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(71) **Applicant**: **CANCER RESEARCH TECHNOLOGY LTD** [GB/GB]; Angel Building, 407 St John Street, London EC1V 4AD (GB).

(72) **Inventors**: **BALKWILL, Frances Rosemary**; 37 The Lexington, 40 City Road, London EC1Y 2AN (GB). **MCCAFFERTY, John**; 47 Church Farm Cottage, Sawston Road, Babraham, Cambridge CB22 3AT (GB). **GRAHAM, Gerard John**; 39 Berryhill Drive, Giffnock, Glasgow G46 7AA (GB). **VELLATT, Aneesh Karatt**; 31 St Thomas Close, Comberton, Cambridge CB23 7DN (GB). **SLAVNY, Peter**; c/o Cancer Research Technology Ltd, Angel Building, 407 St John Street, London EC1V 4AD (GB).

(74) **Agents**: **KIDDLE, Simon** et al.; Mewburn Ellis LLP, City Tower, 40 Basinghall Street, London Greater London EC2V 5DE (GB).

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(54) **Title**: ANTI-CXCL12 ANTIBODY MOLECULES AND THEIR USES

(57) **Abstract**: Anti-CXCL12 antibody molecules and their uses are disclosed, and in particular anti-CXCL12 antibody molecules that are capable of inhibiting a biological activity of CXCL12 in vitro and in vivo and their use for treating CXCL12-mediated disease.

Anti-CXCL12 Antibody Molecules and Their Uses

Field of the Invention

The present invention relates to anti-CXCL12 antibody molecules and their uses, and more particularly to anti-CXCL12 antibody molecules that are capable of inhibiting a biological activity of CXCL12 and their use for treating cancer.

Background of the Invention

The C-X-C motif chemokine 12 (CXCL12), also known as stromal cell-derived factor 1 (SDF-1), is a CXC chemokine protein that in humans is encoded by the CXCL12 gene. It is known to bind to two G-protein-coupled receptors, CXCR4 and CXCR7. It participates in many developmental and physiologic processes, including haematopoiesis and angiogenesis. CXCL12 plays a role in angiogenesis by recruiting endothelial progenitor cells (EPCs) from the bone marrow through a CXCR4 dependent mechanism, making it a significant factor in carcinogenesis and neovascularisation linked to tumour progression. Migration another important way in which CXCL12 influences tumour development and progression. CXCL12 also has a role in organ-specific metastasis of several cancers, where cancer cells that express the receptor CXCR4 are attracted to metastasis target tissues that release the ligand, CXCL12. CXCL12 also acts to recruit CXCR4-positive stromal cells and regulates immune cell infiltration. For example, CXCL12 may aid the formation of pre-metastatic niches through the recruitment of regulatory T cells, producing an immunosuppressive environment (Zhao et al, Oncoimmunology, 1(2): 152-161, 2012). In prostate cancer, cancer associated fibroblasts (CAFs) engage monocyte recruitment and M2 polarization through CXCL12 (Comito et al, Oncogene, 33: 2423 - 2431, 2014). High levels of CXCL12 are associated with low numbers of T cells in a pancreatic cancer model and it was possible to increase T cell infiltration through combined treatment with PD-L1 and CXCR4 inhibitors. This increase in T cell infiltration was accompanied by a significant reduction in tumour volume, highlighting the role of the CXCL12/CXCR4 axis

in immune control of cancer (Feig et al, PNAS, 110(50): p20212-20217, 2013).

The CXCL12/CXCR4/CXCR7 pathway has therefore generated considerable interest as a potential therapeutic target given its role in tumour growth, survival and angiogenesis (Balkwill et al., Seminars in Cancer Biology, 14: 171-179, 2004).

WO 2008/018641 (Ono Pharmaceutical Co. Ltd and Medarex, Inc.) discloses human monoclonal antibodies that specifically bind to SDF-1 and proposes their medical uses for treating various B cell malignancies, including breast cancer, multiple myeloma and non-Hodgkin's lymphoma and autoimmune disorders. Zhong et al. (Clinical Cancer Research, 19: 4433-4445, 2013; DOI: 10.1158/1078-0432.CCR-13-0943) discloses a humanised version of a hamster monoclonal antibody 30D8 and shows that it was capable of binding to human and murine CXCL12 in *in vitro* assays.

Summary of the Invention

Broadly, the present invention is based on the affinity maturation of anti-CXCL12 antibody molecules and their functional validation in cell-based assays and *in vivo* to show that the antibody molecules are capable of inhibiting CXCR4-induced cancer cell migration and/or are capable of inhibiting VEGF-induced angiogenesis *in vitro*. These properties enable the antibody molecules of the present invention to be used in the treatment of cancer, in particular by inhibiting metastasis and or tumour neovascularisation.

Accordingly, in a first aspect, the present invention provides an isolated anti-CXCL12 antibody molecule which specifically binds to human, and optionally murine, CXCL12 and inhibits CXCL12-mediated biological activity, wherein the antibody molecule binds to an epitope of human CXCL12 having the amino acid sequence as set out in SEQ ID NO: 24 that comprises amino acids (a) P10 and R12, and optionally one or more of E15, I28, P32, N45 and/or K54

or (b) P10 and Q48, and optionally one of more of K54 and N45.

In a further aspect, the present invention provides an anti-CXCL12 antibody molecule which comprises: (a) a CDR-H1 having the amino acid sequence of SEQ ID NO: 1, or the amino acid sequence of SEQ ID NO: 1 with one, two, three or more amino acid substitutions, deletions or insertions, (b) a CDR-H2 having the amino acid sequence of SEQ ID NO: 2, or the amino acid sequence of SEQ ID NO: 2 with one, two, three or more amino acid substitutions, deletions or insertions and (c) a CDR-H3 having the amino acid sequence of SEQ ID NO: 3, or the amino acid sequence of SEQ ID NO: 3 with one, two, three or more amino acid substitutions, deletions or insertions; and optionally (d) a CDR-L1 having the amino acid sequence of SEQ ID NO: 4, or the sequences of SEQ ID NO: 4, with one, two, three or more amino acid substitutions, deletions or insertions, (e) a CDR-L2 having the amino acid sequence of SEQ ID NO: 5, or the sequences of SEQ ID NO: 5, with one, two, three or more amino acid substitutions, deletions or insertions and (f) a CDR-L3 having the amino acid sequence of SEQ ID NO: 6, or the sequences of SEQ ID NO: 6, with one, two, three or more amino acid substitutions, deletions or insertions.

In a further aspect, the present invention provides an anti-CXCL12 antibody molecule which comprises (a) a CDR-H1 having the amino acid sequence of SEQ ID NO: 12, or the amino acid sequence of SEQ ID NO: 12 with one, two, three or more amino acid substitutions, deletions or insertions, and (b) a CDR-H2 having the amino acid sequence of SEQ ID NO: 13, or the amino acid sequence of SEQ ID NO: 13 with one, two, three or more amino acid substitutions, deletions or insertions, and (c) a CDR-H3 having the amino acid sequence of SEQ ID NO: 14, or the amino acid sequence of SEQ ID NO: 14 with one, two, three or more amino acid substitutions, deletions or insertions; and optionally (d) a CDR-L1 having the amino acid sequence of SEQ ID NO: 15, or the sequences of SEQ ID NO: 15, with one or more amino acid substitutions, deletions or insertions, and (e) a CDR-L2 having

the amino acid sequence of SEQ ID NO: 16, or the sequences of SEQ ID NO: 16, with one, two, three or more amino acid substitutions, deletions or insertions and (f) a CDR-L3 having the amino acid sequence of SEQ ID NO: 17, or the sequences of SEQ ID NO: 17, with one, two, three or more amino acid substitutions, deletions or insertions.

In a further aspect, the present invention provides a pharmaceutical composition comprising an antibody molecule or immunoconjugate as disclosed herein and a pharmaceutically acceptable excipient.

In a further aspect, the present invention provides an antibody molecule or immunoconjugate as disclosed herein for use in a method of treatment of the human or animal body.

In a further aspect, the present invention provides an antibody molecule or immunoconjugate as disclosed herein for use in a method of treatment of a CXCL12-mediated condition.

In a further aspect, the present invention provides the use of an antibody molecule or immunoconjugate as disclosed herein in the manufacture of a medicament for use in treating a CXCL12-mediated condition.

In a further aspect, the present invention provides a method of treating an individual with a CXCL12-mediated condition comprising administering an antibody molecule or immunoconjugate as disclosed herein to an individual in need thereof.

In a further aspect, the present invention provides an antibody molecule of the present invention for use in a method for the diagnosis or prognosis of a patient having a CXCL12-mediated condition. By way of example, the method may comprise determining the presence or amount of CXCL12 in the sample using the antibody and correlating the presence or amount of CXCL12 with the likely outcome of treating the patient with a CXCL12

inhibitor.

In the medical uses and methods of treatment of the present invention, preferably the CXCL12-mediated condition is cancer, including cancer and/or immune cell migration and/or metastasis. The types of cancer that may be treated using the antibodies or immunoconjugates of the present invention include ovarian cancer, breast cancer, bone cancer, prostate cancer, thyroid cancer, pancreatic cancer, multiple myeloma, non-Hodgkin's lymphoma, intraocular lymphoma, follicular centre lymphoma, CML, colorectal cancer, oral squamous carcinoma, cervical cancer, neuroblastoma, kidney cancer, brain cancers, such as glioma and astrocytoma, rhabdmyosarcoma, lung cancer, such as small cell lung cancer, melanoma, B cell malignancies, such as B-cell chronic lymphocytic leukemia (B-CLL), and leukaemia, such as acute myeloid leukaemia (AML) and acute lymphoblastic leukemia. In other embodiments, the present invention may be used for the treatment of WHIM syndrome.

In other uses, the present invention may be used for the treatment of conditions in which CXCL12 signalling is implicated, for example in the mobilisation of cells such as stem cell mobilisation in bone marrow, e.g. in preparation for cell transplantation, similar to the use of a small molecule CXCR4 inhibitor (Plerixafor, AMD3100).

Embodiments of the present invention will now be described by way of example and not limitation with reference to the accompanying figures. However various further aspects and embodiments of the present invention will be apparent to those skilled in the art in view of the present disclosure.

"and/or" where used herein is to be taken as specific disclosure of each of the two specified features or components with or without the other. For example "A and/or B" is to be taken as specific disclosure of each of (i) A, (ii) B and (iii) A and B, just as if each is set out individually herein.

Unless context dictates otherwise, the descriptions and definitions of the features set out above are not limited to any particular aspect or embodiment of the invention and apply equally to all aspects and embodiments which are described.

Brief Description of the Figures

Figure 1. Vector systems used for expressing anti-CXCL12 antibodies. A) Single chain antibody (scFv) expression using the pSANG10-3F vector. In this plasmid, transcription of the scFv gene is under the control of a bacteriophage T7 promoter. Restriction sites *Nco*I, *Xho*I, *Nhe*I and *Not*I facilitate the sub-cloning of variable heavy (VH) and light (VL) chain genes into the Fab and IgG expression vectors. B) Fab antibody expression using the pBIOCAM-7 vector. This plasmid contains a bicistronic Fab expression cassette under the control of a CMV promoter. P2A sequence present between heavy and light chain genes allows the release of antibody heavy polypeptide chain (VH-CH1) downstream of it by a ribosomal skip mechanism. The P2A peptide is post translationally removed from the antibody light chain by Furin cleavage. C) IgG expression using pBIOCAM1-2 dual plasmid system. Heavy (VH-CH1-CH2-Ch3) and light chain expression cassettes are located in two different plasmids. Plasmid pBIOCAM-1 codes for the light chain genes and the pBIOCAM-2 encodes the heavy chain cassette. Transcription of antibody gene is under the control of CMV promoter in both plasmids. Hexa-histidine (6X-His) and tri-FLAG tags fused to the antibody genes in pSANG10-3F and pBIOCAM7 enable purification and immune-detection of the expressed antibodies.

Figure 2. SDS-PAGE analysis of anti-CXCL12 Fabs and IgGs. Lead anti-CXCL12 antibodies and their parent clones were expressed in HEK-293 cells as Fabs and IgGs. Affinity purified antibodies were visualised on a reducing SDS-PAGE gel using SYPRO® Red staining. Clones 093_2D06, 093_2A02, 114_3H1 (labelled as 114_3H01) and 113_1H12 were loaded respectively as Fabs (lanes 1-4) and IgGs (lanes 5-8). Smearing of VH-CH1 bands in some of the Fab

preparations could be due to the cleavage of the FLAG tag (which often occurs in Flag tagged proteins.

Figure 3. Affinity measurement of anti-CXCL12 antibodies using SPR A) Sensograms of multiple concentrations of lead and parental anti-CXCL12 Fabs binding to biotinylated CXCL12 immobilised on a streptavidin chip (carboxymethylated dextran matrix pre-immobilised with streptavidin). B) Binding constants of 114_3H1 (labelled as 114_3H01) were determined using 1:1 Langmuir binding model. The equilibrium dissociation constant (KD) of the parent clone 093_2D06 was calculated using steady state binding model due to its very fast off-rate. C) Antibody 113_1H12 and its parental clone 093_2A02 showed a biphasic binding profile. The binding constants of these antibodies were determined using two-state binding model. Biacore T100 evaluation software was used for all calculations.

Figure 4. CXCL12 induced migration of ovarian cancer cells. A) Fluorescently labelled human ovarian cancer cells (TOV-21G) in the upper chamber and CXCL12 in the lower chamber were separated by a porous membrane coated with collagen. Migration of cells across the membrane was quantified by fluorescence scanning. B) Optimum human CXCL12 concentration for inducing cell migration was determined by titration of CXCL12 (ranging from 20-1200 ng/ml). 80 ng/ml CXCL12 was chosen for stimulating cell migration in the inhibition assay. All error bars represent the standard deviation.

Figure 5. Inhibition of cancer cell migration by anti-CXCL12 antibodies. Transwell migration of fluorescently labelled TOV-21G cells towards CXCL12 was quantified using fluorescence scanning. Titrations of 114_3H1 (labelled as 114_3H01) and 113_1H12 IgGs ranging from 0.39-500 nM were mixed with 80ng/ml (10nM) human CXCL12 in the lower chamber to test the impact of these antibodies on CXCL12 induced migration. An anti-lysozyme antibody (500 nM) was used as an isotype control. All error bars represent standard deviation.

Figure 6. Inhibition of angiogenesis by anti-CXCL12 antibodies. Human umbilical vein endothelial cells (HUVECs) were plated onto fibroblasts that had been grown for 6 days on gelatin coated chamber slides. These two cell types were co-cultured for 7 days in a media containing VEGF and lead anti-CXCL12 antibodies 114_3H1 and 113_1H12. An IgG that bind to lysozyme (Non-specific IgG) was used as an isotype control for the assay (panel A). After 7 days of co-culture the cells were stained for the platelet/endothelial adhesion molecule-1 (PECAM-1, a marker for angiogenesis) to visualise the formation and branching of tubules by light microscopy. Total number of tubules, number of branch junctions (Figure 6A) and the total tubule length (Figure 6B) was calculated using AngioSys image analysis software.

Figure 7. Heavy and light chain sequence alignments of CXCL12 antibodies 114_3H1 and 113_1H12.

Figure 8. Sequence alignment of heavy and light chain sequence alignments of CXCL12 antibodies 114_3H1 and 113_1H12 with the antibodies of WO 2008/018641.

Figure 9A & B. Migration tracks showing the results of experiments to determine the effectiveness of the antibodies (113_1H12 in a human IgG2 format (hAB113), 113_1H12 in a chimeric murine IgG2a format (mAB113) and 114_3H1 in a chimeric murine IgG2a format (mAB114)) in blocking migration of a murine metastatic melanoma cell line (B16F10) and human ovarian carcinoma cell line (TOV-21) in the presence of human CXCL12.

Figure 10. Results of *in vivo* experimental metastasis model cell migration assay based B16F10 melanoma cells requiring CXCR4 for migration to the lung and initiation of metastasis. B16F10 melanoma cells were introduced into C57Bl mice through tail vein injection on day 0 and treatment commenced on day 1. Treatment regimes were either 5 mg/kg of the clinical CXCR4 inhibitor AMD3100 (Plerixafor) twice daily or twice a week with either 10,

15 or 20 mg/kg of the anti-CXCL12 antibody in a chimeric murine IgG2a format. Mice in the control arm were treated twice a week with 20 mg/kg of a control antibody. All mice were culled on day 14 and the number of metastatic colonies in the lungs quantified. A level of inhibition equivalent to that of AMD3100 was achieved with the 20 mg/kg dose of 113_1H12.

Figure 11. Result of *in vitro* cell transwell migration assays that show that anti-CXCL12 antibodies of the present invention block migration of TOV21G cancer cells induced by human CXCL12 in scFv-Fc and human IgG2 formats.

Detailed Description

Anti-CXCL12 antibody molecules

Unless stated otherwise, antibody residues are numbered herein in accordance with the Kabat numbering scheme.

The full length amino acid sequence of CXCL12 is set out as SEQ ID NO: 23 and consists of 89 amino acids. The amino acid sequence of the synthesized 68 amino acid CXCL12 fragment used to select the exemplified antibodies is set out in SEQ ID No: 24. The epitope mapping studies described in the examples below used a wild type mature CXCL12 polypeptide having the amino acid sequence set out in SEQ ID NO: 25 that include a polyhistidine tag and linker sequence. Preferably, the antibody molecules of the present invention are capable of binding to CXCL12 polypeptides that comprise a polypeptide having at least 90% sequence identity to amino acids 1 to 68 as set out in SEQ ID NO: 23, or a fragment thereof, wherein the fragment is biologically active. Examples of the biological activities of the antibody molecules or immunoconjugates of the present invention include binding to CXCL12, for example to block the interaction of CXCL12 with CXCR4 and optionally also the interaction of CXCL12 with CXCR7. In addition, the biological activities of CXCL12 that may be inhibited (antagonised) by the antibody molecules or immunoconjugates of the present invention include inhibition of VEGF-induced angiogenesis *in vitro* and/or the inhibition of

CXCL12-induced cancer cell migration and/or spreading and/or metastasis. The antibody molecules or immunoconjugates of the present invention may also inhibit the role of CXCL12 in regulating immune cell infiltration. Assays for determining cancer cell migration and metastasis are described in the examples herein.

As described in detail in the examples below, the *in vitro* affinity maturation of primary anti-CXCL12 antibodies was carried out in two steps. Firstly, the primary antibody sequences were diversified by light chain shuffling to create a derivative library. Secondly, tailored selection and screening procedures were used to identify affinity-improved variants from the light chain shuffled library. The rationale for using light chain shuffling for diversifying primary anti-CXCL12 antibodies was as follows. The original diversity of naïve immune repertoires *in vivo* (pre-immune B-cell repertoire) or *in vitro* ("McCafferty library") is derived from the combinatorial rearrangement of germline variable gene segments. A light chain variable region (VL) is encoded by the combination of a long V gene segment and a short joining (J) gene segment. In contrast, the gene encoding heavy chain variable region (VH) is assembled from three gene segments - a V segment, a J segment, and diversity (D) segment and hence is the more diverse of two variable chains. Due to this increased diversity, especially in the CDR3 region, the VH domain tends to play the dominant role in antigen binding and in defining the epitope specificity. As VL domains also make contributions in fine-tuning the binding affinity and antibody expression levels, it is important to have as many VH-VL combinations as possible in a library to identify high affinity antibodies with desirable expression properties. In the examples below, a light chain shuffled library of $2x10^8$ was created by combining the heavy chain variable regions of 20 anti-CXCL12 antibodies with a repertoire of kappa and lambda light chain variable domains. Thus each original heavy chain was paired with approximately 10 million new light chain partners. Three rounds of phage display selection were carried out under stringent

conditions to enrich for high affinity binders from the chain shuffled library. The stringency of the selection conditions was increased at each round by reducing the antigen concentration or using harsher and longer wash steps. Such selection procedures facilitate preferential enrichment of antibody clones with lower dissociation constants.

Affinity maturation selections were carried out in solution-phase, allowing precise control of antigen concentration, which is an important parameter in determining the stringency of a selection. This process identified two antibodies (114_3H1 and 113_1H12) exhibiting the lowest dissociation constants were selected as lead antibodies for detailed characterisation and optimisation. Complete kinetic analysis of 114_3H1 and 113_1H12 and their parent clones (093_2D06 and 093_2A02 respectively) confirmed the improvement in affinity after light chain shuffling. The calculated affinity of 1 nM for 114_3H1 represents a 3800-fold improvement from its parent antibody 093_2D06 (KD = 3.8 μM). Since the affinity of the other parent antibody 093_2A02 (KD = 16.7 nM) was relatively high at the outset, affinity improvement on its daughter clone 113_1H12 led to a KD = 3.7 nM.

In addition to the binding and kinetic studies, the biological properties of the antibody molecules were tested in cell-based functional assays to determine their likely *in vivo* potency as this does not necessarily correlate with binding, especially if the antibody molecule is intended to modulate complex biological functions. In view of the role of CXCL12 in cancer metastasis and establishing a tumour supportive vasculature, the biological properties of the antibody molecules of the present invention include inhibition of CXCL12-induced cancer cell migration and/or inhibition of VEGF-induced angiogenesis *in vitro*.

The biological property of inhibiting CXCL12-induced cancer cell migration may be determined using a transwell migration assay used here was a modified version of the Boyden chamber assay used

to study the chemotactic response of leukocytes (Boyden, J. Exp. Med. *115*, 453-46, 1962) in which migration of fluorescently labelled cancer cells, such as human ovarian cancer cells TOV-21G, seeded in an upper chamber across a porous membrane and into a lower chamber containing CXCL12 is determined.

The biological property of inhibiting angiogenesis may be determined using a cell based assay in which human umbilical vein endothelial cells (HUVECs) and fibroblasts are cultured together in a media containing anti-CXCL12 antibodies and VEGF. The interaction of these two cell types in the presence of VEGF results in the formation of three-dimensional tubes that resemble small capillaries *in vivo*, see Hetheridge et al. (Biochem. Soc. Trans. *39*, 1597-1600, 2011).

The two lead antibodies, 114_3H1 and 113_1H12 were tested using *in vitro* cancer cell migration assay and angiogenesis assay in order to evaluate their functional characteristics. Both antibodies inhibited CXCL12-induced migration of ovarian cancer cells, with 114_3H1 outperforming 113_1H12. For both antibodies the IC_{50}s observed in this assay were comparable to the calculated KD values from SPR analysis. In addition, antibody clone 113_1H12 significantly inhibited VEGF-induced angiogenesis, while antibody clone 114_3H1 partially inhibited VEGF-induced angiogenesis.

Without wishing to be bound by any particular theory, this difference in the properties of the antibodies may be a result of the fact that CXCL12 can induce angiogenesis via interaction with both CXCR4 and CXCR7. Therefore, it may be possible that 114_3H1 only blocks CXCL12/CXCR4 interaction, but not CXCL12/CXCR7 resulting in partial inhibition of angiogenesis. By contrast, 113_1H12 might be blocking CXCL12 binding to both CXCR4 and CXCR7 leading to superior inhibition of CXCL12 induced angiogenesis.

Accordingly, the present invention provides antibody molecules that are based on the antibody clones 113_1H12 or 114_3H1.

In one aspect, the present invention provides an anti-CXCL12 antibody molecule comprising at least one, two, three, four, five, or six of the following CDR sequences based on the CDR sequences of antibody 114_3H1:

(a) a CDR-H1 having the amino acid sequence of SEQ ID NO: 1, or the amino acid sequence of SEQ ID NO: 1 with one, two, three or more amino acid substitutions, deletions or insertions; and/or

(b) a CDR-H2 having the amino acid sequence of SEQ ID NO: 2, or the amino acid sequence of SEQ ID NO: 2 with one, two, three or more amino acid substitutions, deletions or insertions; and/or

(c) a CDR-H3 having the amino acid sequence of SEQ ID NO: 3, or the amino acid sequence of SEQ ID NO: 3 with one, two, three or more amino acid substitutions, deletions or insertions; and/or

(d) a CDR-L1 having the amino acid sequence of SEQ ID NO: 4, or the sequences of SEQ ID NO: 4, with one, two, three or more amino acid substitutions, deletions or insertions; and/or

(e) a CDR-L2 having the amino acid sequence of SEQ ID NO: 5, or the sequences of SEQ ID NO: 5, with one, two, three or more amino acid substitutions, deletions or insertions; and/or

(f) a CDR-L3 having the amino acid sequence of SEQ ID NO: 6, or the sequences of SEQ ID NO: 6, with one, two, three or more amino acid substitutions, deletions or insertions.

In one embodiment, the anti-CXCL12 antibody molecules comprise all six CDRs as defined above optionally with one or more amino acid substitutions, deletions or insertions.

In a further aspect, the present invention provides an anti-CXCL12 antibody molecule comprising at least one, two, three, four, five, or six or more of the following CDR sequences based on the CDR sequences of antibody 113_1H12:

(a) a CDR-H1 having the amino acid sequence of SEQ ID NO: 12, or the amino acid sequence of SEQ ID NO: 12 with one, two, three or more amino acid substitutions, deletions or insertions; and/or

(b) a CDR-H2 having the amino acid sequence of SEQ ID NO:

13, or the amino acid sequence of SEQ ID NO: 13 with one, two, three or more amino acid substitutions, deletions or insertions; and/or

(c) a CDR-H3 having the amino acid sequence of SEQ ID NO: 14, or the amino acid sequence of SEQ ID NO: 14 with one, two, three or more amino acid substitutions, deletions or insertions; and/or

(d) a CDR-L1 having the amino acid sequence of SEQ ID NO: 15, or the sequences of SEQ ID NO: 15, with one or more amino acid substitutions, deletions or insertions; and/or

(e) a CDR-L2 having the amino acid sequence of SEQ ID NO: 16, or the sequences of SEQ ID NO: 16, with one, two, three or more amino acid substitutions, deletions or insertions; and/or

(f) a CDR-L3 having the amino acid sequence of SEQ ID NO: 17, or the sequences of SEQ ID NO: 17, with one, two, three or more amino acid substitutions, deletions or insertions.

In one embodiment, the anti-CXCL12 antibody molecules comprise all six CDRs as defined above (SEQ ID NO: 12-17), optionally with one or more amino acid substitutions, deletions or insertions.

A light chain variable region (VL) is encoded by the combination of a long V gene segment and a short joining (J) gene segment. In contrast, gene encoding heavy chain variable region (VH) is assembled from three gene segments - a V segment, a J segment, and diversity (D) segment and hence is the more diverse of two variable chains. Due to this increased diversity, especially in the CDR3 region, the VH domain tends to play the dominant role in antigen binding and defining the epitope specificity.

Accordingly, in some embodiments, the present invention provides anti-CXCL12 antibody molecules that comprise the CDRs of the heavy chain of the exemplified antibodies as defined herein, optionally each with one, two, three or more amino acid substitutions, deletions or insertions, in combination with light chain derived from a different antibody molecule.

In further aspect, the present invention provides an anti-CXCL12 antibody that comprises (a) a VH domain comprising at least one, at least two, or all three VH CDR sequences selected from (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 2, and (iii) CDR-H3 comprising an amino acid sequence selected from SEQ ID NO: 3; and (b) a VL domain comprising at least one, at least two, or all three VL CDR sequences selected from (i) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 4, (ii) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and (c) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 6.

In a further aspect, the antibody molecules of the present invention comprise a VH domain comprising the amino acid sequence set out in SEQ ID NO 7, or a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 7. In some embodiments, the VH sequence comprises one or more substitutions, insertions, or deletions relative to the reference sequence, while the antibody molecule retains the property of binding to CXCL12, and optionally one or more of the other biological activities of the anti-CXCL12 antibody molecules of the present invention as described herein. Preferably, the VH domain comprises one, two or three CDRs selected from (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 2, and (iii) CDR-H3 comprising an amino acid sequence selected from SEQ ID NO: 3.

In a further aspect, the antibody molecules of the present invention comprise a VL domain comprising the amino acid sequence set out in SEQ ID NO 9, or a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to amino acid sequence of SEQ ID NO: 9. In some embodiments, the VH sequence comprises one or more substitutions, insertions, or deletions relative to the reference sequence, while the antibody molecule retains the property of binding to CXCL12, and optionally one or more of the other biological activities of the

anti-CXCL12 antibody molecules of the present invention as described herein. Preferably, the VL domain comprises one, two or three CDRs selected from (i) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 4, (ii) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 5, and (c) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 6.

In some embodiments, the antibody molecules of the present invention comprise a VH domain comprising the amino acid sequence set out in SEQ ID NO 7, or a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 7, and a VL domain comprising the amino acid sequence set out in SEQ ID NO 9, or a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO: 9.

In further aspect, the present invention provides an anti-CXCL12 antibody of the invention comprises (a) a VH domain comprising at least one, at least two, or all three VH CDR sequences selected from (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 12, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 13, and (iii) CDR-H3 comprising an amino acid sequence selected from SEQ ID NO: 14; and (b) a VL domain comprising at least one, at least two, or all three VL CDR sequences selected from (i) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 15, (ii) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 16, and (c) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 17.

In a further aspect, the antibody molecules of the present invention comprise a VH domain comprising the amino acid sequence set out in SEQ ID NO 18, or a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to the amino acid sequence of SEQ ID NO: 18. In some embodiments, the VH sequence comprises one or more substitutions, insertions, or deletions relative to the reference sequence, while the antibody molecule retains the property of binding to CXCL12, and optionally one or more of the other biological

activities of the anti-CXCL12 antibody molecules of the present invention as described herein. Preferably, the VH domain comprises one, two or three CDRs selected from (i) CDR-H1 comprising the amino acid sequence of SEQ ID NO: 12, (ii) CDR-H2 comprising the amino acid sequence of SEQ ID NO: 13, and (iii) CDR-H3 comprising an amino acid sequence selected from SEQ ID NO: 14.

In a further aspect, the antibody molecules of the present invention comprise a VL domain comprising the amino acid sequence set out in SEQ ID NO 20, or a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to amino acid sequence of SEQ ID NO: 20. In some embodiments, the VH sequence comprises one or more substitutions, insertions, or deletions relative to the reference sequence, while the antibody molecule retains the property of binding to CXCL12, and optionally one or more of the other biological activities of the anti-CXCL12 antibody molecules of the present invention as described herein. Preferably, the VL domain comprises one, two or three CDRs selected from (i) CDR-L1 comprising the amino acid sequence of SEQ ID NO: 15, (ii) CDR-L2 comprising the amino acid sequence of SEQ ID NO: 16, and (c) CDR-L3 comprising the amino acid sequence of SEQ ID NO: 17.

In some embodiments, the antibody molecules of the present invention comprise a VH domain comprising the amino acid sequence set out in SEQ ID NO: 18, or a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 18, and a VL domain comprising the amino acid sequence set out in SEQ ID NO: 20, or a sequence having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% sequence identity to SEQ ID NO: 20.

In a further aspect, the present invention provides anti-CXCL12 antibody molecules capable of binding to an epitope of CXCL12 having the amino acid sequence as set out in SEQ ID NO: 24 or 25 that comprises amino acids (a) P10 and R12, and optionally one or

more of E15, I28, P32, N45 and/or K54 or (b) P10 and Q48, optionally one of more of K54 and N45. The exemplified antibody 114_3H1 binds to epitope (a) and antibody 113_1H12 binds to epitope (b).

E15 is outside of regions that involved in receptor or heparin binding. All other epitope residues are within regions involved in receptor binding, which according to numbering of the full length protein at UniProt P48061 (SDF1_HUMAN)are 29-33, 39-41, 48-50, 60-70).

In one aspect, the present invention provides an isolated antibody molecule which binds CXCL12 and which comprises the 114_3H1 VH domain (SEQ ID NO: 7) and/or the 114_3H1 VL domain (SEQ ID NO: 9). Preferably, the CXCL12 is human CXCL12, and optionally also murine CXCL12.

In a further aspect, the present invention provides an isolated antibody which binds CXCL12 and which comprises the 113_1H12 VH domain (SEQ ID NO: 18) and/or the 113_1H12 VL domain (SEQ ID NO: 20). Preferably, the CXCL12 is human CXCL12, and optionally also murine CXCL12.

Generally, a VH domain is paired with a VL domain to provide an antibody antigen binding site, although as discussed further below a VH domain alone may be used to bind antigen. In preferred embodiments, the 114_3H1 or 113_1H12 VH domain (SEQ ID NO: 7 or 18) is paired with the 114_3H1 or 113_1H12 VL domain (SEQ ID NO: 9 or 20), so that an antibody antigen binding site is formed comprising both the 114_3H1 or 113_1H12 VH and VL domains. In other embodiments, the 114_3H1 or 113_1H12 VH is paired with a VL domain other than the 114_3H1 or 113_1H12 VL. Light-chain promiscuity is well established in the art.

One or more CDRs may be taken from the 114_3H1 or 113_1H12 VH or VL domain and incorporated into a suitable framework. This is discussed further below. 114_3H1 VH CDRs H1, H2 and H3 are shown

in SEQ ID NOs: 1, 2 and 3, respectively. 114_3H1 VL CDRs L1, L2 and L3 are shown in SEQ ID NOs: 4, 5 and 6, respectively. 113_1H12 VH CDRs H1, H2 and H3 are shown in SEQ ID NOs: 12, 13 and 14, respectively. 113_1H12 VL CDRs L1, L2 and L3 are shown in SEQ ID NOs: 15, 16 and 17, respectively.

In one aspect, the present invention provides an anti-CXCL12 antibody that binds CXCL12 and which comprises:

an antibody VH domain selected from the group consisting of the 114_3H1 VH domain (SEQ ID NO:7) and a VH domain comprising a VH CDR3 with the amino acid sequence of SEQ ID NO: 3 and optionally one or more VH CDR's with an amino acid sequence selected from SEQ ID NO: 1 and SEQ ID NO: 2; and/or

an antibody VL domain selected from the group consisting of the 114_3H1 VL domain (SEQ ID NO: 9) and a VL domain comprising one or more VL CDR's with an amino acid sequence selected from SEQ ID NOs: 4, 5 and 6.

In one aspect, the present invention provides an anti-CXCL12 antibody that binds CXCL12 and which comprises:

an antibody VH domain selected from the group consisting of the 113_1H12 VH domain (SEQ ID NO: 18) and a VH domain comprising a VH CDR3 with the amino acid sequence of SEQ ID NO: 14 and optionally one or more VH CDR's with an amino acid sequence selected from SEQ ID NO: 12 and SEQ ID NO: 13; and/or

an antibody VL domain selected from the group consisting of the 113_1H12 VL domain (SEQ ID NO: 20) and a VL domain comprising one or more VL CDR's with an amino acid sequence selected from SEQ ID NOs: 15, 16 and 17.

As shown in the examples, the antibody molecule of the present invention can tolerate a number of amino acid alterations to the sequences of the CDRs, while retaining the properties of the parent antibody. By way of example, the amino acid sequences of the CDRs of the antibody molecule may each comprise 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid substitutions, deletions or insertions as compared to any one of SEQ ID NOs: 1 to 6 and 12 to

17.

As is well known in the art, the CDRs may be present in a range of different antibody types or framework regions, optionally involving one or more further sequence alterations to ensure retention of a useful property of the antibody as disclosed herein. For example, Figure 11 shows that the antibodies of the present invention are functional in scFc-Fv fusion and human IgG2 formats.

Each of the VH and VL domains typically comprise three complementarity determining regions (CDRs) responsible for antigen binding, interspersed by framework regions. In one exemplified embodiment, the present invention provides antibody molecules which comprise a VH domain comprising a CDR-H1, CDR-H2 and CDR-H3 having the sequences of SEQ ID NOs: 1, 2 and 3, respectively, and/or a VL domain comprising a CDR-L1, CDR-L2 and CDR-L3 having the sequences of SEQ ID NOs: 4, 5 and 6, respectively. In a further exemplified embodiment, the present invention provides antibody molecules which comprise a VH domain comprising a CDR-H1, CDR-H2 and CDR-H3 having the sequences of SEQ ID NOs: 12, 13 and 14, respectively, and/or a VL domain comprising a CDR-L1, CDR-L2 and CDR-L3 having the sequences of SEQ ID NOs: 15, 16 and 17, respectively.

The present invention also provides an anti-CXCL12 antibody molecule in an scFv format having at least 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99% or 100% amino acid sequence identity to SEQ ID NO: 11 or SEQ ID NO: 22.

Generally, the present invention relates to antibody molecules that are capable of inhibiting a biological activity of CXCL12, i.e. antagonist antibody molecules as understood by those skilled in the art. By way of example, the properties may be determined in *in vitro* cancer cell migration assay and an *in vivo* angiogenesis assay. Biological activities include inhibiting CXCL12-induced cancer cell growth, inhibiting cancer cell

migration, inhibiting cancer cell adhesion, inhibiting cancer metastasis and/or angiogenesis, e.g. VEGF-induced angiogenesis. Optionally, the antibody molecules of the present invention function by binding and sequestering CXCL12 thereby preventing it from interacting with the receptor in the biological system in which it is present.

Binding kinetics and affinity (expressed as the equilibrium dissociation constant Kd) of the anti-CXCL12 antibody molecules may be determined using standard techniques, such as surface plasmon resonance e.g. using BIAcore analysis, for example as described in the experimental examples below. Alternatively, Kd may be measured using a radiolabeled antigen binding assay (RIA) performed with the Fab version of an antibody of interest and CXCL12.

Anti-CXCL12 antibody molecules may have a dissociation constant for CXCL12 of less than 50nM, less than 40nM, less than 30nM, less than 20nM, less than 10nM, or less than 1nM. For example, an antibody molecule may have an affinity for CXCL12 of 1 to 20 nM, e.g. 9 to 15 nM. Preferably, antibody molecules of the present invention have affinity constants (K_D) of less than 10 nM, more preferably less than 5 nM and most preferably less than 3 nM for human CXCL12. In addition, the antibodies of the present invention preferably also bind to CXCL12 of other species, such as murine CXCL12, making them compatible with animal models of disease. The affinity constants for binding to CXCL12 can be determined using techniques well known in the art, such as Biacore SPR analysis as exemplified in the experimental examples below.

Anti-CXCL12 antibody molecule of the present invention according to any of the above aspects or embodiments is a monoclonal antibody, including a chimeric, humanized or human antibody. The antibody molecule may be an antibody fragment, e.g., a Fv, Fab, Fab', scFv, scFv-Fc, diabody, or $F(ab')_2$ complete antibody, a triabody, a bispecific antibody or a chimeric antibody.

Preferred formats of antibodies according to the present invention include, IgG, scFv-Fc, Fab and scFv. In another embodiment, the anti-CXCL12 antibody molecule may be a whole antibody. For example, an IgG, IgA, IgE or IgM or any of the isotype sub-classes, particularly IgG1 and IgG4. The anti-CXCL12 antibody molecules may be monoclonal antibodies. Antibody molecules and methods for their construction and use are described, in for example Hollinger & Hudson, Nature Biotechnology 23(9): 1126-1136 (2005).

Antibody molecules normally comprise an antigen binding domain comprising an immunoglobulin heavy chain variable domain (VH) and an immunoglobulin light chain variable domain (VL), although antigen binding domains comprising only a heavy chain variable domain (VH) are also possible (e.g. camelid or shark antibodies). Such antibodies are included within the scope of the present invention.

In some instances, antibody molecules of the present invention may be modified to alter the extent to which the antibody molecule glycosylated. This may be accomplished by altering the amino acid sequence such that one or more of the glycosylation sites present in a parent antibody is created or removed. In particular, where an antibody molecule comprises an Fc region, it is known that alteration of the carbohydrates attached to the Fc region can change the properties of the antibody molecule, in particular by reducing the fucosylation of the Fc region, it is possible to increase ADCC function.

Anti-CXCL12 antibody molecules as described herein may be isolated, in the sense of being free from contaminants, such as antibodies able to bind other polypeptides and/or serum components. Monoclonal antibodies are preferred for most purposes, though polyclonal antibodies may also be employed.

Methods of producing anti-CXCL12 antibody molecules include immunising a mammal (e.g. mouse, rat, rabbit, horse, goat, sheep

or monkey) with the protein or a fragment thereof. Antibodies may be obtained from immunised animals using any of a variety of techniques known in the art, and screened, preferably using binding of antibody to antigen of interest. For instance, Western blotting techniques or immunoprecipitation may be used (Armitage et al., 1992, Nature 357: 80-82). Isolation of antibodies and/or antibody-producing cells from an animal may be accompanied by a step of sacrificing the animal.

As an alternative or supplement to immunising a mammal with a peptide, an antibody specific for a protein may be obtained from a recombinantly produced library of expressed immunoglobulin variable domains, e.g. using lambda bacteriophage or filamentous bacteriophage which display functional immunoglobulin binding domains on their surfaces. The library may be naive, that is constructed from sequences obtained from an organism which has not been immunised with any of the proteins (or fragments), or may be one constructed using sequences obtained from an organism which has been exposed to the antigen of interest.

In the present invention, the method described in the examples may be employed to screen for further examples of anti-CXCL12 antibodies having antagonistic properties. After production and/or isolation, the biological activity of an anti-CXCL12 antibody molecule may be tested. For example, one or more biological activities may be determined that are selected from inhibiting CXCL12-induced cancer cell growth, inhibiting cancer cell migration, inhibiting cancer cell adhesion, inhibiting cancer metastasis and/or angiogenesis, e.g. VEGF-induced angiogenesis.

Competition between antibody molecules may be assayed easily in vitro, for example using ELISA and/or by tagging a specific reporter molecule to one antibody molecule which can be detected in the presence of one or more other untagged antibody molecules, to enable identification of antibody molecules which bind the same epitope or an overlapping epitope. Such methods are readily

known to one of ordinary skill in the art.

The present invention also provides nucleic acid molecules encoding the antibody molecules of the present invention. The nucleic acid molecules are useful for expressing the anti-CXCL12 antibody molecules, for example by incorporating the nucleic acid sequences into an expression vector having control sequences operably linked to the nucleic acid encoding the anti-CXCL12 antibody molecule to control its expression. The vectors may include other sequences such as promoters or enhancers to drive the expression of the inserted nucleic acid, nucleic acid sequences so that the anti-CXCL12 antibody molecule is produced as a fusion and/or nucleic acid encoding secretion signals so that the polypeptide produced in the host cell is secreted from the cell. Suitable vectors can be chosen or constructed, containing appropriate regulatory sequences, including promoter sequences, terminator fragments, polyadenylation sequences, enhancer sequences, marker genes and other sequences as appropriate. Vectors may be plasmids or viral, e.g. 'phage, or phagemid, as appropriate. For further details see, for example, Molecular Cloning: a Laboratory Manual: 2nd edition, Sambrook et al., 1989, Cold Spring Harbour Laboratory Press. Many known techniques and protocols for manipulation of nucleic acid, for example in preparation of nucleic acid constructs, mutagenesis, sequencing, introduction of DNA into cells and gene expression, and analysis of proteins, are described in detail in Current Protocols in Molecular Biology, Ausubel et al. eds., John Wiley & Sons, 1992.

Anti-CXCL12 antibody molecules can be obtained by transforming the vectors into host cells in which the vector is functional, culturing the host cells so that the anti-CXCL12 antibody molecule is produced and recovering the anti-CXCL12 antibody molecule from the host cells or the surrounding medium. Prokaryotic and eukaryotic cells are used for this purpose in the art, including strains of E. coli, insect cells (e.g. transformed with baculovirus), yeast, and eukaryotic cells such as COS or CHO

cells. The choice of host cell can be used to control the properties of the anti-CXCL12 antibody molecule expressed in those cells, e.g. controlling where the polypeptide is deposited in the host cells or affecting properties such as its glycosylation and phosphorylation. If the polypeptide is expressed coupled to an appropriate signal leader peptide it may be secreted from the cell into the culture medium. Following production by expression, the antibody molecule of the present invention may be isolated and/or purified from the host cell and/or culture medium, as the case may be, and subsequently used as desired, e.g. in the formulation of a composition which may include one or more additional components such as a carrier as described elsewhere in the present application.

Accordingly, in further aspects, the present invention provides nucleic acid encoding an anti-CXCL12 antibody molecule of the present invention, an expression vector comprising the nucleic acid encoding an anti-CXCL12 antibody molecule, operably linked to control sequences to direct its expression, and host cells transformed with this expression vector. In a still further aspect, the present invention provides a method of producing an anti-CXCL12 antibody molecule of the present invention, the method comprising culturing the host cells and isolating the anti-CXCL12 antibody molecule thus produced.

Derivatised Antibody Molecules

The antibody molecules of the present invention may also be derivatised to modify their properties, and in particular their pharmacological properties, such as half-life (e.g. increasing half-life). An example is the conjugation of antibody molecules to poly(alkylene glycol) molecules, in particular polyethylene glycol (PEG) molecules, that may be used to enhance the half-life or other pharmacological properties of polypeptide therapeutics. Pegylation is a known strategy for modifying the properties of therapeutic polypeptides, such as peptides, proteins and antibodies. In general, the attachment of PEG molecules to polypeptides is used to alter their conformation, electrostatic

or hydrophobic properties, and lead to improvements in their biological and pharmacological properties, such as increasing drug solubility, reducing dosage frequency, modulating (especially increasing) circulating half-life, increasing drug stability and increasing resistance to proteolytic degradation Pegylation works by increasing the molecular weight of the therapeutic polypeptide by conjugating the polypeptide to one or more PEG polymer molecules. This is particularly applicable to types of antibody molecules that are fragments of complete antibodies, such as Fab fragments.

This may be carried out to the antibody molecules of the present invention by reacting suitable functional groups present in the antibody molecules with a reactive poly(alkylene glycol) molecules. Depending on the functional groups available in the antibody molecules of the present invention, it may be possible to pegylate the antibody molecules in a selective way, for example by identifying suitable reactive cysteine residues in the antibody molecules. Poly(alkylene glycol) molecules are interchangeably referred to in the art as poly(alkylene oxide) molecules and are polyethers. Poly(alkylene glycol) molecules may have linear, branched, comb or star structures and generally are highly water soluble. In addition, the basic poly(alkylene glycol) structure may be provided with one or more reactive functional groups such as hydroxy, amine, carboxylic acid, alkyl halide or thiol groups to facilitate the reaction of the poly(alkylene glycol) molecule with other species such as polypeptides. Preferred poly(alkylene glycol) molecules include those substituted at one or more hydroxyl positions with a chemical group, such as an alkyl group having between one and four carbon atoms. Preferred poly(alkylene glycol) molecules for use in accordance with the present invention are polyethylene glycol ("PEG") molecules, although the skilled person would be able to derivatise antibody molecules of the present invention using other poly(alkylene glycol) molecules, such as polypropylene glycol or polyethylene-polypropylene glycol copolymers. Poly(alkylene glycol) molecules, including PEGs,

typically have molecular weights between about 400 Da and about 80 kDa, more preferably between about 1 kDa and about 60 kDa, and more preferably between about 5 kDa and about 50 kDa, e.g. molecular weights of 10 kDa, 20 kDa, 30 kDa or 40 kDa. Poly(alkylene glycol) molecules that may be used in accordance with the present invention are well known in the art and publicly available, for example from commercially available sources such as SigmaAldrich.

The present invention also provides immunoconjugates comprising an anti-CXCL12 antibody molecule as described herein conjugated to one or more cytotoxic agents, such as chemotherapeutic agents or drugs, growth inhibitory agents, toxins (e.g., protein toxins, enzymatically active toxins of bacterial, fungal, plant, or animal origin, or fragments thereof), or radioactive isotopes.

In one aspect, an immunoconjugate of the present invention is an antibody-drug conjugate (ADC) in which an antibody is conjugated to one or more drugs, for example a chemotherapeutic drug. The antibody moiety is optionally linked to the drug via a linker.

Imaging Applications

The antibody molecules of the present invention may additionally be labelled to enable them to be employed for imaging, either in conjunction with or independent of their therapeutic uses. Techniques for labelling antibodies are well known in the art that enable the antibodies to be used in a range of imaging and spectroscopic applications. This might be useful in a number of different medical or research applications, for example in the fields of oncology, cardiovascular medicine or graft rejection.

One particular example of the use of the antibody molecules for imaging involves the use of radionuclide labels in nuclear medicine imaging techniques, such as Single Photon Emission Computed Tomography (SPECT), an imaging technique that detects gamma rays emitted from a radionuclide to produce a two dimensional image of the distribution of the radionuclide in a

sample or subject, and Positron Emission Tomography (PET), an imaging technique that three-dimensional images by detecting pairs of gamma rays emitted indirectly by a positron-emitting radionuclide introduced into a sample or subject. Antibody molecules having radionuclide labels may also be employed for multi-modal studies in which imaging techniques are combined, either by selecting radionuclides that are active in more than one imaging technique or by labelling the antibody molecules with more than one type of label.

The antibody molecules of the present invention may be labelled with a radionuclide, for example a radionuclide provided as a complex, or conjugated to a second molecule, such as a linker, that is can be associated with the label. Examples of radionuclides for use in imaging techniques or therapy include technetium, rhenium, copper, cobalt, gallium and indium isotopes such as Tc-99m, Re-186, Re-188, Co-57, Ga-67, In-111 (SPECT), Cu-64, Cu-60, Cu-61, Cu-62, Cu-67, Tc-94m, Ga-68, Co-55 (PET). In general, technetium isotopes are employed for imaging purposes, rhenium isotopes for therapeutic purposes and copper isotopes for both imaging and therapy.

Medical Uses

CXCL12 has been reported to be involved in angiogenesis by recruiting endothelial progenitor cells (EPCs) from the bone marrow through a CXCR4 dependent mechanism, making it an significant factor in carcinogenesis and neovascularisation linked to tumour progression. CXCL12 also has a role in tumour metastasis where cancer cells that express the receptor CXCR4 are attracted to metastasis target tissues that release the ligand, CXCL12. The CXCL12/CXCR4/CXCR7 pathway has therefore generated considerable interest as a potential therapeutic target given its role in tumour growth, survival and angiogenesis.

Accordingly, CXCL12 has been shown to be important in the organ-specific metastasis of tumours, as reviewed in Balkwill et al., Seminars in Cancer Biology, 14: 171-179, 2004. Tumours that

express CXCL12/CXCR4 include ovarian cancer, breast cancer, bone cancer, prostate cancer, thyroid cancer, pancreatic cancer, multiple myeloma, non-Hodgkin's lymphoma, intraocular lymphoma, follicular center lymphoma, CML, colorectal cancer, oral squamous carcinoma, cervical cancer, neuroblastoma, kidney cancer, brain cancers such as glioma and astrocytoma, rhabdmyosarcoma, lung cancer, such as small cell lung cancer, melanoma, B cell malignancies, such as B-cell chronic lymphocytic leukemia (B-CLL), and leukaemias, such as acute myeloid leukaemia (AML).

CAFs are known to secrete CXCL12 and this increases angiogenesis and tumour growth directly in breast cancer (Orimo et al., Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion, Cell, 121(3): 335-348, 2005) and pancreatic cancer (Feig et al., Targeting CXCL12 from FAP-expressing carcinoma associated fibroblasts synergizes with anti-PD-L1 immunotherapy in pancreatic cancer. P.N.A.S., 110: 50: 20212-20217, 2013). In addition high levels of CXCL12 are associated with low numbers of T cells in a pancreatic cancer model and it was possible to increase T cell infiltration through combined treatment with PD-L1 and CXCR4 inhibitors. This increase in T cell infiltration was accompanied by a significant reduction in tumour volume, highlighting the role of the CXCL12/CXCR4 axis in immune control of cancer (Feig et al, PNAS, 110(50): p20212-20217, 2013).

Certain chemotherapeutics, anti-angiogenic agents and irradiation have been shown to cause additional upregulation of CXCL12/CXCR4, which aids tumour recurrence post-treatment. An increased level of CXCL12 triggers mobilisation of endothelial progenitors (Shaked et al, Cancer Cell, 14(3): 263-273, 2008) and the recruitment of monocytes to the tumour (Hughes et al, Cancer Res, 75(17): OF1 - OF13, 2015), which stimulate tumour invasion, neovascularisation and metastasis as well as suppress anti-tumour immune responses. Therefore, the combination of the anti-CXCL12

antibodies of the present invention with these type of CXCL12/CXCR4-inducing agents could be of clinical benefit.

In other embodiments, the antibody molecules or immunoconjugates of the present invention may be used in the treatment of WHIM Syndrome (Warts, Hypogammaglobulinemia, Infections, and Myelokathexis syndrome) is a rare congenital immunodeficiency disorder characterized by chronic noncyclic neutropenia which results from mutations in the chemokine receptor CXCR4.

In some embodiments, the antibody molecules or immunoconjugates of the present invention may be administered in conjunction with a further cancer therapy or in conjunction with radiotherapy. By way of example, the antibody molecules or immunoconjugates of the present invention may be administered in conjunction with a chemotherapeutic agent, an antibody therapy, immune modulatory therapy, surgery or in conjunction with radiotherapy, or in conjunction with cell mediated therapy. In some embodiments, the antibody molecule and the further cancer therapy are administered together, optionally as a combined formulation. Alternatively, the antibody molecule and the further cancer therapy may be administered by alternation, with either the further cancer therapy administered before the antibody molecule, or the further cancer therapy administered after the antibody molecule. The combination may be administered in accordance with clinical practice, for example being administered at intervals from about one week to three weeks.

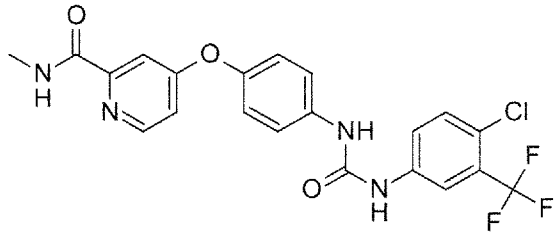
In one particular embodiment, the antibody molecules of the present invention are administered in conjunction with an angiogenesis inhibitor. Based on the mode of action of the antibody molecules of the present invention, combination therapies with angiogenesis inhibitors might provide additive or synergistic effects(see Liang et al., CXCR4/CXCL12 axis promotes VEGF-mediated tumor angiogenesis through Akt signaling pathway, Biochem. Biophys. Res. Commun. 359(3): 716- 722, 2007).

Angiogenesis inhibitors as used herein include agents that inhibit angiogenesis, vasculogenesis, or undesirable vascular permeability, either directly or indirectly. It should be understood that the angiogenesis inhibitors include those agents that bind and block the angiogenic activity of the angiogenic factor or its receptor. See, e.g., Grothey and Galanis (2009) Nat. Rev. Clin. Oncol. 6(9): 507-18 (e.g. Table 1 lists large-molecule VEGF inhibitors), Ivy, Wick and Kaufman (2009) Nat. Rev. Clin. Oncol. 6(9): 569-7 (e.g. Supplementary Table 1 lists small molecule receptor tyrosine kinase inhibitors).

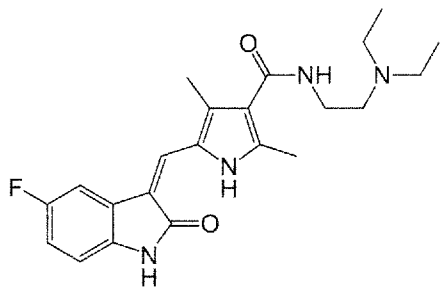
Angiogenesis inhibitors include antibodies or peptide-antibody fusions targeted to angiogenesis-promoting growth factor receptors, e.g. Bevacizumab (Avastin®), Cetuximab (Erbitux®), Ramucirumab (Cyramza®), Icrucumab, HuMV833, 2C3, Aflibercept (Zaltrap®) and IMC-1C11. Other angiogenesis inhibitors include small molecule kinase inhibitors, e.g. Sorafenib (Nexavar®), Sunitinib (Sutent®), Pazopanib (Votrient®), Everolimus (Afinitor®), AEE788, AAL881, AAL993, ZD4190, ABT-869 (Linifanib), PTK787 (Vatalanib), AMG706 (Motesanib), Cediranib (Recentin), Axitinib (Inlyta®), Vandetanib (Caprelsa®), SU6668, ZD1839, Telatinib, Nintedanib (Vargatef®), Brivanib alaninate, BMS-605541, BMS-645737, CEP-7055, Dovitinib, CP-547,632, E7080, GW654652, KRN633, Tivozanib, OSI-930, PD173074, PF-00337210, SU1498, Semaxanib (SU5416), SU5614, SU11657, SU14813, TKI-28, TKI-31 and ZM323881. Examples of native angiogenesis inhibitors are endostatin and angiostatin. Other drugs used to inhibit angiogenesis include thalidomide, squalamine and angiozyme.

Bevacizumab (Avastin®) is a recombinant humanised anti-VEGF monoclonal antibody generated according to Presta et al. (1997) *Cancer Res.* 57(20):4593-4599. Afibercept is a recombinant peptide-antibody fusion consisting of VEGF-binding portions fused to the Fc portion of human IgG1. Sorafenib (Nexavar®) is a multikinase inhibitor that blocks the receptor tyrosine kinases VEGFR, PDGFR (Platelet Derived Growth Factor Receptor), RAF serine/threonine kinases and c-KIT. In chemical terms, sorafenib

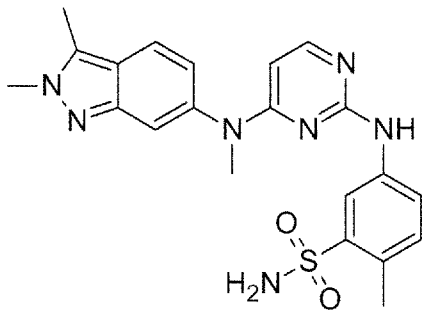
is named 4-[4-[[4-chloro-3-(trifluoromethyl)phenyl]carbamoylamino]phenoxy]-*N*-methyl-pyridine-2-carboxamide and has the structure:



Sunitinib (Sutent®) is a small molecule multi-targeted receptor tyrosine kinase inhibitor used in the treatment of cancer. It inhibits cellular signalling by targeting PDGFR and VEGFR. In chemical terms sunitinib is named *N*-(2-diethylaminoethyl)-5-[(*Z*)-(5-fluoro-2-oxo-1H-indol-3-ylidene)mWethyl]-2,4-dimethyl-1*H*-pyrrole-3-carboxamide and has the structure:

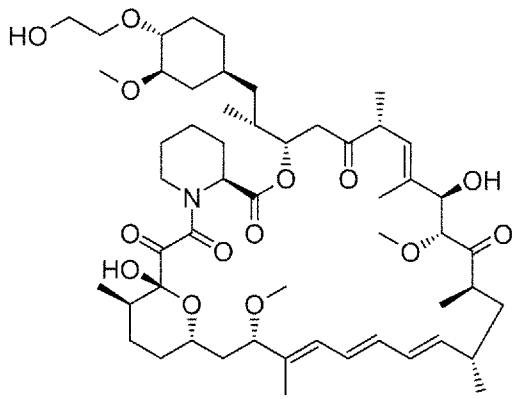


Pazopanib (Votrient®) is a multikinase inhibitor used in the treatment of cancer. It is known to target c-KIT, PDGFR and VEGFR. In chemical terms it is named 5-[[4-[(2,3-Dimethyl-2H-indazol-6-yl)methylamino]-2-pyrimidinyl]amino]-2-methylbenzolsulfonamide and has the structure:

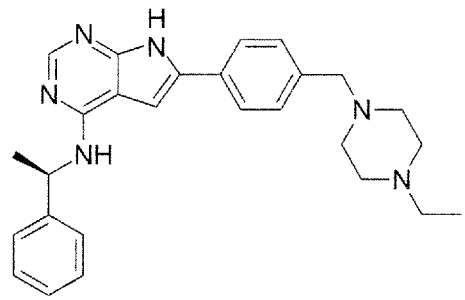


Everolimus (Afinitor®) is a signal transduction inhibitor targeting mTOR (mammalian target of rapamycin). In chemical terms it is named dihydroxy-12-[(2*R*)-1-[(1*S*,3*R*,4*R*)-4-(2-hydroxyethoxy)-3-methoxycyclohexyl]propan-2-yl]-19,30-dimethoxy-

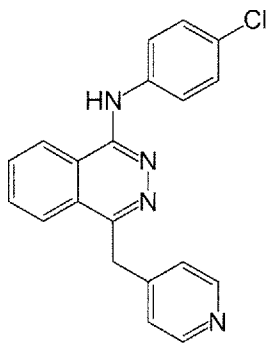
15,17,21,23,29,35-hexamethyl-11,36-dioxa-4-azatricyclo[30.3.1.0 hexatriaconta-16,24,26,28-tetraene-2,3,10,14,20-pentone and has the structure:



AEE788 is a small molecule drug being evaluated for treatment for cancer. It is a combined inhibitor of both the EGFR (epidermal growth factor receptor) and VEGFR family members. In chemical terms it is named 6-[4-[(4-Ethylpiperazin-1-yl)methyl]phenyl]-*N*-[(1*R*)-1-phenylethyl]-7*H*-pyrrolo[2,3-d]pyrimidin-4-amine and has the structure:



PTK787 (Vatalanib) is a protein kinase inhibitor that inhibits angiogenesis being developed for cancer treatment. It inhibits VEGF receptors, PDGFR-beta and c-kit. In chemical terms it is named *N*-(4-chlorophenyl)-4-(pyridin-4-ylmethyl)phthalazin- 1-amine and has the structure:



Examples of additional chemotherapeutic agents include an EGFR pathway inhibitor, such as an anti-EGFR antibody or an EGFR kinase inhibitor, such as cetuximab, panitumumab, Iressa (gefitinib or (*N*-(3-chloro-4-fluoro-phenyl)-7-methoxy-6-(3-morpholin-4-ylpropoxy)quinazolin-4-amine), or Tarceva (erlitonib or *N*-(3-ethynylphenyl)-6,7-bis(2-methoxyethoxy) quinazolin-4-amine), or other agents such as Herceptin™ (trastuzumab). Further examples of chemotherapeutic agents include alkylating agents, such as cisplatin, carboplatin and oxaliplatin, anthracyclines, plant alkaloids such as taxanes and vinca alkaloids, and topoisomerase inhibitors such as irinotecan, topotecan, amsacrine, etoposide, etoposide phosphate and teniposide, or fluorouracil (5FU).

In a further possibility, the antibody molecules of the present invention may be administered with an immune therapeutic, for example an immune pathway agent, small molecule agent or an antibody specific for PD-1, PDL-1, CTLA-4 or OX40.

In a further possibility, the antibody molecules of the present invention may be antibody-drug conjugates in which the antibody molecule is linked to a drug or toxin. This may be done to direct the drug or toxin to a target site in a biological system where CXCL12 is present. This approach may entail engineering the antibody molecule to provide a functional group capable of reacting with the drug or toxin, or alternatively providing the antibody molecule with a linker group that is capable of reacting with the drug or toxin. In this aspect of the present invention, the drug may also be a pro-drug for conversion to active drug at a target site in a patient.

Accordingly, the present invention provides an immunoconjugate which comprises an antibody molecule of the present invention conjugated to a cytotoxic moiety or an immunostimmulatory moiety. By way of illustration, the cytotoxic moiety may be an alkylating agent, an alkaloid, a platinum coordination complex, a cytotoxic peptide, a radioactive agent, or a pro-drug capable of conversion

into a cytotoxic moiety.

In a further aspect, the present invention relates to antibody molecules for use in a method of diagnosis or prognosis of a condition in which CXCL12 is implicated. In some embodiments, the antibody molecules of the present invention may be used in assays for identifying patients who are likely to be more responsive to treatment than a wider class of patients considered as a whole. This in turn may enable therapy, for example using antibody molecules of the present invention, to be directed to those patients most likely to respond, while providing the patients for whom treatment is less likely to be successful with alternative forms of therapy. On related aspect, the present invention provide a method of assaying for the presence of CXCL12 in a sample, the method comprising contacting the sample with antibody molecules of the present invention so that the CXCL12 binds to the antibody molecules to form a complex and detecting the complex thus produced. Alternatively or additionally, the methods may also employ antibody molecules of the present invention as reagents for detecting the binding of CXCL12 to a capture antibody. Preferably, the method comprises determining the presence or amount of CXCL12 in the sample using the antibody and correlating the presence or amount of CXCL12 with the likely outcome of treating the patient with a CXCL12 inhibitor.

In this case, the antibody molecules may be used in an ELISA-type format or otherwise labelled linked to a detectable molecule such as, but not limited to, radioactive or fluorescent labels or to enzymes which utilise a chromogenic substrate. Examples of radiolabels of use in this technique are ^{32}P, ^{3}H or ^{14}C. Examples of fluorescent molecules of use in this technique are green fluorescent protein, Fluorescein IsoThioCyanate (FITC), Rhodamine IsoThioCyanate (TRICT) Cy3 and Cy5 Dyes. Examples of enzymes with chromagenic substrates of possible use in this technique are peroxidase, alkaline phosphatase or glucose oxidase.

Preferably, the method of the present invention is an *in vitro*

method carried out on a sample obtained from said individual. In some embodiments of the present invention, the method may therefore comprise an initial step of obtaining a sample from the individual in question and/or preparing the sample for analysis . Preferred examples of samples for use in the method include blood samples, tissue samples or cell samples.

Additional variations of the above techniques exist that will be apparent to someone skilled in the art.

Pharmaceutical Compositions

The anti-CXCL12 antibody molecules or immunoconjugates of the present invention may be comprised in pharmaceutical compositions with a pharmaceutically acceptable excipient. A pharmaceutically acceptable excipient may be a compound or a combination of compounds entering into a pharmaceutical composition which does not provoke secondary reactions and which allows, for example, facilitation of the administration of the anti-CXCL12 antibody molecule, an increase in its lifespan and/or in its efficacy in the body or an increase in its solubility in solution. These pharmaceutically acceptable vehicles are well known and will be adapted by the person skilled in the art as a function of the mode of administration of the anti-CXCL12 antibody molecule.

In some embodiments, anti-CXCL12 antibody molecules or immunoconjugates may be provided in a lyophilised form for reconstitution prior to administration. For example, lyophilised antibody molecules may be re-constituted in sterile water and mixed with saline prior to administration to an individual.

Anti-CXCL12 antibody molecules will usually be administered in the form of a pharmaceutical composition, which may comprise at least one component in addition to the antibody molecule. Thus pharmaceutical compositions may comprise, in addition to the anti-CXCL12 antibody molecule, a pharmaceutically acceptable excipient, carrier, buffer, stabilizer or other materials well known to those skilled in the art. Such materials should be non-

toxic and should not interfere with the efficacy of the anti-CXCL12 antibody molecule. The precise nature of the carrier or other material will depend on the route of administration, which may be by bolus, infusion, injection or any other suitable route, as discussed below.

For intra-venous administration, e.g. by injection, the pharmaceutical composition comprising the anti-CXCL12 antibody molecule may be in the form of a parenterally acceptable aqueous solution which is pyrogen-free and has suitable pH, isotonicity and stability. Those of relevant skill in the art are well able to prepare suitable solutions using, for example, isotonic vehicles, such as Sodium Chloride Injection, Ringer's Injection, Lactated Ringer's Injection. Preservatives, stabilizers, buffers, antioxidants and/or other additives may be employed as required including buffers such as phosphate, citrate and other organic acids; antioxidants, such as ascorbic acid and methionine; preservatives (such as octadecyldimethylbenzyl ammonium chloride; hexamethonium chloride; benzalkonium chloride; benzethonium chloride; phenol, butyl or benzyl alcohol; alkyl parabens, such as methyl or propyl paraben; catechol; resorcinol; cyclohexanol; 3'-pentanol; and m-cresol); low molecular weight polypeptides; proteins, such as serum albumin, gelatin or immunoglobulins; hydrophilic polymers, such as polyvinylpyrrolidone; amino acids, such as glycine, glutamine, asparagines, histidine, arginine, or lysine; monosaccharides, disaccharides and other carbohydrates including glucose, mannose or dextrins; chelating agents, such as EDTA; sugars, such as sucrose, mannitol, trehalose or sorbitol; salt-forming counter-ions, such as sodium; metal complexes (e.g. Zn-protein complexes); and/or non-ionic surfactants, such as TWEEN™, PLURONICS™ or polyethylene glycol (PEG).

A pharmaceutical composition comprising an anti-CXCL12 antibody molecule may be administered alone or in combination with other treatments, either simultaneously or sequentially dependent upon the condition to be treated.

An anti-CXCL12 antibody molecule as described herein may be used in a method of treatment of the human or animal body, including prophylactic treatment (e.g. treatment before the onset of a condition in an individual to reduce the risk of the condition occurring in the individual; delay its onset; or reduce its severity after onset). The method of treatment may comprise administering an anti-CXCL12 antibody molecule to an individual in need thereof.

Administration is normally in a "therapeutically effective amount", this being sufficient to show benefit to a patient. Such benefit may be at least amelioration of at least one symptom. The actual amount administered, and rate and time-course of administration, will depend on the nature and severity of what is being treated, the particular mammal being treated, the clinical condition of the individual patient, the cause of the disorder, the site of delivery of the composition, the method of administration, the scheduling of administration and other factors known to medical practitioners. Prescription of treatment, e.g. decisions on dosage etc., is within the responsibility of general practitioners and other medical doctors and may depend on the severity of the symptoms and/or progression of a disease being treated. Appropriate doses of antibody molecules are well known in the art (Ledermann J.A. et al. (1991) Int. J. Cancer 47: 659-664; Bagshawe K.D. et al. (1991) Antibody, Immunoconjugates and Radiopharmaceuticals 4: 915-922). Specific dosages may be indicated herein or in the Physician's Desk Reference (2003) as appropriate for the type of medicament being administered may be used. A therapeutically effective amount or suitable dose of an antibody molecule may be determined by comparing its *in vitro* activity and *in vivo* activity in an animal model. Methods for extrapolation of effective dosages in mice and other test animals to humans are known. The precise dose will depend upon a number of factors, including whether the antibody is for prevention or for treatment, the size and location of the area to be treated, the precise nature of the

antibody (e.g. whole antibody, fragment) and the nature of any detectable label or other molecule attached to the antibody.

A typical antibody dose will be in the range 100 µg to 1 g for systemic applications, and 1 µg to 1 mg for topical applications. An initial higher loading dose, followed by one or more lower doses, may be administered. Typically, the antibody will be a whole antibody, e.g. the IgG1, IgG2, IgG3 or IgG4 isotype, according to differences in amino acid sequence in the hinge and Fc regions. These different isotypes affect the *in vivo* half-lives of the antibody molecules and their ability to induce effector functions. Hence, the choice of IgG isotype may be used to engineer the *in vivo* properties of the antibody molecules of the present invention. For neutralising soluble antigens such as CXCL12, effector functions are less critical and so the use of an antibody isotype lacking effector function (such as IgG2) may be preferred to determine the benefits of CXCL12 neutralisation without interference of the host immune system.

This is a dose for a single treatment of an adult patient, which may be proportionally adjusted for children and infants, and also adjusted for other antibody formats in proportion to molecular weight. Treatments may be repeated at daily, twice-weekly, weekly or monthly intervals, at the discretion of the physician. Treatments may be every two to four weeks for subcutaneous administration and every four to eight weeks for intra-venous administration. Treatment may be periodic, and the period between administrations is about two weeks or more, e.g. about three weeks or more, about four weeks or more, or about once a month. Treatment may be given before, and/or after surgery, and/or may be administered or applied directly at the anatomical site of surgical treatment or invasive procedure. Suitable formulations and routes of administration are described above.

In some preferred embodiments, the therapeutic effect of the anti-CXCL12 antibody molecule may persist for several half-lives, depending on the dose. For example, the therapeutic effect of a

single dose of anti-CXCL12 antibody molecule may persist in an individual for 1 month or more, 2 months or more, 3 months or more, 4 months or more, 5 months or more, or 6 months or more.

Material and Methods

GENERATION CXCL12 NEUTRALISING ANTIBODIES BY PHAGE DISPLAY TECHNOLOGY

Panning antibody libraries on CXCL12

The 'McCafferty naïve antibody library', which contains more than 10 billion antibody clones in scFv format, was used for isolating anti-CXCL12 antibodies and the resulting antibodies were then screened in a cell-based assay to assess potential to block the CXCL12-CXCR4 interaction. In more detail, two rounds of panning were carried out on biotinylated CXCL12 immobilised on streptavidin or neutravidin. In order to avoid enriching for antibody clones that bind to streptavidin or neutravidin two strategies were employed. Round 1 panning was carried out on biotinylated CXCL12 immobilised on streptavidin using the phage library that has been depleted of any streptavidin binders (known as 'de-selection; or 'subtractive selection'). For Round 2, neutravidin was used (instead of streptavidin) to immobilise CXCL12. Polyclonal phage prepared from round 2 panning output was tested in a TRF binding assay against CXCL12, streptavidin, neutravidin and a non-specific antigen (NCK1) to confirm specific enrichment. The binding signal observed was specific to CXCL12 and no detectable binding was observed for streptavidin, neutravidin and NCK1.

Anti-CXCL12 antibody screening

The scFv population from the Round 2 panning output was PCR amplified and cloned into the pSANG10-3F expression vector and transformed into BL21. The psANG10-3F encodes a hexa-histidine tag (for Ni-affinity purification) and tri-FLAG tag (for detection) downstream of the scFv gene. 940 individual transformants were picked into 10 x 96 well culture plates and antibody expression was induced using auto-induction media. Recombinant monoclonal antibodies secreted into culture

supernatant after overnight induction were tested for binding to CXCL12. The culture supernatants containing secreted scFvs were used for a TRF binding assay in which scFv binding to biotinylated-CXCL12 (immobilised on streptavidin in 96 well plates) was detected using an anti-FLAG antibody conjugated to europium. Clones with signal above 1000 FU (100 fold above background) were considered as positive for CXCL12 binding.

Approximately 24% (224/940) of the clones screened were found be positive for CXCL12 binding. The top 184 clones were cherry-picked for sequence analysis and further characterisation. Sequences of cherry-picked clones were generated by Sanger sequencing using 4 primers and a consensus sequence was assembled for each clone. CDR and framework regions of the consensus sequences were analysed using BLAZE antibody analysis software. By focusing the analysis on variation in CDR3 of heavy and light chains, 118 unique scFv sequences were found. With in these sequences there were additional changes in other CDRs or framework residues bringing 38 more unique sequences, i.e. 156 unique sequences from 184 sequenced. Detailed analysis of the framework regions revealed a preference for certain heavy chain and light chain germline families. Vh3 (62%) and Vh1 (43%) are the most frequently found heavy chain families while Vκ1 (58%) followed by Vκ2 (17%) dominate the light chain sequences.

CloneID	VH germline	VL germline	VH CDR3	VL CDR3
093_1C03	Vh1_DP-5_(1-24)	Vlambda3_3h	LISGSYRLEDYF.....DH	QAWDSSTG.....YV
093_2G07	Vh3_DP-86_(3-66)	Vk1_DPK1_(O18.O8)	EASDPRYYYDSSGYYYGM....DV	QQYDNLP.....LT
093_2A11	Vh3_DP-42_(3-53)	Vk1_DPK4_(A20)	EASDPRYYYDSSGYYYGM....DV	QKYNSAP.....RT
093_2H09	Vh1_DP-88_(1-e)	Vk2_DPK18_(A17)	DYNDWGAF.....EL	VQGTHWP.....WT
093_1H10	Vh1_DP-5_(1-24)	Vk1_DPK4_(A20)	EGYDSSGYGARPRYYYYGM...DV	QQSYNTP.....RT
093_1E11.093_1E10	Vh3_DP-53_(3-74)	Vk2_DPK18_(A17)	DSLDGNGSGSWDDAF.....DI	VQGTHWP.....WT
093_2G12	Vh1_DP-5_(1-24)	Vlambda6_6a	GSAYYYGSGSYYKAPYYYYYGMDV	QSYDSSN.....QV
093_2B01	Vh3_DP-46_(3-30.3)	Vk1_DPK1_(O18.O8)	GMGYGM.....DL	QQYDNLP.....YT
093_2F10	Vh3_DP-47_(3-23)	Vlambda2_DPL10_(2b2)	EGGDPTTPTTT.....TV	CSYAGPFT.....VI
093_2F12	Vh3_DP-49_(3-30.5)	Vlambda1_DPL3_(1g)	DDSTADL.....DY	AAWDDSLSGP....YV

Table 1. A snapshot of sequence analysis of the primary CXCL12 antibodies. The framework and the CDR regions were analysed using BLAZE software and the antibodies were clustered based on similarity in VH and VL CDR3 sequence (for example, 093_E11 and 093_E10).

Identification of antibodies that block CXCL12 binding to CXCR4

A large panel of unique CXCL12 binders were identified from the primary screening and sequence analysis. In order to identify antibodies that block CXCL12-CXCR4 interaction, a cell based CXCL12-CXCR4 binding assay using flow cytometry was established. A human acute lymphoblastic leukemia cell line (MOLT-4) was identified as an ideal CXCR4 expressing cell line for CXCL12-CXCR4 binding assay as they were found to be negative for CXCR7 expression. In this assay, the binding of biotinylated-CXCL12 to CXCR4 expressing MOLT-4 cells was detected using streptavidin conjugated with phycoerythrin (streptavidin-PE). Anti-CXCL12 antibodies were then tested for their ability to inhibit this interaction.

At first, a number of pilot experiments were carried out to identify optimum assay parameters (such as detection method, amount of CXCL12, concentration of the blocking agent etc.) in

order to achieve the maximum assay sensitivity. A one-step binding detection method was tested and compared to a two-step binding detection method. The one step binding detection method involved direct staining of MOLT-4 cells with biotinylated-CXCL12 that has been pre-complexed with streptavidin-PE and detecting the binding by flow cytometry. In contrast, the two-step binding detection method involved incubation of MOLT-4 cells with biotinylated CXCL12, washing, and then staining with streptavidin-PE prior to binding detection by flow cytometry. The two-step detection method gave a sharp fluorescent peak in comparison to the broad distribution of fluorescence observed with one-step detection method. In addition, the pre-complexing of CXCL12 and streptavidin-PE would result in tetrameric presentation of CXCL12 molecules. This would result in avidity effects which could hinder the blocking of CXCL12-MOLT-4 cell interaction by monomeric scFvs. For these reasons, the two-step method for detection of CXCL12 binding was chosen for the blocking assay. A dose response analysis identified the optimum concentration of CXCL12 for the assay as 7.5 µg/ml.

A two-fold dilution series of biotinylated CXCL12 starting from 40 µg/ml was incubated with MOLT-4 cells expressing CXCR4. The CXCL12 binding to the cells binding was detected by streptavidin-PE using flow cytometry. The mean fluorescence observed for each test sample was plotted against the CXCL12 concentration.

39 clones from the 118 anti-CXCL12 antibodies identified from primary screening for blocking were selected for this assay on the basis of heavy chain CDR3 sequence diversity and binding signal in the primary screen. ScFV antibody was produced from these clones and was purified by immobilised metal ion affinity chromatography. The purified antibodies (and a non-specific antibody) were then tested for blocking activity in CXCL12-MOLT-4 cell binding assay. Based on the percentage inhibition of CXCL12-MOLT-4 cell binding, 20 antibodies with blocking activity greater than 45% were selected for further study (Table 2).

Rank	Clone ID	Percentage blocking in cell-binding assay
1	093_1C03	99
2	093_2A02	96
3	093_2D06	94
4	093_1F01	87
5	093_2G07	86
6	093_2G10	83
7	093_1C04	65
8	093_2C02	64
9	093_2E04	63
10	093_1A10	63
11	093_1A08	59
12	093_1G07	58
13	093_2G09	57
14	093_1A09	54
15	093_1F09	54
16	093_1G10	51
17	093_2E12	51
18	093_2D05	51
19	093_2A10	46
20	093_1A11	45

Table 2. Top 20 blocking antibodies from the cell-based CXCL12-CXCR4 binding assay.

Affinity maturation and functional characterisation of primary anti-CXCL12 antibodies

Primary antibody phage display selections and screening identified several antibodies that block CXCL12 binding to CXCR4. Therapeutic applications often require monoclonal antibodies with affinities in the low-to-sub-nanomolar range to accomplish the desired clinical efficacy. Panning from the "McCafferty library" under low stringency conditions generally yields primary antibodies with affinity ranging from 10nM-1μM. Such antibodies are often affinity matured *in vitro* to attain sufficient affinity for the given application.

In vitro affinity maturation of antibodies can be achieved by mimicking the *in vivo* process that occurs during a humoral immune response. The initial response to antigen stimulation involves the selection of antigen specific B-cells from a large and diverse pre-immune repertoire of B-cells expressing low affinity antibodies. These primary, low affinity antibodies then undergo a process called somatic hypermutation in which they accumulate point mutations in the heavy and light variable regions. The B-cells expressing high affinity antibodies compete with B-cells expressing low affinity antibodies for antigen stimulation to survive and proliferate. By repeated cycles of somatic hypermutation and preferential expansion of B-cells expressing higher affinity antibodies, the immune system gradually establishes an effective response to invading pathogens. Similar to the *in vivo* process, commonly used *in vitro* affinity maturation strategies involve two key steps, diversification of the primary antibody sequence and the selective enrichment of affinity improved antibodies using a selection platform such as phage display technology. Diversification of primary antibody sequence can be done by introducing mutations to the variable regions using random or targeted mutagenesis. Alternatively, new combinations of heavy and light variable regions can be made by recombining selected heavy or light chains with a repertoire of partner chains by a process known as chain shuffling.

Given the modular nature of antibodies, chain shuffled libraries can be easily created by simple cloning hence this was the chosen method for affinity maturing primary anti-CXCL12 antibodies. Since the heavy chain variable domains commonly play the dominant role in antigen binding and defining the epitope specificity, light chain shuffling is preferred over heavy chain shuffling to preserve the binding epitope of the primary antibodies.

Construction of chain shuffled libraries and stringent phage display selections on CXCL12

In order to create a light chain shuffled library, the antibody heavy chain regions from the top 20 blocking antibodies were

amplified by PCR. The resultant PCR products were cloned into a phage display light chain library preparation encompassing a repertoire of naïve lambda and kappa light chain variable region partners (in vector pSANG4). The ensuing plasmid population was transformed into *E.Coli* TG1 to yield a library containing 2.6 x 10^8 scFv clones. Thus each original heavy chain was paired with approximately 10 million new light chain partners. In order to assess the frequency of heavy chain insertion, 20 random clones from the light chain shuffled library were analysed by colony PCR screen. 19 out of 20 clones showed the presence of a full-length scFv gene (not shown) suggesting that approximately 95% of clones in the library are light chain shuffled recombinants.

Successful isolation of high affinity antibodies from any library requires stringent selection conditions that can selectively enrich high affinity binders. Antibodies with high affinities can be enriched by iterative rounds of phage selection using diminishing antigen concentrations. This method relies on the competition for limiting amounts of antigen and the preferential enrichment of variants with lower dissociation constants. For precise control of antigen concentration, phage display selections are carried out in solution phase. The phage antibodies are allowed to bind to a biotinylated antigen in solution and the bound phage is subsequently captured using a streptavidin-coated surface for washing and elution. The stringency of the selection can be further increased at this step by including a number of harsh and long washing steps.

For the isolation of high affinity anti-CXCL12 antibodies from the light chain shuffled library, three rounds of solution phase selections were carried out on biotinylated CXCL12. The optimum antigen concentration for each round was determined empirically by selecting the phage antibodies against a range of antigen concentrations and comparing the output numbers with a "no antigen" control. The third round also included a set of selections in which the captured phage-antigen complex was subjected to 17-hour wash (washed 6 times every hour with

phosphate buffered saline containing 0.2% Tween-20) to further increase the stringency.

Screening the selection outputs for affinity matured monoclonal binders

The scFv populations from round 3 selection outputs were PCR amplified and cloned into the pSANG10-3F expression vector and the resulting plasmid DNA was transformed into *E.coli* BL21 DE3 cells. 960 individual transformants were picked into 10 x 96 well culture plates and the antibody expression was induced using auto-induction media. Recombinant monoclonal antibodies secreted into the culture supernatant after overnight induction, were screened for their ability to bind CXCL12 in a TRF binding assay. 48% (458/960) of the clones screened from various selection outputs were found to be positive for CXCL12 binding. The binding signals exhibited by these clones were significantly better than those of the clones isolated from the naïve library. 37% of the clones screened from the chain shuffled selection outputs showed binding signal above 10,000 fluorescent units (FU). In contrast, only 12% of the clones isolated from the naïve library exhibited binding signals exceeding 10,000 FU. Since clones tested in the screen were not normalised for expression, the observed improvement in the CXCL12 binding could be due to improved expression or improved affinity.

The CXCL12 binding clones were and analysed using BLAZE antibody sequence analysis software. Analysis of the heavy and light chain CDR3 diversity identified 227 unique clones. Although 20 different VH genes were used in the construction of the chain shuffled library, only 9 VH genes were represented amongst the 227 unique clones identified. Within this set there were four major clone families. These were derived from the VH gene of primary clones 093_2A02 (122/227), 093_2D06 (38/227), 093_2G07 (24/227) and 093_2A10 (22/227). Similar to the clones isolated from the naïve library, VL germline usages of these chain shuffled clones were dominated by Vκ1 (63%) and Vκ2 (22%) germline families.

The CXCL12 binding signal observed for a particular clone in the primary screen was dependent on the combined effect of antibody expression and affinity. Since the antibody expression varies significantly from clone-to-clone, ranking antibodies by their binding signal in the primary screen do not necessarily correlate with their affinities. Therefore, an expression-independent secondary screening assay was used to identify high affinity anti-CXCL12 antibodies with superior binding kinetics. In this screening assay, dissociation constants (off-rates) of the top 150 anti-CXCL12 scFv antibodies were analysed using surface plasmon resonance (SPR). The dissociation constant of an antibody-antigen interaction is concentration independent and therefore a normalisation for differential antibody expression was not required. However, due to the tendency of scFv antibodies to dimerise in solution, the accurate measurement of monovalent antibody-antigen (1:1) interactions is complicated and often results in erroneous determination of binding constants. Hence a panel of 24 antibodies showing low dissociation constants in the scFv-SPR screen were reformatted as Fab antibodies for another cycle of 'off-rate screen'. Unlike scFvs, Fab antibodies are stable in monomeric format and are optimal for accurate kinetic analysis. Antibody clones, 114_3H1 (derived from 093_2D06) and 113_1H12 (derived from 093_2A02) showed the best off-rates (i.e. the lowest dissociation constants) amongst the 24 Fab antibodies tested. These two clones were selected as lead anti-CXCL12 antibodies for detailed characterisation. The sequences of these antibodies are provided in the sequence listing.

Expression and purification of anti-CXCL12 antibodies as Fabs and IgGs in mammalian cells

All antibody work up to the second cycle of off-rate screen was performed with antibodies in scFv format. ScFv format is well suited for phage display selections and subsequent screening of large number clones to identify a panel of lead antibodies due to the efficient expression in *E.coli*. However, there are a number

of limitations associated with this format that make it sub-optimal for downstream biophysical and biological characterisation. For example, affinity determination of scFvs is complicated due to their propensity to dimerise in solution. Poor stability of scFv molecules makes them susceptible to aggregation and precipitation thereby limiting their long-term storage. In addition, the presence of high level of endotoxin in scFv preparations from *E.coli*, restricts their usage in number of cell based assays. Hence, scFvs identified from primary screening assays are usually reformatted to larger and more stable antibody formats and are expressed in mammalian cells for downstream characterisation and *in vivo* testing. The two lead anti-CXCL12 antibodies and their parent clones were reformatted to Fabs and IgGs. Fabs are optimal for accurate determination of the binding constants. Whilst the IgG is the preferred format for majority of clinically approved antibodies and the antibodies in development. Their superior *in vivo* half-life, and the ability to engage the host immune system are ideal for the treatment of diseases, such as cancer. In addition, the bivalent nature of an IgG molecule greatly enhances its antigen neutralisation capability both *in vitro* and *in vivo*.

The sequences of the anti-CXCL12 antibodies of the present invention could be used in any appropriate expression systems designed for the generation of antibody molecules. All scFv antibodies discussed in this work were expressed from the pSANG10-3F vector, in which a single T7 promoter drives the expression of heavy and light variable domains that are connected by a glycine-serine linker (Figure 1A). In this system, the scFv gene is transcribed as a single mRNA and translated as a single protein. In contrast, commonly used mammalian Fab and IgG expression systems use expression cassettes that transcribe and translate heavy and light chain genes separately. Here we used a bicistronic vector (pBIOCAM-7) for transient expression of anti-CXCL12 Fabs in HEK-293 cells (Figure 1B). In pBIOCAM7 antibody light (VL+CL) and heavy chain (VH+CH) genes are separated by a gene segment that encodes a "ribosome skipping" peptide from

porcine teschovirus-1 (known as the P2A peptide). This Fab expression system produces a single mRNA transcript for the whole Fab cassette (VL+CL-P2A-VH+CH). However, during translation the ribosome skips the synthesis of the glycyl-prolyl peptide bond at the C-terminus of P2A peptide resulting in the release of the polypeptide chain immediately downstream of it. The heavy and light chain polypeptides are then folded and assembled independently in the endoplasmic reticulum (ER) to form Fab molecules. The furin cleavage site at the C-terminus of light chain facilitates the posttranslational removal of the P2A peptide from the Fab protein.

For the expression of anti-CXCL12 antibodies in IgG format, a dual plasmid system was used in which the heavy and light chain expression cassettes were carried on two different plasmids (Figure 1C). Upon co-transfection of these plasmids into HEK-293 cells, heavy and light chain genes are transcribed and translated separately before being assembled into IgG molecules in ER. IgG antibodies are divided into 4 isotypes (IgG1, IgG2, IgG3 and IgG4) according to differences in amino acid sequence in the hinge and Fc regions. These different isotypes affect the *in vivo* half-lives of the antibody molecules and their ability to induce effector functions. Hence, the choice of IgG isotype may be used to engineer the *in vivo* properties of the antibody molecules of the present invention. For neutralising soluble antigens such as CXCL12, effector functions are less critical. In fact, it is desirable to have antibodies lacking effector functions for future *in vivo* experiments in order to determine the benefits of CXCL12 neutralisation without interference of the host immune system. Therefore, we chose IgG2 Isotype, which exhibits reduced antibody dependent cell-mediated cytotoxicity (ADCC) and complement dependent cytotoxicity (CDC).

For Fab and IgG expression, the variable regions of the lead antibodies (114_3H1 and 113_1H12) and their parent clones (093_2D06 and 093_2A02) were sub-cloned into the pBIOCAM-7 vector and IgG2 expression plasmids (pBIOCAM-1 and pBIOCAM2-IgG2).

Transfection quality DNA was prepared for these plasmids and transfected into HEK-293F cells for transient antibody expression. Fab and IgG antibodies were purified from the cell culture supernatants (6 days post transfection) using Nickel and Protein-G affinity chromatography methods respectively. As illustrated in Figure 2, purified Fabs and IgGs were composed of a heavy and a light chain domains of the expected sizes.

Affinity measurement of Anti-CXCL12 antibodies

The lead anti-CXCL12 antibodies and their parent clones were subjected to a full kinetic analysis to determine the improvement in binding constants after affinity maturation. The binding of the Fab antibodies to biotinylated CXCL12 immobilised on a streptavidin chip was analysed using SPR (Figure 3). Equilibrium dissociation constants (KD) of these antibodies were determined using a binding model appropriate for each binding interaction (Figure 3B and C). Of the four antibodies tested, 114_3H1 had the highest affinity to CXCL12 with a KD of 1 nM. This represents a 3800-fold improvement in affinity from its parent clone 093_2D06 (KD = 3800 nM) after chain shuffling. Both 093_2A02 and its affinity matured variant 113_1H12 exhibited a biphasic binding profile with an initial fast dissociation phase followed by a much slower second dissociation phase. Such binding profiles are generally associated with binding interactions that involve antibody (or any other analyte) induced conformational change of the ligand resulting in a two-step binding. Two different preparations of both 113_1H13 and 092_2A02 were analysed by SPR. Similar results were obtained using both protein batches confirming that the observed binding profile was not an artefact or an issue with a particular protein preparation. The fact that only the antibodies from the same VH lineage exhibited biphasic binding favours the hypothesis that these antibodies might have a two-step binding mechanism. Hence the affinities of these antibodies were determined using a two-state binding model (Figure 3C). The calculated binding affinities of 113_1H12 and 093_2D06 were 3.7nM and 16.7 nM

respectively, which represents a 4.5-fold improvement in affinity following light chain shuffling.

Inhibition of cancer cell migration by lead anti-CXCL12 antibodies

Migration of CXCR4 expressing cancer cells towards CXCL12 rich environments is one of the key factors promoting metastasis in many malignancies. The inhibition of CXCL12/CXCR4 dependent cancer cell migration is an important biological property of the therapeutic anti-CXCL12 antibody molecules of the present invention. Hence the lead antibodies 114_3H1 and 113_1H12 were tested for their ability to block CXCL12 induced migration of ovarian cancer cells using a transwell migration assay. The transwell migration assay used here was a modified version of the Boyden chamber assay used to study the chemotactic response of leukocytes. In this assay, migration of fluorescently labelled human ovarian cancer cells (TOV-21G) seeded in the upper chamber across a porous membrane and into the lower chamber containing CXCL12 was analysed (Figure 4A). A previous study has shown that CXCL12 dimers are formed at higher concentrations, could inhibit cell migration. Therefore, the optimum CXCL12 concentration for stimulating cell migration was determined empirically (Figure 4B).

In order to assess the ability of the lead anti-CXCL12 antibodies to inhibit CXCL12 induced migration of ovarian cancer cells, titration of 114_3H1 IgG and 113_1H12 IgG were carried out in the transwell migration assay described above. Both antibodies inhibit the migration of TOV-21G cells in a dose dependent manner (Figure 5). The half maximal inhibitory concentration (IC_{50}) of 114_H01 and 113_1H12 were 4.6 (± 0.5) nM and 13.2 (± 4.1) nM respectively. These values are in accordance with the equilibrium dissociation constants determined by SPR. Further replicate experiments produced higher quality data that confirmed that the IC50 values for 114_H01 and 113_1H12 were 5 nM and 9 nM respectively.

Inhibition of angiogenesis by lead anti-CXCL12 antibodies

The survival and proliferation of tumours is greatly dependent on a supportive vascular network that provides adequate supply of oxygen and nutrients. The CXCL12/CXCR4 axis plays a key role in promoting the formation of new blood vessels (angiogenesis) to establish a tumour supportive vasculature. CXCL12 and CXCR4 form a positive feed back loop with VEGF, a well-known pro-angiogenic factor. In this loop, VEGF stimulates the expression of both CXCL12 and CXCR4. Conversely, CXCL12 induced activation of CXCR4 up-regulates production of VEGF by endothelial cells. Hence the lead anti-CXCL12 antibodies were tested in an *in vitro* angiogenesis assay to evaluate their ability to inhibit the formation and branching of tubules.

In this assay, human umbilical vein endothelial cells (HUVECs) and fibroblasts were cultured together in a media containing anti-CXCL12 antibodies and VEGF. The interaction of these two cell types in the presence of VEGF results in the formation of three-dimensional tubes that resemble small capillaries *in vivo*. Inhibitory effects of anti-CXCL12 antibodies were analysed after 7 days of co-culture by immunohistochemistry (Figure 6).

The most valuable parameters for analysing angiogenesis are the tubule length and the number branches per tubule. Endothelial cells form long and branched tubules upon VEGF stimulation and this was unaffected by the presence of a non-specific antibody. significant inhibition of tubule formation and branching was observed with 113_1H12 (Figure 6B).

Comparison with Antibodies of WO 2008/018641

WO 2008/018641 describes two pairs of anti-CXCL12 antibodies, 1D3 and 1H2 and 1C6 and 2A5. The pairs of antibodies recognise common epitopes in CXCL12.

The sequences of the heavy and light chains of the antibodies of WO 2008/018641 were aligned with the sequences of the antibodies of the present invention and the alignment is shown in Figure 8. This

shows that there are significant differences in the CDR sequences of these antibodies as compared to antibodies 114_H01 and 113_1H12 of the present invention.

The affinity of the antibodies was also compared. The affinity constant (K_D) for human CXCL12 of antibody 114_H01 was 2.4 nM and that of antibody 113_1H12 was 4.2 nM. This compares to the values reported for the antibodies of WO 2008/018641 of 1D3 K_D = 151 nM; 1H2 K_D = 176 nM; 1C6 K_D = 3.6 nM and 2A5 K_D = 4.6 nM. The affinity data shows that the antibodies 114_H01 and 113_1H12 of the present invention have affinities that are as good or better than the best results reported for the antibodies of WO 2008/018641 despite using Fab format antibodies rather than IgG which would in general lead to an underestimate of the affinity of the antibodies of the present invention relative to the prior art.

Epitope mapping

The epitopes bound by antibodies 113_1H12 and 114_H01 were compared to the epitope bound by the four exemplified antibodies disclosed in WO 2008/018641. These experiments showed that 113_1H12 partially shares an epitope with the antibodies 1D3 and 1H2 of WO 2008/018641, while 114_3H1 has a unique epitope that shares only one residue with the antibodies of WO 2008/018641. E15 is outside of regions that involved in receptor or heparin binding. All other epitope residues are within regions involved in receptor binding, which according to numbering of the full length protein at UniProt P48061 (SDF1_HUMAN)are 29-33, 39-41, 48-50, 60-70). WO 2008/018641 discloses that the residues involved in receptor binding lie between amino acid residues 7-19.

Antibody	***Substitutions that strongly reduce/eliminate binding***	***Substitutions that partially reduce binding***
114_3H1	*P10A, R12A*	*E15A, I28A, P32A, N45A, K54A*

113_1H12	*P10A, Q48A*	*K54A, N45A*
1D3 and 1H2	*P10A, N45A, Q48A*	
2A5 and 1C6	*P10A, E15A, N45A, R47A*	*F13A, I28A, K54A*

Properties of anti-CXCL12 monoclonal antibodies in a chimeric murine IgG2a backbone

The variable regions of the 113_1H12 and 114_3H1 were cloned into an murine IgG2a expression system, permitting production of the chimeric antibodies, with murine constant regions and human variable regions. This type of chimeric antibody could be preferable for *in vivo* testing in immunocompetent animals to reduce immune response towards the antibody which could occur upon administration of a fully human antibody.

A) Migration assays.

The ability of the antibodies in a chimeric murine IgG2a and a fully human IgG2 formats to block cellular migration of human and murine cell lines was investigated using a murine metastatic melanoma cell line (B16F10) and a human ovarian carcinoma cell line (TOV21G).

All antibodies were used at a concentration of 50 µg/ml. The human CXCL12 ligand (Peprotech) was used at a concentration of 500 ng/ml. Antibodies and ligands were prepared in DMEM (for B16F10 studies) or RPMI (for TOV21G studies).For each time-lapse migration assay 3-4 x 10^6 cells were used in a collagen-cell mix inside a coverslip migration chamber. Cell suspensions were combined with a collagen mix in a ratio of 1 (cells):2 (collagen). The collagen mix was composed of sodium bicarbonate. 10X MEM and Collagen (3mg/ml, Sigma) in a 1:2:15 ratio. The antibodies were added to the cell-collagen mix and the resulting mixture was inserted into the migration chamber where the collagen was polymerised by incubation at 37°C for 30 minutes. Once polymerised the CXCL12 ligand was added to produce the chemokine gradient and movement of the cells towards CXCL12 was

determined using time-lapse imaging. Images were taken at an interval of 1 picture/min over a period of 3.5 - 4 hours. Time lapse imagining permit the visualisation of cellular migration tracks which were them analysed using Image J software to determine the extent of cellular migration.

The migration distance for B16F10 cells in the presence of CXCL12 and the 3 blocking antibodies are shown in Figure 9A and B.

All antibodies, 113_1H12 in a human IgG2 format (hAB113), 113_1H12 in a chimeric murine IgG2a format (mAB113) and 114_3H1 in a chimeric murine IgG2a format (mAB114), were effective at substantially blocking cellular migration in B16F10 cells and to some degree also in TOV-21 cells. In this assay 113_1H12 in a human IgG2 format (hAB113) was inactive in terms of blocking TOV21G cell migration whereas mAB113 was fully active and mAB114 somewhat in between. However, from the results in Figure 5 we know that the 113_1H12 antibody in a human IgG2 format can inhibit the migration of the TOV21G cell line towards CXCL12 and so the inactive result here is likely to be an anomaly. These results are summarised in Figure 9A.

In addition, all three antibodies were effective at inhibiting CXCL12 dependent migration of B16F10 cells. This is shown graphically in Figure 9B. Thus, these experiments confirm that the antibodies in a chimeric murine IgG2a format work in *in vitro* migration assays and are capable of inhibiting cancer cell migration.

B) Serum stability testing.

The serum stability of the antibodies 114_3H1 and 113_1H12 in a chimeric murine IgG2a format was tested. Serum stability assays were performed using human serum. Antibodies were incubated in serum at a concentration of 100 µg/ml for 6, 12, 24 and 48 hours. Serum containing antibodies were diluted to a concentration of 50 µg/ml in DMEM or RPMI and used for the time-lapse migration assay as described above in A. These experiments found that the

antibodies in a chimeric murine IgG2a format were stable over 48 hours in serum and were still active at inhibiting CXCL12-dependent B16F10 cell migration after 48 hours in serum. This confirms that both antibodies are very stable in serum.

C) *In vivo* pulmonary metastasis.

B16F10 melanoma cells require CXCR4 for migration to the lung and initiation of metastasis. A simple experimental metastasis *in vivo* model was used to evaluate the ability of the chimeric murine IgG2a antibodies to interfere with CXCL12 and therefore block metastatic development. B16F10 melanoma cells were introduced into C57Bl mice through tail vein injection on day 0 and treatment commenced on day 1. Treatment regimes were either 5 mg/kg of the clinical CXCR4 inhibitor AMD3100 (Plerixafor) twice daily or twice a week with either 10, 15 or 20 mg/kg of the anti-CXCL12 antibody in a murine IgG2a format. Mice in the control arm were treated twice a week with 20 mg/kg of a murine IgG2a control antibody. All mice were culled on day 14 and the number of metastatic colonies in the lungs quantified by manual counting. As shown in Figure 10, both chimeric murine 114_3H1 and 113_1H12 antibodies were active at inhibiting metastatic development. In particular, antibody 113_1H12 was more potent and resulted in and inhibition at 20mg/kg equivalent to that seen with 5 mg/kg of the small molecule antagonist of CXCR4, AMD3100. Thus, both antibodies are active *in vivo* in inhibiting CXCR4 dependent-pulmonary metastasis.

Sequence Listing

Antibody 114_3H1

SEQ ID NO: 1: CDR-H1 amino acid sequence (from Ab114_3H1)

ELSMH

SEQ ID NO: 2: CDR-H2 amino acid sequence (from Ab114_3H1)

GFDPEDGETIYAQKFQG

SEQ ID NO: 3: CDR-H3 amino acid sequence (from Ab114_3H1)

RVWGSYRPNDAFDI

SEQ ID NO: 4: CDR-L1 amino acid sequence (from Ab114_3H1)

RASQSISDYVN

SEQ ID NO: 5: CDR-L2 amino acid sequence (from Ab114_3H1)

AASTSQS

SEQ ID NO: 6: CDR-L3 amino acid sequence (from Ab114_3H1)

QQSYSPPYT

SEQ ID NO: 7: VH domain amino acid sequence

114_3H1 Variable Heavy chain
QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQ
GRVTMTEDTSTDTAYMELSSLGSEDTAVYYCARRVWGSYRPNDAFDIWGQGTLVTVSS

SEQ ID NO: 8: VH domain nucleic acid sequence

>114_3H1_VH
CAGGTCCAGCTGGTACAGTCTGGGGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGTCTCCTG
CAAGGTTTCCGGATACACCCTCACTGAATTATCCATGCACTGGGTGCGACAGGCTCCTGGAAAAG
GGCTTGAGTGGATGGGAGGTTTTGATCCTGAAGATGGTGAAACAATCTACGCACAGAAGTTCCAG
GGCAGAGTCACCATGACCGAGGACACATCTACAGACACAGCCTACATGGAGCTGAGCAGCCTGGG
ATCTGAGGACACGGCCGTGTATTACTGTGCGAGACGCGTTTGGGGGAGTTATCGCCCCAATGATG
CTTTTGATATCTGGGGCCAAGGAACCCTGGTCACCGTCTCCTCA

SEQ ID NO: 9: VL domain amino acid sequence

114_3H1 Variable Light chain
DIQMTQSPSSLSASVGDRVTITCRASQSISDYVNWYQQKPGKAPNLLMFAASTSQSGVPSRFTGS
GSGTDFTLTISSLQPEDFATYFCQQSYSPPYTFGQGTKVEIKR

SEQ ID NO: 10: VL domain nucleic acid sequence

>114_3H1_VL

GACATCCAGATGACCCAGTCTCCATCCTCCCTGTCTGCATCTGTAGGCGACAGAGTCACCATCAC
TTGCCGGGCAAGTCAGAGCATAAGCGACTATGTAAACTGGTATCAGCAGAAACCAGGGAAAGCCC
CCAACCTCCTGATGTTTGCTGCATCCACTTCGCAAAGTGGGGTCCCGTCAAGGTTCACTGGCAGC
GGATCTGGGACAGATTTCACTCTCACCATCAGCAGTCTGCAACCTGAAGATTTTGCAACTTACTT
CTGTCAACAGAGTTACAGTCCGCCCTACACTTTTGGCCAGGGGACCAAGGTGGAGATCAAACGT

SEQ ID NO: 11: 114_3H1 scFv

Lead antibody sequence
>114_3H1
QVQLVQSGAEVKKPGASVKVSCKVSGYTLTELSMHWVRQAPGKGLEWMGGFDPEDGETIYAQKFQ
GRVTMTEDTSTDTAYMELSSLGSEDTAVYYCARRVWGSYRPNDAFDIWGQGTLVTVSSLEGGGGS
GGGGSGGGASDIQMTQSPSSLSASVGDRVTITCRASQSISDYVNWYQQKPGKAPNLLMFAASTSQ
SGVPSRFTGSGSGTDFTLTISSLQPEDFATYFCQQSYSPPYTFGQGTKVEIKRAAASAHHHHHHK
LDYKDHDGDYKDHDIDYKDDDDK

Antibody 113_1H12

SEQ ID NO: 12: CDR-H1 amino acid sequence 113_1H12

NYGIS

SEQ ID NO: 13: CDR-H2 amino acid sequence 113_1H12

WISAYNGNTNYAQKLQG

SEQ ID NO: 14: CDR-H3 amino acid sequence 113_1H12

AGGVYYDYFTDY

SEQ ID NO: 15: CDR-L1 amino acid sequence 113_1H12

SGSRSNIGSNSVN

SEQ ID NO: 16: CDR-L2 amino acid sequence 113_1H12

NNDERPS

SEQ ID NO: 17: CDR-L3 amino acid sequence 113_1H12

AAWDDSLNVGEL

SEQ ID NO: 18: VH domain amino acid sequence

113_1H12 Variable Heavy chain
EVQLVQSGAEVKKPGASVKVSCKTSGYTFTNYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQ
GRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARAGGVYYDYFTDYWGQGTMVTVSS

SEQ ID NO: 19: VH domain nucleic acid sequence

>113_1H12_VH

ATGGCCGAGGTGCAGCTGGTGCAGTCTGGAGCTGAGGTGAAGAAGCCTGGGGCCTCAGTGAAGGT
CTCCTGCAAGACTTCTGGTTACACCTTTACCAACTATGGTATCAGCTGGGTGCGACAGGCCCCTG
GACAAGGGCTTGAGTGGATGGGATGGATCAGCGCTTACAATGGTAACACGAACTATGCACAGAAG
CTCCAGGGCAGAGTCACCATGACCACAGACACATCCACGAGCACAGCCTACATGGAGCTGAGGAG
CCTGAGATCTGACGACACGGCCGTGTATTACTGCGCGAGAGCCGGCGGAGTCTATTACGATTATT
TCACGGACTACTGGGGCCAGGGGACAATGGTCACCGTCTCTTCA

SEQ ID NO: 20: VL domain amino acid sequence

113_1H12 Variable Light chain

QSELTQPPSASGTPGQRVTISCSGSRSNIGSNSVNWYQQLPGTAPKLLIYNNDERPSGVPDRFSG
SKSGTSASLAISGLQSEDEADYFCAAWDDSLNVGELFGGGTKLTVLG

SEQ ID NO: 21: VL domain nucleic acid sequence

>113_1H12_VL

CAGTCTGAGCTGACTCAGCCACCCTCAGCGTCTGGGACCCCCGGGCAGAGGGTCACCATCTCTTG
TTCTGGAAGCCGCTCCAACATCGGAAGTAATTCTGTAAACTGGTACCAGCAGCTCCCAGGAACGG
CCCCCAAACTCCTCATTTATAATAATGATGAGCGGCCCTCAGGGGTCCCTGACCGATTCTCTGGC
TCCAAGTCTGGCACCTCAGCCTCCCTGGCCATCAGTGGGCTCCAGTCTGAGGATGAGGCTGATTA
TTTCTGTGCAGCATGGGATGACAGCCTGAATGTCGGGGAGCTATTCGGCGGAGGGACCAAGCTGA
CCGTCCTAGGT

SEQ ID NO: 22: 113_1H12 scFv

Lead antibody sequence

>113_1H12_scFv

EVQLVQSGAEVKKPGASVKVSCKTSGYTFTNYGISWVRQAPGQGLEWMGWISAYNGNTNYAQKLQ
GRVTMTTDTSTSTAYMELRSLRSDDTAVYYCARAGGVYYDYFTDYWGQGTMVTVSSLEGGGGSGG
GGSGGGASQSELTQPPSASGTPGQRVTISCSGSRSNIGSNSVNWYQQLPGTAPKLLIYNNDERPS
GVPDRFSGSKSGTSASLAISGLQSEDEADYFCAAWDDSLNVGELFGGGTKLTVLGAAASAHHHHH
HKLDYKDHDGDYKDHDIDYKDDDDK

SEQ ID NO: 23 : CXCL12 amino acid sequence (full length sequence)

>sp|P48061-2|SDF1_HUMAN Isoform Alpha of Stromal cell-derived factor 1 OS=Homo sapiens GN=CXCL12

MNAKVVVVLVLVLTALCLSDGKPVSLSYRCPCRFFESHVARANVKHLKILNTPNCALQIVARLKN
NNRQVCIDPKLKWIQEYLEKALNK

SEQ ID NO: 24 : Synthesised CXCL12 amino acid sequence used for antibody selection. Corresponds to amino acids 22 to 89 of full length CXCL12 protein

KPVSLSYRCPCRFFESHVARANVKHLKILNTPNCALQIVARLKNNNRQVCIDPKLKWIQEYLEKA
LNK

SEQ ID NO: 25: recombinant "wild-type" CXCL12 expressed in E.coli for epitope mapping (including His tag and linker)

KPVSLSYRCPCRFFESHVARANVKHLKILNTPNCALQIVARLKNNNRQVCIDPKLKWIQEYLEKA
LNKAAASAHHHHHHKL

References:

All documents mentioned in this specification are incorporated herein by reference in their entirety.

Claims:

1. An isolated anti-CXCL12 antibody molecule which specifically binds to human CXCL12 and inhibits CXCL12-mediated biological activity, wherein the antibody molecule binds to an epitope of CXCL12 having the amino acid sequence as set out in SEQ ID NO: 24 that comprises amino acids (a) P10 and R12, and optionally one or more of E15, I28, P32, N45 and/or K54 or (b) P10 and Q48, and optionally one of more of K54 and N45.

2. The anti-CXCL12 antibody molecule of claim 1, wherein the antibody molecule has one or more biological activities selected from inhibiting CXCL12-induced cancer cell growth, inhibiting cancer cell migration, inhibiting cancer cell adhesion and/or inhibiting cancer metastasis.

3. The anti-CXCL12 antibody molecule of claim 1 or claim 2, wherein the antibody molecule has the biological activity of inhibiting angiogenesis.

4. The anti-CXCL12 antibody molecule of claim 3, wherein the angiogenesis is VEGF-induced angiogenesis.

5. An isolated anti-CXCL12 antibody molecule which specifically binds to human CXCL12 and inhibits CXCL12-mediated biological activity, wherein the antibody molecule comprises (a) a CDR-H1 having the amino acid sequence of SEQ ID NO: 12, or the amino acid sequence of SEQ ID NO: 12 with one, two, three or more amino acid substitutions, deletions or insertions, and (b) a CDR-H2 having the amino acid sequence of SEQ ID NO: 13, or the amino acid sequence of SEQ ID NO: 13 with one, two, three or more amino acid substitutions, deletions or insertions, and (c) a CDR-H3 having the amino acid sequence of SEQ ID NO: 14, or the amino acid sequence of SEQ ID NO: 14 with one, two, three or more amino acid substitutions, deletions or insertions.

6. The anti-CXCL12 antibody molecule of claim 5, further comprising (d) a CDR-L1 having the amino acid sequence of SEQ ID

NO:15, or the sequences of SEQ ID NO: 15, with one or more amino acid substitutions, deletions or insertions, and (e) a CDR-L2 having the amino acid sequence of SEQ ID NO: 16, or the sequences of SEQ ID NO: 16, with one, two, three or more amino acid substitutions, deletions or insertions and (f) a CDR-L3 having the amino acid sequence of SEQ ID NO: 17, or the sequences of SEQ ID NO: 17, with one, two, three or more amino acid substitutions, deletions or insertions.

7. An isolated anti-CXCL12 antibody molecule which specifically binds to human CXCL12 and inhibits CXCL12-mediated biological activity, wherein the antibody molecule comprises: (a) a CDR-H1 having the amino acid sequence of SEQ ID NO: 1, or the amino acid sequence of SEQ ID NO: 1 with one, two, three or more amino acid substitutions, deletions or insertions, (b) a CDR-H2 having the amino acid sequence of SEQ ID NO: 2, or the amino acid sequence of SEQ ID NO: 2 with one, two, three or more amino acid substitutions, deletions or insertions and (c) a CDR-H3 having the amino acid sequence of SEQ ID NO: 3, or the amino acid sequence of SEQ ID NO: 3 with one, two, three or more amino acid substitutions, deletions or insertions.

8. The anti-CXCL12 antibody molecule of claim 7, further comprising (d) a CDR-L1 having the amino acid sequence of SEQ ID NO: 4, or the sequences of SEQ ID NO: 4, with one, two, three or more amino acid substitutions, deletions or insertions, (e) a CDR-L2 having the amino acid sequence of SEQ ID NO: 5, or the sequences of SEQ ID NO: 5, with one, two, three or more amino acid substitutions, deletions or insertions and (f) a CDR-L3 having the amino acid sequence of SEQ ID NO: 6, or the sequences of SEQ ID NO: 6, with one, two, three or more amino acid substitutions, deletions or insertions.

9. The anti-CXCL12 antibody molecule of any one of claims 5 to 8, wherein amino acid sequence of the CDRs of the antibody molecule comprise 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acid

substitutions, deletions or insertions as compared to any one of SEQ ID NOs: 1 to 6 or SEQ ID NOs: 12 to 17.

10. The anti-CXCL12 antibody molecule of any one of the preceding claims, wherein the anti-CXCL12 antibody binds CXCL12 and comprises:

an antibody VH domain selected from the group consisting of the 114_3H1 VH domain (SEQ ID NO: 7) and a VH domain comprising a VH CDR3 with the amino acid sequence of SEQ ID NO: 3 and optionally one or more VH CDRs with an amino acid sequence selected from SEQ ID NO: 1 and SEQ ID NO: 2; and/or

an antibody VL domain selected from the group consisting of the 114_3H1 VL domain (SEQ ID NO: 9) and a VL domain comprising one or more VL CDRs with an amino acid sequence selected from SEQ ID NOs: 4, 5 and 6.

11. The anti-CXCL12 antibody molecule of any one of the preceding claims, wherein the anti-CXCL12 antibody binds CXCL12 and comprises:

an antibody VH domain selected from the group consisting of the 113_1H12 VH domain (SEQ ID NO: 18) and a VH domain comprising a VH CDR3 with the amino acid sequence of SEQ ID NO: 14 and optionally one or more VH CDRs with an amino acid sequence selected from SEQ ID NO: 12 and SEQ ID NO: 13; and/or

an antibody VL domain selected from the group consisting of the 113_1H12 VL domain (SEQ ID NO: 20) and a VL domain comprising one or more VL CDRs with an amino acid sequence selected from SEQ ID NOs: 15, 16 and 17.

12. The anti-CXCL12 antibody molecule according to any one of claims 1 to 11 comprising (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 7, (b)a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 9, or (c) a VH sequence as in (a) and a VL sequence as in (b).

13. The anti-CXCL12 antibody molecule according to any one of

claims 1 to 11 comprising (a) a VH sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 18, (b) a VL sequence having at least 95% sequence identity to the amino acid sequence of SEQ ID NO: 20, or (c) a VH sequence as in (a) and a VL sequence as in (b).

14. The anti-CXCL12 antibody molecule of any one of the preceding claims, wherein the antibody molecule is a complete antibody, a monoclonal antibody, a Fab fragment, a $F(ab')_2$ fragment, a scFv, a scFv-Fc, an intrabody, a nanobody, a diabody, a triabody, a bispecific antibody and a chimeric antibody.

15. The anti-CXCL12 antibody molecule of any one of the preceding claims wherein the antibody molecule is a human antibody or a humanised antibody.

16. An immunoconjugate comprising an antibody molecule according to any one of claims 1 to 15.

17. The immunoconjugate of claim 16, wherein the antibody molecule is conjugated to therapeutically active moiety, such as a cytotoxic moiety or an immunostimmulatory moiety.

18. The immunoconjugate of claim 16 or claim 17, wherein the cytotoxic moiety is an alkylating agent, an alkaloid, a platinum coordination complex, a cytotoxic peptide, a radioactive agent, or a pro-drug capable of conversion into a cytotoxic moiety.

19. A pharmaceutical composition comprising an antibody molecule or an immunoconjugate according to any one of claims 1 to 18 and a pharmaceutically acceptable excipient.

20. An antibody molecule or an immunoconjugate according to any one of claims 1 to 18 for use in a method of treatment of the human or animal body.

21. The antibody molecule or an immunoconjugate for use in a

method of treatment of the human or animal body according to claim 20, wherein the method is for the treatment of CXCL12 mediated disease.

22. An antibody molecule or an immunoconjugate according to any one of claims 1 to 18 for use in a method of treatment of a CXCL12-mediated cancer or for the treatment of WHIM syndrome.

23. The antibody molecule or an immunoconjugate according to claim 22, wherein the CXCL12-mediated cancer overexpresses CXCL12.

24. Use of an antibody molecule or an immunoconjugate according to any one of claims 1 to 18 in the manufacture of a medicament for use in treating a CXCL12-mediated cancer.

25. A method of treating an individual with a CXCL12-mediated cancer comprising administering an antibody molecule or an immunoconjugate according to any one of claims 1 to 18 to an individual in need thereof.

26. The antibody or immunoconjugate for use in a method of treatment, the use or the method of any one of claims 22 to 25, wherein the CXCL12-mediated cancer overexpresses CXCL12.

27. The antibody or immunoconjugate for use in a method of treatment, the use or the method of any one of claims 22 to 26, wherein the antibody inhibits VEGF-induced angiogenesis and/or inhibits of CXCL12-induced cancer cell migration.

28. The antibody for use in a method of treatment, the use or the method of any one of claims 22 to 27, wherein the cancer is ovarian cancer, breast cancer, bone cancer, prostate cancer, thyroid cancer, pancreatic cancer, multiple myeloma, non-Hodgkin's lymphoma, intraocular lymphoma, follicular center lymphoma, CML, colorectal cancer, oral squamous carcinoma, cervical cancer, neuroblastoma, kidney cancer, brain cancers,

such as glioma and astrocytoma, rhabdmyosarcoma, lung cancer, such as small cell lung cancer, melanoma, B cell malignancies, such as B-cell chronic lymphocytic leukemia (B-CLL), and leukaemias, such as acute myeloid leukaemia (AML) or acute lymphoblastic leukaemia.

29. The antibody for use in a method of treatment, the use or the method of any one of claims 22 to 28 wherein the antibody is administered in conjunction with a chemotherapeutic agent, an antibody therapy, immune modulatory therapy, surgery or in conjunction with radiotherapy, or in conjunction with cell mediated therapy.

30. The antibody for use in a method of treatment, the use or the method of any one of claims 22 to 29, wherein the antibody is administered in conjunction with an angiogenesis inhibitor.

31. The antibody for use in a method of treatment, the use or the method of claim 30, wherein the angiogenesis inhibitor is selected from antibodies or peptide-antibody fusions targeted to angiogenesis-promoting growth factor receptors, such as Bevacizumab (Avastin®), Cetuximab (Erbitux®), Ramucirumab (Cyramza®), Icrucumab, HuMV833, 2C3, Aflibercept (Zaltrap®) and IMC-1C11; small molecule kinase inhibitors, such as Sorafenib (Nexavar®), Sunitinib (Sutent®), Pazopanib (Votrient®), Everolimus (Afinitor®), AEE788, AAL881, AAL993, ZD4190, ABT-869 (Linifanib), PTK787 (Vatalanib), AMG706 (Motesanib), Cediranib (Recentin), Axitinib (Inlyta®), Vandetanib (Caprelsa®), SU6668, ZD1839, Telatinib, Nintedanib (Vargatef®), Brivanib alaninate, BMS-605541, BMS-645737, CEP-7055, Dovitinib, CP-547,632, E7080, GW654652, KRN633, Tivozanib, OSI-930, PD173074, PF-00337210, SU1498, Semaxanib (SU5416), SU5614, SU11657, SU14813, TKI-28, TKI-31 and ZM323881; native angiogenesis inhibitors, such as endostatin and angiostatin; and drugs capable of inhibiting angiogenesis such as thalidomide, squalamine and angiozyme.

32. An antibody molecule according to any one of claims 1 to 15

for use in a method for the diagnosis or prognosis of a patient having a CXCL12-mediated condition.

33. The antibody molecule for use in a method for the diagnosis or prognosis of claim 32, wherein the method comprises determining the presence or amount of CXCL12 in the sample using the antibody and correlating the presence or amount of CXCL12 with the likely outcome of treating the patient with a CXCL12 inhibitor.

34. An antibody molecule or an immunoconjugate according to any one of claims 1 to 18 for use in a method of imaging.

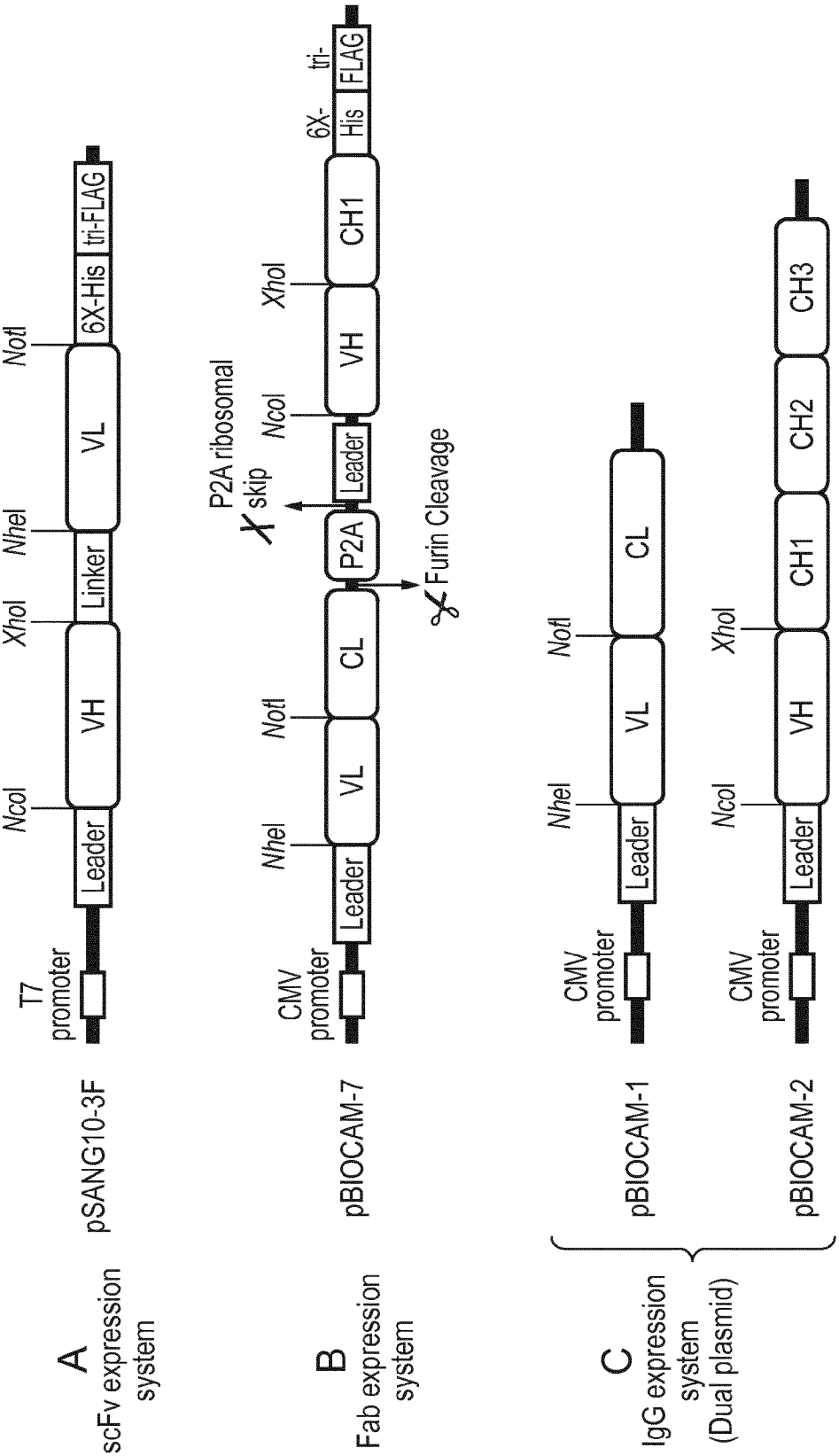

A
scFv expression system
pSANG10-3F
T7 promoter
Leader
NcoI
VH
XhoI
Linker
NheI
VL
NotI
6X-His
tri-FLAG
B
Fab expression system
pBIOCAM-7
CMV promoter
Leader
NheI
VL
NotI
CL
P2A
Furin Cleavage
P2A ribosomal skip
Leader
NcoI
VH
XhoI
CH1
6X-His
tri-FLAG
C
IgG expression system (Dual plasmid)
pBIOCAM-1
CMV promoter
Leader
NheI
VL
NotI
CL
pBIOCAM-2
CMV promoter
Leader
NcoI
VH
XhoI
CH1
CH2
CH3

FIG. 1

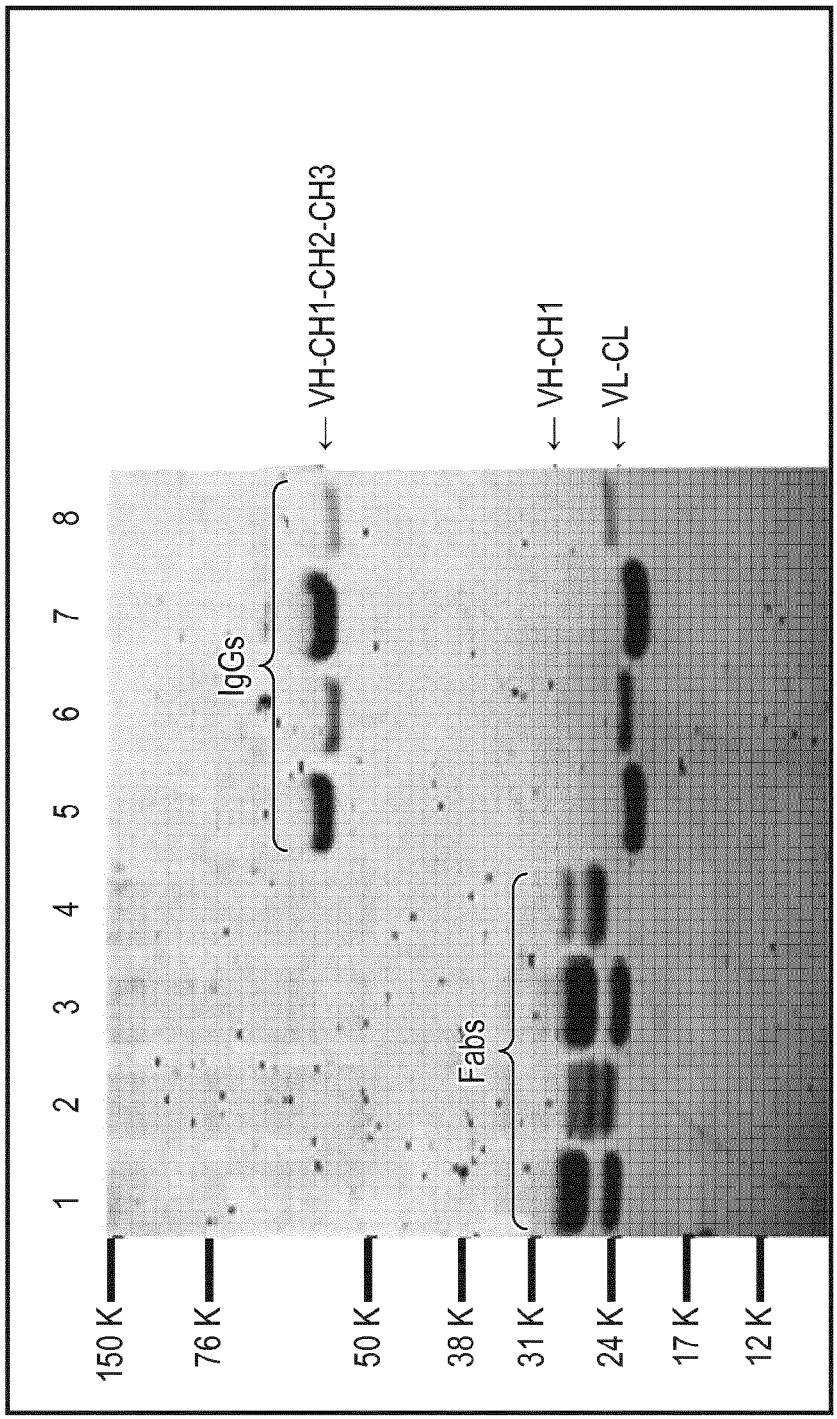
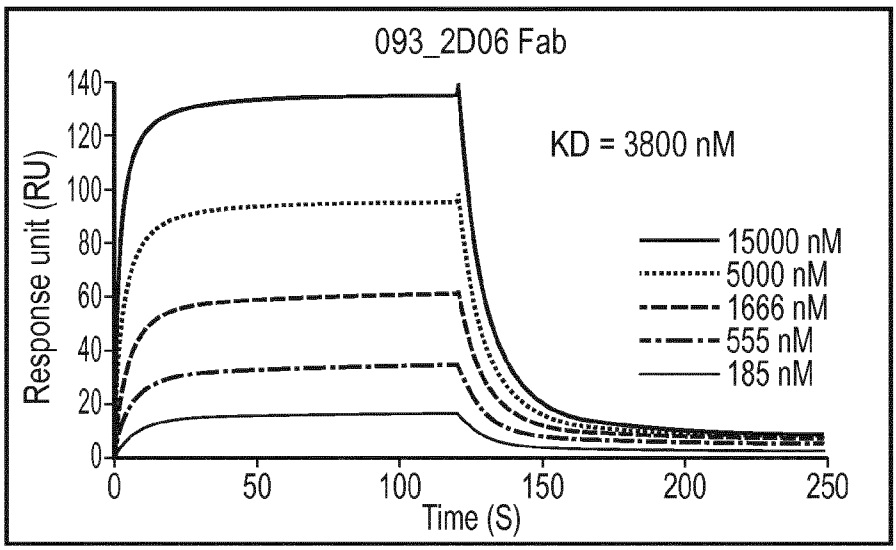
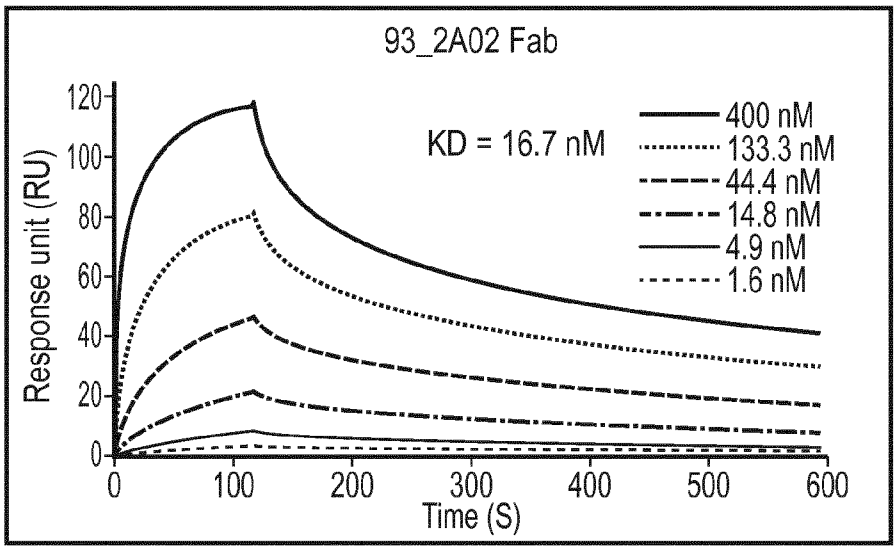

1
2
3
4
5
6
7
8
150 K
76 K
50 K
38 K
31 K
24 K
17 K
12 K
IgGs
Fabs
← VH-CH1-CH2-CH3
← VH-CH1
← VL-CL

FIG. 2

A





B

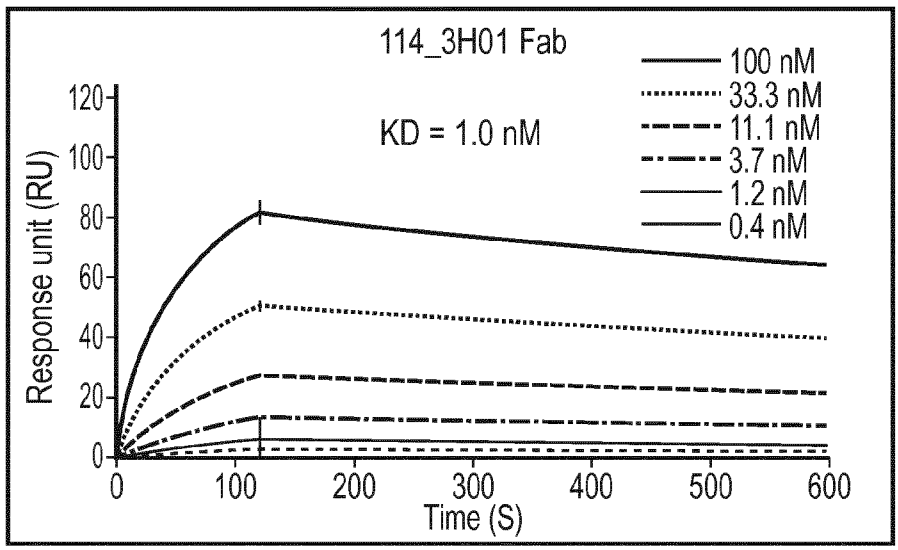
Antibody	ka (1/Ms)
093_2D06	ND
114_3H01	4.9×10^5

C

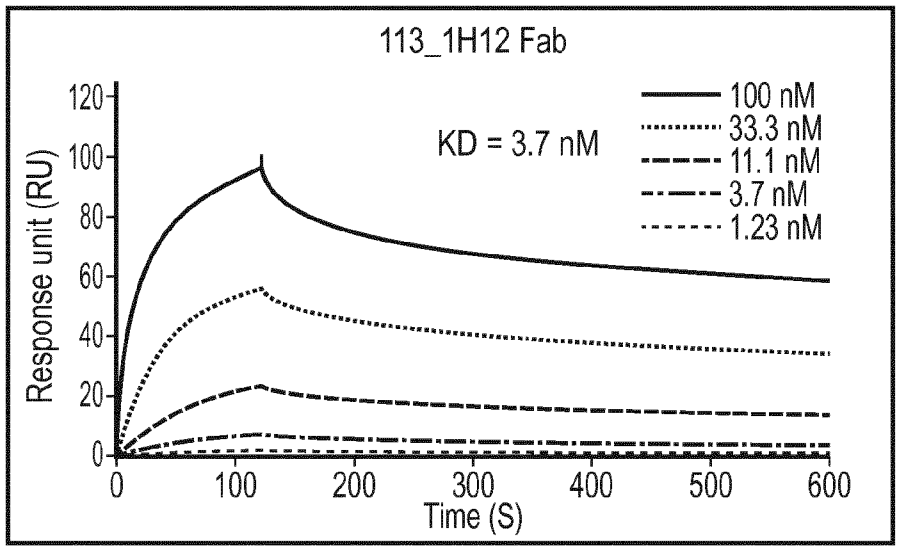
Antibody	ka 1 (1/Ms)	kd 1 (1/s)
093_2A02	2.0×10^5	0.01
113_1H12	5.1×10^5	0.04

FIG. 3

maturation



maturation



kd (1/s)	KD (M)
ND	3.7 x 10-6
5.1 x 10-4	1.0 x 10-9

ka 2 (1/Ms)	kd 2 (1/s)	KD (M)
0.007	22.2 x 10-4	16.7 x 10-9
0.015	7.9 x 10-4	3.7 x 10-9

FIG. 3 (Continued)

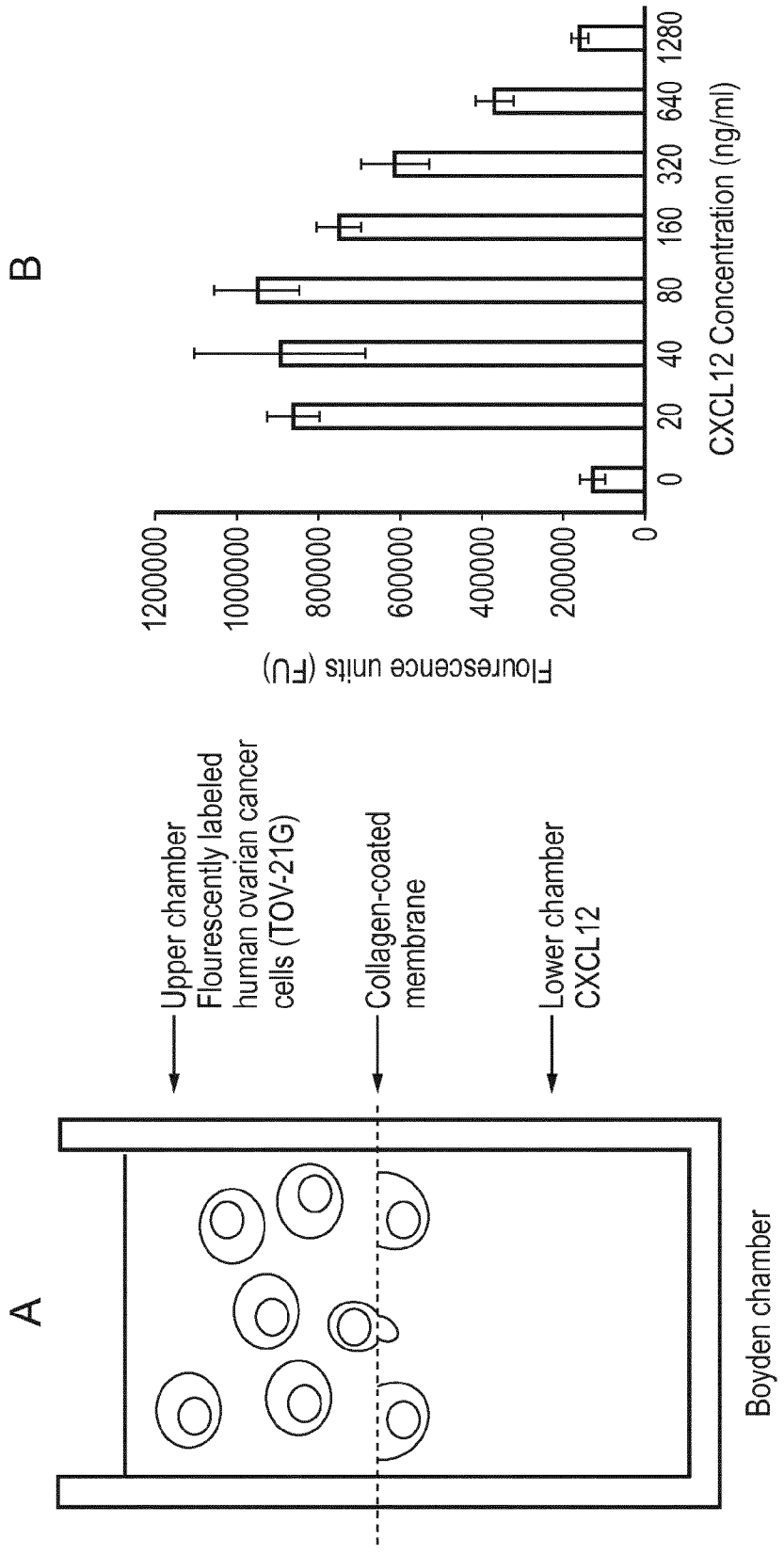

A
Upper chamber
Flourescently labeled
human ovarian cancer
cells (TOV-21G)
Collagen-coated
membrane
Lower chamber
CXCL12
Boyden chamber
B
Flourescence units (FU)
1200000
1000000
800000
600000
400000
200000
0
0
20
40
80
160
320
640
1280
CXCL12 Concentration (ng/ml)

FIG. 4

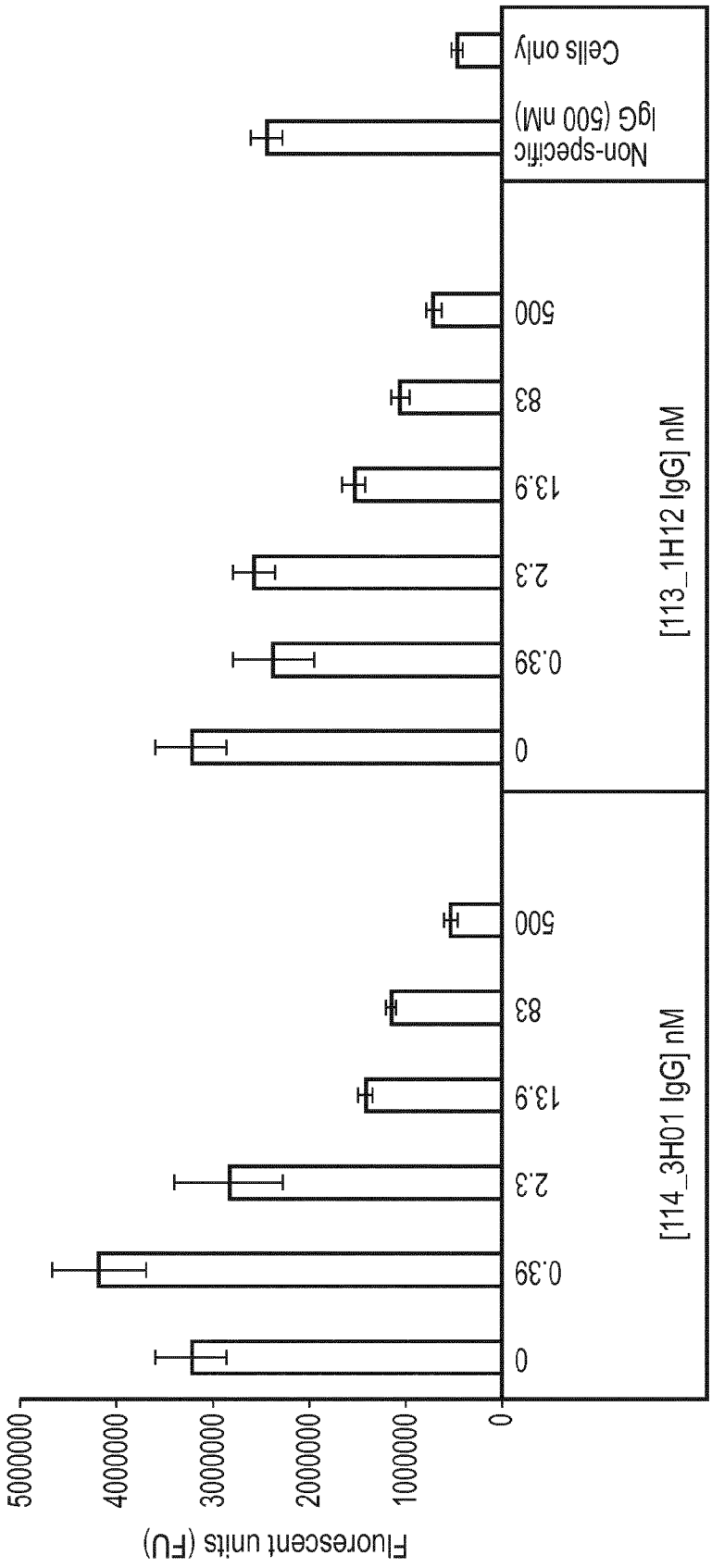

Fluorescent units (FU)
5000000
4000000
3000000
2000000
1000000
0
[114_3H01 IgG] nM
0
0.39
2.3
13.9
83
500
[113_1H12 IgG] nM
0
0.39
2.3
13.9
83
500
Non-specific IgG (500 nM)
Cells only

FIG. 5

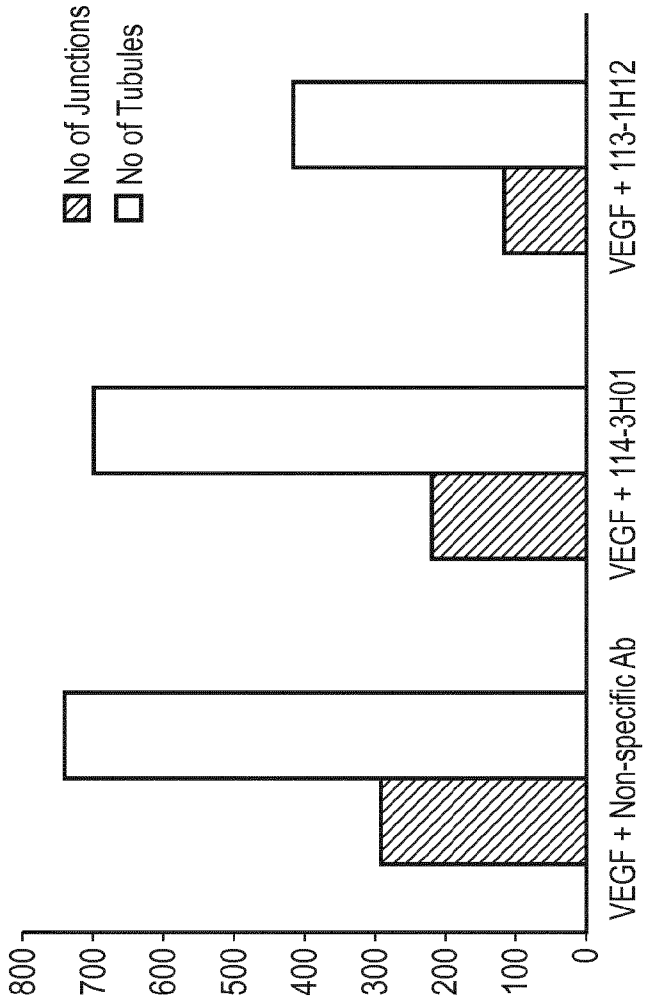

No of Junctions
No of Tubules
800
700
600
500
400
300
200
100
0
VEGF + Non-specific Ab
VEGF + 114-3H01
VEGF + 113-1H12

FIG. 6A

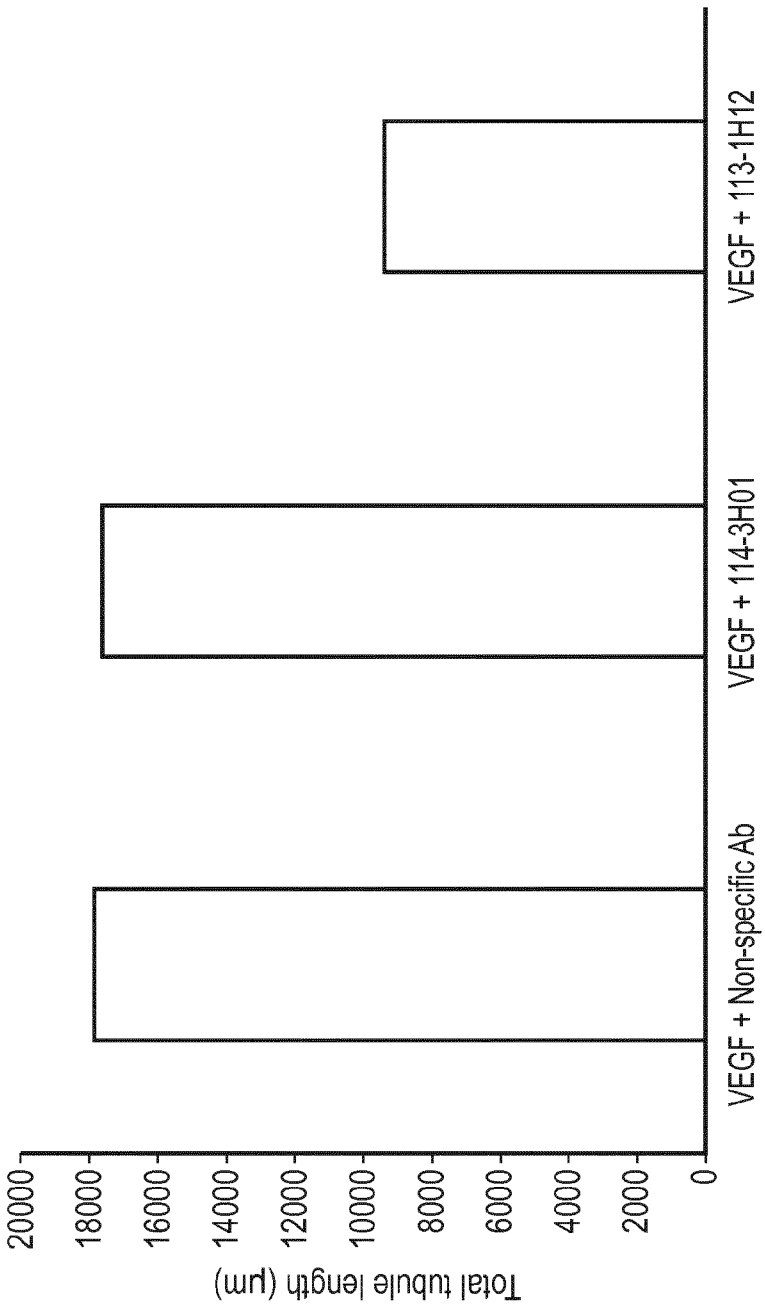

Total tubule length (µm)
0
2000
4000
6000
8000
10000
12000
14000
16000
18000
20000
VEGF + Non-specific Ab
VEGF + 114-3H01
VEGF + 113-1H12

FIG. 6B

CXCL12 Antibody - 114 3H1

Heavy chain sequence alignment

	FW 1	CDR 1	FW 2	CDR 2	FW 3	CDR 3	FW 4
114_3H1	QVQLVQSGAEVKKPGASVKVSCKVSGYTLT**ELSMH**----WVRQAPGKGLEWMG**GFDP**----**EDGETIYAQKFQG**RVTMTEDTSTDTAYMELSSL--GSEDTAVYYCAR**RVWGSYRPNDAF**----------**DIW**GQGTLVTVSS						

Light chain sequence alignment

	FW 1	CDR 1	FW 2	CDR 2	FW 3	CDR 3	FW 4
114_3H1	DIQMTQSPSSLSASVGDRVTITC**RASQ**-------**SISDVVNW**YQQKPGKAPNLLMF**AA**-----**STSQ**SGVPSRFTGSGSG--TDFTLTISSLQPEDFATYFC**QQSVSPP**---------**YT**FGQGTKVEI---KR						

CXCL12 Antibody - 113 1H12

Heavy chain sequence alignment

	FW 1	CDR 1	FW 2	CDR 2	FW 3	CDR 3	FW 4
113_1H12	EVQLVQSGAEVKKPGASVKVSCKTSGYTF**TNYGIS**----**W**VRQAPGQGLEWM**GWISA**-----**YNGNTNYAQKLQG**RVTMTTDTSTSTAYMELRSL--RSDDTAVYYCAR**AGGVYYDYFT**----------**DYW**GQGTMVTVSS						

Light chain sequence alignment

	FW 1	CDR 1	FW 2	CDR 2	FW 3	CDR 3	FW 4
113_1H12	QSELTQPPS-ASGTPGQRVTISCSGSRSN-----IGSNSVNWYQQLPGTAPKLLIY**NN**-----**DERPS**GVPDRFSGSKSG--TSASLAISGLQSEDEADYFC**AAWDDSLNVG**-----**EL**FGGGTKLTV--LG						

FIG. 7

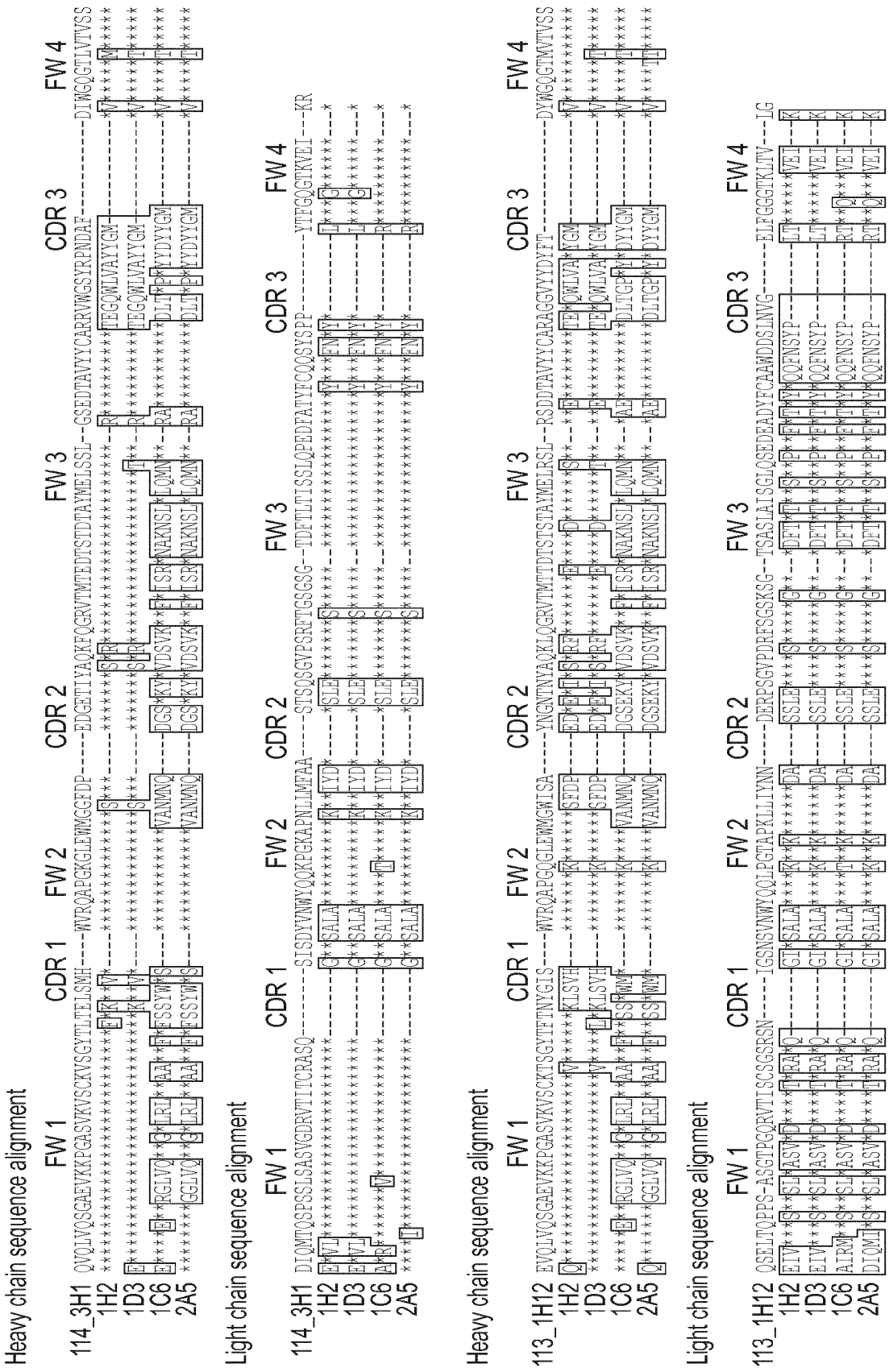

Heavy chain sequence alignment
Light chain sequence alignment
Heavy chain sequence alignment
Light chain sequence alignment
FW 1
CDR 1
FW 2
CDR 2
FW 3
CDR 3
FW 4

FIG. 8

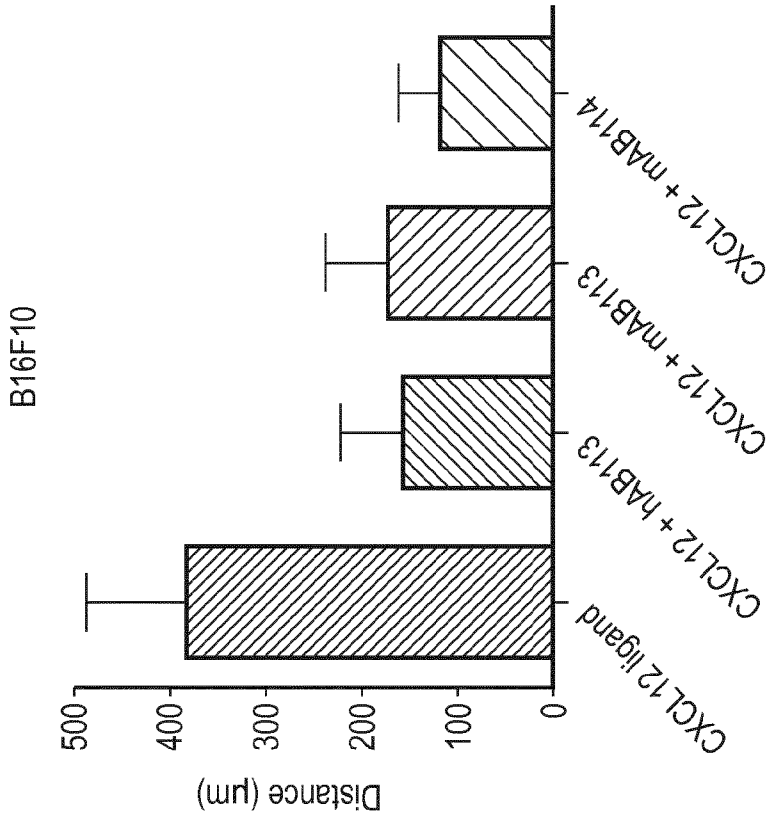

B16F10
Distance (µm)
0
100
200
300
400
500
CXCL12 ligand
CXCL12 + hAB113
CXCL12 + mAB113
CXCL12 + mAB114

FIG. 9B

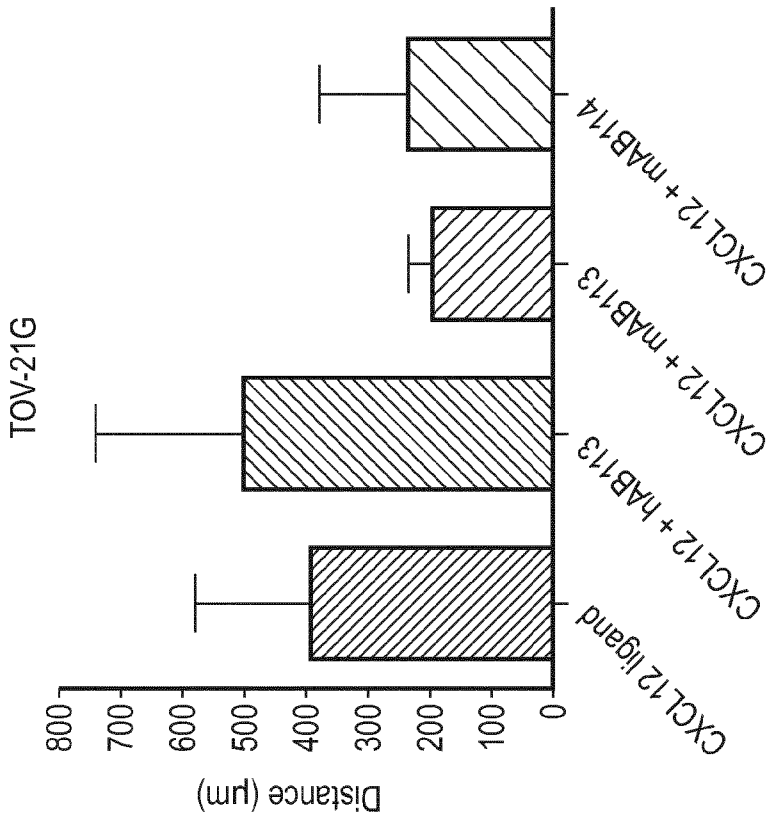

TOV-21G
Distance (µm)
0
100
200
300
400
500
600
700
800
CXCL12 ligand
CXCL12 + hAB113
CXCL12 + mAB113
CXCL12 + mAB114

FIG. 9A

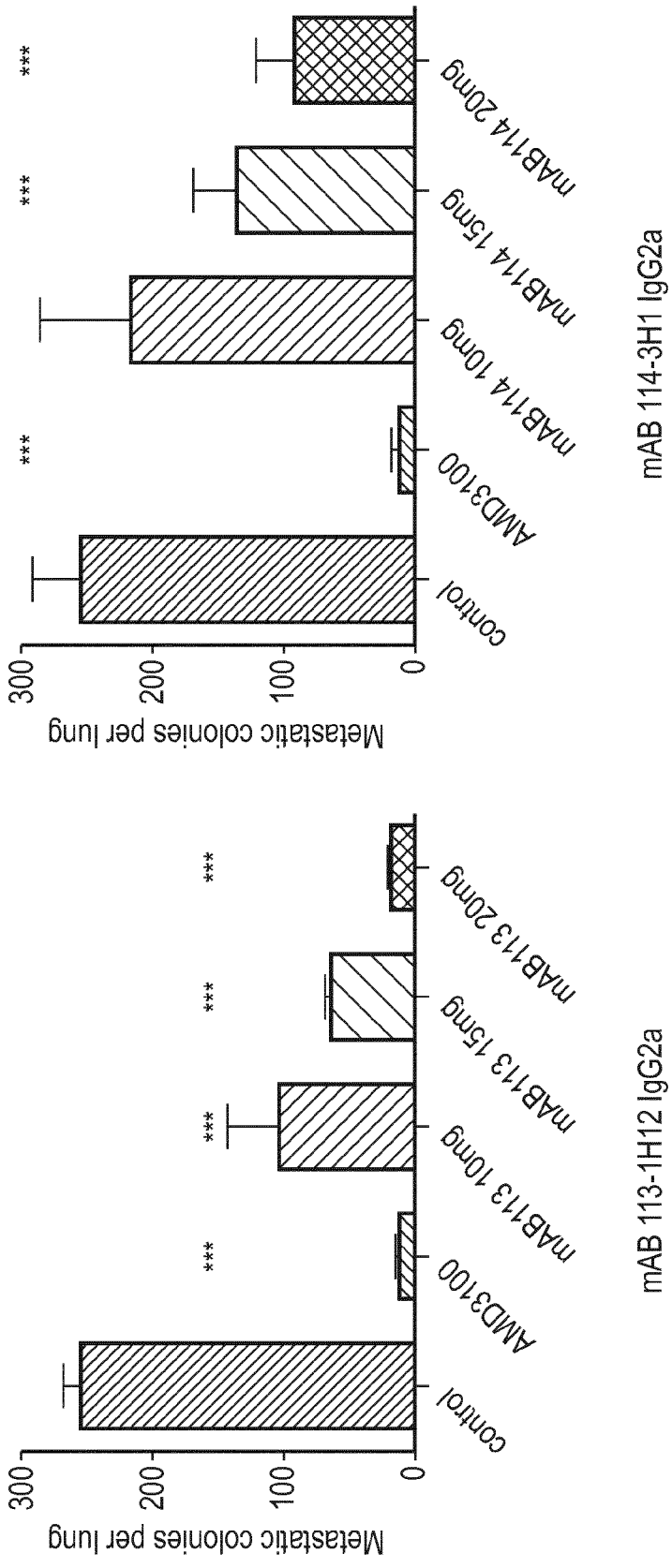

Metastatic colonies per lung
300
200
100
0
control
AMD3100
mAB113 10mg
mAB113 15mg
mAB113 20mg
mAB 113-1H12 IgG2a
***
Metastatic colonies per lung
300
200
100
0
control
AMD3100
mAB114 10mg
mAB114 15mg
mAB114 20mg
mAB 114-3H1 IgG2a
***

FIG. 10

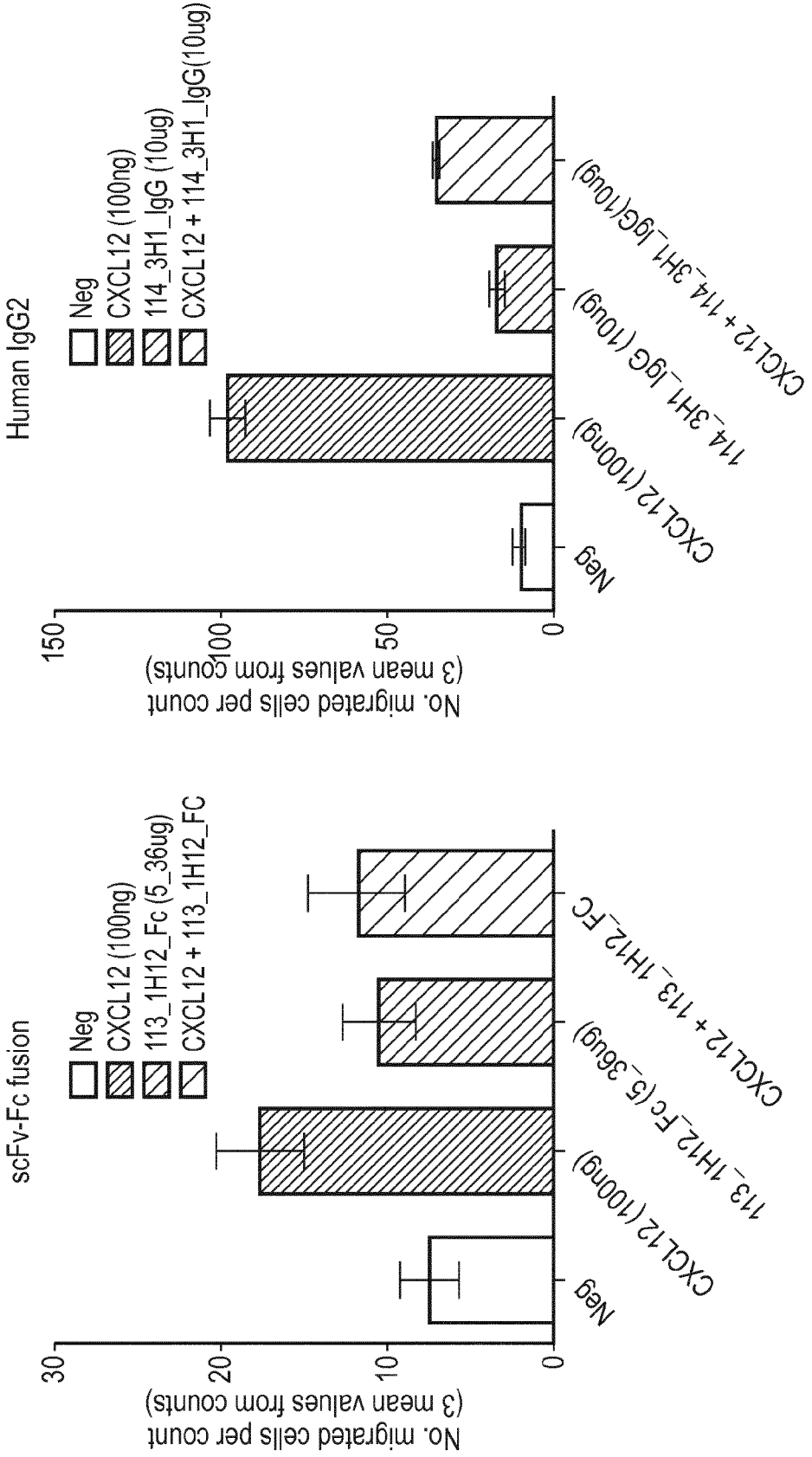

Human IgG2
Neg
CXCL12 (100ng)
114_3H1_IgG (10ug)
CXCL12 + 114_3H1_IgG(10ug)
No. migrated cells per count
(3 mean values from counts)
0
50
100
150
Neg
CXCL12 (100ng)
114_3H1_IgG (10ug)
CXCL12 + 114_3H1_IgG(10ug)
scFv-Fc fusion
Neg
CXCL12 (100ng)
113_1H12_Fc (5_36ug)
CXCL12 + 113_1H12_FC
No. migrated cells per count
(3 mean values from counts)
0
10
20
30
Neg
CXCL12 (100ng)
113_1H12_FC (5_36ug)
CXCL12 + 113_1H12_FC

FIG. 11