(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
International Bureau

(43) International Publication Date
2 August 2012 (02.08.2012)

WIPO | PCT

(10) International Publication Number
WO 2012/101125 Al

(51) International Patent Classification:
C07K 16/24 (2006.01) *A61K 39/395* (2006.01)

(21) International Application Number:
PCT/EP20 12/05 1056

(22) International Filing Date:
24 January 2012 (24.01 .2012)

(25) Filing Language: English

(26) Publication Language: English

(30) Priority Data:
11305068.6 24 January 201 1 (24.01.201 1) EP
61/436,646 27 January 201 1 (27.01.201 1) US

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(81) Designated States *(unless otherwise indicated, for every kind of national protection available):* AE, AG, AL, AM, AO, AT, AU, AZ, BA, BB, BG, BH, BR, BW, BY, BZ, CA, CH, CL, CN, CO, CR, CU, CZ, DE, DK, DM, DO, DZ, EC, EE, EG, ES, FI, GB, GD, GE, GH, GM, GT, HN, HR, HU, ID, IL, IN, IS, JP, KE, KG, KM, KN, KP, KR, KZ, LA, LC, LK, LR, LS, LT, LU, LY, MA, MD, ME, MG, MK, MN, MW, MX, MY, MZ, NA, NG, NI, NO, NZ, OM, PE, PG, PH, PL, PT, QA, RO, RS, RU, RW, SC, SD, SE, SG, SK, SL, SM, ST, SV, SY, TH, TJ, TM, TN, TR, TT, TZ, UA, UG, US, UZ, VC, VN, ZA, ZM, ZW.

(84) Designated States *(unless otherwise indicated, for every kind of regional protection available):* ARIPO (BW, GH, GM, KE, LR, LS, MW, MZ, NA, RW, SD, SL, SZ, TZ, UG, ZM, ZW), Eurasian (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European (AL, AT, BE, BG, CH, CY, CZ, DE, DK, EE, ES, FI, FR, GB, GR, HR, HU, IE, IS, IT, LT, LU, LV, MC, MK, MT, NL, NO, PL, PT, RO, RS, SE, SI, SK, SM, TR), OAPI (BF, BJ, CF, CG, CI, CM, GA, GN, GQ, GW, ML, MR, NE, SN, TD, TG).

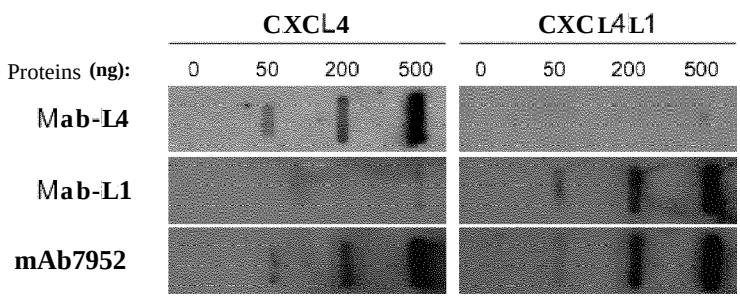
Published:

— *with international search report (Art. 21(3))*

— *with (an) indication^) in relation to deposited biological material furnished under Rule 13bis separately from the description (Rules 13bis.4(d)(i) and 48.2(a)(viii))*

WO 2012/101125 A1

(54) Title: SPECIFIC ANTIBODIES AGAINST HUMAN CXCL4 AND USES THEREOF



(57) Abstract: The invention relates to specific antibodies against human CXCL4 and uses thereof. More particularly,the antibodies of the invention maybe used for specifically detecting CXCL4 in a biological sample or as drug, for instance for treating disease associated with pathological angiogenesis when said antibodies are coupled with a cytotoxic agent.

SPECIFIC ANTIBODIES AGAINST HUMAN CXCL4 AND USES THEREOF

FIELD OF THE INVENTION:

The invention relates to specific antibodies against human CXCL4 and uses thereof.

BACKGROUND OF THE INVENTION:

Angiogenesis, the development of new blood vessels, is a complex biological process which occurs both in physiological conditions, such as during embryonic development, wound healing etc., and in pathological conditions, such as cancer (Bikfalvi and Bicknel, 2002). Angiogenesis is controlled by the net balance between pro-angiogenic and anti-angiogenic factors. Known factors for promoting angiogenesis are for example the fibroblast growth factor (FGF) family, Vascular Endothelial Growth Factor (VEGF), Platelet-Derived Growth Factor (PDGF) and the angiopoetins. Factors which inhibit angiogenesis have been the focus of many anti-cancer research projects since it was postulated that tumors need to be vascularised in order to grow and that diffusible molecules regulate this process. Anti-angiogenic molecules include antibodies such as avastatin, small chemical molecules, and endogenous factors, among which Platelet Factor 4 (PF4).

The CXC-chemokine family consists of pro-inflammatory cytokines, primarily involved in chemoattraction and activation of specific leukocytes in various immuno-inflammatory responses. The CXC chemokine family is unique because it comprises angiogenic and angiostatic chemokines. The Platelet Factor 4 (PF4 or CXCL4) was the first chemokine described as a regulator of angiogenesis (Maione TE *et al.* 1990). PF4 was previously known as oncostatin as described in the international application WO 85/04397.

At the same time, the gene of a nonallelic PF4-variant, called PF4vl or CXCL4L1 was also identified. The mature proteins differ in only three amino acids located in the C-terminus (P58L, K66E, and L67H) in PF4 and PF4vl, respectively (Eisman R *et al.* 1990). PF4vl was characterised as a potent inhibitor of angiogenesis more effective than PF4. Thus, the international patent application WO 2006/029487 relates to PF4vl, fragments, and modified versions of PF4vl and PF4vl fragments for the prevention and/or reduction of angiogenesis, and more particularly for the treatment or prevention of angiogenic disorders or diseases involving angiogenic disorders or pathological angiogenesis such as cancer.

Moreover, an important aspect is the need of additional studies to optimise the use of anti-angiogenesis agents by identifying those patients who most benefit of it and elucidating

the best way of delivering them, either in combination or as sequential single agents. Furthermore, accurate prediction of long-term disease-free survival immediately after surgical resection of clinically localized disease would also be valuable for identifying poor risk group patients who might benefit from enrolment in adjuvant therapy protocols. This underscores the need to identify and validate novel reliable biomarkers for the disease. It needs to be emphasized that currently, the ability to incorporate gene expression signatures into tangible and relevant clinical information for real time patient care currently does not exist. In addition, it would be suitable to develop assays able to quantify biomarkers in patient's fluids (blood, urine, etc. ..) for real-time treatment monitoring.

For instance, in a recent study it has been concluded in a recent study that CXCL4 may represent a potential biomarker of early tumor presence (Cervi *et al.* 2008 and International patent application N° WO 2006/022895) since changes in CXCL4 were detected across a spectrum of human cancers in mice. SELDI-ToF MS and tandem mass spectrometry together with ProteinChip immunoassay identified platelet-associated CXCL4 and possibly CXCL4L1 upregulation in mice in various xenografted human tumors. This suggests that CXCL4 chemokines and/or CXCL4L1 (because there is a difference of 3 amino acids, which may not be detected by MS) are potential markers for diagnosis and prognosis of malignant disease in humans. Moreover, it has also been reported that CXCL4 is upregulated in dendritic cells after severe trauma (Maier *et al.* 2009).

It results therefore that specific antibodies against human CXCL4 which specifically recognizes human CXCL4 are required. Indeed, the inventors have noticed that available commercial polyclonal and monoclonal anti-human CXCL4 antibodies recognize both CXCL4 and CXCL4L1.

SUMMARY OF THE INVENTION:

The invention relates to an isolated CXCL4 antibody which is obtainable from the hybridoma accessible under CNCM deposit number 1-43 10.

The invention also relates to an isolated CXCL4 antibody which comprises the CDRs sequences of the antibody as defined above.

The invention also relates to a method for detecting CXCL4 in a biological sample with an antibody according to the invention.

The invention further relates to an isolated CXCL4 antibody as defined above for use as a drug.

DETAILED DESCRIPTION OF THE INVENTION:

Definitions:

Throughout the specification, several terms are employed and are defined in the following paragraphs.

The term "CXCL4" denotes the CXCL4 protein also named as Platelet Factor 4 (PF4). CXCL4 is a member of CXC-chemokine family and is an inhibitor of angiogenesis.

The term "CXCL4 antibody" refers to an antibody directed against human CXCL4.

The term "specific CXCL4 antibody" refers to an antibody which binds only to CXCL4 and therefore which does not bind to the variant CXCL4L1 .

According to the present invention, "antibody" or "immunoglobulin" have the same meaning, and will be used equally in the present invention. The term "antibody" as used herein refers to immunoglobulin molecules and immunologically active portions of immunoglobulin molecules, i.e., molecules that contain an antigen binding site that immunospecifically binds an antigen. As such, the term antibody encompasses not only whole antibody molecules, but also antibody fragments as well as variants (including derivatives) of antibodies and antibody fragments. In natural antibodies, two heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain by a disulfide bond. There are two types of light chain, lambda (1) and kappa (k). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. Each chain contains distinct sequence domains. The light chain includes two domains, a variable domain (VL) and a constant domain (CL). The heavy chain includes four domains, a variable domain (VH) and three constant domains (CHI, CH2 and CH3, collectively referred to as CH). The variable regions of both light (VL) and heavy (VH) chains determine binding recognition and specificity to the antigen. The constant region domains of the light (CL) and heavy (CH) chains confer important biological properties such as antibody chain association, secretion, trans-placental mobility, complement binding, and binding to Fc receptors (FcR). The Fv fragment is the N-terminal part of the Fab fragment of an immunoglobulin and consists of the variable portions of one light chain and one heavy chain. The specificity of the antibody resides in the structural complementarity between the antibody combining site and the antigenic determinant. Antibody combining sites are made up of residues that are primarily from the hypervariable or complementarity determining regions (CDRs). Occasionally, residues from nonhypervariable or framework regions (FR) influence the overall domain structure and hence the combining site. Complementarity Determining

Regions or CDRs refer to amino acid sequences which together define the binding affinity and specificity of the natural Fv region of a native immunoglobulin binding site. The light and heavy chains of an immunoglobulin each have three CDRs, designated L-CDR1, L-CDR2, L-CDR3 and H-CDR1, H-CDR2, H-CDR3, respectively. An antigen-binding site, therefore, includes six CDRs, comprising the CDR set from each of a heavy and a light chain V region. Framework Regions (FRs) refer to amino acid sequences interposed between CDRs.

The term "chimeric antibody" refers to an antibody which comprises a VH domain and a VL domain of a specific CXCL4 antibody derived from a non-human animal, such as for instance derived from the murine 2G2 antibody, and a CH domain and a CL domain of a human antibody. As the non-human animal, any animal such as mouse, rat, hamster, rabbit or the like can be used.

According to the invention, the term "humanized antibody" refers to an antibody having variable region framework and constant regions from a human antibody but retains for instance the CDRs of the murine 2G2 antibody.

The term "Fab" denotes an antibody fragment having a molecular weight of about 50,000 and antigen binding activity, in which about a half of the N-terminal side of H chain and the entire L chain, among fragments obtained by treating IgG with a protease, papaine, are bound together through a disulfide bond.

The term "F(ab')2" refers to an antibody fragment having a molecular weight of about 100,000 and antigen binding activity, which is slightly larger than the Fab bound via a disulfide bond of the hinge region, among fragments obtained by treating IgG with a protease, pepsin.

The term "Fab"' refers to an antibody fragment having a molecular weight of about 50,000 and antigen binding activity, which is obtained by cutting a disulfide bond of the hinge region of the F(ab')2.

A single chain Fv ("scFv") polypeptide is a covalently linked VH::VL heterodimer which is usually expressed from a gene fusion including VH and VL encoding genes linked by a peptide-encoding linker. "dsFv" is a VH::VL heterodimer stabilised by a disulfide bond. Divalent and multivalent antibody fragments can form either spontaneously by association of monovalent scFvs, or can be generated by coupling monovalent scFvs by a peptide linker, such as divalent sc(Fv)2.

The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (VH) connected to a light-chain variable domain (VL) in the same polypeptide chain (VH-VL). By using a linker that is

too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites.

By "purified" and "isolated" it is meant, when referring to a polypeptide (i.e. an antibody according to the invention) or to a nucleotide sequence, that the indicated molecule is present in the substantial absence of other biological macromolecules of the same type. The term "purified" as used herein preferably means at least 75% by weight, more preferably at least 85% by weight, more preferably still at least 95% by weight, and most preferably at least 98% by weight, of biological macromolecules of the same type are present. An "isolated" nucleic acid molecule which encodes a particular polypeptide refers to a nucleic acid molecule which is substantially free of other nucleic acid molecules that do not encode the polypeptide; however, the molecule may include some additional bases or moieties which do not deleteriously affect the basic characteristics of the composition.

Antibodies of the invention and nucleic acids encoding them:

The present invention provides for isolated antibodies or fragments thereof that are specifically directed against human CXCL4. In particular, the inventors have deposited a murine CXCL4 antibody (2G2) producing hybridoma at the Collection Nationale de Cultures de Microorganismes (CNCM, Institut Pasteur, 25 rue du Docteur Roux, 75724 Paris Cedex 15, France), in accordance with the terms of Budapest Treaty, on the 4th of January 2008. The deposited hybridoma has CNCM deposit number 1-43 10.

A first aspect of the invention thus relates to an isolated specific CXCL4 antibody which does not bind to CXCL4L1.

In a particular embodiment, the antibody is a murine CXCL4 antibody (2G2) obtainable from the hybridoma available under CNCM deposit number 1-43 10.

In another embodiment the antibody of the invention comprises a variable light chain (VL) comprising the CDRs of the VL chain of the antibody obtainable from hybridoma deposited as CNCM 1-43 10 and a variable heavy chain (VH) comprising the CDRs of the VH chain of the antibody obtainable from hybridoma deposited as CNCM 1-43 10.

In another embodiment the antibody of the invention comprises the VL chain of the antibody obtainable from hybridoma deposited as CNCM 1-4310 and the VH chain of the antibody obtainable from hybridoma deposited as CNCM 1-4310.

In another embodiment, the antibody of the invention is a chimeric antibody, which comprises the variable domains of the antibody obtainable from hybridoma deposited as CNCM 1-4310.

In another embodiment, the antibody of the invention is a humanized antibody. In particular, in said humanized antibody, the variable domain comprises human acceptor frameworks regions, and optionally human constant domain where present, and the CDRs of the antibody obtainable from hybridoma deposited as CNCM 1-4310.

The invention further provides fragments directed against CXCL4 of said antibodies which include but are not limited to Fv, Fab, F(ab')2, Fab', dsFv, scFv, sc(Fv)2 and diabodies.

A further object of the invention relates to a nucleic acid sequence encoding an antibody according to the invention.

In a particular embodiment, the invention relates to a nucleic acid sequence encoding the VH domain of the antibody obtainable from hybridoma deposited as CNCM 1-4310 (2G2) or the VL domain of the antibody obtainable from hybridoma deposited as CNCM 1-4310 (2G2).

Typically, said nucleic acid is a DNA or RNA molecule, which may be included in any suitable vector, such as a plasmid, cosmid, episome, artificial chromosome, phage or a viral vector.

The terms "vector", "cloning vector" and "expression vector" mean the vehicle by which a DNA or RNA sequence (e.g. a foreign gene) can be introduced into a host cell, so as to transform the host and promote expression (e.g. transcription and translation) of the introduced sequence.

So, a further object of the invention relates to a vector comprising a nucleic acid of the invention.

Such vectors may comprise regulatory elements, such as a promoter, enhancer, terminator and the like, to cause or direct expression of said antibody upon administration to a subject. Examples of promoters and enhancers used in the expression vector for animal cell include early promoter and enhancer of SV40 (Mizukami T. *et al.* 1987), LTR promoter and enhancer of Moloney mouse leukemia virus (Kuwana Y *et al.* 1987), promoter (Mason JO *et al.* 1985) and enhancer (Gillies SD *et al.* 1983) of immunoglobulin H chain and the like.

Any expression vector for animal cell can be used, so long as a gene encoding the human antibody C region can be inserted and expressed. Examples of suitable vectors include pAGE107 (Miyaji H *et al.* 1990), pAGE103 (Mizukami T *et al.* 1987), pHSG274 (Brady G *et al.* 1984), pKCR (O'Hare K *et al.* 1981), pSGl beta d2-4-(Miyaji H *et al.* 1990) and the like.

Other examples of plasmids include replicating plasmids comprising an origin of replication, or integrative plasmids, such as for instance pUC, pcDNA, pBR, and the like.

Other examples of viral vector include adenoviral, retroviral, herpes virus and AAV vectors. Such recombinant viruses may be produced by techniques known in the art, such as by transfecting packaging cells or by transient transfection with helper plasmids or viruses. Typical examples of virus packaging cells include PA317 cells, PsiCRIP cells, GPenv+ cells, 293 cells, etc. Detailed protocols for producing such replication-defective recombinant viruses may be found for instance in WO 95/14785, WO 96/22378, US 5,882,877, US 6,013,516, US 4,861,719, US 5,278,056 and WO 94/19478.

A further object of the present invention relates to a cell which has been transfected, infected or transformed by a nucleic acid and/or a vector according to the invention.

The term "transformation" means the introduction of a "foreign" (i.e. extrinsic or extracellular) gene, DNA or RNA sequence to a host cell, so that the host cell will express the introduced gene or sequence to produce a desired substance, typically a protein or enzyme coded by the introduced gene or sequence. A host cell that receives and expresses introduced DNA or RNA bas been "transformed".

The nucleic acids of the invention may be used to produce an antibody of the invention in a suitable expression system. The term "expression system" means a host cell and compatible vector under suitable conditions, e.g. for the expression of a protein coded for by foreign DNA carried by the vector and introduced to the host cell.

Common expression systems include E. coli host cells and plasmid vectors, insect host cells and Baculovirus vectors, and mammalian host cells and vectors. Other examples of host cells include, without limitation, prokaryotic cells (such as bacteria) and eukaryotic cells (such as yeast cells, mammalian cells, insect cells, plant cells, etc.). Specific examples include E.coli, Kluyveromyces or Saccharomyces yeasts, mammalian cell lines (e.g., Vero cells, CHO cells, 3T3 cells, COS cells, etc.) as well as primary or established mammalian cell cultures (e.g., produced from lymphoblasts, fibroblasts, embryonic cells, epithelial cells, nervous cells, adipocytes, etc.). Examples also include mouse SP2/0-Agl4 cell (ATCC CRL1581), mouse P3X63-Ag8.653 cell (ATCC CRL1580), CHO cell in which a dihydrofolate reductase gene (hereinafter referred to as "DHFR gene") is defective (Urlaub G *et al.* 1980), rat

YB2/3HL.P2.G11.16Ag.20 cell (ATCC CRL1662, hereinafter referred to as "YB2/0 cell"), and the like.

The present invention also relates to a method of producing a recombinant host cell expressing an antibody according to the invention, said method comprising the steps of: (i) introducing in vitro or ex vivo a recombinant nucleic acid or a vector as described above into a competent host cell, (ii) culturing in vitro or ex vivo the recombinant host cell obtained and (iii), optionally, selecting the cells which express and/or secrete said antibody. Such recombinant host cells can be used for the production of antibodies of the invention.

Methods of producing antibodies of the invention:

Antibodies of the invention may be produced by any technique known in the art, such as, without limitation, any chemical, biological, genetic or enzymatic technique, either alone or in combination.

Knowing the amino acid sequence of the desired sequence, one skilled in the art can readily produce said antibodies, by standard techniques for production of polypeptides. For instance, they can be synthesized using well-known solid phase method, preferably using a commercially available peptide synthesis apparatus (such as that made by Applied Biosystems, Foster City, California) and following the manufacturer's instructions. Alternatively, antibodies of the invention can be synthesized by recombinant DNA techniques well-known in the art. For example, antibodies can be obtained as DNA expression products after incorporation of DNA sequences encoding the antibodies into expression vectors and introduction of such vectors into suitable eukaryotic or prokaryotic hosts that will express the desired antibodies, from which they can be later isolated using well-known techniques.

In particular, the invention further relates to a method of producing an antibody of the invention, which method comprises the steps consisting of: (i) culturing a transformed host cell according to the invention under conditions suitable to allow expression of said antibody; and (ii) recovering the expressed antibody.

In another particular embodiment, the method comprises the steps of:

(i) culturing the hybridoma deposited as CNCM 1-4310 under conditions suitable to allow expression of 2G2 antibody; and

(ii) recovering the expressed antibody.

Antibodies of the invention are suitably separated from the culture medium by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

In a particular embodiment, the human chimeric antibody of the present invention can be produced by obtaining nucleic sequences encoding VL and VH domains as previously described, constructing a human chimeric antibody expression vector by inserting them into an expression vector for animal cell having genes encoding human antibody CH and human antibody CL, and expressing the coding sequence by introducing the expression vector into an animal cell.

As the CH domain of a human chimeric antibody, it may be any region which belongs to human immunoglobulin, but those of IgG class are suitable and any one of subclasses belonging to IgG class, such as IgGl, IgG2, IgG3 and IgG4, can also be used. Also, as the CL of a human chimeric antibody, it may be any region which belongs to Ig, and those of kappa class or lambda class can be used.

Methods for producing chimeric antibodies involve conventional recombinant DNA and gene transfection techniques are well known in the art (Morrison SL. *et al.* 1984 and patent documents U.S. Pat. Nos 5,202,238; and 5,204, 244).

The humanized antibody of the present invention may be produced by obtaining nucleic acid sequences encoding CDR domains, as previously described, constructing a humanized antibody expression vector by inserting them into an expression vector for animal cell having genes encoding (i) a heavy chain constant region identical to that of a human antibody and (ii) a light chain constant region identical to that of a human antibody, and expressing the genes by introducing the expression vector into an animal cell.

The humanized antibody expression vector may be either of a type in which a gene encoding an antibody heavy chain and a gene encoding an antibody light chain exists on separate vectors or of a type in which both genes exist on the same vector (tandem type). In respect of easiness of construction of a humanized antibody expression vector, easiness of introduction into animal cells, and balance between the expression levels of antibody H and L chains in animal cells, humanized antibody expression vector of the tandem type is preferred (Shitara K *et al.* 1994). Examples of tandem type humanized antibody expression vector include pKANTEX93 (WO 97/10354), pEE18 and the like.

Methods for producing humanized antibodies based on conventional recombinant DNA and gene transfection techniques are well known in the art (See, e. g., Riechmann L. *et*

al. 1988; Neuberger MS. *et al.* 1985). Antibodies can be humanized using a variety of techniques known in the art including, for example, CDR-grafting (EP 239,400; PCT publication WO91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089), veneering or resurfacing (EP 592,106; EP 519,596; Padlan EA (1991); Studnicka GM *et al.* 1994; Roguska MA. *et al.* 1994), and chain shuffling (U.S. Pat. No. 5,565,332). The general recombinant DNA technology for preparation of such antibodies is also known (see European Patent Application EP 125023 and International Patent Application WO 96/02576).

The Fab of the present invention can be obtained by treating an antibody which specifically reacts with human CXCL4 with a protease, papaine. Also, the Fab can be produced by inserting DNA encoding Fab of the antibody into a vector for prokaryotic expression system, or for eukaryotic expression system, and introducing the vector into a procaryote or eucaryote (as appropriate) to express the Fab.

The F(ab')2 of the present invention can be obtained treating an antibody which specifically reacts with human CXCL4 with a protease, pepsin. Also, the F(ab')2 can be produced by binding Fab' described below via a thioether bond or a disulfide bond.

The Fab' of the present invention can be obtained treating F(ab')2 which specifically reacts with human CXCL4 with a reducing agent, dithiothreitol. Also, the Fab' can be produced by inserting DNA encoding Fab' fragment of the antibody into an expression vector for prokaryote, or an expression vector for eukaryote, and introducing the vector into a prokaryote or eukaryote (as appropriate) to perform its expression.

The scFv of the present invention can be produced by obtaining cDNA encoding the VH and VL domains as previously described, constructing DNA encoding scFv, inserting the DNA into an expression vector for prokaryote, or an expression vector for eukaryote, and then introducing the expression vector into a prokaryote or eukaryote (as appropriate) to express the scFv. To generate a humanized scFv fragment, a well known technology called CDR grafting may be used, which involves selecting the complementary determining regions (CDRs) from a donor scFv fragment, and grafting them onto a human scFv fragment framework of known three dimensional structure (see, e. g., W098/45322; WO 87/02671; US5,859,205; US5,585,089; US4,816,567; EP0173494).

Amino acid sequence modification(s) of the antibodies described herein are contemplated. For example, it may be desirable to improve the binding affinity and/or other biological properties of the antibody. It is known that when a humanized antibody is produced by simply grafting only CDRs in VH and VL of an antibody derived from a non-human animal in FRs of the VH and VL of a human antibody, the antigen binding activity is reduced

in comparison with that of the original antibody derived from a non-human animal. It is considered that several amino acid residues of the VH and VL of the non-human antibody, not only in CDRs but also in FRs, are directly or indirectly associated with the antigen binding activity. Hence, substitution of these amino acid residues with different amino acid residues derived from FRs of the VH and VL of the human antibody would reduce of the binding activity. In order to resolve the problem, in antibodies grafted with human CDR, attempts have to be made to identify, among amino acid sequences of the FR of the VH and VL of human antibodies, an amino acid residue which is directly associated with binding to the antibody, or which interacts with an amino acid residue of CDR, or which maintains the three-dimensional structure of the antibody and which is directly associated with binding to the antigen. The reduced antigen binding activity could be increased by replacing the identified amino acids with amino acid residues of the original antibody derived from a non-human animal.

Modifications and changes may be made in the structure of the antibodies of the present invention, and in the DNA sequences encoding them, and still obtain a functional molecule that encodes an antibody with desirable characteristics.

In making the changes in the amino sequences, the hydropathic index of amino acids may be considered. The importance of the hydropathic amino acid index in conferring interactive biologic function on a protein is generally understood in the art. It is accepted that the relative hydropathic character of the amino acid contributes to the secondary structure of the resultant protein, which in turn defines the interaction of the protein with other molecules, for example, enzymes, substrates, receptors, DNA, antibodies, antigens, and the like. Each amino acid has been assigned a hydropathic index on the basis of their hydrophobicity and charge characteristics these are: isoleucine (+4.5); valine (+4.2); leucine (+3.8); phenylalanine (+2.8); cysteine/cystine (+2.5); methionine (+1.9); alanine (+1.8); glycine (-0.4); threonine (-0.7); serine (-0.8); tryptophane (-0.9); tyrosine (-1.3); proline (-1.6); histidine (-3.2); glutamate (-3.5); glutamine (-3.5); aspartate (-3.5); asparagine (-3.5); lysine (-3.9); and arginine (-4.5).

A further aspect of the present invention also encompasses function-conservative variants of the antibodies of the present invention.

"Function-conservative variants" are those in which a given amino acid residue in a protein or enzyme has been changed without altering the overall conformation and function of the polypeptide, including, but not limited to, replacement of an amino acid with one having

similar properties (such as, for example, polarity, hydrogen bonding potential, acidic, basic, hydrophobic, aromatic, and the like). Amino acids other than those indicated as conserved may differ in a protein so that the percent protein or amino acid sequence similarity between any two proteins of similar function may vary and may be, for example, from 70 % to 99 % as determined according to an alignment scheme such as by the Cluster Method, wherein similarity is based on the MEGALIGN algorithm. A "function-conservative variant" also includes a polypeptide which has at least 60 % amino acid identity as determined by BLAST or FASTA algorithms, preferably at least 75 %, more preferably at least 85%, still preferably at least 90 %, and even more preferably at least 95%, and which has the same or substantially similar properties or functions as the native or parent protein to which it is compared.

Two amino acid sequences are "substantially homologous" or "substantially similar" when greater than 80 %, preferably greater than 85 %, preferably greater than 90 % of the amino acids are identical, or greater than about 90 %, preferably grater than 95 %, are similar (functionally identical) over the whole length of the shorter sequence. Preferably, the similar or homologous sequences are identified by alignment using, for example, the GCG (Genetics Computer Group, Program Manual for the GCG Package, Version 7, Madison, Wisconsin) pileup program, or any of sequence comparison algorithms such as BLAST, FASTA, etc.

For example, certain amino acids may be substituted by other amino acids in a protein structure without appreciable loss of activity. Since the interactive capacity and nature of a protein define the protein's biological functional activity, certain amino acid substitutions can be made in a protein sequence, and, of course, in its DNA encoding sequence, while nevertheless obtaining a protein with like properties. It is thus contemplated that various changes may be made in the antibodies sequences of the invention, or corresponding DNA sequences which encode said antibodies, without appreciable loss of their biological activity.

It is known in the art that certain amino acids may be substituted by other amino acids having a similar hydropathic index or score and still result in a protein with similar biological activity, i.e. still obtain a biological functionally equivalent protein.

As outlined above, amino acid substitutions are generally therefore based on the relative similarity of the amino acid side-chain substituents, for example, their hydrophobicity, hydrophilicity, charge, size, and the like. Exemplary substitutions which take various of the foregoing characteristics into consideration are well known to those of skill in the art and include: arginine and lysine; glutamate and aspartate; serine and threonine; glutamine and asparagine; and valine, leucine and isoleucine.

Another type of amino acid modification of the antibody of the invention may be useful for altering the original glycosylation pattern of the antibody.

By "altering" is meant deleting one or more carbohydrate moieties found in the antibody, and/or adding one or more glycosylation sites that are not present in the antibody.

Glycosylation of antibodies is typically N-linked. "N-linked" refers to the attachment of the carbohydrate moiety to the side chain of an asparagine residue. The tripeptide sequences asparagine-X-serine and asparagines-X-threonine, where X is any amino acid except proline, are the recognition sequences for enzymatic attachment of the carbohydrate moiety to the asparagine side chain. Thus, the presence of either of these tripeptide sequences in a polypeptide creates a potential glycosylation site. Addition of glycosylation sites to the antibody is conveniently accomplished by altering the amino acid sequence such that it contains one or more of the above-described tripeptide sequences (for N-linked glycosylation sites).

Another type of covalent modification involves chemically or enzymatically coupling glycosides to the antibody. These procedures are advantageous in that they do not require production of the antibody in a host cell that has glycosylation capabilities for N-or O-linked glycosylation. Depending on the coupling mode used, the sugar(s) may be attached to (a) arginine and histidine, (b) free carboxyl groups, (c) free sulfhydryl groups such as thoseof cysteine, (d) free hydroxyl groups such as those of serine, threonine, orhydroxyproline, (e) aromatic residues such as those of phenylalanine, tyrosine, or tryptophan, or (f) the amide group of glutamine. For example, such methods are described in WO 87/05330.

Removal of any carbohydrate moieties present on the antibody may be accomplished chemically or enzymatically. Chemical deglycosylation requires exposure of the antibody to the compound trifluoromethanesulfonic acid, or an equivalent compound. This treatment results in the cleavage of most or all sugars except the linking sugar (N-acetylglucosamine or N-acetylgalactosamine), while leaving the antibody intact. Chemical deglycosylation is described by Edge AS. *et al,* 1981. Enzymatic cleavage of carbohydrate moieties on antibodies can be achieved by the use of a variety of endo-and exo-glycosidases as described by Thotakura NR. *et al,* 1987.

Another type of covalent modification of the antibody comprises linking the antibody to one of a variety of non proteinaceous polymers, eg. , polyethylene glycol, polypropylene glycol, or polyoxyalkylenes, in the manner set forth in US Patent Nos. 4,640, 835; 4,496, 689; 4,301, 144; 4,670, 417; 4,791, 192 or 4,179,337.

It may be also desirable to modify the antibody of the invention with respect to effector function, e.g. so as to enhance antigen-dependent cell-mediated cytotoxicity (ADCC) and/or complement dependent cytotoxicity (CDC) of the antibody. This may be achieved by introducing one or more amino acid substitutions in an Fc region of the antibody. Alternatively or additionally, cysteine residue(s) may be introduced in the Fc region, thereby allowing inter-chain disulfide bond formation in this region. The homodimeric antibody thus generated may have improved internalization capability and/or increased complement-mediated cell killing and/or antibody-dependent cellular cytotoxicity (ADCC) (Caron PC. *et al,* 1992; and Shopes B. 1992).

Detection methods and uses:

A further aspect of the invention thus relates to the use of a specific CXCL4 antibody of the invention or a fragment thereof for the detection of CXCL4 in a biological sample.

In a particular embodiment, antibodies of the invention may be labelled with a detectable molecule or substance, such as a fluorescent molecule, a radioactive molecule or any others labels known in the art. Labels are known in the art that generally provide (either directly or indirectly) a signal.

As used herein, the term "labeled", with regard to the antibody, is intended to encompass direct labeling of the antibody by coupling (i.e., physically linking) a detectable substance, such as a radioactive agent or a fluorophore (e.g. fluorescein isothiocyanate (FITC) or phycoerythrin (PE) or Indocyanine (Cy5)) to the antibody, as well as indirect labeling of the antibody by reactivity with a detectable substance.

An antibody of the invention may be labelled with a radioactive molecule by any method known to the art. For example radioactive molecules include but are not limited radioactive atom for scintigraphic studies such as I^{123}, 1^{124}, In^{111}, Re^{186} and Re^{188}. Antibodies of the invention may be also labelled with a spin label for nuclear magnetic resonance (NMR) imaging (also known as magnetic resonance imaging, mri), such as iodine-123, iodine-13 1, indium-Ill, fluorine-19, carbon-13, nitrogen-15, oxygen-17, gadolinium, manganese or iron.

A "biological sample" encompasses a variety of sample types obtained from a subject and can be used in a diagnostic or monitoring assay. Biological samples include but are not limited to blood and other liquid samples of biological origin, solid tissue samples such as a biopsy specimen or tissue cultures or cells derived there from, and the progeny thereof. Therefore, biological samples encompass clinical samples, cells in culture, cell supernatants, cell lysates, serum, plasma, urine, cerebro-spinal fluid, biological fluid, and tissue samples.

The term "detection" as used herein includes qualitative and/or quantitative detection (measuring levels) with or without reference to a control.

In one aspect, the invention is a method for detecting CXCL4 in a biological sample using an antibody of the invention or a fragment thereof.

In particular, said method may comprise a step of contacting said biological sample with an antibody of the invention or a fragment thereof.

The invention also provides kits comprising at least one antibody of the invention. Kits containing antibodies of the invention find use in detecting CXCL4 expression in biological sample. Kits of the invention can contain an antibody coupled to a solid support, e.g., a tissue culture plate or beads (e.g., sepharose beads). Kits can be provided which contain antibodies for detection and quantification of CXCL4 *in vitro*, e.g. in an ELISA or a Western blot. Such antibody useful for detection may be provided with a label such as a fluorescent or radiolabel.

In another aspect, the invention relates to a method for detecting and/or staging a CXCL4-mediated pathology in a patient comprising determining the expression level of the CXCL4 gene according to methods of the invention is performed by measuring the concentration of the CXCL4 protein in a biological sample obtained from said patient.

Once the biological sample from the patient is prepared, the concentration of CXCL4 may be measured using an antibody of the invention or a fragment thereof.

The term "CXCL4-mediated pathology" as used herein refers to a pathology induced by the modulation of the activity and/or the expression level of CXCL4 protein.

As used herein, the term "modulation" includes both upregulation and downregulation of the activity and/or the expression level of CXCL4.

CXCL4-mediated pathology is selected from the group consisting of cancers such as pancreatic cancer; ophthalmologic diseases such as age-related macular degeneration (AMD); inflammatory diseases such as polyarthritis; cardiovascular diseases, such as atherosclerosis and thromboembolic diseases. Indeed, CXCL4 has been shown to have two important functions in the vasculature. It has a role in atherosclerosis (a pro-atherogenic role) and also has in angiogenesis (an anti-angiogenic role) as described in Aidoudi *et al.* 2010.

The term "patient" as used herein denotes a mammal. Preferably, a patient according to the invention is a human.

Therapeutic uses of the antibodies of the invention:

Another aspect of the invention relates to an isolated specific CXCL4 antibody which does not bind to CXCL4L1 for use as drug.

In a particular embodiment, the antibody is an antibody or fragment thereof according to the invention as described above.

It should be noted that the overexpression of CXCL4 in a number of pathologies enables the specific targeting of pathological cells with an antibody or fragment thereof according to the invention which has been coupled with a cytotoxic agent. Indeed, in a number of diseases, for example cancer; age-related macular dystrophy (AMD) and other hyperproliferative ocular diseases; and chronic inflammatory diseases such as chronic inflammatory, polyarthritis, connective tissue disorders and lupus, there is a local increase in the concentration of secreted CXCL4 in the pathological tissue. Thus, when said pathological tissue is targeted by an antibody or fragment thereof according to the invention coupled with a cytotoxic agent, there is a non-specific but localized destroying of the pathological tissue.

The invention provides a method for treating a disease associated with pathological angiogenesis selected from the group consisting of cancer; age-related macular dystrophy (AMD) and other hyperproliferative ocular diseases; and chronic inflammatory diseases such as chronic inflammatory, polyarthritis, connective tissue disorders and lupus, comprising the step of administering an effective amount of an antibody or fragment thereof according to the invention which has been coupled with a cytotoxic agent to a subject in need thereof.

Also provided is an antibody or fragment thereof according to the invention coupled with a cytotoxic agent for use in the treatment of a disease associated with pathological angiogenesis selected from the group consisting of cancer, age-related macular dystrophy (AMD) and other hyperproliferative ocular diseases and chronic inflammatory diseases.

Typically said antibody or fragment thereof coupled with a cytotoxic agent may be conjugated with a cytokine, a cytotoxic drug or labelled with a cytotoxic radioisotope.

Examples of cytokines which can be coupled with the antibody or fragment thereof according to the invention are IL12 and TNFalpha.

Examples of cytotoxic drugs are platinum salts, taxanes, vinca derivatives and analogues, gemcitabine, methotrexate, doxorubicin, cytotoxin such as *Pseudomonas* exotoxin, g protein, and g protein coupled receptor inhibitors.

Common cytotoxic radioisotopes are, for example, ^{131}I, ^{90}Y, ^{11}Lu, ^{67}Cu, ^{186}Re, ^{188}Re, ^{212}Bi and ^{213}Bi.

In the context of the invention, the term "treating" or "treatment", as used herein, means reversing, alleviating, inhibiting the progress of, the pathology to which such term applies, or one or more symptoms of such pathology

The term "patient" as used herein denotes a mammal. Preferably, a patient according to the invention is a human.

By a "therapeutically effective amount" of an antibody of the invention coupled with a cytotoxic agent is meant a sufficient amount of the antibody coupled with a cytotoxic agent to treat said disease associated with pathological angiogenesis, at a reasonable benefit/risk ratio applicable to any medical treatment.

It will be understood, however, that the total daily usage of the antibodies and compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific antibody employed; the specific composition employed, the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific antibody employed; the duration of the treatment; drugs used in combination or coincidental with the specific polypeptide employed; and like factors well known in the medical arts. For example, it is well known within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

Pharmaceutical compositions:

The antibodies of the invention or fragments thereof may be combined with pharmaceutically acceptable excipients, and optionally sustained-release matrices, such as biodegradable polymers, to form therapeutic compositions.

"Pharmaceutically" or "pharmaceutically acceptable" refers to molecular entities and compositions that do not produce an adverse, allergic or other untoward reaction when administered to a mammal, especially a human, as appropriate. A pharmaceutically acceptable carrier or excipient refers to a non-toxic solid, semi-solid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

The form of the pharmaceutical compositions, the route of administration, the dosage and the regimen naturally depend upon the condition to be treated, the severity of the illness, the age, weight, and sex of the patient, etc.

The pharmaceutical compositions of the invention can be formulated for a topical, oral, parenteral, intranasal, intravenous, intramuscular, subcutaneous or intraocular administration and the like.

Preferably, the pharmaceutical compositions contain vehicles which are pharmaceutically acceptable for a formulation capable of being injected. These may be in particular isotonic, sterile, saline solutions (monosodium or disodium phosphate, sodium, potassium, calcium or magnesium chloride and the like or mixtures of such salts), or dry, especially freeze-dried compositions which upon addition, depending on the case, of sterilized water or physiological saline, permit the constitution of injectable solutions.

The doses used for the administration can be adapted as a function of various parameters, and in particular as a function of the mode of administration used, of the relevant pathology, or alternatively of the desired duration of treatment.

To prepare pharmaceutical compositions, an effective amount of the antibody may be dissolved or dispersed in a pharmaceutically acceptable carrier or aqueous medium.

The pharmaceutical forms suitable for injectable use include sterile aqueous solutions or dispersions; formulations including sesame oil, peanut oil or aqueous propylene glycol; and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In all cases, the form must be sterile and must be fluid to the extent that easy syringability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms, such as bacteria and fungi.

Solutions of the active compounds as free base or pharmacologically acceptable salts can be prepared in water suitably mixed with a surfactant, such as hydroxypropylcellulose. Dispersions can also be prepared in glycerol, liquid polyethylene glycols, and mixtures thereof and in oils. Under ordinary conditions of storage and use, these preparations contain a preservative to prevent the growth of microorganisms.

An antibody of the invention can be formulated into a composition in a neutral or salt form. Pharmaceutically acceptable salts include the acid addition salts (formed with the free amino groups of the protein) and which are formed with inorganic acids such as, for example, hydrochloric or phosphoric acids, or such organic acids as acetic, oxalic, tartaric, mandelic, and the like. Salts formed with the free carboxyl groups can also be derived from inorganic

bases such as, for example, sodium, potassium, ammonium, calcium, or ferric hydroxides, and such organic bases as isopropylamine, trimethylamine, histidine, procaine and the like.

The carrier can also be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), suitable mixtures thereof, and vegetables oils. The proper fluidity can be maintained, for example, by the use of a coating, such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. The prevention of the action of microorganisms can be brought about by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol, sorbic acid, thimerosal, and the like. In many cases, it will be preferable to include isotonic agents, for example, sugars or sodium chloride. Prolonged absorption of the injectable compositions can be brought about by the use in the compositions of agents delaying absorption, for example, aluminium monostearate and gelatin.

Sterile injectable solutions are prepared by incorporating the active compounds in the required amount in the appropriate solvent with various of the other ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the various sterilized active ingredients into a sterile vehicle which contains the basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, the preferred methods of preparation are vacuum-drying and freeze-drying techniques which yield a powder of the active ingredient plus any additional desired ingredient from a previously sterile-filtered solution thereof.

The preparation of more, or highly concentrated solutions for direct injection is also contemplated, where the use of DMSO as solvent is envisioned to result in extremely rapid penetration, delivering high concentrations of the active agents. Upon formulation, solutions will be administered in a manner compatible with the dosage formulation and in such amount as is therapeutically effective. The formulations are easily administered in a variety of dosage forms, such as the type of injectable solutions described above, but drug release capsules and the like can also be employed.

For parenteral administration in an aqueous solution, for example, the solution should be suitably buffered if necessary and the liquid diluent first rendered isotonic with sufficient saline or glucose. These particular aqueous solutions are especially suitable for intravenous, intramuscular, subcutaneous and intraperitoneal administration. In this connection, sterile aqueous media which can be employed will be known to those of skill in the art in light of the

present disclosure. For example, one dosage could be dissolved in 1 ml of isotonic NaCl solution and either added to 1000 ml of hypodermoclysis fluid or injected at the proposed site of infusion, (see for example, "Remington's Pharmaceutical Sciences" 15th Edition, pages 1035-1038 and 1570-1580). Some variation in dosage will necessarily occur depending on the condition of the subject being treated. The person responsible for administration will, in any event, determine the appropriate dose for the individual subject.

The antibodies of the invention may be formulated within a therapeutic mixture to comprise about 0.0001 to 1.0 milligrams, or about 0.001 to 0.1 milligrams, or about 0.1 to 1.0 or even about 10 milligrams per dose or so. Multiple doses can also be administered.

In addition to the compounds formulated for parenteral administration, such as intravenous or intramuscular injection, other pharmaceutically acceptable forms include, e.g. tablets or other solids for oral administration; time release capsules; and any other form currently used.

In certain embodiments, the use of liposomes and/or nanoparticles is contemplated for the introduction of antibodies into host cells. The formation and use of liposomes and/or nanoparticles are known to those of skill in the art.

Nanocapsules can generally entrap compounds in a stable and reproducible way. To avoid side effects due to intracellular polymeric overloading, such ultrafine particles (sized around 0.1 μιη) are generally designed using polymers able to be degraded in vivo. Biodegradable polyalkyl-cyanoacrylate nanoparticles that meet these requirements are contemplated for use in the present invention, and such particles may be are easily made.

Liposomes are formed from phospholipids that are dispersed in an aqueous medium and spontaneously form multilamellar concentric bilayer vesicles (also termed multilamellar vesicles (MLVs)). MLVs generally have diameters of from 25 nm to 4 μιη. Sonication of MLVs results in the formation of small unilamellar vesicles (SUVs) with diameters in the range of 200 to 500 **A,** containing an aqueous solution in the core. The physical characteristics of liposomes depend on pH, ionic strength and the presence of divalent cations.

The invention will be further illustrated by the following figures and examples. However, these examples and figures should not be interpreted in any way as limiting the scope of the present invention.

FIGURE:

Figure 1: Specific monoclonal antibodies MabL4 (anti-CXCL4) and MabLl (anti-CXCL4Ll) allow to distinguish between CXCL4 and CXCL4L1.

A. Western blot of recombinant fusion proteins using hybridoma supernatants (Clone G2) from immunized mice.

B. Slot-blot of CXCL4 and CXCL4L1 using MabL4 and MabLl. mAb7952, which recognizes both proteins, was used as control antibody.

C. SPR analysis of MabL4 and MabLl specificity. The figure depicts the overlay of sensorgrams derived from the injection of rCXCL4 and rCXCL4Ll (500nM) over immobilized MabL4, MabLl and mAb7952 (**R&D** Systems) antibodies (10000 RU).

EXAMPLE:

Material & Methods

Western Blot: Samples were boiled at 95°C for 5 min after addition of reducing agents and 30 µg of total proteins were separated on a NuPAGE 4-12% Bis-Tris gel (Invitrogen). After electrophoretic transfer, the nitrocellulose membranes (Schleicher & Schuell) were blocked with 3% skimmed milk. CXCL4L1, CXCL4 and mutants were immunodetected using the specific mouse anti human CXCL4 monoclonal antibody (Mab-L4) as a primary antibody and peroxidase-conjugated sheep anti-mouse (dilution 1:10000) (Amersham) as a secondary antibody and the ECL detection kit (Amersham).

Slot Blot: Samples were boiled at 95°C for 5 min after addition of reducing agents and 30 µg of total proteins were spotted on the nitrocellulose membranes (Schleicher & Schuell) and blocked with 3% skimmed milk. CXCL4L1, CXCL4 and mutants were immunodetected using the specific mouse anti human CXCL4 monoclonal antibody (Mab-L4), mouse anti CXCL4L1 antibody (Mab-Ll) or mAb7952 (which recognizes both forms) as a primary antibody and peroxidase-conjugated sheep anti-mouse (dilution 1:10000) (Amersham) as a secondary antibody and the ECL detection kit (Amersham).

SPR (surface plasmon resonance): Real-time binding experiments were performed with a BIAcore 3000 biosensor instrument (BIAcore AB) and quantified in terms of resonance units (RU) (1000 RU=1 ng of protein bound/mm^2 of flow cell surface) (Ferjoux *et al,* 2003). Antibodies were immobilized on a carboxymethylated dextran chip (chip CM5, BIAcore AB). Antibodies (7000 RU) were crosslinked on flow cell 2 and flow cell 3, whereas flow cell 1 was activated and deactivated as a nonspecific interaction reference. Soluble ligands (31,2-500 nM) were injected at a flow rate of 30 µl/min, exposed to the surface for

200s (association phase) followed by a 200s flow running during which the dissociation occurred. Sensorgrams are representative of specific interactions (differential response) where non-specific binding that occurred on flow cell 1 was deduced from binding that occurred on flow cell 2 and 3. Results are expressed as resonance units (RU) as a function of time in seconds. A kinetic analysis to determine association, dissociation, and affinity constants (ka, kd, and KD, respectively) was carried out for all recombinants proteins by injecting different concentrations (31,2-500 nM) of proteins over immobilized antibodies. The analysis was carried out by fitting the overlaid sensorgrams with the 1:1 Languimuir binding model with mass transfer of the BIA evaluation 3.1 software.

Results

We generated a novel monoclonal antibody specific for CXCL4 (Mab-L4). The C-terminal peptide of CXCL4 polypeptide was used for immunizing mice. We then tested the purified antibody for reactivity against CXCL4 or CXCL4L1 by western blot (Mab-L4) or slot blot. In comparison Mab-Ll did not recognize CXCL4 when spotted on the membrane. As seen in Figure 1 the monoclonal specific CXCL4 antibody from clone 2G2 only recognized with CXCL4 but not with CXCL4L1. Biacore analysis confirmed the results. The antibodies were immobilized and then CXCL4 and CXCL4L1 were injected at different concentrations. As seen the monoclonal antibody against CXCL4 (MabL4) only recognizes CXCL4 but not CXCL4L1 and the monoclonal antibody against CXCL4L1 (Mab-Ll) only recognizes CXCL4L1. The Mab7952 antibody that regognizes both forms, cross-reacted with both CXCL4 and CXCL4L1. We also determined affinity of the specific CXCL4 antibody. The affinity, when expressed in KD was of 2.6 e^{-8} M similar to that of Mab7952 (that recognizes CXCL4 and CXCL4L1) (1.61 e^{-8} M).

Others available commercial polyclonal and monoclonal anti-human CXCL4 antibodies have been tested and have been shown to recognize both CXCL4 and CXCL4L, namely:

Human CXCL4/PF4 MAb (Clone 170131), Mouse IgG2B ELISA (Capture) [Ref : MAB7951 R&D];

Human CXCL4/PF4 Biotinylated Affinity Purified PAb, Goat IgG ELISA (Detection) and WB [Ref : BAF795 R&D];

Human CXCL4/PF4 Affinity Purified Polyclonal Ab, Goat IgG ELISA (Detection) and WB [Ref : AF795 R&D];

Rabbit polyclonal anti-human CXCL4, ELISA (Detection), [Ref : ab9561 Abeam]

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Throughout this application, various references describe the state of the art to which this invention pertains. The disclosures of these references are hereby incorporated by reference into the present disclosure.

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PCT

Print Out (Original in Electronic Form)

0-1	**Form PCT/RO/134 (SAFE) Indications Relating to Deposited Microorganism(s) or Other Biological Material (PCT Rule 13bis)**	
0-1-1	Prepared Using	PCT Online Filing Version 3.5.000.225 MT/FOP 20020701/0.20.5.20
0-2	**International Application No.**	EP2012051056
0-3	**Applicant's or agent's file reference**	BIKFAL1001AS

1	**The indications made below relate to the deposited microorganism(s) or other biological material referred to in the description on:**	
1-1	**page**	5
1-2	**line**	21
1-3	**Identification of deposit**	
1-3-1	Name of depositary institution	CNCM Collection nationale de cultures de micro-organismes
1-3-2	Address of depositary institution	Institut Pasteur, 28, rue du Dr Roux, 75724 Paris Cedex 15, France
1-3-3	Date of deposit	30 April 2010 (30.04.2010)
1-3-4	Accession Number	CNCM I-4310
1-5	**Designated States for Which Indications are Made**	All designations

FOR RECEIVING OFFICE USE ONLY

0-4	**This form was received with the international application:** (yes or no)	YES
0-4-1	Authorized officer	Cajide, Maria

FOR INTERNATIONAL BUREAU USE ONLY

0-5	**This form was received by the international Bureau on:**	**06 February 2012 (06.02.2012)**
0-5-1	Authorized officer	**Ryad Bentobbal**

CLAIMS:

1. An isolated CXCL4 antibody which is obtainable from the hybridoma accessible under CNCM deposit number 1-4310.

2. An isolated CXCL4 antibody which comprises the CDRs sequences of the antibody according to claim 1.

3. An antibody which comprises a variable light chain (VL) comprising the CDRs of the VL chain of an antibody according to claim 1 and a variable heavy chain (VH) comprising the CDRs of the VH chain of an antibody according to claim 1.

4. The antibody according to claim 3 comprising the VL chain of the antibody according to claim 1 and the VH chain of the antibody according to claim 1.

5. The antibody of claim 4 wherein said antibody is a chimeric antibody.

6. The antibody according to claim 2 or 3, wherein said antibody is a humanized antibody.

7. The antibody according to any of claims 1 to 6 which is an antibody fragment directed against CXCL4.

8. The antibody according to claim 7 wherein said fragment is selected from the group consisting of Fv, Fab, F(ab')2, Fab', dsFv, scFv, sc(Fv)2 and diabodies.

9. A nucleic acid comprising a sequence encoding the antibody according to any of claims 1 to 8.

10. A vector comprising a nucleic acid according to claim 9.

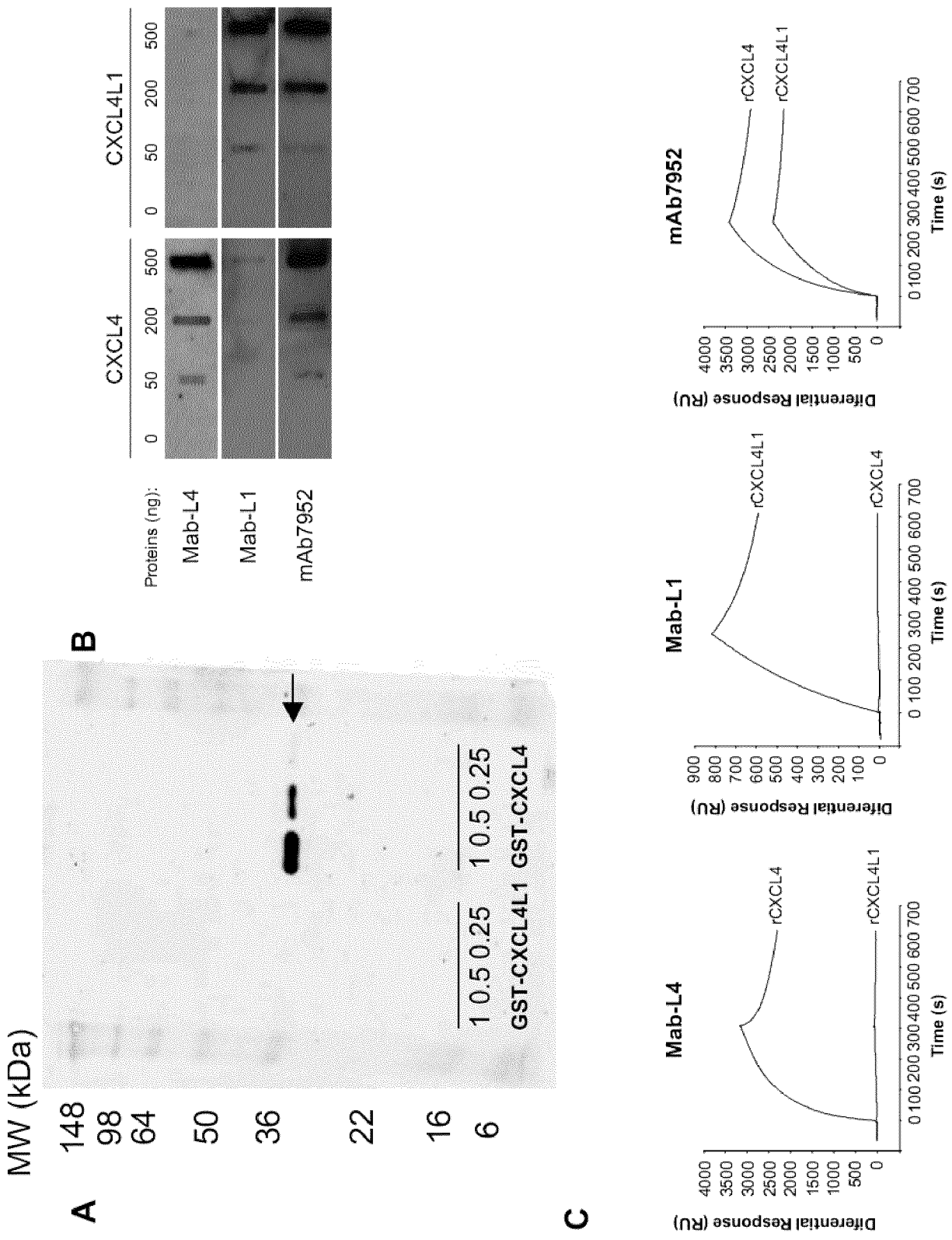
11. A host cell comprising a nucleic acid according to claim 8 or a vector according to claim 9.

12. A pharmaceutical composition comprising the antibody according to any of claims 1 to 8 and a pharmaceutically acceptable carrier.

13. The antibody according to any one claims 1 to 8 which is labelled with a detectable molecule or substance.

14. A method for detecting CXCL4 in a biological sample comprising a step of contacting said biological sample with the antibody according to any one of claims 1 to 8, or a labelled antibody or fragment thereof according to claim 13.

15. The antibody according to any one claims 1 to 8 for use as drug.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MW (kDa)
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98
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GST-CXCL4L1
1 0.5 0.25
GST-CXCL4
B
CXCL4
CXCL4L1
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0 50 200 500
Proteins (ng):
Mab-L4
Mab-L1
mAb7952
C
Mab-L4
rCXCL4
rCXCL4L1
Mab-L1
rCXCL4L1
rCXCL4
mAb7952
rCXCL4
rCXCL4L1
Differential Response (RU)
Time (s)
0 100 200 300 400 500 600 700

Figure 1

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/051056

A. CLASSIFICATION OF SUBJECT MATTER
INV. C07K16/24 A61K39/395
ADD.

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)
C07K A61K

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, Sequence Search , EMBASE, WPI Data

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
Y	EP 0 457 532 Al (FIDIA SPA [IT]) 21 November 1991 (1991-11-21) the whole document in particular abstract page 2, lines 33-58 page 3, line 54 - page 5, line 4 claims 1-26; examples	1-15
Y	WO 2007/007665 Al (SAITAMA MEDICAL SCHOOL [JP] ; SUDA TATSUO [JP] ; KATAGIRI TAKENOBU [JP] ;) 18 January 2007 (2007-01-18) abstract	1-15

-/- .

[X] Further documents are listed in the continuation of Box C.

[X] See patent family annex.

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"A" document defining the general state of the art which is not considered to be of particular relevance

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"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

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Date of the actual completion of the international search	Date of mailing of the international search report
20 February 2012	01/03/2012

Name and mailing address of the ISA/	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Fax: (+31-70) 340-3016	Ferrei ra, Roger

INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/051056

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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A	WO 01/04159 A1 (STC UNM [US] ; AREPALLY GOWTHAMI [US] ; KISIEL WALTER [US] ; KAMEI KEIKO) 18 January 2001 (2001-01-18) the whole document	1-15
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INTERNATIONAL SEARCH REPORT

International application No
PCT/EP2012/051056

C(Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

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A	LASAGNI LAURA ET AL: "PF-4/CXCL4 and CXCLAL1 exhi bi t di sti nct subcel l ular l ocal i zati on and a di fferenti al l y regul ated mechani sm of secreti on" , BLOOD, AMERICAN SOCI ETY OF HEMATOLOGY, US, vol . 109 , no. 10, 1 May 2007 (2007-05-01) , pages 4127-4134, XP002515692 , ISSN: 0006-4971 , D0I : D0I : 10. 1182/BL00D-2006-10-052035 [retri eved on 2007-01-11] the whol e document	1-15
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