Several independent mouse genetic studies have shown a beneficial effect of CX3CR1 deficiency on atherosclerosis. A reduction in lesion area in the aortic arch and thoracic aorta as well as a decrease in monocyte/macrophage accumulation in plaques was seen in two independently derived strains of $CX3CR1^{-/-}$ $apoE^{-/-}$ mice fed a high fat diet (Combadière, 2003; *Circulation*, 107:1009, Lesnik, 2003; *J. Clin. Invest.* 111:333).

This shows that CX3CR1 is involved in cardiovascular diseases and the modulation of its activity could provide promising therapies. There is therefore a need for antagonist molecules against CX3CR1 with beneficial pharmacological properties, which can be used as therapeutic agents to treat diseases, in particular cardiovascular diseases in humans.

Accordingly, one aim of the present disclosure ~~invention~~ is to provide anti-CX3CR1 antagonist molecules, in particular anti-CX3CR1 antagonist molecules, which have high binding affinity to CX3CR1.

A further aim of the present disclosure ~~invention~~ is to provide anti-CX3CR1 antagonist molecules, which have high specificity for CX3CR1.

A further aim of the present disclosure ~~invention~~ is to provide anti-CX3CR1 antagonists, which have potent activity.

A further aim of the present disclosure ~~invention~~ is to provide anti-CX3CR1 antagonists, which have a favorable bioavailability and half-life.

A further aim of the present disclosure ~~invention~~ is to provide anti-CX3CR1 antagonists, which have favorable biophysical properties.

Further aims of the present disclosure ~~invention~~ include combinations of any of the aims set forth above.

SUMMARY OF THE ~~INVENTION~~DISCLOSURE

The ~~invention~~ disclosure provides polypeptides which bind to human CX3CR1 and are capable of blocking the binding of human fractalkine to human CX3CR1.

~~In one aspect,~~ The polypeptide ~~is~~ may be an immunoglobulin comprising an antigen-binding domain comprising three complementarity determining regions CDR1, CDR2 and CDR3, wherein said immunoglobulin binds to human CX3CR1 and is capable of blocking the binding of human fractalkine to human CX3CR1. In

one aspect, there is provided a polypeptide comprising an anti-CX3CR1 (anti-CX3 chemokine receptor 1) immunoglobulin single variable domain, wherein said polypeptide comprises a CDR1, CDR2 and CDR3 having the amino acid sequences set forth in:

- SEQ ID No: 213, 221 and 186, respectively; or
- SEQ ID No: 141, 162 and 186, respectively; or
- SEQ ID No: 141, 163 and 187, respectively; or
- SEQ ID No: 141, 164 and 186, respectively; or
- SEQ ID No: 141, 166 and 186, respectively; or
- SEQ ID No: 141, 167 and 186, respectively; or
- SEQ ID No: 141, 167 and 189, respectively; or
- SEQ ID No: 141, 168 and 186, respectively; or
- SEQ ID No: 141, 168 and 187, respectively; or
- SEQ ID No: 141, 169 and 190, respectively; or
- SEQ ID No: 141, 170 and 186, respectively; or
- SEQ ID No: 141, 171 and 186, respectively; or
- SEQ ID No: 141, 174 and 186, respectively; or
- SEQ ID No: 141, 175 and 187, respectively; or
- SEQ ID No: 142, 165 and 188, respectively; or
- SEQ ID No: 142, 173 and 188, respectively; or
- SEQ ID No: 143, 164 and 186, respectively; or
- SEQ ID No: 144, 172 and 187, respectively; or
- SEQ ID No: 145, 172 and 187, respectively; or
- SEQ ID No: 141, 214 and 186, respectively; or
- SEQ ID No: 141, 215 and 186, respectively; or
- SEQ ID No: 141, 216 and 186, respectively; or
- SEQ ID No: 141, 217 and 186, respectively; or
- SEQ ID No: 141, 218 and 186, respectively; or
- SEQ ID No: 141, 219 and 186, respectively; or
- SEQ ID No: 141, 220 and 186, respectively; or
- SEQ ID No: 213, 214 and 186, respectively.

~~In a further aspect,~~

Tthe polypeptide may comprises one or more anti-CX3CR1 immunoglobulin single variable domain, wherein said polypeptide is capable of blocking the binding of human fractalkine to human CX3CR1.

~~In one aspect, a~~A polypeptide of the present disclosure may be ~~invention is~~ characterized by one or more of the following properties:

- Bind with high affinity to human CX3CR1;
- Block the binding of soluble fractalkine to human CX3CR1;
- Inhibit fractalkine induced chemotaxis;
- Inhibit fractalkine induced human CX3CR1 receptor internalization;
- Cross-react with cyno CX3CR1 within 10-fold of E/IC_{50} for human CX3CR1 for binding and functional inhibition.

~~In a further aspect, a~~A polypeptide of the present disclosure may~~invention~~ comprises an anti-CX3CR1 immunoglobulin single variable domain and may further comprises a half-life extending moiety, for example an albumin binding moiety, a polyethylene glycol molecule or a Fc domain. ~~In a further aspect, a~~A polypeptide of the present disclosure may ~~invention~~ comprises two or more anti-CX3CR1 immunoglobulin single variable domains. ~~In one aspect, t~~The two anti-CX3CR1 immunoglobulin single variable domains ~~are~~ may be covalently linked by a linker peptide. ~~In one aspect, t~~The two anti-CX3CR1 immunoglobulin single variable domains in a polypeptide of the present disclosure ~~invention~~ may have the same amino acid sequence. ~~In another aspect, t~~The two anti-CX3CR1 immunoglobulin single variable domains in a polypeptide of the present disclosure ~~invention~~ may have different amino acid sequences. ~~In one aspect, a~~A polypeptide of the present disclosure ~~invention~~ may comprises two anti-CX3CR1 immunoglobulin single variable domains and further comprises a half-life extending moiety, for example an albumin binding moiety, a polyethylene glycol molecule or a Fc domain.

~~In one aspect, a~~A polypeptide of the present disclosure ~~invention~~ may comprises a first anti-CX3CR1 immunoglobulin single variable domain covalently linked to an albumin binding moiety by a first linker peptide, wherein said albumin binding moiety is further covalently linked to a second anti-CX3CR1 immunoglobulin single variable domain by a second linker peptide.

~~In one aspect, a~~A polypeptide of the present disclosure ~~invention~~ may comprises an anti-CX3CR1 immunoglobulin single variable domain covalently linked to a Fc domain by a

linker peptide. ~~In one aspect, s~~Such polypeptide comprising an anti-CX3CR1 immunoglobulin single variable domain covalently linked to a Fc domain by a linker peptide ~~is~~ may be provided as a dimer, for example through disulfide bridges.

In a further aspect, there is provided a polypeptide comprising a first immunoglobulin single variable domain and a second immunoglobulin single variable domain, each comprising CDR1, CDR2 and CDR3 having amino acid sequences set forth in SEQ ID NO's: 141, 164 and 186, or SEQ ID NO's: 141, 162 and 186, or SEQ ID NO's: 213, 214 and 186, or SEQ ID NO's: 213, 221 and 186.

In a further aspect, there is provided a polypeptide comprising the amino acid sequence of any one of SEQ ID NO: 225-227 or 257-262.

In a further aspect, there is provided a nucleic acid molecule encoding a polypeptide as defined above.

In a further aspect, there is provided an expression vector comprising a nucleic acid molecule as defined above.

In a further aspect, there is provided a host cell comprising a nucleic acid molecule encoding a polypeptide as defined above, wherein the host cell is capable of expressing the polypeptide.

In a further aspect, there is provided a pharmaceutical composition comprising (i) a polypeptide as defined above, and (ii) a pharmaceutically acceptable carrier, and optionally (iii) a diluent, excipient, adjuvant and/or stabilizer.

In a further aspect, there is provided a method of manufacturing a polypeptide as defined above, comprising the steps of

- culturing a host cell under conditions that allow expression of a polypeptide as defined above,

wherein the host cell is carrying an expression vector comprising a nucleic acid molecule, the nucleic acid molecule comprising a region encoding a polypeptide as defined above, and wherein the host cell is a prokaryotic or a eukaryotic cell.

In a further aspect, there is provided a polypeptide as defined above for use in the treatment, prevention or alleviation of a disease, disorder or condition, in a human being.

In a further aspect, there is provided use of a polypeptide as defined above for the manufacture of a medicament for the treatment, prevention or alleviation of a disease, disorder or condition, in a human being.

In a further aspect, there is provided use of polypeptide as defined above in the manufacture of a medicament for the treatment or prevention of CX3CR1-associated disease or disorder.

In a further aspect, there is provided a diagnostic kit or diagnostic method comprising a polypeptide as defined above, or the use thereof

In a further aspect, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 225.

In a further aspect, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 226.

In a further aspect, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 227.

In a further aspect, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 258.

In a further aspect, there is provided a polypeptide comprising the amino acid sequence of SEQ ID NO: 261.

In a further aspect, there is provided a pharmaceutical composition comprising (i) a polypeptide as defined above, and (ii) a pharmaceutically acceptable carrier, and optionally (iii) a diluent, excipient, adjuvant and/or stabilizer.

The polypeptides of the present disclosure ~~invention~~ are used for the prevention, treatment, alleviation and/or diagnosis of CX3CR1-associated diseases, disorders or conditions, in particular cardiovascular diseases, such as atherosclerosis.

~~In a further aspect, t~~The present disclosure ~~invention~~ may provide~~s~~:

Embodiment 1: An immunoglobulin comprising an antigen-binding domain comprising three complementarity determining regions CDR1, CDR2 and CDR3, wherein said immunoglobulin binds to human CX3CR1 and is capable of blocking the binding of human fractalkine to human CX3CR1.

Embodiment 2: A polypeptide comprising one or more anti-CX3CR1 immunoglobulin single variable domain, wherein said polypeptide is capable of blocking the binding of human fractalkine to human CX3CR1.

Embodiment 3: A polypeptide according to embodiment 2, wherein said anti-CX3CR1 immunoglobulin single variable domain consists essentially of four framework regions (FR1, FR2, FR3 and FR4) and three complementary determining regions (CDR1, CDR2 and CDR3).

Embodiment 4: A polypeptide according to embodiment 3, wherein said anti-CX3CR1 immunoglobulin single variable domain has the structure FR1 - CDR1 - FR2 - CDR2 - FR3 - CDR3 - FR4.

Embodiment 5: A polypeptide according to any one of embodiments 2 to 4, wherein said anti-CX3CR1 immunoglobulin single variable domain is an antibody domain.

Embodiment 6: A polypeptide according to embodiment 5, wherein said anti-CX3CR1 immunoglobulin single variable domain is a VH, VL, VHH, camelized

Claims

1. A polypeptide comprising an anti-CX3CR1 immunoglobulin single variable domain, wherein said polypeptide is capable of blocking the binding of human fractalkine to human CX3CR1.

2. The polypeptide according to claim 1, wherein said anti-CX3CR1 immunoglobulin single variable domain consists essentially of four framework regions (FR1, FR2, FR3 and FR4) and three complementary determining regions (CDR1, CDR2 and CDR3).

3. The polypeptide according to claim 1 or 2, wherein said anti-CX3CR1 immunoglobulin single variable domain is a VH, VL, VHH, camelized VH, or VHH that is optimized for stability, potency, manufacturability and/or similarity to human framework regions.

4. The polypeptide according to any one of claims 1 to 3, wherein said polypeptide has an affinity to human CX3CR1 at:

a) an EC50 of less than or equal to 10nM, less than or equal to 5nM, less than or equal to 2.5nM or less than or equal to 1nM, as determined by cell binding FACS; or

b) an IC50 of less than or equal to 10nM, less than or equal to 5nM, less than or equal to 2.5nM or less than or equal to 1nM, as determined by competition FACS.

5. The polypeptide according to any one of claims 1 to 4, wherein said polypeptide blocks the binding of human fractalkine to human CX3CR1 at an IC50 of less than or equal to 300nM, or less than or equal to 100nM, or less than or equal to 20nM, or less than or equal to 10nM, or less than or equal to 5nM, or less than or equal to 2.5nM or less than or equal to 1nM.

~~6. The polypeptide according to any one of claims 1 to 5, wherein said polypeptide comprises a CDR3 that has the amino acid sequence of Asp-Xaa1-Arg-Arg-Gly-Trp-Xaa2-Xaa3-Xaa4-Xaa5 (SEQ ID NO: 197), wherein:~~

- ~~Xaa1 is Pro, Ala or Gly;~~
- ~~Xaa2 is Asp or Asn;~~
- ~~Xaa3 is Thr or Ser;~~
- ~~Xaa4 is Arg, Lys, Ala or Gly; and~~
- ~~Xaa5 is Tyr or Phe.~~

~~7. The polypeptide according to claim 6, wherein said CDR3 has the amino acid sequence of Asp-Pro-Arg-Arg-Gly-Trp-Asp-Thr-Arg-Tyr (SEQ ID NO: 186).~~

Claims

18. ~~The polypeptide according to any of claims 1-6,~~A polypeptide comprising an anti-CX3CR1 (anti-CX3 chemokine receptor 1) immunoglobulin single variable domain, wherein said polypeptide comprises a CDR1, CDR2 and CDR3 having the amino acid sequences set forth in:

- SEQ ID No: 213, 221 and 186, respectively; or
- SEQ ID No: 141, 162 and 186, respectively; or
- SEQ ID No: 141, 163 and 187, respectively; or
- SEQ ID No: 141, 164 and 186, respectively; or
- SEQ ID No: 141, 166 and 186, respectively; or
- SEQ ID No: 141, 167 and 186, respectively; or
- SEQ ID No: 141, 167 and 189, respectively; or
- SEQ ID No: 141, 168 and 186, respectively; or
- SEQ ID No: 141, 168 and 187, respectively; or
- SEQ ID No: 141, 169 and 190, respectively; or
- SEQ ID No: 141, 170 and 186, respectively; or
- SEQ ID No: 141, 171 and 186, respectively; or
- SEQ ID No: 141, 174 and 186, respectively; or
- SEQ ID No: 141, 175 and 187, respectively; or
- SEQ ID No: 142, 165 and 188, respectively; or
- SEQ ID No: 142, 173 and 188, respectively; or
- SEQ ID No: 143, 164 and 186, respectively; or
- SEQ ID No: 144, 172 and 187, respectively; or

- SEQ ID No: 145, 172 and 187, respectively; or
- SEQ ID No: 141, 214 and 186, respectively; or
- SEQ ID No: 141, 215 and 186, respectively; or
- SEQ ID No: 141, 216 and 186, respectively; or
- SEQ ID No: 141, 217 and 186, respectively; or
- SEQ ID No: 141, 218 and 186, respectively; or
- SEQ ID No: 141, 219 and 186, respectively; or
- SEQ ID No: 141, 220 and 186, respectively; or
- SEQ ID No: 213, 214 and 186, respectively.

~~9. The polypeptide according to any of claims 1 to 5, wherein said polypeptide comprises a CDR1, CDR2 and CDR3 having the amino acid sequences set forth in:~~

~~- SEQ ID No: 146, 176 and 191, respectively; or~~
~~- SEQ ID No: 146, 177 and 191, respectively.~~

~~10. The polypeptide according to claim any of claims 1 to 5, wherein said polypeptide comprises a CDR1, CDR2 and CDR3 having the amino acid sequences set forth in:~~

~~- SEQ ID No: 147, 178 and 192, respectively; or~~
~~- SEQ ID No: 147, 179 and 192, respectively; or~~
~~- SEQ ID No: 147, 179 and 194, respectively; or~~
~~- SEQ ID No: 148, 179 and 193, respectively; or~~
~~- SEQ ID No: 149, 179 and 192, respectively; or~~
~~- SEQ ID No: 149, 180 and 192, respectively; or~~
~~- SEQ ID No: 149, 181 and 192, respectively; or~~
~~- SEQ ID No: 149, 183 and 192, respectively; or~~
~~- SEQ ID No: 149, 185 and 192, respectively; or~~
~~- SEQ ID No: 150, 179 and 194, respectively; or~~
~~- SEQ ID No: 150, 182 and 194, respectively; or~~
~~- SEQ ID No: 151, 179 and 193, respectively; or~~
~~- SEQ ID No: 151, 182 and 194, respectively; or~~
~~- SEQ ID No: 151, 184 and 196, respectively; or~~

~~SEQ ID No: 152, 179 and 195, respectively; or~~

~~SEQ ID No: 153, 179 and 194, respectively; or~~

~~SEQ ID No: 154, 182 and 194, respectively; or~~

~~SEQ ID No: 155, 179 and 195, respectively; or~~

~~SEQ ID No: 156, 181 and 192, respectively; or~~

~~SEQ ID No: 157, 179 and 194, respectively; or~~

~~SEQ ID No: 158, 179 and 192, respectively; or~~

~~SEQ ID No: 159, 178 and 192, respectively; or~~

~~SEQ ID No: 160, 179 and 194, respectively; or~~

~~SEQ ID No: 161, 179 and 194, respectively.~~

2~~11~~. The polypeptide according to claim 1, wherein the polypeptide comprises a CDR1, CDR2 and CDR3 having amino acid sequences set forth in: SEQ ID NO's: 141, 164 and 186, respectively, SEQ ID NO's: 141, 162 and 186, respectively, SEQ ID NO's: 213, 214 and 186 respectively, or SEQ ID NO's: 213, 221 and 186 respectively.

3~~12~~. The polypeptide according to any one of claims 1 to 2~~5~~, wherein said anti-CX3CR1 immunoglobulin single variable domain is a VHH domain comprising the sequence set forth in:

a) the amino acid sequence of SEQ ID NO: 3;

b) an amino acid sequence that has at least 90% amino acid identity with the amino acid sequences of SEQ ID NO: 3; or

c) an amino acid sequence of any one of SEQ ID NO: 1-48, 121-140 or 222-224.

~~13. The polypeptide according to any one of claims 1 to 5, wherein said anti-CX3CR1 immunoglobulin single variable domain is a VHH domain comprising the sequence set forth in:~~

~~a) the amino acid sequence of SEQ ID NO: 49;~~

~~b) an amino acid sequence that has at least 95% amino acid identity with the amino acid sequences of SEQ ID NO: 49; or~~

~~c) an amino acid sequence of any one of SEQ ID NO: 49-52.~~

~~14. The polypeptide according to any one of claims 1 to 5, wherein said anti-CX3CR1 immunoglobulin single variable domain is a VHH domain comprising the sequence set forth in:~~

~~a) the amino acid sequence of SEQ ID NO: 67;~~

~~b) an amino acid sequence that has at least 90% amino acid identity with the amino acid sequences of SEQ ID NO: 67; or~~

~~c) an amino acid sequence of any one of SEQ ID NO: 53-120.~~

~~15. The polypeptide according to claim 1, wherein said anti-CX3CR1 immunoglobulin single variable domain comprises the sequence set forth in SEQ ID NO: 1, SEQ ID NO: 3, SEQ ID NO: 121-140 or SEQ ID NO: 222-224.~~

~~16. The polypeptide according to any one of claims 1 to 15, wherein said immunoglobulin single variable domain either~~

~~a) cross-blocks the binding of, or~~

~~b) is cross-blocked from binding by~~

~~at least one of the immunoglobulin single variable domains of SEQ ID NO's: 1-120, 121-140 and 222-224 to CX3CR1.~~

4~~17~~. The polypeptide according to ~~any one of~~ claim~~s~~ 1 ~~to 16~~, wherein the polypeptide further comprises a half-life extending moiety.

5~~18~~. The polypeptide according to claim 4~~17~~, wherein said half-life extending moiety is covalently linked to said polypeptide and is selected from the group consisting of an albumin binding moiety, such as an anti-albumin immunoglobulin domain, a transferrin binding moiety, such as an anti-transferrin immunoglobulin domain, a polyethylene glycol molecule, a recombinant polyethylene glycol molecule, human serum albumin, a fragment of human serum albumin, an albumin binding peptide or a Fc domain.

619. The polypeptide according to claim 417 or 518, wherein said half-life extending moiety consists of an anti-albumin immunoglobulin single variable domain.

720. The polypeptide according to claim 619, wherein the immunoglobulin single variable domain is selected from a VHH domain, a humanized VHH domain, a camelized VH domain, a domain antibody, a single domain antibody and/or "dAb"s.

821. The polypeptide according to claim 720, wherein the anti-albumin immunoglobulin single variable domain comprises a sequence selected from any one of SEQ ID NO's: 230-232.

~~22. The polypeptide according to any one of claims 1 to 18, wherein said polypeptide is linked to an Fc portion, optionally via a suitable linker or hinge region.~~

923. The polypeptide according to ~~any one of~~ claim~~s~~ 1 ~~to 16~~, wherein said polypeptide further comprises a second immunoglobulin single variable domain.

1024. The polypeptide according to claim 923 wherein said second immunoglobulin single variable domain comprises a second anti-CX3CR1 immunoglobulin single variable domain.

1125. The polypeptide according to claim 923 ~~or 24~~, wherein said first and second immunoglobulin single variable domains are a VH, VL, VHH, camelized VH, or VHH that is optimized for stability, potency, manufacturability and/or similiarity to human framework regions.

1226. The polypeptide according to ~~any of~~ claim~~s~~ 1023 ~~to 25~~, wherein said second immunoglobulin single variable domain comprises CDR1, CDR2 and CDR3 amino acid sequences set forth in

- SEQ ID No: 213, 221 and 186, respectively; or
- SEQ ID No: 141, 162 and 186, respectively; or
- SEQ ID No: 141, 163 and 187, respectively; or

- SEQ ID No: 141, 164 and 186, respectively; or
- SEQ ID No: 141, 166 and 186, respectively; or
- SEQ ID No: 141, 167 and 186, respectively; or
- SEQ ID No: 141, 167 and 189, respectively; or
- SEQ ID No: 141, 168 and 186, respectively; or
- SEQ ID No: 141, 168 and 187, respectively; or
- SEQ ID No: 141, 169 and 190, respectively; or
- SEQ ID No: 141, 170 and 186, respectively; or
- SEQ ID No: 141, 171 and 186, respectively; or
- SEQ ID No: 141, 174 and 186, respectively; or
- SEQ ID No: 141, 175 and 187, respectively; or
- SEQ ID No: 142, 165 and 188, respectively; or
- SEQ ID No: 142, 173 and 188, respectively; or
- SEQ ID No: 143, 164 and 186, respectively; or
- SEQ ID No: 144, 172 and 187, respectively; or
- SEQ ID No: 145, 172 and 187, respectively; or
- SEQ ID No: 141, 214 and 186, respectively; or
- SEQ ID No: 141, 215 and 186, respectively; or
- SEQ ID No: 141, 216 and 186, respectively; or
- SEQ ID No: 141, 217 and 186, respectively; or
- SEQ ID No: 141, 218 and 186, respectively; or
- SEQ ID No: 141, 219 and 186, respectively; or
- SEQ ID No: 141, 220 and 186, respectively; or
- SEQ ID No: 213, 214 and 186, respectively.

~~any one of claims 6 to 11.~~

1327. The polypeptide according to claim 1226, wherein said first and said second immunoglobulin single variable domains comprise the same CDR1, CDR2 and CDR3.

1428. The polypeptide according to ~~any of~~ claim~~s~~ 1323 ~~to 27~~, wherein said first and second immunoglobulin single variable domains comprise a VHH domain having an amino acid sequence set forth in

a) the amino acid sequence of SEQ ID NO: 3;

b) an amino acid sequence that has at least 90% amino acid identity with the amino acid sequences of SEQ ID NO: 3; or

c) an amino acid sequence of any one of SEQ ID NO: 1-48, 121-140 or 222-224;

d) the amino acid sequence of SEQ ID NO: 49;

e) an amino acid sequence that has at least 95% amino acid identity with the amino acid sequences of SEQ ID NO: 49;

f) an amino acid sequence of any one of SEQ ID NO: 49-52;

g) the amino acid sequence of SEQ ID NO: 67;

h) an amino acid sequence that has at least 90% amino acid identity with the amino acid sequences of SEQ ID NO: 67; or

i) an amino acid sequence of any one of SEQ ID NO: 53-120.

~~any one of claims 12-15.~~

~~29. The polypeptide according to claim 28, wherein said first and said second immunoglobulin single variable domains comprise the same VHH domain.~~

1530. ~~The polypeptide according to claim 1,~~A polypeptide comprising a first immunoglobulin single variable domain and a second immunoglobulin single variable domain, each comprising CDR1, CDR2 and CDR3 having amino acid sequences set forth in SEQ ID NO's: 141, 164 and 186, or SEQ ID NO's: 141, 162 and 186, or SEQ ID NO's: 213, 214 and 186, or SEQ ID NO's: 213, 221 and 186.~~, and a second immunoglobulin single variable domain as set forth in any one of claims 1 to 16.~~

~~31. The polypeptide according to claim 30, wherein said second immunoglobulin single variable domain comprises CDR1, CDR2 and CDR3 having amino acid sequences set forth SEQ ID NO's: 141, 164 and 186, or SEQ ID NO's: 141, 162 and 186, or SEQ ID NO's: 213, 214 and 186, or SEQ ID NO's: 213, 221 and 186.~~

~~32. The polypeptide according to claim 1, comprising a first immunoglobulin single variable domain, wherein said first immunoglobulin single variable domain is a VHH domain comprising the sequence set forth in SEQ ID NO: 1, SEQ ID NO: 3, or any of SEQ ID NO: 121-140 or 222-224 and a second immunoglobulin single variable domain according to any one of claims 1-16.~~

~~33. The polypeptide according to claim 32, wherein said second immunoglobulin single variable domain is a VHH domain comprising the sequence set forth in SEQ ID NO: 1, SEQ ID NO: 3, or any one of SEQ ID NO: 121-140 or 222-224.~~

~~34. The polypeptide according to any one of claims 23-33, wherein the polypeptide further comprises a half-life extending moiety.~~

~~35. The polypeptide according to claim 34, wherein said half-life extending moiety is covalently linked to said polypeptide and is selected from the group consisting~~

~~of an albumin binding moiety, such as an anti-albumin immunoglobulin domain, a transferrin binding moiety, such as an anti-transferrin immunoglobulin domain, a polyethylene glycol molecule, a recombinant polyethylene glycol molecule, human serum albumin, a fragment of human serum albumin, an albumin binding peptide or a Fc domain.~~

~~36. The polypeptide according to claim 35, wherein said half-life extending moiety consists of an anti-albumin immunoglobulin single variable domain.~~

~~37. The polypeptide according to claim 36, wherein the immunoglobulin single variable domain is selected from a VHH domain, a humanized VHH domain, a camelized VH domain, a domain antibody, a single domain antibody and/or "dAb"s.~~

~~38. The polypeptide according to claim 37, wherein the anti-albumin immunoglobulin single variable domain is selected from SEQ ID NO's: 230-232.~~

~~39. The polypeptide according to claim 35, wherein said polypeptide is linked to an Fc portion, optionally via a suitable linker or hinge region.~~

16~~40~~. A~~The~~ polypeptide ~~according to any one of claims 1 to 5~~ comprising the amino acid sequence of any one of SEQ ID NO: 225-227 or 257-262.

~~41. The polypeptide according to any one of claims 1 to 5 comprising the amino acid sequence of any one of SEQ ID NO: 253, 254, or 263 to 266.~~

~~42. The polypeptide according to any one of claims 1 to 5 comprising the amino acid sequence of any one of SEQ ID NO: 249, 277-281, and 267-276.~~

17~~43~~. A nucleic acid molecule encoding a polypeptide according to any one of claims 1 to 16~~42~~.

18~~44~~. An expression vector comprising a nucleic acid molecule according to claim 17~~43~~.

1945. A host cell comprising a nucleic acid molecule encoding a polypeptide according to any one of claims 1 to 1642, wherein said host cell is capable of expressing said polypeptide.

2046. A pharmaceutical composition comprising (i) a polypeptide according to any one of claims 1 to 1642, and (ii) a pharmaceutically acceptable carrier, and optionally (iii) a diluent, excipient, adjuvant and/or stabilizer.

2147. The pharmaceutical composition according to claim 1746, wherein said pharmaceutical composition is suitable for intravenous or subcutaneous injection in a human being.

2248. A method of manufacturing a polypeptide according to any one of claims 1 to 1642, comprising the steps of

- culturing a host cell under conditions that allow expression of a polypeptide according to any one of claims 1 to 1642, wherein said host cell is carrying an expression vector comprising a nucleic acid molecule, said nucleic acid molecule comprising a region encoding a polypeptide according to any one of claims 1 to 1642, and wherein said host cell is a prokaryotic or a eukaryotic cell.

2349. The method according to claim 2248, further comprising the steps of

- recovering said polypeptide; and
- purifying said polypeptide.

2450. A polypeptide according to any of claims 1 to 1642 for use in the treatment, prevention or alleviation of a disease, disorder or condition, in a human being.

2551. The polypeptide for use according to claim 2450, wherein the disease, disorder or condition is a CX3CR1-associated disease, disorder or condition.

2652. The polypeptide for use according to claim 2551, wherein the disease, disorder or condition is selected from cardio- and cerebrovascular atherosclerotic

disorders, peripheral artery disease, restenosis, diabetic nephropathy, glomerulomephritis, human crescentic glomerulonephritis, IgA nephropathy, membranous nephropathy, lupus nephritis, vasculitis including Henoch-Schonlein purpura and Wegener's granulomatosis, rheumatoid arthritis, osteoarthritis, allograft rejection, systemic sclerosis, neurodegenerative disorders and demyelinating disease, multiple sclerosis (MS), Alzheimer's disease, pulmonary diseases such as COPD, asthma,neuropathic pain, inflammatory pain, or cancer.

2753. The polypeptide for use according to claim 2652, wherein the disease, disorder or condition is atherosclerosis.

2854. Use of a polypeptide according to any of claims 1 to 1642 for the manufacture of a medicament for the treatment, prevention or alleviation of a disease, disorder or condition, in a human being.

2955. Use of a polypeptide according to any one of claims 1 to 16 in the manufacture of a medicament for the ~~A method of~~ treatment or prevention of CX3CR1-associated disease or disorder ~~comprising administering a pharmaceutically active amount of a polypeptide according to any one of embodiments 1 to 42 to a subject in need thereof~~.

3056. The ~~method~~ use according to claim 2955, wherein the disease or disorder is selected from cardio- and cerebrovascular atherosclerotic disorders, peripheral artery disease, restenosis, diabetic nephropathy, glomerulonephritis, human crescentic glomerulonephritis, IgA nephropathy, membranous nephropathy, lupus nephritis, vasculitis including Henoch-Schonlein purpura and Wegener's granulomatosis, rheumatoid arthritis, osteoarthritis, allograft rejection, systemic sclerosis, neurodegenerative disorders and demyelinating disease, multiple sclerosis (MS), Alzheimer's disease, pulmonary diseases such as COPD, asthma,neuropathic pain, inflammatory pain, or cancer.

3157. The ~~method~~ use according to claim 2955, wherein the disease or disorder is atherosclerosis.

3258. A diagnostic kit or diagnostic method comprising a polypeptide according to any one of claims 1 to 1642, or the use thereof.

3359. A diagnostic kit or diagnostic method according to claim 3258, for the diagnosis of at least one of cardio- and cerebrovascular atherosclerotic disorders, peripheral artery disease, restenosis, diabetic nephropathy, glomerulomephritis, human crescentic glomerulonephritis, IgA nephropathy, membranous nephropathy, lupus nephritis, vasculitis including Henoch-Schonlein purpura and Wegener's granulomatosis, rheumatoid arthritis, osteoarthritis, allograft rejection, systemic sclerosis, neurodegenerative disorders and demyelinating disease, multiple sclerosis (MS), Alzheimer's disease, pulmonary diseases such as COPD, asthma,neuropathic pain, inflammatory pain, or cancer.

34. A polypeptide comprising the amino acid sequence of SEQ ID NO: 225.

35. A polypeptide comprising the amino acid sequence of SEQ ID NO: 226.

36. A polypeptide comprising the amino acid sequence of SEQ ID NO: 227.

37. A polypeptide comprising the amino acid sequence of SEQ ID NO: 258.

38. A polypeptide comprising the amino acid sequence of SEQ ID NO: 261.

39. A pharmaceutical composition comprising (i) a polypeptide according to claim 35, and (ii) a pharmaceutically acceptable carrier, and optionally (iii) a diluent, excipient, adjuvant and/or stabilizer.

40. A pharmaceutical composition comprising (i) a polypeptide according to claim 36, and (ii) a pharmaceutically acceptable carrier, and optionally (iii) a diluent, excipient, adjuvant and/or stabilizer.

41. A pharmaceutical composition comprising (i) a polypeptide according to claim 37, and (ii) a pharmaceutically acceptable carrier, and optionally (iii) a diluent, excipient, adjuvant and/or stabilizer.

42. A pharmaceutical composition comprising (i) a polypeptide according to claim 38, and (ii) a pharmaceutically acceptable carrier, and optionally (iii) a diluent, excipient, adjuvant and/or stabilizer.

43. A pharmaceutical composition comprising (i) a polypeptide according to claim 39, and (ii) a pharmaceutically acceptable carrier, and optionally (iii) a diluent, excipient, adjuvant and/or stabilizer.