

CLAIMS

1. A C-X-C motif chemokine ligand 10 (CXCL10) binding protein, wherein the binding protein binds to full-length human CXCL10, N-terminally truncated CXCL10
5 and citrullinated CXCL10.
2. The CXCL10 binding protein of claim 1 which binds the epitope NH₂-LSRTVRCTCISISNQPVNPRSLE-COOH (SEQ ID NO: 26) of full-length human CXCL10, N-terminally truncated CXCL10 and citrullinated CXCL10.
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3. The CXCL10 binding protein of claim 1 or claim 2, wherein the binding protein:
 - (i) binds to full-length human CXCL10 with a K_D of 50 nM or less; and/or
 - (ii) binds to N-terminally truncated human CXCL10 with a K_D of 5 nM or less.
- 15 4. A C-X-C motif chemokine ligand 10 (CXCL10) binding protein, wherein the binding protein binds to full-length human CXCL10 with a K_D of 50 nM or less, but does not bind N-terminally truncated CXCL10 and citrullinated CXCL10.
5. The CXCL10 binding protein of claim 4 which requires the N-terminal valine
20 and/or proline of the epitope NH₂-VPLSRTVRCTCISISNQPVNPRSLE-COOH (SEQ ID NO: 25) to bind to full-length human CXCL10.
6. The CXCL10 binding protein according to any one of claims 1 to 5, wherein the binding protein comprises a variable region or an antigen binding domain.
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7. The CXCL10 binding protein according to any one of claims 1 to 6, wherein the binding protein is selected from the group consisting of:
 - (i) a Fv;
 - (ii) a single chain Fv fragment (scFv);
 - 30 (iii) a dimeric scFv (di-scFv);
 - (iv) a single-domain antibody;
 - (v) a minibody;
 - (vi) a diabody;
 - (vii) a triabody;
 - 35 (viii) a tetrabody;
 - (ix) a Fab;

- (x) a F(ab')₂;
 - (xi) an antibody;
 - (xii) an antibody mimetic;
 - (xiii) a heavy chain only immunoglobulin;
 - 5 (xiv) a T-cell receptor;
 - (xv) an adnectin;
 - (xvi) an anticalin;
 - (xvii) an affibody;
 - (xviii) an avimer;
 - 10 (xix) a designed ankyrin-repeat protein (DARPin); or
 - (xx) one of (i) to (xix) linked to a constant region of an antibody, Fc or a heavy chain constant domain (C_H)₂ and/or C_H3.
8. The CXCL10 binding protein of any one of claims 1 to 7, wherein the binding
- 15 protein competitively inhibits the binding of an antibody or antigen binding fragment thereof to CXCL10 comprising:
- (i) a heavy chain variable region (V_H) comprising an amino acid sequence set forth in SEQ ID NO: 3 and a light chain variable region (V_L) comprising an amino acid sequence set forth in SEQ ID NO: 4; and/or
 - 20 (ii) a V_H comprising an amino acid sequence set forth in SEQ ID NO: 11 and a V_L comprising an amino acid sequence set forth in SEQ ID NO: 12.
9. The CXCL10 binding protein according to any one of claims 1 to 8, wherein the binding protein comprises:
- 25 (i) a V_H comprising a sequence which is at least 90% identical to a sequence set forth in SEQ ID NO: 3 and a V_L comprising a sequence which is at least 90% identical to a sequence set forth in SEQ ID NO: 4; or
 - (ii) a V_H comprising a sequence which is at least 90% identical to a sequence set forth in SEQ ID NO: 11 and a V_L comprising a sequence which is at least 90%
 - 30 identical to a sequence set forth in SEQ ID NO: 12.
10. The CXCL10 binding protein according to any one of claims 1 to 9, wherein the binding protein is an antibody or antigen binding fragment thereof comprising:
- (i) a V_H comprising an amino acid sequence set forth in SEQ ID NO: 3 and a
 - 35 V_L comprising an amino acid sequence set forth in SEQ ID NO: 4; or

- (ii) a V_H comprising an amino acid sequence set forth in SEQ ID NO: 11 and a V_L comprising an amino acid sequence set forth in SEQ ID NO: 12.

11. The CXCL10 binding protein according to any one of claims 1 to 9, wherein the
5 binding protein is an antibody or antigen binding fragment thereof comprising:

(i) a V_H comprising:

- a) a CDR1 comprising a sequence set forth in amino acids 25-34 of SEQ ID NO: 3; and
b) a CDR2 comprising a sequence set forth in amino acids 49-65 of SEQ ID NO: 3; and
10 c) a CDR3 comprising a sequence set forth in amino acids 98-108 of SEQ ID NO: 3; and

a V_L comprising:

- a) a CDR1 comprising a sequence set forth in amino acids 23-33 of SEQ ID NO: 4; and
15 b) a CDR2 comprising a sequence set forth in amino acids 49-55 of SEQ ID NO: 4; and
c) a CDR3 comprising a sequence set forth in amino acids 88-96 of SEQ ID NO: 4; or

20 (ii) a V_H comprising:

- a) a CDR1 comprising a sequence set forth in amino acids 25-34 of SEQ ID NO: 11; and
b) a CDR2 comprising a sequence set forth in amino acids 49-65 of SEQ ID NO: 11; and
25 c) a CDR3 comprising a sequence set forth in amino acids 98-108 of SEQ ID NO: 11; and

a V_L comprising:

- a) a CDR1 comprising a sequence set forth in amino acids 23-33 of SEQ ID NO: 12; and
30 b) a CDR2 comprising a sequence set forth in amino acids 49-55 of SEQ ID NO: 12; and
c) a CDR3 comprising a sequence set forth in amino acids 88-96 of SEQ ID NO: 12.

35 12. The CXCL10 binding protein according to any one of claims 1 to 11, wherein the binding protein is an antibody or antigen binding fragment thereof comprising:

- (i) a V_H comprising:
a) a CDR1 comprising a sequence set forth in SEQ ID NO: 5; and
b) a CDR2 comprising a sequence set forth in SEQ ID NO: 6; and
c) a CDR3 comprising a sequence set forth in SEQ ID NO: 7; and
5 a V_L comprising:
a) a CDR1 comprising a sequence set forth in SEQ ID NO: 8; and
b) a CDR2 comprising a sequence set forth in SEQ ID NO: 9; and
c) a CDR3 comprising a sequence set forth in SEQ ID NO: 10; or
(ii) a V_H comprising:
10 a) a CDR1 comprising a sequence set forth in SEQ ID NO: 13; and
b) a CDR2 comprising a sequence set forth in SEQ ID NO: 14; and
c) a CDR3 comprising a sequence set forth in SEQ ID NO: 15; and
a V_L comprising:
a) a CDR1 comprising a sequence set forth in SEQ ID NO: 16; and
15 b) a CDR2 comprising a sequence set forth in SEQ ID NO: 17; and
c) a CDR3 comprising a sequence set forth in SEQ ID NO: 18.
13. The CXCL10 binding protein according to any one of claims 1 to 12, wherein the binding protein is conjugated to a detectable label.
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14. A composition comprising the binding protein of any one of claims 1 to 13 and a carrier.
15. A polynucleotide encoding the CXCL10 binding protein according to any one of
25 claims 1 to 13.
16. An expression vector comprising the polynucleotide according to claim 15.
17. A cell comprising the expression vector of claim 16 *in vitro*.
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18. Use of the cell of claim 17 for preparing a CXCL10 binding protein.
19. The use of claim 18, comprising culturing the cell of claim 17 and producing the CXCL10 binding protein therefrom; and optionally isolating and purifying the produced
35 binding protein.

20. A method of detecting and/or diagnosing a malignant condition in a subject, the method comprising:
- (i) determining a level of active CXCL10 in the subject and a level of total CXCL10 in the subject; and
 - 5 (ii) determining a CXCL10 ratio of active CXCL10 to total CXCL10 in the subject.
21. The method of claim 20, wherein determining the level of active CXCL10 and total CXCL10 comprises determining the amount of active CXCL10 protein and the amount of total CXCL10 protein in the subject.
- 10 22. The method of claim 20 or claim 21, wherein the method further comprises comparing the CXCL10 ratio in the subject to a CXCL10 ratio in at least one reference.
23. The method of claim 22, wherein the method comprises determining
- 15 a) if the CXCL10 ratio in the subject is higher than the CXCL10 ratio in the reference, or
 - b) if the CXCL10 ratio in the subject is lower than the CXCL10 ratio in the reference.
- 20 24. The method of claim 23, wherein:
- (i) a lower CXCL10 ratio in the subject compared to the CXCL10 ratio in the reference is indicative of the malignant condition; or
 - (ii) a higher CXCL10 ratio in the subject compared to the CXCL10 ratio in the reference is indicative of a benign condition.
- 25 25. The method of any one of claims 20 to 24 wherein the method comprises using:
- (i) a CXCL10 binding protein that specifically binds full-length human CXCL10 and N-terminally truncated CXCL10 and citrullinated CXCL10 to determine the level of total CXCL10 in the subject; and
 - 30 (ii) a CXCL10 binding protein that specifically binds full-length human CXCL10, but does not bind N-terminally truncated CXCL10 and citrullinated CXCL10 to determine the level of active CXCL10 in the subject.
26. The method of claim 25, wherein the method comprises using at least one
- 35 CXCL10 binding protein according to any one of claims 1 to 13.

27. The method of any one of claims 20 to 26, wherein:
- (i) the level of total CXCL10 in the subject is determined using an antibody or antigen binding fragment thereof comprising a V_H comprising an amino acid sequence set forth in SEQ ID NO: 11 and a V_L comprising an amino acid sequence set forth in SEQ ID NO: 12; and/or
 - (ii) the level of active CXCL10 in the subject is determined using an antibody or antigen binding fragment thereof comprising a V_H comprising an amino acid sequence set forth in SEQ ID NO: 3 and a V_L comprising an amino acid sequence set forth in SEQ ID NO: 4.
28. The method of any one of claims 25 to 27, wherein one or more of the CXCL10 binding proteins is conjugated to a detectable label.
29. The method of claim 28, wherein the detectable label is selected from the group consisting of a radiolabel, an enzyme, a fluorescent label, a luminescent label, a bioluminescent label, a magnetic label, a prosthetic group and a contrast agent.
30. The method of any one of claims 20 to 29, wherein the method comprises performing flow cytometry, an enzyme-linked immunosorbent assay or a western blot.
31. The method of any one of claims 20 to 30, wherein the method is performed on at least one biological sample obtained from the subject.
32. The method of claim 31, wherein the biological sample is a biopsy, a fluid sample, a plasma sample or cellular swab.
33. The method of any one of claims 20 to 32, wherein the malignant condition is a reproductive cancer.
34. The method of claim 33, wherein the reproductive cancer is ovarian cancer.
35. The method of any one of claims 20 to 34, wherein the method further comprises determining the level of dipeptidyl peptidase-4 (DPP4) and/or cancer antigen 125 (CA-125) in the subject.

36. The method of any one of claims 20 to 35, wherein the method further comprises determining the level of one or more or all of granulocyte-macrophage colony-stimulating factor (GM-CSF), interleukin 6 (IL-6), tumor necrosis factor receptor II (TNF-RII), human epididymis protein 4 (HE4) and interleukin 8 (IL-8).
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37. A method of monitoring tumour burden in a subject suffering from a malignant condition, the method comprising determining a CXCL10 ratio of active CXCL10 to total CXCL10 in the subject at one or more time points.
- 10 38. A method of monitoring progression of a malignant condition in a subject, the method comprising determining a CXCL10 ratio of active CXCL10 to total CXCL10 in the subject at one or more time points.
39. A method of determining tumour regression in a subject suffering from a
- 15 malignant condition, the method comprising determining a CXCL10 ratio of active CXCL10 to total CXCL10 in the subject at one or more time points.
40. A method of treating a malignant condition in a subject, the method comprising detecting and/or diagnosing the malignant condition in the subject according to any one
- 20 of claims 20 to 36, and administering a treatment to the subject.
41. A method of detecting and/or diagnosing a condition in a subject, the method comprising determining a level of CXCL10 in the subject using at least one CXCL10 binding protein according to any one of claims 1 to 13.
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