

CLAIMS

1. A multimeric binding protein that binds specifically to human CD20, human NKp46, human CD122, and optionally CD16A, in which the protein comprises

(a) a first (I) polypeptide chain comprising the amino acid sequence of SEQ ID NO: 1, and a second (II) polypeptide chain comprising the amino acid sequence of SEQ ID NO: 70;

(b) a first (I) polypeptide chain comprising the amino acid sequence of SEQ ID NO: 1, a second (II) polypeptide chain having an amino acid sequence of SEQ ID NO: 9, and a third (III) polypeptide chain comprising the amino acid sequence of SEQ ID 17;

(c) a first (I) polypeptide chain comprising the amino acid sequence of SEQ ID NO: 1, a second (II) polypeptide chain having an amino acid sequence of SEQ ID NO: 73, and a third (III) polypeptide chain comprising the amino acid sequence of SEQ ID NO: 74, or

(d) a first (I) polypeptide chain comprising the amino acid sequence of SEQ ID NO: 66, a second (II) polypeptide chain having an amino acid sequence of SEQ ID NO: 67, and a third (III) polypeptide chain comprising the amino acid sequence of SEQ ID 17.

2. A binding protein comprising a first and a second antigen binding domains (ABDs), a cytokine moiety and all or part of an immunoglobulin Fc region or variant thereof, in which each of the ABDs comprise an immunoglobulin heavy chain variable domain (VH) and an immunoglobulin light chain variable domain (VL), in which each VH and VL comprises three complementary determining regions (CDR1, CDR2 and CDR3); and in which:

(i) the first ABD binds specifically to human CD20 and comprises:

a VH1 comprising a CDR1, CDR2 and CDR3 corresponding to the amino acid sequences of SEQ ID NO: 29 (HCDR1), SEQ ID NO: 32 (HCDR2), SEQ ID NO: 35 (HCDR3), and

a VL1 comprising a CDR1, CDR2 and CDR3 corresponding to the amino acid sequences of SEQ ID NO: 38 (LCDR1), SEQ ID NO: 41 (LCDR2), SEQ ID NO: 44 (LCDR3);

(ii) the second ABD binds specifically to human NKp46 and comprises:

a VH2 comprising a CDR1, CDR2 and CDR3 corresponding to the amino acid sequences of SEQ ID NO: 47 (HCDR1), SEQ ID NO: 50 (HCDR2), SEQ ID NO: 53 (HCDR3), and

5 a VL2 comprising a CDR1, CDR2 and CDR3 corresponding to the amino acid sequences of SEQ ID NO: 56 (LCDR1), SEQ ID NO: 59 (LCDR2), SEQ ID NO: 62 (LCDR3);

in which the cytokine moiety is a variant IL-2;

and in which all or part of the immunoglobulin Fc region or variant thereof binds to a human FcRn polypeptide.

- 10 3. A binding protein according to Claim 2, in which the first ABD has a Fab structure.
4. A binding protein according to Claim 2, comprising three polypeptide chains (I), (II) and (III) that together comprise the first ABD and the second ABD:

$V_{1A} - C_{1A} - \text{Hinge}_1 - (\text{Fc domain})_A$ (I)

$V_{1B} - C_{1B} - \text{Hinge}_2 - (\text{Fc domain})_B - L_1 - V_{2A} - C_{2A}$ (II)

15 $V_{2B} - C_{2B} - \text{Hinge}_3 - L_2 - \text{IL-2}$ (III)

in which:

V_{1A} and V_{1B} form a binding pair V_1 (V_{H1}/V_{L1}) of the first ABD;

V_{2A} and V_{2B} form a binding pair V_2 (V_{H2}/V_{L2}) of the second ABD;

C_{1A} and C_{1B} form a pair C_1 ($CH1/C_L$) and C_{2A} and C_{2B} form a pair C_2 ($CH1/C_L$)

20 in which CH1 is an immunoglobulin heavy chain constant domain 1 and C_L is an immunoglobulin light chain constant domain;

Hinge₁, Hinge₂ and Hinge₃ are identical or different and correspond to all or part of an immunoglobulin hinge region, in which Hinge₃ is optional;

(Fc domain)_A and (Fc domain)_B are identical or different, and comprise a CH2-CH3 domain;

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L_1 and L_2 are an amino acid linker, in which L_1 and L_2 can be different or the same;

IL-2 is a variant human interleukin-2 polypeptide or portion thereof that binds to CD122 present on NK cells.

5. A binding protein according to Claim 4, in which:

CH1 is an immunoglobulin heavy chain constant domain 1 that comprises the amino acid sequence of SEQ ID NO: 12;

- 5 C_K is an immunoglobulin kappa light chain constant domain (C_K) that comprises the amino acid sequence of SEQ ID NO: 4;

(Fc domain)_A comprises a CH2-CH3 domains corresponding to the amino acid sequence of SEQ ID NO: 6;

(Fc domain)_B comprises a CH2-CH3 domains corresponding to the amino acid sequence of SEQ ID NO: 14;

- 10 Hinge₁ corresponds to the amino acid sequence of SEQ ID NO: 5;

Hinge₂ corresponds to the amino acid sequence of SEQ ID NO: 13;

Hinge₃ corresponds to the amino acid sequence of SEQ ID NO: 19;

L₁ corresponds to the amino acid sequence of SEQ ID NO: 15;

L₂ corresponds to any one of the amino acid sequence of SEQ ID NO: 20-23.

- 15 6. A binding protein according to any of Claims 2 to 5, in which the variant IL-2 comprises an amino acid sequence at least 90% identical to a sequence selected from SEQ ID NOS: 24-28 and 65 or to a contiguous sequence of at least 40, 50, 60, 70, 80 or 100 amino acid residues thereof.

7. A binding protein according to Claim 4, in which:

- 20 polypeptide (I) consists of an amino acid sequence of SEQ ID NO: 1;

polypeptide (II) consists of an amino acid sequence of SEQ ID NO: 9; and

polypeptide (III) consists of an amino acid sequence of SEQ ID NO: 17.

8. A binding protein according to Claim 2, in which the first ABD that binds to CD20 is a Fab and the second ABD that binds to NKp46 is an scFv.

- 25 9. A binding protein according to Claim 2, in which the second ABD and cytokine moiety have an arrangement:

– L1 – V_{2A} – L2 – V_{2B} – L3 – IL-2,

in which V_{2A} and V_{2B} form a binding pair V_2 (V_{H2}/V_{L2}) of the second ABD;

L_1 , L_2 and L_3 are an amino acid linker, in which L_1 , L_2 and L_3 can be different or the same;

IL-2 is a variant human interleukin-2 polypeptide or portion thereof that binds to CD122 present on NK cells.

10. A binding protein according to Claim 2, in which the binding protein comprises two polypeptide chains (I) and (II):

$V_{1A} - C_{1A} - \text{Hinge}_1 - (\text{Fc domain})_A$ (I)

$V_{1B} - C_{1B} - \text{Hinge}_2 - (\text{Fc domain})_B - L_1 - V_{2A} - L_2 - V_{2B} - L_3 - \text{IL-2}$ (II)

in which:

V_{1A} and V_{1B} form a binding pair V_1 (V_{H1}/V_{L1}) of the first ABD;

V_{2A} and V_{2B} form a binding pair V_2 (V_{H2}/V_{L2}) of the second ABD;

C_{1A} and C_{1B} form a pair C_1 ($CH1/C_L$) in which $CH1$ is an immunoglobulin heavy chain constant domain 1 and C_L is an immunoglobulin light chain constant domain;

Hinge_1 and Hinge_2 are identical or different and correspond to all or part of an immunoglobulin hinge region;

$(\text{Fc domain})_A$ and $(\text{Fc domain})_B$ are identical or different, and comprise a $CH2$ - $CH3$ domain;

L_1 , L_2 and L_3 are an amino acid linker, in which L_1 , L_2 and L_3 can be different or the same;

IL-2 is a variant human interleukin-2 polypeptide or portion thereof that binds to CD122 present on NK cells.

11. A binding protein according to Claim 2, in which:

(a) V_{H1} and V_{L1} corresponds to the amino acid sequences of SEQ ID NOS: 11 and 3 respectively,

and/or

(b) V_{H2} and V_{L2} corresponds to the amino acid sequences of SEQ ID NOS: 93 and 95 respectively.

12. A binding protein according to any preceding claim, comprising a variant IL-2 that displays reduced binding to CD25 compared to a wild-type human IL-2 polypeptide.
13. A binding protein according to Claim 1 or 2, in which the binding protein comprises a polypeptide comprising the amino acid sequence of SEQ ID NO: 1, and a polypeptide
5 comprising the amino acid sequence of SEQ ID NO: 70.
14. A pharmaceutical composition comprising a binding protein according to any preceding claim and a pharmaceutically acceptable carrier.
15. An isolated nucleic acid molecule comprising a nucleotide sequence that encodes a binding protein or a polypeptide chain thereof according to any preceding claim.
- 10 16. An isolated cell comprising a nucleic acid molecule according to Claim 15.
17. A binding protein according to any preceding claim for use in the treatment of a disease involving CD20-expressing cells.
18. Use of a binding protein according to any of Claims 1 to 13 in the manufacture of a medicament for treating a disease and/or eliminating CD20-expressing cells in a subject,
15 optionally in which the disease is characterized by CD20-expressing cells, in which the binding protein is to be administered to the subject.
19. A binding protein for use according to Claim 17 or a use according to Claim 18, in which the disease is selected from the group consisting of B cell lymphoma, Hodgkin's or non Hodgkins B cell lymphoma, precursor B cell lymphoblastic leukemia/lymphoma and mature
20 B cell neoplasms, such as B cell chronic lymphocytic leukemia (CLL)/small lymphocytic lymphoma (SLL), B cell prolymphocytic leukemia, lymphoplasmacytic lymphoma, mantle cell lymphoma (MCL), follicular lymphoma (FL), cutaneous follicle center lymphoma, marginal zone B cell lymphoma (MALT type, nodal and splenic type), hairy cell leukemia, diffuse large B cell lymphoma, Burkitt's lymphoma, plasmacytoma, plasma cell myeloma,
25 post-transplant lymphoproliferative disorder, Waldenstrom's macroglobulinemia, and anaplastic large-cell lymphoma (ALCL).
20. A method for making a binding protein according to any of Claims 1 to 13, comprising a step of:

- 5 (a) culturing host cell(s) under conditions suitable for expressing a plurality of recombinant polypeptides, the plurality comprising (i) a polypeptide comprising an amino acid sequence of SEQ ID NO: 1, 66, 68 or 77, and (ii) a polypeptide comprising an amino acid sequence of SEQ ID NO: 9, 67, 69, 70, 71, 72, 73, 75, 76, 78 or 79, and optionally (iii) a polypeptide comprising an amino acid sequence of SEQ ID NO: 17 or 74; and
- (b) optionally recovering the expressed recombinant polypeptides.