CLAIMS

1. A polypeptide comprising or consisting of at least three immunoglobulin single variable domains (ISVDs), wherein each of the at least three ISVDs has the structure FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4 in which FR1 to FR4 refer to framework regions and CDR1 to CDR3 refer to complementarity determining regions; and wherein the polypeptide comprises

(a) a first ISVD specifically binding to fractalkine receptor CX3CR1 and/or a single nucleotide polymorphism (SNP) variant of CX3CR1 and comprising (according to AbM numbering)

a CDR1 that is the amino acid sequence of SEQ ID NO: 10;

a CDR2 that is the amino acid sequence of SEQ ID NO: 12; and

a CDR3 that is the amino acid sequence of SEQ ID NO: 14; and

(b) a second ISVD specifically binding to fractalkine receptor CX3CR1 and/or a single nucleotide polymorphism (SNP) variant of CX3CR1 and comprising (according to AbM numbering)

a CDR1 that is the amino acid sequence of SEQ ID NO: 10;

a CDR2 that is the amino acid sequence of SEQ ID NO: 12; and

a CDR3 that is the amino acid sequence of SEQ ID NO: 14; and

(c) a third ISVD specifically binding to albumin and comprising (according to AbM numbering)

a CDR1 that is the amino acid sequence of SEQ ID NO: 53 or an amino acid sequence with 2 or 1 amino acid difference(s) with SEQ ID NO: 53;

a CDR2 that is the amino acid sequence of SEQ ID NO: 55 or an amino acid sequence with 2 or 1 amino acid difference(s) with SEQ ID NO: 55; and

a CDR3 that is the amino acid sequence of SEQ ID NO: 57 or an amino acid sequence with 2 or 1 amino acid difference(s) with SEQ ID NO: 57; and

wherein (a), (b), and (c) are linked directly or via a peptidic linker.

2. The polypeptide according to the previous claim, wherein the C-terminal ISVD of the polypeptide carries a C-terminal extension of FR4 comprising 1, 2, 3, 4, 5, 6, 7, 8, 9 or 10 amino acids in length; optionally wherein the C-terminal extension of FR4 is a single alanine (A) residue.

3. The polypeptide according to any one of the previous claims, wherein the order of (a), (b), and (c) indicates their relative position to each other within the polypeptide, considered from the N-terminus to the C-terminus of said polypeptide.

4. The polypeptide according to any one of the previous claims, wherein the ISVD in (a), (b) and (c) is selected from a VH domain and a VHH domain; optionally wherein the ISVD in (a), (b) and (c) is selected from a camelized VH domain, a humanized VHH domain, domain antibody and a dAb.

5. The polypeptide according to any one of the previous claims, wherein in (a) and in (b);

(i) the ISVD comprises one or more selected from an aspartic acid (D) at amino acid position 1 according to Kabat numbering, a proline (P) at amino acid position 14 according to Kabat numbering, a glycine (G) at amino acid position 16 according to Kabat numbering, an arginine (R) at amino acid position 83 according to Kabat numbering, a leucine (L) at amino acid position 89 according to Kabat numbering and a leucine (L) at amino acid position 108 according to Kabat numbering; and/or

(ii) the ISVD comprises one or more selected from a serine (S) at amino acid position 11 according to Kabat numbering, and a glutamine (Q) at position 44 according to Kabat numbering; and/or

(iii) the ISVD comprises one or more selected from a threonine (T) at amino acid positions 32 according to Kabat numbering, a serine (S) at amino acid position 52 according to Kabat numbering and a threonine (T) at amino acid position 53 according to Kabat numbering; and/or

(iv) the ISVD comprises one or more selected from a valine (V) at amino acid position 50 according to Kabat numbering, an isoleucine (I) at amino acid position 56 according to Kabat numbering, and an alanine (A) at amino acid position 96 according to Kabat numbering.

6. The polypeptide according to any one of the previous claims, wherein in (a):

(i) the ISVD consists of an amino acid sequence as shown in SEQ ID NO: 3 or an amino acid that has at least 90% sequence identity with SEQ ID NO:3 and in (b) the ISVD consists of an amino acid sequence as shown in SEQ ID NO: 2 or an amino acid that has at least 90% sequence identity with SEQ ID NO: 2, wherein for the purpose of determining the % sequence identity, the amino acid sequence of the CDR regions are disregarded; and/or

(ii) the ISVD consists of an amino acid sequence as shown in SEQ ID NO: 3 or an amino acid sequence having two or one amino acid difference(s) with SEQ ID NO: 3 and in (b) the ISVD consists of an amino acid sequence as shown in SEQ ID NO: 2 or an amino acid sequence having two or one amino acid difference(s) with SEQ ID NO: 2; and/or

(iii) the ISVD consists of an amino acid sequence as shown in SEQ ID NO: 3 and in (b) the ISVD consists of an amino acid sequence as shown in SEQ ID NO: 2; and/or

(iv) the ISVD consists of an amino acid sequence as shown in SEQ ID NO: 3 and in (b) the ISVD consists of an amino acid sequence as shown in SEQ ID NO: 2, and wherein the order of (a), (b),

and (c) indicates their relative position to each other within the polypeptide, considered from the N-terminus to the C-terminus of said polypeptide.

7. The polypeptide according to any one of the previous claims, wherein in (c):

(i) the ISVD specifically binds to human serum albumin; and/or

(ii) the ISVD specifically binds to human serum albumin and comprises (according to AbM numbering)

a CDR1 that is the amino acid sequence of SEQ ID NO: 53;

a CDR2 that is the amino acid sequence of SEQ ID NO: 55; and

a CDR3 that is the amino acid sequence of SEQ ID NO: 57; and/or

(iii) the ISVD comprises or consists of an amino acid sequence as shown in SEQ ID NO: 37 or an amino acid sequence having a sequence identity of more than 90% identity or more than 95% identity with SEQ ID NO: 37; and/or

(iv) the ISVD comprises or consists of an amino acid sequence as shown in SEQ ID NO: 37.

8. The polypeptide according to any one of the previous claims, wherein in (a) and in (b) the ISVD consists of an amino acid sequence as shown in SEQ ID NO: 2 or an amino acid sequence having two or one amino acid difference(s) with SEQ ID NO: 2 and in (c) the ISVD consists of an amino acid sequence as shown in SEQ ID NO: 37; optionally wherein the C-terminal extension of FR4 is a single Ala residue.

9. The polypeptide according to any one of the previous claims, wherein the polypeptide comprises two peptidic linkers, wherein each peptidic linker is located between two ISVDs within the polypeptide; optionally wherein the two peptidic linkers comprise or consist of an amino acid sequence as shown in SEQ ID NO: 69.

10. The polypeptide according to any one of the previous claims, wherein the polypeptide comprises an amino acid sequence having a sequence identity of more than 90% identity or more than 95% identity with SEQ ID NO: 82; optionally wherein the polypeptide comprises or consists of an amino acid sequence as shown in SEQ ID NO: 82.

11. The polypeptide according to any one of the previous claims, wherein the single nucleotide polymorphism (SNP) variant of CX3CR1 comprises at least one SNP selected from the group consisting of SNPs rs938203, rs2669849, rs1050592, rs3732379, and rs3732378.

12. The polypeptide according to any one of the previous claims, wherein:

(i) the polypeptide is characterized by an increased yield after expression in yeast *Pichia pastoris* as compared to a reference polypeptide consisting of an amino acid sequence as shown in SEQ ID NO: 83; and/or

(ii) the polypeptide is characterized by at least one improved biophysical property as compared to a reference polypeptide consisting of an amino acid sequence as shown in SEQ ID NO: 83; wherein the biophysical property is selected from

(a) improved stability after purification of the polypeptide, e.g., as measured by turbidity / opalescence of concentrations of 100 mg/mL of polypeptide;

(b) reduced amount of low molecular weight species after expression of the polypeptide in a suitable host, e.g., as measured by pre-peaks on reverse phase chromatography (e.g., RP-UPLC);

(c) reduced amount of low molecular weight species after purification of the polypeptide, e.g., as measured by capillary gel electrophoresis (CGE);

(d) reduced amount of high molecular weight species after expression of the polypeptide in a suitable host, e.g., as measured by size exclusion high performance liquid chromatography (SE-HPLC);

(e) reduced amount of high molecular weight species after purification of the polypeptide, e.g., as measured by size exclusion high performance liquid chromatography (SE-HPLC); and

(f) combinations of two or more of said biophysical properties; and/or

(iii) the polypeptide is characterized by a higher recovery after a purification step as compared to the recovery of a reference polypeptide consisting of an amino acid sequence as shown in SEQ ID NO: 83, wherein the purification step is selected from:

i. a capture step on a resin;
ii. a pH adjustment step after the capture step on a resin;
iii. a polish step;
iv. an ultrafiltration/diafiltration/ultrafiltration step; and
v. combinations of two or more of said purification steps; and/or

(iv) the polypeptide has a reduced amount of missing disulfide bridges after expression of the polypeptide in a suitable host compared to a reference polypeptide consisting of an amino acid sequence as shown in SEQ ID NO: 83, wherein the amount of polypeptides with the reduced

amount of missing disulfide bridges is measured by reverse phase high performance liquid chromatography (RP-HPLC); and/or

(v) the polypeptide binds cell surface glycoprotein MUC18 with a lower affinity compared to the binding by a reference polypeptide consisting of an amino acid sequence as shown in SEQ ID NO: 83.

13. A polynucleotide encoding a polypeptide according to any one of the previous claims.

14. A host or host cell comprising a polynucleotide according to the previous claim.

15. A composition comprising a polypeptide or polynucleotide according to any one of the previous claims; optionally wherein the composition is a pharmaceutical composition; and further optionally wherein the composition comprises at least one pharmaceutically acceptable carrier, diluent, excipient and/or adjuvant.

16. A method for producing a polypeptide or composition according to any one of the previous claims comprising steps of:

- expressing the polypeptide in a suitable host cell or host organism or in another suitable expression system;

  and further optionally

- isolating and/or purifying the polypeptide.

17. A polypeptide, polynucleotide or composition according to any one of the previous claims for use in inhibiting the binding of CX3CR1 to fractalkine in a mammalian cell.

18. A polypeptide, polynucleotide or composition according to any one of the previous claims for use as a medicament.

19. A polypeptide, polynucleotide or composition according to any one of the previous claims for use in prevention, therapy and diagnosis of a disease or disorder; optionally wherein the disease or disorder is a disease or disorder associated with a fractalkine receptor CX3CR1 or a single nucleotide polymorphism (SNP) variant of CX3CR1; further optionally wherein the disease or disorder is selected from inflammatory disease, cardio- and cerebrovascular atherosclerotic disorder, peripheral artery disease, myocardial infarction, restenosis, diabetic nephropathy, glomerulonephritis, human

crescentic glomerulonephritis, IgA nephropathy, membranous nephropathy, lupus nephritis, vasculitis including Henoch-Schonlein purpura and Wegener's granulomatosis, rheumatoid arthritis, graft versus host disease, atopic dermatitis, inflammatory bowel disease, Crohn's disease, osteoarthritis, allograft rejection, systemic sclerosis, neurodegenerative disorder and demyelinating disease, multiple sclerosis (MS), Alzheimer's disease, pulmonary disease such as COPD, asthma, neuroinflammation, neuropathic pain, inflammatory pain, and cancer, including ovarian cancer.

20. A method for inhibiting the binding of CX3CR1 to fractalkine in a mammalian cell, comprising administering to the cell a polypeptide according to any one of the previous items, whereby signalling mediated by the fractalkine is inhibited.

21. Use of the polypeptide, polynucleotide or composition according to any one of the previous claims in the manufacture of a medicament for treating a disease or disorder selected from inflammatory disease, cardio- and cerebrovascular atherosclerotic disorder, peripheral artery disease, myocardial infarction, restenosis, diabetic nephropathy, glomerulonephritis, human crescentic glomerulonephritis, IgA nephropathy, membranous nephropathy, lupus nephritis, vasculitis including Henoch-Schonlein purpura and Wegener's granulomatosis, rheumatoid arthritis, graft versus host disease, atopic dermatitis, inflammatory bowel disease, Crohn's disease, osteoarthritis, allograft rejection, systemic sclerosis, neurodegenerative disorder and demyelinating disease, multiple sclerosis (MS), Alzheimer's disease, pulmonary disease such as COPD, asthma, neuroinflammation, neuropathic pain, inflammatory pain, and cancer, including ovarian cancer.