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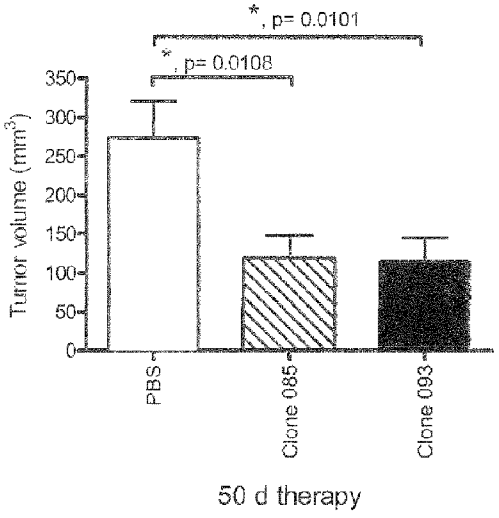
[Continued on next page]

(54) Title: BISPECIFIC ANTI-CXCR7 IMMUNOGLOBULIN SINGLE VARIABLE DOMAINS

Figure 7



(57) Abstract: The present invention relates to particular polypeptides, nucleic acids encoding such polypeptides; to methods for preparing such polypeptides; to host cells expressing or capable of expressing such polypeptides; to compositions and in particular to pharmaceutical compositions that comprise such polypeptides, for prophylactic, therapeutic or diagnostic purposes. In particular, the present invention provides immunoglobulin single variable domains inhibiting CXCR7 mediated tumour growth.

WO 2012/130874 A1

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BISPECIFIC ANTI-CXCR7 IMMUNOGLOBULIN SINGLE VARIABLE DOMAINS

Field of the Invention

The present invention relates to biological materials and methods related to CXCR7 including polypeptides, nucleic acids encoding such polypeptides; methods for preparing such polypeptides; host cells expressing or capable of expressing such polypeptides; compositions including pharmaceutical compositions that comprise such polypeptides, such as for prophylactic, therapeutic or diagnostic purposes.

Background of the Invention

Any discussion of the prior art throughout the specification should in no way be considered as an admission that such prior art is widely known or forms part of common general knowledge in the field.

Although it is suggested in the art i) that the blockage of CXCR7 employed along with CXCR4 blockage may be useful for the treatment of SDF-1-dependent tumor progression and metastasis (RB Maksym et al., 2009, The role of stromal-derived factor-1 – CXCR7 axis in development of cancer, European Journal of Pharmacology, 625 (1-3), pages 31-40) and ii) that some small molecular inhibitors, such as CCX733 or CCX266, siRNA and blocking antibodies (clones Mab 11G8, Mab 9C4 see *e.g.,* US20070167443; clone 358426 (R&D Systems); Mab 8F11 (Biolegend)), may be useful for therapeutic interference with CXCR4-mediated activation of integrins (TN Hartmann et al., 2008, A crosstalk between intracellular CXCR7 and CXCR4 involved in rapid CXCL12-triggered integrin activation but not in chemokine-triggered motility of human T lymphocytes and CD34+ cells, Journal of Leukocyte Biology, 84, pages 11301140), the biology of CXCR7 is still poorly understood as the mechanism(s) of action through which CXCR7 acts is unclear because i) it may act as a kind of decoy or signalling receptor depending on cell type – RM Maksym et al., *supra* and since ii) the interplay between I-TAC and SDF-1 binding to CXCR7 is unclear.

The identification of selective therapeutically effective anti-CXCR7 agents is not only challenging because of its poorly understood biology (such as *e.g.,* mechanism of action, *e.g.,* of the potential agonists CCX733 or CCX266 versus antagonists, interplay with CXCR4, recognition of important epitopes, cross-reactivity of the compounds CCX733 or CCX266 and associated toxicity), it is also acknowledged in the art (see *e.g.,* Naunyn-Schmied Archives Pharmacology 379: 385-388) that the generation of an anti-GPCR therapeutic agent such as an anti-CXCR7 agent is difficult since i) the native conformation of active CXCR7 in cancer cells is not exactly known, and ii) it is expected that

CXCR7 shows low immunogenicity (due to a limited number of extracellular surface exposed amino acid residues that are in addition very conserved, *e.g.,* mouse-human CXCR7 is 96% homologous).

Furthermore, compounds (CCX733, CCX754), which can selectively block binding of CXCL11 and CXCL12 to CXCR7, function like chemokine ligands with respect to homodimerization, *i.e.,* they enhance CXCR7 homodimerization by 2.5 to 3.5 fold with significant increases ($P<0.05$) first detected at 10 and 100 nM (KE Luker et al., 2009, Imaging chemokine receptor dimerization with firefly luciferase complementation, FASEB journal, 23, pages 823-834).

CXCR7 has been attributed a potential role in tumour development because its expression provides cells with a growth and survival advantage. It was recently demonstrated that CXCR7 promotes the growth of breast and lung tumours and enhances lung metastases (Proc. Natl. Acad. Sci. USA 2007 104:15735-15740). Moreover, CXCR7 expression is correlated with tumour aggressiveness in prostate cancer (J.Biol.Chem 2008 283:4283-4294). Administration of a small molecule antagonist to CXCR7 resulted in impediment of tumour growth in animal models, validating CXCR7 as target for development of novel cancer therapeutics (J.Exp.Med. 2006 203:2201-2213).

Head and neck cancers are among the most prevalent tumors in the world. Despite advances in the treatment of head and neck tumors, the survival of patients with these cancers has not markedly improved over the past several decades because of the inability to control and poor understanding of the regional and distant spread of this disease. Head and neck cancers consistently rank among the six most frequently diagnosed cancers in the world. Cancers of the oral cavity and pharynx alone account for some 300,000 new cases worldwide and little under 200,000 deaths annually. Over 90% of head and neck cancers are squamous cell carcinomas of the upper aerodigestive tract, including the oral cavity, pharynx, larynx, and paranasal sinuses. In addition, epithelial head and neck tumors can arise in the salivary and thyroid glands. Despite advances in our understanding and advances in the prevention and treatment of head and neck cancers, the survival of patients with head and neck cancers has not significantly improved over the past several decades.

Summary of the Invention

WO2006/116319 and WO2008/048519 both note that the production of antibodies to G-protein coupled receptors (GPCRs) has been notoriously difficult. Indeed, the generation of a conventional anti-CXCR7 antibody has been described only in a limited number of cases, *e.g.,* in WO2006/116319 for conventional antibodies 11G8, 6E10 and in Zabel et al. for conventional antibody 8F11 (Zabel et al., 2009, Elucidation of CXCR7 mediated signalling events and inhibition of CXCR4 mediated tumor cell transendothelial migration by CXCR7 ligands. J Immunol.; 183 (5):3204-11). However, despite

extensive research, it is unclear at present whether these or similar antibodies are suitable for a medical application.

Zheng *et al.* reports increased CXCR7 expression in hepatocellular carcinoma tissues. Down-regulation of CXCR7 expression leads to a reduction of tumour growth in a xenograft model of HCC. However, the authors used SMMC-7721 cells, which were previously transfected in vitro by CXCR7 shRNA (Zheng et al. 2010 "Chemokine receptor CXCR7 regulates the invasion, angiogenesis and tumour growth of human hepatocellular carcinoma cells" *J Exp.Clin.Cancer Res.* 29:31).

Small molecules are known for side effects and unwanted effects. The small molecule CCX771 blocks CXCL12 binding (cf. Carbajal et al; 2010 "Migration of engrafted neural stem cells is mediated by CXCL12 signaling through CXCR4 in a viral model of multiple sclerosis Proc Natl Acad Sci USA. 107:11068–11073), on the other hand it is described as a synthetic CXCR7 ligand CCX771, which also potently stimulates β-arrestin2 recruitment to CXCR7, with greater potency and efficacy than the endogenous chemokine ligands (Zabel et al. 2009 "Elucidation of CXCR7-Mediated Signaling Events and Inhibition of CXCR4-Mediated Tumor Cell Transendothelial Migration by CXCR7 Ligands" *J.Immun.* 183: 0000–0000). Similarly, the small compound VUF11403 (VU Amsterdam) behaves as an agonist in the β-arrestin assay.

Currently, there is no anti-CXCR7 drug on the market or in the clinic.

There is a need therefore for potent anti-CXCR7 agents that can explore and establish the medical potential of this target. Furthermore, there is a need for diagnostically, preventatively, and/or therapeutically suitable anti-CXCR7 agents.

It is an object of the present invention to overcome or ameliorate at least one of the disadvantages of the prior art, or to provide a useful alternative.

According to a first aspect, the present invention provides a construct comprising at least one immunoglobulin single variable domain (ISVD) that binds to and/or recognizes amino acid residue M33, and optionally amino acid residue V32 and/or amino acid residue M37 in CXCR7 (SEQ ID NO: 1) and at least one ISVD that binds to and/or recognizes amino acid residue W19, and optionally S23 and/or D25 of CXCR7 (SEQ ID NO: 1).

According to a second aspect, the present invention provides an immunoglobulin single variable domain that binds to and/or recognizes amino acid residue M33, and optionally amino acid residue V32 and/or amino acid residue M37 in CXCR7 (SEQ ID NO: 1).

According to a third aspect, the present invention provides a polypeptide comprising an immunoglobulin single variable domain of the invention.

2012234284 01 Sep 2015

According to a fourth aspect, the present invention provides a nucleic acid sequence encoding

i) for an immunoglobulin single variable domain of the invention;

ii) for a polypeptide of the invention, or

iii) for a construct of the invention.

According to a fifth aspect, the present invention provides a pharmaceutical composition comprising

i) an immunoglobulin single variable domain of the invention;

ii) a polypeptide of the invention; or

iii) a construct of the invention;

and optionally a pharmaceutically acceptable excipient.

According to a sixth aspect, the present invention provides the immunoglobulin single variable domain of the invention, a polypeptide of the invention, or a construct of the invention when used in cancer, preferably head or neck cancer, GBM and/or inflammatory diseases

According to a seventh aspect, the present invention provides the immunoglobulin single variable domain of the invention, a polypeptide of the invention, or a construct of the invention when used in rheumatoid arthritis.

According to an eighth aspect, the present invention provides the immunoglobulin single variable domain of the invention, a polypeptide of the invention, or a construct of the invention when used in multiple sclerosis.

According to a ninth aspect, the present invention provides a method for producing an immunoglobulin single variable domain of the invention, a polypeptide of the invention, or a construct of the invention, said method at least comprising the step of:

a) expressing, in a suitable host cell or host organism or in another suitable expression system, a nucleic acid or nucleotide sequence according to the invention;

optionally followed by:

b) isolating and/or purifying the immunoglobulin single variable domain of the invention, a polypeptide of the invention, or a construct of the invention.

According to a tenth aspect, the present invention provides a method for: reducing tumour growth; treating cancer; treating GBM; treating inflammatory disease; treating rheumatoid arthritis; or treating multiple sclerosis, said method comprising administering a construct of the invention, a immunoglobulin single variable domain of the invention, a polypeptide of the invention or a pharmaceutical composition according to the invention.

According to an eleventh aspect, the present invention provides use of a construct of the invention, an immunoglobulin single variable domain of the invention or a polypeptide of the invention in the manufacture of a medicament for: reducing tumour growth; treating cancer; treating GBM; treating inflammatory disease; treating rheumatoid arthritis; or treating multiple sclerosis.

According to a twelfth aspect, the present invention provides an immunoglobulin single variable domain; a polypeptide; or a construct, when produced by the method of the invention.

CXCR7 is expressed on many human tumour cells but not on most healthy cells. In our tumour model systems we found that reduction or inhibition of CXCR7 by immunoglobulin single variable domains reduces or abolishes tumour formation in vivo.

Immunoglobulin sequences, such as antibodies and antigen binding fragments derived there from (*e.g.,* immunoglobulin single variable domains) are used to specifically target their respective antigens in research and therapeutic applications. The generation of immunoglobulin single variable domains such as *e.g.,* VHHs may involve the immunization of an experimental animal such as a Llama, construction of phage libraries from immune tissue, selection of phage displaying antigen binding immunoglobulin single variable domains and screening of said domains and engineered constructs thereof for the desired specificities (WO 94/04678). Alternatively, immunoglobulin single variable domains such as *e.g.,* dAbs can be generated by selecting phage displaying antigen binding immunoglobulin single variable domains directly from naïve or synthetic libraries and subsequent screening of said domains and engineered constructs thereof for the desired specificities (Ward et al, Binding activities of a repertoire of single immunoglobulin variable domains secreted from Escherichia coli, Nature, 1989, Oct 12; 341 (6242): 544-6); Holt et al., Trends Biotechnol., 2003, 21(11):484-490; as well as for example WO 06/030220, WO 06/003388 and other published patent applications of Domantis Ltd.).

Targeting serum albumin to extend the half-life of biological molecules such as *e.g.,* immunoglobulin single variable domains has been described e.g. in WO2008/028977.

In other aspects, the present invention relates to polypeptides that comprise or essentially consist of i) a first building block consisting essentially of one or more immunoglobulin single variable domain(s), wherein said immunoglobulin single variable domain(s) is (are) directed against CXCR7 and in particular against human CXCR7; and ii) a second building block consisting essentially of one or more (preferably one) immunoglobulin single variable domain(s), wherein said immunoglobulin single variable domain(s) is (are) directed against serum albumin and in particular against human serum albumin (and even more preferably wherein said immunoglobulin single variable domain is Alb8 (as herein defined)). Furthermore, the invention also relates to nucleic acids encoding such

polypeptides; to methods for preparing such polypeptides; to host cells expressing or capable of expressing such polypeptides; to compositions, and in particular to pharmaceutical compositions that comprise such polypeptides, nucleic acids and/or host cells; and to uses of such polypeptides, nucleic acids, host cells and/or compositions for prophylactic, therapeutic or diagnostic purposes. Other aspects, embodiments, advantages and applications of the invention will become clear from the further description herein.

Unless the context clearly requires otherwise, throughout the description and the claims, the words "comprise", "comprising", and the like are to be construed in an inclusive sense as opposed to an exclusive or exhaustive sense; that is to say, in the sense of "including, but not limited to".

Brief Description of the Drawings

Figure 1 shows an SDF-1 competition experiment using FACS.

Figure 2 shows an Mab 11G8 competition experiment using FACS.

Figure 3 shows an immunohistochemical analysis of CXCR7 expression in primary tumor sections.

Figure 4 shows profiling of CXCR7 mRNA in head and neck cancer cell lines 11B, 22A, 22B, FaDu, OE and 93-VU-147 by qPCR.

Figure 5 shows a [^{125}I]-CXCL12 competition experiment on head and neck cancer cell lines 11B, 22A, 22B, FaDu, OE and 93-VU-147 with ligand CXCL11, CXCL12, Nanobody 09A04 and negative controls: no competitor (designated by "-", indicating total binding (TB)); and CXCL10 (indicating a-specific competition).

Figure 6 shows in vivo CXCR7 Nanobody therapy with 22A transplants in nude mice: "-" negative control (PBS); polypeptide constructs clone 060, clone 083, clone 085 and clone 093.

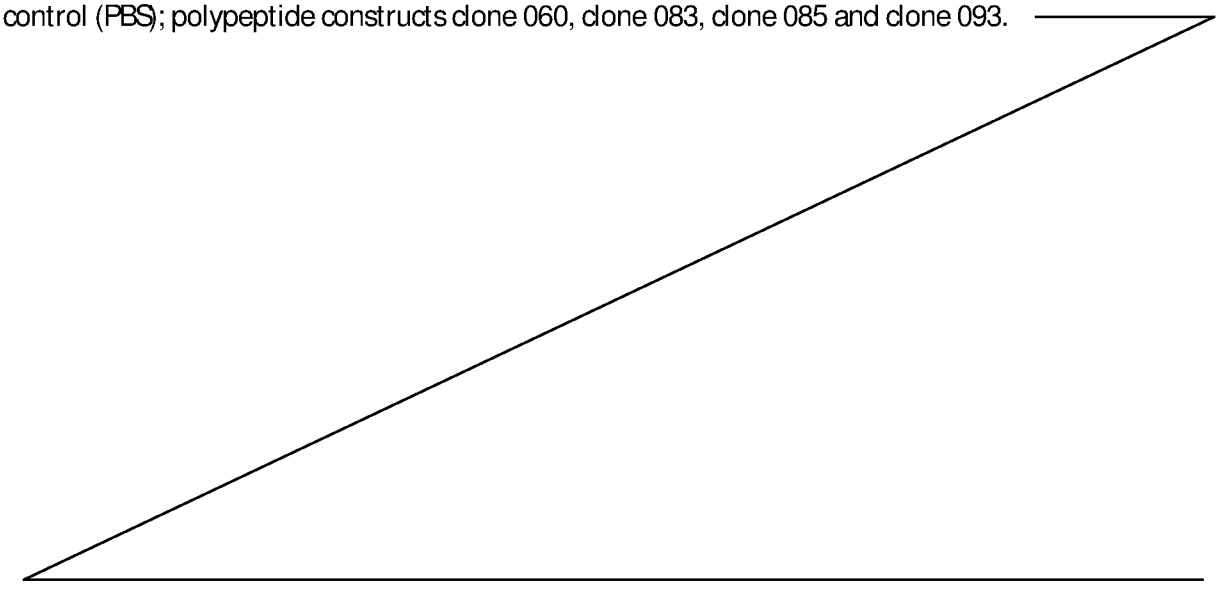


Figure 7 shows tumour volumes after 50 days of treatment with *in vivo* CXCR7 Nanobody therapy with 22A transplants in nude mice: "-" negative control (PBS); polypeptide constructs clone 085 and clone 093.

Figure 8 shows inhibition of SDF-1 binding to HEK293T hCXCR7 in the presence of 2mg/ml HSA.

Description of the invention

Definitions:

a) Unless indicated or defined otherwise, all terms used have their usual meaning in the art, which will be clear to the skilled person. Reference is for example made to the standard handbooks mentioned in paragraph a) on page 46 of WO 08/020079.

b) Unless indicated otherwise, the term "immunoglobulin single variable domain" (ISVD) is used as a general term to include but not limited to antigen-binding domains or fragments such as V_{HH} domains or V_H or V_L domains, respectively. The terms antigen-binding molecules or antigen-binding protein are used interchangeably and include also the term Nanobodies. The immunoglobulin single variable domains further are light chain variable domain sequences (*e.g.*, a V_L-sequence), or heavy chain variable domain sequences (*e.g.*, a V_H-sequence); more specifically, they can be heavy chain variable domain sequences that are derived from a conventional four-chain antibody or heavy chain variable domain sequences that are derived from a heavy chain antibody. Accordingly, the immunoglobulin single variable domains can be domain antibodies, or immunoglobulin sequences that are suitable for use as domain antibodies, single domain antibodies, or immunoglobulin sequences that are suitable for use as single domain antibodies, "dAbs", or immunoglobulin sequences that are suitable for use as dAbs, or Nanobodies, including but not limited to V_{HH} sequences. The invention includes immunoglobulin sequences of different origin, comprising mouse, rat, rabbit, donkey, human and camelid immunoglobulin sequences. The immunoglobulin single variable domain includes fully human, humanized, otherwise sequence optimized or chimeric immunoglobulin sequences. The immunoglobulin single variable domain and structure of an immunoglobulin single variable domain can be considered - without however being limited thereto - to be comprised of four framework regions or "FR's", which are referred to in the art and herein as "Framework region 1" or "FR1"; as "Framework region 2" or "FR2"; as "Framework region 3" or "FR3"; and as "Framework region 4" or "FR4", respectively; which framework regions are interrupted by three complementary determining regions or "CDR's", which are referred to in the art as "Complementarity Determining Region 1"or "CDR1"; as "Complementarity Determining Region

2" or "CDR2"; and as "Complementarity Determining Region 3" or "CDR3", respectively. It is noted that the terms Nanobody or Nanobodies are registered trademarks of Ablynx N.V. and thus may also be referred to as Nanobody® and/or Nanobodies®).

c) Unless indicated otherwise, the terms "immunoglobulin sequence", "sequence", "nucleotide sequence" and "nucleic acid" are as described in paragraph b) on page 46 of WO 08/020079. The term Nanobody is also as defined in WO 08/020079, and as described therein generally refers to an immunoglobulin heavy chain variable domain that has the functional and/or structural characteristics of a V_{HH} domain (*e.g.*, a V_H domain from the "heavy-chain only" antibodies that occur in Camelids), and as such may in particular be a (native) V_{HH}, a humanized V_{HH} or a camelized V_H, such as a camelized human V_H.

d) Unless indicated otherwise, all methods, steps, techniques and manipulations that are not specifically described in detail can be performed and have been performed in a manner known *per se*, as will be clear to the skilled person. Reference is for example again made to the standard handbooks and the general background art mentioned herein and to the further references cited therein; as well as to for example the following reviews Presta, Adv. Drug Deliv. Rev. 2006, 58 (5-6): 640-56; Levin and Weiss, Mol. Biosyst. 2006, 2(1): 49-57; Irving et al., J. Immunol. Methods, 2001, 248(1-2), 31-45; Schmitz et al., Placenta, 2000, 21 Suppl. A, S106-12, Gonzales et al., Tumour Biol., 2005, 26(1), 31-43, which describe techniques for protein engineering, such as affinity maturation and other techniques for improving the specificity and other desired properties of proteins such as immunoglobulins.

e) Amino acid residues will be indicated according to the standard three-letter or one-letter amino acid code. Reference is made to Table A-2 on page 48 of the International application WO 08/020079 of Ablynx N.V. entitled "*Immunoglobulin single variable domains directed against IL-6R and polypeptides comprising the same for the treatment of diseases and disorders associated with Il-6 mediated signalling*".

f) For the purposes of comparing two or more nucleotide sequences, the percentage of "*sequence identity*" between a first nucleotide sequence and a second nucleotide sequence may be calculated or determined as described in paragraph e) on page 49 of WO 08/020079 (incorporated herein by reference), such as by dividing [*the number of nucleotides in the first nucleotide sequence that are identical to the nucleotides at the corresponding positions in the second nucleotide sequence*] by [*the total number of nucleotides in the first nucleotide sequence*] and multiplying by [*100%*], in which each deletion, insertion, substitution or addition of a nucleotide in the second nucleotide sequence - compared to the first nucleotide sequence - is

considered as a difference at a single nucleotide (position); or using a suitable computer algorithm or technique, again as described in paragraph e) on pages 49 of WO 08/020079 (incorporated herein by reference).

g) For the purposes of comparing two or more immunoglobulin single variable domains or other amino acid sequences such *e.g.*, the polypeptides of the invention etc., the percentage of *"sequence identity"* between a first amino acid sequence and a second amino acid sequence (also referred to herein as *"amino acid identity"*) may be calculated or determined as described in paragraph f) on pages 49 and 50 of WO 08/020079 (incorporated herein by reference), such as by dividing [*the number of amino acid residues in the first amino acid sequence that are identical to the amino acid residues at the corresponding positions in the second amino acid sequence*] by [*the total number of amino acid residues in the first amino acid sequence*] and multiplying by [*100%*], in which each deletion, insertion, substitution or addition of an amino acid residue in the second amino acid sequence - compared to the first amino acid sequence - is considered as a difference at a single amino acid residue (position), i.e. as an "amino acid difference" as defined herein; or using a suitable computer algorithm or technique, again as described in paragraph f) on pages 49 and 50 of WO 08/020079 (incorporated herein by reference).

Also, in determining the degree of sequence identity between two immunoglobulin single variable domains, the skilled person may take into account so-called "conservative" amino acid substitutions, as described on page 50 of WO 08/020079.

Any amino acid substitutions applied to the polypeptides described herein may also be based on the analysis of the frequencies of amino acid variations between homologous proteins of different species developed by Schulz et al., Principles of Protein Structure, Springer-Verlag, 1978, on the analyses of structure forming potentials developed by Chou and Fasman, Biochemistry 13: 211, 1974 and Adv. Enzymol., 47: 45-149, 1978, and on the analysis of hydrophobicity patterns in proteins developed by Eisenberg et al., Proc. Natl. Acad Sci. USA 81: 140-144, 1984; Kyte & Doolittle; J Molec. Biol. 157: 105-132, 198 1, and Goldman et al., Ann. Rev. Biophys. Chem. 15: 321-353, 1986, all incorporated herein in their entirety by reference. Information on the primary, secondary and tertiary structure of Nanobodies is given in the description herein and in the general background art cited above. Also, for this purpose, the crystal structure of a V_{HH} domain from a llama is for example given by Desmyter et al., Nature Structural Biology, Vol. 3, 9, 803 (1996); Spinelli et al., Natural Structural Biology (1996); 3, 752-757; and Decanniere et al., Structure, Vol. 7, 4, 361 (1999). Further information about some of

the amino acid residues that in conventional V_H domains form the V_H/V_L interface and potential camelizing substitutions on these positions can be found in the prior art cited above.

h) Immunoglobulin single variable domains and nucleic acid sequences are said to be *"exactly the same"* if they have 100% sequence identity (as defined herein) over their entire length.

i) When comparing two immunoglobulin single variable domains, the term *"amino acid difference"* refers to an insertion, deletion or substitution of a single amino acid residue on a position of the first sequence, compared to the second sequence; it being understood that two immunoglobulin single variable domains can contain one, two or more such amino acid differences.

j) When a nucleotide sequence or amino acid sequence is said to "comprise" another nucleotide sequence or amino acid sequence, respectively, or to "essentially consist of" another nucleotide sequence or amino acid sequence, this has the meaning given in paragraph i) on pages 51-52 of WO 08/020079.

k) The term "in essentially isolated form" has the meaning given to it in paragraph j) on pages 52 and 53 of WO 08/020079.

l) The terms "domain" and "binding domain" have the meanings given to it in paragraph k) on page 53 of WO 08/020079.

m) The terms "antigenic determinant" and "epitope", which may also be used interchangeably herein, have the meanings given to it in paragraph l) on page 53 of WO 08/020079.

n) As further described in paragraph m) on page 53 of WO 08/020079, an amino acid sequence (such as an antibody, a polypeptide of the invention, or generally an antigen binding protein or polypeptide or a fragment thereof) that can (specifically) bind to, that has affinity for and/or that has specificity for a specific antigenic determinant, epitope, antigen or protein (or for at least one part, fragment or epitope thereof) is said to be *"against"* or *"directed against"* said antigenic determinant, epitope, antigen or protein.

o) The term *"specificity"* has the meaning given to it in paragraph n) on pages 53-56 of WO 08/020079; and as mentioned therein refers to the number of different types of antigens or antigenic determinants to which a particular antigen-binding molecule or antigen-binding protein (such as a polypeptide of the invention) molecule can bind. The specificity of an antigen-binding protein can be determined based on affinity and/or avidity, as described on pages 53-56 of WO 08/020079 (incorporated herein by reference), which also describes some preferred techniques for measuring binding between an antigen-binding molecule (such as a polypeptide

of the invention) and the pertinent antigen. Typically, antigen-binding proteins (such as the immunoglobulin single variable domains, and/or polypeptides of the invention) will bind to their antigen with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/liter or less, and preferably 10^{-7} to 10^{-12} moles/liter or less and more preferably 10^{-8} to 10^{-12} moles/liter (*i.e.*, with an association constant (K_A) of 10^5 to 10^{12} liter/ moles or more, and preferably 10^7 to 10^{12} liter/moles or more and more preferably 10^8 to 10^{12} liter/moles). Any K_D value greater than 10^{-4} mol/liter (or any K_A value lower than 10^4 M^{-1}) liters/mol is generally considered to indicate non-specific binding. Preferably, a monovalent immunoglobulin single variable domain of the invention will bind to the desired antigen with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM. Specific binding of an antigen-binding protein to an antigen or antigenic determinant can be determined in any suitable manner known per se, including, for example, Scatchard analysis and/or competitive binding assays, such as radioimmunoassays (RIA), enzyme immunoassays (EIA) and sandwich competition assays, and the different variants thereof known per se in the art; as well as the other techniques mentioned herein. As will be clear to the skilled person, and as described on pages 53-56 of WO 08/020079, the dissociation constant may be the actual or apparent dissociation constant. Methods for determining the dissociation constant will be clear to the skilled person, and for example include the techniques mentioned on pages 53-56 of WO 08/020079.

p) The half-life of an amino acid sequence, compound or polypeptide of the invention can generally be defined as described in paragraph o) on page 57 of WO 08/020079 and as mentioned therein refers to the time taken for the serum concentration of the amino acid sequence, compound or polypeptide to be reduced by 50%, *in vivo*, for example due to degradation of the sequence or compound and/or clearance or sequestration of the sequence or compound by natural mechanisms. The *in vivo* half-life of an amino acid sequence, compound or polypeptide of the invention can be determined in any manner known *per se*, such as by pharmacokinetic analysis. Suitable techniques will be clear to the person skilled in the art, and may for example generally be as described in paragraph o) on page 57 of WO 08/020079. As also mentioned in paragraph o) on page 57 of WO 08/020079, the half-life can be expressed using parameters such as the t1/2-alpha, t1/2-beta and the area under the curve (AUC). Reference is for example made to the Experimental Part below, as well as to the standard handbooks, such as Kenneth, A et al: Chemical Stability of Pharmaceuticals: A Handbook for Pharmacists and Peters et al, Pharmacokinetic analysis: A Practical Approach (1996). Reference is also made to "Pharmacokinetics", M Gibaldi & D Perron, published by Marcel Dekker, 2nd Rev. edition (1982). The terms "increase in half-life" or "increased half-life" as also as defined in paragraph o) on

page 57 of WO 08/020079 and in particular refer to an increase in the t1/2-beta, either with or without an increase in the t1/2-alpha and/or the AUC or both.

q) In respect of a target or antigen, the term "interaction site" on the target or antigen means a site, epitope, antigenic determinant, part, domain or stretch of amino acid residues on the target or antigen that is a site for binding to a ligand, receptor or other binding partner, a catalytic site, a cleavage site, a site for allosteric interaction, a site involved in multimerization (such as homomerization or heterodimerization) of the target or antigen; or any other site, epitope, antigenic determinant, part, domain or stretch of amino acid residues on the target or antigen that is involved in a biological action or mechanism of the target or antigen. More generally, an "interaction site" can be any site, epitope, antigenic determinant, part, domain or stretch of amino acid residues on the target or antigen to which an amino acid sequence or polypeptide of the invention can bind such that the target or antigen (and/or any pathway, interaction, signalling, biological mechanism or biological effect in which the target or antigen is involved) is modulated (as defined herein).

r) An immunoglobulin single variable domain or polypeptide is said to be "*specific for*" a first target or antigen compared to a second target or antigen when is binds to the first antigen with an affinity/avidity (as described above, and suitably expressed as a K_D value, K_A value, K_{off} rate and/or K_{on} rate) that is at least 10 times, such as at least 100 times, and preferably at least 1000 times, and up to 10.000 times or more better than the affinity with which said amino acid sequence or polypeptide binds to the second target or polypeptide. For example, the first antigen may bind to the target or antigen with a K_D value that is at least 10 times less, such as at least 100 times less, and preferably at least 1000 times less, such as 10.000 times less or even less than that, than the K_D with which said amino acid sequence or polypeptide binds to the second target or polypeptide. Preferably, when an immunoglobulin single variable domain or polypeptide is "specific for" a first target or antigen compared to a second target or antigen, it is directed against (as defined herein) said first target or antigen, but not directed against said second target or antigen.

s) The terms "*cross-block*", "*cross-blocked*" and "*cross-blocking*" are used interchangeably herein to mean the ability of an immunoglobulin single variable domain or polypeptide to interfere with the binding directly or indirectly through allosteric modulation of other immunoglobulin single variable domains or polypeptides of the invention to a given target. The extent to which an immunoglobulin single variable domain or polypeptide of the invention is able to interfere with the binding of another to target, and therefore whether it can be said to cross-block according to the invention, can be determined using competition binding assays. One

particularly suitable quantitative cross-blocking assay uses a FACS- or an ELISA-based approach to measure competition between the labelled (*e.g.*, His tagged or radioactive labelled) immunoglobulin single variable domain or polypeptide according to the invention and the other binding agent in terms of their binding to the target. The experimental part generally describes suitable FACS-, ELISA- or radioligand-displacement-based assays for determining whether a binding molecule cross-blocks or is capable of cross-blocking an immunoglobulin single variable domain or polypeptide according to the invention. It will be appreciated that the assay can be used with any of the immunoglobulin single variable domains or other binding agents described herein. Thus, in general, a cross-blocking amino acid sequence or other binding agent according to the invention is for example one which will bind to the target in the above cross-blocking assay such that, during the assay and in the presence of a second amino acid sequence or other binding agent of the invention, the recorded displacement of the immunoglobulin single variable domain or polypeptide according to the invention is between 60% and 100% (*e.g.*, in ELISA/radioligand based competition assay) or between 80% to 100% (*e.g.*, in FACS based competition assay) of the maximum theoretical displacement (*e.g.*, displacement by cold (*e.g.*, unlabeled) immunoglobulin single variable domain or polypeptide that needs to be cross-blocked) by the to be tested potentially cross-blocking agent that is present in an amount of 0.01 mM or less (cross-blocking agent may be another conventional monoclonal antibody such as IgG, classic monovalent antibody fragments (Fab, scFv)) and engineered variants (diabodies, triabodies, minibodies, VHHs, dAbs, VHs, VLs).

t) An amino acid sequence such as e.g. an immunoglobulin single variable domain or polypeptide according to the invention is said to be "*cross-reactive*" for two different antigens or antigenic determinants (such as serum albumin from two different species of mammal, such as human serum albumin and cyno serum albumin) if it is specific for (as defined herein) both these different antigens or antigenic determinants.

u) As further described in paragraph q) on pages 58 and 59 of WO 08/020079 (incorporated herein by reference), the amino acid residues of an immunoglobulin single variable domain are numbered according to the general numbering for V_H domains given by Kabat et al. ("Sequence of proteins of immunological interest", US Public Health Services, NIH Bethesda, MD, Publication No. 91), as applied to V_{HH} domains from Camelids in the article of Riechmann and Muyldermans, J. Immunol. Methods 2000 Jun 23; 240 (1-2): 185-195 (see for example Figure 2 of this publication), and accordingly FR1 of an immunoglobulin single variable domain comprises the amino acid residues at positions 1-30, CDR1 of an immunoglobulin single variable domain comprises the amino acid residues at positions 31-35, FR2 of an immunoglobulin single variable

domain comprises the amino acids at positions 36-49, CDR2 of an immunoglobulin single variable domain comprises the amino acid residues at positions 50-65, FR3 of an immunoglobulin single variable domain comprises the amino acid residues at positions 66-94, CDR3 of an immunoglobulin single variable domain comprises the amino acid residues at positions 95-102, and FR4 of an immunoglobulin single variable domain comprises the amino acid residues at positions 103-113.

v) The Figures, Sequence Listing and the Experimental Part/Examples are only given to further illustrate the invention and should not be interpreted or construed as limiting the scope of the invention and/or of the appended claims in any way, unless explicitly indicated otherwise herein.

1. Polypeptides of the invention and uses thereof

1.1. Anti-CXCR7 building blocks

The polypeptides of the present invention can generally be used to modulate, and in particular inhibit and/or prevent, binding of CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) to CXCL12 (and/or CXCL11) and in particular human CXCL12 (NM_000609) and/or in particular human CXCL11 (U66096), and thus to modulate, and in particular inhibit or prevent, the signalling that is mediated by CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) and/or CXCL12 (and/or CXCL11) and in particular human CXCL12 (NM_000609) and/or in particular human CXCL11 (U66096), to modulate the biological pathways in which CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) and/or CXCL12 (and/or CXCL11) and in particular human CXCL12 (NM_000609) and/or in particular human CXCL11 (U66096) are involved, and/or to modulate the biological mechanisms, responses and effects associated with such signalling or these pathways.

As such, the polypeptides and compositions of the present invention can be used for the diagnosis, prevention and treatment of diseases and disorders of the present invention (herein also "diseases and disorders of the present invention") and include, but are not limited to cancer, *e.g.*, carcinomas, gliomas, mesotheliomas, melanomas, lymphomas, leukemias, adenocarcinomas, breast cancer, ovarian cancer, cervical cancer, glioblastoma, leukemia, lymphoma, prostate cancer, and Burkitt's lymphoma, head and neck cancer, colon cancer, colorectal cancer, non-small cell lung cancer, small cell lung cancer, cancer of the esophagus, stomach cancer, pancreatic cancer, hepatobiliary cancer, cancer of the gallbladder, cancer of the small intestine, rectal cancer, kidney cancer, bladder cancer, prostate cancer, penile cancer, urethral cancer, testicular cancer, cervical cancer, vaginal cancer, uterine cancer, ovarian cancer, thyroid cancer, parathyroid cancer, adrenal cancer, pancreatic

endocrine cancer, carcinoid cancer, bone cancer, skin cancer, retinoblastomas, Hodgkin's lymphoma, non-Hodgkin's lymphoma, Kaposi's sarcoma, multicentric Castleman's disease or AIDS-associated primary effusion lymphoma, neuroectodermal tumors, rhabdomyosarcoma (see, Cancer, Principles and practice (DeVita, V.T. et al. eds 1997) for additional cancers); preferably head and neck cancer, as well as brain and neuronal dysfunction, such as Alzheimer's disease and multiple sclerosis; kidney dysfunction, renal allograft rejection; nasal polyposis; rheumatoid arthritis; cardiac allograft rejection; cardiac dysfunction; atherosclerosis; asthma; glomerulonephritis; contact dermatitis; inflammatory bowel disease; colitis; psoriasis; reperfusion injury; as well as other disorders and diseases described herein. In particular, the polypeptides and compositions of the present invention can be used for the diagnosis, prevention and treatment of diseases involving CXCR7 mediated metastasis, chemotaxis, cell adhesion, trans endothelial migration, cell proliferation and/or survival.

Generally, said "diseases and disorders of the present invention" can be defined as diseases and disorders that can be diagnosed, prevented and/or treated, respectively, by suitably administering to a subject in need thereof (*i.e.*, having the disease or disorder or at least one symptom thereof and/or at risk of attracting or developing the disease or disorder) of either a polypeptide or composition of the invention (and in particular, of a pharmaceutically active amount thereof) and/or of a known active principle active against CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) or a biological pathway or mechanism in which CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) is involved (and in particular, of a pharmaceutically active amount thereof).

In particular, the polypeptides of the present invention can be used for the diagnosis, prevention and treatment of diseases and disorders of the present invention which are characterized by excessive and/or unwanted CXCL12 and in particular human CXCL12 signalling mediated by CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) or by the pathway(s) in which CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) is involved (*e.g.*, CXCL11/I-TAC – CXCR7 axis). Examples of such diseases and disorders of the present invention will again be clear to the skilled person based on the disclosure herein.

Thus, without being limited thereto, the immunoglobulin single variable domains and polypeptides of the invention can for example be used to diagnose, prevent and/or to treat all diseases and disorders that are currently being diagnosed, prevented or treated with active principles that can modulate CXCR7 and in particular human CXCR7 (SEQ ID NO: 1)-mediated signalling, such as those mentioned in the prior art cited herein. It is also envisaged that the polypeptides of the invention can be used to diagnose, prevent and/or to treat all diseases and disorders for which treatment with such active principles is currently being developed, has been proposed, or will be proposed or developed in future. In addition, it is envisaged that, because of their favourable properties as further described

herein, the polypeptides of the present invention may be used for the diagnosis, prevention and treatment of other diseases and disorders than those for which these known active principles are being used or will be proposed or developed; and/or that the polypeptides of the present invention may provide new methods and regimens for treating the diseases and disorders described herein.

Other applications and uses of the immunoglobulin single variable domains and polypeptides of the invention will become clear to the skilled person from the further disclosure herein.

Generally, it is an object of the invention to provide pharmacologically active agents, as well as compositions comprising the same, that can be used in the diagnosis, prevention and/or treatment of diseases and/or disorders of the invention; and to provide methods for the diagnosis, prevention and/or treatment of such diseases and disorders that involve the administration and/or use of such agents and compositions.

In particular, it is an object of the invention to provide such pharmacologically active agents, compositions and/or methods that have certain advantages compared to the agents, compositions and/or methods that are currently used and/or known in the art. These advantages will become clear from the further description below.

More in particular, it is an object of the invention to provide therapeutic proteins that can be used as pharmacologically active agents, as well as compositions comprising the same, for the diagnosis, prevention and/or treatment of diseases and/or disorders of the invention and of the further diseases and disorders mentioned herein; and to provide methods for the diagnosis, prevention and/or treatment of such diseases and disorders that involve the administration and/or the use of such therapeutic proteins and compositions.

Accordingly, it is a specific object of the present invention to provide immunoglobulin single variable domains that are directed against CXCR7, in particular against CXCR7 from a warm-blooded animal, more in particular against CXCR7 from a mammal such as *e.g.,* mouse, and especially against human CXCR7 (SEQ ID NO: 1); and to provide proteins and polypeptides comprising or essentially consisting of at least one such immunoglobulin single variable domain.

In particular, it is a specific object of the present invention to provide such immunoglobulin single variable domains and such proteins and/or polypeptides that are suitable for prophylactic, therapeutic and/or diagnostic use in a warm-blooded animal, and in particular in a mammal, and more in particular in a human being.

More in particular, it is a specific object of the present invention to provide such immunoglobulin single variable domains and such proteins and/or polypeptides that can be used for the prevention, treatment, alleviation and/or diagnosis of one or more diseases, disorders or conditions associated

with CXCR7 and/or mediated by CXCR7 (such as the diseases, disorders and conditions mentioned herein) in a warm-blooded animal, in particular in a mammal, and more in particular in a human being.

It is also a specific object of the invention to provide such immunoglobulin single variable domains and such proteins and/or polypeptides that can be used in the preparation of pharmaceutical or veterinary compositions for the prevention and/or treatment of one or more diseases, disorders or conditions associated with and/or mediated by CXCR7 (such as the diseases, disorders and conditions mentioned herein) in a warm-blooded animal, in particular in a mammal, and more in particular in a human being.

In the invention, generally, these objects are achieved by the use of the immunoglobulin single variable domains, proteins, polypeptides and compositions that are described herein.

In general, the invention provides immunoglobulin single variable domains that are directed against (as defined herein) and/or can specifically bind (as defined herein) to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1); as well as compounds and constructs, and in particular proteins and polypeptides, that comprise at least one such amino acid sequence.

More in particular, the invention provides immunoglobulin single variable domains and polypeptides that can bind to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) with an affinity (suitably measured and/or expressed as a K_D-value (actual or apparent), a K_A-value (actual or apparent), a k_{on}-rate and/or a k_{off}-rate, or alternatively as an IC_{50} value, as further described herein) that is as defined herein; as well as compounds and constructs, and in particular proteins and polypeptides, that comprise at least one such amino acid sequence.

In a particular aspect, the immunoglobulin single variable domains and/or polypeptides of the invention are such that they:

- bind to human CXCR7 (SEQ ID NO: 1) with an EC50 of 100nM or lower, more preferably of 50nM or lower, even more preferably of 20nM or lower, most preferably of 10nM or lower in a binding FACS assay as e.g. described in the experimental part (see Example 8), and wherein the polypeptides comprise only one human CXCR7 binding immunoglobulin single variable domain unit;

and/or such that they:

- fully displace human CXCL12 (SDF-1) from human CXCR7 (SEQ ID NO: 1) at an average Ki value of 100 nM or less, more preferably at an average Ki value of 20nM or less, even more preferably at an average Ki value of 10nM or less in an assay as *e.g.*, described in the experimental part

(Examples 9 and 10), and wherein the polypeptides comprise only one human CXCR7 binding immunoglobulin single variable domain unit, and wherein full displacement means an average CXCL12 displacement of about 60% to 80% and more (*e.g.*, when measured according to the ligand displacement assay of Example 9) or wherein full displacement means an average CXCL12 displacement of about 80% to 100% and more (when measured according to the FACS based competition assay of Example 10) ;

and/or such that they:

- fully displace human CXCL11 (I-TAC) from human CXCR7 (SEQ ID NO: 1) at an average Ki value of 1000nM or less, more preferably at an average Ki value 500 nM or less, even more preferably at an average Ki value 100 nM or less, even more preferably at an average Ki value of 20nM or less, even more preferably at an average Ki value of 10nM or less in an assay as e.g. described in the experimental part (Examples 9 and 10), and wherein the polypeptides comprise only one human CXCR7 binding immunoglobulin single variable domain unit, and wherein full displacement means an average CXCL11 displacement of about 60% to 80% and more (*e.g.*, when measured according to the ligand displacement assay of Example 9) or wherein full displacement means an average CXCL12 displacement of about 80% to 100% and more (when measured according to the FACS based competition assay of Example 10)

and/or such that they:

- partially displace human CXCL12 (SDF-1) from human CXCR7 (SEQ ID NO: 1) at an average Ki value of 100 nM or less, more preferably at an average Ki value of 20nM or less, even more preferably at an average Ki value of 10nM or less in an assay as e.g. described in the experimental part (Examples 9 and 10), and wherein the polypeptides comprise only one human CXCR7 binding immunoglobulin single variable domain unit, and wherein partial displacement means an average CXCL12 displacement of about 40% to 60% (e.g. when measured according to the ligand displacement assay of Example 9) or wherein partial displacement means an average CXCL12 displacement of about 50% to 80% (when measured according to the FACS based competition assay of Example 10) ;

and/or such that they:

- partially displace human CXCL11 (I-TAC) from human CXCR7 (SEQ ID NO: 1) at an average Ki value of 1000nM or less, more preferably at an average Ki value 500nM or less, even more preferably at an average Ki value 100nM or less, even more preferably at an average Ki value of 20nM or less, even more preferably at an average Ki value of 10nM or less in an assay as e.g. described in the experimental part (Examples 9 and 10), and wherein the polypeptides comprise

only one human CXCR7 binding immunoglobulin single variable domain unit, and wherein partial displacement means an average CXCL11 displacement of about 40% to 60% (e.g., when measured according to the ligand displacement assay of Example 9) or wherein partial displacement means an average CXCL12 displacement of about 50% to 80% (when measured according to the FACS based competition assay of Example 10),

and/or such that they:

- bind human CXCR7 (SEQ ID NO: 1) with an average Kd value of 100 nM or less, more preferably at an average Kd value of 50 nM or less, even more preferably at an average Kd value of 40 nM or less, such as less than 30, 25, 20, 15, 10, 9, 8, 7, 6, 5, 4, 3, 3 nM or even less, such as less than 1 nM, or most preferably even less than 0.1 nM.

It should be appreciated that binding of the immunoglobulin single variable domains and/or polypeptides of the invention to (human) CXCR7 may result in displacing (human) CXCL11 and/or CXCL12 from (human) CXCR7 as described herein. It should further be appreciated that binding of the immunoglobulin single variable domains and/or polypeptides of the invention to (human) CXCR7 may result in inhibiting binding of (human) CXCL11 and/or CXCL12 to its cognate receptor, such as, (human) CXCR7 as described herein.

As already mentioned, in some specific, but non-limiting aspects (described in more detail herein), the invention provides:

amino acid sequences that are directed against (as defined herein) CXCR7 and that are capable of inhibiting or blocking (fully or partially, as further described herein) ligand binding, and in particular of inhibiting or blocking (fully or partially, as further described herein) the binding of SDF-1 to CXCR7 (as further described herein). These amino acid sequences are also referred to herein as "*CXCR-7 binding amino acid sequences*" or "*CXCR7 binding blocks*". Preferably, these CXCR7-binding amino acid sequences are ISVD's (as described herein), in which case they are also referred to as "*CXCR7-binding ISVD's*". Preferably, any CXCR7-binding amino acid sequences, CXCR7-binding building blocks or CXCR7-binding ISVD's are such that they have blocking activity, i.e. block SDF-1 binding to CXCR7 partially or completely, which can be determined by any suitable assay known to the person skilled in the art, such as, for instance, by an Alphascreen assay or by a FACS competition assay (e.g. as described herein). Preferably, the blocking activity is determined by a FACS competition assay as described in Example 9. Preferably, the ISVD has a blocking activity or competition capacity in NIH3T3-hCXCR7 cells of blocking or competing SDF-1 binding to CXCR7 with an average Ki of less than 600 nMs, but preferably, 500 nMs, 400 nMs, 300 nMs, 200 nMs, 100 nMs or even less.

- For instance, the **01C10-like ISVD** has a blocking activity or competition capacity in this assay with an average Ki of less than 100 nMs, more preferably, less than 75 nMs, 50 nMs or even less, such as less than 40 nMs or 30 nMs, 25 nMs or 24 nMs or even more preferably of less than 22 nMs.
- For instance, the **14G03-like ISVD** has a blocking activity or competition capacity in this assay with an average Ki of less than 150 nMs, more preferably, less than 100 nMs, 90 nMs, 80 nMs or even less, such as less than 70 nMs or 60 nMs, 50 nMs or 40 nMs, 30 nMs, 20 nMs, 15 nMs or 10 nMs, 5 nMs or even more preferably of less than 4 nMs.

In one specific, but non-limiting aspect, (some of the) *"CXCR-7 binding amino acid sequences"* or *"CXCR7 binding blocks"* may (and preferably also are) be such that they are capable of inhibiting or blocking β-arrestin recruitment (see Example 15). Preferably, any CXCR7-binding amino acid sequences, CXCR7-binding building blocks or CXCR7-binding ISVD's are such that they have blocking activity, i.e. block or inhibit SDF-1 mediated CXCR7 signalling partially or completely, which can be determined by any suitable assay known to the person skilled in the art, such as, for instance, by any suitable β-arrestin recruitment assay, as described herein.

Preferably, the blocking activity or inhibiting capacity is determined by a **β-arrestin assay** as described in Example 15. Preferably, the ISVD has a blocking activity or an inhibition capacity of ligand (e.g.SDF-1) induced β-arrestin in the PathHunter eXpress β-arrestin assay (DiscoverX) with a % inhibition of β-arrestin recruitment of more than 25%, more than 30%, but preferably, 40%, 50%, 60%, 70%, 80% or even more.

- For instance, the **14G03-like ISVD** has a blocking activity or inhibition capacity in this assay with a % inhibition of more than 50%, more preferably, more than 60%, 70% or even more, such as more than 75% or 80%, 85%, or even more preferably of more than 90%.

Some preferred technical values for binding, displacing, migration or other in vivo and/or in vitro potency of the immunoglobulin single variable domains or polypeptides of the invention to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) will become clear from the further description and examples herein.

Also, in the present description and claims, the following terms are defined as follows:

A) **01C10-like sequences:** a *"01C10-like sequence"*, *"01C10-like ISVD"*, *"01C10-like building block"* or *"Group 1 ISVDs"* is defined as an ISVD (as described herein) that comprises:

a) a CDR1 which comprises or essentially consists of either (i) the amino acid sequence NYAMG (SEQ ID NO: 93) or (ii) an amino acid sequence that has only 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence NYAMG (SEQ ID NO: 93); and/or

b) a CDR2 which comprises or essentially consists of either (i) the amino acid sequence AITPRAFTTYYADSVKG (SEQ ID NO: 95) or (ii) an amino acid sequence that has at least 80%, such as at least 85%, for example at least 90% or more than 95% sequence identity with the amino acid sequence AITPRAFTTYYADSVKG (SEQ ID NO: 95); or (iii) an amino acid sequence that has only 7, 6, 5, 4, 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence AITPRAFTTYYADSVKG (SEQ ID NO: 95); and/or

c) a CDR3 which comprises or essentially consists of either (i) the amino acid sequence QLVGSGSNLGRQESYAY (SEQ ID NO: 97) or (ii) an amino acid sequence that has at least 80%, such as at least 85%, for example at least 90% or more than 95% sequence identity with the amino acid sequence QLVGSGSNLGRQESYAY (SEQ ID NO: 97); or (iii) an amino acid sequence that has only 7, 6, 5, 4, 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence QLVGSGSNLGRQESYAY (SEQ ID NO: 97);

in which the framework sequences present in such an ISVD are as further described herein, and in which CDR1, CDR2 and CDR3 are preferably such that the 01C10-like ISVD has blocking activity, e.g. block CXCL11 and/or CXCL12 binding to CXCR7 partially or completely as described above, and/or reducing and/or inhibiting tumorigenesis in a xenograph model, and/or binds and/or recognizes amino acid residue W19, and optionally amino acid residue S23 and/or amino acid residue D25 in CXCR7 (SEQ ID NO: 1), all as described herein.

As also mentioned herein, (some of the) 01C10-like sequences may (and preferably also are) be such that they are capable of inhibiting, blocking or displacing SDF-1 binding (see Examples 9 and 10), for example in the displacement assay used in Example 10. Preferably, in such a 01C10-like sequence, CDR1 and CDR2 are as defined under a) and b), respectively; or CDR1 and CDR3 are as defined under a) and c), respectively; or CDR2 and CDR3 are as defined under b) and c), respectively. More preferably, in such a 01C10-like sequence, CDR1, CDR2 and CDR3 are all as defined under a), b) and c), respectively. Again, in such an 01C10-like sequence, CDR1, CDR2 and CDR3 are preferably such that the 01C10-like ISVD has blocking activity, e.g. block SDF-1 binding to CXCR7 partially or completely as described herein, and/or reducing and/or inhibiting tumorigenesis in a xenograph model, and/or binds and/or recognizes amino acid residue W19, and optionally amino acid residue S23 and/or amino acid residue D25 in CXCR7 (SEQ ID NO: 1), all as described herein.

For example, in such an 01C10-like sequence: CDR1 may comprise or essentially consist of the amino acid sequence NYAMG (SEQ ID NO: 93) (with CDR2 and CDR3 being as defined under b) and c), respectively); and/or CDR2 may comprise or essentially consist of the amino acid sequence AITPRAFTTYYADSVKG (SEQ ID NO: 95) (with CDR1 and CDR3 being as defined under a)

and c), respectively); and/or CDR3 may comprise or essentially consist of the amino acid sequence QLVGSGSNLGRQESYAY (SEQ ID NO: 97) (with CDR1 and CDR2 being as defined under a) and b), respectively). Particularly, when an 01C10-like sequence is according to this aspect: CDR1 may comprise or essentially consist of the amino acid sequence NYAMG (SEQ ID NO: 93) and CDR2 may comprise or essentially consist of the amino acid sequence AITPRAFTTYYADSVKG (SEQ ID NO: 95) (with CDR3 being as defined under c) above); and/or CDR1 may comprise or essentially consist of the amino acid sequence NYAMG (SEQ ID NO: 93) and CDR3 may comprise or essentially consist of the amino acid sequence QLVGSGSNLGRQESYAY (SEQ ID NO: 97) (with CDR2 being as defined under b) above); and/or CDR2 may comprise or essentially consist of the amino acid sequence AITPRAFTTYYADSVKG (SEQ ID NO: 95) and CDR3 may comprise or essentially consist of the amino acid sequence QLVGSGSNLGRQESYAY (SEQ ID NO: 97) (with CDR1 being as defined under a) above). Again, in such 01C10-like sequences, CDR1, CDR2 and CDR3 are preferably such that the 01C10-like ISVD has blocking activity, e.g. block SDF-1 binding to CXCR7 partially or completely as described herein and/or reducing and/or inhibiting tumorigenesis in a xenograph model, and/or binds and/or recognizes amino acid residue W19, and optionally amino acid residue S23 and/or amino acid residue D25 in CXCR7 (SEQ ID NO: 1), all as described herein. In a specifically preferred aspect, a *"01C10-like sequence"*, *"01C10-like ISVD"*, *"01C10-like building block"* or "*Group 1 ISVD*" is an ISVD that comprises:

d) a CDR1 which is either (i) the amino acid sequence NYAMG (SEQ ID NO: 93) or (ii) an amino acid sequence that has only 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence NYAMG (SEQ ID NO: 93); and/or

e) a CDR2 which is either (i) the amino acid sequence AITPRAFTTYYADSVKG (SEQ ID NO: 95) or (ii) an amino acid sequence that has at least 80%, such as at least 85%, for example at least 90% or more than 95% sequence identity with the amino acid sequence AITPRAFTTYYADSVKG (SEQ ID NO: 95); or (iii) an amino acid sequence that has only 7, 6, 5, 4, 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence AITPRAFTTYYADSVKG (SEQ ID NO: 95); and/or

f) a CDR3 which is either (i) the amino acid sequence QLVGSGSNLGRQESYAY (SEQ ID NO: 97) or (ii) an amino acid sequence that has at least 80%, such as at least 85%, for example at least 90% or more than 95% sequence identity with the amino acid sequence QLVGSGSNLGRQESYAY (SEQ ID NO: 97); or (iii) an amino acid sequence that has only 7, 6, 5, 4, 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence QLVGSGSNLGRQESYAY (SEQ ID NO: 97);

in which the framework sequences present in such an ISVD are as further described herein, and in which CDR1, CDR2 and CDR3 are preferably such that the 01C10-like ISVD has blocking activity, *e.g.*, block SDF-1 binding to CXCR7 partially or completely as described herein and/or reducing and/or inhibiting tumorigenesis in a xenograph model, and/or binds and/or recognizes amino acid residue W19, and optionally amino acid residue S23 and/or amino acid residue D25 in CXCR7 (SEQ ID NO: 1), all as described herein. Preferably, in a 01C10-like sequence according to this specifically preferred aspect, CDR1 and CDR2 are as defined under d) and e), respectively; or CDR1 and CDR3 are as defined under d) and f), respectively; or CDR2 and CDR3 are as defined under e) and f), respectively. More preferably, in such a 01C10-like sequence, CDR1, CDR2 and CDR3 are all as defined under d), e) and f), respectively. Again, in such an 01C10-like sequence, CDR1, CDR2 and CDR3 are preferably such that the 01C10-like ISVD has blocking activity, e.g. block SDF-1 binding to CXCR7 partially or completely as described herein, and/or reducing and/or inhibiting tumorigenesis in a xenograph model, and/or binds and/or recognizes amino acid residue W19, and optionally amino acid residue S23 and/or amino acid residue D25 in CXCR7 (SEQ ID NO: 1), all as described herein.

For example, in a 01C10-like sequence according to this specifically preferred aspect: CDR1 is the amino acid sequence NYAMG (SEQ ID NO: 93) (with CDR2 and CDR3 being as defined under e) and f), respectively); and/or CDR2 is the amino acid sequence AITPRAFTTYYADSVKG (SEQ ID NO: 95) (with CDR1 and CDR3 being as defined under d) and f), respectively); and/or CDR3 is the amino acid sequence QLVGSGSNLGRQESYAY (SEQ ID NO: 97) (with CDR1 and CDR2 being as defined under d) and e), respectively). Particularly, when an 01C10-like sequence is according to this aspect: CDR1 is the amino acid sequence NYAMG (SEQ ID NO: 93) and CDR2 is the amino acid sequence AITPRAFTTYYADSVKG (SEQ ID NO: 95) (with CDR3 being as defined under f) above); and/or CDR1 is the amino acid sequence NYAMG (SEQ ID NO: 93) and CDR3 is the amino acid sequence QLVGSGSNLGRQESYAY (SEQ ID NO: 97) (with CDR2 being as defined under e) above); and/or CDR2 is the amino acid sequence AITPRAFTTYYADSVKG (SEQ ID NO: 95) and CDR3 is QLVGSGSNLGRQESYAY (SEQ ID NO: 97) (with CDR1 being as defined under d) above). Again, in such 01C10-like sequences, CDR1, CDR2 and CDR3 are preferably such that the 01C10-like ISVD has blocking activity, *e.g.*, block SDF-1 binding to CXCR7 partially or completely as described herein, and/or reducing and/or inhibiting tumorigenesis in a xenograph model, and/or binds and/or recognizes amino acid residue W19, and optionally amino acid residue S23 and/or amino acid residue D25 in CXCR7 (SEQ ID NO: 1), all as described herein.

In a particularly preferred 01C10-like sequence: CDR1 is the amino acid sequence NYAMG (SEQ ID NO: 93), CDR2 is the amino acid sequence AITPRAFTTYYADSVKG (SEQ ID NO: 95); and CDR3 is the amino acid sequence QLVGSGSNLGRQESYAY (SEQ ID NO: 97).

In all the 01C10-like sequence described in this paragraph A), the framework sequences may be as further described herein. Preferably, the framework sequences are such that the framework sequences have at least 80%, such as at least 85%, for example at least 90%, such as at least 95% sequence identity with the framework sequences of 01C10 (which, for example, can be determined by determining the overall degree of sequence identity of a given sequence with the sequence of 01C10 while disregarding the CDR's in the calculation). Again, the combination of CDR's and frameworks present in a given sequence are preferably such that the resulting 01C10-like ISVD has blocking activity, *e.g.*, block SDF-1 binding to CXCR7 partially or completely as described herein and/or reducing and/or inhibiting tumorigenesis in a xenograph model, and/or binds and/or recognizes amino acid residue W19, and optionally amino acid residue S23 and/or amino acid residue D25 in CXCR7 (SEQ ID NO: 1), all as described herein.

In one specific aspect, a 01C10-like sequence is an ISVD that has at least 70%, such at least 80%, for example at least 85%, such as at least 90% or more than 95% sequence identity with the amino acid sequence 01C10 (SEQ ID NO: 91). For example, in an 01C10-like sequence according to this aspect, the CDR's may be according to the specifically preferred aspect described above, and may in particularly (but without limitation) be NYAMG (SEQ ID NO: 93) (CDR1); AITPRAFTTYYADSVKG (SEQ ID NO: 95) (CDR2); and QLVGSGSNLGRQESYAY (SEQ ID NO: 97) (CDR3). Again, preferably, the combination of CDR's and frameworks present in such a 01C10-like ISVD are preferably such that the resulting 01C10-like ISVD has blocking activity, e.g. block SDF-1 binding to CXCR7 partially or completely as described herein and/or reducing and/or inhibiting tumorigenesis in a xenograph model, and/or binds and/or recognizes amino acid residue W19, and optionally amino acid residue S23 and/or amino acid residue D25 in CXCR7 (SEQ ID NO: 1), all as described herein. In one particular aspect, any 01C10-like sequence may be a humanized and/or sequence optimized sequence, as further described herein.

B) **14G03-like sequences:** a *"14G03-like sequence"*, *"14G03-like ISVD"*, *"14G03-like building block"* or *"Group 2 ISVDs"* is defined as an ISVD (as described herein) that comprises:

a) a CDR1 which comprises or essentially consists of either (i) the amino acid sequence INYMG (SEQ ID NO: 13) or (ii) an amino acid sequence that has only 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence INYMG (SEQ ID NO: 13); and/or

b) a CDR2 which comprises or essentially consists of either (i) the amino acid sequence TLTSGGSTNYAGSVKG (SEQ ID NO: 23) or (ii) an amino acid sequence that has at least 80%,

such as at least 85%, for example at least 90% or more than 95% sequence identity with the amino acid sequence TLTSGGSTNYAGSVKG (SEQ ID NO: 23); or (iii) an amino acid sequence that has only 7, 6, 5, 4, 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence TLTSGGSTNYAGSVKG (SEQ ID NO: 23); and/or

c) a CDR3 which comprises or essentially consists of either (i) the amino acid sequence GGTLYDRRRFES (SEQ ID NO: 33) or (ii) an amino acid sequence that has at least 80%, such as at least 85%, for example at least 90% or more than 95% sequence identity with the amino acid sequence GGTLYDRRRFES (SEQ ID NO: 33); or (iii) an amino acid sequence that has only 7, 6, 5, 4, 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence GGTLYDRRRFES (SEQ ID NO: 33);

in which the framework sequences present in such an ISVD are as further described herein, and in which CDR1, CDR2 and CDR3 are preferably such that the 14G03-like ISVD has blocking activity, e.g. block CXCL11 and/or CXCL12 binding to CXCR7 partially or completely as described above, and/or reducing and/or inhibiting tumorigenesis in a xenograph model, and/or inhibits β-arrestin recruitment, and/or binds and/or recognizes amino acid residue M33, and optionally amino acid residue V32 and/or amino acid residue M37 in CXCR7 (SEQ ID NO: 1), all as described herein.

As also mentioned herein, (some of the) 14G03-like sequences may (and preferably also are) be such that they are capable of inhibiting, blocking or displacing SDF-1 binding (see Examples 9 and 10), for example in the displacement assay used in Example 10. Preferably, in such a 14G03-like sequence, CDR1 and CDR2 are as defined under a) and b), respectively; or CDR1 and CDR3 are as defined under a) and c), respectively; or CDR2 and CDR3 are as defined under b) and c), respectively. More preferably, in such a 14G03-like sequence, CDR1, CDR2 and CDR3 are all as defined under a), b) and c), respectively. Again, in such an 14G03-like sequence, CDR1, CDR2 and CDR3 are preferably such that the 14G03-like ISVD has blocking activity, e.g. block SDF-1 binding to CXCR7 partially or completely as described herein, and/or reducing and/or inhibiting tumorigenesis in a xenograph model, and/or inhibits β-arrestin recruitment, and/or binds and/or recognizes amino acid residue M33, and optionally amino acid residue V32 and/or amino acid residue M37 in CXCR7 (SEQ ID NO: 1), all as described herein.

For example, in such an 14G03-like sequence: CDR1 may comprise or essentially consist of the amino acid sequence INYMG (SEQ ID NO: 13) (with CDR2 and CDR3 being as defined under b) and c), respectively); and/or CDR2 may comprise or essentially consist of the amino acid sequence TLTSGGSTNYAGSVKG (SEQ ID NO: 23) (with CDR1 and CDR3 being as defined under a) and c), respectively); and/or CDR3 may comprise or essentially consist of the amino acid sequence

GGTLYDRRRFES (SEQ ID NO: 33) (with CDR1 and CDR2 being as defined under a) and b), respectively). Particularly, when an 14G03-like sequence is according to this aspect: CDR1 may comprise or essentially consist of the amino acid sequence INYMG (SEQ ID NO: 13) and CDR2 may comprise or essentially consist of the amino acid sequence TLTSGGSTNYAGSVKG (SEQ ID NO: 23) (with CDR3 being as defined under c) above); and/or CDR1 may comprise or essentially consist of the amino acid sequence INYMG (SEQ ID NO: 13) and CDR3 may comprise or essentially consist of the amino acid sequence GGTLYDRRRFES (SEQ ID NO: 33) (with CDR2 being as defined under b) above); and/or CDR2 may comprise or essentially consist of the amino acid sequence TLTSGGSTNYAGSVKG (SEQ ID NO: 23) and CDR3 may comprise or essentially consist of the amino acid sequence GGTLYDRRRFES (SEQ ID NO: 33) (with CDR1 being as defined under a) above). Again, in such 14G03-like sequences, CDR1, CDR2 and CDR3 are preferably such that the 14G03-like ISVD has blocking activity, e.g. block SDF-1 binding to CXCR7 partially or completely as described herein and/or reducing and/or inhibiting tumorigenesis in a xenograph model, and/or inhibits β-arrestin recruitment, and/or binds and/or recognizes amino acid residue M33, and optionally amino acid residue V32 and/or amino acid residue M37 in CXCR7 (SEQ ID NO: 1), all as described herein.

In a specifically preferred aspect, a *"14G03-like sequence"*, *"14G03-like ISVD"*, *"14G03-like building block"* or *"Group 2 ISVD"* is an ISVD that comprises:

d) a CDR1 which is either (i) the amino acid sequence INYMG (SEQ ID NO: 13) or (ii) an amino acid sequence that has only 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence INYMG (SEQ ID NO: 13); and/or

e) a CDR2 which is either (i) the amino acid sequence TLTSGGSTNYAGSVKG (SEQ ID NO: 23) or (ii) an amino acid sequence that has at least 80%, such as at least 85%, for example at least 90% or more than 95% sequence identity with the amino acid sequence TLTSGGSTNYAGSVKG (SEQ ID NO: 23); or (iii) an amino acid sequence that has only 7, 6, 5, 4, 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence TLTSGGSTNYAGSVKG (SEQ ID NO: 23); and/or

f) a CDR3 which is either (i) the amino acid sequence GGTLYDRRRFES (SEQ ID NO: 33) or (ii) an amino acid sequence that has at least 80%, such as at least 85%, for example at least 90% or more than 95% sequence identity with the amino acid sequence GGTLYDRRRFES (SEQ ID NO: 33); or (iii) an amino acid sequence that has only 7, 6, 5, 4, 3, 2 or 1 amino acid difference(s) (as defined herein) with the amino acid sequence GGTLYDRRRFES (SEQ ID NO: 33);

in which the framework sequences present in such an ISVD are as further described herein, and in which CDR1, CDR2 and CDR3 are preferably such that the 14G03-like ISVD has blocking

activity, e.g. block SDF-1 binding to CXCR7 partially or completely as described herein and/or reducing and/or inhibiting tumorigenesis in a xenograph model, and/or inhibits β-arrestin recruitment, and/or binds and/or recognizes amino acid residue M33, and optionally amino acid residue V32 and/or amino acid residue M37 in CXCR7 (SEQ ID NO: 1), all as described herein. Preferably, in a 14G03-like sequence according to this specifically preferred aspect, CDR1 and CDR2 are as defined under d) and e), respectively; or CDR1 and CDR3 are as defined under d) and f), respectively; or CDR2 and CDR3 are as defined under e) and f), respectively. More preferably, in such a 14G03-like sequence, CDR1, CDR2 and CDR3 are all as defined under d), e) and f), respectively. Again, in such an 14G03-like sequence, CDR1, CDR2 and CDR3 are preferably such that the 14G03-like ISVD has blocking activity, e.g. block SDF-1 binding to CXCR7 partially or completely as described herein, and/or reducing and/or inhibiting tumorigenesis in a xenograph model, and/or inhibits β-arrestin recruitment, and/or binds and/or recognizes amino acid residue M33, and optionally amino acid residue V32 and/or amino acid residue M37 in CXCR7 (SEQ ID NO: 1), all as described herein.

For example, in a 14G03-like sequence according to this specifically preferred aspect: CDR1 is the amino acid sequence INYMG (SEQ ID NO: 13) (with CDR2 and CDR3 being as defined under e) and f), respectively); and/or CDR2 is the amino acid sequence TLTSGGSTNYAGSVKG (SEQ ID NO: 23) (with CDR1 and CDR3 being as defined under d) and f), respectively); and/or CDR3 is the amino acid sequence GGTLYDRRRFES (SEQ ID NO: 33) (with CDR1 and CDR2 being as defined under d) and e), respectively). Particularly, when an 14G03-like sequence is according to this aspect: CDR1 is the amino acid sequence INYMG (SEQ ID NO: 13) and CDR2 is the amino acid sequence TLTSGGSTNYAGSVKG (SEQ ID NO: 23) (with CDR3 being as defined under f) above); and/or CDR1 is the amino acid sequence INYMG (SEQ ID NO: 13) and CDR3 is the amino acid sequence GGTLYDRRRFES (SEQ ID NO: 33) (with CDR2 being as defined under e) above); and/or CDR2 is the amino acid sequence TLTSGGSTNYAGSVKG (SEQ ID NO: 23) and CDR3 is GGTLYDRRRFES (SEQ ID NO: 33) (with CDR1 being as defined under d) above). Again, in such 14G03-like sequences, CDR1, CDR2 and CDR3 are preferably such that the 14G03-like ISVD has blocking activity, e.g. block SDF-1 binding to CXCR7 partially or completely as described herein, and/or reducing and/or inhibiting tumorigenesis in a xenograph model, and/or inhibits β-arrestin recruitment, and/or binds and/or recognizes amino acid residue M33, and optionally amino acid residue V32 and/or amino acid residue M37 in CXCR7 (SEQ ID NO: 1), all as described herein.

In a particularly preferred 14G03-like sequence: CDR1 is the amino acid sequence INYMG (SEQ ID NO: 13), CDR2 is the amino acid sequence TLTSGGSTNYAGSVKG (SEQ ID NO: 23); and CDR3 is the amino acid sequence GGTLYDRRRFES (SEQ ID NO: 33).

In all the 14G03-like sequence described in this paragraph A), the framework sequences may be as further described herein. Preferably, the framework sequences are such that the framework sequences have at least 80%, such as at least 85%, for example at least 90%, such as at least 95% sequence identity with the framework sequences of 14G03 (which, for example, can be determined by determining the overall degree of sequence identity of a given sequence with the sequence of 14G03 while disregarding the CDR's in the calculation). Again, the combination of CDR's and frameworks present in a given sequence are preferably such that the resulting 14G03-like ISVD has blocking activity, e.g. block SDF-1 binding to CXCR7 partially or completely as described herein and/or reducing and/or inhibiting tumorigenesis in a xenograph model, and/or inhibits β-arrestin recruitment, and/or binds and/or recognizes amino acid residue M33, and optionally amino acid residue V32 and/or amino acid residue M37 in CXCR7 (SEQ ID NO: 1), all as described herein.

In one specific aspect, a 14G03-like sequence is an ISVD that has at least 70%, such at least 80%, for example at least 85%, such as at least 90% or more than 95% sequence identity with the amino acid sequence 14G03 (SEQ ID NO: 43). For example, in an 14G03-like sequence according to this aspect, the CDR's may be according to the specifically preferred aspect described above, and may in particularly (but without limitation) be INYMG (SEQ ID NO: 13) (CDR1); TLTSGGSTNYAGSVKG (SEQ ID NO: 23) (CDR2); and GGTLYDRRRFES (SEQ ID NO: 33) (CDR3). Again, preferably, the combination of CDR's and frameworks present in such a 14G03-like ISVD are preferably such that the resulting 14G03-like ISVD has blocking activity, *e.g.,* block SDF-1 binding to CXCR7 partially or completely as described herein and/or reducing and/or inhibiting tumorigenesis in a xenograph model, and/or inhibits β-arrestin recruitment, and/or binds and/or recognizes amino acid residue M33, and optionally amino acid residue V32 and/or amino acid residue M37 in CXCR7 (SEQ ID NO: 1), all as described herein. In one particular aspect, any 14G03-like sequence may be a humanized and/or sequence optimized sequence, as further described herein.

For binding to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1), an amino acid sequence or polypeptide of the invention will usually contain within its amino acid sequence one or more amino acid residues or one or more stretches of amino acid residues (*i.e.,* with each "stretch" comprising two or amino acid residues that are adjacent to each other or in close proximity to each other, *i.e.,* in the primary or tertiary structure of the amino acid sequence) via which the amino acid sequence of the invention can bind to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1), which amino acid residues or stretches of amino acid residues thus form the "site" for binding to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) (also referred to herein as the "*antigen binding site*").

The immunoglobulin single variable domains provided by the invention are preferably in essentially isolated form (as defined herein), or form part of a protein or polypeptide of the invention (as defined herein), which may comprise or essentially consist of one or more immunoglobulin single variable domains of the invention and which may optionally further comprise one or more further immunoglobulin single variable domains (all optionally linked via one or more suitable linkers), and/or one or more further binding domains, binding units, amino acid sequences or other (functional) groups or moieties, that preferably also confer one or more desired properties to the constructs (some non-limiting examples of the same will become clear from the further description herein).

The polypeptides or immunoglobulin single variable domains provided by the invention preferentially reduce tumorigenesis *in vivo*.

In a further preferred embodiment, the invention provides constructs comprising at least two immunoglobulin single variable domains against CXCR7. More preferably, said immunoglobulin single variable domains against CXCR7 are selected from variants of polypeptides and immunoglobulin single variable domains against CXCR7 as defined in section 1.5 in respect of Table B-2 *infra* (*e.g.*, Group 2 immunoglobulin single variable domains), wherein said immunoglobulin single variable domains against CXCR7 may be the same or different. Preferably, said two immunoglobulin single variable domains against CXCR7 are chosen from 14G03-like ISVDs, such as 14G03, 08A05, 08A10, 07C03 and 07B11. In another further preferred embodiment, the invention provides constructs comprising at least two immunoglobulin single variable domains against CXCR7 are selected from variants of polypeptides and immunoglobulin single variable domains against CXCR7 which as defined in section 1.5 in respect of Table B-2 *infra* (*e.g.*, Group 1 immunoglobulin single variable domains), wherein said immunoglobulin single variable domains against CXCR7 may be the same or different. Preferably, said two immunoglobulin single variable domains against CXCR7 are chosen from 01C10 (SEQ ID NO: 91), 01B12 (SEQ ID NO: 100), 01F11 (SEQ ID NO: 101) or 01B10 (SEQ ID NO: 102).

It has unexpectedly been demonstrated that bispecific constructs comprising at least one Group 1 immunoglobulin single variable domain and at least one Group 2 ISVD are especially suitable for reducing tumour growth in vivo. In particular, it has been shown that these constructs inhibit SDF-1 binding to CXCR7, inhibit tumour growth in vivo, as well as inhibit β-arrestin recruitment. Moreover, in view of the binding efficacy of the Group 2 ISVDs, for instance as characterized by SDF-1 displacement, these constructs comprising at least one Group 1 ISVD and at least one Group 2 ISVD bind better to the target (see *e.g.*, Example 17). This would result in a lower dose for inhibiting

tumour growth. In addition, the simultaneous inhibition of β-arrestin recruitment would result in a prolonged anti-tumorigenic effect.

Accordingly, in a further preferred embodiment, the invention provides constructs comprising at least two immunoglobulin single variable domains against CXCR7, wherein at least one of said immunoglobulin single variable domains against CXCR7 (*i.e.*, a "first" immunoglobulin single variable domains against CXCR7) is 01C10-like, such as for instance 01C10 (SEQ ID NO: 91), 01B12 (SEQ ID NO: 100), 01F11 (SEQ ID NO: 101) or 01B10 (SEQ ID NO: 102), or variants thereof as defined in section 1.5 in respect of Table B-2 *infra* (*e.g.*, Group 1 immunoglobulin single variable domains), and wherein at least one immunoglobulin single variable domains against CXCR7 (*i.e.*, a "second" immunoglobulin single variable domain against CXCR7) is selected from variants of polypeptides and immunoglobulin single variable domains against CXCR7 as defined in section 1.5 *infra* in respect of Table B-2 different from the "first" immunoglobulin single variable domains against CXCR7 or variants thereof. Preferably, said "first" immunoglobulin single variable domains against CXCR7 is 01C10 and said "second" immunoglobulin single variable domains against CXCR7 is chosen from the group consisting of 14G03-like, such as for instance, 14G03, 08A05, 08A10, 07C03 and 07B11.

As described in Example 11, binding to CXCR7 by the Group 1 immunoglobulin single variable domains as represented by 01C10 was influenced by mutating W19. In contrast, binding of all tested immunoglobulin single variable domains was affected by a M33 mutation, while Group 1 ISVDs were not. It was further shown that Group 1 ISVDs preferably recognize and/or bind also S23 and D25 (data not shown).

Group 1 ISVDs or polypeptides can be characterized by binding/recognizing "Group 1 epitope". Group 1 ISVDs or polypeptides bind and/or recognize amino acid residue W19, and optionally amino acid residue S23 and/or amino acid residue D25 in CXCR7 (SEQ ID NO: 1). Group 1 epitope comprises amino acid residue W19, and optionally amino acid residue S23 and/or amino acid residue D25 in CXCR7 (SEQ ID NO: 1). Group 1 ISVDs is represented by *inter alia* 01C10 (SEQ ID NO: 91), 01B12 (SEQ ID NO: 100), 01F11 (SEQ ID NO: 101) or 01B10 (SEQ ID NO: 102), apparently hitting an epitope distinct from Group 2 epitope;

Group 2 ISVDs or polypeptides can be characterized by binding/recognizing "Group 2 epitope". Group 2 ISVDs or polypeptides do not bind and/or recognize amino acid residue W19, amino acid residue S23 and/or amino acid residue D25 in CXCR7 (SEQ ID NO: 1). Group 2 ISVDs or polypeptides bind and/or recognize amino acid residue M33, and optionally amino acid residue V32 and/or amino acid residue M37 in CXCR7 (SEQ ID NO: 1). Group 2 epitope comprises amino acid residue M33, and optionally amino acid residue V32 and/or amino acid residue M37 in CXCR7 (SEQ ID NO: 1). Group 2

ISVDs are represented by 14G03-like ISVDs, such as for instance, 14G03 (09A04), 08A05, 08A10 and 07C03, apparently hitting an epitope distinct from Group 1. Preferably, Group 2 ISVDs inhibit β-arrestin recruitment, as defined herein; and

Group 3 ISVDs or polypeptides can be characterized by binding/recognizing (part of) "Group 1" epitope as well as (part of) "Group 2" epitope. Group 3 ISVDs or polypeptides is represented by 07B11, apparently intermediary to Group 1 and Group 2.

The person skilled in the art is familiar with methods common in the art for determining epitopes, such as for instance provided in Example 11: "epitope mapping" of the present invention.

Accordingly, the present invention relates to polypeptides ISVDs, as well as (conventional) antibodies, or parts thereof, such as Fc, Fab, minibodies, etc., recognizing and/or binding W19, and optionally S23 and/or D25 in CXCR7.

The above described anti-CXCR7/CXCR7 bispecific constructs may be suitably half-life extended (e.g., by pegylation, fusion to serum albumin, or fusion to a peptide or binding unit that can bind to a serum protein such as serum albumin, as further described herein), and thus may for example further comprise a serum-albumin binding peptide or binding domain (such as those described herein), optionally linked via one or more suitable spacers or linkers.

Again, such further binding domains, binding units, amino acid sequences or other (functional) groups or moieties include one or more other immunoglobulin single variable domains, such as one or more (single) domain antibodies, dAb's or Nanobodies (*e.g.*, a V_{HH}, humanized V_{HH} or camelized V_H, such as a camelized human V_H), so as to provide a "bispecific" protein or polypeptide of the invention (*i.e.*, a polypeptide of the invention that contains at least one - such as one or two - immunoglobulin single variable domain that is directed against CXCR7 and at least one - such as one or two - immunoglobulin single variable domain that is directed against another target).

For example, according to a specific but non-limiting aspect, the constructs, proteins or polypeptides of the invention may have been provided with an increased half-life, for example by functionalisation and/or by including in the construct a moiety or binding unit that increases the half-life of the construct. Examples of such functionalisation, moieties or binding units will be clear to the skilled person and may for example include pegylation, fusion to serum albumin, or fusion to a peptide or binding unit that can bind to a serum protein such as serum albumin.

In the latter constructs (*i.e.*, fusion constructs comprising at least one – such as one or two - amino acid sequence of the invention and at least one – such as one or two - peptide or binding unit that can bind to a serum protein such as serum albumin), the serum-albumin binding peptide or binding domain may be any suitable serum-albumin binding peptide or binding domain capable of increasing

the half-life of the construct (compared to the same construct without the serum-albumin binding peptide or binding domain), and may in particular be serum albumin binding peptides as described in WO 2008/068280 by applicant (and in particular WO 2009/127691 and WO 2011/095545, both by applicant), or a serum–albumin binding immunoglobulin single variable domain (such as a serum-albumin binding Nanobody; for example Alb-1 or a humanized version of Alb-1 such as Alb-8, for which reference is for example made to WO 06/122787).

With respect to half-life, it should be noted that in the invention, and by using the various half-life extending techniques described herein (for example, by suitably choosing a serum-albumin binding peptide according to WO 2008/068280, WO 2009/127691 and/or WO 2011/095545, the half-life of a construct or polypeptide of the invention can (and preferably is) suitably "tailored" for the intended (therapeutic and/or diagnostic) application and/or to obtain the best balance between the desired therapeutic and/or pharmacological effect and possible undesired side-effects.

Thus, for example, and without limitation, a preferred aspect of the invention provides a "bispecific" polypeptide consisting essentially of one immunoglobulin single variable domain directed against human CXCR7 (or, alternatively, of two immunoglobulin single variable domains directed against human CXCR7, which may be the same or different, so as to provide - when they are the same or different - a "bivalent" polypeptide of the invention, or - when they are different - "biparatopic" polypeptide of the invention) and one immunoglobulin single variable domain directed against human serum albumin linked by a peptide linker (as defined herein), so as to provide a bispecific polypeptide of the invention, respectively, all as described herein. Such a protein or polypeptide may also be in essentially isolated form (as defined herein).

In another specific, but non-limiting aspect, an amino acid sequence (such as a Nanobody) of the invention or a polypeptide of the invention (such as a bivalent, biparatopic or bispecific polypeptide of the invention) may be suitably linked (again, chemically or via one or more suitable linkers or spacers) to a toxin or to a (cyto)toxic residue, moiety or payload. Examples of suitable (cyto)toxic moieties, compounds, payloads or residues which can be linked to amino acids sequences or polypeptides of the invention to provide – for example – a cytotoxic compound (*i.e.*, an antibody-drug conjugate or "ADC" based upon an amino acid sequence or polypeptide of the invention) will be clear to the skilled person. Reference is for example made to the review by Ducry and Stump, Bioconjugate Chem., 2010, 21 (1), pp. 5–13. Such cytotoxic amino acid sequences or polypeptides of the invention may in particular be useful/suitable for those applications in which it is intended to kill a cell that expresses the target against which the amino acid sequences or polypeptides of the invention are directed (e.g. in the treatment of cancer), or to reduce or slow the growth and/or proliferation such a cell. Usually, but without limitation, (cyto)toxic polypeptides of the invention will

either not be half-life extended or will have only a limited and/or tightly controlled half-life extension.

In another aspect, at least one amino acid sequence of the invention (*i.e.*, immunoglobulin single variable domain against CXCR7) may be suitably linked to at least one immunoglobulin single variable domain that is directed against CXCR4, so as to provide a bispecific polypeptide of the invention that is directed against both CXCR7 and CXCR4.

For example, in this aspect, at least one – such as one or two – amino acid sequences of the invention may be suitably linked to at least one – such as one or two –immunoglobulin single variable domains against CXCR4.

Some preferred but non-limiting examples of immunoglobulin single variable domains against CXCR4 that can be used in such constructs are (or may be suitably chosen from)

- the immunoglobulin single variable domains (and in particular one of the Nanobodies) against CXCR4 from the international application WO 09/138519 by Ablynx N.V. (for example and without limitation, 238D2/SEQ ID NO: 238 and 238D4/SEQ ID NO: 239 in Table B-1.1 of WO 09/138519); and/or
- the sequence-optimized/improved variants of the amino acid sequences 238D2 and 238D4 described in the non-prepublished US application 61/358,495 by Ablynx N.V. filed on June 25, 2010; and/or
- the immunoglobulin single variable domains that are capable of binding to the same epitope as 238D2 and/or 238D4 as described in the PCT application PCT/EP2010/064766 by Ablynx N.V. filed on October 4, 2010; and/or
- the 10E9-type sequences, 281E10-type sequences, 10E12-type sequences, 10A10-type sequences, 10G10-type sequences, 14A2-type sequences, 15A1-type sequences, 15H3-type sequences and/or 283B6-type sequences described on pages 7-13 of the PCT application PCT/EP2011/050156 by Ablynx N.V. filed on January 7, 2011; and/or
- the 10E9-type sequences, 281E10-type sequences, 10E12-type sequences, 10A10-type sequences, 10G10-type sequences, 14A2-type sequences, 15A1-type sequences, 15H3-type sequences and/or 283B6-type sequences described on pages 15-47 of the PCT application PCT/EP2011/050156 by Ablynx N.V. filed on January 7, 2011.

The above described anti-CXCR7/CXCR4 bispecific constructs (as well as other bispecific constructs comprising at least one amino acid sequence of the invention) may be suitably half-life extended (*e.g.*, by pegylation, fusion to serum albumin, or fusion to a peptide or binding unit that can bind to a

serum protein such as serum albumin, as further described herein), and thus may for example further comprise a serum-albumin binding peptide or binding domain (such as those described herein), optionally linked via one or more suitable spacers or linkers.

Thus, one specific but non-limiting aspect of the invention is a polypeptide that comprises one or two (and preferably one) immunoglobulin single variable domains (as defined herein, and preferably one or two Nanobodies) against CXCR7, one or two (and preferably one) immunoglobulin single variable domains (as defined herein, and preferably one or two Nanobodies) against CXCR4, and a peptide or immunoglobulin single variable domain against (human) serum albumin, optionally suitably linked via one or more spacers or linkers.

The above anti-CXCR7/CXCR4 bispecific constructs (as well as other bispecific constructs comprising at least one amino acid sequence of the invention) may also be suitably linked (again, chemically or via one or more suitable linkers or spacers) to a toxin or to a (cyto)toxic residue, moiety or payload (as further described herein). Again, such (cyto)toxic bispecfic polypeptides of the invention will either not be half-life extended or will have only a limited and/or tightly controlled half-life extension.

The invention in its broadest sense also comprises derivatives of the amino acid sequences (*e.g.*, Nanobodies) of the invention and of the polypeptides of the invention. Such derivatives can generally be obtained by modification, and in particular by chemical and/or biological (e.g. enzymatical) modification, of the amino acid sequences (*e.g.*, Nanobodies) of the invention and polypeptides of the invention and/or of one or more of the amino acid residues that form the Nanobodies of the invention.

Examples of such modifications, as well as examples of amino acid residues within the amino acid sequences (*e.g.*, Nanobodies) of the invention and polypeptides that can be modified in such a manner (*i.e.*, either on the protein backbone but preferably on a side chain), methods and techniques that can be used to introduce such modifications and the potential uses and advantages of such modifications will be clear to the skilled person.

For example, such a modification may involve the introduction (*e.g.*, by covalent linking or in another suitable manner) of one or more functional groups, residues or moieties into or onto the amino acid sequences (*e.g.*, Nanobodies) of the invention and polypeptides of the invention, and in particular of one or more functional groups, residues or moieties that confer one or more desired properties or functionalities to the Nanobody of the invention. Example of such functional groups will be clear to the skilled person.

For example, such modification may comprise the introduction (*e.g.*, by covalent binding or in any other suitable manner) of one or more functional groups that increase the half-life, the solubility and/or the absorption of the Nanobody of the invention, that reduce the immunogenicity and/or the toxicity of the Nanobody of the invention, that eliminate or attenuate any undesirable side effects of the Nanobody of the invention, and/or that confer other advantageous properties to and/or reduce the undesired properties of the Nanobodies and/or polypeptides of the invention; or any combination of two or more of the foregoing. Examples of such functional groups and of techniques for introducing them will be clear to the skilled person, and can generally comprise all functional groups and techniques mentioned in the general background art cited hereinabove as well as the functional groups and techniques known per se for the modification of pharmaceutical proteins, and in particular for the modification of antibodies or antibody fragments (including ScFv's and single domain antibodies), for which reference is for example made to Remington's Pharmaceutical Sciences, 16th ed., Mack Publishing Co., Easton, PA (1980). Such functional groups may for example be linked directly (for example covalently) to a Nanobody of the invention, or optionally via a suitable linker or spacer, as will again be clear to the skilled person.

One of the most widely used techniques for increasing the half-life and/or reducing the immunogenicity of pharmaceutical proteins comprises attachment of a suitable pharmacologically acceptable polymer, such as poly(ethyleneglycol) (PEG) or derivatives thereof (such as methoxypoly(ethyleneglycol) or mPEG). Generally, any suitable form of pegylation can be used, such as the pegylation used in the art for antibodies and antibody fragments (including but not limited to (single) domain antibodies and ScFv's); reference is made to for example Chapman, Nat. Biotechnol., 54, 531-545 (2002); by Veronese and Harris, Adv. Drug Deliv. Rev. 54, 453-456 (2003), by Harris and Chess, Nat. Rev. Drug. Discov., 2, (2003) and in WO 04/060965. Various reagents for pegylation of proteins are also commercially available, for example from Nektar Therapeutics, USA.

Preferably, site-directed pegylation is used, in particular via a cysteine-residue (see for example Yang et al., Protein Engineering, 16, 10, 761-770 (2003). For example, for this purpose, PEG may be attached to a cysteine residue that naturally occurs in a Nanobody of the invention, a Nanobody of the invention may be modified so as to suitably introduce one or more cysteine residues for attachment of PEG, or an amino acid sequence comprising one or more cysteine residues for attachment of PEG may be fused to the N- and/or C-terminus of a Nanobody of the invention, all using techniques of protein engineering known per se to the skilled person.

Preferably, for the Nanobodies and proteins of the invention, a PEG is used with a molecular weight of more than 5000, such as more than 10,000 and less than 200,000, such as less than 100,000; for example in the range of 20,000-80,000.

Another, usually less preferred modification comprises N-linked or O-linked glycosylation, usually as part of co-translational and/or post-translational modification, depending on the host cell used for expressing the Nanobody or polypeptide of the invention.

Yet another modification may comprise the introduction of one or more detectable labels or other signal-generating groups or moieties, depending on the intended use of the labelled Nanobody. Suitable labels and techniques for attaching, using and detecting them will be clear to the skilled person, and for example include, but are not limited to, the fluorescent labels, phosphorescent labels, chemiluminescent labels, bioluminescent labels, radio-isotopes, metals, metal chelates, metallic cations, chromophores and enzymes, such as those mentioned on page 109 of WO 08/020079. Other suitable labels will be clear to the skilled person, and for example include moieties that can be detected using NMR or ESR spectroscopy.

Such labelled Nanobodies and polypeptides of the invention may for example be used for *in vitro, in vivo* or *in situ* assays (including immunoassays known per se such as ELISA, RIA, EIA and other "sandwich assays", etc.) as well as *in vivo* diagnostic and imaging purposes, depending on the choice of the specific label.

As will be clear to the skilled person, another modification may involve the introduction of a chelating group, for example to chelate one of the metals or metallic cations referred to above. Suitable chelating groups for example include, without limitation, diethyl-enetriaminepentaacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA).

Yet another modification may comprise the introduction of a functional group that is one part of a specific binding pair, such as the biotin-(strept)avidin binding pair. Such a functional group may be used to link the Nanobody of the invention to another protein, polypeptide or chemical compound that is bound to the other half of the binding pair, *i.e.*, through formation of the binding pair. For example, a Nanobody of the invention may be conjugated to biotin, and linked to another protein, polypeptide, compound or carrier conjugated to avidin or streptavidin. For example, such a conjugated Nanobody may be used as a reporter, for example in a diagnostic system where a detectable signal-producing agent is conjugated to avidin or streptavidin. Such binding pairs may for example also be used to bind the Nanobody of the invention to a carrier, including carriers suitable for pharmaceutical purposes. One non-limiting example are the liposomal formulations described by Cao and Suresh, Journal of Drug Targetting, 8, 4, 257 (2000). Such binding pairs may also be used to link a therapeutically active agent to the Nanobody of the invention.

Other potential chemical and enzymatical modifications will be clear to the skilled person. Such modifications may also be introduced for research purposes (*e.g.*, to study function-activity

relationships). Reference is for example made to Lundblad and Bradshaw, Biotechnol. Appl. Biochem., 26, 143-151 (1997).

The immunoglobulin single variable domains and polypeptides of the invention as such preferably essentially consist of a single amino acid chain that is not linked via disulphide bridges to any other amino acid sequence or chain (but that may or may not contain one or more intramolecular disulphide bridges. For example, it is known that agent of the invention – as described herein - may sometimes contain a disulphide bridge between CDR3 and CDR1 or FR2). However, it should be noted that one or more immunoglobulin single variable domains of the invention may be linked to each other and/or to other immunoglobulin single variable domains (*e.g.*, via disulphide bridges) to provide peptide constructs that may also be useful in the invention (for example Fab' fragments, $F(ab')_2$ fragments, ScFv constructs, "diabodies" and other multispecific constructs. Reference is for example made to the review by Holliger and Hudson, Nat Biotechnol. 2005 Sep;23(9):1126-36).

Generally, when an amino acid sequence of the invention (or a compound, construct or polypeptide comprising the same) is intended for administration to a subject (for example for therapeutic and/or diagnostic purposes as described herein), it is preferably either an amino acid sequence that does not occur naturally in said subject; or, when it does occur naturally in said subject, is in essentially isolated form (as defined herein).

It will also be clear to the skilled person that for pharmaceutical use, the immunoglobulin single variable domains of the invention (as well as compounds, constructs and polypeptides comprising the same) are preferably directed against human CXCR7 and in particular human CXCR7 (SEQ ID NO: 1); whereas for veterinary purposes, the immunoglobulin single variable domains and polypeptides of the invention are preferably directed against CXCR7 from the species to be treated, or at least cross-reactive with CXCR7 from the species to be treated.

Furthermore, an amino acid sequence of the invention may optionally, and in addition to the at least one binding site for binding against CXCR7 and in particular human CXCR7 (SEQ ID NO: 1), contain one or more further binding sites for binding against other antigens, proteins or targets.

The efficacy of the immunoglobulin single variable domains and polypeptides of the invention, and of compositions comprising the same, can be tested using any suitable *in vitro* assay, cell-based assay, *in vivo* assay and/or animal model known *per se*, or any combination thereof, depending on the specific disease or disorder involved. Suitable assays and animal models will be clear to the skilled person, and for example include ligand displacement assays (Burns et al, J.Exp.Med. 2006 4;203(9):2201-13), beta arrestin recruitment assays (Zabel et al., J. Immunol. 2009 1;183(5):3204-11), dimerization assays (Luker et al, Faseb J. 2009 23(3):823-34), signaling assays (Wang et al, J

Immunol. 2009 Sep 1;183(5):3204-11) proliferation assays (Wang et al, J Immunol. 2009 Sep 1;183(5):3204-11; Odemis et al., J Cell Sign. 2010 April 1; 123(Pt 7): 1081-8), survival assays (Burns et al, J.Exp.Med. 2006 4;203(9):2201-13), cell adhesion assays (Burns et al, J.Exp.Med. 2006 4;203(9):2201-13) and transendothelial migration assays (Mazzinghi et al, J.Exp.Med. 2008 Feb 18;205(2):479-90), endothelial cell sprouting assays (Wang et al, J Immunol. 2009 Sep 1;183(5):3204-11), myogenic differentiation (Melchionna et al., Muscle Nerve, 2010 Feb 11) and *in vivo* xenograft models (Burns et al, J.Exp.Med. 2006 4;203(9):2201-13), collagen induced arthritis models (Hegen et al, Ann Rheum Dis. 2008 Nov;67(11):1505-15) and experimental autoimmune encephalomyelitis models (Wekerle, Ann Rheum Dis. 2008 Dec;67 Suppl 3:iii56-60) as well as the assays and animal models used in the experimental part below and in the prior art cited herein.

Also, according to the invention, immunoglobulin single variable domains and polypeptides that are directed against CXCR7 from a first species of warm-blooded animal may or may not show cross-reactivity with CXCR7 from one or more other species of warm-blooded animal. For example, immunoglobulin single variable domains and polypeptides directed against human CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) may or may not show cross reactivity with CXCR7 from one or more other species of primates (such as, without limitation, monkeys from the genus *Macaca* (such as, and in particular, cynomolgus monkeys (*Macaca fascicularis*) and/or rhesus monkeys (*Macaca mulatta*)) and baboon (*Papio ursinus*)) and/or with CXCR7 from one or more species of animals that are often used in animal models for diseases (for example mouse, rat, rabbit, pig or dog), and in particular in animal models for diseases and disorders associated with CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) (such as the species and animal models mentioned herein). In this respect, it will be clear to the skilled person that such cross-reactivity, when present, may have advantages from a drug development point of view, since it allows the immunoglobulin single variable domains and polypeptides against human CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) to be tested in such disease models (see *e.g.*, Example 12).

More generally, immunoglobulin single variable domains and polypeptides of the invention that are cross-reactive with CXCR7 from multiple species of mammal will usually be advantageous for use in veterinary applications, since it will allow the same amino acid sequence or polypeptide to be used across multiple species. Thus, it is also encompassed within the scope of the invention that immunoglobulin single variable domains and polypeptides directed against CXCR7 from one species of animal (such as immunoglobulin single variable domains and polypeptides against human CXCR7 (SEQ ID NO: 1)) can be used in the treatment of another species of animal, as long as the use of the immunoglobulin single variable domains and/or polypeptides provide the desired effects in the species to be treated.

The present invention is in its broadest sense also not particularly limited to or defined by a specific antigenic determinant, epitope, part, domain, subunit or confirmation (where applicable) of CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) against which the immunoglobulin single variable domains and polypeptides of the invention are directed. For example, the immunoglobulin single variable domains and polypeptides may or may not be directed against the CXCL11/CXCL12 interaction site and/or CXCR7/CXCR7 homodimerization site and/or CXCR4/CXCR7 heterodimerization site (or heterodimerization of CXCR7 to other chemokine receptor such as e.g. CXCR3), and are as further defined herein.

As further described herein, a polypeptide of the invention may contain two or more immunoglobulin single variable domains of the invention that are directed against CXCR7 and in particular human CXCR7 (SEQ ID NO: 1). Generally, such polypeptides will bind to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) with increased avidity compared to a single amino acid sequence of the invention. Such a polypeptide may for example comprise two immunoglobulin single variable domains of the invention that are directed against the same antigenic determinant, epitope, part, domain, subunit or confirmation (where applicable) of CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) (which may or may not be an interaction site); or comprise at least one "first" amino acid sequence of the invention that is directed against a first same antigenic determinant, epitope, part, domain, subunit or confirmation (where applicable) of CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) (which may or may not be an interaction site), such as for instance Group 1 epitopes; and at least one "second" amino acid sequence of the invention that is directed against a second antigenic determinant, epitope, part, domain, subunit or confirmation (where applicable) different from the first (and which again may or may not be an interaction site) , such as for instance Group 2 epitopes. Preferably, in such "biparatopic" polypeptides of the invention, at least one amino acid sequence of the invention is directed against an interaction site (as defined herein), although the invention in its broadest sense is not limited thereto. For instance, polypeptides of the invention may be formatted *e.g.*, in a biparatopic way such as to combine monovalent building blocks directed against different epitopes as characterized in the experimental part (see Examples 9 to 17). Although the binding constants, *e.g.*, association and dissociation constants, of individual immunoglobulin single variable domains of a "bivalent" polypeptide are wholly favourable over the binding constants of the individual immunoglobulin single variable domains of a "biparatopic" polypeptide, the present invention demonstrates completely unexpectedly that a "biparatopic" polypeptide of the invention is more effective in biological assays, *e.g.*, β-arrestin assay, than "bivalent" polypeptides.

Also, when the target is part of a binding pair (for example, a receptor-ligand binding pair), the immunoglobulin single variable domains and polypeptides may be such that they compete with the

cognate binding partners, *e.g.*, CXCL11 (also referred to as I-TAC) and/or CXCL12 (also referred to as SDF-1), for binding to CXCR7, and/or such that they (fully or partially) neutralize binding of the binding partner to the target.

It is also expected that the immunoglobulin single variable domains and polypeptides of the invention will generally bind to all naturally occurring or synthetic analogs, variants, mutants, alleles, parts and fragments of CXCR7 and in particular human CXCR7 (SEQ ID NO: 1); or at least to those analogs, variants, mutants, alleles, parts and fragments of CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) that contain one or more antigenic determinants or epitopes that are essentially the same as the antigenic determinant(s) or epitope(s) to which the immunoglobulin single variable domains and polypeptides of the invention bind to CXCR7 and in particular to human CXCR7 (SEQ ID NO: 1). Again, in such a case, the immunoglobulin single variable domains and polypeptides of the invention may bind to such analogs, variants, mutants, alleles, parts and fragments with an affinity and/or specificity that are the same as, or that are different from (*i.e.*, higher than or lower than), the affinity and specificity with which the immunoglobulin single variable domains of the invention bind to (wild-type) CXCR7.

As CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) exists in a monomeric form and in one or more multimeric forms, *e.g.*, in homodimeric as well in heterodimeric form with CXCR4, *e.g.*, human CXCR4 (RM Maksym et al., supra; KE Luker et al. supra), it is within the scope of the invention that the immunoglobulin single variable domains and polypeptides of the invention i) only bind to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) in monomeric form, ii) only bind to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) in multimeric/dimeric (homo- and/or heterodimeric) form, or iii) bind to both the monomeric and the multimeric form. In a preferred aspect of the invention, the polypeptides of the invention prevent formation of homodimeric human CXCR7 complexes and/or heterodimeric human CXCR4/CXCR7 complexes. In another preferred aspect of the invention, the polypeptides of the invention do not induce (even at higher concentration such as 10nM or less, 50nM or less, 100nM or less, or 500nM or less) formation of homodimeric human CXCR7 complexes and/or heterodimeric human CXCR4/CXCR7 complexes. Again, in such a case, the polypeptides of the invention may bind to the monomeric form with an affinity and/or specificity that are the same as, or that are different from (*i.e.*, higher than or lower than), the affinity and specificity with which the immunoglobulin single variable domains of the invention bind to the multimeric form.

Also, when CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) can associate with other proteins or polypeptides to form protein complexes (*e.g.*, with CXCL12/SDF-1 or CXCL11/I-TAC), it is within the scope of the invention that the immunoglobulin single variable domains and polypeptides of the invention bind to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) in its non-associated state

(and e.g. prevent the ligand binding), bind to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) in its associated state, or bind to both (preferably to the non-associated state). In all these cases, the immunoglobulin single variable domains and polypeptides of the invention may bind to such associated protein complexes with an affinity and/or specificity that may be the same as or different from (*i.e.*, higher than or lower than) the affinity and/or specificity with which the immunoglobulin single variable domains and polypeptides of the invention bind to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) in its non-associated state.

Also, as will be clear to the skilled person, proteins or polypeptides that contain two or more immunoglobulin single variable domains directed against CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) may bind with higher avidity to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) than the corresponding monomeric amino acid sequence(s). For example, and without limitation, proteins or polypeptides that contain two or more immunoglobulin single variable domains directed against different epitopes of CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) may (and usually will) bind with higher avidity than each of the different monomers, and proteins or polypeptides that contain two or more immunoglobulin single variable domains directed against CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) may (and usually will) bind also with higher avidity to a multimer (e.g. homodimer, heterodimer with CXCR4) of CXCR7 and in particular to a multimer (e.g. homodimer, heterodimer with human CXCR4) of human CXCR7 (SEQ ID NO: 1).

Generally, immunoglobulin single variable domains and polypeptides of the invention will at least bind to those forms of CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) (including monomeric, multimeric, associated and different conformational forms) that are the most relevant from a biological and/or therapeutic point of view, as will be clear to the skilled person.

It is also within the scope of the invention to use parts, fragments, analogs, mutants, variants, alleles and/or derivatives of the immunoglobulin single variable domains and polypeptides of the invention, and/or to use proteins or polypeptides comprising or essentially consisting of one or more of such parts, fragments, analogs, mutants, variants, alleles and/or derivatives, as long as these are suitable for the uses envisaged herein. Such parts, fragments, analogs, mutants, variants, alleles and/or derivatives will usually contain (at least part of) a functional antigen-binding site for binding against CXCR7 and in particular human CXCR7 (SEQ ID NO: 1); and more preferably will be capable of specific binding to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1), and even more preferably capable of binding to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) with an EC50 value, average Ki, IC_{50} value concerning binding, migration, displacing and/or proliferation blocking and/or other measures for potency, as further described herein, *e.g.*, in the experimental part) that is as defined herein and such parts, fragments, analogs, mutants, variants, alleles and/or derivatives may be more

potent, more stable, more soluble and may have the same epitope. Some non-limiting examples of such parts, fragments, analogs, mutants, variants, alleles, derivatives, proteins and/or polypeptides will become clear from the further description herein. Additional fragments or polypeptides of the invention may also be provided by suitably combining (i.e. by linking or genetic fusion) one or more (smaller) parts or fragments as described herein.

For a general description of immunoglobulin single variable domains, reference is made to the further description below, as well as to the prior art cited herein. In this respect, it should however be noted that this description and the prior art mainly describes immunoglobulin single variable domains of the so-called "V_H3 class" (*i.e.*, immunoglobulin single variable domains with a high degree of sequence homology to human germline sequences of the V_H3 class, such as DP-47, DP-51 or DP-29), which form a preferred aspect of this invention. It should however be noted that the invention in its broadest sense generally covers any type of immunoglobulin single variable domains directed against CXCR7 and in particular human CXCR7 (SEQ ID NO: 1), and for example also covers the immunoglobulin single variable domains belonging to the so-called "V_H4 class" (*i.e.*, immunoglobulin single variable domains with a high degree of sequence homology to human germline sequences of the V_H4 class such as DP-78), as for example described in WO 07/118670.

Generally, immunoglobulin single variable domains (in particular V_{HH} sequences and sequence optimized immunoglobulin single variable domains) can in particular be characterized by the presence of one or more "*Hallmark residues*" (as described herein) in one or more of the framework sequences (again as further described herein).

Thus, generally, an immunoglobulin single variable domain can be defined as an amino acid sequence with the (general) structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively.

In a preferred aspect, the invention provides polypeptides comprising at least an immunoglobulin single variable domain that is an amino acid sequence with the (general) structure

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively, and in which:

i) at least one of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table A-1 below; and/or in which:

ii) said amino acid sequence has at least 80%, more preferably 90%, even more preferably 95% amino acid identity with at least one of the immunoglobulin single variable domains as shown in WO 2009/138519 (see SEQ ID NOs: 1 to 125 in WO 2009/138519), in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences (indicated with X in the sequences) are disregarded; and/or in which:

iii) the CDR sequences are generally as further defined herein (e.g. the CDR1, CDR2 and CDR3 in a combination as provided in Table B-2, note that the CDR definitions are calculated according to the Kabat numbering system); and/or in which:

iv) the FR sequences are generally as further defined herein, such as, for instance, the FR1, FR2, FR3 and FR4 in a combination as provided in Table B-2, and/or FR1, FR2, FR3 and FR4 has at least 80%, more preferably 90%, even more preferably 95% amino acid identity with at least one of FR1, FR2, FR3 and FR4, respectively, of the FRs as provided in Table B-2 (wherein the FR definitions are calculated according to the Kabat numbering system).

Table A-1: Hallmark Residues in VHHs

Position	Human V_H3	Hallmark Residues
11	L, V; predominantly L	L, S, V, M, W, F, T, Q, E, A, R, G, K, Y, N, P, I; preferably L
37	V, I, F; usually V	F(1), Y, V, L, A, H, S, I, W, C, N, G, D, T, P, preferably F(1) or Y
44(8)	G	E(3), Q(3), G(2), D, A, K, R, L, P, S, V, H, T, N, W, M, I; preferably G(2), E(3) or Q(3);most preferably G(2) or Q(3).
45(8)	L	L(2), R(3), P, H, F, G, Q, S, E, T, Y, C, I, D, V; preferably L(2) or R(3)
47(8)	W, Y	F(1), L(1) or W(2) G, I, S, A, V, M, R, Y, E, P, T, C, H, K, Q, N, D; preferably W(2), L(1) or F(1)
83	R or K; usually R	R, K(5), T, E(5), Q, N, S, I, V, G, M, L, A, D, Y, H; preferably K or R; most preferably K
84	A, T, D; predominantly A	P(5), S, H, L, A, V, I, T, F, D, R, Y, N, Q, G, E; preferably P
103	W	W(4), R(6), G, S, K, A, M, Y, L, F, T, N, V, Q, P(6), E, C; preferably W
104	G	G, A, S, T, D, P, N, E, C, L; preferably G
108	L, M or T; predominantly L	Q, L(7), R, P, E, K, S, T, M, A, H; preferably Q or L(7)

(1) In particular, but not exclusively, in combination with KERE or KQRE at positions 43-46.

(2) Usually as GLEW at positions 44-47.

(3) Usually as KERE or KQRE at positions 43-46, e.g. as KEREL, KEREF, KQREL, KQREF, KEREG, KQREW or KQREG at positions 43-47. Alternatively, also sequences such as TERE (for example TEREL), TQRE (for example TQREL), KECE (for example KECEL or KECER), KQCE (for example KQCEL), RERE (for example REREG), RQRE (for example RQREL, RQREF or RQREW), QERE (for example QEREG), QQRE, (for example QQREW, QQREL or QQREF), KGRE (for example KGREG), KDRE (for example KDREV) are possible. Some other possible, but less preferred sequences include for example DECKL and NVCEL.

(4) With both GLEW at positions 44-47 and KERE or KQRE at positions 43-46.

(5) Often as KP or EP at positions 83-84 of naturally occurring V_{HH} domains.

(6) In particular, but not exclusively, in combination with GLEW at positions 44-47.

(7) With the proviso that when positions 44-47 are GLEW, position 108 is always Q in (non-humanized) V_{HH} sequences that also contain a W at 103.

(8) The GLEW group also contains GLEW-like sequences at positions 44-47, such as for example GVEW, EPEW, GLER, DQEW, DLEW, GIEW, ELEW, GPEW, EWLP, GPER, GLER and ELEW.

Again, such immunoglobulin single variable domains may be derived in any suitable manner and from any suitable source, and may for example be naturally occurring V_{HH} sequences (*i.e.*, from a suitable species of Camelid, *e.g.*, llama) or synthetic or semi-synthetic VHs or VLs (*e.g.*, from human). Such immunoglobulin single variable domains may include "humanized" or otherwise "sequence optimized" VHHs, "camelized" immunoglobulin sequences (and in particular camelized heavy chain variable domain sequences, *i.e.*, camelized VHs), as well as human VHs, human VLs, camelid VHHs that have been altered by techniques such as affinity maturation (for example, starting from synthetic, random or naturally occurring immunoglobulin sequences), CDR grafting, veneering, combining fragments derived from different immunoglobulin sequences, PCR assembly using overlapping primers, and similar techniques for engineering immunoglobulin sequences well known to the skilled person; or any suitable combination of any of the foregoing as further described herein.

In a further preferred aspect, the invention provides polypeptides comprising one immunoglobulin single variable domain with amino acid sequence selected from the group consisting of amino acid sequences with SEQ ID NOs: 39 to 43 and 91 as well as 99-102 (see Table B-3) and one immunoglobulin single variable domain with amino acid sequence selected from the group consisting of moieties providing an increased half-life (see below).

In a further preferred aspect, the invention provides polypeptides comprising at least one immunoglobulin single variable domain with amino acid sequence selected from the group consisting of amino acid sequences that essentially consist of 4 framework regions (FR1 to FR4, respectively) and 3 complementarity determining regions (CDR1 to CDR3, respectively), in which the CDR sequences of said amino acid sequences have at least 70% amino acid identity, preferably at least 80% amino acid identity, more preferably at least 90% amino acid identity, such as 95% amino acid identity or more or even essentially 100% amino acid identity with the CDR sequences of at least one of the immunoglobulin single variable domains of SEQ ID NOs: 39 to 43 and 91 as well as 99-102 (see Tables B-2 and B-3). This degree of amino acid identity can for example be determined by determining the degree of amino acid identity (in a manner described herein) between said amino acid sequence and one or more of the sequences of SEQ ID NOs: 39 to 43 and 91 as well as 99-102 (see Tables B-2 and B-3), in which the amino acid residues that form the framework regions are disregarded. Such polypeptides and/or immunoglobulin single variable domains of the invention may further provide the following:

1. polypeptides comprising at least one immunoglobulin single variable domain that is directed against (as defined herein) CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) and that has at least 80%, preferably at least 85%, such as 90% or 95% or more sequence identity with at least

one of the immunoglobulin single variable domains of SEQ ID NOs: 39 to 43 and 91 as well as 99-102 (see Table B-3);

2. polypeptides comprising at least one immunoglobulin single variable domain that is directed against (as defined herein) CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) and that cross-block (as defined herein) the binding of at least one of the immunoglobulin single variable domains of SEQ ID NOs: 39 to 43 and 91 as well as 99-102 (see Table B-3) to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) and/or that compete with at least one of the immunoglobulin single variable domains of SEQ ID NOs: 39 to 43 and 91 as well as 99-102 (see Table B-3) for binding to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1); and

3. which immunoglobulin single variable domains may be as further described herein; as well as polypeptides of the invention that comprise one or more of such immunoglobulin single variable domains (which may be as further described herein, and may for example be bispecific (*e.g.*, also bind to serum albumin) and/or biparatopic polypeptides as described herein), and nucleic acid sequences that encode such immunoglobulin single variable domains and polypeptides. Such immunoglobulin single variable domains and polypeptides do not include any naturally occurring ligands.

The polypeptides of the invention comprise or essentially consist of at least one immunoglobulin single variable domain of the invention. Some preferred, but non-limiting examples of immunoglobulin single variable domains of the invention are given in SEQ ID NOs: 39 to 43 and 91 as well as 99-102 (see Table B-3).

1.2. Serum albumin binding building blocks or other building blocks increasing half-life

In another aspect, the invention relates to a compound or construct, and in particular to a protein or polypeptide (also referred to herein as a "*compound of the invention*" or "*polypeptide of the invention*", respectively) that comprises or essentially consists of one or more (preferably one) immunoglobulin single variable domains directed to human CXCR7 (or suitable fragments thereof), and optionally further comprises one or more other groups, residues, moieties or binding units. As will become clear to the skilled person from the further disclosure herein, such further groups, residues, moieties, binding units or immunoglobulin single variable domains may or may not provide further functionality to the amino acid sequence of the invention (and/or to the compound or construct in which it is present) and may or may not modify the properties of the amino acid sequence of the invention.

As will be clear from the further description above and herein, this means that the immunoglobulin single variable domains of the invention can be used as "building blocks" to form polypeptides of the invention, i.e. by suitably combining them with other groups, residues, moieties or binding units, in order to form compounds or constructs as described herein (such as, without limitations, the biparatopic, bi/multivalent and bi/multispecific polypeptides of the invention described herein) which combine within one molecule one or more desired properties or biological functions.

The compounds or polypeptides of the invention can generally be prepared by a method which comprises at least one step of suitably linking the one or more immunoglobulin single variable domains of the invention to the one or more further groups, residues, moieties or binding units, optionally via the one or more suitable linkers, so as to provide the compound or polypeptide of the invention. Polypeptides of the invention can also be prepared by a method which generally comprises at least the steps of providing a nucleic acid that encodes a polypeptide of the invention, expressing said nucleic acid in a suitable manner, and recovering the expressed polypeptide of the invention. Such methods can be performed in a manner known per se, which will be clear to the skilled person, for example on the basis of the methods and techniques further described herein.

The process of designing/selecting and/or preparing a compound or polypeptide of the invention, starting from an amino acid sequence of the invention, is also referred to herein as "*formatting*" said amino acid sequence of the invention; and an amino acid of the invention that is made part of a compound or polypeptide of the invention is said to be "*formatted*" or to be "*in the format of*" said compound or polypeptide of the invention. Examples of ways in which an amino acid sequence of the invention can be formatted and examples of such formats will be clear to the skilled person based on the disclosure herein; and such formatted immunoglobulin single variable domains form a further aspect of the invention.

For example, such further groups, residues, moieties or binding units may be one or more additional immunoglobulin single variable domains, such that the compound or construct is a (fusion) protein or (fusion) polypeptide. In a preferred but non-limiting aspect, said one or more other groups, residues, moieties or binding units are immunoglobulin sequences. Even more preferably, said one or more other groups, residues, moieties or binding units are chosen from the group consisting of domain antibodies, immunoglobulin single variable domains that are suitable for use as a domain antibody, single domain antibodies, immunoglobulin single variable domains that are suitable for use as a single domain antibody, "dAb's", immunoglobulin single variable domains that are suitable for use as a dAb, or Nanobodies. Alternatively, such groups, residues, moieties or binding units may for example be chemical groups, residues, moieties, which may or may not by themselves be biologically and/or pharmacologically active. For example, and without limitation, such groups may be linked to

the one or more immunoglobulin single variable domains of the invention so as to provide a "derivative" of an amino acid sequence or polypeptide of the invention, as further described herein.

Also within the scope of the present invention are compounds or constructs, that comprises or essentially consists of one or more derivatives as described herein, and optionally further comprises one or more other groups, residues, moieties or binding units, optionally linked via one or more linkers. Preferably, said one or more other groups, residues, moieties or binding units are immunoglobulin single variable domains. In the compounds or constructs described above, the one or more immunoglobulin single variable domains of the invention and the one or more groups, residues, moieties or binding units may be linked directly to each other and/or via one or more suitable linkers or spacers. For example, when the one or more groups, residues, moieties or binding units are immunoglobulin single variable domains, the linkers may also be immunoglobulin single variable domains, so that the resulting compound or construct is a fusion (protein) or fusion (polypeptide).

In one specific, but non-limiting aspect of the invention, which will be further described herein, the polypeptides of the invention have an increased half-life in serum (as further described herein) compared to the immunoglobulin single variable domain from which they have been derived. For example, an immunoglobulin single variable domain of the invention may be linked (chemically or otherwise) to one or more groups or moieties that extend the half-life (such as PEG), so as to provide a derivative of an amino acid sequence of the invention with increased half-life.

In one specific aspect of the invention, a compound of the invention or a polypeptide of the invention may have an increased half-life, compared to the corresponding amino acid sequence of the invention. Some preferred, but non-limiting examples of such compounds and polypeptides will become clear to the skilled person based on the further disclosure herein, and for example comprise immunoglobulin single variable domains or polypeptides of the invention that have been chemically modified to increase the half-life thereof (for example, by means of pegylation); immunoglobulin single variable domains of the invention that comprise at least one additional binding site for binding to a serum protein (such as serum albumin); or polypeptides of the invention that comprise at least one amino acid sequence of the invention that is linked to at least one moiety (and in particular at least one amino acid sequence) that increases the half-life of the amino acid sequence of the invention. Examples of polypeptides of the invention that comprise such half-life extending moieties or immunoglobulin single variable domains will become clear to the skilled person based on the further disclosure herein; and for example include, without limitation, polypeptides in which the one or more immunoglobulin single variable domains of the invention are suitably linked to one or more serum proteins or fragments thereof (such as (human) serum albumin or suitable fragments thereof)

or to one or more binding units that can bind to serum proteins (such as, for example, domain antibodies, immunoglobulin single variable domains that are suitable for use as a domain antibody, single domain antibodies, immunoglobulin single variable domains that are suitable for use as a single domain antibody, "dAb's", immunoglobulin single variable domains that are suitable for use as a dAb, or Nanobodies that can bind to serum proteins such as serum albumin (such as human serum albumin), serum immunoglobulins such as IgG, or transferrin; reference is made to the further description and references mentioned herein); polypeptides in which an amino acid sequence of the invention is linked to an Fc portion (such as a human Fc) or a suitable part or fragment thereof; or polypeptides in which the one or more immunoglobulin single variable domains of the invention are suitable linked to one or more small proteins or peptides that can bind to serum proteins (such as, without limitation, the proteins and peptides described in WO 91/01743, WO 01/45746, WO 02/076489, WO2008/068280, WO2009/127691).

Generally, the compounds or polypeptides of the invention with increased half-life preferably have a half-life that is at least 1.5 times, preferably at least 2 times, such as at least 5 times, for example at least 10 times or more than 20 times, greater than the half-life of the corresponding amino acid sequence of the invention per se. For example, the compounds or polypeptides of the invention with increased half-life may have a half-life *e.g.*, in humans that is increased with more than 1 hours, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the corresponding amino acid sequence of the invention per se.

In a preferred, but non-limiting aspect of the invention, such compounds or polypeptides of the invention have a serum half-life *e.g.*, in humans that is increased with more than 1 hours, preferably more than 2 hours, more preferably more than 6 hours, such as more than 12 hours, or even more than 24, 48 or 72 hours, compared to the corresponding amino acid sequence of the invention *per se*.

In another preferred, but non-limiting aspect of the invention, such compounds or polypeptides of the invention exhibit a serum half-life in human of at least about 12 hours, preferably at least 24 hours, more preferably at least 48 hours, even more preferably at least 72 hours or more. For example, compounds or polypeptides of the invention may have a half-life of at least 5 days (such as about 5 to 10 days), preferably at least 9 days (such as about 9 to 14 days), more preferably at least about 10 days (such as about 10 to 15 days), or at least about 11 days (such as about 11 to 16 days), more preferably at least about 12 days (such as about 12 to 18 days or more), or more than 14 days (such as about 14 to 19 days).

In a particular preferred but non-limiting aspect of the invention, the invention provides a polypeptide of the invention comprising i) one CXCR7 binding immunoglobulin single variable domain as described herein; and ii) one or more (preferably one) serum albumin binding immunoglobulin single variable domain as described herein.

In a further preferred aspect, the invention provides a polypeptide of the invention comprising i) one or more CXCR7 binding immunoglobulin single variable domain as described herein; and ii) one or more (preferably one) serum albumin binding immunoglobulin single variable domain of SEQ ID NO: 2 (Table B-1).

In a further preferred aspect, the invention provides a polypeptide of the invention comprising i) one or more CXCR7 binding immunoglobulin single variable domain as described herein; and ii) one or more (preferably one) serum albumin binding immunoglobulin single variable domain with CDRs (defined according to the Kabat numbering) of SEQ ID NO: 2 (Table B-2, B-1).

Thus, for example, further reference (and thus incorporated by reference) is made in particular to the experimental part and further description of WO2008/068280, wherein further details on SEQ ID NO: 2 is made and *e.g.*, the half-life of a immunoglobulin single variable domain construct containing said sequence in rhesus monkeys is disclosed.

Generally, proteins or polypeptides that comprise or essentially consist of a single immunoglobulin single variable domain will be referred to herein as "monovalent" proteins or polypeptides or as "monovalent constructs". Proteins and polypeptides that comprise or essentially consist of two or more immunoglobulin single variable domains (such as at least two immunoglobulin single variable domains of the invention or at least one immunoglobulin single variable domain of the invention and at least one other immunoglobulin single variable domain) will be referred to herein as "multivalent" proteins or polypeptides or as "multivalent constructs", and these may provide certain advantages compared to the corresponding monovalent immunoglobulin single variable domains of the invention. Some non-limiting examples of such multivalent constructs will become clear from the further description herein.

According to another specific, but non-limiting aspect, a polypeptide of the invention comprises or essentially consists of at least one immunoglobulin single variable domain of the invention and at least one other binding unit (i.e. directed against another epitope, antigen, target, protein or polypeptide), which is preferably also a immunoglobulin single variable domain. Such proteins or polypeptides are also referred to herein as "multispecific" proteins or polypeptides or as "multispecific constructs", and these may comprise of two immunoglobulin single variable domains of the invention, such as one immunoglobulin single variable domain directed against CXCR7 and one

immunoglobulin single variable domain against serum albumin. Such multispecific constructs will be clear to the skilled person based on the disclosure herein; some preferred, but non-limiting examples of such multispecific immunoglobulin single variable domains are the constructs of SEQ ID NOs: 44 to 48, 80-81, 83-85 and 88-89 as well as 131-140 (see Table B-4), as well as clones 009, 013, 018-029, 031-038, 044, 046, 048-053, 055-058, 060, 061, 063, 065, 068, 069, 072, 081-086 and 093 (Tables B-12 to B-14).

According to yet another specific, but non-limiting aspect, a polypeptide of the invention comprises or essentially consists of at least one immunoglobulin single variable domain of the invention, optionally one or more further immunoglobulin single variable domains, and at least one other amino acid sequence (such as a protein or polypeptide) that confers at least one desired property to the immunoglobulin single variable domain of the invention and/or to the resulting fusion protein. Again, such fusion proteins may provide certain advantages compared to the corresponding monovalent immunoglobulin single variable domains of the invention such as e.g. may provide an increased half-life.

In the above constructs, the one or more immunoglobulin single variable domains and/or other immunoglobulin single variable domains may be directly linked to each other and/or suitably linked to each other via one or more linker sequences. Some suitable but non-limiting examples of such linkers will become clear from the further description herein.

In one embodiment, the linker sequence joining the immunoglobulin single variable domains are SEQ ID NOs: 49 to 58 – see Table B-5, or a combination of both, or as known in the art.

According to yet another specific, but non-limiting aspect, a polypeptide of the invention may for example be chosen from the group consisting of immunoglobulin single variable domains that have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more "sequence identity" (as defined herein) with one or more of the immunoglobulin single variable domains of SEQ ID NOs: 39 to 43 and 91 as well as 99-102 (see Table B-3), in which the polypeptides are preferably as further defined herein, *i.e.*, in the preferred format of one immunoglobulin single variable domain directed against CXCR7 and one immunoglobulin singe variable domain directed against serum albumin.

According to yet another specific, but non-limiting aspect, a polypeptide of the invention may for example be chosen from the group consisting of polypeptides that have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more "sequence identity" (as defined herein) with one or more of the polypeptides of SEQ ID NOs: 44 to 48 (see Table B-4). Some illustrative non-limiting examples of biparatopic and bispecific polypeptides of the invention are

given in SEQ ID NOs: 78 to 89 as well as SEQ ID NOs: 131-140, or clones 009, 013, 018-029, 031-038, 044, 046, 048-053, 055-058, 060, 061, 063, 065, 068, 069, 072, 081-086 and 093 (Tables B-12 to B-14).

1.3. Compositions of the invention

Generally, for pharmaceutical use, the polypeptides of the invention may be formulated as a pharmaceutical preparation or composition comprising at least one polypeptide of the invention and at least one pharmaceutically acceptable carrier, diluent or excipient and/or adjuvant, and optionally one or more further pharmaceutically active polypeptides and/or compounds. By means of non-limiting examples, such a formulation may be in a form suitable for oral administration, for parenteral administration (such as by intravenous, intramuscular or subcutaneous injection or intravenous infusion), for topical administration, for administration by inhalation, by a skin patch, by an implant, by a suppository, etc. wherein which the parenteral administration is preferred. Such suitable administration forms - which may be solid, semi-solid or liquid, depending on the manner of administration - as well as methods and carriers for use in the preparation thereof, will be clear to the skilled person, and are further described herein. Such a pharmaceutical preparation or composition will generally be referred to herein as a "pharmaceutical composition". A pharmaceutical preparation or composition for use in a non-human organism will generally be referred to herein as a "veterinary composition".

Thus, in a further aspect, the invention relates to a pharmaceutical composition that contains at least one amino acid of the invention, at least one polypeptide of the invention or at least one polypeptide of the invention and at least one suitable carrier, diluent or excipient (*i.e.*, suitable for pharmaceutical use), and optionally one or more further active substances.

Generally, the polypeptides of the invention can be formulated and administered in any suitable manner known *per se*. Reference is for example made to the general background art cited above (and in particular to WO 04/041862, WO 04/041863, WO 04/041865, WO 04/041867 and WO 08/020079) as well as to the standard handbooks, such as Remington's Pharmaceutical Sciences, 18th Ed., Mack Publishing Company, USA (1990), Remington, the Science and Practice of Pharmacy, 21th Edition, Lippincott Williams and Wilkins (2005); or the Handbook of Therapeutic Antibodies (S. Dubel, Ed.), Wiley, Weinheim, 2007 (see for example pages 252-255).

The polypeptides of the invention may be formulated and administered in any manner known per se for conventional antibodies and antibody fragments (including ScFv's and diabodies) and other pharmaceutically active proteins. Such formulations and methods for preparing the same will be

clear to the skilled person, and for example include preparations suitable for parenteral administration (for example intravenous, intraperitoneal, subcutaneous, intramuscular, intraluminal, intra-arterial or intrathecal administration) or for topical (*i.e.,* transdermal or intradermal) administration.

Preparations for parenteral administration may for example be sterile solutions, suspensions, dispersions or emulsions that are suitable for infusion or injection. Suitable carriers or diluents for such preparations for example include, without limitation, those mentioned on page 143 of WO 08/020079. In one embodiment, the preparation is an aqueous solution or suspension.

The polypeptides of the invention can be administered using gene therapy methods of delivery. See, *e.g.,* U.S. Patent No. 5,399,346, which is incorporated by reference for its gene therapy delivery methods. Using a gene therapy method of delivery, primary cells transfected with the gene encoding an amino acid sequence, polypeptide of the invention can additionally be transfected with tissue specific promoters to target specific organs, tissue, grafts, tumors, or cells and can additionally be transfected with signal and stabilization sequences for subcellularly localized expression.

Thus, the polypeptides of the invention may be systemically administered, *e.g.,* orally, in combination with a pharmaceutically acceptable vehicle such as an inert diluent or an assimilable edible carrier. They may be enclosed in hard or soft shell gelatin capsules, may be compressed into tablets, or may be incorporated directly with the food of the patient's diet. For oral therapeutic administration, the polypeptides of the invention may be combined with one or more excipients and used in the form of ingestible tablets, buccal tablets, troches, capsules, elixirs, suspensions, syrups, wafers, and the like. Such compositions and preparations should contain at least 0.1% of the polypeptide of the invention. Their percentage in the compositions and preparations may, of course, be varied and may conveniently be between about 2 to about 60% of the weight of a given unit dosage form. The amount of the polypeptide of the invention in such therapeutically useful compositions is such that an effective dosage level will be obtained.

For local administration at the site of tumor resection, the polypeptides of the invention may be used in biodegradable polymeric drug delivery systems, slow release poly(lactic-co-glycolic acid formulations and the like (Hart et al., Cochrane Database Syst Rev. 2008 Jul 16; (3): CD007294).

In a further preferred aspect of the invention, the polypeptides of the invention, such as a polypeptide consisting essentially of one monovalent anti-human CXCR7 immunoglobulin single variable domain and of one monovalent anti-human serum albumin immunoglobulin single variable domain linked by a GS linker, may have a beneficial distribution and kinetics profile in solid tumors compared to conventional antibodies such as *e.g.,* IgG.

The tablets, troches, pills, capsules, and the like may also contain binders, excipients, disintegrating agents, lubricants and sweetening or flavoring agents, for example those mentioned on pages 143-144 of WO 08/020079. When the unit dosage form is a capsule, it may contain, in addition to materials of the above type, a liquid carrier, such as a vegetable oil or a polyethylene glycol. Various other materials may be present as coatings or to otherwise modify the physical form of the solid unit dosage form. For instance, tablets, pills, or capsules may be coated with gelatin, wax, shellac or sugar and the like. A syrup or elixir may contain the polypeptides of the invention, sucrose or fructose as a sweetening agent, methyl and propylparabens as preservatives, a dye and flavoring such as cherry or orange flavor. Of course, any material used in preparing any unit dosage form should be pharmaceutically acceptable and substantially non-toxic in the amounts employed. In addition, the polypeptides of the invention may be incorporated into sustained-release preparations and devices.

Preparations and formulations for oral administration may also be provided with an enteric coating that will allow the constructs of the invention to resist the gastric environment and pass into the intestines. More generally, preparations and formulations for oral administration may be suitably formulated for delivery into any desired part of the gastrointestinal tract. In addition, suitable suppositories may be used for delivery into the gastrointestinal tract.

The polypeptides of the invention may also be administered intravenously or intraperitoneally by infusion or injection. Particular examples are as further described on pages 144 and 145 of WO 08/020079.

For topical administration, the polypeptides of the invention may be applied in pure form, i.e., when they are liquids. However, it will generally be desirable to administer them to the skin as compositions or formulations, in combination with a dermatologically acceptable carrier, which may be a solid or a liquid. Particular examples are as further described on page 145 of WO 08/020079.

Generally, the concentration of the polypeptides of the invention in a liquid composition, such as a lotion, will be from about 0.1-25 wt-%, preferably from about 0.5-10 wt-%. The concentration in a semi-solid or solid composition such as a gel or a powder will be about 0.1-5 wt-%, preferably about 0.5-2.5 wt-%.

The amount of the polypeptides of the invention required for use in treatment will vary not only with the particular polypeptide selected but also with the route of administration, the nature of the condition being treated and the age and condition of the patient and will be ultimately at the discretion of the attendant physician or clinician. Also the dosage of the polypeptides of the invention varies depending on the target cell, tumor, tissue, graft, or organ.

The desired dose may conveniently be presented in a single dose or as divided doses administered at appropriate intervals, for example, as two, three, four or more sub-doses per day. The sub-dose itself may be further divided, *e.g.*, into a number of discrete loosely spaced administrations.

An administration regimen could include long-term, daily treatment. By "long-term" is meant at least two weeks and preferably, several weeks, months, or years of duration. Necessary modifications in this dosage range may be determined by one of ordinary skill in the art using only routine experimentation given the teachings herein. See Remington's Pharmaceutical Sciences (Martin, E.W., ed. 4), Mack Publishing Co., Easton, PA. The dosage can also be adjusted by the individual physician in the event of any complication.

In another aspect, the invention relates to a method for the prevention and/or treatment of at least one diseases and disorders associated with CXCR7, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same.

In the context of the present invention, the term "prevention and/or treatment" not only comprises preventing and/or treating the disease, but also generally comprises preventing the onset of the disease, slowing or reversing the progress of disease, preventing or slowing the onset of one or more symptoms associated with the disease, reducing and/or alleviating one or more symptoms associated with the disease, reducing the severity and/or the duration of the disease and/or of any symptoms associated therewith and/or preventing a further increase in the severity of the disease and/or of any symptoms associated therewith, preventing, reducing or reversing any physiological damage caused by the disease, and generally any pharmacological action that is beneficial to the patient being treated.

The subject to be treated may be any warm-blooded animal, but is in particular a mammal, and more in particular a human being. As will be clear to the skilled person, the subject to be treated will in particular be a person suffering from, or at risk of, the diseases and disorders mentioned herein.

The invention relates to a method for the prevention and/or treatment of at least one disease or disorder that is associated with CXCR7, with its biological or pharmacological activity, and/or with the biological pathways or signaling in which CXCR7 is involved, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of an amino acid sequence of the invention, of a Polypeptide of the invention, of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same. In one embodiment, the invention relates to a method for the prevention and/or treatment of at least one disease or disorder that can be treated by modulating CXCR7, its biological or pharmacological activity, and/or the biological pathways or

signaling in which CXCR7 is involved, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of a polypeptide of the invention, and/or of a pharmaceutical composition comprising the same. In one embodiment, said pharmaceutically effective amount may be an amount that is sufficient to modulate CXCR7, its biological or pharmacological activity, and/or the biological pathways or signaling in which CXCR7 is involved; and/or an amount that provides a level of the polypeptide of the invention in the circulation that is sufficient to modulate CXCR7, its biological or pharmacological activity, and/or the biological pathways or signaling in which CXCR7 is involved.

In one embodiment the invention relates to a method for the prevention and/or treatment of at least one disease or disorder that can be prevented and/or treated by administering a polypeptide of the invention, or a nucleotide construct of the invention encoding the same, and/or of a pharmaceutical composition comprising the same, to a patient. In one embodiment, the method comprises administering a pharmaceutically active amount of a polypeptide of the invention, or a nucleotide construct of the invention encoding the same, and/or of a pharmaceutical composition comprising the same to a subject in need thereof.

In one embodiment the invention relates to a method for the prevention and/or treatment of at least one disease or disorder that can be prevented and/or treated by inhibiting binding of CXCL12 and/or CXCL11 to CXCR7 in specific cells or in a specific tissue of a subject to be treated (and in particular, by inhibiting binding of CXCL12 and/or CXCL11 to CXCR7 in cancer cells or in a tumor present in the subject to be treated), said method comprising administering a pharmaceutically active amount of a polypeptide of the invention, or a nucleotide construct of the invention encoding the same, and/or of a pharmaceutical composition comprising the same, to a subject in need thereof.

In one embodiment, the invention relates to a method for the prevention and/or treatment of at least one disease or disorder chosen from the group consisting of the diseases and disorders listed herein, said method comprising administering, to a subject in need thereof, a polypeptide of the invention, or a nucleotide construct of the invention encoding the same, and/or of a pharmaceutical composition comprising the same.

In one embodiment, the invention relates to a method for immunotherapy, and in particular for passive immunotherapy, which method comprises administering, to a subject suffering from or at risk of the diseases and disorders mentioned herein, a pharmaceutically active amount of a polypeptide of the invention, or a nucleotide construct of the invention encoding the same, and/or of a pharmaceutical composition comprising the same.

In the above methods, the amino acid sequences, polypeptides of the invention and/or the compositions comprising the same can be administered in any suitable manner, depending on the specific pharmaceutical formulation or composition to be used. Thus, the polypeptides of the invention and/or the compositions comprising the same can for example be administered orally, intraperitoneally (e.g. intravenously, subcutaneously, intramuscularly, or via any other route of administration that circumvents the gastrointestinal tract), intranasally, transdermally, topically, by means of a suppository, by inhalation, again depending on the specific pharmaceutical formulation or composition to be used. The clinician will be able to select a suitable route of administration and a suitable pharmaceutical formulation or composition to be used in such administration, depending on the disease or disorder to be prevented or treated and other factors well known to the clinician.

The polypeptides of the invention and/or the compositions comprising the same are administered according to a regime of treatment that is suitable for preventing and/or treating the disease or disorder to be prevented or treated. The clinician will generally be able to determine a suitable treatment regimen, depending on factors such as the disease or disorder to be prevented or treated, the severity of the disease to be treated and/or the severity of the symptoms thereof, the polypeptide of the invention to be used, the specific route of administration and pharmaceutical formulation or composition to be used, the age, gender, weight, diet, general condition of the patient, and similar factors well known to the clinician.

Generally, the treatment regimen will comprise the administration of one or more polypeptides of the invention, or of one or more compositions comprising the same, in one or more pharmaceutically effective amounts or doses. The specific amount(s) or doses to be administered can be determined by the clinician, again based on the factors cited above.

Generally, for the prevention and/or treatment of the diseases and disorders mentioned herein and depending on the specific disease or disorder to be treated, the potency of the specific polypeptide of the invention to be used, the specific route of administration and the specific pharmaceutical formulation or composition used, the polypeptides of the invention will generally be administered in an amount between 1 gram and 0.01 microgram per kg body weight per day, preferably between 0.1 gram and 0.1 microgram per kg body weight per day, such as about 1, 10, 100 or 1000 microgram per kg body weight per day, either continuously (*e.g.*, by infusion), as a single daily dose or as multiple divided doses during the day. The clinician will generally be able to determine a suitable daily dose, depending on the factors mentioned herein. It will also be clear that in specific cases, the clinician may choose to deviate from these amounts, for example on the basis of the factors cited above and his expert judgment. Generally, some guidance on the amounts to be administered can be obtained from the amounts usually administered for comparable conventional antibodies or antibody

fragments against the same target administered via essentially the same route, taking into account however differences in affinity/avidity, efficacy, biodistribution, half-life and similar factors well known to the skilled person.

In one embodiment, a single contiguous polypeptide of the invention will be used. In one embodiment two or more polypeptides of the invention are provided in combination.

The polypeptides of the invention may be used in combination with one or more further pharmaceutically active compounds or principles, *i.e.*, as a combined treatment regimen, which may or may not lead to a synergistic effect. Again, the clinician will be able to select such further compounds or principles, as well as a suitable combined treatment regimen, based on the factors cited above and his expert judgment.

In particular, the polypeptides of the invention may be used in combination with other pharmaceutically active compounds or principles that are or can be used for the prevention and/or treatment of the diseases and disorders cited herein, as a result of which a synergistic effect may or may not be obtained. Examples of such compounds and principles, as well as routes, methods and pharmaceutical formulations or compositions for administering them will be clear to the clinician, and generally include the cytostatic active principles usually applied for the treatment of the tumor to be treated.

Specific contemplated combinations for use with the polypeptides of the invention for oncology include, but are not limited to, *e.g.*, CXCR4 antagonists such as *e.g.*, AMD3100, other chemokine receptor antagonists, taxol; gemcitobine; cisplatin; cIAP inhibitors (such as inhibitors to cIAP1, cIAP2 and/or XIAP); MEK inhibitors including but not limited to, *e.g.*, U0126, PD0325901; bRaf inhibitors including but not limited to, *e.g.*, RAF265; and mTOR inhibitors including but not limited to, *e.g.*, RAD001; VEGF inhibitors including but not limited to e.g. bevacizumab, sutinib and sorafenib; Her 2 inhibitors including but not limited to *e.g.*, trastuzumab and lapatinib; PDGFR, FGFR, src, JAK, STAT and/or GSK3 inhibitors; selective estrogen receptor modulators including but not limited to tamoxifen; estrogen receptor downregulators including but not limited to fulvestrant. Specific contemplated combinations for use with the polypeptides of the invention for inflammatory conditions include, but are not limited to, *e.g.*, interferon beta 1 alpha and beta, natalizumab; TNF alpha antagonists including but not limited to *e.g.*, infliximab, adalimumab, certolizumab pegol, etanercept; disease-modifying antirheumatic drugs such as *e.g.*, methotrexate (MTX); glucocortioids including but not limited to e.g. hydrocortisone; Nonsteroidal anti-inflammatory drugs including but not limited to *e.g.*, ibuprofen, sulindac.

Other specific compounds/polypeptides that could be used in combination (therapy) with the compounds/polypeptides of the invention are the amino acid sequences and polypeptides directed against CXCR4 that are described in the international application WO 09/138519 by Ablynx N.V. , the non-prepublished US application 61/358,495 by Ablynx N.V. filed on June 25, 2010; the PCT application PCT/EP210/064766 by Ablynx N.V. filed on October 4, 2010; and/or the PCT application PCT/EP2011/050156 by Ablynx N.V. filed on January 7, 2011.

When two or more substances or principles are to be used as part of a combined treatment regimen, they can be administered via the same route of administration or via different routes of administration, at essentially the same time or at different times (*e.g.*, essentially simultaneously, consecutively, or according to an alternating regime). When the substances or principles are to be administered simultaneously via the same route of administration, they may be administered as different pharmaceutical formulations or compositions or part of a combined pharmaceutical formulation or composition, as will be clear to the skilled person.

Also, when two or more active substances or principles are to be used as part of a combined treatment regimen, each of the substances or principles may be administered in the same amount and according to the same regimen as used when the compound or principle is used on its own, and such combined use may or may not lead to a synergistic effect. However, when the combined use of the two or more active substances or principles leads to a synergistic effect, it may also be possible to reduce the amount of one, more or all of the substances or principles to be administered, while still achieving the desired therapeutic action. This may for example be useful for avoiding, limiting or reducing any unwanted side-effects that are associated with the use of one or more of the substances or principles when they are used in their usual amounts, while still obtaining the desired pharmaceutical or therapeutic effect.

The effectiveness of the treatment regimen used according to the invention may be determined and/or followed in any manner known per se for the disease or disorder involved, as will be clear to the clinician. The clinician will also be able, where appropriate and on a case-by-case basis, to change or modify a particular treatment regimen, so as to achieve the desired therapeutic effect, to avoid, limit or reduce unwanted side-effects, and/or to achieve an appropriate balance between achieving the desired therapeutic effect on the one hand and avoiding, limiting or reducing undesired side effects on the other hand.

Generally, the treatment regimen will be followed until the desired therapeutic effect is achieved and/or for as long as the desired therapeutic effect is to be maintained. Again, this can be determined by the clinician.

In another aspect, the invention relates to the use of polypeptide of the invention in the preparation of a pharmaceutical composition for prevention and/or treatment of at least one of the diseases and disorders associated with CXCR7; and/or for use in one or more of the methods of treatment mentioned herein.

The subject to be treated may be any warm-blooded animal, but is in particular a mammal, and more in particular a human being. In veterinary applications, the subject to be treated includes any animal raised for commercial purposes or kept as a pet. As will be clear to the skilled person, the subject to be treated will in particular be a person suffering from, or at risk of, the diseases and disorders mentioned herein.

The invention relates to the use of a polypeptide of the invention, or a nucleotide encoding the same, in the preparation of a pharmaceutical composition for the prevention and/or treatment of at least one disease or disorder that can be prevented and/or treated by administering a polypeptide of the invention, or a nucleotide encoding the same, and/or a pharmaceutical composition of the same to a patient.

More in particular, the invention relates to the use of a polypeptide of the invention, or a nucleotide encoding the same, in the preparation of a pharmaceutical composition for the prevention and/or treatment of diseases and disorders associated with CXCR7, and in particular for the prevention and treatment of one or more of the diseases and disorders listed herein.

Again, in such a pharmaceutical composition, the one or more polypeptide of the invention, or nucleotide encoding the same, and/or a pharmaceutical composition of the same, may also be suitably combined with one or more other active principles, such as those mentioned herein.

The invention also relates to a composition (such as, without limitation, a pharmaceutical composition or preparation as further described herein) for use, either *in vitro* (*e.g.*, in an in vitro or cellular assay) or *in vivo* (*e.g.*, in an a single cell or multicellular organism, and in particular in a mammal, and more in particular in a human being, such as in a human being that is at risk of or suffers from a disease or disorder of the invention).

In the context of the present invention, "modulating" or "to modulate" generally means reducing or inhibiting the activity of CXCR7 and in particular human CXCR7 (SEQ ID NO: 1), as measured using a suitable in vitro, cellular or in vivo assay (such as those mentioned herein). In particular, reducing or inhibiting the activity of CXCR7 and in particular human CXCR7 (SEQ ID NO: 1), as measured using a suitable in vitro, cellular or in vivo assay (such as those mentioned herein), by at least 1%, preferably at least 5%, such as at least 10% or at least 25%, for example by at least 50%, at least 60%, at least 70%, at least 80%, or 90% or more, compared to activity of CXCR7 and in particular human CXCR7

(SEQ ID NO: 1) in the same assay under the same conditions but without the presence of the polypeptide of the invention.

Modulating may for example involve reducing or inhibiting the binding CXCR7 to one of its substrates or ligands and/or competing with natural ligands (CXCL11 and/or CXCL12), substrate for binding to CXCR7.

1.4. Generation of the polypeptides of the invention

The invention further relates to methods for preparing or generating the immunoglobulin single variable domains, polypeptides, nucleic acids, host cells, products and compositions described herein. Some preferred but non-limiting examples of such methods will become clear from the further description herein.

Generally, these methods may comprise the steps of:

a) providing a set, collection or library of immunoglobulin single variable domains; and

b) screening said set, collection or library of immunoglobulin single variable domains for immunoglobulin single variable domains that can bind to and/or have affinity for CXCR7 and in particular human CXCR7 (SEQ ID NO: 1); and

c) isolating the amino acid sequence(s) that can bind to and/or have affinity for CXCR7 and in particular human CXCR7 (SEQ ID NO: 1).

In such a method, the set, collection or library of immunoglobulin single variable domains may be any suitable set, collection or library of immunoglobulin single variable domains. For example, the set, collection or library of immunoglobulin single variable domains may be a set, collection or library of immunoglobulin sequences (as described herein), such as a naïve set, collection or library of immunoglobulin sequences; a synthetic or semi-synthetic set, collection or library of immunoglobulin sequences; and/or a set, collection or library of immunoglobulin sequences that have been subjected to affinity maturation.

Also, in such a method, the set, collection or library of immunoglobulin single variable domains may be a set, collection or library of heavy or light chain variable domains (such as VL-, VH- or VHH domains). For example, the set, collection or library of immunoglobulin single variable domains may be a set, collection or library of domain antibodies or single domain antibodies, or may be a set, collection or library of immunoglobulin single variable domains that are capable of functioning as a domain antibody or single domain antibody.

In a preferred aspect of this method, the set, collection or library of immunoglobulin single variable domains may be an immune set, collection or library of immunoglobulin sequences, for example derived from a mammal that has been suitably immunized with CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) or with a suitable antigenic determinant based thereon or derived therefrom, such as an antigenic part, fragment, region, domain, loop or other epitope thereof. In one particular aspect, said antigenic determinant may be an extracellular part, region, domain, loop or other extracellular epitope(s).

In the above methods, the set, collection or library of immunoglobulin single variable domains may be displayed on a phage, phagemid, ribosome or suitable micro-organism (such as yeast), such as to facilitate screening. Suitable methods, techniques and host organisms for displaying and screening (a set, collection or library of) immunoglobulin single variable domains will be clear to the person skilled in the art, for example on the basis of the further disclosure herein. Reference is also made to the review by Hoogenboom in Nature Biotechnology, 23, 9, 1105-1116 (2005).

In another aspect, the method for generating immunoglobulin single variable domains comprises at least the steps of:

a) providing a collection or sample of cells expressing immunoglobulin single variable domains;

b) screening said collection or sample of cells for cells that express an amino acid sequence that can bind to and/or have affinity for CXCR7 and in particular human CXCR7 (SEQ ID NO: 1); and

c) either (i) isolating said amino acid sequence; or (ii) isolating from said cell a nucleic acid sequence that encodes said amino acid sequence, followed by expressing said amino acid sequence.

In another aspect, the method for generating an amino acid sequence directed against CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) may comprise at least the steps of:

a) providing a set, collection or library of nucleic acid sequences encoding immunoglobulin single variable domains;

b) screening said set, collection or library of nucleic acid sequences for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for CXCR7 and in particular human CXCR7 (SEQ ID NO: 1); and

c) isolating said nucleic acid sequence, followed by expressing said amino acid sequence.

In such a method, the set, collection or library of nucleic acid sequences encoding immunoglobulin single variable domains may for example be a set, collection or library of nucleic acid sequences encoding a naïve set, collection or library of immunoglobulin sequences; a set, collection or library of nucleic acid sequences encoding a synthetic or semi-synthetic set, collection or library of

immunoglobulin sequences; and/or a set, collection or library of nucleic acid sequences encoding a set, collection or library of immunoglobulin sequences that have been subjected to affinity maturation.

In another aspect, the method for generating an amino acid sequence directed against CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) may comprise at least the steps of:

a) providing a set, collection or library of nucleic acid sequences encoding immunoglobulin single variable domains;

b) screening said set, collection or library of nucleic acid sequences for nucleic acid sequences that encode an amino acid sequence that can bind to and/or has affinity for CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) and that is cross-blocked or is cross blocking a immunoglobulin single variable domain or polypeptide of the invention, *e.g.*, SEQ ID NOs: 39 to 43, 91 or 99-102 (Table B-3); and

c) isolating said nucleic acid sequence, followed by expressing said amino acid sequence.

The invention also relates to immunoglobulin single variable domains that are obtained by the above methods, or alternatively by a method that comprises the one of the above methods and in addition at least the steps of determining the nucleotide sequence or amino acid sequence of said immunoglobulin sequence; and of expressing or synthesizing said amino acid sequence in a manner known per se, such as by expression in a suitable host cell or host organism or by chemical synthesis.

Also, following the steps above, one or more immunoglobulin single variable domains of the invention may be suitably humanized, camelized or otherwise sequence optimized (e.g. sequence optimized for manufacturability, stability and/or solubility); and/or the amino acid sequence(s) thus obtained may be linked to each other or to one or more other suitable immunoglobulin single variable domains (optionally via one or more suitable linkers) so as to provide a polypeptide of the invention. Also, a nucleic acid sequence encoding an amino acid sequence of the invention may be suitably humanized, camelized or otherwise sequence optimized (*e.g.*, sequence optimized for manufacturability, stability and/or solubility) and suitably expressed; and/or one or more nucleic acid sequences encoding an amino acid sequence of the invention may be linked to each other or to one or more nucleic acid sequences that encode other suitable immunoglobulin single variable domains (optionally via nucleotide sequences that encode one or more suitable linkers), after which the nucleotide sequence thus obtained may be suitably expressed so as to provide a polypeptide of the invention.

The invention further relates to applications and uses of the immunoglobulin single variable domains, compounds, constructs, polypeptides, nucleic acids, host cells, products and compositions described

herein, as well as to methods for the diagnosis, prevention and/or treatment for diseases and disorders associated with CXCR7 and in particular human CXCR7 (SEQ ID NO: 1). Some preferred but non-limiting applications and uses will become clear from the further description herein.

The invention also relates to the immunoglobulin single variable domains, compounds, constructs, polypeptides, nucleic acids, host cells, products and compositions described herein for use in therapy.

In particular, the invention also relates to the immunoglobulin single variable domains, compounds, constructs, polypeptides, nucleic acids, host cells, products and compositions described herein for use in therapy of a disease or disorder that can be prevented or treated by administering, to a subject in need thereof, of (a pharmaceutically effective amount of) an amino acid sequence, compound, construct or polypeptide as described herein.

More in particular, the invention relates to the immunoglobulin single variable domains, compounds, constructs, polypeptides, nucleic acids, host cells, products and compositions described herein for use in therapy of cancer.

1.5. Variants of polypeptides and immunoglobulin single variable domains of the invention

Polypeptides of the invention and immunoglobulin single variable domains (that form part of the polypeptides of the invention) may be altered in order to further improve potency or other desired properties.

Generally, an immunoglobulin single variable domain can be defined as a polypeptide with the formula 1:

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4

in which FR1 to FR4 refer to framework regions 1 to 4, respectively, and in which CDR1 to CDR3 refer to the complementarity determining regions 1 to 3, respectively.

Some particularly preferred, but non-limiting combinations of CDR sequences, as well as preferred combinations of CDR sequences and framework sequences, are mentioned in Table B-2, which lists the CDR sequences and framework sequences that are present in a number of preferred (but non-limiting) immunoglobulin single variable domains of the invention. As will be clear to the skilled person, a combination of CDR1, CDR2 and CDR3 sequences that occur in the same clone (i.e. CDR1, CDR2 and CDR3 sequences that are mentioned on the same line or row in Table B-2) will usually be preferred (although the invention in its broadest sense is not limited thereto, and also comprises other suitable combinations of the CDR sequences mentioned in Table B-2). Also, a combination of

CDR sequences and framework sequences that occur in the same clone (*i.e.*, CDR sequences and framework sequences that are mentioned on the same line or row in Table B-2) will usually be preferred (although the invention in its broadest sense is not limited thereto, and also comprises other suitable combinations of the CDR sequences and framework sequences mentioned in Table B-2, as well as combinations of such CDR sequences and other suitable framework sequences, *e.g.*, as further described herein).

Also, in the immunoglobulin single variable domains of the invention that comprise the combinations of CDR's mentioned in Table B-2, each CDR can be replaced by a CDR chosen from the group consisting of immunoglobulin single variable domains that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity (as defined herein) with the mentioned CDR's; in which:

i) any amino acid substitution in such a CDR is preferably, and compared to the corresponding CDR sequence mentioned in Table B-2, a conservative amino acid substitution (as defined herein); and/or

ii) any such CDR sequence preferably only contains amino acid substitutions, and no amino acid deletions or insertions, compared to the corresponding CDR sequence mentioned in Table B-2; and/or

iii) any such CDR sequence is a CDR that is derived by means of a technique for affinity maturation known per se, and in particular starting from the corresponding CDR sequence mentioned in Table B-2.

However, as will be clear to the skilled person, the (combinations of) CDR sequences, as well as (the combinations of) CDR sequences and framework sequences mentioned in Table B-2 will generally be preferred.

Thus, in the immunoglobulin single variable domains of the invention, at least one of the CDR1, CDR2 and CDR3 sequences present is suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-2; or from the group of CDR1, CDR2 and CDR3 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% "sequence identity" (as defined herein) with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-2; and/or from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, that have 3, 2 or only 1 "amino acid difference(s)" (as defined herein) with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-2.

In this context, by "suitably chosen" is meant that, as applicable, a CDR1 sequence is chosen from suitable CDR1 sequences (i.e. as defined herein), a CDR2 sequence is chosen from suitable CDR2 sequences (i.e. as defined herein), and a CDR3 sequence is chosen from suitable CDR3 sequence (i.e. as defined herein), respectively. More in particular, the CDR sequences are preferably chosen such that the Nanobodies of the invention bind to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) with an affinity (suitably measured and/or expressed as a EC50 value, or alternatively as an IC_{50} value, as further described herein in various in vitro and/or in vivo potency or other assays) that is as defined herein.

In particular, in the immunoglobulin single variable domains of the invention, at least the CDR3 sequence present is suitably chosen from the group consisting of the CDR3 sequences listed in Table B-2 or from the group of CDR3 sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR3 sequences listed in Table B-2; and/or from the group consisting of the CDR3 sequences that have 3, 2 or only 1 amino acid difference(s) with at least one of the CDR3 sequences listed in Table B-2.

Preferably, in the immunoglobulin single variable domains of the invention, at least two of the CDR1, CDR2 and CDR3 sequences present are suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-2 or from the group consisting of CDR1, CDR2 and CDR3 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-2; and/or from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, that have 3, 2 or only 1 "amino acid difference(s)" with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-2.

In particular, in the immunoglobulin single variable domains of the invention, at least the CDR3 sequence present is suitably chosen from the group consisting of the CDR3 sequences listed in Table B-2 or from the group of CDR3 sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR3 sequences listed in Table B-2, respectively; and at least one of the CDR1 and CDR2 sequences present is suitably chosen from the group consisting of the CDR1 and CDR2 sequences, respectively, listed in Table B-2 or from the group of CDR1 and CDR2 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR1 and CDR2 sequences, respectively, listed in Table B-2; and/or from the group consisting of the CDR1 and CDR2 sequences, respectively, that have 3, 2 or

only 1 amino acid difference(s) with at least one of the CDR1 and CDR2 sequences, respectively, listed in Table B-2.

Most preferably, in the immunoglobulin single variable domains of the invention, all three CDR1, CDR2 and CDR3 sequences present are suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-2 or from the group of CDR1, CDR2 and CDR3 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-2; and/or from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, that have 3, 2 or only 1 amino acid difference(s) with at least one of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-2.

Even more preferably, in the immunoglobulin single variable domains of the invention, at least one of the CDR1, CDR2 and CDR3 sequences present is suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-2. Preferably, in this aspect, at least one or preferably both of the other two CDR sequences present are suitably chosen from CDR sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the corresponding CDR sequences, respectively, listed in Table B-2; and/or from the group consisting of the CDR sequences that have 3, 2 or only 1 amino acid difference(s) with at least one of the corresponding sequences, respectively, listed in Table B-2.

In particular, in the immunoglobulin single variable domains of the invention, at least the CDR3 sequence present is suitably chosen from the group consisting of the CDR3 listed in Table B-2. Preferably, in this aspect, at least one and preferably both of the CDR1 and CDR2 sequences present are suitably chosen from the groups of CDR1 and CDR2 sequences, respectively, that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the CDR1 and CDR2 sequences, respectively, listed in Table B-2; and/or from the group consisting of the CDR1 and CDR2 sequences, respectively, that have 3, 2 or only 1 amino acid difference(s) with at least one of the CDR1 and CDR2 sequences, respectively, listed in Table B-2.

Even more preferably, in the immunoglobulin single variable domains of the invention, at least two of the CDR1, CDR2 and CDR3 sequences present are suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-2. Preferably, in this aspect, the remaining CDR sequence present is suitably chosen from the group of CDR sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the corresponding CDR sequences listed in Table B-2; and/or

from the group consisting of CDR sequences that have 3, 2 or only 1 amino acid difference(s) with at least one of the corresponding sequences listed in Table B-2.

In particular, in the immunoglobulin single variable domains of the invention, at least the CDR3 sequence is suitably chosen from the group consisting of the CDR3 sequences listed in Table B-2, and either the CDR1 sequence or the CDR2 sequence is suitably chosen from the group consisting of the CDR1 and CDR2 sequences, respectively, listed in Table B-2. Preferably, in this aspect, the remaining CDR sequence present is suitably chosen from the group of CDR sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with at least one of the corresponding CDR sequences listed in Table B-2; and/or from the group consisting of CDR sequences that have 3, 2 or only 1 amino acid difference(s) with the corresponding CDR sequences listed in Table B-2.

Even more preferably, in the immunoglobulin single variable domains of the invention, all three CDR1, CDR2 and CDR3 sequences present are suitably chosen from the group consisting of the CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-2.

Also, generally, the combinations of CDR's listed in Table B-2 (*i.e.*, those mentioned on the same line or row in Table B-2) are preferred. Thus, it is generally preferred that, when a CDR in a immunoglobulin single variable domain of the invention is a CDR sequence mentioned in Table B-2 or is suitably chosen from the group of CDR sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with a CDR sequence listed in Table B-2; and/or from the group consisting of CDR sequences that have 3, 2 or only 1 amino acid difference(s) with a CDR sequence listed in Table B-2, that at least one and preferably both of the other CDR's are suitably chosen from the CDR sequences that belong to the same combination in Table B-2 (*i.e.*, mentioned on the same line or row in Table B-2) or are suitably chosen from the group of CDR sequences that have at least 80%, preferably at least 90%, more preferably at least 95%, even more preferably at least 99% sequence identity with the CDR sequence(s) belonging to the same combination and/or from the group consisting of CDR sequences that have 3, 2 or only 1 amino acid difference(s) with the CDR sequence(s) belonging to the same combination. The other preferences indicated in the above paragraphs also apply to the combinations of CDR's mentioned in Table B-2.

Thus, by means of non-limiting examples, a polypeptide of the invention can for example comprise a CDR1 sequence that has more than 80 % sequence identity with one of the CDR1 sequences mentioned in Table B-2, a CDR2 sequence that has 3, 2 or 1 amino acid difference with one of the

CDR2 sequences mentioned in Table B-2 (but belonging to a different combination), and a CDR3 sequence.

Some preferred immunoglobulin single variable domains of the invention may for example comprise: (1) a CDR1 sequence that has more than 80 % sequence identity with one of the CDR1 sequences mentioned in Table B-2; a CDR2 sequence that has 3, 2 or 1 amino acid difference with one of the CDR2 sequences mentioned in Table B-2 (but belonging to a different combination); and a CDR3 sequence that has more than 80 % sequence identity with one of the CDR3 sequences mentioned in Table B-2 (but belonging to a different combination); or (2) a CDR1 sequence that has more than 80 % sequence identity with one of the CDR1 sequences mentioned in Table B-2; a CDR2 sequence, and one of the CDR3 sequences listed in Table B-2; or (3) a CDR1 sequence; a CDR2 sequence that has more than 80% sequence identity with one of the CDR2 sequence listed in Table B-2; and a CDR3 sequence that has 3, 2 or 1 amino acid differences with the CDR3 sequence mentioned in Table B-2 that belongs to the same combination as the CDR2 sequence.

Some particularly preferred immunoglobulin single variable domains of the invention may for example comprise: (1) a CDR1 sequence that has more than 80 % sequence identity with one of the CDR1 sequences mentioned in Table B-2; a CDR2 sequence that has 3, 2 or 1 amino acid difference with the CDR2 sequence mentioned in Table B-2 that belongs to the same combination; and a CDR3 sequence that has more than 80 % sequence identity with the CDR3 sequence mentioned in Table B-2 that belongs to the same combination; (2) a CDR1 sequence; a CDR2 listed in Table B-2 and a CDR3 sequence listed in Table B-2 (in which the CDR2 sequence and CDR3 sequence may belong to different combinations).

Some even more preferred immunoglobulin single variable domains of the invention may for example comprise: (1) a CDR1 sequence that has more than 80 % sequence identity with one of the CDR1 sequences mentioned in Table B-2; the CDR2 sequence listed in Table B-2 that belongs to the same combination; and a CDR3 sequence mentioned in Table B-2 that belongs to a different combination; or (2) a CDR1 sequence mentioned in Table B-2; a CDR2 sequence that has 3, 2 or 1 amino acid differences with the CDR2 sequence mentioned in Table B-2 that belongs to the same combination; and a CDR3 sequence that has more than 80% sequence identity with the CDR3 sequence listed in Table B-2 that belongs to the same or a different combination.

Particularly preferred immunoglobulin single variable domains of the invention may for example comprise a CDR1 sequence mentioned in Table B-2, a CDR2 sequence that has more than 80 % sequence identity with the CDR2 sequence mentioned in Table B-2 that belongs to the same combination; and the CDR3 sequence mentioned in Table B-2 that belongs to the same combination.

In the most preferred immunoglobulin single variable domains of the invention, the CDR1, CDR2 and CDR3 sequences present are suitably chosen from one of the combinations of CDR1, CDR2 and CDR3 sequences, respectively, listed in Table B-2.

According to another preferred, but non-limiting aspect of the invention (a) CDR1 has a length of between 1 and 12 amino acid residues, and usually between 2 and 9 amino acid residues, such as 5, 6 or 7 amino acid residues; and/or (b) CDR2 has a length of between 13 and 24 amino acid residues, and usually between 15 and 21 amino acid residues, such as 16 and 17 amino acid residues; and/or (c) CDR3 has a length of between 2 and 35 amino acid residues, and usually between 3 and 30 amino acid residues, such as between 6 and 23 amino acid residues.

In another preferred, but non-limiting aspect, the invention relates to a immunoglobulin single variable domain in which the CDR sequences (as defined herein) have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more sequence identity (as defined herein) with the CDR sequences of at least one of the immunoglobulin single variable domains of SEQ ID NOs: 39 to 43 or 91 as well as 99-102 (see Table B-3).

Another preferred, but non-limiting aspect of the invention relates to humanized variants of the immunoglobulin single variable domains of SEQ ID NOs: 39 to 43 and 91 as well as 99-102 (see Table B-3), that comprise, compared to the corresponding native V_{HH} sequence, at least one humanizing substitution (as defined herein), and in particular at least one humanizing substitution in at least one of its framework sequences (as defined herein).

It will be clear to the skilled person that the immunoglobulin single variable domains that are mentioned herein as "preferred" (or "more preferred", "even more preferred", etc.) are also preferred (or more preferred, or even more preferred, etc.) for use in the polypeptides described herein. Thus, polypeptides that comprise or essentially consist of one or more "preferred" immunoglobulin single variable domains of the invention will generally be preferred, and polypeptides that comprise or essentially consist of one or more "more preferred" immunoglobulin single variable domains of the invention will generally be more preferred, etc.

1.6. Nucleotides, host cells of the invention

Another aspect of this invention relates to a nucleic acid that encodes an amino acid sequence of the invention (such as an immunoglobulin single variable domain of the invention) or a polypeptide of the invention comprising the same. Again, as generally described herein for the nucleic acids of the invention, such a nucleic acid may be in the form of a genetic construct, as defined herein. Specific

embodiments of this aspect of the invention are provided in Table B-6, SEQ ID NOs: 59 to 63 and 73 to 77.

In another preferred, but non-limiting aspect, the invention relates to nucleic acid sequences of immunoglobulin single variable domain in which the sequences (as defined herein) have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more sequence identity (as defined herein) with the sequences of at least one of nucleic acid sequence of the immunoglobulin single variable domains of SEQ ID NOs: 59 to 63 and 73 to 77 (see Table B-6).

In another aspect, the invention relates to nucleic acid sequences that comprise the nucleic acid sequences of immunoglobulin single variable domain in which the sequences (as defined herein) have more than 80%, preferably more than 90%, more preferably more than 95%, such as 99% or more sequence identity (as defined herein) with the sequences of at least one of nucleic acid sequence of the immunoglobulin single variable domains of SEQ ID NOs: 59 to 63 and 73 to 77 (see Table B-6).

In another aspect, the invention relates to host or host cell that expresses or that is capable of expressing an amino acid sequence (such as an immunoglobulin single variable domain) of the invention and/or a polypeptide of the invention comprising the same; and/or that contains a nucleic acid of the invention. Some preferred but non-limiting examples of such hosts or host cells will become clear from the further description herein.

As will be clear to the skilled person, one particularly useful method for preparing a polypeptide of the invention generally comprises the steps of:

i) the expression, in a suitable host cell or host organism (also referred to herein as a "host of the invention") or in another suitable expression system of a nucleic acid that encodes said amino acid sequence, polypeptide of the invention (also referred to herein as a "*nucleic acid of the invention*"), optionally followed by:

ii) isolating and/or purifying the polypeptide of the invention thus obtained.

In particular, such a method may comprise the steps of:

i) cultivating and/or maintaining a host of the invention under conditions that are such that said host of the invention expresses and/or produces at least one polypeptide of the invention; optionally followed by:

ii) isolating and/or purifying the polypeptide of the invention thus obtained.

A nucleic acid of the invention can be in the form of single or double stranded DNA or RNA, and is preferably in the form of double stranded DNA. For example, the nucleotide sequences of the

invention may be genomic DNA, cDNA or synthetic DNA (such as DNA with a codon usage that has been specifically adapted for expression in the intended host cell or host organism).

According to one aspect of the invention, the nucleic acid of the invention is in essentially isolated from, as defined herein.

The nucleic acid of the invention may also be in the form of, be present in and/or be part of a vector, such as for example a plasmid, cosmid or YAC, which again may be in essentially isolated form.

The nucleic acids of the invention can be prepared or obtained in a manner known per se, based on the information on the immunoglobulin single variable domains for the polypeptides of the invention given herein, and/or can be isolated from a suitable natural source. To provide analogs, nucleotide sequences encoding naturally occurring V_{HH} domains can for example be subjected to site-directed mutagenesis, so at to provide a nucleic acid of the invention encoding said analog. Also, as will be clear to the skilled person, to prepare a nucleic acid of the invention, also several nucleotide sequences, such as at least one nucleotide sequence encoding a polypeptide of the invention and for example nucleic acids encoding one or more linkers can be linked together in a suitable manner.

Techniques for generating the nucleic acids of the invention will be clear to the skilled person and may for instance include, but are not limited to, automated DNA synthesis; site-directed mutagenesis; combining two or more naturally occurring and/or synthetic sequences (or two or more parts thereof), introduction of mutations that lead to the expression of a truncated expression product; introduction of one or more restriction sites (e.g. to create cassettes and/or regions that may easily be digested and/or ligated using suitable restriction enzymes), and/or the introduction of mutations by means of a PCR reaction using one or more "mismatched" primers, using for example a sequence of a naturally occurring form of CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) as a template. These and other techniques will be clear to the skilled person, and reference is again made to the standard handbooks, such as Sambrook et al. and Ausubel et al., mentioned above, as well as the Examples below.

The nucleic acid of the invention may also be in the form of, be present in and/or be part of a genetic construct, as will be clear to the person skilled in the art and as described on pages 131-134 of WO 08/020079 (incorporated herein by reference). Such genetic constructs generally comprise at least one nucleic acid of the invention that is optionally linked to one or more elements of genetic constructs known per se, such as for example one or more suitable regulatory elements (such as a suitable promoter(s), enhancer(s), terminator(s), etc.) and the further elements of genetic constructs referred to herein. Such genetic constructs comprising at least one nucleic acid of the invention will also be referred to herein as "genetic constructs of the invention".

The genetic constructs of the invention may be DNA or RNA, and are preferably double-stranded DNA. The genetic constructs of the invention may also be in a form suitable for transformation of the intended host cell or host organism, in a form suitable for integration into the genomic DNA of the intended host cell or in a form suitable for independent replication, maintenance and/or inheritance in the intended host organism. For instance, the genetic constructs of the invention may be in the form of a vector, such as for example a plasmid, cosmid, YAC, a viral vector or transposon. In particular, the vector may be an expression vector, *i.e.*, a vector that can provide for *expression in vitro* and/or *in vivo* (*e.g.*, in a suitable host cell, host organism and/or expression system).

In a preferred but non-limiting aspect, a genetic construct of the invention comprises

i) at least one nucleic acid of the invention; operably connected to

ii) one or more regulatory elements, such as a promoter and optionally a suitable terminator;

and, optionally,

iii) one or more further elements of genetic constructs known *per se*;

in which the terms "operably connected" and "operably linked" have the meaning given on pages 131-134 of WO 08/020079; and in which the "regulatory elements", "promoter", "terminator" and "further elements" are as described on pages 131-134 of WO 08/020079; and in which the genetic constructs may further be as described on pages 131-134 of WO 08/020079.

The nucleic acids of the invention and/or the genetic constructs of the invention may be used to transform a host cell or host organism, *i.e.*, for expression and/or production of the polypeptide of the invention. Suitable hosts or host cells will be clear to the skilled person, and may for example be any suitable fungal, prokaryotic or eukaryotic cell or cell line or any suitable fungal, prokaryotic or eukaryotic organism, for example those described on pages 134 and 135 of WO 08/020079.; as well as all other hosts or host cells known *per se* for the expression and production of antibodies and antibody fragments (including but not limited to (single) domain antibodies and ScFv fragments), which will be clear to the skilled person. Reference is also made to the general background art cited hereinabove, as well as to for example WO 94/29457; WO 96/34103; WO 99/42077.

The immunoglobulin single variable domains, and polypeptides of the invention can for example also be produced in the milk of transgenic mammals, for example in the milk of rabbits, cows, goats or sheep (see for example US-A-6,741,957, US-A-6,304,489 and US-A-6,849,992 for general techniques for introducing transgenes into mammals), in plants or parts of plants including but not limited to their leaves, flowers, fruits, seed, roots or turbers (for example in tobacco, maize, soybean or alfalfa) or in for example pupae of the silkworm *Bombix mori*.

Furthermore, the immunoglobulin single variable domains, and polypeptides of the invention can also be expressed and/or produced in cell-free expression systems, and suitable examples of such systems will be clear to the skilled person. Some preferred, but non-limiting examples include expression in the wheat germ system; in rabbit reticulocyte lysates; or in the *E. coli* Zubay system.

As mentioned above, one of the advantages of the use of immunoglobulin single variable domains is that the polypeptides based thereon can be prepared through expression in a suitable bacterial system, and suitable bacterial expression systems, vectors, host cells, regulatory elements, etc., will be clear to the skilled person, for example from the references cited above. It should however be noted that the invention in its broadest sense is not limited to expression in bacterial systems.

Preferably, in the invention, an (*in vivo* or *in vitro*) expression system, such as a bacterial expression system, is used that provides the polypeptides of the invention in a form that is suitable for pharmaceutical use, and such expression systems will again be clear to the skilled person. As also will be clear to the skilled person, polypeptides of the invention suitable for pharmaceutical use can be prepared using techniques for peptide synthesis.

For production on industrial scale, preferred heterologous hosts for the (industrial) production of immunoglobulin single variable domains or immunoglobulin single variable domain-containing protein therapeutics include strains of *E. coli*, *Pichia pastoris*, *S. cerevisiae* that are suitable for large scale expression/production/fermentation, and in particular for large scale pharmaceutical (*i.e.*, GMP grade) expression/production/ fermentation. Suitable examples of such strains will be clear to the skilled person. Such strains and production/expression systems are also made available by companies such as Richter Helm (Hamburg, Germany) or CMC Biologics (Soeborg, Denmark).

Alternatively, mammalian cell lines, in particular Chinese hamster ovary (CHO) cells, can be used for large scale expression/production/fermentation, and in particular for large scale pharmaceutical expression/production/fermentation. Again, such expression/production systems are also made available by some of the companies mentioned above.

The choice of the specific expression system would depend in part on the requirement for certain post-translational modifications, more specifically glycosylation. The production of a immunoglobulin single variable domain-containing recombinant protein for which glycosylation is desired or required would necessitate the use of mammalian expression hosts that have the ability to glycosylate the expressed protein. In this respect, it will be clear to the skilled person that the glycosylation pattern obtained (*i.e.*, the nature of the saccharide, number and position of residues attached) will depend on the cell or cell line that is used for the expression. Preferably, either a human cell or cell line is used (*i.e.*, leading to a protein that essentially has a human glycosylation pattern) or another

mammalian cell line is used that can provide a glycosylation pattern that is essentially and/or functionally the same as human glycosylation or at least mimics human glycosylation. Generally, prokaryotic hosts such as *E. coli* do not have the ability to glycosylate proteins, and the use of lower eukaryotes such as yeast usually leads to a glycosylation pattern that differs from human glycosylation. Nevertheless, it should be understood that all the foregoing host cells and expression systems can be used in the invention, depending on the desired polypeptide to be obtained.

Thus, according to one non-limiting aspect of the invention, the polypeptide of the invention is glycosylated. According to another non-limiting aspect of the invention, the polypeptide of the invention is non-glycosylated.

According to one preferred, but non-limiting aspect of the invention, the polypeptide of the invention is produced in a bacterial cell, in particular a bacterial cell suitable for large scale pharmaceutical production, such as cells of the strains mentioned above.

According to another preferred, but non-limiting aspect of the invention, the polypeptide of the invention is produced in a yeast cell, in particular a yeast cell suitable for large scale pharmaceutical production, such as cells of the species mentioned above.

According to yet another preferred, but non-limiting aspect of the invention, the polypeptide of the invention is produced in a mammalian cell, in particular in a human cell or in a cell of a human cell line, and more in particular in a human cell or in a cell of a human cell line that is suitable for large scale pharmaceutical production, such as the cell lines mentioned hereinabove.

As further described on pages 138 and 139 of WO 08/020079, when expression in a host cell is used to produce the immunoglobulin single variable domains, and the polypeptides of the invention, the immunoglobulin single variable domains, and polypeptides of the invention can be produced either intracellularly (*e.g.*, in the cytosol, in the periplasma or in inclusion bodies) and then isolated from the host cells and optionally further purified; or can be produced extracellularly (*e.g.*, in the medium in which the host cells are cultured) and then isolated from the culture medium and optionally further purified. Thus, according to one non-limiting aspect of the invention, the polypeptide of the invention is an amino acid sequence, polypeptide that has been produced intracellularly and that has been isolated from the host cell, and in particular from a bacterial cell or from an inclusion body in a bacterial cell. According to another non-limiting aspect of the invention, the amino acid sequence, or polypeptide of the invention is an amino acid sequence, or polypeptide that has been produced extracellularly, and that has been isolated from the medium in which the host cell is cultivated.

Some preferred, but non-limiting promoters for use with these host cells include those mentioned on pages 139 and 140 of WO 08/020079.

Some preferred, but non-limiting secretory sequences for use with these host cells include those mentioned on page 140 of WO 08/020079.

Suitable techniques for transforming a host or host cell of the invention will be clear to the skilled person and may depend on the intended host cell/host organism and the genetic construct to be used. Reference is again made to the handbooks and patent applications mentioned above.

After transformation, a step for detecting and selecting those host cells or host organisms that have been successfully transformed with the nucleotide sequence/genetic construct of the invention may be performed. This may for instance be a selection step based on a selectable marker present in the genetic construct of the invention or a step involving the detection of the amino acid sequence of the invention, *e.g.*, using specific antibodies.

The transformed host cell (which may be in the form or a stable cell line) or host organisms (which may be in the form of a stable mutant line or strain) form further aspects of the present invention.

Preferably, these host cells or host organisms are such that they express, or are (at least) capable of expressing (*e.g.*, under suitable conditions), a polypeptide of the invention (and in case of a host organism: in at least one cell, part, tissue or organ thereof). The invention also includes further generations, progeny and/or offspring of the host cell or host organism of the invention that may for instance be obtained by cell division or by sexual or asexual reproduction.

To produce/obtain expression of the immunoglobulin single variable domains of the invention, the transformed host cell or transformed host organism may generally be kept, maintained and/or cultured under conditions such that the (desired) amino acid sequence, or polypeptide of the invention is expressed/produced. Suitable conditions will be clear to the skilled person and will usually depend upon the host cell/host organism used, as well as on the regulatory elements that control the expression of the (relevant) nucleotide sequence of the invention. Again, reference is made to the handbooks and patent applications mentioned above in the paragraphs on the genetic constructs of the invention.

Generally, suitable conditions may include the use of a suitable medium, the presence of a suitable source of food and/or suitable nutrients, the use of a suitable temperature, and optionally the presence of a suitable inducing factor or compound (*e.g.*, when the nucleotide sequences of the invention are under the control of an inducible promoter); all of which may be selected by the skilled person. Again, under such conditions, the immunoglobulin single variable domains of the invention may be expressed in a constitutive manner, in a transient manner, or only when suitably induced.

It will also be clear to the skilled person that the amino acid sequence, or polypeptide of the invention may (first) be generated in an immature form (as mentioned above), which may then be

subjected to post-translational modification, depending on the host cell/host organism used. Also, the amino acid sequence, or polypeptide of the invention may be glycosylated, again depending on the host cell/host organism used.

The amino acid sequence, or polypeptide of the invention may then be isolated from the host cell/host organism and/or from the medium in which said host cell or host organism was cultivated, using protein isolation and/or purification techniques known per se, such as (preparative) chromatography and/or electrophoresis techniques, differential precipitation techniques, affinity techniques (*e.g.*, using a specific, cleavable amino acid sequence fused with the amino acid sequence, or polypeptide of the invention) and/or preparative immunological techniques (i.e. using antibodies against the amino acid sequence to be isolated).

The entire contents of all of the references (including literature references, issued patents, published patent applications, and co-pending patent applications) cited throughout this application are hereby expressly incorporated by reference, in particular for the teaching that is referenced hereinabove.

1.7 Modulators of CXCR7

A number of different screening protocols can be utilized to identify agents that modulate the level of activity or function of CXCR7 in cells, particularly in mammalian cells, and especially in human cells. In general terms, the screening methods involve screening an agent or a plurality of agents to identify one or more agents that interacts with (human) CXCR7 (SEQ ID NO:1), for example, by binding to a CXCR7 or a fragment thereof and preventing the polypeptides or ISVDs of the invention, such as, for instance, comprising any one of SEQ ID NOs: 39-48, 78-89, 91, 99-102 or 132-140, from binding to CXCR7 (SEQ ID NO: 1). In some embodiments, an agent binds CXCR7 with at least about 1.5, 2, 3, 4, 5, 10, 20, 50, 100, 300, 500, or 1000 times the affinity of the agent for another protein. In some embodiments, the fragment of CXCR7 comprising the epitopes described herein (and optionally comprising further non-CXCR7 amino acids at the N and/or C termini) is no more than, e.g., 300, 250, 200, 150, 100, 50, 40, 30, 20 or fewer amino acids. In some embodiments, the CXCR7 fragment is any fragment having less than all of the amino acids in the full length CXCR7 polypeptide.

In some embodiments, CXCR7 modulators are identified by screening for molecules that compete with the polypeptide or ISVD of the invention from binding to a CXCR7 polypeptide, or fragment thereof. Those of skill in the art will recognize that there are a number of ways to perform competition analyses, for instance, such as disclosed herein. In some embodiments, samples with CXCR7 are pre-incubated with a labeled polypeptides or ISVDs of the invention, such as, for instance, comprising any one of SEQ ID NOs: 39-48, 78-89, 91, 99-102 or 132-140 and then contacted with a

potential competitor molecule. Alteration (*e.g.,* a decrease) of the quantity of polypeptide or ISVD bound to CXCR7 in the presence of a test compound indicates that the test compound is a potential CXCR7 modulator.

1.8 Kits for use in diagnostic and/or prognostic applications

For use in the diagnostic, research, and therapeutic applications suggested above, kits are also provided by the invention. In the diagnostic and research applications such kits may include any or all of the following: assay reagents, buffers, and the anti-CXCR7 polypeptides or ISVDs of the invention. A therapeutic product may include sterile saline or another pharmaceutically acceptable emulsion and suspension base.

In addition, the kits may include instructional materials containing directions (i.e., protocols) for the practice of the methods of this invention. While the instructional materials typically comprise written or printed materials they are not limited to such. Any medium capable of storing such instructions and communicating them to an end user is contemplated by this invention. Such media include, but are not limited to electronic storage media (*e.g.,* magnetic discs, tapes, cartridges, chips), optical media (*e.g.,* CD ROM), and the like. Such media may include addresses to internet sites that provide such instructional materials.

The invention will now be further described by means of the following non-limiting preferred aspects, figures and examples:

Preferred Non-limiting Aspects:

Aspect A-1: An immunoglobulin single variable domain that is directed against and/or that can specifically bind to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1).

Aspect A-2: An immunoglobulin single variable domain according to aspect A-1, that is in essentially isolated form.

Aspect A-3: An immunoglobulin single variable domain according to aspect A-1 or A-2, for administration to a subject, wherein said immunoglobulin single variable domain does not naturally occur in said subject.

Aspect A-4: An immunoglobulin single variable domain that can specifically bind to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/litre or less, and preferably 10^{-7} to 10^{-12} moles/litre or less and more

preferably 10^{-8} to 10^{-12} moles/litre. Such an immunoglobulin single variable domain may in particular be an immunoglobulin single variable domain according to any of the preceding aspects.

Aspect A-5: An immunoglobulin single variable domain that can specifically bind to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) with a rate of association (k_{on}-rate) of between 10^2 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, preferably between 10^3 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, more preferably between 10^4 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, such as between 10^5 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$. Such an immunoglobulin single variable domain may in particular be an immunoglobulin single variable domain according to any of the preceding aspects.

Aspect A-6: An immunoglobulin single variable domain that can specifically bind to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) with a rate of dissociation (k_{off} rate) between 1 s^{-1} and 10^{-6} s^{-1}, preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1}, more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1}, such as between 10^{-4} s^{-1} and 10^{-6} s^{-1}. Such an immunoglobulin single variable domain may in particular be an immunoglobulin single variable domain according to any of the preceding aspects.

Aspect A-7: An immunoglobulin single variable domain that can specifically bind to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM. Such an immunoglobulin single variable domain may in particular be an immunoglobulin single variable domain according to any of the preceding aspects.

Aspect A-8: An immunoglobulin single variable domain that can specifically displace SDF-1 and/or I-TAC (CXCL11 and/or CXCL12) on CXCR7 and in particular on human CXCR7 (SEQ ID NO: 1) with an average Ki of less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 1 nM and an average SDF-1 and/or I-TAC displacement of 50% or more, more preferably of 75% or more, even more preferably of 80% or more. Such an average Ki and/or average displacement value may be determined e.g. in an assay as described in Example 9 or 10.

Aspect A-9: An immunoglobulin single variable domain that can specifically displace SDF-1 and/or I-TAC (CXCL11 and/or CXCL12) on CXCR7 and in particular on human CXCR7 (SEQ ID NO: 1) with an average Ki of less than 20 nM and an average SDF-1 and/or I-TAC displacement of 70% or more. Such an average Ki and/or average displacement value may be determined e.g. in an assay as described in Example 9 or 10.

Aspect A-10: An immunoglobulin single variable domain according to any of the preceding aspects, that essentially consists of 4 framework regions (FR1 to FR4 respectively) and 3 complementarity determining regions (CDR1 to CDR3 respectively).

Aspect A-11: An immunoglobulin single variable domain according to any of the preceding aspects, that is an immunoglobulin sequence.

Aspect A-12: An immunoglobulin single variable domain according to any of the preceding aspects, that is a naturally occurring immunoglobulin sequence (from any suitable species) or a synthetic or semi-synthetic immunoglobulin sequence.

Aspect A-13: An immunoglobulin single variable domain according to any of the preceding aspects that is a humanized immunoglobulin sequence, a camelized immunoglobulin sequence or an immunoglobulin sequence that has been obtained by techniques such as affinity maturation.

Aspect A-14: An immunoglobulin single variable domain according to any of the preceding aspects, that essentially consists of a light chain variable domain sequence (*e.g.*, a VL-sequence); or of a heavy chain variable domain sequence (*e.g.*, a VH-sequence).

Aspect A-15: An immunoglobulin single variable domain according to any of the preceding aspects, that essentially consists of a heavy chain variable domain sequence that is derived from a conventional four-chain antibody or that essentially consist of a heavy chain variable domain sequence that is derived from heavy chain antibody.

Aspect A-16: An immunoglobulin single variable domain according to any of the preceding aspects, that essentially consists of a domain antibody (or an immunoglobulin single variable domain that is suitable for use as a domain antibody), of a single domain antibody (or an immunoglobulin single variable domain that is suitable for use as a single domain antibody), of a "dAb" (or an immunoglobulin single variable domain that is suitable for use as a dAb) or of a Nanobody (including but not limited to a VHH sequence).

Aspect A-17: An immunoglobulin single variable domain according to any of the preceding aspects, that essentially consists of a Nanobody.

Aspect A-18: An immunoglobulin single variable domain according to any of the preceding aspects, that essentially consists of a Nanobody that

i) has at least 80% amino acid identity with at least one of the immunoglobulin single variable domains of SEQ ID NOs: 1 to 22 of WO 2009/138519, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded;

and in which:

ii) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table A-1.

Aspect A-19: An immunoglobulin single variable domain according to any of the preceding aspects, that essentially consists of an immunoglobulin single variable domain that

i) has at least 80% amino acid identity with at least one of the immunoglobulin single variable domains of SEQ ID NOs: 39 to 43, 91 or 99-102, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded;

and in which:

ii) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table A-1.

Aspect A-20: An immunoglobulin single variable domain according to any of the preceding aspects, that essentially consists of a polypeptide that comprises of

i) a first immunoglobulin single variable domain that has at least 80% amino acid identity with an immunoglobulin single variable domain selected from the group of immunoglobulin single variable domain having SEQ ID NOs: 39 to 43 91 or 99-102, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded; and that comprises of

ii) a second immunoglobulin single variable domain that has at least 80% amino acid identity with the immunoglobulin single variable domain having SEQ ID NO: 2, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded; and, optionally, comprises

iii) a linker.

Aspect A-21: An immunoglobulin single variable domain according to any of the preceding aspects, that essentially consists of a humanized or otherwise sequence optimized immunoglobulin single variable domain.

Aspect A-22: An immunoglobulin single variable domain according to any of the preceding aspects, that, in addition to the at least one binding site for binding against CXCR7 and in particular human CXCR7 (SEQ ID NO: 1), contains one or more further binding sites for binding against other antigens, proteins or targets.

CDR-based aspects

Aspect B-1: An immunoglobulin single variable domain that is directed against and/or that can specifically bind CXCR7 and in particular human CXCR7 (SEQ ID NO: 1), and that

comprises one or more (preferably one) stretches of amino acid residues chosen from the group consisting of:

a) the immunoglobulin single variable domains of SEQ ID NOs: 9 to 13, 93 or 107-110;

b) immunoglobulin single variable domains that have at least 80% amino acid identity with at least one of the immunoglobulin single variable domains of SEQ ID NOs 9 to 13, 93 or 107-110;

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with at least one of the immunoglobulin single variable domains of SEQ ID NOs: 9 to 13, 93 or 107-110;

d) the immunoglobulin single variable domains of SEQ ID NOs: 19 to 23, 95, or 115-118;

e) immunoglobulin single variable domains that have at least 80% amino acid identity with at least one of the immunoglobulin single variable domains of SEQ ID NOs: 19 to 23, 95, or 115-118;

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with at least one of the immunoglobulin single variable domains of SEQ ID NOs: 19 to 23, 95, or 115-118;

g) the immunoglobulin single variable domains of SEQ ID NOs: 29 to 33, 97 or 123-126;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with at least one of the immunoglobulin single variable domains of SEQ ID NOs: 29 to 33, 97 or 123-126;

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with at least one of the immunoglobulin single variable domains of SEQ ID NOs: 29 to 33, 97 or 123-126;

or any suitable combination thereof.

Such an immunoglobulin single variable domain may in particular be VHH or sequence optimized VHH such as humanized, stabilized and/or solubilized VHH.

Aspect B-2: An immunoglobulin single variable domain according to aspect B-1, in which at least one of said stretches of amino acid residues forms part of the antigen binding site for binding against CXCR7 and in particular human CXCR7 (SEQ ID NO: 1).

Aspect B-3: An immunoglobulin single variable domain sequence that is directed against and/or that can specifically bind CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) and

that comprises two or more stretches of amino acid residues chosen from the group consisting of:

a) the immunoglobulin single variable domains of SEQ ID NOs: 9 to 13, 93 or 107-110;

b) immunoglobulin single variable domains that have at least 80% amino acid identity with at least one of the immunoglobulin single variable domains of SEQ ID NOs: 9 to 13, 93 or 107-110;

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with at least one of the immunoglobulin single variable domains of SEQ ID NOs: 9 to 13, 93 or 107-110;

d) the immunoglobulin single variable domains of SEQ ID NOs: 19 to 23, 95, or 115-118;

e) immunoglobulin single variable domains that have at least 80% amino acid identity with at least one of the immunoglobulin single variable domains of SEQ ID NOs: 19 to 23, 95, or 115-118;

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with at least one of the immunoglobulin single variable domains of SEQ ID NOs: 19 to 23, 95, or 115-118;

g) the immunoglobulin single variable domains of SEQ ID NOs: 29 to 33, 97 or 123-126;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with at least one of the immunoglobulin single variable domains of SEQ ID NOs: 29 to 33, 97 or 123-126;

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with at least one of the immunoglobulin single variable domains of SEQ ID NOs: 29 to 33, 97 or 123-126;

such that (i) when the first stretch of amino acid residues corresponds to one of the immunoglobulin single variable domains according to a), b) or c), the second stretch of amino acid residues corresponds to one of the immunoglobulin single variable domains according to d), e), f), g), h) or i); (ii) when the first stretch of amino acid residues corresponds to one of the immunoglobulin single variable domains according to d), e) or f), the second stretch of amino acid residues corresponds to one of the immunoglobulin single variable domains according to a), b), c), g), h) or i); or (iii) when the first stretch of amino acid residues corresponds to one of the immunoglobulin single variable domains according to g), h) or i), the second stretch

of amino acid residues corresponds to one of the immunoglobulin single variable domains according to a), b), c), d), e) or f).

Such an immunoglobulin single variable domain may in particular be VHH or sequence optimized VHH such as humanized, stabilized and/or solubilized VHH.

Aspect B-4: An immunoglobulin single variable domain according to aspect B-3, in which the at least two stretches of amino acid residues forms part of the antigen binding site for binding against CXCR7 and in particular human CXCR7 (SEQ ID NO: 1).

Aspect B-5: An immunoglobulin single variable domain sequence that is directed against and/or that can specifically bind CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) and that comprises three or more stretches of amino acid residues, in which the first stretch of amino acid residues is chosen from the group consisting of:

a) the immunoglobulin single variable domains of SEQ ID NOs: 9 to 13, 93 or 107-110;

b) immunoglobulin single variable domains that have at least 80% amino acid identity with at least one of the immunoglobulin single variable domains of SEQ ID NOs: 9 to 13, 93 or 107-110;

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with at least one of the immunoglobulin single variable domains of SEQ ID NOs: 9 to 13, 93 or 107-110;

the second stretch of amino acid residues is chosen from the group consisting of:

d) the immunoglobulin single variable domain of SEQ ID NOs: 19 to 23, 95, or 115-118;

e) immunoglobulin single variable domains that have at least 80% amino acid identity with at least one of the immunoglobulin single variable domains of SEQ ID NOs: 19 to 23, 95, or 115-118;

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with at least one of the immunoglobulin single variable domains of SEQ ID NOs: 19 to 23, 95, or 115-118;

and the third stretch of amino acid residues is chosen from the group consisting of:

g) the immunoglobulin single variable domains of SEQ ID NOs: 29 to 33, 97 or 123-126;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with at least one of the immunoglobulin single variable domains of SEQ ID NOs: 29 to 33, 97 or 123-126;

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with at least one of the immunoglobulin single variable domains of SEQ ID NOs: 29 to 33, 97 or 123-126.

Such an immunoglobulin single variable domain may in particular be VHH or sequence optimized VHH such as humanized, stabilized and/or solubilized VHH.

Aspect B-6: An immunoglobulin single variable domain according to aspect B-5, in which the at least three stretches of amino acid residues forms part of the antigen binding site for binding against CXCR7 and in particular human CXCR7 (SEQ ID NO: 1).

Aspect B-7: An immunoglobulin single variable domain that is directed against and/or that can specifically bind CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) in which the CDR sequences of said immunoglobulin single variable domain have at least 70% amino acid identity, preferably at least 80% amino acid identity, more preferably at least 90% amino acid identity, such as 95% amino acid identity or more or even essentially 100% amino acid identity with the CDR sequences of at least one of the immunoglobulin single variable domains of SEQ ID NOs: 39 to 43, 91 or 99-102.

The CDR sequences are preferentially determined via Kabat as defined herein.

Such an immunoglobulin single variable domain may in particular be VHH or sequence optimized VHH such as humanized, stabilized and/or solubilized VHH.

Aspect C-1: An immunoglobulin single variable domain or polypeptide that is directed against CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) and that cross-blocks the binding of at least one of the immunoglobulin single variable domains of SEQ ID NOs: 39 to 43, 91 or 99-102, or polypeptides of SEQ ID NOs: 44 to 48, 78-89 or 131-140 to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1). Such an immunoglobulin single variable domain may in particular be an immunoglobulin single variable domain according to any of the aspects A-1 to A-22 and/or according to aspects B-1 to B-7. Also, preferably, such an immunoglobulin single variable domain is able to specifically bind to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1).

Aspect C-2: An immunoglobulin single variable domain or polypeptide, such as an antibody or fragment thereof, that is directed against CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) and that is cross-blocked from binding to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) by at least one of the immunoglobulin single variable domains of SEQ ID NOs: 39 to 43, 91 or 99-102, or polypeptides of SEQ ID NOs: 44 to 48, 78-89 or 131-140. Such an immunoglobulin single variable domain may in particular be an immunoglobulin single variable domain according to any of the aspects A-1 to A-22

and/or according to aspects B-1 to B-7. Also, preferably, such an immunoglobulin single variable domain is able to specifically bind to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1).

Aspect C-3: An immunoglobulin single variable domain or polypeptide according to any of aspects C-1 or C-2, wherein the ability of said immunoglobulin single variable domain to cross-block or to be cross-blocked is detected in a displacement assay (*e.g.*, as described in Examples 9 and/or 10 below).

Aspect C-4: An immunoglobulin single variable domain or polypeptide according to any of aspects C-1 to C-3 wherein the ability of said immunoglobulin single variable domain to cross-block or to be cross-blocked is detected in an ELISA assay.

Aspect D-1: An immunoglobulin single variable domain according to any of aspects B-1 to B-7 or C-1 to C-7, that is in essentially isolated form.

Aspect D-2: An immunoglobulin single variable domain according to any of aspects B-1 to B-7, C-1 to C-7, and/or D1 for administration to a subject, wherein said immunoglobulin single variable domain does not naturally occur in said subject.

Aspect D-3: An immunoglobulin single variable domain according to any of aspects B-1 to B-7, C-1 to C-7, and/or D1 to D-2 that can specifically bind to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) with a dissociation constant (K_D) of 10^{-5} to 10^{-12} moles/litre or less, and preferably 10^{-7} to 10^{-12} moles/litre or less and more preferably 10^{-8} to 10^{-12} moles/litre.

Aspect D-4: An immunoglobulin single variable domain according to any of aspects B-1 to B-7, C-1 to C-7, and/or D-1 to D-3 that can specifically bind to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) with a rate of association (k_{on}-rate) of between 10^2 $M^{-1}s^{-1}$ to about 10^7 $M^{-1}s^{-1}$, preferably between 10^3 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, more preferably between 10^4 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$, such as between 10^5 $M^{-1}s^{-1}$ and 10^7 $M^{-1}s^{-1}$.

Aspect D-5: An immunoglobulin single variable domain according to any of aspects B-1 to B-7, C-1 to C-7, and/or D-1 to D-4 that can specifically bind to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) with a rate of dissociation (k_{off} rate) between 1 s^{-1} and 10^{-5} s^{-1} preferably between 10^{-2} s^{-1} and 10^{-6} s^{-1}, more preferably between 10^{-3} s^{-1} and 10^{-6} s^{-1}, such as between 10^{-4} s^{-1} and 10^{-6} s^{-1}.

Aspect D-6: An immunoglobulin single variable domain according to any of aspects B-1 to B-7, C-1 to C-7, and/or D-1 to D-5 that can specifically bind to CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) with an affinity less than 500 nM, preferably less than 200 nM, more preferably less than 10 nM, such as less than 500 pM.

The immunoglobulin single variable domains according to aspects D-1 to D-6 may in particular be an immunoglobulin single variable domain according to any of the aspects A-1 to A-22.

Aspect E-1: An immunoglobulin single variable domain according to any of aspects B-1 to B-7, C-1 to C-7 and/or D1 to D-6, that is a naturally occurring immunoglobulin single variable domain (from any suitable species) or a synthetic or semi-synthetic immunoglobulin single variable domain.

Aspect E-2: An immunoglobulin single variable domain according to any of aspects B-1 to B-7, C-1 to C-7, D1 to D-6, and/or E-1 that is sequence optimized

Aspect E-3: An immunoglobulin single variable domain according to any of aspects B-1 to B-7, C-1 to C-7, D1 to D-6, and/or D-1 or D-2 that is stabilized.

Aspect E-4: An immunoglobulin single variable domain according to any of aspects B-1 to B-7, C-1 to C-7, D1 to D-6, and/or E-1 to E-3, that is a naturally occurring immunoglobulin sequence (from any suitable species) or a synthetic or semi-synthetic immunoglobulin sequence.

Aspect E-5: An immunoglobulin single variable domain according to any of aspects B-1 to B-7, C-1 to C-7, D1 to D-6, and/or E-1 to E-4 that is a humanized immunoglobulin sequence, a camelized immunoglobulin sequence or an immunoglobulin sequence that has been obtained by techniques such as affinity maturation.

Aspect E-6: An immunoglobulin single variable domain according to any of aspects B-1 to B-7, C-1 to C-7, D1 to D-6, and/or E-1 to E-5 that essentially consists of a light chain variable domain sequence (*e.g.*, a V_L-sequence); or of a heavy chain variable domain sequence (*e.g.*, a V_H-sequence).

Aspect E-7: An immunoglobulin single variable domain according to any of aspects B-1 to B-7, C-1 to C-7, D1 to D-6, and/or E-1 to E-6, that essentially consists of a heavy chain variable domain sequence that is derived from a conventional four-chain antibody or that essentially consist of a heavy chain variable domain sequence that is derived from heavy chain antibody.

Aspect E-8: An immunoglobulin single variable domain according to any of aspects B-1 to B-7, C-1 to C-7, D1 to D-6, and/or E-1 to E-7, that essentially consists of a domain antibody (or an immunoglobulin single variable domain that is suitable for use as a domain antibody), of a single domain antibody (or an immunoglobulin single variable domain that is suitable for use as a single domain antibody), of a "dAb" (or an

immunoglobulin single variable domain that is suitable for use as a dAb) or of a Nanobody (including but not limited to a V_{HH} sequence).

Aspect E-9: An immunoglobulin single variable domain according to any of aspects B-1 to B-7, C-1 to C-7, D1 to D-6, and/or E-1 to E-8 that essentially consists of a Nanobody.

Aspect E-10: An immunoglobulin single variable domain according to any of aspects B-1 to B-7, C-1 to C-7, D1 to D-6, and/or E-1 to E-9 that essentially consists of a immunoglobulin single variable domain that

i) has at least 80% amino acid identity with at least one of the immunoglobulin single variable domains described herein, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded;

and in which:

ii) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table B-2.

Aspect E-11: An immunoglobulin single variable domain according to any of aspects B-1 to B-7, C-1 to C-7, D1 to D-6, and/or E-1 to E-10, that essentially consists of an immunoglobulin single variable domain that

i) has at least 80% amino acid identity with at least one of the An immunoglobulin single variable domains of SEQ ID NOs: 39 to 43, 91 or 99-102, in which for the purposes of determining the degree of amino acid identity, the amino acid residues that form the CDR sequences are disregarded;

and in which:

ii) preferably one or more of the amino acid residues at positions 11, 37, 44, 45, 47, 83, 84, 103, 104 and 108 according to the Kabat numbering are chosen from the Hallmark residues mentioned in Table B-2.

Aspect E-12: An immunoglobulin single variable domain according to any of aspects B-1 to B-7, C-1 to C-7, D1 to D-6, and/or E-1 to E-11 that essentially consists of a humanized immunoglobulin single variable domain.

Aspect E-13: An immunoglobulin single variable domain according to any of the aspects B-1 to B-7, C-1 to C-7, D1 to D-6, and/or E-1 to E-11, that in addition to the at least one binding site for binding formed by the CDR sequences, contains one or more further binding sites for binding against other antigens, proteins or targets.

The immunoglobulin single variable domains according to aspects E-1 to E-13 may in particular be an immunoglobulin single variable domain according to any of the aspects A-1 to A-22.

Polypeptides

Aspect K-1: Polypeptide that comprises of one or more immunoglobulin single variable domains according to any of aspects A-1 to A-22, B-1 to B-7, C-1 to C-4, D-1 to D-6, and/or E-1 to E-13, and optionally further comprises one or more peptidic linkers.

Aspect K-2: Polypeptide according to aspect K-1, which additionally comprises one or more (preferably one) immunoglobulin single variable domain directed against serum albumin.

Aspect K-3: Polypeptide according to any of aspects K-1 or K-2, in which said immunoglobulin single variable domain directed against serum albumin is directed against human serum albumin.

Aspect K-4: Polypeptide according to any of aspects K-1 to K-3, in which said one or more immunoglobulin single variable domain directed against serum albumin is an immunoglobulin single variable domain with SEQ ID NO: 2.

Aspect K-5: Polypeptide that comprises of one or more immunoglobulin single variable domains according to any of aspects A-1 to A-22, B-1 to B-7, C-1 to C-4, D-1 to D-6, and/or E-1 to E-13, one or more cytotoxic payloads, and optionally further comprises one or more peptidic linkers.

Aspect K-6: Polypeptide that comprises or essentially consists of one or more immunoglobulin single variable domains according to any of aspects A-1 to A-22, B-1 to B-7, C-1 to C-4, D-1 to D-6, and/or E-1 to E-13, one or more (and preferably one) immunoglobulin single variable domains (preferably Nanobody) directed against CXCR4 and optionally further comprises one or more peptidic linkers.

Aspect K-7: Polypeptide that comprises or essentially consists of at least one (preferably one) immunoglobulin single variable domain (preferably Nanobody) directed against (human) CXCR7 and at least one (cyto)toxic group, moiety or payload (optionally linked chemically or via one or more suitable linkers or spacers).

Aspect K-8: Polypeptide that comprises or essentially consists of at least one (preferably one) immunoglobulin single variable domain (preferably Nanobody) directed against (human) CXCR7, at least one (preferably one) immunoglobulin single variable domain (preferably Nanobody) directed against (human) CXCR4 and at least one (cyto)toxic

group, moiety or payload (optionally linked chemically or via one or more suitable linkers or spacers).

Aspect K-9: Polypeptide that comprises or essentially consists of at least one (preferably one) immunoglobulin single variable domain (preferably Nanobody) directed against (human) CXCR7 and at least one (preferably one) immunoglobulin single variable domain (preferably Nanobody) directed against (human) CXCR4 (optionally linked chemically or via one or more suitable linkers or spacers).

Aspect K-10: Polypeptide that comprises or essentially consists of at least one (preferably one) immunoglobulin single variable domain (preferably Nanobody) directed against (human) CXCR7, at least one (preferably one) immunoglobulin single variable domain (preferably Nanobody) directed against (human) CXCR4, and a peptide or immunoglobulin single variable domain (preferably Nanobody) directed against (human) serum albumin (optionally linked chemically or via one or more suitable linkers or spacers).

Aspect K-11: Polypeptide that comprises or essentially consists of two immunoglobulin single variable domains (preferably Nanobody) directed against (human) CXCR7, which are the same (optionally linked chemically or via one or more suitable linkers or spacers).

Aspect K-12: Polypeptide that comprises or essentially consists of two immunoglobulin single variable domains (preferably Nanobody) directed against (human) CXCR7, which are different from each other (optionally linked chemically or via one or more suitable linkers or spacers).

Aspect K-13: Polypeptide that comprises or essentially consists of two immunoglobulin single variable domains (preferably Nanobody) directed against (human) CXCR7, which are the same, and a peptide or immunoglobulin single variable domain (preferably Nanobody) directed against (human) serum albumin (optionally linked chemically or via one or more suitable linkers or spacers).

Aspect K-14: Polypeptide that comprises or essentially consists of two immunoglobulin single variable domains (preferably Nanobody) directed against (human) CXCR7, which are different from each other, and a peptide or immunoglobulin single variable domain (preferably Nanobody) directed against (human) serum albumin (optionally linked chemically or via one or more suitable linkers or spacers).

Nucleic acids

Aspect M-1: Nucleic acid or nucleotide sequence, that encodes an immunoglobulin single variable domain according to any of aspects A-1 to A-22, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, a polypeptide according to any of aspects K-1 to K-4.

Aspect M-2: Nucleic acid or nucleotide sequence with SEQ ID NOs: 59-63, 73-77 or 99 (Table B-6).

Host cells

Aspect N-1: Host or host cell that expresses, or that under suitable circumstances is capable of expressing, an immunoglobulin single variable domain according to any of aspects A-1 to A-22, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, a polypeptide according to any of aspects K-1 to K-4; and/or that comprises a nucleic acid or nucleotide sequence according to aspect M-1 or M-2.

Compositions

Aspect O-1: Composition comprising at least one immunoglobulin single variable domain according to any of aspects A-1 to A-22, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, or at least one polypeptide according to any of aspects K-1 to K-4, or nucleic acid or nucleotide sequence according to aspects M-1 or M-2.

Aspect O-2: Composition according to aspect O-1, which is a pharmaceutical composition.

Aspect O-3: Composition according to aspect O-2, which is a pharmaceutical composition, that further comprises at least one pharmaceutically acceptable carrier, diluent or excipient and/or adjuvant, and that optionally comprises one or more further pharmaceutically active polypeptides and/or compounds.

Making of an agent and composition of the invention

Aspect P-1: Method for producing an immunoglobulin single variable domain according to any of aspects A-1 to A-22, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, a polypeptide according to any of aspects K-1 to K-4, said method at least comprising the steps of:

a) expressing, in a suitable host cell or host organism or in another suitable expression system, a nucleic acid or nucleotide sequence according to aspect M-1, or aspect M-2;

optionally followed by:

b) isolating and/or purifying the immunoglobulin single variable domain according to any of aspects A-1 to A-22, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, a polypeptide according to any of aspects K-1 to K-4.

Aspect P-2: Method for producing an immunoglobulin single variable domain according to any of aspects A-1 to A-22, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, a polypeptide according to any of aspects K-1 to K-4, said method at least comprising the steps of:

a) cultivating and/or maintaining a host or host cell according to aspect N-1 under conditions that are such that said host or host cell expresses and/or produces at least one immunoglobulin single variable domain according to any of aspects A-1 to A-22, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, a polypeptide according to any of aspects K-1 to K-4;

optionally followed by:

b) isolating and/or purifying the immunoglobulin single variable domain according to any of aspects A-1 to A-22, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, a polypeptide according to any of aspects K-1 to K-4.

Method of screening

Aspect Q-1: Method for screening immunoglobulin single variable domains directed against CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) that comprises at least the steps of:

a) providing a set, collection or library of nucleic acid sequences encoding immunoglobulin single variable domains;

b) screening said set, collection or library of nucleic acid sequences for nucleic acid sequences that encode an immunoglobulin single variable domain that can bind to and/or has affinity for CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) and that is cross-blocked or is cross blocking a Nanobody of the invention, *e.g.*, SEQ ID NO: 39 to 43, 91 or 99-102 (Table-B-3), or a polypeptide or construct of the invention, *e.g.*, SEQ ID NO: 44 to 48, 78-89 or 131-140 (see Table B-4); and

c) isolating said nucleic acid sequence, followed by expressing said immunoglobulin single variable domain.

Use of agents of the invention

Aspect R-1: Method for the prevention and/or treatment of cancer and of inflammatory diseases (such as *e.g.*, mentioned herein), said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of at least one immunoglobulin single variable domain according to any of aspects A-1 to A-22, B-1 to B-7, C-1 to C-4,

D-1 to D-6, E-1 to E-13, a polypeptide according to any of aspects K-1 to K-4; or composition according to aspect O-2 or O-3.

Aspect R-2: Method for the prevention and/or treatment of at least one disease or disorder that is associated with CXCR7 and in particular human CXCR7 (SEQ ID NO: 1), with its biological or pharmacological activity, and/or with the biological pathways or signalling in which CXCR7 and in particular human CXCR7 (SEQ ID NO: 1) is involved, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of at least one immunoglobulin single variable domain according to any of aspects A-1 to A-22, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, a polypeptide according to any of aspects K-1 to K-4; or composition according to aspect O-2 or O-3.

Aspect R-3: Method for the prevention and/or treatment of at least one disease or disorder that can be prevented and/or treated by administering, to a subject in need thereof, at least one immunoglobulin single variable domain according to any of aspects A-1 to A-22, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, a polypeptide according to any of aspects K-1 to K-4; or composition according to aspect O-2 or O-3, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of at least one at least one immunoglobulin single variable domain according to any of aspects A-1 to A-22, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, a polypeptide according to any of aspects K-1 to K-4; or composition according to aspect O-2 or O-3.

Aspect R-4: Method for immunotherapy, said method comprising administering, to a subject in need thereof, a pharmaceutically active amount of at least one immunoglobulin single variable domain according to any of aspects A-1 to A-22, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, a polypeptide according to any of aspects K-1 to K-4; or composition according to aspect O-2 or O-3.

Aspect R-5: An immunoglobulin single variable domain according to any of aspects A-1 to A-22, B-1 to B-7, C-1 to C-4, D-1 to D-6, E-1 to E-13, a polypeptide according to any of aspects K-1 to K-4, a pharmaceutical composition according to aspect O-2 or O-3 for use in one or more of the methods according to aspects R-1 to R-3.

Aspect R-6: A polypeptide according to any of aspects K-1 to K-4, for the diagnosis, prevention and/or treatment of cancer.

Further aspects:

1. A construct comprising at least one immunoglobulin single variable domain (ISVD) that binds to and/or recognizes amino acid residue M33, and optionally amino acid residue V32 and/or amino acid residue M37 in CXCR7 (SEQ ID NO: 1) and at least one ISVD that binds to and/or recognizes amino acid residue WF19, and optionally S23 and/or D25 of CXCR7 (SEQ ID NO: 1).

2. The construct according to aspect 1 for use as a medicament to reduce tumour growth and/or to treat cancer, preferably head and neck cancer or GBM.

3. An immunoglobulin single variable domain that can specifically displace SDF-1 and I-TAC on human CXCR7 (SEQ ID NO: 1) with an average Ki of less than 100 nM and an average SDF-1 and I-TAC displacement of 50% or more.

4. An immunoglobulin single variable domain that can specifically displace SDF-1 on human CXCR7 (SEQ ID NO: 1) with an average Ki of less than 100 nM and an average SDF-1 displacement of 50% or more.

5. An immunoglobulin single variable domain that can specifically displace I-TAC on human CXCR7 (SEQ ID NO: 1) with an average Ki of less than 100 nM and an average I-TAC displacement of 50% or more.

6. The immunoglobulin single variable domain of any of aspects 3-5, wherein the average Ki is 50 nM or less.

7. The immunoglobulin single variable domain of any of aspects 3-5, wherein the average Ki is 10 nM or less.

8. The immunoglobulin single variable domain of any of aspects 3-7, wherein the average SDF-1 or I-TAC displacement is 80% or more.

9. An immunoglobulin single variable domain that can bind human CXCR7 (SEQ ID NO: 1) with a Kd of less than 50nM.

10. An immunoglobulin single variable domain that binds to and/or recognizes amino acid residue M33, and optionally amino acid residue V32 and/or amino acid residue M37 in CXCR7 (SEQ ID NO: 1).

11 An immunoglobulin single variable domain that binds to and/or recognizes amino acid residue WF19, and optionally S23 and/or D25 of CXCR7 (SEQ ID NO: 1).

12. The immunoglobulin single variable domain according to aspect 10 or 11 for use as a medicament to reduce tumour growth and/or to treat cancer, preferably head and neck cancer or GBM.

13. The immunoglobulin single variable domain of any of aspects 3-12, wherein the immunoglobulin single variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain; and

wherein CDR1 is chosen from the group consisting of:

a) the immunoglobulin single variable domain of SEQ ID NO: 9,

b) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 9,

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 9,

and wherein CDR2 is chosen from the group consisting of:

d) the immunoglobulin single variable domain of SEQ ID NO: 19;

e) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 19;

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 19;

and wherein CDR3 is chosen from the group consisting of:

g) the immunoglobulin single variable domain of SEQ ID NO: 29;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 29;

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 29.

14. The immunoglobulin single variable domain of any of aspects 3-12, wherein the immunoglobulin single variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain; and

wherein CDR1 is chosen from the group consisting of:

a) the immunoglobulin single variable domain of SEQ ID NO: 10,

b) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 10,

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 10,

and wherein CDR2 is chosen from the group consisting of:

d) the immunoglobulin single variable domain of SEQ ID NO: 20;

e) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 20;

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 20;

and wherein CDR3 is chosen from the group consisting of:

g) the immunoglobulin single variable domain of SEQ ID NO: 30;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 30;

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 30.

15. The immunoglobulin single variable domain of any of aspects 3-12, wherein the immunoglobulin single variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain; and

wherein CDR1 is chosen from the group consisting of:

a) the immunoglobulin single variable domain of SEQ ID NO: 11,

b) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 11,

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 11,

and wherein CDR2 is chosen from the group consisting of:

d) the immunoglobulin single variable domain of SEQ ID NO: 21;

e) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 21;

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 21;

and wherein CDR3 is chosen from the group consisting of:

g) the immunoglobulin single variable domain of SEQ ID NO: 31;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 31;

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 31.

16. The immunoglobulin single variable domain of any of aspects 3-12, wherein the immunoglobulin single variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain; and

wherein CDR1 is chosen from the group consisting of:

a) the immunoglobulin single variable domain of SEQ ID NO: 12,

b) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 12,

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 12,

and wherein CDR2 is chosen from the group consisting of:

d) the immunoglobulin single variable domain of SEQ ID NO: 22;

e) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 22;

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 22;

and wherein CDR3 is chosen from the group consisting of:

g) the immunoglobulin single variable domain of SEQ ID NO: 32;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 32;

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 32.

17. The immunoglobulin single variable domain of any of aspects 3-12, wherein the immunoglobulin single variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain; and

wherein CDR1 is chosen from the group consisting of:

a) the immunoglobulin single variable domain of SEQ ID NO: 13,

b) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 13,

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 13,

and wherein CDR2 is chosen from the group consisting of:

d) the immunoglobulin single variable domain of SEQ ID NO: 23;

e) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 23;

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 23;

and wherein CDR3 is chosen from the group consisting of:

g) the immunoglobulin single variable domain of SEQ ID NO: 33;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 33;

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 33.

18. The immunoglobulin single variable domain of any of aspects 3-12, wherein the immunoglobulin single variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain; and wherein CDR1 is chosen from the group consisting of:

a) the immunoglobulin single variable domain of SEQ ID NO: 93,

b) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 93,

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 93;

and wherein CDR2 is chosen from the group consisting of:

d) the immunoglobulin single variable domain of SEQ ID NO: 95;

e) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 95;

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 95;

and wherein CDR3 is chosen from the group consisting of:

g) the immunoglobulin single variable domain of SEQ ID NO: 97;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 97;

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 97.

19. The immunoglobulin single variable domain of any of aspects 3-12, wherein the immunoglobulin single variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain; and

wherein CDR1 is chosen from the group consisting of:

a) the immunoglobulin single variable domain of SEQ ID NO: 107,

b) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 107,

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 107,

and wherein CDR2 is chosen from the group consisting of:

d) the immunoglobulin single variable domain of SEQ ID NO: 115;

e) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 115;

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 115;

and wherein CDR3 is chosen from the group consisting of:

g) the immunoglobulin single variable domain of SEQ ID NO: 123;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 123;

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 123.

20. The immunoglobulin single variable domain of any of aspects 3-12, wherein the immunoglobulin single variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain; and

wherein CDR1 is chosen from the group consisting of:

a) the immunoglobulin single variable domain of SEQ ID NO: 108,

b) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 108,

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 108,

and wherein CDR2 is chosen from the group consisting of:

d) the immunoglobulin single variable domain of SEQ ID NO: 116;

e) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 116;

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 116;

and wherein CDR3 is chosen from the group consisting of:

g) the immunoglobulin single variable domain of SEQ ID NO: 124;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 124;

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 124.

21. The immunoglobulin single variable domain of any of aspects 3-12, wherein the immunoglobulin single variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain; and

wherein CDR1 is chosen from the group consisting of:

a) the immunoglobulin single variable domain of SEQ ID NO: 110,

b) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 110,

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 110,

and wherein CDR2 is chosen from the group consisting of:

d) the immunoglobulin single variable domain of SEQ ID NO: 118;

e) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 118;

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 118;

and wherein CDR3 is chosen from the group consisting of:

g) the immunoglobulin single variable domain of SEQ ID NO: 126;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 126;

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 126.

22. The immunoglobulin single variable domain according to any of aspects 1-21, wherein the framework regions (FRs) have a sequence identity of more than 80% with the FRs of SEQ ID NOs: 4 to 8, 92, 103, 104 or 106 (FR1), 14 to 18, 94, 111, 112 or 114 (FR2), 24 to 28, 96, 119, 120 or 122 (FR3), and/or 34 to 38, 98, 127, 128 or 130 (FR4).

23. A polypeptide comprising an immunoglobulin single variable domain of any of aspects 3-22.

24. The polypeptide according to aspect 23, wherein the immunoglobulin single variable domain is selected from the group consisting of immunoglobulin single variable domains that have an amino acid sequence with a sequence identity of more than 80% with the immunoglobulin single variable domains of SEQ ID NOs: 39 to 43, 91 or 99-102.

25. The polypeptide according to any of aspects 23-24 and additionally comprising at least one human serum albumin binding immunoglobulin single variable domain and optionally comprising a linker selected from the group of linkers with SEQ ID NOs: 49 to 58.

26. The polypeptide according to any of aspects 23 - 25 and additionally comprising ALB8 (SEQ ID NO: 2), and optionally comprising a linker selected from the group of linkers with SEQ ID NOs: 49 to 58.

27. The polypeptides according to any of aspects 23 - 26, wherein the polypeptide is selected from the group consisting of polypeptides that have an amino acid sequence with a sequence identity of more than 80% with the polypeptides of SEQ ID NOs: 44 to 48, 78 to 89 and 131 to 140.

28. A construct chosen from the group consisting of:

- constructs comprising at least two ISVDs that bind to and/or recognize amino acid residue WF19, and optionally S23 and/or D25 of CXCR7 (SEQ ID NO: 1), wherein said at least two ISVDs can be the same or different;

- constructs comprising at least two ISVDs that bind to and/or recognize amino acid residue M33, and optionally amino acid residue V32 and/or amino acid residue M37 in CXCR7 (SEQ ID NO: 1), wherein said at least two ISVDs can be the same or different;
- constructs comprising at least one group 1 ISVD and at least one group 2 ISVD;
- constructs comprising at least one group 1 ISVD and at least one group 3 ISVD;
- constructs comprising at least one group 2 ISVD and at least one group 3 ISVD; and
- constructs comprising at least one 01C10-like sequence and at least one 14G03-like sequence.

29. The construct according to aspect 28 for use as a medicament to reduce tumour growth and/or to treat cancer, preferably head and neck cancer or GBM.

30. A nucleic acid sequence encoding
    i) for an immunoglobulin single variable domain according to any of aspects 3-22;
    ii) for a polypeptide according to any of aspects 23-27, or
    iii) for a construct according to any of aspects 1, 2, 28 or 29.

31. A pharmaceutical composition comprising
    i) an immunoglobulin single variable domain according to any of aspects 3-22;
    ii) a polypeptide according to any of aspects 23-27; or
    iii) a construct according to any of aspects 1, 2, 28 or 29;
    and optionally a pharmaceutically acceptable excipient.

32. An immunoglobulin single variable domain according to any of aspects 3-22, a polypeptide according to any of aspects 23-27, or a construct according to any of aspects 1, 2, 28 or 29 for use in cancer, preferably head or neck cancer, GBM and/or inflammatory diseases.

33. An immunoglobulin single variable domain according to any of aspects 3-22, a polypeptide according to any of aspects 23-27, or a construct according to any of aspects 1, 2, 28 or 29for use in rheumatoid arthritis.

34. An immunoglobulin single variable domain according to any of aspects 3-22, a polypeptide according to any of aspects 23-27, or a construct according to any of aspects 1, 2, 28 or 29 for use in multiple sclerosis.

35. Method for producing an immunoglobulin single variable domain according to any of aspects 3-22, a polypeptide according to any of aspects 23-27, or a construct according to any of aspects 1, 2, 28 or 29, said method at least comprising the steps of:

a) expressing, in a suitable host cell or host organism or in another suitable expression system, a nucleic acid or nucleotide sequence according to aspect 30; optionally followed by:

b) isolating and/or purifying the immunoglobulin single variable domain according to any of aspects 3-22, a polypeptide according to any of aspects 23-27, or a construct according to any of aspects 1, 2, 28 or 29.

36. An immunoglobulin single variable comprising an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain;

wherein CDR1 is the immunoglobulin single variable domain of SEQ ID NO: 9;

wherein CDR2 is the immunoglobulin single variable domain of SEQ ID NO: 19; and

wherein CDR3 is the immunoglobulin single variable domain of SEQ ID NO: 29.

37. An immunoglobulin single variable comprising an amino acid sequence with the formula 1 variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain;

wherein CDR1 is the immunoglobulin single variable domain of SEQ ID NO: 10;

wherein CDR2 is the immunoglobulin single variable domain of SEQ ID NO: 20; and

wherein CDR3 is the immunoglobulin single variable domain of SEQ ID NO: 30.

38. An immunoglobulin single variable comprising an amino acid sequence with the formula 1 variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain;

wherein CDR1 is the immunoglobulin single variable domain of SEQ ID NO: 11;

wherein CDR2 is the immunoglobulin single variable domain of SEQ ID NO: 21; and

wherein CDR3 is the immunoglobulin single variable domain of SEQ ID NO: 31.

39. An immunoglobulin single variable comprising an amino acid sequence with the formula 1 variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an

immunoglobulin single variable domain;

wherein CDR1 is the immunoglobulin single variable domain of SEQ ID NO: 12;

wherein CDR2 is the immunoglobulin single variable domain of SEQ ID NO: 22; and

wherein CDR3 is the immunoglobulin single variable domain of SEQ ID NO: 32.

40. An immunoglobulin single variable comprising an amino acid sequence with the formula 1 variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain;

wherein CDR1 is the immunoglobulin single variable domain of SEQ ID NO: 13;

wherein CDR2 is the immunoglobulin single variable domain of SEQ ID NO: 23; and

wherein CDR3 is the immunoglobulin single variable domains of SEQ ID NO: 33.

41. An immunoglobulin single variable comprising an amino acid sequence with the formula 1 variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain;

wherein CDR1 is the immunoglobulin single variable domain of SEQ ID NO: 93;

wherein CDR2 is the immunoglobulin single variable domain of SEQ ID NO: 95; and

wherein CDR3 is the immunoglobulin single variable domain of SEQ ID NO: 97.

42. An immunoglobulin single variable comprising an amino acid sequence with the formula 1 variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain;

wherein CDR1 is the immunoglobulin single variable domain of SEQ ID NO: 107;

wherein CDR2 is the immunoglobulin single variable domain of SEQ ID NO: 115; and

wherein CDR3 is the immunoglobulin single variable domain of SEQ ID NO: 123.

43. An immunoglobulin single variable comprising an amino acid sequence with the formula 1 variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an

immunoglobulin single variable domain;

wherein CDR1 is the immunoglobulin single variable domain of SEQ ID NO: 108;

wherein CDR2 is the immunoglobulin single variable domain of SEQ ID NO: 116; and

wherein CDR3 is the immunoglobulin single variable domains of SEQ ID NO: 124.

44. An immunoglobulin single variable comprising an amino acid sequence with the formula 1 variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain;

wherein CDR1 is the immunoglobulin single variable domain of SEQ ID NO: 110;

wherein CDR2 is the immunoglobulin single variable domain of SEQ ID NO: 118; and

wherein CDR3 is the immunoglobulin single variable domain of SEQ ID NO: 126.

Experimental Part:

Sequences:

Table B-1: Prior art sequences

Name	SEQ ID NO:	Amino acid sequences
Human CXCR7 or hCXCR7	1	MDLHLFDYSEPGNFSDISWPCNSSDCIVVDTVMCPNMPN KSVLLYTLSFIYIFIFVIGMIANSVVVWVNIQAKTTGYD THCYILNLAIADLWVVLTIPVWVVSLVQHNQWPMGELTC KVTHLIFSINLFGSIFFLTCMSVDRYLSITYFTNTPSSR KKMVRRVVCILVWLLAFCVSLPDTYYLKTVTSASNNETY CRSFYPEHSIKEWLIGMELVSVVLGFAVPFSIIAVFYFL LARAISASSDQEKHSSRKIIFSYVVVFLVCWLPYHVAVL LDIFSILHYIPFTCRLEHALFTALHVTQCLSLVHCCVNP VLYSFINRNYRYELMKAFIFKYSAKTGLTKLIDASRVSE TEYSALEQSTK
Alb8	2	EVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQ APGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTT LYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
Mouse CXCR7 or mCXCR7	3	MDVHLFDYAEPGNYSDINWPCNSSDCIVVDTVQCPTMPN KNVLLYTLSFIYIFIFVIGMIANSVVVWVNIQAKTTGYD THCYILNLAIADLWVVITIPVWVVSLVQHNQWPMGELTC KITHLIFSINLFGSIFFLACMSVDRYLSITYFTGTSSYK KKMVRRVVCILVWLLAFFVSLPDTYYLKTVTSASNNETY CRSFYPEHSIKEWLIGMELVSVILGFAVPFTIIAIFYFL LARAMSASGDQEKHSSRKIIFSYVVVFLVCWLPYHFVVL LDIFSILHYIPFTCQLENVLFTALHVTQCLSLVHCCVNP VLYSFINRNYRYELMKAFIFKYSAKTGLTKLIDASRVSE TEYSALEQNTK
Tag-1	71	AAAHHHHHHGAAEQKLISEEDLNGAA
Tag-2	72	AAAEQKLISEEDLNGAAHHHHHH
Tag-3	105	GAAEQKLISEEDLNGAAHHHHHH
Cynomolgus CXCR7 or cCXCR7	90	MDLHVFDYSEPGNFSDISWPCNSSDCIVVDTVMCPNMPN KSVLLYTLAFIYIFIFVIGMIANSVVVWVNIQAKTTGYD THCYILNLAIADLWVVLTIPVWVVSLVQHNQWPMGELTC KVTHLIFSINLFGSIFFLTCMSVDRYLSITYFTNTSSSR KKMVRRVVCVLVWLLAFCVSLPDTYYLKTVTSASNNETY CRSFYPEHSIKEWLIGMELVSVVLGFAVPFSVIAVFYFL LARAISASGDQEKHSSRKIIFSYVVVFLVCWLPYHVAVL LDIFSILHYIPFTCRLEHALFTALHVTQCLSLVHCCVNP VLYSFINRNYRYELMKAFIFKYSAKTGLTKLIDASRVSE TEYSALEQSTK

5

Table B-2: Sequences for CDRs and frameworks, plus preferred combinations as provided in for formula I, namely FR1-CDR1-FR2-CDR2-FR3-CDR3-FR4

(Terms: "ID" refers to the given SEQ ID NO. Preferred combination of FR and CDR sequences for each NB construct are used interchangeably through-out the application)

Clone	ID	FR1	ID	CDR1	ID	FR2
07B11	4	EVQLVESGGNLVQAGGSLGLSCAAS VSISS	9	IHIMG	14	WYRQAPGKQRDLVA
07C03	5	EVQLVESGGGLVQAGESLTLSCAAS GRTLS	10	AYIMG	15	WFRQAPGKEREFVA
08A05	6	EVQLVESGGGLVQAGDSLRLSCAAS GLTFS	11	NYDMG	16	WFRQAPGKEREFVG
08A10	7	EVQLVESGGGLVQAGGSLRLSCAAS GSIFS	12	IAAMG	17	WYRQATGKQRELVA
14G03 (09A04)	8	EVQLVESGGGLVQPGGSLRISCAAS GSIYL	13	INYMG	18	WYRQAPGKQRELVA
Alb8	64	EVQLVESGGGLVQPGNSLRLSCAAS GFTFS	65	SFGMS	66	WVRQAPGKGLEWVS
01C10	92	EVQLVESGGGLVQTGASLRLSCAAS GRTFS	93	NYAMG	94	WFRQAPGKERERVA
01C12	103	EVQLVESGGGLVQAGASLRLSCAAS GRTFS	107	NYAMG	111	WFRQAPGKERERVA
01B12	104	EVQLVESGGGLVQAGASLRLSCAAS GRTFS	108	NYAMG	112	WFRQAPGKEREPVA
01F11	105	EVQLVESGGGLVQAGASLRLSCAAS GRTFS	109	NYAMG	113	WFRQAPGKEREPVA
01B10	106	EVQLVESGGGLVQAGASLRLSCAAS GRTFG	110	NYAMG	114	WFRQAPGKEREPVA

Table B-2 (cont.)

Clone	ID	CDR2	ID	FR3
07B11	19	TITSGGSTAYADSVKG	24	RFTVSKDNAKNTVYLQMDSLKPEDTSVYYCAA
07C03	20	GIWSGGYTHLADSAKG	25	RFSISRDNAKNTVYLQMNGLKPEDTAVYYCAA
08A05	21	ASWWSGGAPYYSDSVKG	26	RFTISRDNAKNTVYLQANSLRPEDTAVYYCAA
08A10	22	TITDGGTTTYADSVKG	27	RVTISRDRSANTVYLAMNNLKPDDTAVYYCYA
14G03 (09A04)	23	TLTSGGSTNYAGSVKG	28	RFAISRDNAKNTVYLQMNSLKPEDTAVYYCNI
Alb8	67	SISGSGSDTLYADSVKG	68	RFTISRDNAKTTLYLQMNSLRPEDTAVYYCTI
01C10	95	AITPRAFTTYYADSVKG	96	RFTISRDNAKNTAYLQMVSLKPEDTAVYYCAA
01C12	115	AISPSAVTTYYADSVKG	119	RFTISRDNAKNTAYLQMVSLKPEDTAVYYCAA
01B12	116	AISPAALTTYYADFVKG	120	RFTISRDNAKNTAYLQMVSLKPEDTAVYYCAA
01F11	117	AISPAALTTYYADFVKG	121	RFTISRDNAKNTAYLQMVSLKPEDTAVYYCAA
01B10	118	AISPAAVTTYYADFVKG	122	RFTISRDNAKNTAYLQMVSLKPEDTAVYYCAA

Table B-2 (cont.)

Clone	ID	CDR3	ID	FR4
07B11	29	EVRNGVFGKWNHY	34	WGQGTQVTVSS
07C03	30	GLRGRQYSN	35	WGQGTQVTVSS
08A05	31	KRLRSFASGGSYDY	36	WGQGTQVTVSS
08A10	32	YLRYTSRVPGDNY	37	WGQGTQVTVSS
14G03 (09A04)	33	GGTLYDRRRFES	38	WGQGTQVTVSS
Alb8	69	GGSLSR	70	SSQGTLVTVSS
01C10	97	QLVGSGSNLGRQESYAY	98	WGQGTQVTVSS
01C12	123	QLPGRGSNLGRQASYAY	127	WGQGTQVTVSS
01B12	124	QLVGSGSNLGRQQSYAY	128	WGQGTQVTVSS
01F11	125	QLVGSGSNLGRQQSYAY	129	WGQGTQVTVSS
01B10	126	QLVGSGSNLGRQQSYAY	130	WGQGTQVTVSS

Table B-3: Amino acid sequences of immunoglobulin single variable sequences of the invention

Name of clone	SEQ ID NO:	Amino acid sequences
07B11	39	EVQLVESGGNLVQAGGSLGLSCAASVSISSIHIMGWYRQ APGKQRDLVATITSGGSTAYADSVKGRFTVSKDNAKNTV YLQMDSLKPEDTSVYYCAAEVRNGVFGKWNHYWGQGTQV TVSS
07C03	40	EVQLVESGGGLVQAGESLTLSCAASGRTLSAYIMGWFRQ APGKEREFVAGIWSGGYTHLADSAKGRFSISRDNAKNTV YLQMNGLKPEDTAVYYCAAGLRGRQYSNWGQGTQVTVSS
08A05	41	EVQLVESGGGLVQAGDSLRLSCAASGLTFSNYDMGWFRQ APGKEREFVGASWWSGGAPYYSDSVKGRFTISRDNAKNT VYLQANSLRPEDTAVYYCAAKRLRSFASGGSYDYWGQGT QVTVSS
08A10	42	EVQLVESGGGLVQAGGSLRLSCAASGSIFSIAAMGWYRQ ATGKQRELVATITDGGTTTYADSVKGRVTISRDRSANTV YLAMNNLKPDDTAVYYCYAYLRYTSRVPGDNYWGQGTQV TVSS
14G03 (09A04)*	43	EVQLVESGGGLVQPGGSLRISCAASGSIYLINYMGWYRQ APGKQRELVATLTSGGSTNYAGSVKGRFAISRDNAKNTV YLQMNSLKPEDTAVYYCNIGGTLYDRRRFESWGQGTQVT VSS
01C10	91	EVQLVESGGGLVQTGASLRLSCAASGRTFSNYAMGWFRQ APGKERERVAAITPRAFTTYYADSVKGRFTISRDNAKNT AYLQMVSLKPEDTAVYYCAAQLVGSGSNLGRQESYAYWG QGTQVTVSS
01C12	99	EVQLVESGGGLVQAGASLRLSCAASGRTFSNYAMGWFRQ APGKERERVAAISPSAVTTYYADSVKGRFTISRDNAKNT AYLQMVSLKPEDTAVYYCAAQLPGRGSNLGRQASYAYWG QGTQVTVSS
01B12	100	EVQLVESGGGLVQAGASLRLSCAASGRTFSNYAMGWFRQ

		APGKEREPVAAISPAALTTYYADFVKGRFTISRDNAKNT AYLQMVSLKPEDTAVYYCAAQLVGSGSNLGRQQSYAYWG QGTQVTVSS
01F11	101	EVQLVESGGGLVQAGASLRLSCAASGRTFSNYAMGWFRQ APGKEREPVAAISPAALTTYYADFVKGRFTISRDNAKNT AYLQMVSLKPEDTAVYYCAAQLVGSGSNLGRQQSYAYWG QGTQVTVSS
01B10	102	EVQLVESGGGLVQAGASLRLSCAASGRTFGNYAMGWFRQ APGKEREPVAAISPAAVTTYYADFVKGRFTISRDNAKNT AYLQMVSLKPEDTAVYYCAAQLVGSGSNLGRQQSYAYWG QGTQVTVSS

* The sequences of 14G03is identical to the sequence of 09A04; 14G03 is used interchangeably with 09A04.

Table B-4: Polypeptide sequences of the invention

Name of clone	SEQ ID NO:	Amino acid sequences
07B11-9GS-Alb8	44	EVQLVESGGNLVQAGGSLGLSCAASVSISSIHIMGWYRQ APGKQRDLVATITSGGSTAYADSVKGRFTVSKDNAKNTV YLQMDSLKPEDTSVYYCAAEVRNGVFGKWNHYWGQGTQV TVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASG FTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVK GRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSR SSQGTLVTVSS
07C03-9GS-Alb8	45	EVQLVESGGGLVQAGESLTLSCAASGRTLSAYIMGWFRQ APGKEREFVAGIWSGGYTHLADSAKGRFSISRDNAKNTV YLQMNGLKPEDTAVYYCAAGLRGRQYSNWGQGTQVTVSS GGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFS SFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFT ISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQG TLVTVSS
08A05-9GS-Alb8	46	EVQLVESGGGLVQAGDSLRLSCAASGLTFSNYDMGWFRQ APGKEREFVGASWWSGGAPYYSDSVKGRFTISRDNAKNT VYLQANSLRPEDTAVYYCAAKRLRSFASGGSYDYWGQGT QVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAA SGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADS VKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSL SRSSQGTLVTVSS
08A10-9GS-Alb8	47	EVQLVESGGGLVQAGGSLRLSCAASGSIFSIAAMGWYRQ ATGKQRELVATITDGGTTTYADSVKGRVTISRDRSANTV YLAMNNLKPDDTAVYYCYAYLRYTSRVPGDNYWGQGTQV TVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASG FTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVK GRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSR SSQGTLVTVSS
14G03-9GS-Alb8	48	EVQLVESGGGLVQPGGSLRISCAASGSIYLINYMGWYRQ APGKQRELVATLTSGGSTNYAGSVKGRFAISRDNAKNTV YLQMNSLKPEDIAVYYCNIGGTLYDRRRFESWGQGTQVT

		VSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGF TFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKG RFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRS SQGTLVTVSS
07B11-9GS-07C03	78	EVQLVESGGNLVQAGGSLGLSCAASVSISSIHIMGWYRQ APGKQRDLVATITSGGSTAYADSVKGRFTVSKDNAKNTV YLQMDSLKPEDTSVYYCAAEVRNGVFGKWNHYWGQGTQV TVSSGGGGSGGGSEVQLVESGGGLVQAGESLTLSCAASG RTLSAYIMGWFRQAPGKEREFVAGIWSGGYTHLADSAKG RFSISRDNAKNTVYLQMNGLKPEDTAVYYCAAGLRGRQY SNWGQGTQVTVSS
07C03-9GS-07B11	79	EVQLVESGGGLVQAGESLTLSCAASGRTLSAYIMGWFRQ APGKEREFVAGIWSGGYTHLADSAKGRFSISRDNAKNTV YLQMNGLKPEDTAVYYCAAGLRGRQYSNWGQGTQVTVSS GGGGSGGGSEVQLVESGGNLVQAGGSLGLSCAASVSISS IHIMGWYRQAPGKQRDLVATITSGGSTAYADSVKGRFTV SKDNAKNTVYLQMDSLKPEDTSVYYCAAEVRNGVFGKWN HYWGQGTQVTVSS
07B11-9GS-Alb8-9GS-07C03	80	EVQLVESGGNLVQAGGSLGLSCAASVSISSIHIMGWYRQ APGKQRDLVATITSGGSTAYADSVKGRFTVSKDNAKNTV YLQMDSLKPEDTSVYYCAAEVRNGVFGKWNHYWGQGTQV TVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASG FTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVK GRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSR SSQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQAGESLT LSCAASGRTLSAYIMGWFRQAPGKEREFVAGIWSGGYTH LADSAKGRFSISRDNAKNTVYLQMNGLKPEDTAVYYCAA GLRGRQYSNWGQGTQVTVSS
07B11-9GS-07C03-9GS-Alb8	81	EVQLVESGGNLVQAGGSLGLSCAASVSISSIHIMGWYRQ APGKQRDLVATITSGGSTAYADSVKGRFTVSKDNAKNTV YLQMDSLKPEDTSVYYCAAEVRNGVFGKWNHYWGQGTQV TVSSGGGGSGGGSEVQLVESGGGLVQAGESLTLSCAASG RTLSAYIMGWFRQAPGKEREFVAGIWSGGYTHLADSAKG RFSISRDNAKNTVYLQMNGLKPEDTAVYYCAAGLRGRQY SNWGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQPGNS LRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGS DTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYY CTIGGSLSRSSQGTLVTVSS
08A05-9GS-08A10	82	EVQLVESGGGLVQAGDSLRLSCAASGLTFSNYDMGWFRQ APGKEREFVGASWWSGGAPYYSDSVKGRFTISRDNAKNT VYLQANSLRPEDTAVYYCAAKRLRSFASGGSYDYWGQGT QVTVSSGGGGSGGGSEVQLVESGGGLVQAGGSLRLSCAA SGSIFSIAAMGWYRQATGKQRELVATITDGGTTTYADSV KGRVTISRDRSANTVYLAMNNLKPDDTAVYYCYAYLRYT SRVPGDNYWGQGTQVTVSS
08A10-9GS-Alb8-9GS-	83	EVQLVESGGGLVQAGGSLRLSCAASGSIFSIAAMGWYRQ ATGKQRELVATITDGGTTTYADSVKGRVTISRDRSANTV YLAMNNLKPDDTAVYYCYAYLRYTSRVPGDNYWGQGTQV

08A10		TVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASG FTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVK GRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSR SSQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQAGGSLR LSCAASGSIFSIAAMGWYRQATGKQRELVATITDGGTTT YADSVKGRVTISRDRSANTVYLAMNNLKPDDTAVYYCYA YLRYTSRVPGDNYWGQGTQVTVSS
08A10-9GS-08A10-9GS-Alb8	84	EVQLVESGGGLVQAGGSLRLSCAASGSIFSIAAMGWYRQ ATGKQRELVATITDGGTTTYADSVKGRVTISRDRSANTV YLAMNNLKPDDTAVYYCYAYLRYTSRVPGDNYWGQGTQV TVSSGGGGSGGGSEVQLVESGGGLVQAGGSLRLSCAASG SIFSIAAMGWYRQATGKQRELVATITDGGTTTYADSVKG RVTISRDRSANTVYLAMNNLKPDDTAVYYCYAYLRYTSR VPGDNYWGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQ PGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSIS GSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDT AVYYCTIGGSLSRSSQGTLVTVSS
08A05-9GS-08A10-9GS-Alb8	85	EVQLVESGGGLVQAGDSLRLSCAASGLTFSNYDMGWFRQ APGKEREFVGASWWSGGAPYYSDSVKGRFTISRDNAKNT VYLQANSLRPEDTAVYYCAAKRLRSFASGGSYDYWGQGT QVTVSSGGGGSGGGSEVQLVESGGGLVQAGGSLRLSCAA SGSIFSIAAMGWYRQATGKQRELVATITDGGTTTYADSV KGRVTISRDRSANTVYLAMNNLKPDDTAVYYCYAYLRYT SRVPGDNYWGQGTQVTVSSGGGGSGGGSEVQLVESGGGL VQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSS ISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPE DTAVYYCTIGGSLSRSSQGTLVTVSS
07B11-9GS-238D2 (238D2 is directed against CXCR4)	86	EVQLVESGGNLVQAGGSLGLSCAASVSISSIHIMGWYRQ APGKQRDLVATITSGGSTAYADSVKGRFTVSKDNAKNTV YLQMDSLKPEDTSVYYCAAEVRNGVFGKWNHYWGQGTQV TVSSGGGGSGGGSEVQLVESGGGLVQTGGSLRLSCAASG FTFSSYAMSWVRQAPGKGLEWVSGIKSSGDSTRYAGSVK GRFTISRDNAKNMLYLQMYSLKPEDTAVYYCAKSRVSRT GLYTYDNRGQGTQVTVSS
07C03-9GS-238D4 (238D4 is directed against CXCR4)	87	EVQLVESGGGLVQAGESLTLSCAASGRTLSAYIMGWFRQ APGKEREFVAGIWSGGYTHLADSAKGRFSISRDNAKNTV YLQMNGLKPEDTAVYYCAAGLRGRQYSNWGQGTQVTVSS GGGGSGGGSEVQLMESGGGLVQAGGSLRLSCAASGRTFN NYAMGWFRRAPGKEREFVAAITRSGVRSGVSAIYGDSVK DRFTISRDNAKNTLYLQMNSLKPEDTAVYTCAASAIGSG ALRRFEYDYSGQGTQVTVSS
08A10-9GS-Alb8-9GS-238D2 (238D2 is directed against	88	EVQLVESGGGLVQAGGSLRLSCAASGSIFSIAAMGWYRQ ATGKQRELVATITDGGTTTYADSVKGRVTISRDRSANTV YLAMNNLKPDDTAVYYCYAYLRYTSRVPGDNYWGQGTQV TVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASG FTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVK GRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSR SSQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQTGGSLR

CXCR4)		LSCAASGFTFSSYAMSWVRQAPGKGLEWVSGIKSSGDST RYAGSVKGRFTISRDNAKNMLYLQMYSLKPEDTAVYYCA KSRVSRTGLYTYDNRGQGTQVTVSS
08A05-9GS-238D4-9GS-Alb8 (238D4 is directed against CXCR4)	89	EVQLVESGGGLVQAGDSLRLSCAASGLTFSNYDMGWFRQ APGKEREFVGASWWSGGAPYYSDSVKGRFTISRDNAKNT VYLQANSLRPEDTAVYYCAAKRLRSFASGGSYDYWGQGT QVTVSSGGGGSGGGSEVQLMESGGGLVQAGGSLRLSCAA SGRTFNNYAMGWFRRAPGKEREFVAAITRSGVRSGVSAI YGDSVKDRFTISRDNAKNTLYLQMNSLKPEDTAVYTCAA SAIGSGALRRFEYDYSGQGTQVTVSSGGGGSGGGSEVQL VESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGK GLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQ MNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
clone 060	131	EVQLVESGGGLVQAGDSLRLSCAASGLTFSNYDMGWFRQ APGKEREFVGASWWSGGAPYYSDSVKGRFTISRDNAKNT VYLQANSLRPEDTAVYYCAAKRLRSFASGGSYDYWGQGT LVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGG GSEVQLVESGGGLVQAGDSLRLSCAASGLTFSNYDMGWF RQAPGKEREFVGASWWSGGAPYYSDSVKGRFTISRDNAK NTVYLQANSLRPEDTAVYYCAAKRLRSFASGGSYDYWGQ GTLVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSC AASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYA DSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGG SLSRSSQGTLVTVSS
clone 083	132	EVQLVESGGGLVQAGDSLRLSCAASGLTFSNYDMGWFRQ APGKEREFVGASWWSGGAPYYSDSVKGRFTISRDNAKNT VYLQANSLRPEDTAVYYCAAKRLRSFASGGSYDYWGQGT LVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQ LVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPG KGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYL QMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
clone 085	133	EVQLVESGGGLVQPGGSLRISCAASGSIYLINYMGWYRQ APGKQRELVATLTSGGSTNYAGSVKGRFAISRDNAKNTV YLQMNSLKPEDTAVYYCNIGGTLYDRRRFESWGQGTLVT VSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSE VQLVESGGGLVQTGASLRLSCAASGRTFSNYAMGWFRQA PGKERERVAAITPRAFTTYYADSVKGRFTISRDNAKNTA YLQMVSLKPEDTAVYYCAAQLVGSGSNLGRQESYAYWGQ GTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSG GGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMS WVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDN AKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTV SS
clone 093	134	EVQLVESGGGLVQTGASLRLSCAASGRTFSNYAMGWFRQ APGKERERVAAITPRAFTTYYADSVKGRFTISRDNAKNT AYLQMVSLKPEDTAVYYCAAQLVGSGSNLGRQESYAYWG QGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS GGGGSEVQLVESGGGLVQTGASLRLSCAASGRTFSNYAM GWFRQAPGKERERVAAITPRAFTTYYADSVKGRFTISRD NAKNTAYLQMVSLKPEDTAVYYCAAQLVGSGSNLGRQES YAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGS GGGGSGGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTF SSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRF

		TISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQ GTLVTVSS
clone 021	135	EVQLVESGGGLVQAGGSLRLSCAASGSIFSIAAMGWYRQ ATGKQRELVATITDGGTTTYADSVKGRVTISRDRSANTV YLAMNNLKPDDTAVYYCYAYLRYTSRVPGDNYWGQGTLV TVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS EVQLVESGGGLVQTGASLRLSCAASGRTFSNYAMGWFRQ APGKERERVAAITPRAFTTYYADSVKGRFTISRDNAKNT AYLQMVSLKPEDTAVYYCAAQLVGSGSNLGRQESYAYWG QGTLVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLS CAASGFTFSSFCMSWVRQAPGKGLEWVSSISGSGSDTLY ADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIG GSLSRSSQGTLVTVSS
clone 023	136	EVQLVESGGGLVQAGDSLRLSCAASGLTFSNYDMGWFRQ APGKEREFVGASWWSGGAPYYSDSVKGRFTISRDNAKNT VYLQANSLRPEDTAVYYCAAKRLRSFASGGSYDYWGQGT LVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGG GSEVQLVESGGGLVQTGASLRLSCAASGRTFSNYAMGWF RQAPGKERERVAAITPRAFTTYYADSVKGRFTISRDNAK NTAYLQMVSLKPEDTAVYYCAAQLVGSGSNLGRQESYAY WGQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLR LSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDT LYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCT IGGSLSRSSQGTLVTVSS
clone 038	137	EVQLVESGGGLVQPGGSLRISCAASGSIYLINYMGWYRQ APGKQRELVATLTSGGSTNYAGSVKGRFAISRDNAKNTV YLQMNSLKPEDTAVYYCNIGGTLYDRRRFESWGQGTLVT VSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSE VQLVESGGGLVQTGASLRLSCAASGRTFSNYAMGWFRQA PGKERERVAAITPRAFTTYYADSVKGRFTISRDNAKNTA YLQMVSLKPEDTAVYYCAAQLVGSGSNLGRQESYAYWGQ GTLVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSC AASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYA DSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGG SLSRSSQGTLVTVSS
clone 049	138	EVQLVESGGGLVQTGASLRLSCAASGRTFSNYAMGWFRQ APGKERERVAAITPRAFTTYYADSVKGRFTISRDNAKNT AYLQMVSLKPEDTAVYYCAAQLVGSGSNLGRQESYAYWG QGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS GGGGSEVQLVESGGGLVQAGDSLRLSCAASGLTFSNYDM GWFRQAPGKEREFVGASWWSGGAPYYSDSVKGRFTISRD NAKNTVYLQANSLRPEDTAVYYCAAKRLRSFASGGSYDY WGQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLR LSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDT LYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCT IGGSLSRSSQGTLVTVSS
clone 052	139	EVQLVESGGGLVQTGASLRLSCAASGRTFSNYAMGWFRQ APGKERERVAAITPRAFTTYYADSVKGRFTISRDNAKNT AYLQMVSLKPEDTAVYYCAAQLVGSGSNLGRQESYAYWG QGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS GGGGSEVQLVESGGGLVQPGGSLRISCAASGSIYLINYM GWYRQAPGKQRELVATLTSGGSTNYAGSVKGRFAISRDN AKNTVYLQMNSLKPEDTAVYYCNIGGTLYDRRRFESWGQ GTLVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSC AASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYA

		DSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGG SLSRSSQGTLVTVSS
clone C86	140	EVQLVESGGGLVQPGGSLRISCAASGSIYLINYMGWYRQ APGKQRELVATITSGGSTNYAGSVKGRFAISRDNAKNTV YLQMNSLKPEDTAVYYCNIGGTLYDRRRFESWGQGTLVT VSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSE VQLVESGGGLVQAGESLTLSCAASGRTLSAYIMGWFRQA PGKEREFVAGIWSGGYTHLADSAKGRFSISRDNAKNTVY LQMNGLKPEDTAVYYCAAGLRGRQYSNWGQGTLVTVSSG GGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSS FGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTI SRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGT LVTVSS

Table B-5: Linker sequences of the invention

Name of linker	SEQ ID NO:	Amino acid sequences
5GS	49	GGGGS
6GS	50	SGGSGGS
9GS	51	GGGGSGGGS
10GS	52	GGGGSGGGGS
15GS	53	GGGGSGGGGSGGGGS
18GS	54	GGGGSGGGGSGGGGGGGS
20GS	55	GGGGSGGGGSGGGGSGGGGS
25GS	56	GGGGSGGGGSGGGGSGGGGSGGGGS
30GS	57	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGS
35GS	58	GGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGS

Table B-6: Nucleic acid sequences of the invention

Name of clone	SEQ ID NO:	Nucleic acid sequences
07B11	59	GAGGTGCAATTGGTGGAGTCTGGGGGAAACTTGGTGCAG GCTGGGGGGTCTCTGGGACTCTCCTGTGCAGCCTCTGTA AGCATCTCCAGTATCCATATCATGGGCTGGTACCGGCAG GCTCCAGGCAAACAGCGCGACTTGGTCGCTACTATTACT AGTGGTGGTAGCACAGCATATGCAGACTCCGTGAAGGGA CGATTCACCGTCTCCAAAGACAACGCCAAGAACACGGTG TATCTGCAAATGGACAGCCTGAAACCTGAGGACACATCC GTCTATTACTGTGCAGCCGAGGTCAGAAATGGGGTGTTT GGAAAATGGAATCACTACTGGGGCCAGGGGACCCAGGTC ACCGTCTCCTCA
07C03	60	GAGGTGCAATTGGTGGAGTCTGGGGGAGGATTGGTGCAG GCTGGGGAGTCTCTGACTCTCTCCTGTGCAGCCTCTGGA CGCACCTTAAGTGCCTATATCATGGGCTGGTTCCGCCAG GCTCCAGGGAAGGAGCGGGAGTTTGTAGCCGGTATCTGG

		AGTGGTGGTTACACACACCTTGCAGACTCCGCGAAGGGC CGATTCAGCATCTCTAGAGACAACGCCAAGAACACTGTA TATCTGCAAATGAACGGCCTGAAACCTGAGGACACGGCC GTCTATTACTGTGCAGCAGGTCTGAGAGGCCGCCAGTAT AGTAACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA
08A05	61	GAGGTGCAATTGGTGGAGTCTGGGGGAGGATTGGTGCAG GCTGGGGACTCTCTGAGACTCTCCTGTGCAGCCTCTGGA CTCACTTTCAGTAACTATGACATGGGCTGGTTCCGCCAG GCTCCAGGGAAGGAGCGTGAATTTGTAGGGGCTAGTTGG TGGAGTGGTGGTGCCCCATACTATTCAGACTCCGTGAAG GGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACG GTGTATCTGCAAGCGAACAGCCTGAGACCTGAGGACACG GCCGTTTATTACTGTGCAGCCAAAAGGCTGCGTAGTTTC GCCTCCGGTGGGTCGTATGATTACTGGGGTCAGGGGACC CAGGTCACCGTCTCCTCA
08A10	62	GAGTCTGGGGGAGGCTTGGTGCAGGCTGGAGGGTCTCTG AGACTCTCCTGTGCAGCTTCTGGAAGCATCTTCAGTATC GCTGCCATGGGCTGGTACCGCCAGGCTACAGGGAAGCAG CGCGAGTTGGTCGCAACTATCACTGATGGCGGTACGACA ACCTATGCAGACTCCGTGAAGGGCCGAGTCACCATCTCC AGGGACAGGTCTGCGAACACGGTGTATCTGGCAATGAAC AATTTGAAACCTGATGACACAGCCGTCTATTATTGTTAT GCGTATCTGCGCTATACAAGCAGAGTACCTGGCGATAAC TACTGGGGCCAGGGGACCCAGGTCACCGTCTCCTCA
14G03	63	GAGGTGCAATTGGTGGAGTCTGGGGGAGGCTTGGTGCAG CCTGGGGGGTCTCTGAGAATTTCCTGTGCAGCCTCTGGA AGCATCTACCTTATCAATTACATGGGCTGGTACCGCCAG GCTCCAGGGAAGCAGCGCGAGTTGGTCGCAACGCTTACT AGTGGTGGTAGTACCAACTATGCAGGCTCCGTGAAGGGC CGATTCGCCATCTCCAGAGACAACGCCAAGAACACGGTT TATCTGCAAATGAACAGCCTGAAACCTGAGGACACGGCC GTCTATTACTGTAATATAGGAGGAACGCTATACGACAGA AGGCGGTTTGAATCCTGGGGCCAGGGGACCCAGGTCACC GTCTCCTCAG
01C10	99	GAGGTGCAATTGGTGGAGTCTGGGGGAGGGTTGGTGCAG ACTGGAGCCTCTCTGAGACTCTCCTGTGCAGCCTCTGGA CGCACCTTCAGTAACTATGCCATGGGCTGGTTCCGCCAG GCTCCAGGGAAGGAGCGTGAGCGTGTAGCAGCTATTACA CCGAGAGCATTTACCACATATTATGCAGACTCCGTGAAG GGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACG GCGTATCTACAAATGGTCAGCCTGAAACCTGAGGACACG GCCGTTTATTACTGTGCAGCTCAACTGGTTGGCAGCGGT AGTAATTTAGGACGTCAGGAGTCCTATGCCTACTGGGGC CAGGGGACCCAGGTCACCGTCTCCTC
07B11-9GS-Alb8	73	GAGGTGCAATTGGTGGAGTCTGGGGGAAACTTGGTGCAG GCTGGGGGGTCTCTGGGACTCTCCTGTGCAGCCTCTGTA AGCATCTCCAGTATCCATATCATGGGCTGGTACCGGCAG GCTCCAGGCAAACAGCGCGACTTGGTCGCTACTATTACT AGTGGTGGTAGCACAGCATATGCAGACTCCGTGAAGGGA CGATTCACCGTCTCCAAAGACAACGCCAAGAACACGGTG TATCTGCAAATGGACAGCCTGAAACCTGAGGACACATCC GTCTATTACTGTGCAGCCCGAGGTCAGAAATGGGGTGTTT GGAAAATGGAATCACTACTGGGGCCAGGGGACCCAGGTC ACGGTCTCCTCAGGAGGTGGCGGGTCCGGAGGCGGATCC GAGGTACAGCTGGTGGAGTCTGGGGGTGGCTTGGTGCAA

		CCGGGTAACAGTCTGCGCCTTAGCTGCGCAGCGTCTGGC TTTACCTTCAGCTCCTTTGGCATGAGCTGGGTTCGCCAG GCTCCGGGAAAAGGACTGGAATGGGTTTCGTCTATTAGC GGCAGTGGTAGCGATACGCTCTACGCGGACTCCGTGAAG GGCCGTTTCACCATCTCCCGCGATAACGCCAAAACTACA CTGTATCTGCAAATGAATAGCCTGCGTCCTGAAGACACG GCCGTTTATTACTGTACTATTGGTGGCTCGTTAAGCCGT TCTTCACAGGGTACCCTGGTCACCGTCTCCTCA
07C03-9GS-Alb8	74	GAGGTGCAATTGGTGGAGTCTGGGGGAGGATTGGTGCAG GCTGGGGAGTCTCTGACTCTCTCCTGTGCAGCCTCTGGA CGCACCTTAAGTGCCTATATCATGGGCTGGTTCCGCCAG GCTCCAGGGAAGGAGCGGGAGTTTGTAGCCGGTATCTGG AGTGGTGGTTACACACACCTTGCAGACTCCGCGAAGGGC CGATTCAGCATCTCTAGAGACAACGCCAAGAACACTGTA TATCTGCAAATGAACGGCCTGAAACCTGAGGACACGGCC GTCTATTACTGTGCAGCAGGTCTGAGAGGCCGCCAGTAT AGTAACTGGGGCCAGGGGACCCAGGTCACGGTCTCCTCA GGAGGTGGCGGGTCCGGAGGCGGATCCGAGGTACAGCTG GTGGAGTCTGGGGGTGGCTTGGTGCAACCGGGTAACAGT CTGCGCCTTAGCTGCGCAGCGTCTGGCTTTACCTTCAGC TCCTTTGGCATGAGCTGGGTTCGCCAGGCTCCGGGAAAA GGACTGGAATGGGTTTCGTCTATTAGCGGCAGTGGTAGC GATACGCTCTACGCGGACTCCGTGAAGGGCCGTTTCACC ATCTCCCGCGATAACGCCAAAACTACACTGTATCTGCAA ATGAATAGCCTGCGTCCTGAAGACACGGCCGTTTATTAC TGTACTATTGGTGGCTCGTTAAGCCGTTCTTCACAGGGT ACCCTGGTCACCGTCTCCTCA
08A05-9GS-Alb8	75	GAGGTGCAATTGGTGGAGTCTGGGGGAGGATTGGTGCAG GCTGGGGACTCTCTGAGACTCTCCTGTGCAGCCTCTGGA CTCACTTTCAGTAACTATGACATGGGCTGGTTCCGCCAG GCTCCAGGGAAGGAGCGTGAATTTGTAGGGGCTAGTTGG TGGAGTGGTGGTGCCCCATACTATTCAGACTCCGTGAAG GGCCGATTCACCATCTCCAGAGACAACGCCAAGAACACG GTGTATCTGCAAGCGAACAGCCTGAGACCTGAGGACACG GCCGTTTATTACTGTGCAGCCAAAAGGCTGCGTAGTTTC GCCTCCGGTGGGTCGTATGATTACTGGGGTCAGGGGACC CAGGTCACGGTCTCCTCAGGAGGTGGCGGGTCCGGAGGC GGATCCGAGGTACAGCTGGTGGAGTCTGGGGGTGGCTTG GTGCAACCGGGTAACAGTCTGCGCCTTAGCTGCGCAGCG TCTGGCTTTACCTTCAGCTCCTTTGGCATGAGCTGGGTT CGCCAGGCTCCGGGAAAAGGACTGGAATGGGTTTCGTCT ATTAGCGGCAGTGGTAGCGATACGCTCTACGCGGACTCC GTGAAGGGCCGTTTCACCATCTCCCGCGATAACGCCAAA ACTACACTGTATCTGCAAATGAATAGCCTGCGTCCTGAA GACACGGCCGTTTATTACTGTACTATTGGTGGCTCGTTA AGCCGTTCTTCACAGGGTACCCTGGTCACCGTCTCCTCA
08A10-9GS-Alb8	76	GAGGTGCAATTGGTGGAGTCTGGGGGAGGCTTGGTGCAG GCTGGAGGGTCTCTGAGACTCTCCTGTGCAGCTTCTGGA AGCATCTTCAGTATCGCTGCCATGGGCTGGTACCGCCAG GCTACAGGGAAGCAGCGCGAGTTGGTCGCAACTATCACT GATGGCGGTACGACAACCTATGCAGACTCCGTGAAGGGC CGAGTCACCATCTCCAGGGACAGGTCTGCGAACACGGTG TATCTGGCAATGAACAATTTGAAACCTGATGACACAGCC GTCTATTATTGTTATGCGTATCTGCGCTATACAAGCAGA GTACCTGGCGATAACTACTGGGGCCAGGGGACCCAGGTC

		ACGGTCTCCTCAGGAGGTGGCGGGTCCGGAGGCGGATCC GAGGTACAGCTGGTGGAGTCTGGGGGTGGCTTGGTGCAA CCGGGTAACAGTCTGCGCCTTAGCTGCGCAGCGTCTGGC TTTACCTTCAGCTCCTTTGGCATGAGCTGGGTTCGCCAG GCTCCGGGAAAAGGACTGGAATGGGTTTCGTCTATTAGC GGCAGTGGTAGCGATACGCTCTACGCGGACTCCGTGAAG GGCCGTTTCACCATCTCCCGCGATAACGCCAAAACTACA CTGTATCTGCAAATGAATAGCCTGCGTCCTGAAGACACG GCCGTTTATTACTGTACTATTGGTGGCTCGTTAAGCCGT TCTTCACAGGGTACCCTGGTCACCGTCTCCTCA
14G03-9GS-Alb8	77	GAGGTGCAATTGGTGGAGTCTGGGGGAGGCTTGGTGCAG CCTGGGGGGTCTCTGAGAATTTCCTGTGCAGCCTCTGGA AGCATCTACCTTATCAATTACATGGGCTGGTACCGCCAG GCTCCAGGGAAGCAGCGCGAGTTGGTCGCAACGCTTACT AGTGGTGGTAGTACCAACTATGCAGGCTCCGTGAAGGGC CGATTCGCCATCTCCAGAGACAACGCCAAGAACACGGTT TATCTGCAAATGAACAGCCTGAAACCTGAGGACACGGCC GTCTATTACTGTAATATAGGAGGAACGCTATACGACAGA AGGCGGTTTGAATCCTGGGGCCAGGGGACCCAGGTCACG GTCTCCTCAGGAGGTGGCGGGTCCGGAGGCGGATCCGAG GTACAGCTGGTGGAGTCTGGGGGTGGCTTGGTGCAACCG GGTAACAGTCTGCGCCTTAGCTGCGCAGCGTCTGGCTTT ACCTTCAGCTCCTTTGGCATGAGCTGGGTTCGCCAGGCT CCGGGAAAAGGACTGGAATGGGTTTCGTCTATTAGCGGC AGTGGTAGCGATACGCTCTACGCGGACTCCGTGAAGGGC CGTTTCACCATCTCCCGCGATAACGCCAAAACTACACTG TATCTGCAAATGAATAGCCTGCGTCCTGAAGACACGGCC GTTTATTACTGTACTATTGGTGGCTCGTTAAGCCGTTCT TCACAGGGTACCCTGGTCACCGTCTCCTCA

Example 1: Cloning

Human CXCR7 (hCXCR7), mouse CXCR7 (Open Biosystems) and cynomolgus encoding cDNA (Table B-1) were cloned into pVAX-1 (Invitrogen) and/or pCDNA3.1 (Invitrogen). Transfection of pVAX1-hCXCR7 and pCDNA3.1-human(mouse)(cyno)CXCR7 constructs in Hek293 cells resulted in CXCR7 cell surface expression as shown by FACS analysis using the human CXCR7 specific monoclonal antibody (Mab) 11G8 (R&D Systems) and a PE-labeled goat anti-mouse IgG detecting antibody (Jackson ImmunoResearch Inc.).

Example 2: Immunizations

For genetic immunization, endotoxin-free pVAX1-CXCR7 plasmid was produced, dissolved to a concentration of 2 mg/mL in 0.9% saline and stored at -20°C. Four llamas (391, 395, 396 and 397) were immunized with 2 mg pVAX1-hCXCR7 via intradermal Jet injection (Akra DermoJet France) for four times with two weeks intervals. Three weeks after the final DNA immunization, the 4 animals received a boost with camel kidney (CAKI) cells (Nguyen et al. 2001. Adv. Immunol. 79: 261-296) (2×10^7 cells) stably expressing hCXCR7.

Three llamas (385, 387 and 404) were immunized with four injections of 2 x 10^7 HEK293 cells transfected with pCDNA3.1-hCXCR7 with two weeks intervals. From llamas 391, 395, 396 and 397, peripheral blood lymphocytes were collected 4 days and 10 days after the last DNA immunization and 3 days and 9 days after the cell boost. From llamas 385, 387 and 404, peripheral blood lymphocytes were collected 4 and 8 days after the final cell injection. Additionally, a biopsy of the palpable bow lymph node (LN) was collected from each llama via local surgery 3 days after the last cell boost. From all lymphocyte harboring immune tissues total RNA was extracted and used as template to prepare cDNA.

Example 3: Library construction

Libraries were constructed from immune tissues collected from all llamas. In short, cDNA was prepared from the extracted total RNA samples (example 2) and used to amplify the cDNA repertoire via nested PCR as previously described (WO 02/085945 and WO 04/049794). The PCR products were digested with *Sfi*I (introduced via nested PCR in the FR1 primer region) and *Bst*EII (restriction site naturally occurring in FR4) and following gel electrophoresis, the DNA fragment of approximately 400 bps was purified from gel. The amplified cDNA repertoire was ligated into the corresponding restriction sites of *Sfi*I- *Bst*EII digested phage display vector (pAX50) to obtain a library after electroporation of *Escherichia coli* TG1. This display vector allows the production of phage particles, expressing the individual VHHs (hereinforth also referred to as Nanobodies) as a fusion protein with a C-terminal Myc-His6-tag (hereinforth also TAG-1 or SEQ ID NO: 71) and with the geneIII product.

Libraries were rescued by growing the bacteria to logarithmic phase (OD_{600}= 0.5), followed by infection with helper phage to obtain recombinant phage expressing the cloned Nanobodies on tip of the phage as a pIII fusion protein. Phage was stored after filter sterilization at 4°C for further use.

Example 4: Selections of phage displaying human CXCR7 binding Nanobodies

Phage from the above libraries were used for selections on hCXCR7 virus-like particles (VLP; Integral Molecular), intact CXCR7 expressing cells, membrane extracts from CXCR7 expressing cells and peptides.

In a first selection round, 10 units of VLPs derived from hCXCR7 transfected HEK293 cells were coated in 96-well Maxisorp plate (Nunc) and blocked with low-fat milk powder (Marvell 4% in PBS). After 2 hours of incubation with rescued phage, trypsin elution (1 mg/ml) was allowed for 15 minutes at room temperature subsequent to 20 PBS washes. Protease activity was immediately neutralized by applying 16 mM protease inhibitor ABSF. The round 1 phage outputs were rescued and a second selection round was performed on 10 or 1 units of plate-immobilized hCXCR7 VLPs. The round 2

phage outputs selected on 10 or 1 units plate immobilized hCXCR7 VLPs were infected into TG1 cells and plated on agar plates (LB + Amp + 2% glucose).

Individual colonies of *E. coli* TG1 infected with the eluted phage pools obtained after selections were picked up and grown in 96-deep-well plates to produce monoclonal phage after addition of helper phage. The production of monoclonal Nanobodies was induced by the addition of isopropyl-b-D-thiogalactopyranoside (IPTG). The perisplasmic fraction containing Nanobodies was then prepared by freezing-thawing of the bacterial pellet in PBS and subsequent centrifugation to remove cell fragments.

Example 5: Identification of CXCR7 specific Nanobodies by phage ELISA.

From all round 2 selection outputs clones were screened in phage ELISA on 2 units of immobilized CXCR7 VLPs applying 10-fold dilutions of phage supernatant. After incubation with HRP-conjugated monoclonal-anti-M13 antibody (GE, Cat# 363761) and several washings, phage binding was revealed using TMB substrate (Pierce). The reaction was stopped with H_2SO_4 and the absorbance was measured at 450 nm using Sunrise TECAN spectrophotometer (TECAN). Nanobodies, showing a minimally 2-fold increased ELISA signal on hCXCR7 VLPs over non-transfected control VLPs, were considered to be CXCR7 specific. CXCR7 specific Nanobodies were sequenced and redundant Nanobodies (identical AA sequence) were removed. This resulted in the identification of 78 unique sequences, belonging to 45 distinct Nanobody B-cell lineages. Phage ELISA data for representative clones from distinct Nanobody B-cell lineages are represented in Table B-7 and indicate that the Nanobodies do bind to human CXCR7 on VLP. Notably, all Nanobodies were derived from PBLs after cell boost, except for Nanobody 01C10 (see Example 2). Evaluated against the other CXCR7 specific Nanobodies, Nanobody 01C10 was a notorious weak binder, which in first instance was used for comparative reasons (data not shown).

Table B-7: CXCR7 screening results-ELISA.

Clones with Tag-1	CXCR7-LP 2U/well [OD]	LP Null-LP [OD]	Fold CXCR7-LP/ Null-LP
08A05	0.019	0.008	2.4
08A10	0.104	0.006	17.3
14G03	0.316	0.043	7.3
07B11	0.041	0.010	4.1
07C03	0.053	0.012	4.4
01C10	0.145	0.034	4.2

Example 6: Identification of CXCR7 specific Nanobodies by FACS analysis.

Clones representing distinct Nanobody B-cell lineages were tested as periplasmic extracts for their binding to cell surface exposed CXCR7. In this assay, 5-fold dilutions of periplasmic extract were incubated with Hek293 hCXCR7 and Hek293wt cells. Binding of the Nanobodies was detected using mouse anti-myc (Serotec), followed by anti-mouse IgG-PE (Jackson Immununoresearch). Binding signals of selected Nanobody clones (mcf values and ratios of binding) are represented in Table B-8 and indicate that the Nanobodies do bind to cellular human CXCR7.

Table B-8: CXCR7 screening results-FACS analysis.

Clones with Tag-1	Family	Llama	Hek-CXCR7 [MCF]	Hek wt [MCF]	Fold Hek CXCR7/CXCR4
08A05	**14**	396	18621	310	60.1
08A10	**20**	397	27411	322	85.1
14G03	**23**	385	45811	381	120.2
07B11	**34**	395	42877	389	110.2
07C03	**37**	391	23359	319	73.2
01C10	**1**	395	No data		

Example 7: Expression of CXCR7 specific Nanobodies.

Selected Nanobodies were recloned in *E. coli* expression vector pAX100 and expressed as C-terminal linked myc, His6 (hereforth also Tag-2 or SEQ ID NO: 72) -tagged proteins. Various Nanobodies were also expressed as fusion proteins comprising Alb8 (Nanobody-linker-Alb8-myc-His6) (see sequences SEQ ID NOs: 44 to 48 – Table B-4) or as tagless Nanobodies. Expression was induced by IPTG and allowed to continue for 4h at 37°C. After spinning the cell cultures, periplasmic extracts were prepared by freeze-thawing the pellets. Nanobodies were purified from these extracts using immobilized metal affinity chromatography (IMAC) and a buffer exchange to D-PBS.

Example 8: Binding FACS analysis of CXCR7 specific Nanobodies.

Serial dilutions of purified proteins (concentration range: 400 nM - 180 pM) were incubated with stable HEK-CXCR7 cells for 30 min at 4°C and binding was detected using anti-mouse anti-myc (Serotec) and anti-mouse IgG-PE (Jackson Immunoresearch). The half maximal effective concentration (EC50) values and upper plateau levels of selected clones are depicted in Table B-9. These data confirm the screening data and underscore that the indicated Nanobodies bind to cellular human CXCR7.

Table B-9: Binding FACS analysis

Clones with Tag-2	EC50	Plateau [mcf]
08A05	8.9	28474
08A10	11.9	34896
14G03	10.2	23807
07B11	30.5	24898
07C03	3.3	33113
01C10	No data	No data

Example 9: Nanobodies compete with SDF-1 for CXCR7 binding (displacement assay)

In order to assess the competition capacity, Nanobodies were evaluated in SDF-1 ligand displacement assays using stable NIH3T3-hCXCR7 cells. 24h after seeding the cells, the cells were pre-incubated for 1 h at 4°C with a dilution series of purified monovalent Nanobodies and the corresponding C-terminal Tag-2 tagged fusion proteins to the human serum albumin binding Nanobody Alb8 (see Table B-4: SEQ ID NOs 44 to 48 wherein the polypeptides are all C-terminal tagged with Tag-2). Also reference molecules Mab 8F11 (Biolegend), Mab 11G8 (R&D) and unlabelled SDF-1 were included in the assay. Radiolabeled [^{125}I]-CXCL12 was diluted and added to the cells to reach a final concentration of 75 pM and cells were incubated for 3 h at 4°C. After incubation, cells were washed twice, lysed with RIPA buffer and the ^{125}I signal was measured. Average Ki values and the percentage of displacement relative to the displacement of cold SDF-1, are shown in Table B-10. The competition of tested Nanobodies of Group 1 and Mab 8F11 is between 73 and 83%, relative to competition with unlabelled SDF-1. This level of displacement correspond to a 100% blocking of the CXCR7 protein, as the remaining SDF-1 binding is believed not to be CXCR7 mediated, but due to the SDF-1 interaction with heparin sulfate proteoglycans. Fusion to the human serum albumin-binding Nanobody Alb8 has no significant effect on Ki values.

Table B-10: Displacement assay

Clones with Tag-2	Average Ki whole 3T3 [nMs]	Average SDF-1 displacement (%)	n	SEM Ki	SEM SDF-1 displacement (%)
08A05	13.6	77	8	2.5	6.4
08A05-9GS-Alb8	17.9		1		
08A10	12.1	75	8	1.8	3.3
08A10-9GS-Alb8	14.1		1		
14G03	3.0	73	6	0.6	3.3
14G03-9GS-Alb8	3.5		1		
07B11	96.1	75	2	1.3	1.5
07B11-9GS-Alb8	82.4		1		
07C03	12.2	78	2	6.6	15.0
07C03-9GS-Alb8	10.2		1		
01C10	20.7	31	3	10.7	15.5
SDF-1	0.121	100	15	0.019	0.0
Mab 11G8	4.4	24	3	2.7	2.0
Mab 8F11	5.9	73	6	2.4	4.1

Example 10: Nanobodies compete with SDF-1 for CXCR7 binding (FACS assay)

The potency of Nanobody 07C03 and Mab 8F11 (Biolegend) to compete with SDF-1 was evaluated in competition FACS with HEK-hCXCR7 cells. Cells were incubated simultaneously with 4 nM biotinylated SDF-1 (R&D) and with diluted test molecules, for 2 h at 4°C. Binding of biotinylated SDF-1 was detected using streptavidin-PE. Competition curves are depicted in Figure 1. In this assay, Mab 8F11 and 07C03 competition is complete (>95%), relative to competition with unlabelled SDF-1, underscoring the complete inhibition of the SDF-1-CXCR7 interaction.

Example 11: Epitope mapping

The minimal epitope of Mab 11G8 is known to be F14SDISWP20 located at the CXCR7 N-terminus (see *e.g.*, WO2008/048519). Cells were incubated simultaneously with 20 nM Mab 11G8 APC (R&D) and with diluted test molecules for 2 h at 4°C. Competition curves are depicted at Figure 2. The level of competition with Mab 11G8 APC ranges from ~20 to 100%, suggesting that the respective

Nanobody epitopes match to a high degree (high % of competition) with the Mab 11G8 epitope or to a low degree (low % of competition) or induce allosteric changes affecting the Mab 11G8 binding. These data indicate that the selected Nanobodies bind to divergent, but probably overlapping epitopes.

Nanobodies 08A05, 08A10, 07C03, 07B11, 01C10 and 14G03, Mab 8F11 (Biolegend), Mab 11G8 (R&D) and Mab 9C4 (MBL) were further tested for competition with Alexa647-labelled 14G03 in FACS analysis. Nanobodies 08A05, 08A10, 07C03, 07B11, Mab 8F11, Mab 11G8 and Mab 9C4 compete with 14G03 binding to CXCR7, while 01C10 does not, suggesting that 01C10 hits an epitope distinct from the epitope(s) hit by the other selected Nanobodies.

In a third approach, Nanobodies were tested for their binding to a set of 10 point mutants of CXCR7 (S9A, F14Y, I17L, S18N, W19A, S23G, D25A, V32A, M33Q, N36T), which yielded information on the individual Nanobody epitopes. For Nanobodies 08A05, 08A10, 07C03, 07B11 and 14G03, the epitope included residue M33, while that of 01C10 did not. The binding of 01C10 (and 07B11) was affected by the W19A mutation, while this mutation did not affect the binding of 08A05, 08A10, 07C03 and 14G03. Again, these data indicate that 01C10 hits a distinct epitope.

Example 12: Mouse/Cyno cross-reactivity

HEK293 cells transfected respectively with pCDNA3.1-hCXCR7 and pCDNA3.1-mCXCR7 were used to test cross-reactive binding of Nanobodies to mouse CXCR7 in FACS analysis. Cells were incubated with 32 nM Mab 11G8 (R&D), Mab 9C4 (MBL), Mab 8F11 (Biolegend) or with 800 nM Nanobody for 2 h at 4°C. Nanobody binding was detected using mouse anti-myc (Serotec) and anti-mouse IgG-PE (Jackson Immunoresearch) and Mab binding by goat anti-mouse IgG-PE (Jackson Immunoresearch). Nanobodies 08A10, 14G03, 07B11 and Mab9C4 are not cross-reactive to mouse CXCR7, Nanobodies 08A05 and 07C03 are partially cross-reactive with mouse CXCR7 and Mab 8F11, Mab 11G8 and 01C10 are cross-reactive with mouse CXCR7 (Table B-11).

Cross-reactive binding to cynomolgus CXCR7 was assessed in the same way. Nanobodies 08A10, 14G03, 07B11, 08A05, 07C03, 01C10 and Mab 9C4, Mab 8F11 and Mab 11G8 are all cross-reactive to cynomolgus CXCR7 (Table B-11).

Table B-11: Cross-reactivity to mouse CXCR7

Clones with Tag-2	Family	Llama	Mouse crossreactivity	Cyno crossreactivity
01C10	1	395	Yes	Yes
08A05	14	396	Partial	Yes
08A10	20	397	No	Yes
14G03	23	385	No	Yes
07B11	34	395	No	Yes
07C03	37	391	Partial	Yes
Mab 8F11			Yes	Yes
Mab 11G8			Yes	Yes
Mab 9C4			No	Yes

Example 13: Construction of bivalent and trivalent Nanobodies

Bivalent Nanobodies were constructed with one N-terminal CXCR7-specific building block (either 01C10, 14G03, 08A05, 08A10 or 07C03 but also even less potent building blocks like 08C02, 01C07, 01D04, which were not listed in the examples above) and a C-terminal human serum albumin (HSA)-specific building block (ALB8), providing the Nanobodies with an extended half-life in vivo. Trivalent Nanobodies consisted of one more CXCR7-specific building block in order to improve the potency and efficacy of the Nanobody to displace SDF-1 from the receptor. Bivalent and trivalent Nanobodies were expressed with Tag-2 extension in Pichia.

Example 14: Competition with SDF-1 binding to CXCR7of bivalent and trivalent Nanobodies

Bivalent and trivalent Nanobodies were screened in the SDF-1 displacement assay as described in Example 9. Samples were incubated in the presence or absence of HSA to estimate the effect of HSA binding to the Nanobodies during the assay. While potencies of bivalent Nanobodies were dramatically lowered in the presence of HSA, they are much better conserved for trivalent Nanobodies (Table B-12).

Table B-12: competition with SDF-1 binding to CXCR7 of bivalent and trivalent Nanobodies

Clones with Tag-3	construct	SDF-1 Displacement in absence of HSA[Ki]	SDF-1 Displacement in presence of HSA[Ki]
033	14G03-35GS-07C03-9GS-ALB8	0.82	1.74
035	14G03-35GS-14G03-9GS-ALB8	0.95	4.7
036	14G03-35GS-08C02-9GS-ALB8	1.34	6.19
032	14G03-35GS-08A05-9GS-ALB8	1.51	5.93
026	07C03-35GS-14G03-9GS-ALB8	1.75	33.03
034	14G03-35GS-07B11-9GS-ALB8	1.90	6.93
028	07C03-35GS-01C10-9GS-ALB8	2.2	ND
037	14G03-35GS-01C07-9GS-ALB8	2.28	4.48
013*	14G03-9GS-ALB8	3.1	311
055	01C10-35GS-01C10-9GS-ALB8	3.42	ND
038	14G03-35GS-01C10-9GS-ALB8	3.47	5.85
052	01C10-35GS-14G03-9GS-ALB8	3.65	6.32
049	01C10-35GS-08A05-9GS-ALB8	3.72	ND
018	08A10-35GS-14G03-9GS-ALB8	4.07	ND
053	01C10-35GS-08C02-9GS-ALB8	4.15	ND
048	01C10-35GS-08A10-9GS-ALB8	4.87	ND
050	01C10-35GS-07C03-9GS-ALB8	6.945	ND
025	07C03-35GS-07C03-9GS-ALB8	7.91	ND
009*	07C03-9GS-ALB8	9.50	66.59
056	01D04-35GS-14G03-9GS-ALB8	ND	182.29
Mab 8F11		10.8	

* bears tag-2

Example 15: Inhibition of β-arrestin recruitment of bivalent and trivalent Nanobodies

The PathHunter eXpress β-arrestin assay (DiscoverX) was used to assess the antagonistic effect of trivalent Nanobodies on recruitment of β-arrestin. A panel of 37 trivalent Nanobodies (clones) was screened at a 100 nM concentration in the assay. Results are ranked in Table B-13 on the basis of efficiency of inhibition. The most efficient trivalent molecules constitute combinations with 01C10, the Nanobody that hits a distinct epitope (cf. Example 11). These Nanobodies (clones) can bind in a double mode to one CXCR7 monomer.

Based on the foregoing results, the Nanobodies may be classified into 3 groups:

- Group 1: represented by 01C10, apparently hitting an epitope distinct from Group 2;
- Group 2: represented by 14G03, 08A05, 08A10 and 07C03, apparently hitting an epitope distinct from Group 1; and

- Group 3: represented by 07B11, apparently intermediary to Group 1 and Group 2.

Although Nanobodies of Group 2 (and Group 3) either monovalently or bivalently demonstrate superior binding and competition characteristics than the corresponding Nanobodies of Group 1, Nanobodies of Group 1 combined with Nanobodies of Group 2 gave wholly unexpectedly the best results in the β-arrestin recruitment assay.

Table B-13: Inhibition of β-arrestin recruitment of bivalent and trivalent Nanobodies

Clones with Tag-3	construct	% inhibition of β-arrestin recruitment
038	14G03-35GS-01C10-9GS-ALB8	94.1
052	01C10-35GS-14G03-9GS-ALB8	93.7
021	08A10-35GS-01C10-9GS-ALB8	89.5
023	08A05-35GS-01C10-9GS-ALB8	92.8
049	01C10-35GS-08A05-9GS-ALB8	89.3
022	08A05-35GS-07C03-9GS-ALB8	88.9
058	08A10-35GS-08A05-9GS-ALB8	87.8
060	08A05-35GS-08A05-9GS-ALB8	86.5
032	14G03-35GS-08A05-9GS-ALB8	76.9
048	01C10-35GS-08A10-9GS-ALB8	76.6
029	07B11-35GS-08A05-9GS-ALB8	73.8
018	08A10-35GS-14G03-9GS-ALB8	68.1
044	01C07-35GS-08A05-9GS-ALB8	66.1
020	08A10-35GS-02C08-9GS-ALB8	62.1
019	08A10-35GS-08C02-9GS-ALB8	61.6
028	07C03-35GS-01C10-9GS-ALB8	60.6
053	01C10-35GS-08C02-9GS-ALB8	58.8
061	08A05-35GS-02C08-9GS-ALB8	58.6
025	07C03-35GS-07C03-9GS-ALB8	54.5
027	07C03-35GS-02C08-9GS-ALB8	49.3
034	14G03-35GS-07B11-9GS-ALB8	43.5
050	01C10-35GS-07C03-9GS-ALB8	41.8
033	14G03-35GS-07C03-9GS-ALB8	41.2
026	07C03-35GS-14G03-9GS-ALB8	35.2
037	14G03-35GS-01C07-9GS-ALB8	34.0
065	02C08-35GS-08C02-9GS-ALB8	31.3
046	02C08-35GS-07B11-9GS-ALB8	29.6
051	01C10-35GS-07B11-9GS-ALB8	28.3
057	07B11-35GS-14G03-9GS-ALB8	26.0
063	01C07-35GS-08C02-9GS-ALB8	25.8
035	14G03-35GS-14G03-9GS-ALB8	24.9

036	14G03-35GS-08C02-9GS-ALB8	22.0
031	07B11-35GS-01C10-9GS-ALB8	4.3
055	01C10-35GS-01C10-9GS-ALB8	-8.5
056	01D04-35GS-14G03-9GS-ALB8	-51.3

Example 16: Optimization of bivalent and trivalent Nanobodies

Selected bivalent and trivalent Nanobodies were further characterized in the β-arrestin recruitment assay and potencies were assessed. The assay was run in the presence and absence of HSA to estimate the effect of HSA binding to the Nanobody during the assay. Longer linkers preceding the ALB8 building block were evaluated to minimize sterical interference of HSA binding to the Nanobody (Table B-14).

Table B-14: Optimization of bivalent and trivalent Nanobodies

Clones with Tag-3	construct	β-arrestin recruitment in absence of HSA [IC50]	β-arrestin recruitment in presence of HSA [IC50]
038	14G03-35GS-01C10-9GS-ALB8	3.28	19.38
052	01C10-35GS-14G03-9GS-ALB8	18.3	86.8
055	01C10-35GS-01C10-9GS-ALB8	no antagonism	no antagonism
056	01D04-35GS-14G03-9GS-ALB8	no antagonism	no antagonism
068	07C03-9GS-ALB8	279.6	inefficient antagonism
069	08A05-9GS-ALB8	120.2	inefficient antagonism
072	14G03-9GS-ALB8	inefficient antagonism	inefficient antagonism
081	07C03-30GS-ALB8	296.9	inefficient antagonism
082	14G03-30GS-ALB8	578	inefficient antagonism
083	08A05-30GS-ALB8	45.46	179.1
084	14G03-35GS-01C10-35GS-ALB8	6.3	10.0

Example 17: Characterization of tagless Nanobodies

To exclude any influence of Tag-3 on Nanobody potencies, selected Nanobodies were expressed without Tag-3 and characterized in both the β-arrestin recruitment assay and in the SDF-1

competition FACS in the presence of 2mg/ml HSA (further essentially as described in Example 10) and potencies were assessed (Table B-15 and Figure 8). Constructs comprising "Group 2 ISVD" - "Group 2 ISVD" (represented by e.g. clone 086) and constructs comprising "Group 2 ISVD " - "Group 1 ISVD" (represented by *e.g.,* clone 085) are more efficacious in SDF-1 displacement than constructs comprising "Group 1 ISVD" - "Group 1 ISVD" (represented by *e.g.,* clone 093). Competition with constructs comprising "Group 1 ISVD" - "Group 1 ISVD" (represented by *e.g.,* clone 093) is less effective.

These data corroborate the radioligand competition assays, in which the monovalent 01C10 was tested (cf. Table B-10: 31% for 01C10).

Thus, Group 2 ISVDs are excellent SDF-1 displacers.

Table B-15: Characterization of tagless Nanobodies

Clones	construct	β-arrestin recruitment in absence of HSA [IC50]	β-arrestin recruitment in presence of HSA [IC50]	SDF-1 displacement FACS [IC50]
085	14G03-35GS-01C10-35GS-ALB8	4.36	22.31	7.83
086	14G03-35GS-07C03-9GS-ALB8	weak antagonism	no antagonism	5.02
093	01C10-35GS-01C10-35GS-ALB8	no antagonism	no antagonism	20.6
Mab 8F11		12.9	34.8	34.2

Example 18: Immunohistochemical analysis of CXCR7 expression in primary tumor sections

Tumor sections that were analyzed for CXCR7 expression originated from human primary tumors of variable cancer types that had been passaged one time in nude mice. Paraffin embedded tumors were cut into 5 µm sections (with a Leica RM 2135 microtome), dried, dewaxed and stained with hematoxylin and eosin. Thereafter, one representative region was marked on these tumor sections so that a 1 mm diameter cone for assembling the Tissue Micro Array (TMA) could be punched out. The TMA was then prepared according to Mirlacher and Storz using a Beecher Instruments Micro Tissuearrayer (Mirlacher M. and Storz M., 2000, Gewebe-Chips für die molekulare Untersuchung von Tumoren, *Labmed.*, 293-297). Array sections were cut using the Instrumedics Sectioning Aid System and specifically coated using "Starfrost" slides.

Immunohistochemical staining of CXCR7-expressing tissue was performed as follows: (1) paraffin was removed from the tissue, tissues were dehydrated and washed; (2) endogenous peroxidase was

inactivated by addition of 3% H2O2 in distilled water; (3) the specimen was dried upon washing; (4) unspecific binding was blocked by 10% BSA in PBS; (5) the anti-human/mouse CXCR7 monoclonal antibody (Biolegend, clone Mab 8F11) or an isotype control antibody (Biolegend, IgG2b) was incubated at a concentration of 25 µg/mL and subsequently the tissue was washed; (6) the secondary antibody goat anti-mouse biotinylated IgG (JacksonImmunoResearch) was incubated at a final concentration of 2.8 µg/mL and the tissue was washed afterwards; (7) the detection was performed with the ABC solution and peroxidase substrate of the Vectastain ABC kit (Vector), each step followed by a washing step; (8) counterstaining with hematoxylin and (9) dehydration of the tissue.

The TMA (170 tumor models) was evaluated semi-quantitatively using a Zeiss Axiovert 35 microscope. Photographs were taken with a Zeiss AxioCam MRc camera. All tumor samples were evaluated in duplicate. Staining was interpreted based on the proportion of positively-stained cells as well as on the signal intensity. Samples were grouped in the following categories: 0, no staining (antigen absent); 1, weak staining; 2, moderate staining; 3, strong staining.

Figure 3 gives an overview of the scores assigned to the different tumor types. A high CXCR7 expression (score = 3) in at least one of the two tissue patches was found in 55 out of the 170 tumors tested (= 32.4%). Nine tumors did not show any CXCR7 expression (staining score = 0) and for the rest of the xenograft tissues a weak or intermediate expression (scores 1 and 2) was found. Notably, the majority of colon cancer tumors (19 out of 23 or 82.6%) and gastric cancer tumors (8 out of 12 or 66.7%) displayed no or only weak staining with a score of ≤ 1, whereas all of the head and neck cancer tumors (7 out of 7 or 100%) tested showed a relatively high CXCR7 expression with a score of ≥ 2. In the other histotypes, however, CXCR7 staining was highly variable between the individual tumor models.

For some tumor samples, staining intensity was confirmed on whole tumor sections.

Example 19: CXCR7 Nanobodies reduce head and neck cancer xenograft tumour growth in vivo

19.1 ***Materials and Methods***

19.1.1 Cell lines.

Cell line UM-SCC-11B (11B) was cultured from a biopsy of a primary laryngeal cancer, after the patient got chemotherapy. Cell line UM-SCC-22A (22A) was derived from a primary squamous cell carcinoma of the oropharynx. Cell line UM-SCC-22B (22B) was derived from a metastatic squamous cell carcinoma of the oropharynx. The human head and neck squamous cell carcinoma (HNSCC) cell lines FaDu and HNX-OE have been described earlier (Hermsen et al., (1996) "Centromeric breakage

as a major cause of cytogenetic abnormalities in oral squamous cell carcinoma" Genes Chromosomes Cancer 15:1–9; Ranger (1972) "A new human cell line (FaDu) from a hypopharyngeal carcinoma" Cancer 29:117~121). The HNX-OE and 93-VU-147T cell lines were established at Vrije Universiteit Amsterdam (Hermsen et al. ibid), whereas the FaDu line was obtained from Karl-Heinz Heider (Boehringer Ingelheim Austria).

19.1.2 Quantitative RT-PCR analysis.

Total RNA was extracted from head and neck cancer cell lines with the RNeasy kit from Qiagen according to the manufacturer protocol. Messenger (m)RNA was converted into cDNA using the BioRad iScript cDNA synthesis kit. Subsequently, mRNA expression levels were detected with SyberGreen (BioRad) using CXCR7 and β-actin-specific primers from Origene. CXCR7 expression levels were normalized against those of β-actin to allow comparison of the different cell lines.

19.1.3 Radioligand binding.

Head and neck cancer cell lines were seeded on poly-L-lysine-coated 96-well plates and grown overnight. The following day, binding buffer (50 mM Hepes pH 7.4, 1 mM $CaCl_2$, 5 mM $MgCl_2$, 0.1 M NaCl) supplemented with 0.5 % BSA was added to the cells in the absence or presence of either chemokine (10^{-7} M) or CXCR7-specific Nanobody 9A4 (10^{-6} M). Subsequently, radiolabelled [^{125}I]-CXCL12 (Perkin-Elmer) was added to reach a final concentration of 75 pM. Cells were incubated for 3 h at 4°C, washed twice with binding buffer containing 0.5 M NaCl. After harvesting the samples with lysis buffer, the remaining cell-bound radioactivity was counted.

19.1.4 Animal experiment.

All animal experiments were conducted according to the NIH principles of laboratory animal care and Dutch national law ["Wet op de Dierproeven" (Stb 1985, 336)], approved by the Dierexperimentencommissie from the VU University Medical Center and performed in compliance with the protocol FaCh 10-01. Head and neck cancer cells 22A were injected s.c. in the flanks of 8- to 10-week old female donor nude mice (Hsd, athymic nu/nu, Harlan laboratories). Xenograft tumors were grown to a size of 200-500 mm^3, and were subsequently excised, cut in smaller pieces of equal size and transplanted s.c. in the flanks of recipient nude mice. When transplanted tumors properly engrafted, mice were injected i.p. bi-weekly with either PBS, or 1 mg bivalent Nanobody or 1.5 mg trivalent Nanobody.

19.2 *Results*

19.2.1 mRNA expression of CXCR7.

Head and neck cancer cell lines were first tested for CXCR7 mRNA expression. Out of 6 cell lines tested, 4 cell lines showed mRNA expression of CXCR7, namely 22A, 22B, OE and 93-VU-147 cell lines (*Figure 4*).

19.2.2 Protein expression of CXCR7.

CXCR7 mRNA is expressed in a wide range of tissues in humans. However, mRNA expression does not always correlate with cell surface expression of the protein. Therefore, in order to further assess the presence of CXCR7 protein, protein expression of CXCR7 was confirmed in a [^{125}I]-CXCL12 radioligand binding assay. CXCR7-specific expression was determined by displacing the radioligand with the cold chemokines CXCL12 and CXCL11, but not CXCL10. Additionally, the monovalent Nanobody 09A04 displaced [^{125}I]-CXCL12 to a similar extent than CXCL11 and CXCL12 (*Figure 5*).

These data confirmed that mRNA and protein CXCR7 were expressed in 4 head and neck cancer cell lines.

19.2.3 CXCR7 Nanobodies are able to inhibit tumour growth.

CXCR7-expressing cell lines were used in a xenograft model *in vivo* where tumour growth was measured. The 22A cell line was chosen as xenograft tumour model since nude mice s.c. injected with 2 x 10^6 cells per flank allowed for xenograft tumor formation. Next, to ensure that mice from different groups (treated vs. non-treated) presented similar initial tumour sizes for the therapy experiment, we performed tumour transplantation. First, donor nude mice were initially injected with 2 x 10^6 22A cells s.c. in their flanks. Tumours were grown to a size of 200-500 mm^3 and subsequently extracted, cut in smaller pieces of equal size, and transplanted s.c. in recipient nude mice. When engrafted tumours started growing, mice were randomly distributed into five groups that were injected bi-weekly with 400 ul PBS without or with Nanobodies. The constructs tested for therapy were clone 060, clone 083, clone 085 and clone 093. Bivalent and trivalent Nanobodies were dosed at 1 and 1.5 mg per injection, respectively. Over a period of 50 days of therapy, the control (PBS) and clone 060 and clone 083 groups grew tumors to a similar extent (no significant different sizes)*(Figure 6)*. Mice treated with clone 085 and clone 093 displayed a slower tumour growth and significant smaller size compared to PBS-injected mice at the end of the therapy experiment (tumour volumes PBS = 274 ± 47 mm^3, clone 085= 119 ± 30 mm^3 and clone 093 = 114 ± 32 mm^3) (*Figure 7*).

Thus, CXCR7 Nanobodies reduce head and neck cancer cell growth *in vivo*.

This study supports not only the anti-tumour efficacy of the Nanobodies, but also an excellent safety profile, a reflection of its highly targeted and specific activity profile, which is fundamentally different from many other cytotoxic drugs in development or on the market.

Example 20: CXCR7 Nanobodies reduce xenograft tumour growth *in vivo*

In Example 19, it has been demonstrated that CXCR7 Nanobodies are able to inhibit tumours as exemplified by head and neck cancers.

In a first phase to demonstrate in vivo that the Nanobodies are also effective in other tumours in which CXCR7 is (over)-expressed than head and neck cancers, further xenograph models can be used.

Gliomas are the most common forms of primary human brain tumors, and they are often classified into four clinical grades. The most aggressive tumors, grade 4 tumors, also known as glioblastoma multiforme (GBM), are associated with high mortality and morbidity. Survival of patients affected by GBM has remained virtually unchanged during the last decades (*i.e.,* 6–12 months postdiagnosis) despite advances in surgery, radiation, and chemotherapy. GBM xenograph models can be used essentially as described, for instance, by Yi et al. (*EGFR* Gene Overexpression Retained in an Invasive Xenograft Model by Solid Orthotopic Transplantation of Human Glioblastoma Multiforme Into Nude Mice" Cancer Invest. 2011 29: 229–239).

Essentially, the xenograph set up as described in Example 19 is employed, but using xenographs derived from primary tumours, which are obtained from patients who undergo surgical treatment. Cells derived from these tumours are injected into 4-6 weeks old, congenitally athymic nude mice, female, on Balb/c nu/nu background. Mice are maintained under specific pathogen-free barrier environment. For grafting and imaging, the mice are anesthetized intraperitoneally with a 0.10 mg ketamine hydrochloride solution per gram body weight. If necessary, the tumours are excised and retransplanted into other mice, as described in Example 19.

Therapy is started with biweekly injections of 1.5 mg of either PBS, clone 060, clone 083, clone 085 and clone 093. Tumour size is measured every 4 days. The tumour size is measured by a caliper, and the tumour volume is calculated using the formula (length × width^2)/2. The development of the tumour volumes of the mice is followed for 30 days. At 30 days the mice are sacrificed. The tumours are weighed and fixed in 4% polyformaldehyde. The tumour sections are excised for immunohistochemical analysis.

The tumours listed in Figure 3 are tested similarly, either by xenographs of established cell lines or derived from primary tumours. Tumours having a high percentage of CXCR7 are preferred for initial testing.

Example 21: Group 1 immunoglobulin single variable domains

In view of binding, competition and/or β-arrestin results, various ISVDs were not further assessed after initial screening. However, the in vivo results of Examples 19-20 prompted us to further evaluate the presence of other family members of Group 1 ISVDs.

After reassessing the sequences, at least the following 4 Group 1 ISVDs were identified: 01C12 (SEQ ID NO: 99), 01B12 (SEQ ID NO: 100), 01F11 (SEQ ID NO: 101) and 01B10 (SEQ ID NO: 102) (Table B-3).

Example 22: CXCR7 Nanobodies reduce head and neck cancer xenograft tumour growth in vivo

In Example 19 it was demonstrated that CXCR7 Nanobodies reduce head and neck cancer cell growth in vivo. In Example 19, mice received 1.5 mg of either clone 085 (Group 1 ISVD-Group 2 ISVD) or clone 093 (Group 1 ISVD-Group 1 ISVD).

In view of the binding efficacies of Group 2 ISVDs, it is expected that constructs comprising Group 1 ISVD-Group 2 ISVD (e.g. clone 085) would be more efficient than Group 1 ISVD-Group 1 ISVD (e.g. clone 093).

Accordingly, the in vivo xenograft model of Example 19 is used to test this hypothesis. Again, the mice are randomly distributed into 11 groups of 5 mice each that are injected bi-weekly with 400 ul PBS without or with the constructs. The constructs tested for therapy are clone 085 and clone 093.

The dosing is according to the following scheme:

construct	dose/biweekly/5 mice				
clone 085	1.5 mg	0.75 mg	0.375 mg	0.17 mg	0.085 mg
clone 093	1.5 mg	0.75 mg	0.375 mg	0.17 mg	0.085 mg
PBS (negative control)	-	-	-	-	-

Tumour size is measured every 4 days. The tumour size is measured by a caliper, and the tumour volume is calculated using the formula (length × width^2)/2. The development of the tumour volumes

of the mice is followed for 30 days. At 50 days the mice are sacrificed. The tumours are weighed and fixed in 4% polyformaldehyde. The tumour sections are excised for immunohistochemical analysis.

Claims

1. A construct comprising at least one immunoglobulin single variable domain (ISVD) that binds to and/or recognizes amino acid residue M33, and optionally amino acid residue V32 and/or amino acid residue M37 in CXCR7 (SEQ ID NO: 1) and at least one ISVD that binds to and/or recognizes amino acid residue W19, and optionally S23 and/or D25 of CXCR7 (SEQ ID NO: 1).

2. The construct of claim 1 when used as a medicament to reduce tumour growth and/or to treat cancer, preferably head and neck cancer or GBM.

3. The construct of claim 1 or claim 2 that can displace SDF-1 and I-TAC on human CXCR7 (SEQ ID NO: 1) with an average Ki of less than 100 nM and an average SDF-1 and I-TAC displacement of 50% or more.

4. The construct of claim 1 or claim 2 that can displace SDF-1 on human CXCR7 (SEQ ID NO: 1) with an average Ki of less than 100 nM and an average SDF-1 displacement of 50% or more.

5. The construct of claim 1 or claim 2 that can displace I-TAC on human CXCR7 (SEQ ID NO: 1) with an average Ki of less than 100 nM and an average I-TAC displacement of 50% or more.

6. The construct of any one of claims 3-5, wherein the average Ki is 50 nM or less.

7. The construct of any one of claims 3-5, wherein the average Ki is 10 nM or less.

8. The construct of any one of claims 3-7, wherein the average SDF-1 or I-TAC displacement is 80% or more.

9. The construct of claim 1 or claim 2 that can bind human CXCR7 (SEQ ID NO: 1) with a Kd of less than 50nM.

10. An immunoglobulin single variable domain that binds to and/or recognizes amino acid residue M33, and optionally amino acid residue V32 and/or amino acid residue M37 in CXCR7 (SEQ ID NO: 1).

11. The immunoglobulin single variable domain of claim 10 when used as a medicament to reduce tumour growth and/or to treat cancer, preferably head and neck cancer or GBM.

12. The immunoglobulin single variable domain of claim 10 or claim 11, wherein the immunoglobulin single variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain; and

wherein CDR1 is chosen from the group consisting of:

a) the immunoglobulin single variable domain of SEQ ID NO: 9;

b) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 9; and

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 9,

and wherein CDR2 is chosen from the group consisting of:

d) the immunoglobulin single variable domain of SEQ ID NO: 19;

e) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 19; and

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 19,

and wherein CDR3 is chosen from the group consisting of:

g) the immunoglobulin single variable domain of SEQ ID NO: 29;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 29; and

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 29.

13. The immunoglobulin single variable domain of claim 10 or claim 11, wherein the immunoglobulin single variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain; and

wherein CDR1 is chosen from the group consisting of:

a) the immunoglobulin single variable domain of SEQ ID NO: 10;

b) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 10; and

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 10,

and wherein CDR2 is chosen from the group consisting of:

d) the immunoglobulin single variable domain of SEQ ID NO: 20;

e) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 20; and

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 20,

and wherein CDR3 is chosen from the group consisting of:

g) the immunoglobulin single variable domain of SEQ ID NO: 30;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 30; and

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 30.

14. The immunoglobulin single variable domain of claim 10 or claim 11, wherein the immunoglobulin single variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain; and

wherein CDR1 is chosen from the group consisting of:

a) the immunoglobulin single variable domain of SEQ ID NO: 11;

b) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 11; and

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 11,

and wherein CDR2 is chosen from the group consisting of:

d) the immunoglobulin single variable domain of SEQ ID NO: 21;

e) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 21; and

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 21,

and wherein CDR3 is chosen from the group consisting of:

g) the immunoglobulin single variable domain of SEQ ID NO: 31;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 31; and

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 31.

15. The immunoglobulin single variable domain of claim 10 or claim 11, wherein the immunoglobulin single variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain; and

wherein CDR1 is chosen from the group consisting of:

a) the immunoglobulin single variable domain of SEQ ID NO: 12;

b) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 12; and

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 12,

and wherein CDR2 is chosen from the group consisting of:

d) the immunoglobulin single variable domain of SEQ ID NO: 22;

e) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 22; and

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 22,

and wherein CDR3 is chosen from the group consisting of:

g) the immunoglobulin single variable domain of SEQ ID NO: 32;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 32; and

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 32.

16. The immunoglobulin single variable domain of claim 10 or claim 11, wherein the immunoglobulin single variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain; and

wherein CDR1 is chosen from the group consisting of:

a) the immunoglobulin single variable domain of SEQ ID NO: 13;

b) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 13; and

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 13,

and wherein CDR2 is chosen from the group consisting of:

d) the immunoglobulin single variable domain of SEQ ID NO: 23;

e) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 23; and

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 23,

and wherein CDR3 is chosen from the group consisting of:

g) the immunoglobulin single variable domain of SEQ ID NO: 33;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 33; and

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 33.

17. The immunoglobulin single variable domain of claim 10 or claim 11, wherein the immunoglobulin single variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain; and wherein CDR1 is chosen from the group consisting of:

a) the immunoglobulin single variable domain of SEQ ID NO: 93;

b) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 93; and

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 93,

and wherein CDR2 is chosen from the group consisting of:

d) the immunoglobulin single variable domain of SEQ ID NO: 95;

e) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 95; and

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 95,

and wherein CDR3 is chosen from the group consisting of:

g) the immunoglobulin single variable domain of SEQ ID NO: 97;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 97; and

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 97.

18. The immunoglobulin single variable domain of claim 10 or claim 11, wherein the immunoglobulin single variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain; and

wherein CDR1 is chosen from the group consisting of:

a) the immunoglobulin single variable domain of SEQ ID NO: 107;

b) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 107; and

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 107,

and wherein CDR2 is chosen from the group consisting of:

d) the immunoglobulin single variable domain of SEQ ID NO: 115;

e) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 115; and

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 115,

and wherein CDR3 is chosen from the group consisting of:

g) the immunoglobulin single variable domain of SEQ ID NO: 123;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 123; and

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 123.

19. The immunoglobulin single variable domain of claim 10 or claim 11, wherein the immunoglobulin single variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain; and

wherein CDR1 is chosen from the group consisting of:

a) the immunoglobulin single variable domain of SEQ ID NO: 108;

b) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 108; and

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 108,

and wherein CDR2 is chosen from the group consisting of:

d) the immunoglobulin single variable domain of SEQ ID NO: 116;

e) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 116; and

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 116,

and wherein CDR3 is chosen from the group consisting of:

g) the immunoglobulin single variable domain of SEQ ID NO: 124;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 124; and

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 124.

20. The immunoglobulin single variable domain of claim 10 or claim 11, wherein the immunoglobulin single variable domain comprises an amino acid sequence with the formula 1

FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 (1);

wherein FR1 to FR4 refer to framework regions 1 to 4 and are framework regions of an immunoglobulin single variable domain; and

wherein CDR1 is chosen from the group consisting of:

a) the immunoglobulin single variable domain of SEQ ID NO: 110;

b) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 110; and

c) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 110,

and wherein CDR2 is chosen from the group consisting of:

d) the immunoglobulin single variable domain of SEQ ID NO: 118; and

e) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 118,

f) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 118;

and wherein CDR3 is chosen from the group consisting of:

g) the immunoglobulin single variable domain of SEQ ID NO: 126;

h) immunoglobulin single variable domains that have at least 80% amino acid identity with the immunoglobulin single variable domain of SEQ ID NO: 126; and

i) immunoglobulin single variable domains that have 3, 2, or 1 amino acid difference with the immunoglobulin single variable domain of SEQ ID NO: 126.

21. The immunoglobulin single variable domain of any one of claims 10-20, wherein the framework regions (FRs) have a sequence identity of more than 80% with the FRs of SEQ ID NOs: 4 to 8, 92, 103, 104 or 106 (FR1), 14 to 18, 94, 111, 112 or 114 (FR2), 24 to 28, 96, 119, 120 or 122 (FR3), and/or 34 to 38, 98, 127, 128 or 130 (FR4).

22. A polypeptide comprising an immunoglobulin single variable domain of any one of claims 10-21.

23. The polypeptide of claim 22, wherein the immunoglobulin single variable domain is selected from the group consisting of immunoglobulin single variable domains that have an amino acid sequence with a sequence identity of more than 80% with the immunoglobulin single variable domains of SEQ ID NOs: 39 to 43, 91 or 99-102.

24. The polypeptide of claim 22 or claim 23 and additionally comprising at least one human serum albumin binding immunoglobulin single variable domain and optionally comprising a linker selected from the group of linkers with SEQ ID NOs: 49 to 58.

25. The polypeptide of any one of claims 22-24 and additionally comprising ALB8 (SEQ ID NO: 2), and optionally comprising a linker selected from the group of linkers with SEQ ID NOs: 49 to 58.

26. The polypeptide of any one of claims 22-25, wherein the polypeptide is selected from the group consisting of polypeptides that have an amino acid sequence with a sequence identity of more than 80% with the polypeptides of SEQ ID NOs: 44 to 48, 78 to 89 and 131 to 140.

27. A nucleic acid sequence encoding

i) for an immunoglobulin single variable domain of any one of claims 10-21;

ii) for a polypeptide of any one of claims 22-26, or

iii) for a construct of any one of claims 1-9.

28. A pharmaceutical composition comprising

i) an immunoglobulin single variable domain of any one of claims 10-21;

ii) a polypeptide of any one of claims 22-26; or

iii) a construct of any one of claims 1-9;

and optionally a pharmaceutically acceptable excipient.

29. The immunoglobulin single variable domain of any one of claims 10-21, a polypeptide of any one of claims 22-26, or a construct of any one of claims 1-9 when used in cancer, preferably head or neck cancer, GBM and/or inflammatory diseases.

30. The immunoglobulin single variable domain of any one of claims 10-21, a polypeptide of any one of claims 22-26, or a construct of any one of claims 1-9 when used in rheumatoid arthritis.

31. The immunoglobulin single variable domain of any one of claims 10-21, a polypeptide of any one of claims 22-26, or a construct of any one of claims 1-9 when used in multiple sclerosis.

32. A method for producing an immunoglobulin single variable domain of any one of claims 10-21, a polypeptide of any one of claims 22-26, or a construct of any one of claims 1-9, said method at least comprising the step of:

a) expressing, in a suitable host cell or host organism or in another suitable expression system, a nucleic acid or nucleotide sequence according to claim 27;

optionally followed by:

b) isolating and/or purifying the immunoglobulin single variable domain of any one of claims 10-21, a polypeptide of any one of claims 22-26, or a construct of any one of claims 1-9.

33. A method for: reducing tumour growth; treating cancer; treating GBM; treating inflammatory disease; treating rheumatoid arthritis; or treating multiple sclerosis, said method comprising administering a construct of any one of claims 1-9, a immunoglobulin single variable domain of any one of claims 10-21, a polypeptide of any one of claims 22-26 or a pharmaceutical composition of claim 28.

34. Use of a construct of any one of claims 1-9, an immunoglobulin single variable domain of any one of claims 10-21 or a polypeptide of any one of claims 22-26 in the manufacture of a medicament for: reducing tumour growth; treating cancer; treating GBM; treating inflammatory disease; treating rheumatoid arthritis; or treating multiple sclerosis.

35. The method of claim 33; or the use of claim 34, wherein said cancer is neck or head cancer.

36. An immunoglobulin single variable domain; a polypeptide; or a construct, when produced by the method of claim 32.

37. A construct of any one of claims 1-9; an immunoglobulin single variable domain of any one of claims 10-21, 29-31 or 36; a polypeptide of any one of claims 22-26; a nucleic acid sequence of to claim 27; a pharmaceutical composition of claim 28; a method of claim 32 or claim 33; or use of claim 34, substantially as herein described with reference to any one or more of the examples but excluding comparative examples.

Figure 1

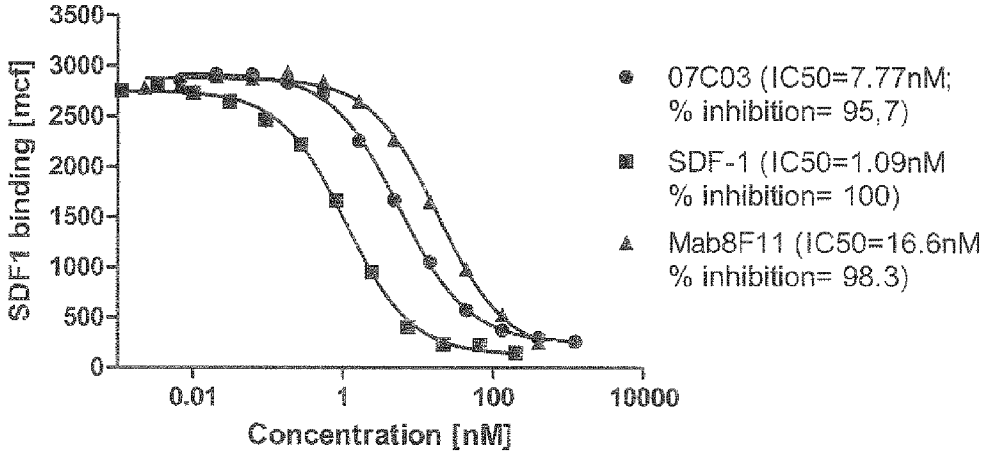
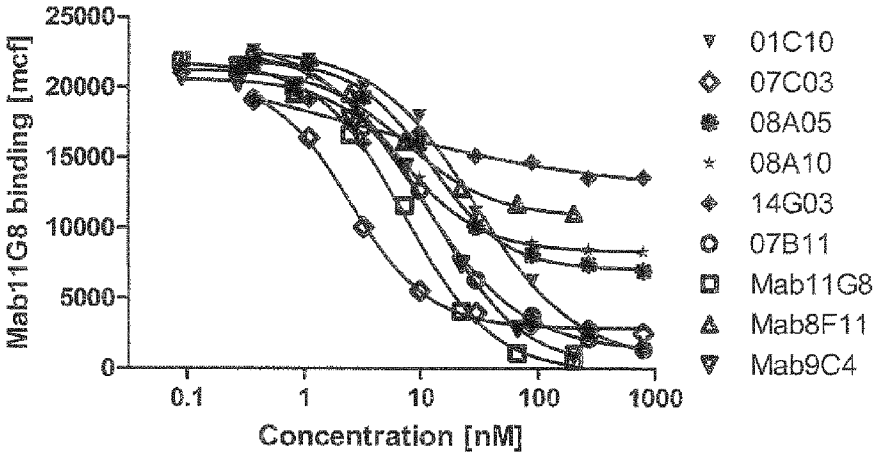


Figure 2



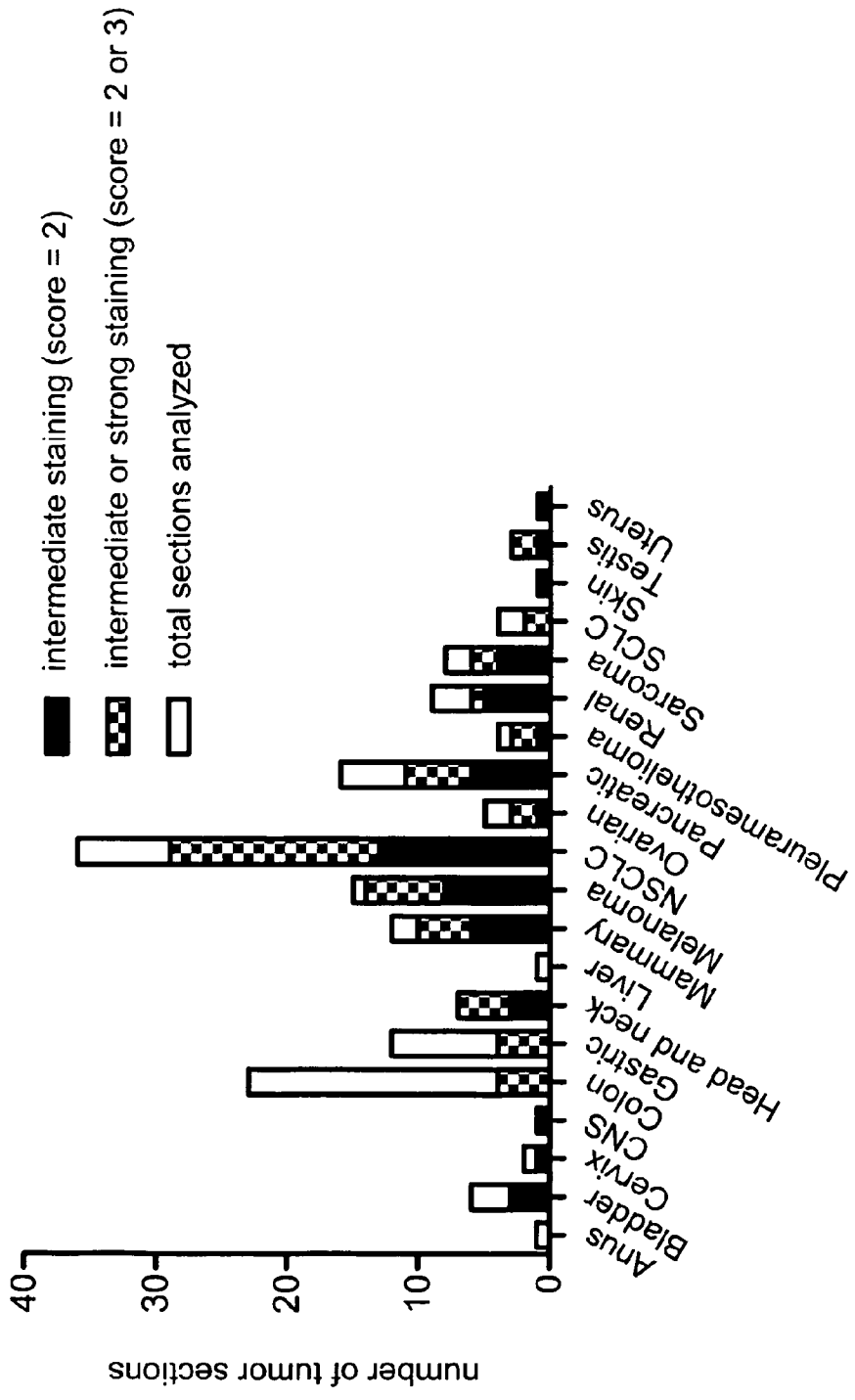

intermediate staining (score = 2)
intermediate or strong staining (score = 2 or 3)
total sections analyzed
number of tumor sections
0
10
20
30
40
Anus
Bladder
Cervix
CNS
Colon
Gastric
Head and neck
Liver
Mammary
Melanoma
NSCLC
Ovarian
Pancreatic
Pleuramesothelioma
Renal
Sarcoma
SCLC
Skin
Testis
Uterus

Figure 3

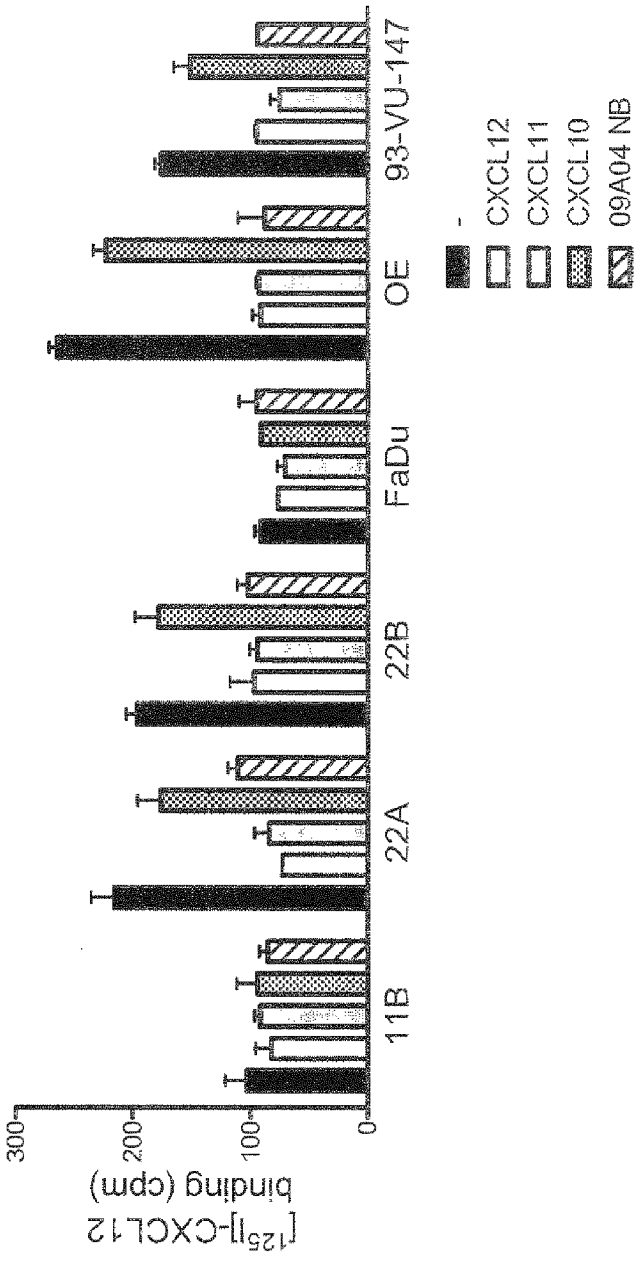

[125I]-CXCL12 binding (cpm)
300
200
100
0
11B
22A
22B
FaDu
OE
93-VU-147
-
CXCL12
CXCL11
CXCL10
09A04 NB

Figure 5

Fu

Figure 4

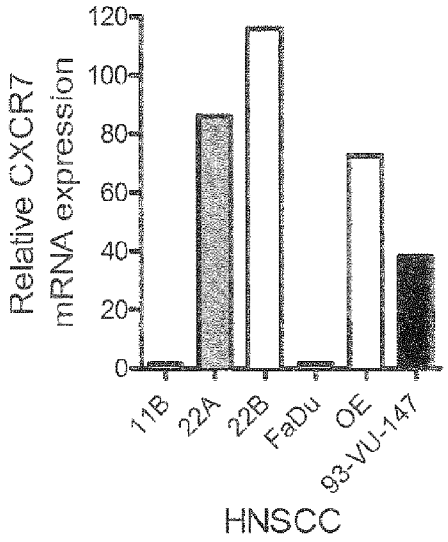


Figure 6

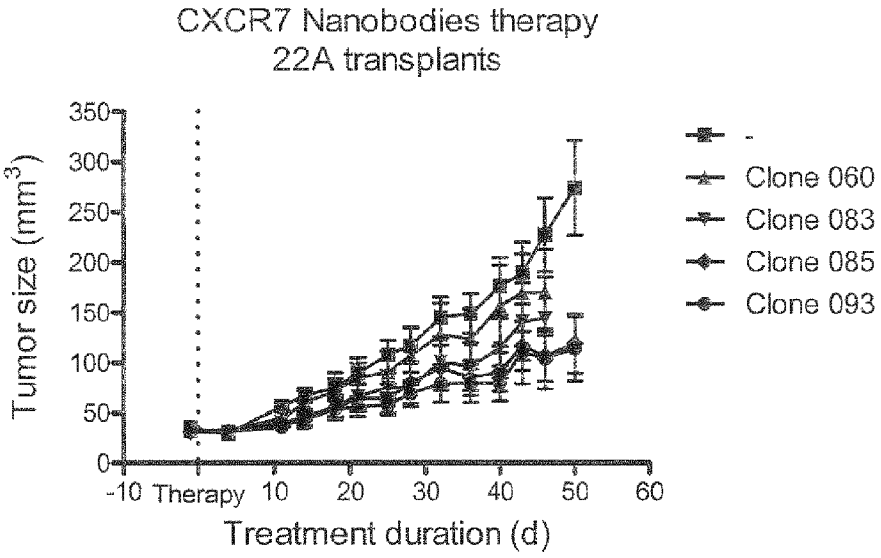


Figure 7

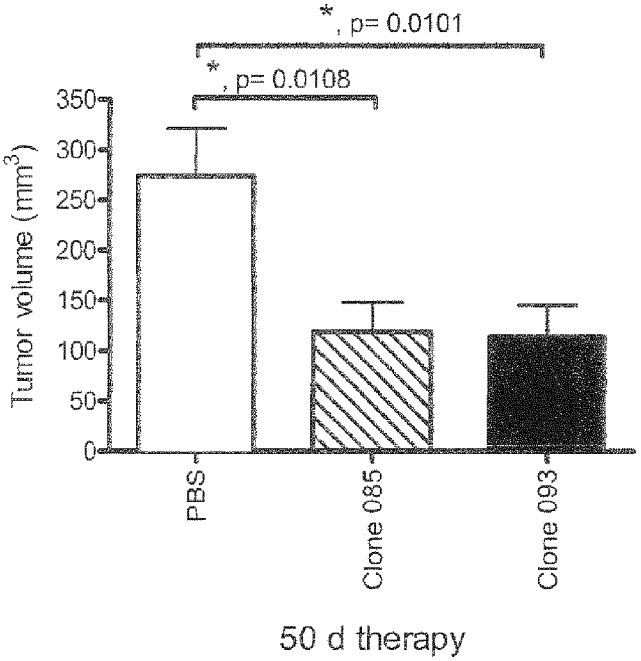
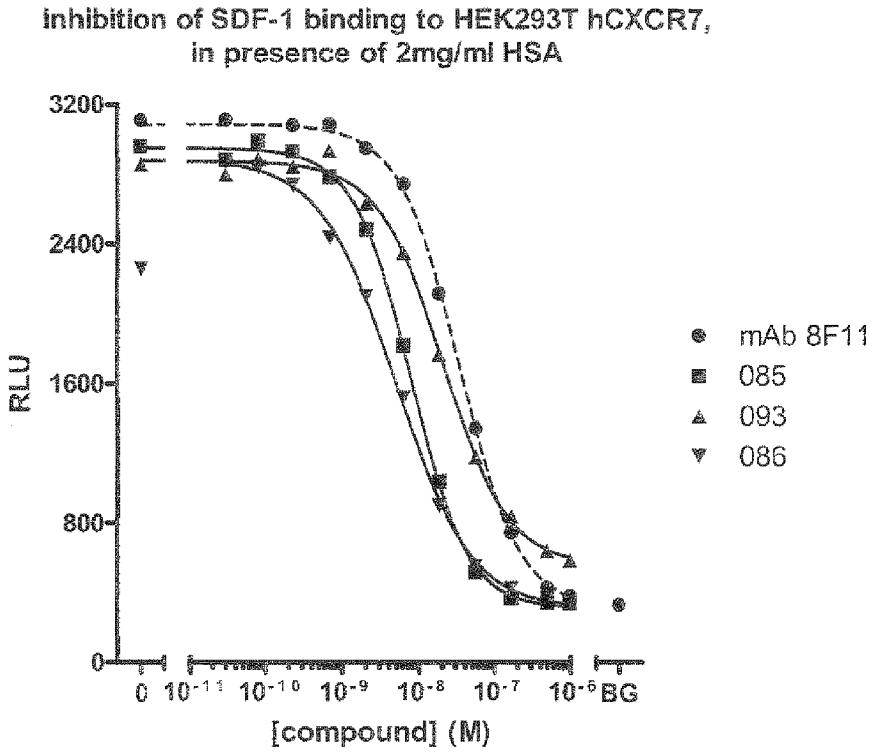


Figure 8



Organization Applicant

Street : Technologiepark 21
City : Gent
State :
Country : Belgium
PostalCode : 9052
PhoneNumber : +3292620000
FaxNumber :
EmailAddress :
<110> OrganizationName : Ablynx NV

Application Project

<120> Title : Biological materials related to CXCR7
<130> AppFileReference : P11-007-PCT-1
<140> CurrentAppNumber :
<141> CurrentFilingDate : ____-__-__

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ANSVVVWVNI QAKTTGYDTH CYILNLAIAD LWVVLTIPVW VVSLVQHNQW PMGELTCKVT 120

HLIFSINLFG SIFFLTCMSV DRYLSITYFT NTPSSRKKMV RRVVCILVWL LAFCVSLPDT 180

YYLKTVTSAS NNETYCRSFY PEHSIKEWLI GMELVSVVLG FAVPFSIIAV FYFLLARAIS 240

ASSDQEKHSS RKIIFSYVVV FLVCWLPYHV AVLLDIFSIL HYIPFTCRLE HALFTALHVT 300

QCLSLVHCCV NPVLYSFINR NYRYELMKAF IFKYSAKTGL TKLIDASRVS ETEYSALEQS 360

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<212> Type : PRT
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SequenceName : Human CXCR7 , SEQ ID NO: 1
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
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ADSVKGRFTI SRDNAKTTLY LQMNSLRPED TAVYYCTIGG SLSRSSQGTL VTVSS 115

<212> Type : PRT
<211> Length : 115
SequenceName : Alb8 , SEQ ID NO: 2
SequenceDescription :

Sequence

<213> OrganismName : Mus musculus
<400> PreSequenceString :
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HLIFSINLFG SIFFLACMSV DRYLSITYFT GTSSYKKKMV RRVVCILVWL LAFFVSLPDT 180

YYLKTVTSAS NNETYCRSFY PEHSIKEWLI GMELVSVILG FAVPFTIIAI FYFLLARAMS 240

ASGDQEKHSS RKIIFSYVVV FLVCWLPYHF VVLLDIFSIL HYIPFTCQLE NVLFTALHVT 300

QCLSLVHCCV NPVLYSFINR NYRYELMKAF IFKYSAKTGL TKLIDASRVS ETEYSALEQN 360

TK 362
<212> Type : PRT
<211> Length : 362
SequenceName : Mouse CXCR7 , SEQ ID NO: 3
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
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EVQLVESGGN LVQAGGSLGL SCAASVSISS 30
<212> Type : PRT
<211> Length : 30
SequenceName : 07B11 FR1 , SEQ ID NO: 4
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
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EVQLVESGGG LVQAGESLTL SCAASGRTLS 30
<212> Type : PRT
<211> Length : 30
SequenceName : 07C03 FR1 , SEQ ID NO: 5
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGG LVQAGDSLRL SCAASGLTFS 30
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<211> Length : 30
SequenceName : 08A05 FR1 , SEQ ID NO: 6
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
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EVQLVESGGG LVQAGGSLRL SCAASGSIFS 30
<212> Type : PRT
<211> Length : 30
SequenceName : 08A10 FR1 , SEQ ID NO: 7
SequenceDescription :

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EVQLVESGGG LVQPGGSLRI SCAASGSIYL 30
<212> Type : PRT
<211> Length : 30
SequenceName : 14G03 FR1 , SEQ ID NO: 8
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
IHIMG 5
<212> Type : PRT
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SequenceName : 07B11 CDR1 , SEQ ID NO: 9
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
AYIMG 5
<212> Type : PRT
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SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
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NYDMG 5
<212> Type : PRT
<211> Length : 5
SequenceName : 08A05 CDR1 , SEQ ID NO: 11
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
IAAMG 5
<212> Type : PRT
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SequenceName : 08A10 CDR1 , SEQ ID NO: 12
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
INYMG 5
<212> Type : PRT
<211> Length : 5
SequenceName : 14G03 CDR1 , SEQ ID NO: 13
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
WYRQAPGKQR DLVA 14
<212> Type : PRT
<211> Length : 14
SequenceName : 07B11 FR2 , SEQ ID NO: 14
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
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WFRQAPGKER EFVA 14
<212> Type : PRT
<211> Length : 14
SequenceName : 07C03 FR2 , SEQ ID NO: 15
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
WFRQAPGKER EFVG 14
<212> Type : PRT
<211> Length : 14

SequenceName : 08A05 FR2 , SEQ ID NO: 16
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
WYRQATGKQR ELVA 14
<212> Type : PRT
<211> Length : 14
SequenceName : 08A10 FR2 , SEQ ID NO: 17
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
WYRQAPGKQR ELVA 14
<212> Type : PRT
<211> Length : 14
SequenceName : 14G03 FR2 , SEQ ID NO: 18
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
TITSGGSTAY ADSVKG 16
<212> Type : PRT
<211> Length : 16
SequenceName : 07B11 CDR2 , SEQ ID NO: 19
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
GIWSGGYTHL ADSAKG 16
<212> Type : PRT
<211> Length : 16
SequenceName : 07C03 CDR2 , SEQ ID NO: 20
SequenceDescription :

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<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
ASWMSGGAPY YSDSVKG 17
<212> Type : PRT
<211> Length : 17
SequenceName : 08A05 CDR2 , SEQ ID NO: 21
SequenceDescription :

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<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
TITDGGTTTY ADSVKG 16
<212> Type : PRT
<211> Length : 16
SequenceName : 08A10 CDR2 , SEQ ID NO: 22
SequenceDescription :

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<213> OrganismName : Artificial Sequence
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TLTSGGSTNY AGSVKG 16

<212> Type : PRT
<211> Length : 16
SequenceName : 14G03 CDR2 , SEQ ID NO: 23
SequenceDescription :

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<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
RFTVSKDNAK NTVYLQMDSL KPEDTSVYYC AA 32
<212> Type : PRT
<211> Length : 32
SequenceName : 07B11 FR3 , SEQ ID NO: 24
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
RFSISRDNAK NTVYLQMNGL KPEDTAVYYC AA 32
<212> Type : PRT
<211> Length : 32
SequenceName : 07C03 FR3 , SEQ ID NO: 25
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
RFTISRDNAK NTVYLQANSL RPEDTAVYYC AA 32
<212> Type : PRT
<211> Length : 32
SequenceName : 08A05 FR3 , SEQ ID NO: 26
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
RVTISRDRSA NTVYLAMNNL KPDDTAVYYC YA 32
<212> Type : PRT
<211> Length : 32
SequenceName : 08A10 FR3 , SEQ ID NO: 27
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
RFAISRDNAK NTVYLQMNSL KPEDTAVYYC NI 32
<212> Type : PRT
<211> Length : 32
SequenceName : 14G03 FR3 , SEQ ID NO: 28
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVRNGVFGKW NHY 13
<212> Type : PRT
<211> Length : 13
SequenceName : 07B11 CDR3 , SEQ ID NO: 29
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence

<400> PreSequenceString :
GLRGRQYSN 9
<212> Type : PRT
<211> Length : 9
SequenceName : 07C03 CDR3 , SEQ ID NO: 30
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
KRLRSFASGG SYDY 14
<212> Type : PRT
<211> Length : 14
SequenceName : 08A05 CDR3 , SEQ ID NO: 31
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
YLRYTSRVPG DNY 13
<212> Type : PRT
<211> Length : 13
SequenceName : 08A10 CDR3 , SEQ ID NO: 32
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
GGTLYDRRRF ES 12
<212> Type : PRT
<211> Length : 12
SequenceName : 14G03 CDR3 , SEQ ID NO: 33
SequenceDescription :

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<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
WGQGTQVTVS S 11
<212> Type : PRT
<211> Length : 11
SequenceName : 07B11 FR4 , SEQ ID NO: 34
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
WGQGTQVTVS S 11
<212> Type : PRT
<211> Length : 11
SequenceName : 07C03 FR4 , SEQ ID NO: 35
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
WGQGTQVTVS S 11
<212> Type : PRT
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SequenceName : 08A05 FR4 , SEQ ID NO: 36
SequenceDescription :

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<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
WGQGTQVTVS S 11
<212> Type : PRT
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SequenceName : 08A10 FR4 , SEQ ID NO: 37
SequenceDescription :

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<213> OrganismName : Artificial Sequence
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WGQGTQVTVS S 11
<212> Type : PRT
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SequenceName : 14G03 FR4 , SEQ ID NO: 38
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
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QMDSLKPEDTSVYYCAAEVRNGVFGKWNHYWGQGTQVTVSS
<212> Type : PRT
<211> Length : 121
SequenceName : 7CXCR7B11 (07B11) , SEQ ID NO: 39
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
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<212> Type : PRT
<211> Length : 117
SequenceName : 7CXCR7C3 (07C03) , SEQ ID NO: 40
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
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LQANSLRPEDTAVYYCAAKRLRSFASGGSYDYWGQGTQVTVSS
<212> Type : PRT
<211> Length : 123
SequenceName : 7CXCR8A5 (08A05) , SEQ ID NO: 41
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
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EVQLVESGGGLVQAGGSLRLSCAASGSIFSIAAMGWYRQATGKQRELVATITDGGTTTYADSVKGRVTISRDRSANTVYL
AMNNLKPDDTAVYYCYAYLRYTSRVPGDNYWGQGTQVTVSS
<212> Type : PRT
<211> Length : 121
SequenceName : 7CXCR8A10 (08A10) , SEQ ID NO: 42
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
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<212> Type : PRT
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SequenceName : 7CXCR14G3 (14G03) , SEQ ID NO: 43
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
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QMDSLKPEDTSVYYCAAEVRNGVFGKVWNHYWGQGTQVTVSSGGGGSGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFS
SFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTL
VTVSS
<212> Type : PRT
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SequenceName : 7CXCR008 (07B11-9GS-Alb8) , SEQ ID NO: 44
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
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SWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVS
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<212> Type : PRT
<211> Length : 241
SequenceName : 7CXCR009 (07C03-9GS-Alb8) , SEQ ID NO: 45
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
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LQANSLRPEDTAVYYCAAKRLRSFASGGSYDYWGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFT
FSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQG
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<212> Type : PRT
<211> Length : 247
SequenceName : 7CXCR010 (08A05-9GS-Alb8) , SEQ ID NO: 46
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
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AMNNLKPDDTAVYYCYAYLRYTSRVPGDNYWGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFS
SFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTL
VTVSS
<212> Type : PRT
<211> Length : 245
SequenceName : 7CXCR011 (08A10-9GS-Alb8) , SEQ ID NO: 47
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQPGGSLRISCAASGSIYLINYMGWYRQAPGKQRELVATLTSGGSTNYAGSVKGRFAISRDNAKNTVYL
QMNSLKPEDTAVYYCNIGGTLYDRRRFESWGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSS
FGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLV
TVSS
<212> Type : PRT
<211> Length : 244

SequenceName : 7CXCR013 (14G03-9GS-Alb8) , SEQ ID NO: 48
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
GGGGS 5
<212> Type : PRT
<211> Length : 5
SequenceName : 5GS , SEQ ID NO: 49
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
SGGSGGS 7
<212> Type : PRT
<211> Length : 7
SequenceName : 6GS , SEQ ID NO: 50
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
GGGGSGGGS 9
<212> Type : PRT
<211> Length : 9
SequenceName : 9GS , SEQ ID NO: 51
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
GGGGSGGGGS 10
<212> Type : PRT
<211> Length : 10
SequenceName : 10GS , SEQ ID NO: 52
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
GGGGSGGGGS GGGGS 15
<212> Type : PRT
<211> Length : 15
SequenceName : 15GS , SEQ ID NO: 53
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
GGGGSGGGGS GGGGGGGS 18
<212> Type : PRT
<211> Length : 18
SequenceName : 18GS , SEQ ID NO: 54
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
GGGGSGGGGS GGGGSGGGGS 20

<212> Type : PRT
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SequenceName : 20GS , SEQ ID NO: 55
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
GGGGSGGGGS GGGGSGGGGS GGGGS 25
<212> Type : PRT
<211> Length : 25
SequenceName : 25GS , SEQ ID NO: 56
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS 30
<212> Type : PRT
<211> Length : 30
SequenceName : 30GS , SEQ ID NO: 57
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
GGGGSGGGGS GGGGSGGGGS GGGGSGGGGS GGGGS 35
<212> Type : PRT
<211> Length : 35
SequenceName : 35GS , SEQ ID NO: 58
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
gaggtgcaattggtggagtctgggggaaacttggtgcaggctgggggtctctgggactctcctgtgcagcctctgtaag
catctccagtatccatatcatgggctggtaccggcaggctccaggcaaacagcgcgacttggtcgctactatttactagtg
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caaatggacagcctgaaacctgaggacacatccgtctatttactgtgcagccgaggtcagaaatggggtgttttggaaaatg
gaatcactactggggccaggggacccaggtcaccgtctcctca
<212> Type : DNA
<211> Length : 363
SequenceName : 7CXCR7B11 (07B11) , SEQ ID NO: 59
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
gaggtgcaattggtggagtctgggggaggattggtgcaggctggggagtctctgactctcctcctgtgcagcctctggacg
cacctttaagtgcctatatcatgggctggttccgccaggctccagggaaggagcgggagttttgtagccggtatctggagtg
gtggttacacacacctttgcagactccgcgaagggccgattcagcatctctagagacaacgccaagaacactgtatatctg
caaatgaacggcctgaaacctgaggacacggccgtctatttactgtgcagcaggtctgagaggccgccagtatagtaactg
gggccaggggacccaggtcaccgtctcctca
<212> Type : DNA
<211> Length : 351
SequenceName : 7CXCR7C3 (07C03) , SEQ ID NO: 60
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
gaggtgcaattggtggagtctgggggaggattggtgcaggctggggactctctgagactctcctgtgcagcctctggact

cactttcagtaactatgacatgggctggttccgccaggctccagggaaggagcgtgaatttgtaggggctagttggtgga
gtggtggtgccccatactattcagactccgtgaagggccgattcaccatctccagagacaacgccaagaacacggtgtat
ctgcaagcgaacagcctgagacctgaggacacggccgttattactgtgcagccaaaaggctgcgtagttcgcctccgg
tgggtcgtatgattactggggtcaggggacccaggtcaccgtctcctca
<212> Type : DNA
<211> Length : 369
SequenceName : 7CXCR8A5 (08A05) , SEQ ID NO: 61
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
gagtctgggggaggcttggtgcaggctggagggtctctgagactcctcctgtgcagcttctggaagcatcttcagtatcgc
tgccatgggctggtaccgccaggctacagggaagcagcgcgagttggtcgcaactatcactgatggcggtacgacaacct
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ccaggggacccaggtcaccgtctcctca
<212> Type : DNA
<211> Length : 348
SequenceName : 7CXCR8A10 (08A10) , SEQ ID NO: 62
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
gaggtgcaattggtggagtctgggggaggcttggtgcagcctgggggtctctgagaatttcctgtgcagcctctggaag
catctacctttatcaatttacatgggctggtaccgccaggctccagggaagcagcgcgagttggtcgcaacgcttactagtg
gtggtagtaccaactatgcaggctccgtgaagggccgattcgccatctccagagacaacgccaagaacacggtttatctg
caaatgaacagcctgaaacctgaggacacggccgtctatttactgtaatatataggaggaacgctatacgacagaaggcggtt
tgaatcctggggccaggggacccaggtcaccgtctcctcag
<212> Type : DNA
<211> Length : 361
SequenceName : 7CXCR14G3 (14G03) , SEQ ID NO: 63
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQPGNSLRLSCAASGFTFS 30
<212> Type : PRT
<211> Length : 30
SequenceName : Alb8 FR1 , SEQ ID NO: 64
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
SFGMS 5
<212> Type : PRT
<211> Length : 5
SequenceName : Alb8 CDR1 , SEQ ID NO: 65
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
WVRQAPGKGLEWVS 14
<212> Type : PRT
<211> Length : 14
SequenceName : Alb8 FR2 , SEQ ID NO: 66
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
SISGSGSDTL YADSVKG 17
<212> Type : PRT
<211> Length : 17
SequenceName : Alb8 CDR2 , SEQ ID NO: 67
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
RFTISRDNAK TTLYLQMNSL RPEDTAVYYC TI 32
<212> Type : PRT
<211> Length : 32
SequenceName : Alb8 FR3 , SEQ ID NO: 68
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
GGSLSR 6
<212> Type : PRT
<211> Length : 6
SequenceName : Alb8 CDR3 , SEQ ID NO: 69
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
SSQGTLVTVS S 11
<212> Type : PRT
<211> Length : 11
SequenceName : Alb8 FR4 , SEQ ID NO: 70
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
AAAHHHHHHG AAEQKLISEE DLNGAA 26
<212> Type : PRT
<211> Length : 26
SequenceName : Tag-1 , SEQ ID NO: 71
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
AAAEQKLISE EDLNGAAHHH HHH 23
<212> Type : PRT
<211> Length : 23
SequenceName : Tag-2 , SEQ ID NO: 72
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
gaggtgcaattggtggagtctgggggaaacttggtgcaggctgggggtctctgggactctcctgtgcagcctctgtaag
catctccagtatccatatcatgggctggtaccggcaggctccaggcaaacagcgcgacttggtcgctactattactagtg
gtggtagcacagcatatgcagactccgtgaagggacgattcaccgtctccaaagacaacgccaagaacacggtgtatctg
caaatggacagcctgaaacctgaggacacatccgtctattactgtgcagccgaggtcagaaatggggtgttttggaaaatg
gaatcactactggggccaggggacccaggtcacggtctcctcaggaggtggcgggtccggaggcggatccgaggtacagc

tggtggagtctgggggtggcttggtgcaaccgggtaacagtctgcgccttagctgcgcagcgtctggctttacctcagc
tcctttggcatgagctgggttcgccaggctccgggaaaaggactggaatgggttcgtctattagcggcagtggtagcga
tacgctctacgcggactccgtgaagggccgttttcaccatctcccgcgataacgccaaaactacactgtatctgcaaatga
atagcctgcgtcctgaagacacggccgttttattactgtactattggtggctcgttaagccgttcttcacagggtaccctg
gtcaccgtctcctca
<212> Type : DNA
<211> Length : 735
SequenceName : 07B11-9GS-Alb8 , SEQ ID NO: 73
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
gaggtgcaattggtggagtctgggggaggattggtgcaggctggggagtctctgactctcctgtgcagcctctggacg
cacctaagtgcctatatcatgggctggttccgccaggctccagggaaggagcgggagttttgtagccggtatctggagtg
gtggttacacacacctgcagactccgcgaagggccgattcagcatctctagagacaacgccaagaacactgtatatctg
caaatgaacggcctgaaacctgaggacacggccgtctattactgtgcagcaggtctgagaggccgccagtatagtaactg
ggccaggggacccaggtcacggtctcctcaggaggtggcgggtccggaggcggatccgaggtacagctggtggagtctg
gggtggctttggtgcaaccgggtaacagtctgcgcctagctgcgcagcgtctggctttacctcagctcctttggcatg
agctgggttcgccaggctccgggaaaaggactggaatgggttcgtctattagcggcagtggtagcgatacgctctacgc
ggactccgtgaagggccgttttcaccatctcccgcgataacgccaaaactacactgtatctgcaaatgaatagcctgcgtc
ctgaagacacggccgttttattactgtactattggtggctcgttaagccgttcttcacagggtaccctggtcaccgtctcc
tca
<212> Type : DNA
<211> Length : 723
SequenceName : 07C03-9GS-Alb8 , SEQ ID NO: 74
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
gaggtgcaattggtggagtctgggggaggattggtgcaggctggggactctctgagactctcctgtgcagcctctggact
cacttttcagtaactatgacatgggctggttccgccaggctccagggaaggagcgtgaatttgtaggggctagttggtgga
gtggtggtgccccatactattcagactccgtgaagggccgattcaccatctccagagacaacgccaagaacacggtgtat
ctgcaagcgaacagcctgagacctgaggacacggccgtttattactgtgcagccaaaaggctgcgtagttttcgcctccgg
tgggtcgtatgattactggggtcaggggacccaggtcacggtctcctcaggaggtggcgggtccggaggcggatccgagg
tacagctggtggagtctgggggtggcttggtgcaaccgggtaacagtctgcgcctagctgcgcagcgtctggctttacc
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tagcgatacgctctacgcggactccgtgaagggccgttttcaccatctcccgcgataacgccaaaactacactgtatctgc
aaatgaatagcctgcgtcctgaagacacggccgttttattactgtactattggtggctcgttaagccgttcttcacagggt
accctggtcaccgtctcctca
<212> Type : DNA
<211> Length : 741
SequenceName : 08A05-9GS-Alb8 , SEQ ID NO: 75
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
gaggtgcaattggtggagtctgggggaggcttggtgcaggctggagggtctctgagactctcctgtgcagcttctggaag
catcttcagtatcgctgccatgggctggtaccgccaggctacagggaagcagcgcgagttggtcgcaactatcactgatg
gcggtacgacaacctatgcagactccgtgaagggccgagtcaccatctccagggacaggtctgcgaacacggtgtatctg
gcaatgaacaattttgaaacctgatgacacagccgtctattattgttatgcgtatctgcgctatacaagcagagtacctgg
cgataactactggggccaggggacccaggtcacggtctcctcaggaggtggcgggtccggaggcggatccgaggtacagc
tggtggagtctgggggtggcttggtgcaaccgggtaacagtctgcgcctagctgcgcagcgtctggctttacctcagc
tcctttggcatgagctgggttcgccaggctccgggaaaaggactggaatgggttcgtctattagcggcagtggtagcga
tacgctctacgcggactccgtgaagggccgttttcaccatctcccgcgataacgccaaaactacactgtatctgcaaatga
atagcctgcgtcctgaagacacggccgttttattactgtactattggtggctcgttaagccgttcttcacagggtaccctg
gtcaccgtctcctca
<212> Type : DNA
<211> Length : 735
SequenceName : 08A10-9GS-Alb8 , SEQ ID NO: 76
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
gaggtgcaattggtggagtctgggggaggcttggtgcagcctgggggtctctgagaatttcctgtgcagcctctggaag
catctaccttatcaattacatgggctggtaccgccaggctccagggaagcagcgcgagttggtcgcaacgcttactagtg
gtggtagtaccaactatgcaggctccgtgaagggccgattcgccatctccagagacaacgccaagaacacggtttatctg
caaatgaacagcctgaaacctgaggacacggccgtctatttactgtaatataggaggaacgctatacgacagaaggcggtt
tgaatcctggggccaggggacccaggtcacggtctcctcaggaggtggcgggtccggaggcggatccgaggtacagctgg
tggagtctgggggtggcttggtgcaaccgggtaacagtctgcgccttagctgcgcagcgtctggctttacctcagctcc
ttttggcatgagctgggttcgccaggctccgggaaaaggactggaatgggttcgtctattagcggcagtggtagcgatac
gctctacgcggactccgtgaagggccgttttcaccatctcccgcgataacgccaaaactacactgtatctgcaaatgaata
gcctgcgtcctgaagacacggccgttttatttactgtactattggtggctcgttaagccgttcttcacagggtaccctggtc
accgtctcctca
<212> Type : DNA
<211> Length : 732
SequenceName : 14G03-9GS-Alb8 , SEQ ID NO: 77
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGNLVQAGGSLGLSCAASVSISSIHIMGWYRQAPGKQRDLVATITSGGSTAYADSVKGRFTVSKDNAKNTVYL
QMDSLKPEDTSVYYCAAEVRNGVFGKWNHYWGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQAGESLTLSCAASGRTLS
AYIMGWFRQAPGKEREFVAGIWSGGYTHLADSAKGRFSISRDNAKNTVYLQMNGLKPEDTAVYYCAAGLRGRQYSNWGQG
TQVTVSS
<212> Type : PRT
<211> Length : 247
SequenceName : 07B11-9GS-07C03 , SEQ ID NO: 78
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQAGESLTLSCAASGRTLSAYIMGWFRQAPGKEREFVAGIWSGGYTHLADSAKGRFSISRDNAKNTVYL
QMNGLKPEDTAVYYCAAGLRGRQYSNWGQGTQVTVSSGGGGSGGGSEVQLVESGGNLVQAGGSLGLSCAASVSISSIHIM
GWYRQAPGKQRDLVATITSGGSTAYADSVKGRFTVSKDNAKNTVYLQMDSLKPEDTSVYYCAAEVRNGVFGKWNHYWGQG
TQVTVSS
<212> Type : PRT
<211> Length : 247
SequenceName : 07C03-9GS-07B11 , SEQ ID NO: 79
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGNLVQAGGSLGLSCAASVSISSIHIMGWYRQAPGKQRDLVATITSGGSTAYADSVKGRFTVSKDNAKNTVYL
QMDSLKPEDTSVYYCAAEVRNGVFGKWNHYWGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFS
SFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTL
VTVSSGGGGSGGGSEVQLVESGGGLVQAGESLTLSCAASGRTLSAYIMGWFRQAPGKEREFVAGIWSGGYTHLADSAKGR
FSISRDNAKNTVYLQMNGLKPEDTAVYYCAAGLRGRQYSNWGQGTQVTVSS
<212> Type : PRT
<211> Length : 371
SequenceName : 07B11-9GS-Alb8-9GS-07C03 , SEQ ID NO: 80
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGNLVQAGGSLGLSCAASVSISSIHIMGWYRQAPGKQRDLVATITSGGSTAYADSVKGRFTVSKDNAKNTVYL
QMDSLKPEDTSVYYCAAEVRNGVFGKWNHYWGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQAGESLTLSCAASGRTLS
AYIMGWFRQAPGKEREFVAGIWSGGYTHLADSAKGRFSISRDNAKNTVYLQMNGLKPEDTAVYYCAAGLRGRQYSNWGQG
TQVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSV
KGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
<212> Type : PRT

<211> Length : 371
SequenceName : 07B11-9GS-07C03-9GS-Alb8 , SEQ ID NO: 81
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQAGDSLRLSCAASGLTFSNYDMGWFRQAPGKEREFVGASWWSGGAPYYSDSVKGRFTISRDNAKNTVY
LQANSLRPEDTAVYYCAAKRLRSFASGGSYDYWGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQAGGSLRLSCAASGSI
FSIAAMGWYRQATGKQRELVATITDGGTTTYADSVKGRVTISRDRSANTVYLAMNNLKPDDTAVYYCYAYLRYTSRVPGD
NYWGQGTQVTVSS
<212> Type : PRT
<211> Length : 253
SequenceName : 08A05-9GS-08A10 , SEQ ID NO: 82
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQAGGSLRLSCAASGSIFSIAAMGWYRQATGKQRELVATITDGGTTTYADSVKGRVTISRDRSANTVYL
AMNNLKPDDTAVYYCYAYLRYTSRVPGDNYWGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFS
SFGMSWWRQAPGKGLEWWSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTL
VTVSSGGGGSGGGSEVQLVESGGGLVQAGGSLRLSCAASGSIFSIAAMGWYRQATGKQRELVATITDGGTTTYADSVKGR
VTISRDRSANTVYLAMNNLKPDDTAVYYCYAYLRYTSRVPGDNYWGQGTQVTVSS
<212> Type : PRT
<211> Length : 375
SequenceName : 08A10-9GS-Alb8-9GS-08A10 , SEQ ID NO: 83
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQAGGSLRLSCAASGSIFSIAAMGWYRQATGKQRELVATITDGGTTTYADSVKGRVTISRDRSANTVYL
AMNNLKPDDTAVYYCYAYLRYTSRVPGDNYWGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQAGGSLRLSCAASGSIFS
IAAMGWYRQATGKQRELVATITDGGTTTYADSVKGRVTISRDRSANTVYLAMNNLKPDDTAVYYCYAYLRYTSRVPGDNY
WGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWWRQAPGKGLEWWSSISGSGSDTLY
ADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
<212> Type : PRT
<211> Length : 375
SequenceName : 08A10-9GS-08A10-9GS-Alb8 , SEQ ID NO: 84
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQAGDSLRLSCAASGLTFSNYDMGWFRQAPGKEREFVGASWWSGGAPYYSDSVKGRFTISRDNAKNTVY
LQANSLRPEDTAVYYCAAKRLRSFASGGSYDYWGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQAGGSLRLSCAASGSI
FSIAAMGWYRQATGKQRELVATITDGGTTTYADSVKGRVTISRDRSANTVYLAMNNLKPDDTAVYYCYAYLRYTSRVPGD
NYWGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWWRQAPGKGLEWWSSISGSGSDT
LYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
<212> Type : PRT
<211> Length : 377
SequenceName : 08A05-9GS-08A10-9GS-Alb8 , SEQ ID NO: 85
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGNLVQAGGSLGLSCAASVSISSIHIMGWYRQAPGKQRDLVATITSGGSTAYADSVKGRFTVSKDNAKNTVYL
QMDSLKPEDTSVYYCAAEVRNGVFGKVWNHYWGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQTGGSLRLSCAASGFTFS
SYAMSWWRQAPGKGLEWWSGIKSSGDSTRYAGSVKGRFTISRDNAKNMLYLQMYSLKPEDTAVYYCAKSRVSRTGLYTYD
NRGQGTQVTVSS
<212> Type : PRT

<211> Length : 252
SequenceName : 07B11-9GS-238D2 , SEQ ID NO: 86
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQAGESLTLSCAASGRTLSAYIMGWFRQAPGKEREFVAGIWSGGYTHLADSAKGRFSISRDNAKNTVYL
QMNGLKPEDTAVYYCAAGLRGRQYSNWGQGTQVTVSSGGGGSGGGSEVQLMESGGGLVQAGGSLRLSCAASGRTFNNYAM
GWFRRAPGKEREFVAAITRSGVRSGVSAIYGDSVKDRFTISRDNAKNTLYLQMNSLKPEDTAVYTCAASAIGSGALRRFE
YDYSGQGTQVTVSS
<212> Type : PRT
<211> Length : 254
SequenceName : 07C03-9GS-238D4 , SEQ ID NO: 87
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQAGGSLRLSCAASGSIFSIAAMGWYRQATGKQRELVATITDGGTTTYADSVKGRVTISRDRSANTVYL
AMNNLKPDDTAVYYCYAYLRYTSRVPGDNYWGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFS
SFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTL
VTVSSGGGGSGGGSEVQLVESGGGLVQTGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSGIKSSGDSTRYAGSVKG
RFTISRDNAKNMLYLQMYSLKPEDTAVYYCAKSRVSRTGLYTYDNRGQGTQVTVSS
<212> Type : PRT
<211> Length : 376
SequenceName : 08A10-9GS-Alb8-9GS-238D2 , SEQ ID NO: 88
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQAGDSLRLSCAASGLTFSNYDMGWFRQAPGKEREFVGASWWSGGAPYYSDSVKGRFTISRDNAKNTVY
LQANSLRPEDTAVYYCAAKRLRSFASGGSYDYWGQGTQVTVSSGGGGSGGGSEVQLMESGGGLVQAGGSLRLSCAASGRT
FNNYAMGWFRRAPGKEREFVAAITRSGVRSGVSAIYGDSVKDRFTISRDNAKNTLYLQMNSLKPEDTAVYTCAASAIGSG
ALRRFEYDYSGQGTQVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSI
SGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
<212> Type : PRT
<211> Length : 384
SequenceName : 08A05-9GS-238D4-9GS-Alb8 , SEQ ID NO: 89
SequenceDescription :

Sequence

<213> OrganismName : Macaca fascicularis
<400> PreSequenceString :
MDLHVFDYSE PGNFSDISWP CNSSDCIVVD TVMCPNMPNK SVLLYTLAFI YIFIFVIGMI 60

ANSVVVWVNI QAKTTGYDTH CYILNLAIAD LWVVLTIPVW VVSLVQHNQW PMGELTCKVT 120

HLIFSINLFG SIFFLTCMSV DRYLSITYFT NTSSSRKKMV RRVVCVLVWL LAFCVSLPDT 180

YYLKTVTSAS NNETYCRSFY PEHSIKEWLI GMELVSVVLG FAVPFSVIAV FYFLLARAIS 240

ASGDQEKHSS RKIIFSYVVV FLVCWLPYHV AVLLDIFSIL HYIPFTCRLE HALFTALHVT 300

QCLSLVHCCV NPVLYSFINR NYRYELMKAF IFKYSAKTGL TKLIDASRVS ETEYSALEQS 360

TK 362
<212> Type : PRT
<211> Length : 362
SequenceName : Cynomolgus CXCR7 or cCXCR7 , SEQ ID NO: 90
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQTGASLRLSCAASGRTFSNYAMGWFRQAPGKERERVAAITPRAFTTYYADSVKGRFTISRDNAKNTAY
LQMVSLKPEDTAVYYCAAQLVGSGSNLGRQESYAYWGQGTQVTVSS
<212> Type : PRT
<211> Length : 126
SequenceName : 01C10 , SEQ ID NO: 91
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGG LVQTGASLRL SCAASGRTFS 30
<212> Type : PRT
<211> Length : 30
SequenceName : 01C10 FR1 , SEQ ID NO: 92
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
NYAMG 5
<212> Type : PRT
<211> Length : 5
SequenceName : 01C10 CDR1 , SEQ ID NO: 93
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
WFRQAPGKER ERVA 14
<212> Type : PRT
<211> Length : 14
SequenceName : 01C10 FR2 , SEQ ID NO: 94
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
AITPRAFTTY YADSVKG 17
<212> Type : PRT
<211> Length : 17
SequenceName : 01C10 CDR2 , SEQ ID NO: 95
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
RFTISRDNAK NTAYLQMVSL KPEDTAVYYC AA 32
<212> Type : PRT
<211> Length : 32
SequenceName : 01C10 FR3 , SEQ ID NO: 96
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
QLVGSGSNLG RQESYAY 17
<212> Type : PRT
<211> Length : 17
SequenceName : 01C10 CDR3 , SEQ ID NO: 97

SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
WGQGTQVTVS S 11
<212> Type : PRT
<211> Length : 11
SequenceName : 01C10 FR4 , SEQ ID NO: 98
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQAGASLRLSCAASGRTFSNYAMGWFRQAPGKEREREVAAISPSAVTTYYADSVKGRFTISRDNAKNTAY
LQMVSLKPEDTAVYYCAAQLPGRGSNLGRQASYAYWGQGTQVTVSS
<212> Type : PRT
<211> Length : 126
SequenceName : 01C12 , SEQ ID NO: 99
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQAGASLRLSCAASGRTFSNYAMGWFRQAPGKEREPVAAISPAALTTYYADFVKGRFTISRDNAKNTAY
LQMVSLKPEDTAVYYCAAQLVGSGSNLGRQQSYAYWGQGTQVTVSS
<212> Type : PRT
<211> Length : 126
SequenceName : 01B12 , SEQ ID NO: 100
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQAGASLRLSCAASGRTFSNYAMGWFRQAPGKEREPVAAISPAALTTYYADFVKGRFTISRDNAKNTAY
LQMVSLKPEDTAVYYCAAQLVGSGSNLGRQQSYAYWGQGTQVTVSS
<212> Type : PRT
<211> Length : 126
SequenceName : 01F11 , SEQ ID NO: 101
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQAGASLRLSCAASGRTFGNYAMGWFRQAPGKEREPVAAISPAAVTTYYADFVKGRFTISRDNAKNTAY
LQMVSLKPEDTAVYYCAAQLVGSGSNLGRQQSYAYWGQGTQVTVSS
<212> Type : PRT
<211> Length : 126
SequenceName : 01B10 , SEQ ID NO: 102
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGG LVQAGASLRL SCAASGRTFS 30
<212> Type : PRT
<211> Length : 30
SequenceName : 01C12 FR1 , SEQ ID NO: 103
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGG LVQAGASLRL SCAASGRTFS 30
<212> Type : PRT
<211> Length : 30
SequenceName : 01B12 FR1 , SEQ ID NO: 104
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGG LVQAGASLRL SCAASGRTFS 30
<212> Type : PRT
<211> Length : 30
SequenceName : 01F11 FR1 , SEQ ID NO: 105
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGG LVQAGASLRL SCAASGRTFG 30
<212> Type : PRT
<211> Length : 30
SequenceName : 01B10 FR1 , SEQ ID NO: 106
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
NYAMG 5
<212> Type : PRT
<211> Length : 5
SequenceName : 01C12 CDR1 , SEQ ID NO: 107
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
NYAMG 5
<212> Type : PRT
<211> Length : 5
SequenceName : 01B12 CDR1 , SEQ ID NO: 108
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
NYAMG 5
<212> Type : PRT
<211> Length : 5
SequenceName : 01F11 CDR1 , SEQ ID NO: 109
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
NYAMG 5
<212> Type : PRT
<211> Length : 5
SequenceName : 01B10 CDR1 , SEQ ID NO: 110
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
WFRQAPGKER ERVA 14
<212> Type : PRT
<211> Length : 14
SequenceName : 01C12 FR2 , SEQ ID NO: 111
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
WFRQAPGKER EPVA 14
<212> Type : PRT
<211> Length : 14
SequenceName : 01B12 FR2 , SEQ ID NO: 112
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
WFRQAPGKER EPVA 14
<212> Type : PRT
<211> Length : 14
SequenceName : 01F11 FR2 , SEQ ID NO: 113
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
WFRQAPGKER EPVA 14
<212> Type : PRT
<211> Length : 14
SequenceName : 01B10 FR2 , SEQ ID NO: 114
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
AISPSAVTTY YADSVKG 17
<212> Type : PRT
<211> Length : 17
SequenceName : 01C12 CDR2 , SEQ ID NO: 115
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
AISPAALTTY YADFVKG 17
<212> Type : PRT
<211> Length : 17
SequenceName : 01B12 CDR2 , SEQ ID NO: 116
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
AISPAALTTY YADFVKG 17
<212> Type : PRT
<211> Length : 17
SequenceName : 01F11 CDR2 , SEQ ID NO: 117

SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
AISPAAVTTY YADFVKG 17
<212> Type : PRT
<211> Length : 17
SequenceName : 01B10 CDR2 , SEQ ID NO: 118
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
RFTISRDNAK NTAYLQMVSL KPEDTAVYYC AA 32
<212> Type : PRT
<211> Length : 32
SequenceName : 01C12 FR3 , SEQ ID NO: 119
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
RFTISRDNAK NTAYLQMVSL KPEDTAVYYC AA 32
<212> Type : PRT
<211> Length : 32
SequenceName : 01B12 FR3 , SEQ ID NO: 120
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
RFTISRDNAK NTAYLQMVSL KPEDTAVYYC AA 32
<212> Type : PRT
<211> Length : 32
SequenceName : 01F11 FR3 , SEQ ID NO: 121
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
RFTISRDNAK NTAYLQMVSL KPEDTAVYYC AA 32
<212> Type : PRT
<211> Length : 32
SequenceName : 01B10 FR3 , SEQ ID NO: 122
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
QLPGRGSNLG RQASYAY 17
<212> Type : PRT
<211> Length : 17
SequenceName : 01C12 CDR3 , SEQ ID NO: 123
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
QLVGSGSNLG RQQSYAY 17
<212> Type : PRT

<211> Length : 17
SequenceName : 01B12 CDR3 , SEQ ID NO: 124
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
QLVGSGSNLG RQQSYAY 17
<212> Type : PRT
<211> Length : 17
SequenceName : 01F11 CDR3 , SEQ ID NO: 125
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
QLVGSGSNLG RQQSYAY 17
<212> Type : PRT
<211> Length : 17
SequenceName : 01B10 CDR3 , SEQ ID NO: 126
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
WGQGTQVTVS S 11
<212> Type : PRT
<211> Length : 11
SequenceName : 01C12 FR4 , SEQ ID NO: 127
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
WGQGTQVTVS S 11
<212> Type : PRT
<211> Length : 11
SequenceName : 01B12 FR4 , SEQ ID NO: 128
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
WGQGTQVTVS S 11
<212> Type : PRT
<211> Length : 11
SequenceName : 01F11 FR4 , SEQ ID NO: 129
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
WGQGTQVTVS S 11
<212> Type : PRT
<211> Length : 11
SequenceName : 01B10 FR4 , SEQ ID NO: 130
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :

EVQLVESGGGLVQAGDSLRLSCAASGLTFSNYDMGWFRQAPGKEREFVGASWWSGGAPYYSDSVKGRFTISRDNAKNTVY
LQANSLRPEDTAVYYCAAKRLRSFASGGSYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEV
QLVESGGGLVQAGDSLRLSCAASGLTFSNYDMGWFRQAPGKEREFVGASWWSGGAPYYSDSVKGRFTISRDNAKNTVYLQ
ANSLRPEDTAVYYCAAKRLRSFASGGSYDYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFS
SFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTL
VTVSS
<212> Type : PRT
<211> Length : 405
SequenceName : clone 060 , SEQ ID NO: 131
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQAGDSLRLSCAASGLTFSNYDMGWFRQAPGKEREFVGASWWSGGAPYYSDSVKGRFTISRDNAKNTVY
LQANSLRPEDTAVYYCAAKRLRSFASGGSYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLVES
GGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLR
PEDTAVYYCTIGGSLSRSSQGTLVTVSS
<212> Type : PRT
<211> Length : 268
SequenceName : clone 083 , SEQ ID NO: 132
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQPGGSLRISCAASGSIYLINYMGWYRQAPGKQRELVATLTSGGSTNYAGSVKGRFAISRDNAKNTVYL
QMNSLKPEDTAVYYCNIGGTLYDRRRFESWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLV
ESGGGLVQTGASLRLSCAASGRTFSNYAMGWFRQAPGKERERVAAITPRAFTTYYADSVKGRFTISRDNAKNTAYLQMVS
LKPEDTAVYYCAAQLVGSGSNLGRQESYAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQL
VESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMN
SLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
<212> Type : PRT
<211> Length : 431
SequenceName : clone 085 , SEQ ID NO: 133
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQTGASLRLSCAASGRTFSNYAMGWFRQAPGKERERVAAITPRAFTTYYADSVKGRFTISRDNAKNTAY
LQMVSLKPEDTAVYYCAAQLVGSGSNLGRQESYAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGG
SEVQLVESGGGLVQTGASLRLSCAASGRTFSNYAMGWFRQAPGKERERVAAITPRAFTTYYADSVKGRFTISRDNAKNTA
YLQMVSLKPEDTAVYYCAAQLVGSGSNLGRQESYAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGG
GSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTT
LYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
<212> Type : PRT
<211> Length : 437
SequenceName : clone 093 , SEQ ID NO: 134
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQAGGSLRLSCAASGSIFSIAAMGWYRQATGKQRELVATITDGGTTTYADSVKGRVTISRDRSANTVYL
AMNNLKPDDTAVYYCYAYLRYTSRVPGDNYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQL
VESGGGLVQTGASLRLSCAASGRTFSNYAMGWFRQAPGKERERVAAITPRAFTTYYADSVKGRFTISRDNAKNTAYLQMV
SLKPEDTAVYYCAAQLVGSGSNLGRQESYAYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTF
SSFGMSWVRQAPGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGT
LVTVSS
<212> Type : PRT
<211> Length : 406
SequenceName : clone 021 , SEQ ID NO: 135
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQAGDSLRLSCAASGLTFSNYDMGWFRQAPGKEREFVGASWWSGGAPYYSDSVKGRFTISRDNAKNTVY
LQANSLRPEDTAVYYCAAKRLRSFASGGSYDYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEV
QLVESGGGLVQTGASLRLSCAASGRTFSNYAMGWFRQAPGKERERVAAITPRAFTTYYADSVKGRFTISRDNAKNTAYLQ
MWSLKPEDTAVYYCAAQLVGSGSNLGRQESYAYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGF
TFSSFGMSWWRQAPGKGLEWWSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQ
GTLVTVSS
<212> Type : PRT
<211> Length : 408
SequenceName : clone 023 , SEQ ID NO: 136
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQPGGSLRISCAASGSIYLINYMGWYRQAPGKQRELVATLTSGGSTNYAGSVKGRFAISRDNAKNTVYL
QMNSLKPEDTAVYYCNIGGTLYDRRRFESWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLV
ESGGGLVQTGASLRLSCAASGRTFSNYAMGWFRQAPGKERERVAAITPRAFTTYYADSVKGRFTISRDNAKNTAYLQMWS
LKPEDTAVYYCAAQLVGSGSNLGRQESYAYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFS
SFGMSWWRQAPGKGLEWWSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTL
VTVSS
<212> Type : PRT
<211> Length : 405
SequenceName : clone 038 , SEQ ID NO: 137
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQTGASLRLSCAASGRTFSNYAMGWFRQAPGKERERVAAITPRAFTTYYADSVKGRFTISRDNAKNTAY
LQMWSLKPEDTAVYYCAAQLVGSGSNLGRQESYAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGG
SEVQLVESGGGLVQAGDSLRLSCAASGLTFSNYDMGWFRQAPGKEREFVGASWWSGGAPYYSDSVKGRFTISRDNAKNTV
YLQANSLRPEDTAVYYCAAKRLRSFASGGSYDYWGQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGF
TFSSFGMSWWRQAPGKGLEWWSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQ
GTLVTVSS
<212> Type : PRT
<211> Length : 408
SequenceName : clone 049 , SEQ ID NO: 138
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQTGASLRLSCAASGRTFSNYAMGWFRQAPGKERERVAAITPRAFTTYYADSVKGRFTISRDNAKNTAY
LQMWSLKPEDTAVYYCAAQLVGSGSNLGRQESYAYWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGG
SEVQLVESGGGLVQPGGSLRISCAASGSIYLINYMGWYRQAPGKQRELVATLTSGGSTNYAGSVKGRFAISRDNAKNTVY
LQMNSLKPEDTAVYYCNIGGTLYDRRRFESWGQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFS
SFGMSWWRQAPGKGLEWWSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTL
VTVSS
<212> Type : PRT
<211> Length : 405
SequenceName : clone 052 , SEQ ID NO: 139
SequenceDescription :

Sequence

<213> OrganismName : Artificial Sequence
<400> PreSequenceString :
EVQLVESGGGLVQPGGSLRISCAASGSIYLINYMGWYRQAPGKQRELVATLTSGGSTNYAGSVKGRFAISRDNAKNTVYL
QMNSLKPEDTAVYYCNIGGTLYDRRRFESWGQGTLVTVSSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSGGGGSEVQLV
ESGGGLVQAGESLTLSCAASGRTLSAYIMGWFRQAPGKEREFVAGIWSGGYTHLADSAKGRFSISRDNAKNTVYLQMNGL

KPEDTAVYYCAAGLRGRQYSNWGQGTLVTVSSGGGGSGGGSEVQLVESGGGLVQPGNSLRLSCAASGFTFSSFGMSWVRQ
APGKGLEWVSSISGSGSDTLYADSVKGRFTISRDNAKTTLYLQMNSLRPEDTAVYYCTIGGSLSRSSQGTLVTVSS
<212> Type : PRT
<211> Length : 396
SequenceName : clone 086 , SEQ ID NO: 140
SequenceDescription :