
</table>

Due to data integration issues this family listing may not include 10 digit Australian applications filed since May 2001.

END OF ANNEX