

5 **MULTIFUNCTIONAL NATURAL KILLER (NK) CELL ENGAGERS BINDING
TO NKp46 AND CD123**

TECHNICAL FIELD

The disclosure relates to multifunctional binding proteins comprising a first and a second antigen binding domains (ABDs) and all or part of an immunoglobulin Fc region
10 or variant thereof, wherein the first ABD binds specifically to human CD123 and the second ABD binds specifically to human NKp46 and wherein all or part of the immunoglobulin Fc region or variant thereof to a human Fc- γ receptor.

The disclosure also relates to methods for making said binding proteins, compositions thereof, and their uses including the treatment or prevention of proliferative
15 disorders, including Acute Myeloid Leukemia (AML) and myelodysplastic syndromes (MDS).

BACKGROUND

Acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS) are heterogeneous clonal neoplastic diseases, which are thought to arise from subpopulations of
20 leukemic stem cells, which tend to be resistant to conventional chemotherapy, and which may be further responsible for disease relapse.

Natural killer (NK) cells are a subpopulation of lymphocytes that are involved in non-conventional immunity. NK cells provide an efficient immunosurveillance mechanism by which undesired cells such as tumor- or virally-infected cells can be
25 eliminated. Characteristics and biological properties of NK cells include the expression of surface antigens including CD16, CD56 and/or CD57, the absence of the α/β or γ/δ TCR complex on the cell surface, the ability to bind to and kill cells in a MHC-unrestrictive manner and in particular cells that fail to express "self" MHC/HLA antigens by the activation of specific cytolytic enzymes, the ability to kill tumor cells or other diseased cells that
30 express a ligand for NK activating receptors, and the ability to release protein molecules called cytokines that stimulate the immune response.

Interest has also focused on natural killer (NK) cells due to their potential anti-tumor properties.

5 Still, there is an urgent need for active agents for treating or preventing proliferative disorders such as Acute myeloid leukemia (AML) and myelodysplastic syndromes (MDS).

 There is also a need for novel NK engagers with a therapeutic effect.

 There is also a need for novel compounds which are easier to manufacture and/or
10 administer, with no or decreased side-effects. In particular, there is a need for novel compounds with no or decreased risk of cytokine release syndrome in patients (e.g. no or decreased IL-6 associated cytokine release).

SUMMARY

15 In one embodiment, the disclosure relates to a binding protein comprising a first and a second antigen binding domain (ABD) and all or part of an immunoglobulin Fc region or variant thereof, wherein each of said ABD comprises an immunoglobulin heavy chain variable domain (V_H) and an immunoglobulin light chain variable domain (V_L), wherein each V_H and V_L comprises three complementary determining regions (CDR-1 to CDR-3);
20 and wherein:

 (i) the first ABD binds specifically to human CD123 and comprises:

 - a V_{H1} comprising a CDR-H1, H2 and H3 corresponding to the amino acid sequences of SEQ ID NO:1 to 3 respectively or corresponding to the amino acid sequences of SEQ ID NO: 4 to 6 respectively, and

25 - a V_{L1} comprising a CDR-L1, L2 and L3 corresponding to the amino acid sequences of SEQ ID NO: 7 to 9 respectively or corresponding to the amino acid sequences of SEQ ID NO: 10 to 12 respectively;

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

 - a V_{H2} comprising a CDR-H1, 2 and 3 corresponding to:

30 the amino acid sequences of SEQ ID NO:13 to 15 respectively;

 the amino acid sequences of SEQ ID NO:16 to 18 respectively;

 the amino acid sequences of SEQ ID NO:19 to 21 respectively;

 the amino acid sequences of SEQ ID NO:22 to 24 respectively; or

 the amino acid sequences of SEQ ID NO:16, 25 and 26 respectively;

35 and

- 5 - a V_{L2} comprising a CDR-L1, 2 and 3 corresponding to:
 the amino acid sequences of SEQ ID NO:27 to 29 respectively;
 the amino acid sequences of SEQ ID NO:30 to 32 respectively;
 the amino acid sequences of SEQ ID NO:33 to 35 respectively;
 the amino acid sequences of SEQ ID NO:36 to 38 respectively; or
 10 the amino acid sequences of SEQ ID NO:39, 31 and 40 respectively;
 and wherein all or part of the immunoglobulin Fc region or variant thereof binds to a human
 Fc- γ receptor.

In certain embodiments, the binding protein comprises three polypeptide chains (I),
 (II) and (III) that form two ABDs, as defined below:

- 15 $V_{1A} - C_{1A} - \text{Hinge}_1 - (C_{H2}-C_{H3})_A$ (I)
 $V_{1B} - C_{1B} - \text{Hinge}_2 - (C_{H2}-C_{H3})_B - L_1 - V_{2A} - C_{2A} - \text{Hinge}_3$ (II)
 $V_{2B} - C_{2B}$ (III)

wherein :

- V_{1A} and V_{1B} form a binding pair V_1 (V_{H1}/V_{L1});
 20 V_{2A} and V_{2B} form a binding pair V_2 (V_{H2}/V_{L2});
 C_{1A} and C_{1B} form a pair C_1 (C_{H1}/C_L) and C_{2A} and C_{2B} form a pair C_2 (C_{H1}/C_L) wherein
 C_{H1} is an immunoglobulin heavy chain constant domain 1 and C_L is an
 immunoglobulin light chain constant domain;
 Hinge_1 , Hinge_2 and Hinge_3 are identical or different and correspond to all or part of an
 25 immunoglobulin hinge region;
 $(C_{H2}-C_{H3})_A$ and $(C_{H2}-C_{H3})_B$ are identical or different, and comprise an
 immunoglobulin heavy chain constant domain 2 (C_{H2}) and an immunoglobulin heavy
 chain constant domain 3 (C_{H3});
 L_1 is an amino acid linker.
 30 In certain embodiments, C_{1B} is an immunoglobulin heavy chain constant domain 1
 (C_{H1}); C_{2A} is an immunoglobulin heavy chain constant domain 1 (C_{H1}); C_L corresponds to
 an immunoglobulin kappa light chain constant domain (C_K); $(C_{H2}-C_{H3})_A$ corresponds to the
 amino acid sequence of SEQ ID NO: 69; $(C_{H2}-C_{H3})_B$ corresponds to the amino acid sequence
 of SEQ ID NO: 70; Hinge_1 corresponds to the amino acid sequence of SEQ ID NO:74;
 35 Hinge_2 corresponds to the amino acid sequence of SEQ ID NO:75; Hinge_3 corresponds to

5 the amino acid sequence of SEQ ID NO: 77; L₁ corresponds to the amino acid sequence of
SEQ ID NO: 76.

In certain embodiments, the residue N297 of the Fc region or variant thereof according
to EU numbering comprises a N-linked glycosylation.

In certain embodiments, the all or part of the Fc region or variant thereof binds to a
10 human CD16A (FcγRIII) polypeptide.

In certain embodiments, the binding protein comprises at least two polypeptide chains
linked by at least one disulfide bridge.

In certain embodiments, the polypeptide chains (I) and (II) are linked by at least one
disulfide bridge between C_{1A} and Hinge₂ and/or wherein the polypeptide chains (II) and (III)
15 are linked by at least one disulfide bridge between Hinge₃ and C_{2B}.

In certain embodiments, V_{1A} is V_{L1} and V_{1B} is V_{H1}. In certain embodiments, V_{2A} is
V_{H2} and V_{2B} is V_{L2}.

In certain embodiments, (a) V_{H1} comprises a CDR-H1 comprising the amino acid
sequence of SEQ ID NO: 1; a CDR-H2 comprising the amino acid sequence of SEQ ID NO:
20 2; a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 3; V_{L1} comprises a CDR-
L1 comprising the amino acid sequence of SEQ ID NO: 7; a CDR-L2 comprising the amino
acid sequence of SEQ ID NO: 8; a CDR-L3 comprising the amino acid sequence of SEQ ID
NO: 9; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 13;
a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 14; a CDR-H3 comprising
25 the amino acid sequence of SEQ ID NO: 15; V_{L2} comprises a CDR-L1 comprising the amino
acid sequence of SEQ ID NO: 27; a CDR-L2 comprising the amino acid sequence of SEQ
ID NO: 28; a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 29; (b) V_{H1}
comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1; a CDR-H2
comprising the amino acid sequence of SEQ ID NO: 2; a CDR-H3 comprising the amino
30 acid sequence of SEQ ID NO: 3; V_{L1} comprises a CDR-L1 comprising the amino acid
sequence of SEQ ID NO: 7; a CDR-L2 comprising the amino acid sequence of SEQ ID NO:
8; a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 9; V_{H2} comprises a CDR-
H1 comprising the amino acid sequence of SEQ ID NO: 16; a CDR-H2 comprising the
amino acid sequence of SEQ ID NO: 17; a CDR-H3 comprising the amino acid sequence of
35 SEQ ID NO: 18; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID
NO: 30; a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 31; a CDR-L3

- 5 comprising the amino acid sequence of SEQ ID NO: 32; (c) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1; a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 2; a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 3; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 7; a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 8; a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 9; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 19; a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 20; a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 21; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 33; a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 34; a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 35; (d) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1; a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 2; a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 3; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 7; a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 8; a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 9; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 22; a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 23; a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 24; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 36; a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 37; a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 38; (e) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 1; a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 2; a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 3; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 7; a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 8; a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 9; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 16; a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 25; a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 26; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 39; a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 31; a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 40; (f) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 4; a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 5; a CDR-H3 comprising the amino acid sequence of SEQ ID

- 5 NO: 6; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 10; a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 11; a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 12; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 13; a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 14; a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 15; V_{L2}
- 10 comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 27; a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 28; a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 29; (g) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 4; a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 5; a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 6; V_{L1} comprises a CDR-
- 15 L1 comprising the amino acid sequence of SEQ ID NO: 10; a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 11; a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 12; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 16; a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 17; a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 18; V_{L2} comprises a CDR-L1
- 20 comprising the amino acid sequence of SEQ ID NO: 30; a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 31; a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 32; (h) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 4; a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 5; a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 6; V_{L1} comprises a CDR-L1 comprising
- 25 the amino acid sequence of SEQ ID NO: 10; a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 11; a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 12; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 19; a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 20; a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 21; V_{L2} comprises a CDR-L1 comprising the amino acid
- 30 sequence of SEQ ID NO: 33; a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 34; a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 35; (i) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 4; a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 5; a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 6; V_{L1} comprises a CDR-L1 comprising the amino acid
- 35 sequence of SEQ ID NO: 10; a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 11; a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 12; V_{H2} comprises

5 a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 22; a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 23; a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 24; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 36; a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 37; a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 38; or (j) V_{H1} comprises a CDR-H1
10 comprising the amino acid sequence of SEQ ID NO: 4; a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 5; a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 6; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 10; a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 11; a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 12; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of SEQ ID NO: 16; a CDR-H2 comprising the amino acid sequence of SEQ ID NO: 25; a CDR-H3 comprising the amino acid sequence of SEQ ID NO: 26; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of SEQ ID NO: 39; a CDR-L2 comprising the amino acid sequence of SEQ ID NO: 31; a CDR-L3 comprising the amino acid sequence of SEQ ID NO: 40.

20 In certain embodiments, (a) V_{H1} and V_{L1} corresponds to the amino acid sequences of SEQ ID NO: 41 and 43 respectively or corresponds to the amino acid sequences of SEQ ID NO: 42 and 44 respectively;
and/or (b) V_{H2} and V_{L2} corresponds to the amino acid sequences of SEQ ID NO: 45 and 53 respectively; the amino acid sequences of SEQ ID NO: 46 and 54 respectively; the amino acid sequences of SEQ ID NO: 47 and 55 respectively; the amino acid sequences of SEQ ID NO: 48 and 56 respectively; the amino acid sequences of SEQ ID NO: 49 and 57 respectively; the amino acid sequences of SEQ ID NO: 50 and 58 respectively; the amino acid sequences of SEQ ID NO: 51 and 59 respectively; or the amino acid sequences of SEQ ID NO: 52 and 60 respectively.

30 In certain embodiments,

(a) V_{H1} comprises the amino acid sequence of SEQ ID NO: 41; V_{L1} comprises the amino acid sequence of SEQ ID NO: 43; V_{H2} comprises the amino acid sequence of SEQ ID NO: 45; V_{L2} comprises the amino acid sequence of SEQ ID NO: 53;

(b) V_{H1} comprises the amino acid sequence of SEQ ID NO: 41; V_{L1} comprises the amino acid sequence of SEQ ID NO: 43; V_{H2} comprises the amino acid sequence of SEQ ID NO: 46; V_{L2} comprises the amino acid sequence of SEQ ID NO: 54;

- 5 (c) V_{H1} comprises the amino acid sequence of SEQ ID NO: 41; V_{L1} comprises the amino acid sequence of SEQ ID NO: 43; V_{H2} comprises the amino acid sequence of SEQ ID NO: 47; V_{L2} comprises the amino acid sequence of SEQ ID NO: 55;
- (d) V_{H1} comprises the amino acid sequence of SEQ ID NO: 41; V_{L1} comprises the amino acid sequence of SEQ ID NO: 43; V_{H2} comprises the amino acid sequence of SEQ ID NO: 48; V_{L2} comprises the amino acid sequence of SEQ ID NO: 56;
- 10 (e) V_{H1} comprises the amino acid sequence of SEQ ID NO: 41; V_{L1} comprises the amino acid sequence of SEQ ID NO: 43; V_{H2} comprises the amino acid sequence of SEQ ID NO: 49; V_{L2} comprises the amino acid sequence of SEQ ID NO: 57;
- (f) V_{H1} comprises the amino acid sequence of SEQ ID NO: 41; V_{L1} comprises the amino acid sequence of SEQ ID NO: 43; V_{H2} comprises the amino acid sequence of SEQ ID NO: 50; V_{L2} comprises the amino acid sequence of SEQ ID NO: 58;
- 15 (g) V_{H1} comprises the amino acid sequence of SEQ ID NO: 41; V_{L1} comprises the amino acid sequence of SEQ ID NO: 43; V_{H2} comprises the amino acid sequence of SEQ ID NO: 51; V_{L2} comprises the amino acid sequence of SEQ ID NO: 59;
- 20 (h) V_{H1} comprises the amino acid sequence of SEQ ID NO: 41; V_{L1} comprises the amino acid sequence of SEQ ID NO: 43; V_{H2} comprises the amino acid sequence of SEQ ID NO: 52; V_{L2} comprises the amino acid sequence of SEQ ID NO: 60;
- (i) V_{H1} comprises the amino acid sequence of SEQ ID NO: 42; V_{L1} comprises the amino acid sequence of SEQ ID NO: 44; V_{H2} comprises the amino acid sequence of SEQ ID NO: 45; V_{L2} comprises the amino acid sequence of SEQ ID NO: 53;
- 25 (j) V_{H1} comprises the amino acid sequence of SEQ ID NO: 42; V_{L1} comprises the amino acid sequence of SEQ ID NO: 44; V_{H2} comprises the amino acid sequence of SEQ ID NO: 46; V_{L2} comprises the amino acid sequence of SEQ ID NO: 54;
- (k) V_{H1} comprises the amino acid sequence of SEQ ID NO: 42; V_{L1} comprises the amino acid sequence of SEQ ID NO: 44; V_{H2} comprises the amino acid sequence of SEQ ID NO: 47; V_{L2} comprises the amino acid sequence of SEQ ID NO: 55;
- 30 (l) V_{H1} comprises the amino acid sequence of SEQ ID NO: 42; V_{L1} comprises the amino acid sequence of SEQ ID NO: 44; V_{H2} comprises the amino acid sequence of SEQ ID NO: 48; V_{L2} comprises the amino acid sequence of SEQ ID NO: 56;

5 (m) V_{H1} comprises the amino acid sequence of SEQ ID NO: 42; V_{L1} comprises the amino acid sequence of SEQ ID NO: 44; V_{H2} comprises the amino acid sequence of SEQ ID NO: 49; V_{L2} comprises the amino acid sequence of SEQ ID NO: 57;

(n) V_{H1} comprises the amino acid sequence of SEQ ID NO: 42; V_{L1} comprises the amino acid sequence of SEQ ID NO: 44; V_{H2} comprises the amino acid sequence of SEQ ID
10 NO: 50; V_{L2} comprises the amino acid sequence of SEQ ID NO: 58.

(o) V_{H1} comprises the amino acid sequence of SEQ ID NO: 42; V_{L1} comprises the amino acid sequence of SEQ ID NO: 44; V_{H2} comprises the amino acid sequence of SEQ ID NO: 51; V_{L2} comprises the amino acid sequence of SEQ ID NO: 59;

(p) V_{H1} comprises the amino acid sequence of SEQ ID NO: 42; V_{L1} comprises the
15 amino acid sequence of SEQ ID NO: 44; V_{H2} comprises the amino acid sequence of SEQ ID NO: 52; V_{L2} comprises the amino acid sequence of SEQ ID NO: 60.

In certain embodiments, polypeptide (I) comprises an amino acid sequence of SEQ ID NO: 64; polypeptide (II) comprises an amino acid sequence of SEQ ID NO: 65; and polypeptide (III) comprises an amino acid sequence of SEQ ID NO: 66.

20 In certain embodiments, polypeptide (I) consists of an amino acid sequence of SEQ ID NO: 64; polypeptide (II) consists of an amino acid sequence of SEQ ID NO: 65; and polypeptide (III) consists of an amino acid sequence of SEQ ID NO: 66.

In one embodiment, the disclosure relates to a pharmaceutical composition comprising the binding protein defined above, and a pharmaceutically acceptable carrier.

25 In one embodiment, the disclosure relates to an isolated nucleic acid molecule comprising a nucleotide sequence that encodes the binding protein defined above.

In one embodiment, the disclosure relates to an expression vector comprising the nucleic acid molecule defined above.

30 In one embodiment, the disclosure relates to an isolated cell comprising the nucleic acid molecule defined above.

In one embodiment, the disclosure relates to an isolated cell comprising the expression vector defined above. In certain embodiments, the host cell is a mammalian cell.

In one embodiment, the disclosure relates to a method for making the binding protein defined above, comprising the steps of: (a) introducing the expression vector defined above
35 into a host cell; (b) culturing the host cell under conditions suitable for expressing the binding protein; and (c) optionally recovering the expressed binding protein.

- 5 In one embodiment, the disclosure relates to an isolated nucleic acid molecule comprising a nucleotide sequence that encodes a binding protein as defined above, and characterized in that it comprises a first and a second antigen binding domain (ABD) and all or part of an immunoglobulin Fc region or variant thereof, wherein each of said ABD comprises an immunoglobulin heavy chain variable domain (V_H) and an immunoglobulin
- 10 light chain variable domain (V_L), wherein each V_H and V_L comprises three complementary determining regions (CDR-1 to CDR-3); wherein:
- (i) the first ABD binds specifically to human CD123 and comprises:
- a V_{H1} comprising a CDR-H1, H2 and H3 corresponding to the amino acid sequences of **SEQ ID NO:1** to **3** respectively or corresponding to the amino acid sequences of **SEQ**
- 15 **ID NO: 4** to **6** respectively, and
- a V_{L1} comprising a CDR-L1, L2 and L3 corresponding to the amino acid sequences of **SEQ ID NO: 7** to **9** respectively or corresponding to the amino acid sequences of **SEQ ID NO: 10** to **12** respectively;
- (ii) the second ABD binds specifically to human NKp46 and comprises:
- 20 - a V_{H2} comprising a CDR-H1, 2 and 3 corresponding to:
- the amino acid sequences of **SEQ ID NO:13** to **15** respectively;
- the amino acid sequences of **SEQ ID NO:16** to **18** respectively;
- the amino acid sequences of **SEQ ID NO:19** to **21** respectively;
- the amino acid sequences of **SEQ ID NO:22** to **24** respectively; or
- 25 the amino acid sequences of **SEQ ID NO:16, 25** and **26** respectively;
- and
- a V_{L2} comprising a CDR-L1, 2 and 3 corresponding to:
- the amino acid sequences of **SEQ ID NO:27** to **29** respectively;
- the amino acid sequences of **SEQ ID NO:30** to **32** respectively;
- 30 the amino acid sequences of **SEQ ID NO:33** to **35** respectively;
- the amino acid sequences of **SEQ ID NO:36** to **38** respectively; or
- the amino acid sequences of **SEQ ID NO:39, 31** and **40** respectively;
- and wherein all or part of the immunoglobulin Fc region or variant thereof binds to a human Fc-γ receptor.

5 In one embodiment, the disclosure relates to an expression vector comprising a nucleic acid molecule comprising a nucleotide sequence that encodes of or more polypeptide chains of the binding protein, as defined above.

 In one embodiment, the disclosure relates to an isolated cell comprising the nucleic acid molecule as defined above.

10 In one embodiment, the disclosure relates to an isolated cell comprising the expression vector as defined above.

 In one embodiment, the disclosure relates to a method for making the binding protein, comprising a step of:

 (a) culturing host cell(s) under conditions suitable for expressing a **plurality** of
15 recombinant polypeptides, said plurality comprising (i) a polypeptide comprising an amino acid sequence of **SEQ ID NO: 64**, and (ii) a polypeptide comprising an amino acid sequence of **SEQ ID NO: 65**, and (iii) a polypeptide comprising an amino acid sequence of **SEQ ID NO: 66**;

 (b) optionally recovering the expressed recombinant polypeptides.
20

 In one embodiment, the disclosure relates to a method of treating or preventing a blood cancer, the method comprising administering to a subject in need of said treatment or prevention the pharmaceutical composition defined above.

 In one embodiment, the disclosure relates to a method of treating or preventing a
25 myelodysplastic syndrome (MDS) or a lymphoproliferative disorder, the method comprising administering to a subject in need of said treatment or prevention the pharmaceutical composition defined above.

 In one embodiment, the disclosure relates to a method of treating or preventing an Acute Myeloid Leukemia (AML), the method comprising administering to a subject in need
30 of said treatment or prevention the pharmaceutical composition defined above.

 In one embodiment, the disclosure relates to a method of treating or preventing a CD64-positive and CD64-negative Acute Myeloid Leukemia (AML), the method comprising administering to a subject in need of said treatment or prevention the pharmaceutical composition defined above.

35 In one embodiment, the disclosure relates to a method of treating or preventing a CD64-positive Acute Myeloid Leukemia (AML), the method comprising administering to a

5 subject in need of said treatment or prevention a binding protein comprising a first and a second antigen binding domain (ABD) and all or part of an immunoglobulin Fc region or variant thereof, wherein the first ABD binds specifically to human CD123, the second ABD binds specifically to human NKp46, and wherein all or part of the immunoglobulin Fc region or variant thereof binds to a human Fc- γ receptor.

10

BRIEF DESCRIPTION OF THE FIGURES

If not specified otherwise, the binding proteins of the present disclosure are oriented with the amino terminal direction (“N-terminal end” or “N-term”) on the left-hand side and the carboxyl-terminal direction (“C-terminal end” or “C-term”) on the right-hand side, in accordance with standard usage and convention.

15 **Figure 1.** Tri-dimensional schematic representation of the F25 format which is a variant of the bispecific F5 format, including one human NKp46 binding site and one human CD123 binding site. On figure 1, the C-term of the polypeptide is on the left-hand side and the N-term is on the right-hand side.

20 **Figure 2A - Figure 2D** show two-dimensional schematic representations of the F25, F5, F26 and F6 formats respectively including the relevant domains for each polypeptide chain. In the figures 2A to 2D, the C-term of the polypeptide is on the left-hand side and the N-term is on the right-hand side. The human NKp46 binding domain is formed by the VH/VL pair on the left side. The human CD123 binding domain is formed by the

25 VH/VL pair on the right side.

Figure 2A shows a two-dimensional schematic representation of the F25 format. This representation represents the claimed “**NKp46-CD123_F25**” binding protein.

Figure 2B shows a two-dimensional schematic representation of the F5 format. When compared to **F25**, the **F5** differs in that the C_L and C_H pair of the NKp46 binding domain are swapped, with the third polypeptide chain comprising a C_{H1} domain and a V_L domain.

30 **Figure 2C** shows a two-dimensional schematic representation of the F26 format. This F26 differs from F25 of figure 2A in that it includes a Fc-silent N297S mutation on each C_{H2} domain.

5 **Figure 2D** shows a two-dimensional schematic representation of the F6 format. This F6 differs from F5 of figure 2B in that it includes a Fc-silent N297S mutation on each C_H2 domain.

Figure 2E shows a two-dimensional detailed representation of a variant of the F25 format. This F25 format representation corresponds to the one in Figure 2A.

10 **Figure 3** shows a proposed mechanism of action of a NK Cell Engager (NKCE) for killing, following joint binding of a tumor cell (i.e. an AML cell line; i.e. MOLM-13) expressing CD123 and a NK cell expressing NKp46 and a Fcγ receptor (CD16a). Reproduced and adapted from **Gauthier, L. et al.** (“Multifunctional natural killer cell engagers targeting NKp46 trigger protective tumor immunity”. *Cell* 177, 1701–1713
15 (2019)).

Figure 4A – Figure 4B report the *in vitro* cytotoxicity of the NKp46-CD123_F25 binding protein of the present disclosure against AML cell line (MOLM-13) or primary AML blast cells respectively. **Figure 4A** shows the *in vitro* cytotoxicity of the NKp46-CD123_F25 binding protein of the present disclosure and negative isotype control
20 variant of format F25 binding NKp46 only (NKp46-IC_F25) against AML cell line (MOLM-13). **Figure 4B** reproduces the same experiment with *ex vivo* patient samples where the cytotoxicity of NKp46-CD123_F25, NKp46-IC_F25, an anti-CD123 ADCC-enhanced antibody with no specificity for NKp46 (Reference-1) and a negative isotype control Fc-optimized antibody with increased ADCC activity and no specificity for NKp46 nor CD123
25 (IC-hIgG1-ADCC-enh) is assessed against primary AML blast cells.

Figure 5 provides *in-vitro* cytotoxicity data using fresh healthy donor NK cells against MOLM-13 AML cell line (EC₅₀ data) of the NKp46-CD123_F25 binding protein of the present disclosure which is capable of activating human NK cells by engaging both NKp46 and CD16a, inducing ADCC activity with its Fc competent format (F25), and a
30 NKp46-CD123_F6 binding protein activating human NK cells by engaging NKp46 only and not CD16a, inducing reduced ADCC-activity with its Fc silent format (F6).

Figures 6A – Figure 6B upper panels report the *in vitro* cytotoxicity of the NKp46-CD123_F25 binding protein of the present disclosure, an anti-CD123 ADCC-enhanced antibody with no binding for NKp46 (Reference-1) and a negative isotype control
35 Fc-optimized antibody with increased ADCC activity with no specificity for NKp46 nor CD123 (IC_hIgG1-ADCC-enh) against different AML cell lines. The AML cell lines tested

5 are: THP-1, a cell line expressing CD64(+) and CD32(+) (**Figure 6A**); MOLM-13, a cell line not expressing CD64(-) but expressing CD32(+) (**Figure 6A**), THP-1 CD64KO, a cell line knockout for CD64(-) expressing CD32(+) (**Figure 6B**); and THP-1 CD32KO, a cell line expressing CD64(+) and knockout for CD32(-) (**Figure 6B**). **Figure 6A and 6B** lower panels reports the phenotype of the malignant AML cell lines and sub-clones and the
 10 expression of CD123, CD64, CD32a/b by flow cytometry analysis. Background staining of AML cells with mouse-IgG2a (IC_mouse IgG2a) and mouse-IgG1 (IC_mouse IgG1) isotype controls is also shown.

Figures 7A – Figure 7B report the *ex vivo* induction of NK degranulation measured through the percentage of CD107a-positive NK cells against primary AML blasts
 15 from two different donors. Primary AML blasts from donor #1 are CD64(+) (**Figure 7A**) and primary AML blasts from donor #2 are CD64(-) (**Figure 7B**). The tested binding proteins are the NKp46-CD123_F25 binding protein of the present disclosure, an anti-CD123 ADCC-enhanced antibody with no specificity for NKp46 (Reference-1), a negative isotype control variant of format F25 not binding CD123 but binding NKp46 and CD16a
 20 (NKp46-IC_F25) and a negative isotype control Fc-optimized antibody with increased ADCC activity with no specificity for NKp46 nor CD123 (IC_hIgG1-ADCC-enh).

Figure 8 reports dose-dependent anti-tumor activity with the muNKp46-huCD123_F25 binding protein (carrying anti-murineNKp46 and anti-humanCD123 binding domains and also known as moNKp46-huCD123) against MOLM-13 human cells in a
 25 Severe Combined ImmunoDeficient mice (SCID) mouse model.

Figure 9A – Figure 9C report the CD123-positive basophil depletion for up to 28 days after the administration NKp46-CD123_F25 binding protein of the present disclosure in non-human primates (M1, M2, M3, M4 and M6) at 3000 µg/kg or 3 mg/kg (**Figure 9A**), at 3 µg/kg (**Figure 9B**), and at 0.5 µg/kg (**Figure 9C**).

30 **Figure 10A – Figure 10B** reports the *in-vitro* cytotoxicity of two NKp46-CD123 NKCE Fc-competent binding proteins (the NKp46-CD123_F25 of the present disclosure and NKp46-CD123_F5) and a control variant of format F5 binding CD123 and CD16a but not binding NKp46 (CD123-IC_F5) against two AML cell lines, MOLM-13 (**Figure 10A**) and THP-1 (**Figure 10B**) in presence of NK cell healthy donor sample (D648).

35 **Figure 11** left panel reports CD123-positive basophil depletion in healthy donor PBMCs (N=10) *in vitro* induced by the treatment with NKp46-CD123_F25 (CD123-NKCE;

5 dose range 0.001 to 10 $\mu\text{g/mL}$), NKCE isotype control NKp46-IC_F25 (IC-NKCE; not binding CD123 but binding NKp46 and CD16a) (dose range 0.001 to 10 $\mu\text{g/mL}$), or CD3-CD123 bispecific T-cell engager (CD123-TCE tool; 0.001 to 0.1 $\mu\text{g/mL}$). Central panel reports NKp46-CD123_F25 maximum depletion activity at the highest dose tested (10 $\mu\text{g/mL}$, 68 nM). Right panel reports the EC_{50} (pM) for CD123-positive basophil depletion
10 calculated from NKp46-CD123_F25 dose responses for the PBMCs of six healthy donors.

Figure 12 displays the IL-1 β , TNF- α , IFN- γ (written as INF γ on the graph), and IL-6 cytokine release *in vitro* by healthy donor PBMCs ($N=10$) following the treatment with NKp46-CD123_F25, NKCE isotype control NKp46-IC_F25 (not binding CD123 but binding NKp46 and CD16a) at doses of 0.1, 1 and 10 $\mu\text{g/mL}$ or a bispecific T Cell Engager
15 tool (TCE tool) co-targeting CD123 and CD3 binding sites at dose of 0.1 $\mu\text{g/mL}$.

Figure 13A - Figure 13F show the individual IL-6 and IL-10 plasma concentration *versus* time profiles in correlation with NKp46-CD123_F25 binding protein concentrations at 3000 $\mu\text{g/kg}$ (**Figure 13A and Figure 13B**), 3 $\mu\text{g/kg}$ (**Figure 13C and Figure 13D**), 0.5 $\mu\text{g/kg}$ (**Figure 13E**) and <0.5 $\mu\text{g/kg}$ (**Figure 13F**) for 6 male Cynomolgus
20 monkeys (M1 to M6).

Figure 14A displays the cytotoxicity of NKp46-CD123_F25 binding protein of the present disclosure as compared to an anti-CD123 ADCC-enhanced antibody with no specificity for NKp46 (Reference-1) and a negative isotype control Fc-optimized antibody with increased ADCC activity and no specificity for NKp46 nor CD123 (IC-hIgG1-ADCC-enh)
25 against AML blasts from patients expressing (#AML5, #AML6) or not (#AML1, #AML2) CD64. Malignant cells from AML patients were used as targets and purified healthy donor NK cells were used as effectors. Results are shown for all healthy donor NK cells tested.

Figure 14B reports the phenotype of the malignant AML cells from patients used in Figure 14A showing the expression of CD33, CD123, CD32a/b, and CD64 by flow
30 cytometry analysis.

Figure 15A is a comparison of the cytotoxicities of NKCEs targeting CD123 on tumor cells and not engaging NK cells (IC-CD123_F6) or engaging NK cells by CD16a only (IC-CD123_F25) or NKp46 only (NKp46-CD123_F6) or co-engaging NKp46+CD16
35 (NKp46-CD123_F25). MOLM-13 cells were used as the target cells and purified resting healthy donor NK cells as effectors. Two NK donors are shown.

5 **Figure 15B** reports the cytotoxicity of NKp46-CD123_F25 binding protein of the present disclosure as compared to a negative isotype control NKCE molecule not binding to CD123 (NKp46-IC_F25) against AML cell line MOLM-13. Results for five healthy NK-cell donors are shown.

10 **Figure 16A** is a flow cytometric analysis of NK and malignant cells from AML patients (AML #8-#10). Upper panels display the expression of CD123 on AML blasts (gated on the CD33-positive population); middle panels display the expression of CD64 (CD64 staining in black and isotype control in gray) on CD123-positive AML blasts; and lower panels display expression of NKp46 and CD16a on NK AML sample NK cells.

15 **Figure 16B** is an analysis of CD107a/b expression by NK cells after the overnight treatment with NKp46-CD123_F25 (CD123-NKCE), anti-CD123 ADCC-enhanced antibody with no specificity for NKp46 (Reference-1 or CD123-IgG1+ in the graph), a control isotype NKp46-IC_F25 not binding CD123 but binding NKp46 and CD16a (600 and 120 ng/mL) (IC-NKCE) and an IgG1 isotype control (600 ng/ml) (IC-IgG1+) on PBMCs from AML patient samples expressing CD64 (AML #8 and #9) and not expressing
20 CD64 (AML #10) at the cell surface of their blasts.

Figure 17 shows percent of marker expression (CD107, CD69, TNF- α , IFN- γ , and MIP-1 β) expression by NK cells treated with increasing concentrations of NKp46-CD123_F25 in comparison to controls, including one engaging NKp46 and CD16 only (NKp46-IC_F25) in an experimental setting using NK cells co-cultured or not with MOLM-
25 13 cells (NK + MOLM-13 versus NK alone). Results for three NK cell donors are shown.

Figure 18 shows the activity of the surrogate muNKp46-huCD123_F25 bispecific antibody (also known as moNKp46-huCD123) against disseminated human AML, MOLM-13, in SCID mice. MOLM-13 cells were injected intravenously at day 0 in a single administration. Treatments were administered on day 1 post tumor implantation by
30 intraperitoneal route. An isotype control antibody binding muNKp46 and murine Fc γ Rs but not huCD123 (muNKp46-IC) was administered at 0.5 mg/kg. muNKp46-huCD123_F25 and Reference-1 were administered at 5, 0.5, 0.25 and 0.05 mg/kg. The control group was left untreated. Graphs represent Kaplan-Meier curves for animals treated by muNKp46-huCD123_F25 bispecific antibody, Reference-1 and controls at 5, 0.5, 0.25 and 0.05 mg/kg.
35 ***: p <0.001 vs control group; **: p <0.01 vs control group; *: p <0.05 vs control group; ###: p <0.001 vs Reference-1; #: p <0.05 vs Reference-1. n = 5 to 10 mice / group.

5 **Figure 19** evaluates the impact of NK depletion on the in vivo efficacy of surrogate muNKP46-huCD123_F25 bispecific antibody in SCID mice bearing disseminated human MOLM-13 tumor cells. NK depletion was induced by 2 intraperitoneal administrations of anti-asialo GM1 serum one day before tumor cell implantation and at day 5 after implantation. MOLM-13 cells were injected intravenously at day 0 in a single
10 administration. Treatments were administered intraperitoneally on day 1 post tumor implantation. Controls including an isotype control antibody binding muNKP46 and murine FcγRs but not huCD123 (muNKP46-IC) and a second isotype control antibody binding huCD123 and murine FcγRs but not murine NKP46 (IC-huCD123) were also evaluated. Graphs represent Kaplan-Meier curves for animals treated by muNKP46-huCD123_F25
15 bispecific antibody and controls (muNKP46-IC, IC-huCD123) at 0.5, 0.25 and 0.05 mg/kg. n = 10 mice / group. ***: p < 0.001 vs control group; **: p < 0.01 vs control group; ###: p < 0.001 vs same treatment + NK depletion; #: p < 0.05 vs same treatment + NK depletion.

Figure 20A depicts the depletion of CD123-positive basophils from the blood of monkeys M3 and M4 treated at the low dose of 3 μg/kg as single 1-hour intravenous
20 infusion. Blood samples were collected before dosing (pre-dose) and 24 hours after the start of the infusion and analyzed by flow cytometry. CD123-positive basophils are shown in the gate.

Figure 20B displays the numbers of circulating CD123-positive basophils (open symbols) and total CD123-positive leukocytes (close symbols) at time of study in monkeys
25 M1 (orange) and M2 (purple) treated with 3 mg/kg as single 1-hour intravenous infusion, and monkeys M3 (red) and M4 (blue) treated with 3 μg/kg as single 1-hour intravenous infusion.

Figure 20C reports cytokine production in cynomolgus monkeys treated with the high and low doses of 3 mg/kg and 3 μg/kg as single 1-hour intravenous infusion, respectively. Plasma IL-6 and IL-10 concentrations are shown before dosing (0), and 1.5, 5
30 and 24 hours after the start of the treatment.

Figure 20D reports the plasma NKP46-CD123_F25 (CD123-NKCE) concentration monitored 1.5, 5, 24, 48, 72, 168, 240, 336, 504 and 672 hours (i.e., 0.04, 0.06, 0.21, 1, 2, 3, 7, 10, 14, 21 and 28 days) after the start of the 1-hour infusion of cynomolgus monkeys
35 treated with the high and low doses of 3 mg/kg and 3 μg/kg. The lower limit of quantification (LLOQ; 0.25 ng/mL) is indicated by the horizontal dotted line.

5 **Figure 21A** shows the toxicokinetics (TK) of the NKp46-CD123_F25 (CD123-NKCE) molecule in male monkey M5 weekly treated at a dose of 3 mg/kg/administration for four weeks (on Days 1, 8, 15 and 22). Plasma CD123-NKCE concentrations were determined before dosing (predose) and 1, 1.5, 5, 24, and/or 72 hours after the start of the 1-hour infusion on days 1, 8, 15 and before dosing and, 1, 1.5, 5, 24, and 168 hours after the
10 start of the last fourth 1-hour infusion on day 22. Values below the lower limit of quantification (LLOQ: 0.25 ng/mL) are not reported on the graphs. Infusion days are indicated by vertical dotted lines.

Figure 21B is an analysis of the interleukin-6 production in monkey M5 weekly treated with the high dose of 3 mg/kg/administration for four weeks. Plasma IL-6
15 concentrations were monitored before dosing and 1, 1.5, 5 and 24 hours after the start of the one-hour infusion on days 1, 8, 15 and before dosing and 1, 1.5, 5, 24, and 168 hours after the start of the last fourth one-hour infusion on day 22.

Figure 21C quantifies the number of circulating CD123-positive basophils (open symbols) and total CD123-positive leukocytes (closed symbols) in blood (left panel) or bone
20 marrow (right panel), by timepoint in the study, for monkey M5, treated at a dose of 3 mg/kg/week.

Figure 22 graphically depicts THP1 cytotoxic activity in an assay with human peripheral blood mononuclear cells (PBMC) from 2 healthy donors (HD). NK cells and THP1 GFP target cells were incubated in presence of NKp46-CD123_F25 or its isotype
25 control IC-CD123_F6 engaging CD123 and inducing reduced ADCC-activity with its Fc silent format (F6) at 0.1, 1, 10 and 100 ng/mL.

DETAILED DESCRIPTION

 The disclosure provides multifunctional binding proteins that bind one surface
30 biomarker on immune NK cells, i.e., NKp46 and one antigen of interest on tumoral target cells i.e., CD123 and is capable of redirecting NK cells to lyse a target cell that expresses the CD123 surface biomarker. The multifunctional binding proteins of the present disclosure further comprises all or part of a Fc region or variant thereof which binds a Fc- γ receptor (Fc γ R), in particular an activating Fc- γ receptor (Fc γ R), for example Fc γ RIIIa also called
35 CD16a.

5 The exemplified multifunctional binding proteins of the present disclosure also possess a dimeric Fc domain that comprises N-linked glycosylation and bind an activating Fc- γ receptor (Fc γ R) such as receptor CD16a thereby providing advantageous immune enhancing activity.

10 The inventors provide experimental evidence that optimal NK cell modulation, in particular NK cell activation, can be achieved with a better safety profile, both *in vitro* on AML cell lines MOLM-13 and THP1 and *ex vivo* on primary samples from AML patients (e.g., peripheral blood lymphocytes from AML patients), by engaging NKp46, a Fc γ R such as CD16a, and the cell surface biomarker CD123.

15 Importantly, the *in vitro* cytotoxic activity of the NKp46-CD123 binding protein of the present disclosure, characterized by a format reported herein as the “F25” and comprising a central Fragment crystallizable (Fc) region retaining binding to a human CD16 polypeptide, was reproduced *ex vivo*.

20 Accordingly, the inventors provide experimental support of the therapeutic properties of a bispecific NKp46/CD16-CD123 binding protein in particular for use in treating and preventing AML and other proliferative disorders.

 The inventors further provide experimental evidence that NKp46-CD123 binding protein activate NK cells in primary samples from AML patients irrespective of their CD64 expression status.

25 Hence, engagement of NK cells through the binding of cell surface markers NKp46/CD16 is validated as a robust and reproducible strategy for use as a medicament.

I. Definitions

30 As used herein, the “CD123” marker, or “*Cluster of Differentiation 123*” is also known as “*Interleukin 3 receptor, alpha (IL3RA)*” or “*IL3R*”, “*IL3RX*”, “*IL3RY*”, “*IL3RAY*”, “*hIL-3Ra*” and denotes an interleukin 3 specific subunit of a heterodimeric cytokine receptor. The functional interleukin 3 receptor is a heterodimer that comprises a specific alpha chain (IL-3A; CD123) and the IL-3 receptor beta chain ($\beta\theta$; CD131) that is shared with the receptors for granulocyte macrophage colony stimulating factor (GM-CSF) and interleukin 5 (IL-5). CD123 is a type I integral transmembrane protein with a deduced

5 Molecular Weight of about 43kDa containing an extracellular domain involved in IL-3 binding, a transmembrane domain and a short cytoplasmic tail of about 50 amino acids. The extracellular domain is composed of two regions: a N-terminal region of about 100 amino acids, the sequence of which exhibits similarity to equivalent regions of the GM-CSF and IL-5 receptor alpha-chains; and a region proximal to the transmembrane domain that
10 contains four conserved cysteine residues and a motif, common to other members of this cytokine receptor family. The IL-3 binding domain comprises about 200 amino acid residue cytokine receptor motifs (CRMs) made up of two Ig-like folding domains. The extracellular domain of CD123 is highly glycosylated, with N-glycosylation necessary for both ligand binding and receptor signaling. The protein family gathers three members: IL3RA
15 (CD123A), CSF2RA and IL5RA. The overall structure is well conserved between the three members, but sequence homologies are very low. One 300 amino-acid long isoform of CD123 has been discovered so far, but only on the RNA level which is accessible on the Getentry database under the accession number ACM241 16.1. A reference sequence of full-length human CD123 protein, including signal peptide, is available from the NCBI database
20 under the accession number NP_002174.1 and under the Uniprot accession number P26951. The extracellular domain of human CD123 (ECD) consists of the amino acid sequence of **SEQ ID NO: 86**. CD123 (the interleukin-3 receptor alpha chain IL-3Ra) is a tumor antigen overexpressed in a variety of hematological neoplasms. The majority of AML blasts express surface CD123 and this expression does not vary by subtype of AML. Higher expression of
25 CD123 on AML at diagnosis has been reported to be associated with poorer prognosis. CD123 expression has been reported in other hematological malignancies including myelodysplasia, systemic mastocytosis, blastic plasmacytoid dendritic cell neoplasm (BPDCN), ALL and hairy cell leukemia.

As used herein, “**Natural killer**” or “**NK cells**” refers to a sub-population of
30 lymphocytes that is involved in non-conventional immunity. NK cells can be identified by virtue of certain characteristics and biological properties, such as the expression of specific surface antigens including CD16, CD56 and/or CD57, NKp46 for human NK cells, the absence of the alpha/beta or gamma/delta TCR complex on the cell surface, the ability to bind to and kill cells that fail to express “self” MHC/HLA antigens by the activation of
35 specific cytolytic machinery, the ability to kill tumor cells or other diseased cells that express a ligand for NK activating receptors, and the ability to release protein molecules called

5 cytokines that stimulate or inhibit the immune response. Any of these characteristics and activities can be used to identify NK cells, using methods well known in the art. Any subpopulation of NK cells will also be encompassed by the term NK cells. Within the context herein “active” NK cells designate biologically active NK cells, including NK cells having the capacity of lysing target cells or enhancing the immune function of other cells. NK cells
10 can be obtained by various techniques known in the art, such as isolation from blood samples, cytophoresis, tissue or cell collections, etc. Useful protocols for assays involving NK cells can be found in Natural Killer Cells Protocols (edited by Campbell KS and Colonna M). Human Press. pp. 219-238 (2000).

As used herein, the “*NKp46*” marker, or “*Natural cytotoxicity triggering receptor 1*”, also known as “*CD335*” or “*NKP46*” or “*NK-p46*” or “*LY94*” refers to a protein
15 - or polypeptide – encoded by the *Ncr1* gene. A reference sequence of full-length human NKp46 protein is available from the NCBI database under the accession number NP_004820. The human NKp46 extracellular domain (ECD) corresponds to the amino acid sequence of **SEQ ID NO: 84**. The human NKp46 mRNA sequence is described in NCBI
20 accession number NM_004829.

As used herein, the term “*Fc-γ receptor*” or “*FcγR*” or “*Fc-gamma receptor*” may refer to both activating and inhibitory FcγRs. Fc-gamma receptors (FcγR) are cellular receptors for the Fc region of an Immunoglobulin G (IgG). Upon binding of complexed IgG, FcγRs can modulate cellular immune effector functions, thereby linking the adaptive and
25 innate immune systems, including ADCC-mediated immune responses. In humans, six classic FcγRs are currently reported: one high-affinity receptor (FcγRI) and five low-to-medium-affinity FcγRs (FcγRIIA, -B and -C, FcγRIIIA and -B). All FcγRs bind the same region on IgG Fc, yet with differing high (FcγRI) and low (FcγRII and FcγRIII) affinities. On a functional level, most of the FcγRs are activating receptors that can induce the cellular
30 responses mentioned above, including ADCC-mediated immune response. Whereas FcγRI, FcγRIIA, FcγRIIc, and FcγRIIIa are activating receptors characterized by an intracellular immunoreceptor tyrosine-based activation motif (ITAM), FcγRIIb has an inhibition motif (ITIM) and is therefore inhibitory. Unless specified otherwise, the term FcγRs encompasses activating receptors, including FcγRI (CD64), FcγRIIA (CD32a), FcγRIIIa (CD16a) and
35 FcγRIIb (CD16b), and preferably FcγRIIIa (CD16a).

5 As used herein, the term “*FcγRIIIa (CD16a)*” or “*FcγRIIIa*” or “*CD16a*” or “*CD16*” or “*Cluster of Differentiation 16*” may refer to a 50-65 kDa cell surface molecule expressed on mast cells, macrophages, and natural killer cells as a transmembrane receptor. FcγRIIIa is an activating receptor containing immunoreceptor tyrosine activating motifs (ITAMs) in the associated FcR γ-chain, ITAMs being necessary for receptor expression,
10 surface assembly and signaling. CD16a is a low affinity receptor for IgG and is an important receptor mediating ADCC (antibody dependent cell mediated cytotoxicity) by NK cells. The high affinity receptor CD16a is preferentially found on NK cells and monocytes and induces antibody-dependent cellular cytotoxicity (ADCC) upon IgG binding.

 As used herein, the term “*FcγRII CD32*”, “*FcγRII*”, “*FCGR2*” or “*CD32a*” or
15 “*CD32A*” or “*CD32*” or “*cluster of differentiation 32*” is a surface receptor glycoprotein belonging to the Ig gene superfamily. CD32A is expressed on all myeloid cells but not on lymphocytes. CD32 has a low-affinity for the Fc region of IgG antibodies in monomeric form, but high affinity for IgG immune complexes. CD32 has two major functions: cellular response regulation, and the uptake of immune complexes. Cellular responses regulated by
20 CD32 include phagocytosis, cytokine stimulation, and endocytic transport. Dysregulated CD32 is associated with different forms of autoimmunity, including systemic lupus erythematosus. In humans, there are three major CD32 subtypes: CD32A, CD32B, and CD32C. While CD32A and CD32C are involved in activating cellular responses, CD32B is inhibitory and balances the activating properties of CD32A. CD32A is an activating subtype
25 of CD32 that can be found on a variety of immune cells. Notably, CD32A (FcγRIIA) mediates effector functions of granulocytes, monocytes, B cells, platelets, and dendritic cells upon low affinity binding of aggregated IgG. When bound to an IgG immune complex, the cytosolic ITAM can promote phagocytic activity and cytokine secretion in neutrophils and macrophages.

30 As used herein, the term “*hFcγRICD64*”, “*hFcγRI*”, or “*CD64*” or “*Cluster of Differentiation 64*” is a surface receptor expressed constitutively only on monocytes and macrophages, but is upregulated on granulocytes upon cytokine stimulation.

 As used herein, the terms “*Format 5*” or “*F5*”, “*Format 25*” or “*F25*”, “*Format F6*” or “*F6*” and “*Format 26*” or “*F26*” refer to specific binding protein configurations of
35 bispecific or multispecific antibodies specifically designed to engineer multiple antigen binding domains into a single antibody molecule. The multifunctional binding proteins of

5 the present disclosure which comprise a NKp46-binding domain and a CD123-binding domain, are made based on the F25 format, as exemplified in **Figure 1** and **Figure 2**. F25 and format F26 respectively differ from format F5 and F6 in that one C_{H1}/C_L pair between the second and third polypeptide chain are swapped to form a C_L/C_{H1} pair. The F5 and F6 format have been previously described in the international patent application
10 WO2017114694, incorporated herein by reference.

As used herein, the term “*bispecific binding protein*” refers to a binding protein that specifically binds to two different antigen targets (e.g. human NKp46 and human CD123) through two distinct antigen-binding domains (ABDs).

As used herein, the term “*specifically binds to*” or “*binds specifically to*” refers
15 to the ability of an antigen-binding domain (ABD) to bind to an antigen (e.g. human NKp46 and/or human CD123) containing an epitope with an K_d of at least about 1×10^{-6} M, 1×10^{-7} M, 1×10^{-8} M, 1×10^{-9} M, 1×10^{-10} M, 1×10^{-11} M, 1×10^{-12} M, or more, and/or to bind to an epitope with an affinity that is at least twofold greater than its affinity for a nonspecific antigen.

20 As used herein, the term “*specifically binds to human NK46 polypeptide*” may refer to a specific binding toward a polypeptide comprising an amino acid sequence of **SEQ ID NO: 84**.

As used herein, the term “*specifically to a human CD123 polypeptide*” may refer to a specific binding toward a polypeptide comprising an amino acid sequence of **SEQ ID**
25 **NO: 86**.

As used herein, the term “*binds to a human Fc- γ receptor polypeptide*” may refer to a binding toward a polypeptide comprising an amino acid sequence of **SEQ ID NO: 87 or SEQ ID NO: 88**.

Competitive binding assays and other methods for determining specific binding
30 are further described below and are well known in the art. Expressions such as “specifically binds to”, or “with specificity for” are used interchangeably. Those terms are not construed to refer exclusively to those antibodies, polypeptides and/or multichain polypeptides which actually bind to the recited target/binding partner, but also to those which, although provided in a non-bound form, retain the specificity to the recited target. Binding specificity can be
35 quantitatively determined by an affinity constant K_A (or K_A) and a dissociation constant K_D (or K_D).

5 As used herein, the term “*affinity*”, concentration (EC50) or the equilibrium dissociation constant (KD) means the strength of the binding of an antibody or polypeptide to an epitope. The affinity of an antibody is given by a specific type of equilibrium constant, which is the dissociation constant KD, defined as $[Ab] \times [Ag] / [Ab-Ag]$, where [Ab-Ag] is the molar concentration of the antibody-antigen complex, [Ab] is the molar concentration of
10 the unbound antibody and [Ag] is the molar concentration of the unbound antigen. The affinity constant KA is defined by 1/KD. Preferred methods for determining the affinity of mAbs can be found in Harlow, et al., *Antibodies: A Laboratory Manual*, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1988), Coligan et al., eds., *Current Protocols in Immunology*, Greene Publishing Assoc. and Wiley Interscience, N.Y., (1992, 1993), and
15 Muller, Meth. Enzymol. 92:589-601 (1983), which references are entirely incorporated herein by reference. One preferred and standard method well known in the art for determining the affinity of mAbs is the use of surface plasmon resonance (SPR) screening (such as by analysis with a BIAcore™ SPR analytical device). In a non-limitative manner, a KD of less than 50 nM as determined by SPR, and under physiological conditions (e.g. at
20 a pH ranging from 6 to 8 under normal buffer conditions), may generally be considered as indicative of specificity of binding for antigen-antigen binding domain (ABD) interactions. As an illustration, and according to some particular and exemplified embodiments, binding proteins reported herein comprise:

- an antigen binding domain which binds specifically to human CD123 with a KD of
25 less than 10 nM, in particular with a KD of less than 0.5 nM, as determined by SPR, under physiological conditions;

- an antigen binding domain which binds specifically to human NKp46 with a KD of less than 50 nM, in particular with a KD of less than 20 nM, as determined by SPR, under physiological conditions.

30 As used herein, the term “*and/or*” is a grammatical conjunction that is to be interpreted as encompassing that one or more of the cases it connects may occur. For example, the wording “such native sequence proteins can be made using standard recombinant and/or synthetic methods” indicates that native sequence proteins can be made using standard recombinant and synthetic methods or native sequence proteins can be made
35 using standard recombinant methods or native sequence proteins can be made using synthetic methods.

5 As used herein, “*treating*” refers to a therapeutic use (i.e., on a subject having a given disease) and means reversing, alleviating, inhibiting the progress of one or more symptoms of such disorder or condition. Therefore, treatment does not only refer to a treatment that leads to a complete cure of the disease, but also to treatments that slow down the progression of the disease and/or prolong the survival of the subject.

10 As used herein, “*preventing*” means a prophylactic use (i.e., on a subject susceptible of developing a given disease and encompasses the treatment of relapsed AML patient.

 As used herein, the terms “*therapeutically effective amount*” of the multifunctional binding protein or pharmaceutical composition thereof is meant a sufficient
15 amount of the antibody-like multifunctional binding protein to treat said cancer disease, at a reasonable benefit/risk ratio applicable to any medical treatment. It will be understood, however, that the total daily usage of the polypeptides and compositions of the present disclosure will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective dose level for any particular patient will
20 depend upon a variety of factors including the disorder being treated and the severity of the disorder; activity of the specific polypeptide employed; the specific composition employed, the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific polypeptide employed; the
25 duration of the treatment; drugs used in combination or coincidental with the specific polypeptide employed; and like factors well known in the medical arts. For example, it is well known within the skill of the art to start doses of the compound at levels lower than those required to achieve the desired therapeutic effect and to gradually increase the dosage until the desired effect is achieved.

 As used herein, the term “*subject*” or “*individual*” or “*patient*” are used
30 interchangeably and may encompass a human or a non-human mammal, rodent or non-rodent. The term includes, but is not limited to, mammals, e.g., humans including man, woman and child, other primates (monkey), pigs, rodents such as mice and rats, rabbits, guinea pigs, hamsters, cows, horses, cats, dogs, sheep and goats.

 As used herein, the singular form “*a*”, “*an*” and “*the*” include plural references
35 unless the context clearly dictates otherwise. For example, the term “*a pharmaceutically*

5 *acceptable carrier*” encompasses a plurality of pharmaceutically acceptable carriers, including mixtures thereof.

As used herein, “*a plurality of*” may thus include « *two* » or « *two or more* ».

As used herein, “*antibody*” or “*immunoglobulin*” may refer to a natural or conventional antibody in which two heavy chains are linked to each other by disulfide bonds and each heavy chain is linked to a light chain by a disulfide bond. There are two types of
10 light chain, lambda (λ) and kappa (κ). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. Each chain contains distinct sequence domains. The light chain includes two domains or regions, a variable domain (V_L) and a constant domain (C_L). The heavy chain generally
15 includes four domains, a variable domain (V_H) and three constant domains (C_{H1} , C_{H2} and C_{H3} , collectively referred to as C_H). In particular, classes IgG, IgA, and IgD have three heavy chain constant region domains, which are designated C_{H1} , C_{H2} , and C_{H3} ; and the IgM and IgE classes have four heavy chain constant region domains, C_{H1} , C_{H2} , C_{H3} , and C_{H4} . The variable regions of both light (V_L) and heavy (V_H) chains determine binding recognition
20 and specificity to the antigen. The constant region domains of the light (C_L) and heavy (C_H) chains confer important biological properties such as antibody chain association, secretion, trans-placental mobility, complement binding, and binding to Fc receptors (FcR). The Fv fragment is the N-terminal part of the antigen-binding fragment (Fab) of an immunoglobulin and consists of the variable portions of one light chain and one heavy chain.

25 As used herein, when referring to “*IgG*” or “*Immunoglobulin G*” in general, IgG1, IgG2, IgG3 and IgG4 are included, unless defined otherwise. In particular, IgG is IgG1.

As used herein, the term “*antibody-like*” or “*immunoglobulin-like*” polypeptide may also refer to non-conventional or synthetic antigen-binding polypeptides or
30 binding protein, including single domain antibodies and fragments thereof, in particular variable heavy chain of single domain antibodies, and chimeric, humanized, bispecific or multimeric antibodies.

As used herein, the term “*multifunctional binding protein*” encompass a multi-chain protein, including but not limited to antibody-like polypeptide or protein formats,
35 which comprises at least one first variable region (e.g. a first immunoglobulin heavy chain variable domain (V_H) and/or an immunoglobulin light chain variable domain (V_L)) binding

5 specifically to a human CD123 polypeptide, and at least one second variable region (e.g. a second immunoglobulin heavy chain variable domain (V_H) and/or immunoglobulin light chain variable domain (V_L)) binding specifically to a human NKp46 polypeptide. Although not limited specifically to a particular type of construct, one general embodiment is particularly considered throughout the specification: the polypeptide constructs reported in
10 **WO2015197593** and **WO2017114694**, each of which is incorporated herein by reference. In particular, the multifunctional binding protein such as those reported in **WO2015197593** and **WO2017114694**, may encompass any construct comprising one or more polypeptide chains.

As used herein, the term “*humanized*”, as in “*humanized antibody*” refers to a
15 polypeptide (i.e., an antibody or an antibody-like polypeptide) which is wholly or partially of non-human origin and which has been modified to replace certain amino acids, in particular in the framework regions of the heavy and light chains, in order to avoid or minimize an immune response in humans. The constant domains of a humanized antibody are most of the time human C_H and C_L domains. Numerous methods for humanization of an
20 antibody sequence are known in the art; see e.g., the review by Almagro & Fransson (2008) Front Biosci. 13: 1619-1633. One commonly used method is CDR grafting, or antibody reshaping, which involves grafting of the CDR sequences of a donor antibody, generally a mouse antibody, into the framework scaffold of a human antibody of different specificity.

For chimeric antibodies, humanization typically involves modification of the
25 framework regions of the variable region sequences. Amino acid residues that are part of a CDR will typically not be altered in connection with humanization, although in certain cases it may be desirable to alter individual CDR amino acid residues, for example to remove a glycosylation site, a deamidation site or an undesired cysteine residue. N-linked glycosylation occurs by attachment of an oligosaccharide chain to an asparagine residue in
30 the tripeptide sequence Asn-X-Ser or Asn-X-Thr, where X may be any amino acid except Pro. Removal of an N-glycosylation site may be achieved by mutating either the Asn or the Ser/Thr residue to a different residue, in particular by way of conservative substitution. Deamidation of asparagine and glutamine residues can occur depending on factors such as pH and surface exposure. Asparagine residues are particularly susceptible to deamidation,
35 primarily when present in the sequence Asn-Gly, and to a lesser extent in other dipeptide sequences such as Asn- Ala. When such a deamidation site, in particular Asn-Gly, is present

5 in a CDR sequence, it may therefore be desirable to remove the site, typically by conservative substitution to remove one of the implicated residues. Substitution in a CDR sequence to remove one of the implicated residues is also intended to be encompassed by the claimed multifunctional binding protein.

As used herein, the term “*conservative amino acid substitution*” refers to
 10 substitutions in which an amino acid residue is replaced with an amino acid residue having a side chain with similar physicochemical properties. Families of amino acid residues having similar side chains are known in the art, and include amino acids with **basic side chains** (e.g., lysine, arginine, histidine), **acidic side chains** (e.g., aspartic acid, glutamic acid), **uncharged polar side chains** (e.g., glycine, asparagine, glutamine, serine, threonine,
 15 tyrosine, cysteine), **nonpolar side chains** (e.g., alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine), and **aromatic side chains** (e.g., tyrosine, phenylalanine, tryptophan). When an amino acid belongs to two different classes (i.e., tyrosine & phenylalanine), both can be accepted. As a reference, the following classification will be followed throughout the specification, unless stated otherwise.

20

<u>Conservative Substitution</u>	<u>Type of Amino acid</u>
Ala, Val, Leu, Ile, Met, Pro, Phe, Trp	Amino acids with non-polar side chains
Ser, Thr, Tyr, Asn, Gln, Cys, Gly	Amino acids with uncharged polar side chains
Asp, Glu	Amino acids with acidic side chains
Lys, Arg, His	Amino acids with basic side chains
Tyr, Phe, Trp,	Aromatic amino acids

As used herein, the term “*domain*” may be any region of a protein, generally defined on the basis of sequence homologies or identities, which is related to a specific
 25 structural or functional entity. Accordingly, the term “*region*”, as used in the context of the present disclosure, is broader in that it may comprise additional regions beyond the corresponding domain.

As used herein, the terms “*linker region*”, “*linker peptide*” or “*linker polypeptide*” or “*amino acid linker*” or “*linker*” refer to any amino acid sequence suitable
 30 for covalently linking two polypeptide domains, such as two antigen-binding domains

5 together and/or a Fc region to one or more variable regions, such as one or more antigen-binding domains. Although the term is not limited to a particular size or polypeptide length, such amino acid linkers are generally less than 50 amino acids in length, preferably less than 30 amino acids in length, for instance 20 or less than 20 amino acids in length, for instance 15 or less than 15 amino acids in length. Such amino acid linkers may optionally comprise
10 all or part of an immunoglobulin polypeptide chain, such as all or part of a hinge region of an immunoglobulin. Alternatively, the amino acid linker may comprise a polypeptide sequence that is not derived from a hinge region of an immunoglobulin, or even that is not derived from an immunoglobulin heavy or light polypeptide chain.

As used herein, an immunoglobulin hinge region, or a fragment thereof, may
15 thus be considered as a particular type of linker, which is derived from an immunoglobulin polypeptide chain.

As used herein, the term “*hinge region*” or “*hinge*” refers to a generally flexible region and born by the corresponding heavy chain polypeptides, and which separates the Fc and Fab portions of certain isotypes of immunoglobulins, more particularly of the IgG, IgA
20 or IgD isotypes. Such hinge regions are known in the Art to depend upon the isotype of immunoglobulin which is considered. For native IgG, IgA and IgD isotypes, the hinge region thus separates the C_H1 domain and the C_H2 domain and is generally cleaved upon papain digestion. On the other hand, the region corresponding to the hinge in IgM and IgE heavy chains is generally formed by an additional constant domain with lower flexibility.
25 Additionally, the hinge region may comprise one or more cysteines involved in interchain disulfide bonds. The hinge region may also comprise one or more binding sites to a Fcγ receptor, in addition to FcγR binding sites born by the C_H2 domain, when applicable. Additionally, the hinge region may comprise one or more post-translational modification, such as one or more glycosylated residues depending on the isotype which is considered.
30 Thus, it will be readily understood that the reference to the term “*hinge*” throughout the specification is not limited to a particular set of hinge sequences or to a specific location on the structure. Unless instructed otherwise, the hinge regions which are still particularly considered comprise all or part of a hinge from an immunoglobulin belonging to one isotype selected from: the IgG isotype, the IgA isotype and the IgD isotype; in particular the IgG
35 isotype.

5 As used herein, the terms “*CH domain*”, or “*C_H domain*”, or “*constant domain*”, can be used interchangeably and refer to any one or more heavy chain immunoglobulin constant domain(s). Such C_H domains are natively folded as immunoglobulin-like domains, although they may be partly disordered in an isolated form (e.g., C_{H1} domains when not associated with the constant domain of a light chain (C_L)).

10 Unless instructed otherwise, the term may thus refer to a C_{H1} domain, a C_{H2} domain, a C_{H3} domain; or any combinations thereof.

 As used herein, the terms “*CH1 domain*”, or “*C_{H1} domain*”, or “*constant domain 1*”, can be used interchangeably and refer to the corresponding heavy chain immunoglobulin constant domain 1.

15 As used herein, the term “*CH2 domain*”, or “*C_{H2} domain*”, or “*constant domain 2*” can be used interchangeably and refer to the corresponding heavy chain immunoglobulin constant domain 2.

 As used herein, the term “*CH3 domain*”, or “*C_{H3} domain*”, or “*constant domain 3*” can be used interchangeably and refer to the corresponding heavy chain immunoglobulin
20 constant domain 3.

 As used herein, the term “*C_{H2}-C_{H3}*”, as in (C_{H2}-C_{H3})_A and (C_{H2}-C_{H3})_B, thus refers to a polypeptide sequence comprising an immunoglobulin heavy chain constant domain 2 (C_{H2}) and an immunoglobulin heavy chain constant domain 3 (C_{H3}).

 As used herein, the term “*CL domain*”, or “*C_L domain*” can be used
25 interchangeably and refer to the corresponding light chain immunoglobulin constant domain. Unless instructed otherwise, this term may thus encompass a C_L domain of the kappa (κ or κ) or lambda (λ) class of immunoglobulin light chains, including all known subtypes (e.g. λ₁, λ₂, λ₃, and λ₇). In particular, when the C_L domain is of the kappa class, it may also be referred herein as a C_κ or C_κ or C_κ domain.

30 As used herein, the terms “*pair C (C_{H1}/C_L)*”, or “*paired C (C_{H1}/C_L)*” “refers to one constant heavy chain domain 1 and one constant light chain domain (e.g., a kappa (κ or κ) or lambda (λ) class of immunoglobulin light chains) bound to one another by covalent or non-covalent bonds, preferably non-covalent bonds; thus forming a heterodimer. Unless specified otherwise, when the constant chain domains forming the pair are not present on a
35 same polypeptide chain, this term may thus encompass all possible combinations.

- 5 Preferably, the corresponding C_{H1} and C_L domains will thus be selected as complementary to each other, such that they form a stable pair C (C_{H1}/C_L).

Advantageously, when the binding protein comprises a plurality of paired C domains, such as one “*pair C₁ (C_{H1}/C_L)*” and one “*pair C₂ (C_{H1}/C_L)*”, each C_{H1} and C_L domain forming the pairs will be selected so that they are formed between complementary
10 C_{H1} and C_L domains. Examples of complementary C_{H1} and C_L domains have been previously described in the international patent applications **WO2006064136** or **WO2012089814** or **WO2015197593A1**.

Unless instructed otherwise, the terms “*pair C₁ (C_{H1}/C_L)*” or “*pair C₂ (C_{H1}/C_L)*” may refer to distinct constant pair domains (C₁ and C₂) formed by identical or distinct
15 constant heavy 1 domains (C_{H1}) and identical or distinct constant light chain domains (C_L). Preferably, the terms “*pair C₁ (C_{H1}/C_L)*” or “*pair C₂ (C_{H1}/C_L)*” may refer to distinct constant pair domains (C₁ and C₂) formed by identical constant heavy 1 domains (C_{H1}) and identical constant light chain domains (C_L).

As used herein, the term “*Fc region*” or “*fragment crystallizable region*”, or
20 alternatively “*Fc portion*”, encompasses all or parts of the “*Fc domain*”, which may thus include all or parts of an immunoglobulin hinge region (which natively bears a first binding site to FcγRs), a C_{H2} domain (which natively bears a second binding site to FcγRs), and a C_{H3} domain of an immunoglobulin (e.g. of an IgG, IgA or IgD immunoglobulin), and/or when applicable of a C_{H4} domain of an immunoglobulin (e.g. for IgM and IgE). Preferably,
25 the Fc region includes all or parts of, at least, a C_{H2} domain and a C_{H3} domain, and optionally all or parts of an immunoglobulin hinge region. The term may thus refer to a molecule comprising the sequence of a non-antigen-binding fragment resulting from digestion of an antibody or produced by other means, whether in monomeric or multimeric form, and can contain the hinge region. The original immunoglobulin source of the native Fc is, in
30 particular, of human origin and can be any of the immunoglobulins, although IgG1 are preferred. Native Fc molecules are made up of monomeric polypeptides that can be linked into dimeric or multimeric forms by covalent (i.e., disulfide bonds) and non-covalent association. The number of intermolecular disulfide bonds between monomeric subunits of native Fc molecules ranges from 1 to 13 depending on class (e.g., IgG, IgA, and IgE) or
35 subclass (e.g., IgG1, IgG2, IgG3, IgGA1, and IgGA2). One example of a native Fc is a disulfide-bonded dimer resulting from papain digestion of an IgG. The term “native Fc” as

5 used herein is generic to the monomeric, dimeric, and multimeric forms. Under that terminology, a “Fc region” may thus comprise or consist of $\text{CH}_2\text{-CH}_3$ (e.g., $(\text{CH}_2\text{-CH}_3)_A$ or $(\text{CH}_2\text{-CH}_3)_B$) or a binding pair thereof, and optionally all or part of an immunoglobulin hinge region, comprising a binding site to a human $\text{Fc}\gamma\text{R}$. Unless specified otherwise, the term “*Fc region*” may refer to either a native or variant Fc region.

10 The term “*Fc variant*” as used herein refers to a molecule or sequence that is modified from a native Fc but still comprises a binding site for the receptor, FcRn (neonatal Fc receptor). Exemplary Fc variants, and their interaction with the receptor, are known in the art. Thus, the term “Fc variant” can comprise a molecule or sequence that is humanized from a non-human native Fc. Furthermore, a native Fc comprises regions that can be
15 removed because they provide structural features or biological activity that are not required for the antibody-like binding proteins of the invention. Thus, the term “Fc variant” comprises a molecule or sequence that lacks one or more native Fc sites or residues, or in which one or more Fc sites or residues has been modified, that affect or are involved in: (1) disulfide bond formation, (2) incompatibility with a selected host cell, (3) N-terminal heterogeneity upon
20 expression in a selected host cell, (4) glycosylation, (5) interaction with complement, (6) binding to an Fc receptor other than a salvage receptor, or (7) antibody-dependent cellular cytotoxicity (ADCC).

The fragment crystallizable (Fc) regions (e.g., native or variant) according to the present disclosure retain a capacity to bind to a human $\text{Fc}\gamma$ receptor polypeptide ($\text{Fc}\gamma$) which
25 generally occurs on native Fc regions through binding of the antibody Fc-hinge region. As a reference, overall structures of IgG1, IgG2, and IgG4 are similar with more than 90% sequence homology, the major differences residing in the hinge region and CH_2 domain, which form primary binding sites to $\text{Fc}\gamma\text{Rs}$. The hinge region also functions as a flexible linker between the Fab and Fc portion.

30 Fc regions having one or more amino acid modifications (e.g., substitutions, deletions, insertions) in one or more portions, which modifications increase the affinity and avidity of the variant Fc region for an $\text{Fc}\gamma\text{R}$ (including activating and inhibitory $\text{Fc}\gamma\text{Rs}$) are further considered as Fc regions. In some embodiments, said one or more amino acid modifications increase the affinity of the Fc region for $\text{Fc}\gamma\text{RIIIA}$ and/or $\text{Fc}\gamma\text{RIIA}$. In another
35 embodiment, the variant Fc region further specifically binds $\text{Fc}\gamma\text{RIIB}$ with a lower affinity than does the Fc region of the reference parent antibody (e.g., an antibody having the same

5 amino acid sequence as the antibody except for the one or more amino acid modifications in the Fc region). Hence, native and variant Fc regions considered herein generally comprise a domain (i.e., a C_H2 domain) capable of binding to human CD16, e.g., a human Fc domain comprising N-linked glycosylation at amino acid residue N297 (according to EU numbering).

10 As used herein, the term “*Fc-competent*” thus refers to a binding protein that is capable of binding specifically to a FcγR, in particular of an activating FcγR, in particular to one selected from FcγRI (CD64a), FcγRIIa (CD32a), and FcγRIIIa (CD16a), and more particularly to FcγRIIIa (CD16a).

Alternatively, several modifications are reported to directly affect the binding to
15 FcγRs, including mutation on residues 297 (according to EU numbering), or alternatively on residues 234 and 235 in the lower hinge region (according to the EU numbering system).

As used herein, the term “*Fc-silent*” refers to a binding protein with a Fc region, wherein the Fc region lacks a binding site to a FcγR (e.g., a Fc region lacking a C_H2 domain with said binding site and hinge region with said binding site); in particular FcγRI, FcγRIIa,
20 and FcγRIIIa, and more particularly to FcγRIIIa (CD16a).

As used herein, the term “*variable*”, as in “variable domain”, refers to certain portions of the relevant binding protein which differ extensively in sequence between and among antibodies and are used in the specific recognition and binding of a particular antibody for its particular target. However, the variability is not evenly distributed
25 throughout the entire variable domains of antibodies. The variability is concentrated in three segments called complementarity determining regions (CDRs; i.e., CDR1, CDR2, and CDR3) also known as hypervariable regions, both in the light chain and the heavy chain variable domains. The more highly conserved portions of variable domains are called the framework (FR) regions or sequences.

30 As used herein, the term “*VH domain*”, or “*V_H domain*” can be used interchangeably and refer to the corresponding heavy chain immunoglobulin variable domain.

As used herein, the term “*VL domain*”, or “*V_L domain*” can be used interchangeably and refer to the corresponding light chain immunoglobulin variable domain.

5 When the V_H or V_L domains are associated to a first antigen-binding domain (ABD) or to a second antigen-binding domain, they may also be respectively referred herein as “V_{H1}” and “V_{L1}”, or “V_{H2}” and “V_{L2}”.

The terms “*binding pair V (V_H/V_L)*”, “*V_H/V_L pair*” or “*(V_H/V_L) pair*” or “*V_L/V_H pair*” or “*(V_L/V_H) pair*” can be used interchangeably. Heavy chain and light chain variable
 10 domain can pair in parallel to form the antigen binding domains (ABDs). Each binding pair includes both a V_H and a V_L region. Unless instructed otherwise, these terms do not specify which immunoglobulin variable regions are V_H or V_L regions and which ABD will bind specifically the protein expressed on the surface of an immune effector cell or a target cell (e.g., Nkp46 and CD123).

15 As used herein, the term “*hypervariable region*” when used herein refers to the amino acid residues of an antibody that are responsible for antigen binding. This term may be substituted by the terms “*Complementarity Determining Regions*” or “*CDRs*”.

Thus, as used herein “*Complementarity Determining Regions*” or “*CDRs*” refer to amino acid sequences that together define the binding affinity and specificity of the natural
 20 Fv region of a native immunoglobulin binding site. The light and heavy chains of an immunoglobulin each have three CDRs, designated CDR-L1, CDR-L2, CDR-L3 and CDR-H1, CDR-H2, CDR-H3, respectively. A conventional antibody antigen-binding domain, therefore, includes six CDRs, comprising the CDR set from each of a heavy and a light chain variable region. Also, as used herein, “*Framework Regions*” (FRs) refer to amino acid
 25 sequences interposed between CDRs, i.e., to those portions of immunoglobulin light and heavy chain variable regions that are relatively conserved among different immunoglobulins in a single species. The light and heavy chains of an immunoglobulin each have four FRs, designated FR-L1, FR-L2, FR-L3, FR-L4, and FR-H1, FR-H2, FR-H3, FR-H4, respectively. Accordingly, the light chain variable domain may thus be designated as (FR-L1)-(CDR-L1)-
 30 (FR-L2)-(CDR-L2)-(FR-L3)-(CDR-L3)-(FR-L4) and the heavy chain variable domain may thus be designated as (FR-H1)-(CDR-H1)-(FR-H2)-(CDR-H2)-(FR-H3)-(CDR-H)-(FR-H4)-(CDR-H3).

The hypervariable region generally comprises amino acid residues from a “complementarity-determining region” or “CDR” (e.g. residues 24-34 (L1), 50-56 (L2) and
 35 89-97 (L3) in the light-chain variable domain and 31-35 (H1), 50-65 (H2) and 95-102 (H3) in the heavy-chain variable domain; Kabat et al. 1991) and/or those residues from a

5 "hypervariable loop" (e.g. residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the light-chain variable domain and 26-32 (H1), 53-55 (H2) and 96-101 (H3) in the heavy-chain variable domain; Chothia and Lesk, J. Mol. Biol 1987;196:901-917). The numbering of amino acid residues in this region is performed by the method described in Kabat et al., supra. Accordingly, phrases such as "Kabat position", "variable domain residue numbering as in
10 Kabat" and "according to Kabat" herein refer to this numbering system for heavy chain variable domains or light chain variable domains. Using the Kabat numbering system, the actual linear amino acid sequence of a peptide may contain fewer or additional amino acids corresponding to a shortening of, or insertion into, a FR or CDR of the variable domain. For example, a heavy chain variable domain may include a single amino acid insert (residue 52a
15 according to Kabat) after residue 52 of CDR H2 and inserted residues (e.g., residues 82a, 82b, and 82c, etc. according to Kabat) after heavy chain FR residue 82. The Kabat numbering of residues may be determined for a given antibody by alignment at regions of homology of the sequence of the antibody with a "standard" Kabat numbered sequence.

Optionally, CDRs are as defined by EU, Kabat, Chotia or IMGT numbering.
20 Correspondances between those classifications are known in the Art, by reference to the IMGT®, or international ImMunoGeneTics information system® (CNRS and Montpellier University), and as further detailed in Lefranc (Biomolecules; 2014; 4, 1102-1139) and Dondelinger (Frontiers in Immunology; 2018; 9, 2278).

Unless instructed otherwise, the numbering of residues will be considered herein
25 by reference to the EU, Kabat, Chotia or IMGT numbering convention. In case of conflict regarding the exact position of hypervariable regions within a reference sequence, the Kabat numbering convention will prevail. In case of conflict regarding the exact position of constant regions within a reference sequence, the EU numbering convention will prevail.

As used herein, the term "*cytotoxicity*" refers to the quality of a compound, such
30 as the multifunctional binding protein according to the present disclosure, to be toxic to tumoral cells. Cytotoxicity may be induced by different mechanisms of action and can thus be divided into cell-mediated cytotoxicity, apoptosis, antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) or complement-dependent cytotoxicity (CDC).

35 As used herein, the term "*antibody-dependent cell-mediated cytotoxicity*" or "ADCC" refers to a mechanism of cell-mediated immune defence whereby an effector cell

5 of the immune system actively lyses a target cell, whose membrane-surface antigens have been bound by specific antibodies or the multifunctional binding protein of the present disclosure.

As used herein, the terms “*proliferative disorders*”, “*hyper-proliferative disorders*” and/or “*cancer*” not only refer to solid tumors, such as cancers of the breast,
10 respiratory tract, brain, reproductive organs, digestive tract, urinary tract, eye, liver, skin, head and neck, thyroid, parathyroid and their distant metastases, but also include blood cancers, including tumors of the hematopoietic and lymphoid tissues, such as lymphomas, myelomas, and leukemias. Leukemias include, but are not limited to acute myeloid leukemia, acute lymphoblastic leukemia, chronic lymphocytic leukemia, chronic
15 myelogenous leukemia, and hairy cell leukemia.

As used herein, “*Acute myelogenous leukemia (AML)*” is a clonal disorder clinically presenting as increased proliferation of heterogeneous and undifferentiated myeloid blasts. Without wishing to be bound by the theory, the leukemic hierarchy is maintained by a small population of LSCs (Leukemic Stem Cells) (AML-LSCs), which have
20 the distinct ability for self-renewal, and are able to differentiate into leukemic progenitors. These progenitors generate the large numbers of leukemic blasts readily detectable in patients at diagnosis and relapse, leading ultimately to mortality. AML-LSC have been commonly reported as quiescent cells, in contrast to rapidly dividing clonogenic progenitors.

Within the context of AML, the term “*relapse*” may in particular be defined as
25 the reoccurrence of AML after complete remission. In that sense “*complete remission*” or “**CR**” may be defined as follows: normal values for neutrophil ($>1.0 \times 10^9/L$), haemoglobin level of 10g/dl and platelet count ($>100 \times 10^9/L$) and independence from red cell transfusion; blast cells less than 5%, no clusters or collections of blasts, and absence of Auer rods on bone marrow examination; and normal maturation of blood cells (morphology;
30 myelogramme) and absence of extramedullary leukemia.

As used herein, “*myelodysplastic syndromes*” (“**MDS**”), formerly known as preleukemia, are a collection of hematological conditions that involve ineffective production (or dysplasia) of the myeloid class of blood cells. They represent a spectrum of clonal hematopoietic stem cell disorders characterized by progressive bone marrow failure and
35 increased risk of progression to acute myeloid leukemia (“**AML**”, also known as “*acute myelogenous leukemia*”). The International Prognostic Scoring System (“**IPSS**”) is widely

5 used to identify patients with high-risk features based on the severity of their cytopenias, bone marrow myeloblast percentage, and cytogenetic abnormalities.

As used herein, a “*pharmaceutically acceptable carrier*” is intended to include any and all carrier (such as any solvents, dispersion media, coatings, antibacterial and antifungal agents, isotonic and absorption delaying agents, and the like) which is compatible
10 with pharmaceutical administration, in particular parenteral administration. The use of such media and agents for pharmaceutically active substances are known. Except insofar as any conventional media or agent is incompatible with the active compound, such media can be used in the compositions of the present disclosure. For example, preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and
15 emulsions. Examples of non-aqueous solvents are propylene glycol, polyethylene glycol, vegetable oils such as olive oil, and injectable organic esters such as ethyl oleate. Aqueous carriers include water, alcoholic/aqueous solutions, emulsions or suspensions, including saline and buffered media. In a non-exhaustive manner, pharmaceutically acceptable carriers include, but are not limited to, 0.01-0.1M (e.g., 0.05M) phosphate buffer or 0.8% saline.
20 Other common parenteral vehicles include sodium phosphate solutions, Ringer's dextrose, dextrose and sodium chloride, lactated Ringer's, or fixed oils. Intravenous vehicles include fluid and nutrient replenishers, electrolyte replenishers, such as those based on Ringer's dextrose, and the like. Preservatives and other additives may also be present such as for example, antimicrobials, antioxidants, chelating agents, and inert gases and the like. More
25 particularly, pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersions. In such cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It should be stable under the conditions of manufacture and storage and will in an embodiment be preserved
30 against the contaminating action of microorganisms, such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (e.g., glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, for example, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion
35 and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal agents, for example, parabens, chlorobutanol, phenol,

5 ascorbic acid, thimerosal and the like. In certain embodiments, isotonic agents are included, for example, sugars, polyalcohols, such as mannitol, sorbitol, or sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition an agent which delays absorption, for example, aluminum monostearate and gelatin.

10 As used herein, and unless instructed otherwise, the term “*at least one*” may encompass “*one or more*”, or even “*two or more*” (or “*a plurality*”). For instance, it may encompass 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 15 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, or more than 100.

As used herein, and unless instructed otherwise, the term “*less than...*” may encompass all values from 0 to the corresponding threshold. For instance, it may encompass less than 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 20 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, or less than 100, when applicable.

As used herein, the term “*cell*” may encompass any prokaryotic cell or eukaryotic 25 cell. Cell types which are particularly considered are those suitable for the production and/or engineering of recombinant antibodies, or fragments, or polypeptide chains thereof. In a non-exhaustive manner, such cells may be selected from the group consisting of: bacterial cells, yeast cells, mammalian cells, non-mammalian cells, insect cells, and plant cells.

The terms “*host cell*,” “*host cell line*,” and “*host cell culture*” are used 30 interchangeably and refer to cells into which exogenous nucleic acid has been introduced, including the progeny of such cells. Host cells include “*transformants*” and “*transformed cells*,” which include the primary transformed cell and progeny derived therefrom without regard to the number of passages. Progeny may not be completely identical in nucleic acid content to a parent cell but may contain mutations. Mutant progeny that have the same 35 function or biological activity as screened or selected for in the originally transformed cell are included herein. A host cell is any type of cellular system that can be used to generate

5 binding proteins of the present disclosure. Host cells may thus include cultured cells, e.g., mammalian cultured cells, such as CHO cells, HEK cells, BHK cells, NS0 cells, SP2/0 cells, YO myeloma cells, P3X63 mouse myeloma cells, PER cells, PER.C6 cells or hybridoma cells, bacterial cells, yeast cells, insect cells, and plant cells, to name only a few.

By “*isolated*” nucleic acid molecule or polynucleotide is intended a nucleic acid
10 molecule, DNA or RNA, which has been removed from its native environment. For example, a recombinant polynucleotide encoding a polypeptide contained in a vector is considered isolated for the purposes of the present disclosure. Further examples of an isolated polynucleotide include recombinant polynucleotides maintained in heterologous host cells or purified (partially or substantially) polynucleotides in solution. An isolated
15 polynucleotide includes a polynucleotide molecule contained in cells that ordinarily contain the polynucleotide molecule, but the polynucleotide molecule is present extrachromosomally or at a chromosomal location that is different from its natural chromosomal location. Isolated RNA molecules include *in vivo* or *in vitro* RNA transcripts of the present disclosure, as well as positive and negative strand forms, and double-stranded
20 forms. Isolated polynucleotides or nucleic acids according to the present disclosure further include such molecules produced synthetically. In addition, a polynucleotide or a nucleic acid may be or may include a regulatory element such as a promoter, ribosome binding site, or a transcription terminator.

The term “*vector*” or “*expression vector*” is intended to mean the vehicle by
25 which a nucleic acid, in particular a DNA or RNA sequence (e.g., a foreign gene), can be introduced into a host cell, so as to transform the host and promote expression (e.g., transcription and translation) of the introduced sequence.

II. Binding protein

In one embodiment, the disclosure relates to a binding protein characterized in
30 that it comprises:

(i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, (ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide, and (iii) all or part of an immunoglobulin Fc region or variant thereof which binds to a human
35 Fc- γ receptor.

5 In some embodiments, the binding protein is characterized in that it comprises:

- (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide and comprising at least one CDR selected from **SEQ ID NO: 1** to **SEQ ID NO: 12**, or a variant thereof with one or more conservative substitution(s);
- 10 (ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide, and (iii) all or part of an immunoglobulin Fc region or variant thereof which binds to a human Fc- γ receptor polypeptide.

In some embodiments, the disclosure relates to a binding protein characterized in that it comprises:

- 15 (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide,
- (ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide and comprising at least one CDR selected from **SEQ ID NO: 13** to **SEQ ID NO: 40**, or a variant thereof with one or more conservative
- 20 substitution(s);
- and (iii) all or part of an immunoglobulin Fc region or variant thereof which binds to a human Fc- γ receptor polypeptide.

In some embodiments, the binding protein is characterized in that it comprises:

- (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide and comprising at least one CDR selected from **SEQ ID NO: 1** to **SEQ ID NO: 12**, or a variant thereof with one or more conservative substitution(s); **and**
- 25 (ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide and comprising at least one CDR selected from **SEQ ID NO: 13** to **SEQ ID NO: 40**, or a variant thereof with one or more conservative
- 30 substitution(s);
- and (iii) all or part of an immunoglobulin Fc region or variant thereof which binds to a human Fc- γ receptor polypeptide.

In some embodiments, the binding protein is characterized in that it comprises:

- 35 (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide and comprising three CDRs selected from **SEQ**

5 **ID NO: 1 to SEQ ID NO: 12**, or a variant thereof with one or more conservative substitution(s), **and**

 (ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide and comprising three CDRs selected from **SEQ ID NO: 13 to SEQ ID NO: 40**, or a variant thereof with one or more conservative substitution(s),
10 and

 (iii) all or part of an immunoglobulin Fc region or variant thereof which binds to a human Fc- γ receptor polypeptide.

 In some embodiments, the binding protein is characterized in that it comprises:

15 (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, and comprising an immunoglobulin heavy chain variable region (V_H) and an immunoglobulin light chain variable region (V_L), wherein each V_H and V_L comprises three complementary determining regions (CDR-1 to CDR-3 respectively), **and**

20 (ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide, and comprising an immunoglobulin light chain variable region (V_L), wherein each V_H and V_L comprises three complementary determining regions (CDR-1 to CDR-3 respectively),
and

25 (iii) all or part of an immunoglobulin Fc region or variant thereof which binds to a human Fc- γ receptor polypeptide.

 In some embodiments, the binding protein is characterized in that it comprises:

 (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, and comprising an immunoglobulin heavy chain variable region (V_H) with three complementary determining regions, at least one being
30 selected from **SEQ ID NO: 1 to SEQ ID NO: 6**, and an immunoglobulin light chain variable region (V_L) with three complementary determining regions, at least one being selected from **SEQ ID NO: 7 to SEQ ID NO: 12**;

 (ii) a second antigen-binding domain (ABD) comprising a variable region which binds
35 specifically to a human NKp46 polypeptide, and comprising an immunoglobulin heavy chain variable region (V_H) with three complementary determining regions at least one being

5 selected from **SEQ ID NO: 13** to **SEQ ID NO: 26**, and an immunoglobulin light chain variable region (V_L) with three complementary determining regions at least one being selected from **SEQ ID NO: 27** to **SEQ ID NO: 40**;
and

(iii) all or part of an immunoglobulin Fc region or variant thereof which binds to a
10 human Fc- γ receptor polypeptide.

In some embodiments, the binding protein is characterized in that it comprises:

(i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, and comprising an immunoglobulin heavy chain variable region (V_H) with three complementary determining regions, at least two being
15 selected from **SEQ ID NO: 1** to **SEQ ID NO: 6**, and an immunoglobulin light chain variable region (V_L) with three complementary determining regions at least two being selected from **SEQ ID NO: 7** to **SEQ ID NO: 12**;

(ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide, and comprising an immunoglobulin heavy
20 chain variable region (V_H) with three complementary determining regions at least two being selected from **SEQ ID NO: 13** to **SEQ ID NO: 26**, and an immunoglobulin light chain variable region (V_L) with three complementary determining regions at least two being selected from **SEQ ID NO: 27** to **SEQ ID NO: 40**;
and

25 (iii) all or part of an immunoglobulin Fc region or variant thereof which binds to a human Fc- γ receptor polypeptide.

In some embodiments, the binding protein is characterized in that it comprises:

(i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, and comprising an immunoglobulin heavy chain
30 variable region (V_H) with three complementary determining regions, selected from **SEQ ID NO: 1** to **SEQ ID NO: 6**, and an immunoglobulin light chain variable region (V_L) with three complementary determining regions selected from **SEQ ID NO: 7** to **SEQ ID NO: 12**;

(ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide, and comprising an immunoglobulin heavy
35 chain variable region (V_H) with three complementary determining regions selected from **SEQ ID NO: 13** to **SEQ ID NO: 26**, and an immunoglobulin light chain variable region

5 (V_L) with three complementary determining regions selected from **SEQ ID NO: 27** to **SEQ ID NO: 40**;

and

(iii) all or part of an immunoglobulin Fc region or variant thereof which binds to a human Fc-γ receptor polypeptide.

10 In some embodiments, the binding protein is characterized in that the first ABD binds specifically to human CD123 and comprises an immunoglobulin heavy chain variable domain (V_H) comprising a CDR-H1, H2 and H3 corresponding to the amino acid sequences of **SEQ ID NO: 1** to **SEQ ID NO: 3** respectively or corresponding to the amino acid sequences of **SEQ ID NO: 4** to **SEQ ID NO: 6** respectively.

15 According to some particular embodiments of this first general object, the binding protein is characterized in that the first ABD binds specifically to human CD123 and comprises an immunoglobulin light chain variable domain (V_L) comprising a CDR-L1, L2 and L3 corresponding to the amino acid sequences of **SEQ ID NO: 7** to **SEQ ID NO: 9** respectively or corresponding to the amino acid sequences of **SEQ ID NO: 10** to **SEQ ID NO: 12** respectively.

20 According to some particular embodiments of this first general object, the binding protein is characterized in that the second ABD binds specifically to human NKp46 and comprises an immunoglobulin heavy chain variable domain (V_H) comprising a CDR-H1, H2 and H3 corresponding to the amino acid sequences of **SEQ ID NO: 13** to **SEQ ID NO: 15** respectively.

25 According to some particular embodiments of this first general object, the binding protein is characterized in that the second ABD binds specifically to human NKp46 and comprises an immunoglobulin heavy chain variable domain (V_H) comprising a CDR-H1, H2 and H3 corresponding to the amino acid sequences of **SEQ ID NO: 16** to **SEQ ID NO: 18** respectively.

30 According to some particular embodiments of this first general object, the binding protein is characterized in that the second ABD binds specifically to human NKp46 and comprises an immunoglobulin heavy chain variable domain (V_H) comprising a CDR-H1, H2 and H3 corresponding to the amino acid sequences of **SEQ ID NO: 19** to **SEQ ID NO: 21** respectively.

35

- 5 According to some particular embodiments of this first general object, the binding protein is characterized in that the second ABD binds specifically to human NKp46 and comprises an immunoglobulin heavy chain variable domain (V_H) comprising a CDR-H1, H2 and H3 corresponding to the amino acid sequences of **SEQ ID NO: 22 to SEQ ID NO: 24** respectively.
- 10 According to some particular embodiments of this first general object, the binding protein is characterized in that the second ABD binds specifically to human NKp46 and comprises an immunoglobulin heavy chain variable domain (V_H) comprising a CDR-H1, H2 and H3 corresponding to the amino acid sequences of **SEQ ID NO: 16, SEQ ID NO: 25 and SEQ ID NO: 26** respectively.
- 15 According to some particular embodiments of this first general object, the binding protein is characterized in that the second ABD binds specifically to human NKp46 and comprises an immunoglobulin light chain variable domain (V_L) comprising a CDR-L1, L2 and L3 corresponding to the amino acid sequences of **SEQ ID NO: 27 to SEQ ID NO: 29** respectively.
- 20 According to some particular embodiments of this first general object, the binding protein is characterized in that the second ABD binds specifically to human NKp46 and comprises an immunoglobulin light chain variable domain (V_L) comprising a CDR-L1, L2 and L3 corresponding to the amino acid sequences of **SEQ ID NO: 30 to SEQ ID NO: 32** respectively.
- 25 According to some particular embodiments of this first general object, the binding protein is characterized in that the second ABD binds specifically to human NKp46 and comprises an immunoglobulin light chain variable domain (V_L) comprising a CDR-L1, L2 and L3 corresponding to the amino acid sequences of **SEQ ID NO: 33 to SEQ ID NO: 35** respectively.
- 30 According to some particular embodiments of this first general object, the binding protein is characterized in that the second ABD binds specifically to human NKp46 and comprises an immunoglobulin light chain variable domain (V_L) comprising a CDR-L1, L2 and L3 corresponding to the amino acid sequences of **SEQ ID NO: 36 to SEQ ID NO: 38** respectively.
- 35 According to some particular embodiments of this first general object, the binding protein is characterized in that the second ABD binds specifically to human NKp46 and

- 5 comprises an immunoglobulin light chain variable domain (V_L) comprising a CDR-L1, L2 and L3 corresponding to the amino acid sequences of **SEQ ID NO: 39, SEQ ID NO: 31 and SEQ ID NO: 40** respectively.

According to one embodiment, the disclosure relates to a binding protein comprising a first and a second antigen binding domains (ABDs) and all or part of an immunoglobulin Fc region or variant thereof, wherein each of said ABDs comprises an immunoglobulin heavy chain variable domain (V_H) and an immunoglobulin light chain variable domain (V_L), wherein each V_H and V_L comprises three complementary determining regions (CDR-1 to CDR-3); and wherein:

- (i) the first ABD binds specifically to human CD123 and comprises:
- 15 - a V_{H1} comprising a CDR-H1, H2 and H3 corresponding to the amino acid sequences of **SEQ ID NO: 1 to SEQ ID NO: 3** respectively or corresponding to the amino acid sequences of **SEQ ID NO: 4 to SEQ ID NO: 6** respectively, and
- a V_{L1} comprising a CDR-L1, L2 and L3 corresponding to the amino acid sequences of **SEQ ID NO: 7 to SEQ ID NO: 9** respectively or corresponding to the amino acid sequences of **SEQ ID NO: 10 to SEQ ID NO: 12** respectively;
- 20 (ii) the second ABD binds specifically to human NKp46 and comprises:
- a V_{H2} comprising a CDR-H1, 2 and 3 corresponding to:
- the amino acid sequences of **SEQ ID NO: 13 to SEQ ID NO: 15** respectively;
- the amino acid sequences of **SEQ ID NO: 16 to SEQ ID NO: 18** respectively;
- 25 the amino acid sequences of **SEQ ID NO: 19 to SEQ ID NO: 21** respectively;
- the amino acid sequences of **SEQ ID NO: 22 to SEQ ID NO: 24** respectively; or
- the amino acid sequences of **SEQ ID NO: 16, SEQ ID NO: 25 and SEQ ID NO: 26** respectively;
- and
- 30 - a V_{L2} comprising a CDR-L1, 2 and 3 corresponding to:
- the amino acid sequences of **SEQ ID NO: 27 to SEQ ID NO: 29** respectively;
- the amino acid sequences of **SEQ ID NO: 30 to SEQ ID NO: 32** respectively;
- the amino acid sequences of **SEQ ID NO: 33 to SEQ ID NO: 35** respectively;
- the amino acid sequences of **SEQ ID NO: 36 to SEQ ID NO: 38** respectively; or
- 35 the amino acid sequences **SEQ ID NO: 39, SEQ ID NO: 31 and SEQ ID NO: 40** respectively;

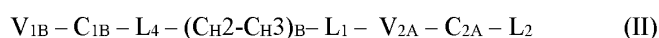
- 5 and wherein all or part of the immunoglobulin Fc region or variant thereof binds to a human Fc- γ receptor.

It will be readily understood by the skilled in the Art that the above described binding protein may consist of one single polypeptide chain, or be a multimeric binding protein, and hence comprise a plurality (two or more) polypeptide chains.

- 10 According to some particular embodiments, the binding protein is a multimeric binding protein, and the two antigen-binding domains may be born at least in part by distinct polypeptide chains.

- Optionally, when the binding protein comprises a plurality of polypeptide chains, (e.g., two or three polypeptide chains), some of those polypeptide chains may be linked covalently. When two polypeptide chains are linked covalently, the covalent linker(s) may advantageously be selected from disulfide bridges, or any other covalent linker, including peptide bond(s) bridging one polypeptide chain with another, and/or linker peptide(s) bridging one polypeptide chain with another.

- 20 According to some particular embodiments, the binding protein is characterized in that it comprises three polypeptide chains (I), (II) and (III) that form two ABDs:



wherein :

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

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

- 25 C_{1A} and C_{1B} form a pair C_1 (C_{H1}/C_L) and C_{2A} and C_{2B} form a pair C_2 (C_{H1}/C_L) wherein C_{H1} is an immunoglobulin heavy chain constant domain 1 and C_L is an immunoglobulin light chain constant domain;

$(CH_2-CH_3)_A$ and $(CH_2-CH_3)_B$ are identical or different, and comprise an immunoglobulin heavy chain constant domain 2 (C_{H2}) and an immunoglobulin heavy chain constant domain 3 (C_{H3});

- 30 L_1 , L_2 , L_3 , L_4 are optional independent amino acid linkers, which may be identical or different.

5 In some embodiments, the $(\text{CH}_2\text{-CH}_3)_\text{A}$ and $(\text{CH}_2\text{-CH}_3)_\text{B}$ comprise each at least one identical CH_2 domain, such as a CH_2 domain corresponding to the amino acid sequence of **SEQ ID NO: 71**.

 In some embodiments, the $(\text{CH}_2\text{-CH}_3)_\text{A}$ and $(\text{CH}_2\text{-CH}_3)_\text{B}$ are identical or different and may comprise a polypeptide sequence selected from amino acid sequences of **SEQ ID NO: 69 or SEQ ID NO: 70**.

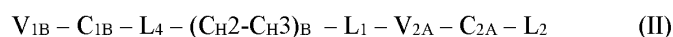
10 In some embodiments, some of L_1 , L_2 , L_3 and L_4 may be identical or different, and may comprise all or part of an amino acid sequence selected from **SEQ ID NO: 74 to SEQ ID NO: 79**; for example, one or more than four consecutive amino acids of an amino acid sequence selected from **SEQ ID NO: 74 to SEQ ID NO: 79**.

15 According to some particular embodiments, some of L_1 , L_2 , L_3 and L_4 may be identical or different, and may comprise all or part of an immunoglobulin hinge region, such as one selected from amino acid sequences **SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 78** and/or **SEQ ID NO: 79**; for example four or more than four consecutive amino acids of an immunoglobulin hinge region, such as one selected from amino acid
20 sequences **SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 78** and/or **SEQ ID NO: 79**.

 According to some more particular embodiments, L_2 , L_3 and L_4 may be identical or different, and may comprise all or part of an immunoglobulin hinge region, such as one selected from sequences **SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 78** and/or **SEQ ID NO: 79**; for example, four or more than four consecutive amino acids of
25 an immunoglobulin hinge region, such as one selected from sequences **SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 78** and/or **SEQ ID NO: 79**.

 According to some more particular embodiments, L_2 , L_3 and L_4 may be identical or different, and may comprise all or part of an immunoglobulin hinge region, such as one
30 selected from sequences **SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 78** and/or **SEQ ID NO: 79** (for example four or more than four consecutive amino acids of an immunoglobulin hinge region, such as one selected from sequences **SEQ ID NO: 74, SEQ ID NO: 75, SEQ ID NO: 77, SEQ ID NO: 78** and/or **SEQ ID NO: 79**), and L_1 may comprise all or part of the linker which corresponds to the amino acid sequence of **SEQ ID NO: 76**.
35

5 According to some particular embodiments, the binding protein is characterized in that it comprises three polypeptide chains (I), (II) and (III) that form two ABDs, as defined below:



wherein:

V_{1A} and V_{1B} form a binding pair V_1 (V_{H1}/V_{L1}), which binds specifically to a human CD123 polypeptide;

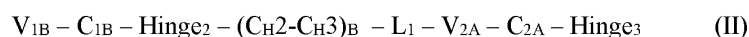
15 V_{2A} and V_{2B} form a binding pair V_2 (V_{H2}/V_{L2}), which binds specifically to a human NKp46 polypeptide;

C_{1A} and C_{1B} form a pair C_1 (C_{H1}/C_L) and C_{2A} and C_{2B} form a pair C_2 (C_{H1}/C_L) wherein C_{H1} is an immunoglobulin heavy chain constant domain 1 and C_L is an immunoglobulin light chain constant domain;

20 $(CH_2-CH_3)_A$ and $(CH_2-CH_3)_B$ are identical or different, and comprise an immunoglobulin heavy chain constant domain 2 (CH_2) and an immunoglobulin heavy chain constant domain 3 (CH_3);

L_1, L_2, L_3, L_4 are optional independent amino acid linkers, which may be identical or different.

25 In one embodiment, the binding protein is characterized in that it comprises three polypeptide chains (I), (II) and (III) that form two ABDs, as defined below:



wherein :

30 V_{1A} and V_{1B} form a binding pair V_1 (V_{H1}/V_{L1});

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

C_{1A} and C_{1B} form a pair C_1 (C_{H1}/C_L) and C_{2A} and C_{2B} form a pair C_2 (C_{H1}/C_L) wherein C_{H1} is an immunoglobulin heavy chain constant domain 1 and C_L is an immunoglobulin light chain constant domain;

35 $\text{Hinge}_1, \text{Hinge}_2$ and Hinge_3 are identical or different and correspond to all or part of an immunoglobulin hinge region;

5 (C_{H2}-C_{H3})_A and (C_{H2}-C_{H3})_B are identical or different, and comprise an immunoglobulin heavy chain constant domain 2 (C_{H2}) and an immunoglobulin heavy chain constant domain 3 (C_{H3});

L₁ is an amino acid linker.

In one embodiment, the binding protein is characterized in that it comprises three
10 polypeptide chains (I), (II) and (III) that form two ABDs, as defined below:

$$V_{1A} - C_{1A} - \text{Hinge}_1 - (C_{H2}-C_{H3})_A \quad (I)$$

$$V_{1B} - C_{1B} - \text{Hinge}_2 - (C_{H2}-C_{H3})_B - L_1 - V_{2A} - C_{2A} - \text{Hinge}_3 \quad (II)$$

$$V_{2B} - C_{2B} \quad (III)$$

wherein :

15 V_{1A} and V_{1B} form a binding pair V₁ (V_{H1}/V_{L1}) which binds specifically to a human CD123 polypeptide;

V_{2A} and V_{2B} form a binding pair V₂ (V_{H2}/V_{L2}) which binds specifically to a human NKp46 polypeptide;

C_{1A} and C_{1B} form a pair C₁ (C_{H1}/C_L) and C_{2A} and C_{2B} form a pair C₂ (C_{H1}/C_L) wherein
20 C_{H1} 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;

(C_{H2}-C_{H3})_A and (C_{H2}-C_{H3})_B are identical or different, and comprise an
25 immunoglobulin heavy chain constant domain 2 (C_{H2}) and an immunoglobulin heavy chain constant domain 3 (C_{H3});

L₁ is an amino acid linker.

In some embodiments of the binding protein, the polypeptide chains (I), (II) and (III) are characterized in that:

30 C_{1A} comprises a C_L domain;

C_{1B} comprises a C_{H1} domain;

C_{2A} comprises a C_{H1} domain;

C_{2B} comprises a C_L domain.

In some embodiments of the binding protein, the polypeptide chains (I), (II) and
35 (III) are characterized in that:

C_{1A} comprises a C_{H1} domain;

- 5 C_{1B} comprises a C_L domain;
 C_{2A} comprises a C_L domain;
 C_{2B} comprises a C_{H1} domain.

 According to some of those particular embodiments of the binding protein, the polypeptide chains (I), (II) and (III) are characterized in that:

- 10 C_{1A} comprises a C_{H1} domain;
 C_{1B} comprises a C_L domain;
 C_{2A} comprises a C_{H1} domain;
 C_{2B} comprises a C_L domain.

- According to some of those particular embodiments of the binding protein, the
15 polypeptide chains (I), (II) and (III) are characterized in that:

- C_{1A} comprises a C_L domain;
 C_{1B} comprises a C_{H1} domain;
 C_{2A} comprises a C_L domain;
 C_{2B} comprises a C_{H1} domain.

- 20 In some embodiments, C_L and C_{H1} domains which form C_{1A}, C_{1B}, C_{2A}, and C_{2B} may be identical or different. Hence in some embodiments of the binding protein, the polypeptide chains (I), (II) and (III) are characterized in that:

- C_{1A} and C_{2A} are identical and comprise a C_L domain; **or**
 - C_{1A} and C_{2B} are identical and comprise a C_L domain; **or**
25 - C_{1B} and C_{2A} are identical and comprise a C_L domain; **or**
 - C_{1B} and C_{2B} are identical and comprise a C_L domain.

 In some embodiments of the binding protein, the polypeptide chains (I), (II) and (III) are characterized in that:

- 30 - C_{1A} and C_{2A} are identical and comprise a C_{H1} domain; **or**
 - C_{1A} and C_{2B} are identical and comprise a C_{H1} domain; **or**
 - C_{1B} and C_{2A} are identical and comprise a C_{H1} domain; **or**
 - C_{1B} and C_{2B} are identical and comprise a C_{H1} domain.

- In some embodiments of polypeptide chains (I), (II) and (III): V_{1A} is a V_H and V_{1B}
35 is a V_L.

5 In some embodiments of polypeptide chains (I), (II) and (III): V_{1A} is a V_L and V_{1B} is a V_H .

In some embodiments of polypeptide chains (I), (II) and (III): V_{2A} is a V_H and V_{2B} is a V_L .

10 In some embodiments of polypeptide chains (I), (II) and (III): V_{2A} is a V_L and V_{2B} is a V_H .

In some embodiments of polypeptide chains (I), (II) and (III): V_{1A} is a V_H and V_{1B} is a V_L ; **and** V_{2A} is a V_H and V_{2B} is a V_L .

In some embodiments of polypeptide chains (I), (II) and (III): V_{1A} is a V_L and V_{1B} is a V_H ; **and** V_{2A} is a V_H and V_{2B} is a V_L .

15 In some embodiments of polypeptide chains (I), (II) and (III): V_{1A} is a V_H and V_{1B} is a V_L ; **and** V_{2A} is a V_L and V_{2B} is a V_H .

In some embodiments of polypeptide chains (I), (II) and (III): V_{1A} is a V_L and V_{1B} is a V_H ; **and** V_{2A} is a V_L and V_{2B} is a V_H .

20 In some embodiment of the binding protein, V_{1A} is V_{L1} and V_{1B} is V_{H1} ; **and** V_{2A} is V_{H2} and V_{2B} is V_{L2} .

In some embodiment of the binding protein, V_{1A} is V_{L1} and V_{1B} is V_{H1} .

In some embodiment of the binding protein, V_{2A} is V_{H2} and V_{2B} is V_{L2} .

In some embodiment of the binding protein, the polypeptide chains (I), (II) and (III) are characterized in that:

25 C_{1B} is an immunoglobulin heavy chain constant domain 1 (C_{H1});

C_{2A} is an immunoglobulin heavy chain constant domain 1 (C_{H1});

C_L corresponds to an immunoglobulin kappa light chain constant domain (C_k);

$(C_{H2}-C_{H3})_A$ corresponds to the amino acid sequence of **SEQ ID NO: 69**;

$(C_{H2}-C_{H3})_B$ corresponds to the amino acid sequence of **SEQ ID NO: 70**;

30 L_2 or Hinge₁ corresponds to the amino acid sequence of **SEQ ID NO: 74**;

L_3 or Hinge₂ corresponds to the amino acid sequence of **SEQ ID NO: 75**;

L_4 or Hinge₃ corresponds to the amino acid sequence of **SEQ ID NO: 77**.

L_1 corresponds to the amino acid sequence of **SEQ ID NO: 76**.

35 In some embodiment of the binding protein, the polypeptide chains (I), (II) and (III) are characterized in that:

C_{1B} is an immunoglobulin heavy chain constant domain 1 (C_{H1});

- 5 C_{2A} is an immunoglobulin heavy chain constant domain 1 (C_{H1});
 C_L corresponds to an immunoglobulin kappa light chain constant domain (C_{κ});
 $(C_{H2}-C_{H3})_A$ corresponds to the amino acid sequence of **SEQ ID NO: 69** ;
 $(C_{H2}-C_{H3})_B$ corresponds to the amino acid sequence of **SEQ ID NO: 70**;
 Hinge₁ corresponds to the amino acid sequence of **SEQ ID NO: 74**;
 10 Hinge₂ corresponds to the amino acid sequence of **SEQ ID NO: 75**;
 Hinge₃ corresponds to the amino acid sequence of **SEQ ID NO: 77**;
 L_1 corresponds to the amino acid sequence of **SEQ ID NO: 76**.

In some embodiment of the binding protein:

- (a) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 1**;
 15 a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 2**; a CDR-H3 comprising
 the amino acid sequence of **SEQ ID NO: 3**; V_{L1} comprises a CDR-L1 comprising the amino
 acid sequence of **SEQ ID NO: 7**; a CDR-L2 comprising the amino acid sequence of **SEQ**
 ID NO: 8; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 9**; V_{H2} comprises
 a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 13**; a CDR-H2 comprising
 20 the amino acid sequence of **SEQ ID NO: 14**; a CDR-H3 comprising the amino acid sequence
 of **SEQ ID NO: 15**; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of **SEQ**
 ID NO: 27; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 28**; a CDR-L3
 comprising the amino acid sequence of **SEQ ID NO: 29**;
- (b) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 1**;
 25 a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 2**; a CDR-H3 comprising
 the amino acid sequence of **SEQ ID NO: 3**; V_{L1} comprises a CDR-L1 comprising the amino
 acid sequence of **SEQ ID NO: 7**; a CDR-L2 comprising the amino acid sequence of **SEQ**
 ID NO: 8; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 9**; V_{H2} comprises
 a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 16**; a CDR-H2 comprising
 30 the amino acid sequence of **SEQ ID NO: 17**; a CDR-H3 comprising the amino acid sequence
 of **SEQ ID NO: 18**; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of **SEQ**
 ID NO: 30; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 31**; a CDR-L3
 comprising the amino acid sequence of **SEQ ID NO: 32**;
- (c) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 1**;
 35 a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 2**; a CDR-H3 comprising
 the amino acid sequence of **SEQ ID NO: 3**; V_{L1} comprises a CDR-L1 comprising the amino

5 acid sequence of **SEQ ID NO: 7**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 8**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 9**; **V_{H2}** comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 19**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 20**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 21**; **V_{L2}** comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 33**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 34**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 35**;

(d) **V_{H1}** comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 1**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 2**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 3**; **V_{L1}** comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 7**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 8**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 9**; **V_{H2}** comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 22**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 23**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 24**; **V_{L2}** comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 36**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 37**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 38**;

(e) **V_{H1}** comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 1**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 2**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 3**; **V_{L1}** comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 7**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 8**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 9**; **V_{H2}** comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 16**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 25**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 26**; **V_{L2}** comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 39**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 31**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 40**;

(f) **V_{H1}** comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 4**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 5**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 6**; **V_{L1}** comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 10**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 11**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 12**; **V_{H2}**

- 5 comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 13**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 14**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 15**; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 27**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 28**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 29**;
- 10 (g) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 4**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 5**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 6**; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 10**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 11**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 12**; V_{H2}
- 15 comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 16**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 17**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 18**; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 30**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 31**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 32**;
- 20 (h) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 4**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 5**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 6**; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 10**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 11**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 12**; V_{H2}
- 25 comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 19**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 20**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 21**; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 33**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 34**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 35**;
- 30 (i) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 4**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 5**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 6**; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 10**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 11**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 12**; V_{H2}
- 35 comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 22**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 23**; a CDR-H3 comprising the amino

5 acid sequence of **SEQ ID NO: 24**; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 36**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 37**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 38**;

(j) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 4**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 5**; a CDR-H3 comprising
10 the amino acid sequence of **SEQ ID NO: 6**; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 10**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 11**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 12**; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 16**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 25**; a CDR-H3 comprising the amino
15 acid sequence of **SEQ ID NO: 26**; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 39**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 31**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 40**.

In some embodiments of the binding protein:

(a) V_{H1} and V_{L1} corresponds to the amino acid sequences of **SEQ ID NO: 41** and **43**
20 respectively or corresponds to the amino acid sequences of **SEQ ID NO: 42** and **44** respectively;
and/or

(b) V_{H2} and V_{L2} corresponds to
the amino acid sequences of **SEQ ID NO: 45** and **53** respectively;
25 the amino acid sequences of **SEQ ID NO: 46** and **54** respectively;
the amino acid sequences of **SEQ ID NO: 47** and **55** respectively;
the amino acid sequences of **SEQ ID NO: 48** and **56** respectively;
the amino acid sequences of **SEQ ID NO: 49** and **57** respectively;
the amino acid sequences of **SEQ ID NO: 50** and **58** respectively;
30 the amino acid sequences of **SEQ ID NO: 51** and **59** respectively; or
the amino acid sequences of **SEQ ID NO: 52** and **60** respectively.

In some embodiments of the binding protein:

(a) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 41**; V_{L1} comprises the
35 amino acid sequence of **SEQ ID NO: 43**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 45**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 53**;

- 5 (b) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 41**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 43**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 46**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 54**;
- (c) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 41**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 43**; V_{H2} comprises the amino acid sequence of **SEQ**
- 10 **ID NO: 47**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 55**;
- (d) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 41**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 43**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 48**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 56**;
- (e) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 41**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 43**; V_{H2} comprises the amino acid sequence of **SEQ**
- 15 **ID NO: 49**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 57**;
- (f) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 41**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 43**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 50**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 58**;
- 20 (g) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 41**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 43**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 51**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 59**;
- (h) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 41**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 43**; V_{H2} comprises the amino acid sequence of **SEQ**
- 25 **ID NO: 52**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 60**;
- (i) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 42**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 44**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 45**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 53**;
- (j) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 42**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 44**; V_{H2} comprises the amino acid sequence of **SEQ**
- 30 **ID NO: 46**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 54**;
- (k) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 42**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 44**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 47**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 55**;

- 5 (l) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 42**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 44**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 48**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 56**;
- (m) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 42**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 44**; V_{H2} comprises the amino acid sequence of **SEQ**
- 10 **ID NO: 49**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 57**;
- (n) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 42**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 44**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 50**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 58**.
- (o) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 42**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 44**; V_{H2} comprises the amino acid sequence of **SEQ**
- 15 **ID NO: 51**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 59**;
- (p) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 42**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 44**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 52**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 60**.

20

In some embodiments, the binding protein comprises at least two polypeptide chains linked by at least one disulfide bridge.

In some embodiments of the binding protein, the polypeptide chains (I), (II) and (III) are characterized in that: polypeptide chain (I) is covalently linked to polypeptide chain (II), in particular covalently linked to polypeptide (II) by one or more disulfide bonds.

25

According to some of those particular embodiments of the binding protein, the polypeptide chains (I), (II) and (III) are characterized in that: polypeptide chain (II) is covalently linked to polypeptide chain (III), by one or more disulfide bonds.

In some embodiments, the polypeptide chains (I) and (II) are linked by at least one disulfide bridge between C_{1A} and $Hinge_2$ and/or wherein the polypeptide chains (II) and (III) are linked by at least one disulfide bridge between $Hinge_3$ and C_{2B} .

30

In some embodiments, the binding protein is characterized in that the Fc region or variant thereof (e.g. $(CH_2-CH_3)_A$ or $(CH_2-CH_3)_B$ or $Hinge_1 - (CH_2-CH_3)_A$ or $Hinge_2 - (CH_2-CH_3)_B$) which binds to a human Fc- γ receptor polypeptide, comprises a CH_2 heavy chain constant domain with a N-linked glycosylation at residue N297 according to EU numbering.

35

5 In some embodiments, the binding protein is characterized in that the residue N297 of the Fc region or variant thereof according to EU numbering comprises a N-linked glycosylation.

 In some embodiments, the binding protein is characterized in that all or part of the Fc region or variant thereof binds to a human Fc- γ receptor polypeptide. In some
10 embodiments, the binding protein is characterized in that all or part of the Fc region or variant thereof binds to a human CD16A (Fc γ RIII) polypeptide.

 In one embodiment, the binding protein comprises:

 - a polypeptide comprising an amino acid sequence of **SEQ ID NO: 61**, a
15 polypeptide comprising an amino acid sequence of **SEQ ID NO: 62** and a polypeptide comprising an amino acid sequence of **SEQ ID NO: 63**, or a variant thereof with at least 80% of sequence identity; **or**

 - a polypeptide comprising an amino acid sequence of **SEQ ID NO: 64**, a
polypeptide comprising an amino acid sequence of **SEQ ID NO: 65** and a polypeptide
20 comprising an amino acid sequence of **SEQ ID NO: 66** or a variant thereof with at least 80% of sequence identity; **and/or**

 - a polypeptide comprising an amino acid sequence of **SEQ ID NO: 61** or **64**,
a polypeptide comprising an amino acid sequence of **SEQ ID NO: 62** or **65** and a
polypeptide comprising an amino acid sequence of **SEQ ID NO: 63** or **66**, or a variant
25 thereof with at least 80% of sequence identity.

 In some embodiments, the binding protein comprises:

 - a polypeptide comprising an amino acid sequence **SEQ ID NO: 61**, a
polypeptide comprising an amino acid sequence **SEQ ID NO: 62** and a polypeptide
30 comprising an amino acid sequence **SEQ ID NO: 63** or a variant thereof with at least 80% of sequence identity; **or**

 - a polypeptide comprising an amino acid sequence of **SEQ ID NO: 64**, a
polypeptide comprising an amino acid sequence of **SEQ ID NO: 65** and a polypeptide
comprising an amino acid sequence of **SEQ ID NO: 66** or a variant thereof with at least
35 80% of sequence identity.

5

In some embodiments, the binding protein comprises: a polypeptide comprising an amino acid sequence of **SEQ ID NO: 61**, a polypeptide comprising a sequence **SEQ ID NO: 62** and a polypeptide comprising an amino acid sequence of **SEQ ID NO: 63** or a variant thereof with at least 80% of sequence identity.

10

In some embodiments, the binding protein comprises: a polypeptide comprising an amino acid sequence of **SEQ ID NO: 64**, a polypeptide comprising an amino acid sequence of **SEQ ID NO: 65** and a polypeptide comprising an amino acid sequence of **SEQ ID NO: 66** or a variant thereof with at least 80% of sequence identity.

15

In some embodiments, the binding protein comprises: a polypeptide comprising an amino acid sequence of **SEQ ID NO: 61**, a polypeptide comprising an amino acid sequence of **SEQ ID NO: 62** and a polypeptide comprising an amino acid sequence of **SEQ ID NO: 63** or a variant thereof with at least 90% of sequence identity.

20

In some embodiments, the binding protein comprises: a polypeptide comprising an amino acid sequence of **SEQ ID NO: 64**, a polypeptide comprising a amino acid sequence of **SEQ ID NO: 65** and a polypeptide comprising an amino acid sequence of **SEQ ID NO: 66** or a variant thereof with at least 90% of sequence identity.

25

In some embodiments, the binding protein comprises: a polypeptide comprising an amino acid sequence of **SEQ ID NO: 61**, a polypeptide comprising an amino acid sequence of **SEQ ID NO: 62** and a polypeptide comprising an amino acid sequence of **SEQ ID NO: 63** or a variant thereof with at least 95% of sequence identity.

30

In some embodiments, the binding protein comprises: a polypeptide comprising an amino acid sequence of **SEQ ID NO: 64**, a polypeptide comprising a amino acid sequence of **SEQ ID NO: 65** and a polypeptide comprising an amino acid sequence of **SEQ ID NO: 66** or a variant thereof with at least 95% of sequence identity.

35

In some embodiments, the binding protein comprises: a polypeptide (I) comprising an amino acid sequence of **SEQ ID NO: 61**, a polypeptide (II) comprising an amino acid sequence of **SEQ ID NO: 62** and a polypeptide (III) comprising an amino acid sequence of **SEQ ID NO: 63**.

- In some embodiments, the binding protein comprises:
- polypeptide (I) consisting of an amino acid sequence of **SEQ ID NO: 61**;

- 5 - polypeptide (II) consisting of an amino acid sequence of **SEQ ID NO: 62**; and
 - polypeptide (III) consisting of an amino acid sequence of **SEQ ID NO: 63**.

 In some embodiments, the binding protein comprises: a polypeptide (I) comprising an amino acid sequence of **SEQ ID NO: 64**, a polypeptide (II) comprising an
10 amino acid sequence of **SEQ ID NO: 65** and a polypeptide (III) comprising an amino acid sequence of **SEQ ID NO: 66**.

 In some embodiments, the binding protein comprises:

- polypeptide (I) consisting of an amino acid sequence of **SEQ ID NO: 64**;
 - polypeptide (II) consisting of an amino acid sequence of **SEQ ID NO: 65**; and
15 - polypeptide (III) consisting of an amino acid sequence of **SEQ ID NO: 66**.

 In some variants of those embodiments, the binding protein comprises polypeptide sequences derived from immunoglobulins chains (in particular immunoglobulins of the IgG type), and/or amino acid sequences selected from any one of **SEQ ID NO: 1 to SEQ ID**
20 **NO: 79**, which may thus include any variant sequence with conservative substitutions, and/or any variant with a degree of percent sequence identity with a reference sequence; especially a reference sequence derived from an immunoglobulin chain.

 In some embodiments, the binding protein comprises polypeptide sequences derived from immunoglobulins chains of the IgG type, in particular of the IgG1, IgG2, IgG3
25 or IgG4 type, preferably of the IgG1 type.

 When variants of Fc and constant regions and non-CDRs polypeptide sequences from a variable region are considered herein, they may consist of Fc and constant regions and non-CDRs polypeptide sequences having at least 80% of sequence identity with a reference polypeptide sequence; more particularly having at least 90% of sequence identity
30 with a reference polypeptide sequence; and preferably having at least 95% of sequence identity with a reference polypeptide sequence.

 Alternatively, when variants of polypeptide sequences include CDR polypeptide sequences (e.g., CDR1, CDR2, and CDR3 from either one of a VH or VL domain), it will be understood herein that those variants do not have modifications on their CDR polypeptide
35 sequences.

5 In some embodiments, the binding protein comprises an amino acid sequence having at least 80% of sequence identity with an amino acid sequence selected from **SEQ ID NO: 67 to 73**.

 In some embodiments, the binding protein comprises a amino acid sequence having at least 90% of sequence identity with an amino acid sequence selected from **SEQ**
10 **ID NO: 67 to 73**.

 In some embodiments, the binding protein comprises an amino acid sequence having at least 95% of sequence identity with an amino acid sequence selected from **SEQ**
ID NO: 67 to 73.

 In some embodiments, the binding protein comprises a Fc region or variant thereof
15 having at least 80% of sequence identity with an amino acid sequence selected from **SEQ**
ID NO: 69 to 73.

 In some embodiments, the binding protein comprises a Fc region or variant thereof having at least 90% of sequence identity with an amino acid sequence selected from **SEQ**
ID NO: 69 to 73.

20 In some embodiments, the binding protein comprises a Fc region or variant thereof having at least 95% of sequence identity with an amino acid s sequence selected from **SEQ ID NO: 69 to 73**.

 In some embodiments, the binding protein comprises a Fc region or variant thereof with a CH_2 - CH_3 domain having at least 80% of sequence identity with an amino acid
25 sequence selected from **SEQ ID NO: 69 or 70**; or alternatively comprises a Fc region or variant thereof with a CH_2 domain having at least 80% of sequence identity with an amino acid sequence of **SEQ ID NO: 71**; or alternatively comprises a Fc region or variant thereof with a CH_3 domain having at least 80% of sequence identity with an amino acid sequence of **SEQ ID NO: 72 or 73**.

30 In some embodiments, the binding protein comprises a Fc region or variant thereof with a CH_2 - CH_3 domain having at least 90% of sequence identity with an amino acid sequence selected from **SEQ ID NO: 69 or 70**; or alternatively comprises a Fc region or variant thereof with a CH_2 domain having at least 90% of sequence identity with an amino acid sequence **SEQ ID NO: 71**; or alternatively comprises a Fc region or variant thereof
35 with a CH_3 domain having at least 90% of sequence identity with an amino acid sequence of **SEQ ID NO: 72 or 73**.

5 In some embodiments, the binding protein comprises a Fc region or variant thereof with a C_{H2}-C_{H3} domain having at least 95% of sequence identity with an amino acid sequence selected from **SEQ ID NO: 69 or 70**; or alternatively comprises a Fc region or variant thereof with a C_{H2} domain having at least 95% of sequence identity with an amino acid sequence of **SEQ ID NO: 71**; or alternatively comprises a Fc region or variant thereof
10 with a C_{H3} domain having at least 95% of sequence identity with an amino acid sequence of **SEQ ID NO: 72 or 73**.

 Preferably, the multispecific binding proteins of the present disclosure are bispecific binding proteins.

15

 The disclosure further relates to a pharmaceutical composition comprising a binding protein as defined above, and a pharmaceutically acceptable carrier.

 Hence, in one embodiment, the disclosure relates to a pharmaceutical composition
20 comprising a binding protein, and a pharmaceutically acceptable carrier, said binding protein comprising:

 (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, (ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46
25 polypeptide, and (iii) all or part of an immunoglobulin fragment crystallizable (Fc) region or variant thereof which binds to a human Fc- γ receptor polypeptide.

 Hence, in one embodiment, the disclosure relates to a pharmaceutical composition comprising the binding protein defined above, and a pharmaceutically acceptable carrier,
30 said binding protein comprising a first and a second antigen binding domains (ABDs) and all or part of an immunoglobulin Fc region or variant thereof, wherein each of said ABDs comprises an immunoglobulin heavy chain variable domain (V_H) and an immunoglobulin light chain variable domain (V_L), wherein each V_H and V_L comprises three complementary determining regions (CDR-1 to CDR-3); and wherein:

35 (i) the first ABD binds specifically to human CD123 and comprises:

- 5 - a V_{H1} comprising a CDR-H1, H2 and H3 corresponding to the amino acid sequences of SEQ ID NO:1 to 3 respectively or corresponding to the amino acid sequences of SEQ ID NO: 4 to 6 respectively, and
- a V_{L1} comprising a CDR-L1, L2 and L3 corresponding to the amino acid sequences of SEQ ID NO: 7 to 9 respectively or corresponding to the amino acid sequences of SEQ ID NO: 10 to 12 respectively;
- 10 (ii) the second ABD binds specifically to human NKp46 and comprises:
- a V_{H2} comprising a CDR-H1, 2 and 3 corresponding to:
- the amino acid sequences of SEQ ID NO:13 to 15 respectively;
- the amino acid sequences of SEQ ID NO:16 to 18 respectively;
- 15 the amino acid sequences of SEQ ID NO:19 to 21 respectively;
- the amino acid sequences of SEQ ID NO:22 to 24 respectively; or
- the amino acid sequences of SEQ ID NO:16, 25 and 26 respectively;
- and
- a V_{L2} comprising a CDR-L1, 2 and 3 corresponding to:
- 20 the amino acid sequences of of SEQ ID NO:27 to 29 respectively;
- the amino acid sequences of SEQ ID NO:30 to 32 respectively;
- the amino acid sequences of SEQ ID NO:33 to 35 respectively;
- the amino acid sequences of SEQ ID NO:36 to 38 respectively; or
- the amino acid sequences of SEQ ID NO:39, 31 and 40 respectively;
- 25 and wherein all or part of the immunoglobulin Fc region or variant thereof to a human Fc-γ receptor

 Preferably, the binding protein according to the disclosure, and pharmaceutical compositions thereof, are sterile and suitable for parenteral use.

30

III. Medical applications

 The disclosed binding protein, and compositions thereof, are particularly suitable for use as a medicament. Methods and uses for the preparation of such medicament are further disclosed herein.

35 Hence, in one embodiment, the disclosure relates to a binding protein comprising:

5 (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, (ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide, and (iii) all or part of an immunoglobulin fragment crystallizable (Fc) region which binds to a human Fc- γ receptor polypeptide; for use as a medicament.

10

According to some particular embodiments of this third general object, the disclosure relates to a binding protein comprising:

(i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, (ii) a second antigen-binding domain
15 (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide, and (iii) all or part of an immunoglobulin fragment crystallizable (Fc) region which binds to a human Fc- γ receptor polypeptide; for use in a method for the treatment or prevention of cancer.

20 According to some particular embodiments of this third general object, the disclosure relates to a binding protein comprising:

(i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, (ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide, and (iii) all or part of an immunoglobulin fragment crystallizable (Fc) region
25 which binds to a human Fc- γ receptor polypeptide; for use in a method for the treatment or prevention of blood cancer.

According to some particular embodiments of this third general object, the disclosure relates to a binding protein comprising:

30 (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, (ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide, and (iii) all or part of an immunoglobulin fragment crystallizable (Fc) region which binds to a human Fc- γ receptor polypeptide; for use in a method for the treatment or
35 prevention of a myelodysplastic syndrome (MDS) or of a lymphoproliferative disorder.

5 According to some particular embodiments of this third general object, the disclosure relates to a binding protein comprising:

 (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, (ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46
10 polypeptide, and (iii) all or part of an immunoglobulin fragment crystallizable (Fc) region which binds to a human Fc- γ receptor polypeptide; for use in a method for the treatment or prevention of Acute Myeloid Leukemia (AML).

 According to some particular embodiments of this third general object, the disclosure relates to a binding protein comprising:

15 (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, (ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide, and (iii) all or part of an immunoglobulin fragment crystallizable (Fc) region which binds to a human Fc- γ receptor polypeptide; for use in a method for the treatment or
20 prevention of CD64-positive and CD64-negative Acute Myeloid Leukemia (AML).

 In some embodiment, the disclosure relates to a binding protein comprising a first and a second antigen binding domains (ABDs) and all or part of an immunoglobulin Fc region or variant thereof, wherein each of said ABDs comprises an immunoglobulin heavy
25 chain variable domain (V_H) and an immunoglobulin light chain variable domain (V_L), wherein each V_H and V_L comprises three complementary determining regions (CDR-1 to CDR-3); and wherein:

 (i) the first ABD binds specifically to human CD123 and comprises:
 - a V_{H1} comprising a CDR-H1, H2 and H3 corresponding to the amino acid sequences
30 of **SEQ ID NO: 1 to 3** respectively or corresponding to the amino acid sequences of **SEQ ID NO: 4 to 6** respectively, and
 - a V_{L1} comprising a CDR-L1, L2 and L3 corresponding to the amino acid sequences of **SEQ ID NO: 7 to 9** respectively or corresponding to the amino acid sequences of **SEQ ID NO: 10 to 12** respectively;
35 (ii) the second ABD binds specifically to human NKp46 and comprises:
 - a V_{H2} comprising a CDR-H1, 2 and 3 corresponding to:

5 the amino acid sequences of SEQ ID NO:13 to 15 respectively;
the amino acid sequences of SEQ ID NO:16 to 18 respectively;
the amino acid sequences of SEQ ID NO:19 to 21 respectively;
the amino acid sequences of SEQ ID NO:22 to 24 respectively; or
the amino acid sequences of SEQ ID NO:16, 25 and 26 respectively;
10 and
- a V_{L2} comprising a CDR-L1, 2 and 3 corresponding to:
the amino acid sequences of SEQ ID NO:27 to 29 respectively;
the amino acid sequences of SEQ ID NO:30 to 32 respectively;
the amino acid sequences of SEQ ID NO:33 to 35 respectively;
15 the amino acid sequences of SEQ ID NO:36 to 38 respectively; or
the amino acid sequences of SEQ ID NO:39, 31 and 40 respectively;
and wherein all or part of the immunoglobulin Fc region or variant thereof binds
to a human Fc-γ receptor; for use as a medicament.

20 In some embodiment, the disclosure relates to a binding protein comprising a first
and a second antigen binding domains (ABDs) and all or part of an immunoglobulin Fc
region or variant thereof, wherein each of said ABDs comprises an immunoglobulin heavy
chain variable domain (V_H) and an immunoglobulin light chain variable domain (V_L),
wherein each V_H and V_L comprises three complementary determining regions (CDR-1 to
25 CDR-3); and wherein:

(i) the first ABD binds specifically to human CD123 and comprises:
- a V_{H1} comprising a CDR-H1, H2 and H3 corresponding to the amino acid sequences
of SEQ ID NO:1 to 3 respectively or corresponding to the amino acid sequences of SEQ
ID NO: 4 to 6 respectively, and
30 - a V_{L1} comprising a CDR-L1, L2 and L3 corresponding to the amino acid sequences
of SEQ ID NO: 7 to 9 respectively or corresponding to the amino acid sequences of SEQ
ID NO: 10 to 12 respectively;
(ii) the second ABD binds specifically to human NKp46 and comprises:
- a V_{H2} comprising a CDR-H1, 2 and 3 corresponding to:
35 the amino acid sequences of SEQ ID NO:13 to 15 respectively;
the amino acid sequences of SEQ ID NO:16 to 18 respectively;

- 5 the amino acid sequences of **SEQ ID NO:19** to **21** respectively;
the amino acid sequences of **SEQ ID NO:22** to **24** respectively; or
the amino acid sequences of **SEQ ID NO:16, 25** and **26** respectively;
and
- a V_{L2} comprising a CDR-L1, 2 and 3 corresponding to:
10 the amino acid sequences of **SEQ ID NO:27** to **29** respectively;
the amino acid sequences of **SEQ ID NO:30** to **32** respectively;
the amino acid sequences of **SEQ ID NO:33** to **35** respectively;
the amino acid sequences of **SEQ ID NO:36** to **38** respectively; or
the amino acid sequences of **SEQ ID NO:39, 31** and **40** respectively;
15 and wherein all or part of the immunoglobulin Fc region or variant thereof binds
to a human Fc- γ receptor; for use in a method for the treatment or prevention of cancer.

According to some particular embodiments of this third main object, the disclosure relates to a binding protein comprising a first and a second antigen binding
20 domains (ABDs) and all or part of an immunoglobulin Fc region or variant thereof, wherein
each of said ABDs comprises an immunoglobulin heavy chain variable domain (V_H) and an
immunoglobulin light chain variable domain (V_L), wherein each V_H and V_L comprises three
complementary determining regions (CDR-1 to CDR-3); and wherein:

- (i) the first ABD binds specifically to human CD123 and comprises:
25 - a V_{H1} comprising a CDR-H1, H2 and H3 corresponding to the amino acid sequences
SEQ ID NO:1 to **3** respectively or corresponding to the amino acid sequences of **SEQ ID**
NO: 4 to **6** respectively, and
- a V_{L1} comprising a CDR-L1, L2 and L3 corresponding to the amino acid sequences
of **SEQ ID NO: 7** to **9** respectively or corresponding to the amino acid sequences of **SEQ**
30 **ID NO: 10** to **12** respectively;
(ii) the second ABD binds specifically to human NKp46 and comprises:
- a V_{H2} comprising a CDR-H1, 2 and 3 corresponding to:
the amino acid sequences of **SEQ ID NO:13** to **15** respectively;
the amino acid sequences of **SEQ ID NO:16** to **18** respectively;
35 the amino acid sequences of **SEQ ID NO:19** to **21** respectively;
the amino acid sequences of **SEQ ID NO:22** to **24** respectively; or

5 the amino acid sequences of **SEQ ID NO:16, 25 and 26** respectively;
and
- a V_{L2} comprising a CDR-L1, 2 and 3 corresponding to:
the amino acid sequences of **SEQ ID NO:27 to 29** respectively;
the amino acid sequences of **SEQ ID NO:30 to 32** respectively;
10 the amino acid sequences of **SEQ ID NO:33 to 35** respectively;
the amino acid sequences of **SEQ ID NO:36 to 38** respectively; or
the amino acid sequences of **SEQ ID NO:39, 31 and 40** respectively;
and wherein all or part of the immunoglobulin Fc region or variant thereof binds
to a human Fc- γ receptor; for use in a method for the treatment or prevention of blood cancer.

15

According to some particular embodiments of this third main object, the disclosure relates to a binding protein comprising a first and a second antigen binding domains (ABDs) and all or part of an immunoglobulin Fc region or variant thereof, wherein each of said ABDs comprises an immunoglobulin heavy chain variable domain (V_H) and an
20 immunoglobulin light chain variable domain (V_L), wherein each V_H and V_L comprises three complementary determining regions (CDR-1 to CDR-3); and wherein:

(i) the first ABD binds specifically to human CD123 and comprises:
- a V_{H1} comprising a CDR-H1, H2 and H3 corresponding to the amino acid sequences of **SEQ ID NO:1 to 3** respectively or corresponding to the amino acid sequences of **SEQ**
25 **ID NO: 4 to 6** respectively, and
- a V_{L1} comprising a CDR-L1, L2 and L3 corresponding to the amino acid sequences of **SEQ ID NO: 7 to 9** respectively or corresponding to the amino acid sequences of **SEQ ID NO: 10 to 12** respectively;

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

30 - a V_{H2} comprising a CDR-H1, 2 and 3 corresponding to:
the amino acid sequences of **SEQ ID NO:13 to 15** respectively;
the amino acid sequences of **SEQ ID NO:16 to 18** respectively;
the amino acid sequences of **SEQ ID NO:19 to 21** respectively;
the amino acid sequences of **SEQ ID NO:22 to 24** respectively; or
35 the amino acid sequences of **SEQ ID NO:16, 25 and 26** respectively;
and

- 5 - a V_{L2} comprising a CDR-L1, 2 and 3 corresponding to:
the amino acid sequences of **SEQ ID NO:27** to **29** respectively;
the amino acid sequences of **SEQ ID NO:30** to **32** respectively;
the amino acid sequences of **SEQ ID NO:33** to **35** respectively;
the amino acid sequences of **SEQ ID NO:36** to **38** respectively; or
10 the amino acid sequences of **SEQ ID NO:39**, **31** and **40** respectively;
and wherein all or part of the immunoglobulin Fc region or variant thereof binds
to a human $Fc\text{-}\gamma$ receptor; for use in a method for the treatment or prevention of a
myelodysplastic syndrome (MDS) or of a lymphoproliferative disorder.
- 15 According to some particular embodiments of this third main object, the
disclosure relates to a binding protein comprising a first and a second antigen binding
domains (ABDs) and all or part of an immunoglobulin Fc region or variant thereof, wherein
each of said ABDs comprises an immunoglobulin heavy chain variable domain (V_H) and an
immunoglobulin light chain variable domain (V_L), wherein each V_H and V_L comprises three
20 complementary determining regions (CDR-1 to CDR-3); and wherein:
(i) the first ABD binds specifically to human CD123 and comprises:
- a V_{H1} comprising a CDR-H1, H2 and H3 corresponding to the amino acid sequences
of **SEQ ID NO:1** to **3** respectively or corresponding to the amino acid sequences of **SEQ**
ID NO: 4 to **6** respectively, and
25 - a V_{L1} comprising a CDR-L1, L2 and L3 corresponding to the amino acid sequences
SEQ ID NO: 7 to **9** respectively or corresponding to the amino acid sequences **SEQ ID NO:**
10 to **12** respectively;
(ii) the second ABD binds specifically to human NKp46 and comprises:
- a V_{H2} comprising a CDR-H1, 2 and 3 corresponding to:
30 the amino acid sequences of **SEQ ID NO:13** to **15** respectively;
the amino acid sequences of **SEQ ID NO:16** to **18** respectively;
the amino acid sequences of **SEQ ID NO:19** to **21** respectively;
the amino acid sequences of **SEQ ID NO:22** to **24** respectively; or
the amino acid sequences of **SEQ ID NO:16**, **25** and **26** respectively;
35 and
- a V_{L2} comprising a CDR-L1, 2 and 3 corresponding to:

- 5 the amino acid sequences of **SEQ ID NO:27** to **29** respectively;
the amino acid sequences of **SEQ ID NO:30** to **32** respectively;
the amino acid sequences of **SEQ ID NO:33** to **35** respectively;
the amino acid sequences of **SEQ ID NO:36** to **38** respectively; or
the amino acid sequences of **SEQ ID NO:39, 31** and **40** respectively;
- 10 and wherein all or part of the immunoglobulin Fc region or variant thereof binds to a human Fc- γ receptor; for use in a method for the treatment or prevention of Acute Myeloid Leukemia (AML).

According to some particular embodiments of this third main object, the disclosure relates to a binding protein comprising a first and a second antigen binding domains (ABDs) and all or part of an immunoglobulin Fc region or variant thereof, wherein each of said ABDs comprises an immunoglobulin heavy chain variable domain (V_H) and an immunoglobulin light chain variable domain (V_L), wherein each V_H and V_L comprises three complementary determining regions (CDR-1 to CDR-3); and wherein:

- 20 (i) the first ABD binds specifically to human CD123 and comprises:
- a V_{H1} comprising a CDR-H1, H2 and H3 corresponding to the amino acid sequences of **SEQ ID NO:1** to **3** respectively or corresponding to the amino acid sequences of **SEQ ID NO: 4** to **6** respectively, and
- a V_{L1} comprising a CDR-L1, L2 and L3 corresponding to the amino acid sequences of **SEQ ID NO: 7** to **9** respectively or corresponding to the amino acid sequences of **SEQ ID NO: 10** to **12** respectively;
- (ii) the second ABD binds specifically to human NKp46 and comprises:
- a V_{H2} comprising a CDR-H1, 2 and 3 corresponding to:
the amino acid sequences of **SEQ ID NO:13** to **15** respectively;
30 the amino acid sequences of **SEQ ID NO:16** to **18** respectively;
the amino acid sequences of **SEQ ID NO:19** to **21** respectively;
the amino acid sequences of **SEQ ID NO:22** to **24** respectively; or
the amino acid sequences of **SEQ ID NO:16, 25** and **26** respectively;
and
35 - a V_{L2} comprising a CDR-L1, 2 and 3 corresponding to:
the amino acid sequences of **SEQ ID NO:27** to **29** respectively;

5 the amino acid sequences of SEQ ID NO:30 to 32 respectively;
the amino acid sequences of SEQ ID NO:33 to 35 respectively;
the amino acid sequences of SEQ ID NO:36 to 38 respectively; or
the amino acid sequences of SEQ ID NO:39, 31 and 40 respectively;
and wherein all or part of the immunoglobulin Fc region or variant thereof binds
10 to a human Fc- γ receptor; for use in a method for the treatment or prevention of CD64-
positive and CD64-negative Acute Myeloid Leukemia (AML).

The disclosure further relates to a use of the above-mentioned binding proteins for
the preparation of a medicament.

15 The disclosure further relates to a use of the above-mentioned binding proteins as
a medicament.

The disclosure further relates to a use of the above-mentioned binding proteins for
the preparation of a medicament for the treatment or prevention of cancer.

20 The disclosure further relates to a use of the above-mentioned binding proteins for
the preparation of a medicament for the treatment or prevention of cancer characterized by
tumor cells that express CD123 at their surface.

The disclosure further relates to a use of the above-mentioned binding proteins for
the preparation of a medicament for the treatment or prevention of cancer characterized by
tumor cells that express CD123 and CD64 at their surface.

25 The disclosure further relates to a use of the above-mentioned binding proteins for
the preparation of a medicament for the treatment or prevention of blood cancer.

The disclosure further relates to a use of the above-mentioned binding proteins for
the preparation of a medicament for the treatment or prevention of blood cancer
characterized by tumor cells that express CD123 at their surface.

30 The disclosure further relates to a use of the above-mentioned binding proteins for
the preparation of a medicament for the treatment or prevention of blood cancer
characterized by tumor cells that express CD123 and CD64 at their surface.

35 The disclosure further relates to a use of the above-mentioned binding proteins for
the preparation of a medicament for the treatment or prevention of a myelodysplastic
syndrome (MDS) or of a lymphoproliferative disorder.

5 The disclosure further relates to a use of the above-mentioned binding proteins for the preparation of a medicament for the treatment or prevention of Acute Myeloid Leukemia (AML).

 The disclosure further relates to a use of the above-mentioned binding proteins for the preparation of a medicament for the treatment or prevention of CD64-positive and CD64-
10 negative Acute Myeloid Leukemia (AML).

 In one aspect, provided is a method for treating a cancer characterized by tumor cells that express CD123 and CD64 at their surface, the method comprising administering to and individual having such cancer a binding protein comprising: (i) a first antigen-binding
15 domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, (ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide, and (iii) all or part of an immunoglobulin Fc region or variant thereof which binds to a human Fc- γ receptor polypeptide.

20 In one aspect, provided is method for treating a CD123-expressing tumor (e.g. a hematological malignancy, AML) in an individual who is susceptible to having tumor cells that express CD64 at their surface, the method comprising administering to the individual a binding protein comprising: (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, (ii) a second antigen-binding
25 domain (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide, and (iii) all or part of an immunoglobulin Fc region or variant thereof which binds to a human Fc- γ receptor polypeptide.

 In one aspect, provided is method for treating a hematological malignancy (e.g. AML) in an individual, the method comprising administering to the individual a binding
30 protein comprising: (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, (ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide, and (iii) all or part of an immunoglobulin Fc region or variant thereof which binds to a human Fc- γ receptor polypeptide.

35 In another aspect, provided is a method of treating a hematological malignancy (e.g., AML) in an individual, the method comprising: (a) assessing or determining whether

5 malignant cells (e.g. AML cells) from the individual express CD64 at their surface; and (b) if the individual is determined to have malignant cells (e.g., AML cells) expressing CD64 at their surface (e.g., at a predetermined level), administering to the individual a binding protein comprising: (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, (ii) a second antigen-binding domain
10 (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide, and (iii) all or part of an immunoglobulin Fc region or variant thereof which binds to a human Fc- γ receptor polypeptide.

In another aspect, provided is a method for depleting malignant cells in an individual, and/or directing NK cell-mediated cytotoxicity toward CD64-expressing
15 malignant cells (e.g., an individual having AML), the method comprising administering, to an individual having malignant cells (e.g. AML cells) expressing CD64 at their surface, a binding protein comprising: (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, (ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46
20 polypeptide, and (iii) all or part of an immunoglobulin Fc region or variant thereof which binds to a human Fc- γ receptor polypeptide.

In another aspect, provided is a method of causing NK cells to eliminate malignant cells that express both CD123 and CD64, the method comprising bringing the malignant cells (e.g. AML cells) into contact, in the presence of NK cells, with a binding
25 protein comprising: (i) a first antigen-binding domain (ABD) comprising a variable region which binds to a human CD123 polypeptide, (ii) a second antigen-binding domain (ABD) comprising a variable region which binds to a human NKp46 polypeptide, and (iii) all or part of an Fc region or variant thereof which binds to a human Fc- γ receptor polypeptide.

Assessing the expression of CD64 by malignant cells (e.g., AML cells), e.g. at
30 their surface, can be carried out by any suitable method. Generally, a biological sample from an individual, for example from a blood sample or suitable biopsy, can be obtained and assessed and expression of CD64 in tumor cells can be determined using assays such as immunohistochemistry (IHC) assays, fluorescence activated cell sorting (FACS) assays, for example quantitative FACS, ELISA, immunoblotting (e.g., western blotting, dot blotting, or
35 in-cell western blotting), and other immunoassays. Anti-CD64 antibodies for use in such assays are available in the art.

5

IV. Means for making the binding protein

Means for making the binding protein of the present disclosure *in vitro* are further disclosed herein. As used herein, a “*binding protein of present disclosure*” refers to multifunctional binding proteins comprising a first and a second antigen binding domains (ABDs) and all or part of an immunoglobulin Fc region or variant thereof, wherein the first
10 (ABDs) and all or part of an immunoglobulin Fc region or variant thereof, wherein the first ABD binds specifically to human CD123 and the second ABD binds specifically to human NKp46 and wherein all or part of the immunoglobulin Fc region or variant thereof to a human Fc- γ receptor. It also refers to all particular embodiments of the binding protein which are described throughout the disclosure.

15 More particularly, the provided means may refer to the making of a binding protein comprising a first and a second antigen binding domains (ABDs) and all or part of an immunoglobulin Fc region or variant thereof, wherein each of said ABDs comprises an immunoglobulin heavy chain variable domain (VH) and an immunoglobulin light chain variable domain (VL), wherein each VH and VL comprises three complementary
20 determining regions (CDR-1 to CDR-3); and wherein:

(i) the first ABD binds specifically to human CD123 and comprises:

- a VH1 comprising a CDR-H1, H2 and H3 corresponding to the amino acid sequences of SEQ ID NO: 1 to 3 respectively or corresponding to the amino acid sequences of SEQ ID NO: 4 to 6 respectively, and

25 - a VL1 comprising a CDR-L1, L2 and L3 corresponding to the amino acid sequences of SEQ ID NO: 7 to 9 respectively or corresponding to the amino acid sequences of SEQ ID NO: 10 to 12 respectively;

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

- a VH2 comprising a CDR-H1, 2 and 3 corresponding to:

30 the amino acid sequences of SEQ ID NO:13 to 15 respectively;

the amino acid sequences of SEQ ID NO:16 to 18 respectively;

the amino acid sequences of SEQ ID NO:19 to 21 respectively;

the amino acid sequences of SEQ ID NO:22 to 24 respectively; or

the amino acid sequences of SEQ ID NO:16, 25 and 26 respectively;

35 and

- 5 - a VL2 comprising a CDR-L1, 2 and 3 corresponding to:
the amino acid sequences of SEQ ID NO:27 to 29 respectively;
the amino acid sequences of SEQ ID NO:30 to 32 respectively;
the amino acid sequences of SEQ ID NO:33 to 35 respectively;
the amino acid sequences of SEQ ID NO:36 to 38 respectively; or
10 the amino acid sequences SEQ ID NO:39, 31 and 40 respectively;
and wherein all or part of the immunoglobulin Fc region or variant thereof binds to a
human Fc- γ receptor.

Hence, in one embodiment, the disclosure relates to an isolated nucleic acid
15 molecule comprising a nucleotide sequence that encodes a binding protein of the present
disclosure.

Hence, in one embodiment, the disclosure relates to an expression vector
comprising a nucleic acid molecule comprising a nucleotide sequence that encodes the
binding protein of the present disclosure.

20 Hence, in one embodiment, the disclosure relates to an isolated cell comprising
the nucleic acid molecules of the present disclosure.

Hence, in one embodiment, the disclosure relates to an isolated cell comprising
the expression vector of the present disclosure.

25 According to a particular embodiment, the cell is an eukaryotic cell, in particular
an insect cell or a mammalian cell. In one embodiment, the cell is a mammalian cell and the
expression vector is a mammalian expression vector.

Hence, in one embodiment, the disclosure relates to a method for making the
binding protein of the present disclosure, comprising a step of making a binding protein
30 comprising (i) a first antigen-binding domain (ABD) comprising a variable region which
binds specifically to a human CD123 polypeptide, (ii) a second antigen-binding domain
(ABD) comprising a variable region which binds specifically to a human NKp46
polypeptide, and (iii) all or part of an immunoglobulin Fc region or variant thereof which
binds to a human Fc- γ receptor polypeptide.

35 According to some particular embodiments, the disclosure relates to a method for
making the binding protein, comprising a step of:

- 5 (a) culturing host cell(s) under conditions suitable for expressing **one or more** recombinant polypeptide(s) comprising (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, **and/or** (ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide, **and/or** (iii) all or part of an immunoglobulin
- 10 Fc region or variant thereof which binds to a human Fc- γ receptor polypeptide;
- (b) optionally recovering the expressed recombinant polypeptide(s).

According to some particular embodiments, the disclosure relates to a method for making the binding protein, comprising a step of:

- 15 (a) culturing host cell(s) under conditions suitable for expressing **one or more** recombinant polypeptide(s) comprising (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, **and** (ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide, **and** (iii) all or part of an immunoglobulin Fc region or variant
- 20 thereof which binds to a human Fc- γ receptor polypeptide;
- (b) optionally recovering the expressed recombinant polypeptide(s).

According to some particular embodiments, the disclosure relates to a method for making the binding protein, comprising a step of:

- 25 (a) culturing host cell(s) under conditions suitable for expressing **a plurality** of recombinant polypeptides, said plurality comprising (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, **and** (ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide, **and** (iii) all or part of an
- 30 immunoglobulin Fc region or variant thereof which binds to a human Fc- γ receptor polypeptide;
- (b) optionally recovering the expressed recombinant polypeptides.

- According to some particular embodiments, the disclosure relates to a method for
- 35 making the binding protein, comprising a step of:

- 5 (a) culturing host cell(s) under conditions suitable for expressing **a plurality** of recombinant polypeptides, said plurality comprising (i) a polypeptide comprising an amino acid sequence of **SEQ ID NO: 61 or 64, and** (ii) a polypeptide comprising an amino acid sequence of **SEQ ID NO: 62 or 65, and** (iii) a polypeptide comprising an amino acid sequence of **SEQ ID NO: 63 or 66;**
- 10 (b) optionally recovering the expressed recombinant polypeptides.

According to some particular embodiments, the disclosure relates to a method for making the binding protein, comprising a step of:

- 15 (a) culturing host cell(s) under conditions suitable for expressing **a plurality** of recombinant polypeptides, said plurality comprising (i) a polypeptide comprising an amino acid sequence of **SEQ ID NO: 64, and** (ii) a polypeptide comprising an amino acid sequence of **SEQ ID NO: 65, and** (iii) a polypeptide comprising an amino acid sequence of **SEQ ID NO: 66;**
- 20 (b) optionally recovering the expressed recombinant polypeptides.

According to some particular embodiments, the disclosure relates to a method for making the binding protein, comprising a step of:

- 25 (a) culturing host cell(s) under conditions suitable for **co-expressing a plurality** of recombinant polypeptides, said plurality comprising (i) a polypeptide comprising an amino acid sequence of **SEQ ID NO: 61 or 64, and** (ii) a polypeptide comprising an amino acid sequence of **SEQ ID NO: 62 or 65, and** (iii) a polypeptide comprising an amino acid sequence of **SEQ ID NO: 63 or 66;**
- 30 (b) optionally recovering the co-expressed recombinant polypeptides.

According to some particular embodiments, the disclosure relates to a method for making the binding protein, comprising a step of:

- 35 (a) culturing host cell(s) under conditions suitable for **co-expressing a plurality** of recombinant polypeptides, said plurality comprising (i) a polypeptide comprising an amino acid sequence of **SEQ ID NO: 64, and** (ii) a polypeptide comprising an amino acid sequence of **SEQ ID NO: 65, and** (iii) a polypeptide comprising an amino acid sequence of **SEQ ID NO: 66;**

- 5 (b) optionally recovering the expressed recombinant polypeptides.

According to some particular embodiments, the disclosure relates to a method for making the binding protein, comprising a step of:

- 10 (a) culturing host cell(s) under conditions suitable for **expressing a plurality** of recombinant polypeptides, said plurality comprising (i) a first polypeptide chain (I), (ii) a second polypeptide chain (II), and (iii) a third polypeptide (III), that form two antigen-binding domains (ABD), one ABD which binds specifically to a human CD123 polypeptide and the one other ABD which binds specifically to a human NKp46 polypeptide; characterized in that the three polypeptide chains (I), (II) and (III) consist of:



- 15 wherein :

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

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

- 20 C_{1A} and C_{1B} form a pair C_1 (C_{H1}/C_L) and C_{2A} and C_{2B} form a pair C_2 (C_{H1}/C_L) wherein C_{H1} is an immunoglobulin heavy chain constant domain 1 and C_L is an immunoglobulin light chain constant domain;

$(CH_2-CH_3)_A$ and $(CH_2-CH_3)_B$ are identical or different, and comprise an immunoglobulin heavy chain constant domain 2 (C_{H2}) and an immunoglobulin heavy chain constant domain 3 (C_{H3});

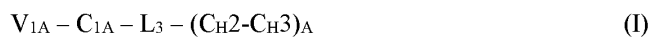
- 25 L_1 , L_2 , L_3 , L_4 are optional independent amino acid linkers, which may be identical or different;

- (b) optionally recovering the expressed polypeptide chains (I), (II) and (III).

According to some particular embodiments, the disclosure relates to a method for making the binding protein, comprising a step of:

- 30 (a) culturing host cell(s) under conditions suitable for **co-expressing a plurality** of recombinant polypeptides, said plurality comprising (i) a first polypeptide chain (I), (ii) a second polypeptide chain (II), and (iii) a third polypeptide (III), that form two antigen-binding domains (ABD), one ABD which binds specifically to a human CD123 polypeptide

- 5 and the one other ABD which binds specifically to a human NKp46 polypeptide; characterized in that it the three polypeptide chains (I), (II) and (III) consist of:



wherein :

- 10 V_{1A} and V_{1B} form a binding pair V_1 (V_{H1}/V_{L1});
 V_{2A} and V_{2B} form a binding pair V_2 (V_{H2}/V_{L2});
 C_{1A} and C_{1B} form a pair C_1 (C_{H1}/C_L) and C_{2A} and C_{2B} form a pair C_2 (C_{H1}/C_L) wherein C_{H1} is an immunoglobulin heavy chain constant domain 1 and C_L is an immunoglobulin light chain constant domain;
 $(CH_2-CH_3)_A$ and $(CH_2-CH_3)_B$ are identical or different, and comprise an immunoglobulin heavy chain constant domain 2 (C_{H2}) and an immunoglobulin heavy
 15 chain constant domain 3 (C_{H3});
 L_1 , L_2 , L_3 , L_4 are optional independent amino acid linkers, which may be identical or different;

(b) optionally recovering the **co-expressed** polypeptide chains (I), (II) and (III).

- 20 Hence, according to some particular embodiment, the disclosure relates to a method for making the binding protein, comprising a step of:

- (a) culturing host cell(s) under conditions suitable for **expressing** a plurality of recombinant polypeptides, said plurality comprising (i) a first polypeptide chain (I) comprising an amino acid sequence of **SEQ ID NO: 61** or **64**, (ii) a second polypeptide
 25 chain (II) comprising an amino acid sequence of **SEQ ID NO: 62** or **65**, and (iii) a third polypeptide (III) comprising an amino acid sequence of **SEQ ID NO: 63** or **66**;

(b) optionally recovering the expressed polypeptide chains (I), (II) and (III).

- 30 Hence, according to some particular embodiments, the disclosure relates to a method for making the binding protein, comprising a step of:

(a) culturing host cell(s) under conditions suitable for **co-expressing** a plurality of recombinant polypeptides, said plurality comprising (i) a first polypeptide chain (I) comprising an amino acid sequence of **SEQ ID NO: 61** or **64**, (ii) a second polypeptide

- 5 chain (II) comprising an amino acid sequence of **SEQ ID NO: 62** or **65**, and (iii) a third polypeptide (III) comprising an amino acid sequence of **SEQ ID NO: 63** or **66**;
- (b) optionally recovering the co-expressed polypeptide chains (I), (II) and (III).

10 Methods for making the binding protein of the disclosure, such as those defined above, may further include a prior step of providing host cell(s) with a nucleic acid, in particular an isolated nucleic acid (i.e., a recombinant nucleic acid), encoding all or part(s) of the said binding protein. In particular, such step may comprise or consist of transfecting said host cell(s) with a nucleic acid, in particular an isolated nucleic acid, encoding all or part(s) of the said binding protein.

15 Hence, according to some particular embodiments, the disclosure relates to a method for making the binding protein, comprising a step of:

- (a) providing host cell(s) with a nucleic acid encoding all or part(s) of the said binding protein;
- (b) culturing said host cell(s) under conditions suitable for expressing **one or**
- 20 **more** recombinant polypeptide(s) comprising (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human CD123 polypeptide, **and/or** (ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide, **and/or** (iii) all or part of an immunoglobulin Fc region or variant thereof which binds to a human Fc- γ receptor
- 25 polypeptide;
- (c) optionally recovering the expressed recombinant polypeptide(s).

In one embodiment, the method for making the binding protein of the present disclosure comprises a step of

- 30 (a) providing one or more nucleic acid(s) encoding a first polypeptide chain (I), a second polypeptide chain (II), and a third polypeptide chain (III);
- (b) transfecting host cell(s) with the one or more nucleic acid(s);
- (c) culturing the host cell(s) under conditions suitable to express (or co-express) the said polypeptide chain(s); and
- 35 (d) optionally recovering the expressed (or co-expressed) polypeptide chain(s) (I), (II) and (III).

5

In one embodiment, the method for making the binding protein of the present disclosure comprises a step of

- (a) providing one or more nucleic acid(s) encoding a first polypeptide chain (I) comprising an amino acid sequence of **SEQ ID NO: 61** or **64**, a second polypeptide chain (II) comprising an amino acid sequence of **SEQ ID NO: 62** or **65**, and a third polypeptide chain (III) comprising an amino acid sequence of **SEQ ID NO: 63** or **66**;
- (b) transfecting host cell(s) with the one or more nucleic acid(s);
- (c) culturing the host cell(s) under conditions suitable to express (or co-express) the said polypeptide chain(s); and
- (d) optionally recovering the expressed (or co-expressed) polypeptide chain(s) (I), (II) and (III).

15

In some particular embodiments, a method of making the binding protein of the present disclosure comprises:

20

(a) providing a first nucleic acid encoding a first polypeptide chain according to any of amino acid sequences of **SEQ ID NO: 61** or **64**, a second nucleic acid encoding a second polypeptide according to any of amino acid sequences of **SEQ ID NO: 62** or **65**, and a third nucleic acid encoding a third polypeptide chain according to any of amino acid sequences of **SEQ ID NO: 63** or **66**; and

25

(b) expressing said first, second and third nucleic acids in the one or more host cell(s) to produce a binding protein comprising said first, second and third polypeptide chains, respectively;

(c) optionally loading the protein produced onto an affinity purification support, optionally a Protein-A support, and recovering the binding protein.

30

It will thus be readily understood by the skilled in the Art that such method of making the binding protein of the disclosure may encompass the production and assembly of some or all of the above-mentioned polypeptides, polypeptide chains and/or regions (e.g. variable regions and Fc region or variants thereof) within one or more host cell(s), as part of an *in vitro* method of production.

35

5 Alternatively, the method may encompass the production of some or all of the above-mentioned polypeptides, polypeptide chains and/or regions within one or more host cell(s), and their assembly outside of the host cell(s). The step of bringing into contact said polypeptides, polypeptide chains and/or regions can thus be achieved simultaneously or sequentially.

10 According to some embodiments, one or more of said regions may be present in distinct polypeptide chain(s) or fragments thereof.

As a reference, the “*F25*” format of binding proteins which is described herein in the examples section possesses four predicted interchain disulfide bridges:

- 15 - one disulfide bridge connecting a cysteine within the CL domain of polypeptide (I) to the first cysteine within the Hinge region of polypeptide chain (II);
- two disulfide bridges connecting two cysteines within the Hinge regions of polypeptide chain (I) and (II);
- one disulfide bridge connecting a C-terminal cysteine at the CL domain of
- 20 polypeptide chain (III) to a C-terminal cysteine on polypeptide chain (II).

In some embodiment, the disclosure relates to a method for making the binding protein of the present disclosure, comprising a step of bringing into contact (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human

25 CD123 polypeptide, said variable region comprising at least one complementary determining region (CDR) selected from the group of amino acid sequences consisting of **SEQ ID NO: 1 to 12**, (ii) a second antigen-binding domain (ABD) comprising a variable region which binds specifically to a human NKp46 polypeptide, said variable region comprising at least one complementary determining region (CDR) selected from the group

30 of amino acid sequences consisting of **SEQ ID NO: 13 to 40** and (iii) all or part of a Fc region or variant thereof which binds to a human Fc- γ receptor polypeptide, especially which binds to a human CD16a Fc- γ receptor polypeptide.

In some embodiment, the disclosure relates to a method for making the binding protein of the present disclosure, comprising a step of bringing into contact (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a human

35 CD123 polypeptide, said variable region comprising at least one complementary

5 determining region (CDR) selected from the group of amino acid sequences consisting of
SEQ ID NO: 1 to 6 and at least one complementary determining region (CDR) selected
from the group of amino acid sequences consisting of **SEQ ID NO: 7 to 12**, (ii) a second
antigen-binding domain (ABD) comprising a variable region suitable which binds
specifically to a human NKp46 polypeptide, said variable region comprising at least one
10 complementary determining region (CDR) selected from the group of amino acid sequences
consisting of **SEQ ID NO: 13 to 26** and at least one complementary determining region
(CDR) selected from the group of amino acid sequences consisting of **SEQ ID NO: 27 to
40**, and (iii) all or part of an immunoglobulin Fc region or variant thereof which binds to a
human Fc- γ receptor polypeptide, especially which binds to a human CD16a Fc- γ receptor
15 polypeptide.

In some embodiments, the disclosure relates to a method for making the binding
protein of the present disclosure, comprising a step of bringing into contact (i) a first antigen-
binding domain (ABD) comprising a variable region which binds specifically to a human
CD123 polypeptide, said variable region comprising at least two complementary
20 determining region (CDR) selected from the group of amino acid sequences consisting of
SEQ ID NO: 1 to 6 and at least two complementary determining region (CDR) selected
from the group of amino acid sequences consisting of **SEQ ID NO: 7 to 12**, (ii) a second
antigen-binding domain (ABD) comprising a variable region which binds specifically to a
human NKp46 polypeptide, said variable region comprising at least two complementary
25 determining region (CDR) selected from the group of amino acid sequences consisting of
SEQ ID NO: 13 to 26 and at least two complementary determining region (CDR) selected
from the group of amino acid sequences consisting of **SEQ ID NO: 27 to 40**, and (iii) all or
part of an immunoglobulin Fc region or variant thereof which binds to a human Fc- γ receptor
polypeptide, especially which binds to a human CD16 Fc- γ receptor polypeptide.

30 In some embodiments, the disclosure relates to a method for making the binding
protein related to the present disclosure, comprising a step of bringing into contact (i) a first
antigen-binding domain (ABD) comprising a variable region which binds specifically to a
human CD123 polypeptide, said variable region comprising three complementary
determining region (CDR) selected from the group of amino acid sequences consisting of
35 **SEQ ID NO: 1 to 6** and three complementary determining region (CDR) selected from the
group of amino acid sequences consisting of **SEQ ID NO: 7 to 12**, (ii) a second antigen-

5 binding domain (ABD) comprising variable region which binds specifically to a human NKp46 polypeptide, said variable region comprising three complementary determining region (CDR) selected from the group of amino acid sequences consisting of **SEQ ID NO: 13 to 26** and three complementary determining region (CDR) selected from the group of amino acid sequences consisting of **SEQ ID NO: 27 to 40**, and (iii) all or part of an
10 immunoglobulin Fc region or variant thereof which binds to a human Fc- γ receptor polypeptide, especially which binds to a human CD16 Fc- γ receptor polypeptide.

In some embodiments, the disclosure relates to a method for making the binding protein related to the present disclosure, comprising a step of bringing into contact (i) a first antigen-binding domain (ABD) comprising a variable region which binds specifically to a
15 human CD123 polypeptide, (ii) a second antigen-binding domain (ABD) comprising a variable region suitable which binds specifically to a human NKp46 polypeptide, and (iii) all or part of an immunoglobulin Fc region or variant thereof which binds to a human Fc- γ receptor polypeptide, especially which binds to a human CD16 Fc- γ receptor polypeptide; characterized in that the step of bringing into contact said regions comprises bringing into
20 contact a plurality of polypeptide chains selected from of amino acid sequences **SEQ ID NO: 61 to 66**.

All or parts of the above-mentioned antigen-binding domain(s) and immunoglobulin Fc region or variant thereof may be expressed *in vitro*, through recombinant
25 means, in an isolated cell or population of cells, in particular in a eukaryotic cell, and preferably in a mammalian or insect cell. Most preferably, the expression system relates to a mammalian cell.

According to alternative embodiments, parts of the above-mentioned antigen-binding domain(s) and Fc region or variant thereof may be expressed in a first population of
30 isolated cells, whereas other parts of the above-mentioned antigen-binding domain(s) and Fc region or variant thereof may be expressed in a second population of isolated cells.

According to alternative embodiments, all the parts of the above-mentioned antigen-binding domain(s) and immunoglobulin Fc region or variant thereof may be expressed in a same population of isolated cells, and then recovered, thereby brought into
35 contact during or at the end of the recovery step.

Hence, the method for making the binding protein may comprise the steps of :

- 5 (a) expressing at least one of said first antigen-binding domain **and/or** said second antigen-binding domain **and/or** said all or part of immunoglobulin Fc region or variant thereof in an isolated cell or population of cells, most preferably in a mammalian cell;
- (b) recovering said first antigen-binding domain and/or said second antigen-binding domain and/or said all or part of Fc region or variant thereof.
- 10

According to one of said preferred embodiment, the disclosure relates to a method for making the binding protein related to the present disclosure, which comprises the steps of :

- 15 (a) expressing at least one of said first antigen-binding domain **and/or** said second antigen-binding domain **and/or** said all or part of Fc region or variant thereof in an isolated cell or population of cells, most preferably in a mammalian cell;
- (b) recovering said first antigen-binding domain and/or said second antigen-binding domain and/or said all or part of Fc region or variant thereof;
- 20 (c) bringing into contact the said first antigen-binding domain **and/or** said second antigen-binding domain **and/or** said all or part of Fc region or variant thereof, steps (b) and (c) being achieved simultaneously or sequentially.

Preferably, the method for making the binding protein comprises the steps of :

- 25 (a) expressing said first antigen-binding domain **and** said second antigen-binding domain **and** said all or part of immunoglobulin Fc region or variant thereof in an isolated cell;
- (b) recovering said first antigen-binding domain and said second antigen-binding domain and said all or part of immunoglobulin Fc region or variant thereof;
- 30 (c) bringing into contact the said first antigen-binding domain **and** said second antigen-binding domain **and** said all or part of immunoglobulin Fc region or variant thereof, steps (b) and (c) being achieved simultaneously or sequentially.

Advantageously, when the said first antigen-binding domain **and** said second antigen-binding domain **and** said all or part of immunoglobulin Fc region or variant thereof

35 are expressed in the same isolated cell or population of cells and/or the same cell culture

5 thereof, they may be brought into contact during the recovery step, thereby making the binding protein.

Alternatively, when the said first antigen-binding domain **and/or** said second antigen-binding domain **and/or** said all or part of immunoglobulin Fc region or variant thereof are expressed in different isolated cells or population of cells, they may be brought
10 into contact after the recovery step.

The recovery step may consist of any method known in the Art. In a non-exhaustive manner, the recovery of the expressed polypeptides bearing all or part of the antigen-binding domain(s) and Fc region or variant thereof (e.g. the expressed one or more
15 polypeptide chain(s)) may comprise the following steps:

(b1) recovering the isolated cell or cell culture thereof,

(b2) optionally centrifugating, depth filtering, membrane filtering, ultrafiltering and/or diafiltering the isolated cell or cell culture thereof.

20 SEQUENCE LISTING

In the protein sequences notation used herein, the left-hand direction is the amino terminal direction (the “N terminus” or “N-term”) and the right-hand direction is the carboxyl-terminal direction (the “C terminus” or “C-term”), in accordance with standard usage and convention.

25

SEQ	Name	Sequence
1	V_{H1} CDR-H1 Anti-CD123 CD123-1	GYSFTDYMK
2	V_{H1} CDR-H2 Anti-CD123 CD123-1	DIIPSSGATF
3	V_{H1} CDR-H3 Anti-CD123 CD123-1	SHLLRASWFAY
4	Anti-CD123	GFTFSHYN

	CD123-2	
5	V_{H1} CDR-H2 Anti-CD123 CD123-2	ITYDDHST
6	V_{H1} CDR-H3 Anti-CD123 CD123-2	ARLVNYAFAY
7	V_{L1} CDR-L1 Anti-CD123 CD123-1	ESSQSLSSGNQKNYLT
8	V_{L1} CDR-L2 Anti-CD123 CD123-1	WASTRES
9	V_{L1} CDR-L3 Anti-CD123 CD123-1	QNDYSYPYT
10	V_{L1} CDR-L1 Anti-CD123 CD123-2	QTVGNN
11	V_{L1} CDR-L2 Anti-CD123 CD123-2	YAS
12	V_{L1} CDR-L3 Anti-CD123 CD123-2	QRMYSNPT
13	V_{H2} CDR-H1 Anti-NKp46 NKp46-1	DYVIN
14	V_{H2} CDR-H2 Anti-NKp46 3D9 and NKp46-1	EIYPGSGTNYYNEKFA
15	V_{H2} CDR-H3 Anti-NKp46 3D9 and NKp46-1	RGRYGLYAMDY
16	V_{H2} CDR-H1 Anti-NKp46 NKp46-2 13G4	SDYAWN

17	V_{H2} CDR-H2 Anti-NKp46 NKp46-2	YITYSGSTSYNPSLES
18	V_{H2} CDR-H3 Anti-NKp46 NKp46-2	GGYYGSSWGVFAY
19	V_{H2} CDR-H1 Anti-NKp46 NKp46-3	EYTMH
20	V_{H2} CDR-H2 Anti-NKp46 NKp46-3	GISPNIGGTSYNQKFKG
21	V_{H2} CDR-H3 Anti-NKp46 NKp46-3	RGGSFDY
22	V_{H2} CDR-H1 Anti-NKp46 NKp46-4	SFTMH
23	V_{H2} CDR-H2 Anti-NKp46 NKp46-4	YINPSSGYTEYNQKFKD
24	V_{H2} CDR-H3 Anti-NKp46 NKp46-4	GSSRGFDY
25	V_{H2} CDR-H2 Anti-NKp46 I3G4	YITYSGSTNYNPSLKS
26	V_{H2} CDR-H3 Anti-NKp46 I3G4	CWDYALYAMDC
27	V_{L2} CDR-H1 Anti-NKp46 3D9 and NKp46-1	RASQDISNYLN
28	V_{L2} CDR-H2 Anti-NKp46 3D9 and NKp46-1	YTSLHS
29	V_{L2} CDR-H3 Anti-NKp46	QQGNTRPWT

	3D9 and NKp46-1	
30	V_{L2} CDR-H1 Anti-NKp46 NKp46-2	RVSENIYSYLA
31	V_{L2} CDR-H2 Anti-NKp46 NKp46-2 I3G4	NAKTLAE
32	V_{L2} CDR-H3 Anti-NKp46 NKp46-2	QHHYGTPWT
33	V_{L2} CDR-H1 Anti-NKp46 NKp46-3	RASQSIDYLH
34	V_{L2} CDR-H2 Anti-NKp46 NKp46-3	YASQSIG
35	V_{L2} CDR-H3 Anti-NKp46 NKp46-3	QNGHSFPLT
36	V_{L2} CDR-H1 Anti-NKp46 NKp46-4	RASENIYSNLA
37	V_{L2} CDR-H2 Anti-NKp46 NKp46-4	AATNLAD
38	V_{L2} CDR-H3 Anti-NKp46 NKp46-4	QHFWGTPRT
39	V_{L2} CDR-H2 Anti-NKp46 I3G4	RTSENIYSYLA
40	V_{L2} CDR-H3 Anti-NKp46 I3G4	QHHYDTPLT
41	VH-full length-anti- CD123	EVQLVQSGAEVKKPGESLKISCKGSGYSFTDYMKWARQMPGKG LEWMGDIIPSSGATFYNQKFKGQVTISADKSISTTYLQWSSLKASDT AMYYCARSHLLRASWFAYWGQGTMTVTVSS

42	VH-full length-anti-CD123	EVQLVESGGGLVQPGRSLKLSAASGFTFSHYNMAWVRQAPK KGLEWVATITYDDHSTYYRDSVKGRFTISRDTAKSTLYLQMDS LRSEDATYYCARLVNYAFAYWGQGTLLTVSS
43	VL-full length-anti-CD123	DIVMTQSPDSLAVSLGERATINCESSQSLLSSGNQKNYLTWYQQKP GQPPKPLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYY CQNDYSYPYTFGQGTKEIK
44	VL-full length-anti-CD123	NIVMTQSPKSMISVGDRTVMNCKASQTVGNNAWYQQKPLSPQ LLIDYASNRYTGVNRFTHGGYGTDFILTINSVQAEDAAFYQCQR MYNSPTFGGGTKLEIK
45	VH-full length-anti-NKp46	QVQLVQSGAEVKKPGSSVKVSKASGYTFSDYVINWVRQAPGQG LEWMGEIYPGSGTNYNEKFKAKATITADKSTSTAYMELSSLRSED TAVYYCARRGRYGLYAMDYWGQGTITVTVSS
46	VH-full length-anti-NKp46 NKp46-1	QVQLQQSGPELVKPGASVKMSCKASGYTFDYYVINWVGKQRSGQG LEWIGEIYPGSGTNYNEKFKAKATLTADKSSNIAYMQLSSLTSED SAVYFCARRGRYGLYAMDYWGQGTSTVTVSS
47	VH-full length-anti-NKp46 NKp46-2	EVQLQESGPGLVKPSQSLSLTCTVTGYSTSDYAWNWRQFPGNKL EWMGYITYSGSTSYNPSLESISRITRDTSTNQFFLQLNSVTTEDAT YYCARGGYYGSSWGVFAYWGQGTLLTVSA
48	VH-full length-anti-NKp46 NKp46-3	EVQLQQSGPELVKPGASVKISCKTSGYTFEYTMHWWKQSHGKSL EWIGGISPNIGGSTYNQKFKGKATLTVDKSSSTAYMELRSLTSEDS AVYYCARRGGSFYWGQGTLLTVSS
49	VH-full length-anti-NKp46 NKp46-4	QVQLQQSAVELARPGASVKMSCKASGYTFTSFTMHWWKQRPQGQ LEWIGYINPSSGYTEYNQKFKDKTTLTADKSSSTAYMQLDSLTSDD SAVYYCVRGSSRGFDYWGQGTLLTVSA
50	VH-full length-anti-NKp46 10B8	QVQLQESGPGLVKPSQSLSLTCTVSGYSISDDYAWNWRQPPGKGL EWIGYITYSGSTSYNPSLESRTISRDTSKNQFSLKLSVTAADTAV YYCARGGYYGSSWGVFAYWGQGTLLTVSS
51	VH-full length-anti-NKp46 12E12	QVQLVQSGAEVKKPGASVKVSKASGYTFTSFTMHWWVRQAPGQG LEWIGYINPSSGYTEYNQKFKDRVTITADKSTSTAYMELSSLRSED TAVYYCVRGSSRGFDYWGQGTLLTVSS
52	VH-full length-anti-NKp46 13G4	QVQLQESGPGLVKPSQSLSLTCTVSGYSISDDYAWNWRQPPGKGL EWIGYITYSGSTNYNPSLKSRTISRDTSKNQFSLKLSVTAADTAV YYCARCWDYALYAMDCWGQGTITVTVSS
53	VL-full length-anti-NKp46 3D9	DIQMTQSPSSLSASVGDRTITCRASQDISNYLNWYQQKPGKAPKL LIYYTSRLHSGVPSRFSGSGSGTDFTFTISSLQPEDATYFCQQGNTRP WTFGGGTKEIK
54	VL-full length-anti-NKp46 NKp46-1	DIQMTQTSSLSASLGDRVTISCRASQDISNYLNWYQQKPDGTVKL LIYYTSRLHSGVPSRFSGSGSGTDYSLTINNLEQEDATYFCQQGNT RPWTFGGGTKEIK
55	VL-full length-anti-NKp46	DIQMTQSPASLSASVGETVTITCRVSENIYSYLAWYQQKQKSPQL LVYNAKTLAEGVPSRFSGSGSGTQFSLKINSQPEDFGSYQCQHHY GTPWTFGGGTKEIK

	NKp46-2	
56	VL-full length-anti-NKp46 NKp46-3	DIVMTQSPATLSVTPGDRVSLSCRASQSISDYLHWYQQKSHESPRL LIKYASQSIGPSRFSGSGSGDFTLSINSVEPEDVGVIYQCQNGHSF PLTFGAGTKLELK
57	VL-full length-anti-NKp46 NKp46-4	DIQMIQSPASLSVSVGETVTITCRASENIYSNLAWFQQKQKSPQLL VYAATNLADGVPSRFSGSGSGTQYSLKINSLSQSEDFGIYYCQHFHW TPRTFGGGTKLEIK
58	VL-full length-anti-NKp46 10B8	DIQMTQSPSSLSASVGDRVITITCRVSENIYSYLAWYQQKPGKAPKL LVYNAKTLAEGVPSRFSGSGSGTDFLTISLQPEDFATYYCQHHY GTPWTFGGGKVEIK
59	VL-full length-anti-NKp46 12E12	DIQMTQSPSSLSASVGDRVITITCRASENIYSNLAWFQQKPGKAPKL LVYAATNLADGVPSRFSGSGSGTDTYLTISLQPEDFATYYCQHFHW GTPRTFGGGTKVEIK
60	VL-full length-anti-NKp46 13G4	DIQMTQSPSSLSASVGDRVITITCRSENIYSYLAWCQQKPGKAPKL LIYNAKTLAEGVPSRFSGSGSGTDFLTISLQPEDFATYYCQHHYD TPLTFGQGTKLEIK
61	Polypeptide chain (I)	MSVPTQVLGLLLLWLTARCDIVMTQSPDSLAVSLGERATINCESS QSLSSGNQKNYL TWYQQKPGQPPKPLIYWASTRESGVPDRFSGS GSGTDFLTISLQAEDVA VYVCQNDYSYPYTFGQGTKLEIKRTVA APSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQS GNSQESVTEQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGL SSPVTKSFNRGEC DKTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMIS RTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNS TYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPEN NYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMEALHN HYTQKSLSLSPGK
62	Polypeptide chain (II)	MEWSWVFLFSLVTTGVHSEVQLVQSGAEVKKPGESLKISKKGSG YSFTDYIMKWARQMPPGKGLEWMGDIIPSSGATFYNQKFKGQVTI SADKSISTTYLQWSSLKASDTAMYCARSHLLRASWFAYWGQGT MVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVS WNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVN HKPSNTKVDKRVPEKSCDKTHTCPPCPAPPELLGGPSVFLFPPKPKD TLMISRTPETCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISK AKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWES NGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV HEALHNHYTQKSLSLSPGSTGSQVQLVQSGAEVKKPGSSVKVCSCK ASGYTFSDYVINWVRQAPGQGLEWMGEIYPGSGTNYNNEKFAK ATTADKSTSTAYMELSSLRSEDTAVYYCARRGRYGLYAMDYWG QGTTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYIC NVNHNKPSNTKVDKRVPEKSCDKTHS
63	Polypeptide chain (III)	MSVPTQVLGLLLLWLTARCDIQMTQSPSSLSASVGDRVITITCRAS QDISNYLWYQQKPGKAPKLLIYYTSRLHSGVPSRFSGSGSGTDF FTISLQPEDIAITYFCQQGNTRPWTFGGGTKVEIKRTVAAPSVFIFPP SDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT EQDSKDYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFN RGEK

64	F25 polypeptide chain (I) Cleaved (without leader peptide)	DIVMTQSPDSLAVSLGERATINCESSQSLSSGNQKNYLTWYQQKP GQPPKPLIYWASTRESGVDPDRFSGSGSGTDFTLTISSLQAEDVAVY YQNDYSYPYTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSLSS TLTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGECDKTHTCPP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKL TVDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPGK
65	F25 polypeptide chain (II) Cleaved (without leader peptide)	EVQLVQSGAEVKKPGESLKISCKGSGYSFTDYMKWARQMPGKG LEWMGDIIPSSGATFYNQKFKGQVTISADKSISTTYLQWSSLKASD TAMYYCARSHLLRASWFAYWGQGTMTVTVSSASTKGPSVFPLAP SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQ SGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV THTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDV DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGS FLYSKLTVDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPGSGT SQVQLVQSGAEVKKPGSSVKVSKASGYTFSDYVINWVVRQAPGQ GLEWMGEIYPGSGTNYNEKFKAKATITADKSTSTAYMELSSLR EDTAVYYCARRGRYGLYAMDYWGQGTITVTVSSASTKGPSVFPLA PSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL QSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKRV DKTHS
66	F25 polypeptide chain (III) Cleaved (without leader peptide)	DIQMTQSPSSLSASVGDRTVITCRASQDISNYLNWYQQKPGKAPKL LIYYTSRLHSGVPSRFRSGSGSGTDFTFTISSLQPEDIAFYFCQQGNTR PWTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDSSTLSLSSTLTLKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC
67	C _K	RTVAAPSVFIFPPSDEQLKSGTASVCLLNNFYPREAKVQWKVDN ALQSGNSQESVTEQDSKDSSTLSLSSTLTLKADYEKHKVYACEVT HQLSSPVTKSFNRGEC
68	C _{H1}	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGAL TSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTK VDKRV
69	(C _{H2} -C _{H3}) _A	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPGK
70	(C _{H2} -C _{H3}) _B	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLT VDKSRWQQGNVVFSCSVMEALHNHYTQKSLSLSPG
71	C _{H2}	APELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFN WYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEY KCKVSNKALPAPIEKTISKAK
72	C _{H3} of (C _{H2} -C _{H3}) _A	GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVME ALHNHYTQKSLSLSPGK
73	C-term truncated C _{H3} of (C _{H2} -C _{H3}) _B	GQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVVFSCSVME ALHNHYTQKSLSLSPG

74	Hinge ₁	DKTHTCPPCP
75	Hinge ₂	EPKSCDKTHTCPPCP
76	Linker ₁	STGS
77	Hinge ₃	EPKSCDKTHS
78	Hinge _{ALT1}	EPKSCDKTH
79	Hinge _{ALT2}	EPKSCDKTHT
80	Single guide RNA	CUUGAGGUGUCAUGCGUGGA
81	Single guide RNA	AAGCAUCGCUACACAUCAGC
82	DNA primer 1	TACGACTCACAAGCTTGCCGCCACCATGTCTTCCACACTCCCTG C
83	DNA primer 2	CCGCCCCGACTCTAGATCAATGGTGATGGTGGTGATGATTCTGG GCAGTGTGATCCC
84	Human NKp46 extracellular domain (ECD)	MSSTLPALLCVGLCLSQRISAQQQTLPKPFIWAEPHFMVPKEKQVT ICCGQNYGAVEYQLHFEGLSFAVDRPKPPERINKVKFYIPDMNSRM AGQYSCIYRVGELWSEPSNLLDLVVTEMYDTPTLSVHPGPEVISGE KVTFYCRLDTATSMFLLLKEGRSSHVQRGYGVQAEFPLGPVTTA HRGTYRCFGSYNNHAWSPSEPVKLLVTGDIENSLAPEDPTFPAD TWGTYLLTTETGLQKDHALWDHTAQN
85	Cynomolgus NKp46-FlagM2 extracellular domain (ECD)	MSSTLRALLCLGLCLSQRISAPKQTLPKPIRAESTYMPVPEKQATL CCQGSYGAVEYQLHFEGLSFAVERPKPPERINGVKFHIPDMNSRKA GRYSCIYRVGELWSESDLLDLVVTEMYDTPTLSVHPGPEVTSGE KVTFYCRLDTATSMFLLLKEGRSRDVQRSYGVQAEFPMGPVTTA HRGSYRCFGSYNNYAWSFPSEPVKLLVTGDIENSLAPDPTFPDS WDTCLLTRETGLQKDLALWDHTAQNDYKDDDDK
86	Human CD123 extracellular domain (ECD)	TKEDPNPPITNLRMKAKAQQLTWDLNRNVTDIECVKDADYSMPA VNNSYQCQGAISLCEVTNYTVRVANPPFSTWILFPENSGKPWAGAE NLTCWIHDVDFLSCSWAVGPGAPADVQYDLYLNVANRRQQYECL HYKTDAQGTRIGCRFDDISRLSSGSQSSHILVRGRSAAFGIPCTDKF VVFSQIEILTPPNMTAKCNKTHSFMHWKMRSHFNKFRYELQIQK RMQPVITEQVRDRTSFQLLNPGTYTVQIRARERVYEFLSAWSTPQR FECQEEGANTRAWR
87	Human Fc-gamma- receptor 3A (CD16A).	MWQLLLPTALLLVVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVT LKCQGAYSPEDNSTQWFHNESLISSQASSYFIDAATVDDSGEYRCQ TNLSTLSDPVQLEVHIGWLLQAPRWVFKEEDPIHLRCHSWKNTA LHKVTYLQNGKGRKYFHHNSDFYIPKATLKDSGSYFCRGLVGSKN VSSETVNITITQGLAVSTISSFFPPGYQVSFCLVMVLLFAVDTGLYFS VKTNIRSSTRDWKDHKFKWRKDPQDK
88	Human Fc-gamma- receptor 3A (CD16A) (V176F polymorphic variant)	MWQLLLPTALLLVVSAGMRTEDLPKAVVFLEPQWYRVLEKDSVT LKCQGAYSPEDNSTQWFHNESLISSQASSYFIDAATVDDSGEYRCQ TNLSTLSDPVQLEVHIGWLLQAPRWVFKEEDPIHLRCHSWKNTA LHKVTYLQNGKGRKYFHHNSDFYIPKATLKDSGSYFCRGLVGSKN VSSETVNITITQGLAVSTISSFFPPGYQVSFCLVMVLLFAVDTGLYFS VKTNIRSSTRDWKDHKFKWRKDPQDK
89	DNA primer 3	TACGACTCACAAGCTTGCCGCCACCATGTCTTCCACACTCCGTG C
90	DNA primer 4	CCGCCCCGACTCTAGATCACTTGTGCATCGTCATCTTTGTAATCAT TCTGGGCAGTGTGGTCC

91	NKp46-IC_F25 Fragment 1	EIVLTQSPATLSLSPGERATLSCRASQSVRSYLAWYQQKPGQAPRL LFSDASNRAITGIPARFSGSGSGTDFTLTISLSEPEDFAVYYCQQYRY SPRTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGECDKTHTCCPCPAPELLG GPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDG VEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSN KALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKG FYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRW QQGNVFCFSVMHEALHNHYTQKSLSLSPGK
92	NKp46-IC_F25 Fragment 2	EVQLVESGGGLVQPGSRSLRSCAASGFTFDNYAMHWVRQAPGKG LEWVSGISRSSGDIYADSVKGRFTISRDNKNSLYLQMNSLRRAED TALYYCARGGVGSFDTWGQGTMTVTVSSASTKGPSVFPLAPSSKST SGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLY SLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHTC PPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEV KFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNG KEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQ VSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYS KLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGSTGSQV QLVQSGAEVKKPKGSSVKVSKASGYTFSDYVINWVRQAPGQGLE WMGEIYPGSGTNYNEKFKAKATITADKSTSTAYMELSSLRSED AVYYCARRGRYGLYAMDYWGQGTITVTVSSASTKGPSVFPLAPSS KSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS GLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKT HS
93	NKp46-IC_F25 Fragment 3	DIQMTQSPSSLSASVGDRTITCRASQDISNYLNWYQQKPGKAPKL LIYYTSRLHSGVPSRFSGSGSGTDFTFTISLQPEDIAITYFCQQGNTR PWTFGGGTKEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFY PREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLSSTLTLSKAD YEKHKVYACEVTHQGLSSPVTKSFNRGEC
94	moNKp46- huCD123_F25 Fragment 1	DIVMTQSPDSLAVSLGERATINCESSQSLSSGNQKNYLTWYQQKP GQPPKPLIYWASTRESGVDRFSGSGSGTDFTLTISLQAEDVAVY YCQNDYSYPYTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSYSTLS TLTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGECDKTHTCCP CPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKF NWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQV LTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKL TVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
95	moNKp46- huCD123_F25 Fragment 2	EVQLVQSGAEVKKPGESLKISCKGSGYSFTDYMKWARQMPGKG LEWMGDIIPSSGATFYNQKFKGQVTISADKISSTYTLQWSSLKASD TAMYYCARSHLLRASWFAYWGQGTMTVTVSSASTKGPSVFPLAPS SKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQS SGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK THTCCPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSH DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSF FLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGSTG SEVQLVESGGGLVKPGGSLKLSAASGFTFDYGMHWVRQAPEK GLEWVAYISSGSSTIYADTVKGRFTISRDNKNTLFLQMTSLRSE DTAMYYCARGTTIFNYFEYWGQGTSTVTVSSASTKGPSVFPLAPSSK STSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSG LYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTH S

96	moNKp46- huCD123_F25 Fragment 3	DIVMSQSPSSLAVSVGEKVTMSCKSSQSLLYSSNQKNYLAWYQQK PGQSPKLLIYWASTRESGVDPDRFTGSGSGTDFTLTISVKAEDLAVY YCQQYYEIPPTFGAGTKLELKRTVAAPSVFIFPPSDEQLKSGTASVV CLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYLSST LTLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
97	NKp46-CD123_F5 Fragment 1	DIVMTQSPDSLAVSLGERATINCESSQSLLSSGNQKNYLWYQQKP GQPPKPLIYWASTRESGVDPDRFTGSGSGTDFTLTISLQAEDVAVY YCQNDYSYPYTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYLSST LTLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGECDKTHTCPP CPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKF NWKYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKL TVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK
98	NKp46-CD123_F5 Fragment 2	EVQLVQSGAEVKKPGESLKISCKGSGYSFTDYIMKWARQMPGKG LEWMGDIIPSSGATFYNQKFKGQVTISADKSISTTYLQWSSLKASD TAMYYCARSHLLRASWFAYWGQGTMTVTVSSASTKGPSVFPLAPS SKSTSGGTAALGCLVKDYFPEPVTWNSGALTSGVHTFPAVLQSS SGLYSLSVSVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHED DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSF FLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGSTG SQVQLVQSGAEVKKPGSSVKVSKASGYFTSDYINWVRQAPGQ GLEWMGEIYPGSGTNYYNEKFKAKATITADKSTSTAYMELSSLS EDTAVYYCARRGRYGLYAMDYWGQGTITVTVSSRTVAAPSVFIFP PSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESV TEQDSKDSSTYLSSTLTLISKADYEKHKVYACEVTHQGLSSPVTKSF NRGEC
99	NKp46-CD123_F5 Fragment 3	DIQMTQSPSSLASVGDRTITCRASQDISNYLNWYQQKPGKAPKL LIYYTSRLHSGVPSRFTGSGSGTDFTFTISLQPEDIAFYFCQQGNTR PWTFTGGGKVEIKASTKGPSVFPLAPSSKSTSGGTAAALGCLVKDYF PEPVTWNSGALTSGVHTFPAVLQSSGLYSLSVSVTPSSSLGTQ TYICNVNHKPSNTKVDKRVEPKSCDKTHS
100	IC-CD123_F5 Fragment 1	DIVMTQSPDSLAVSLGERATINCESSQSLLSSGNQKNYLWYQQKP GQPPKPLIYWASTRESGVDPDRFTGSGSGTDFTLTISLQAEDVAVY YCQNDYSYPYTFGQGTKEIKRTVAAPSVFIFPPSDEQLKSGTASV VCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYLSST LTLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGECDKTHTCPP CPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKF NWKYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKE YKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVS LTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKL TVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGK
101	IC-CD123_F5 Fragment 2	EVQLVQSGAEVKKPGESLKISCKGSGYSFTDYIMKWARQMPGKG LEWMGDIIPSSGATFYNQKFKGQVTISADKSISTTYLQWSSLKASD TAMYYCARSHLLRASWFAYWGQGTMTVTVSSASTKGPSVFPLAPS SKSTSGGTAALGCLVKDYFPEPVTWNSGALTSGVHTFPAVLQSS SGLYSLSVSVTPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDK THTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHED DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQD WLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEM TKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSF FLYSKLTVDKSRWQQGNVFCSSVMHEALHNHYTQKSLSLSPGSTG SEVQLVQSGAEVKKSGESLKISCKGSGYSFTSYWIGWVRQMPGKG LEWMGIFYPGDSSTRYSPSFQGVYVTVSSADKSVNTAYLQWSSLKASD

		TAMYYCARRRNWGNAFDIWGQGTMTVSSRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
102	IC-CD123_F5 Fragment 3	EIVLTQSPGTLSPGERATLSCRASQSVSSSYLAWEYQKPGQAPRLLIYGASSRATGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQQYG SSTWTFGQGTKVEIKASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTWNSGALTSGVHTFPAVLQSSGLYSLSSVVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHS
103	IC-hIgG1-ADCC- enh Light Chain	EIVLTQSPATLSLSPGERATLSCRASQSVRSYLAWEYQKPGQAPRL LFSDASNRATGIPARFSGSGSGTDFTLTISSELPEDFAVYYCQQYRYSPTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
104	IC-hIgG1-ADCC- enh Heavy Chain	EVQLVESGGGLVQPGGSLRLSCAASGFTFDNYAMHWVRQAPGKGLEWVSGISRSSGDDYADSVKGRFTISRDNANKNSLYLQMNSLRAEDTALYYCARGGVGSFDTWGQGTMTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTWNSGALTSGVHTFPAVLQSSGLYSLSSVVPSSSLGTQTYICNVNHKPSNTKVDKRVEPKSCDKTHSTPCPAPELLGGPDVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAAPEEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSPGK

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EXAMPLES

MATERIALS & METHODS

A.1. NKp46-CD123 NKCE expression by transient transfection of EXPI-293F™ cells.

The sequences encoding each polypeptide chain of NKp46-CD123_F25 binding proteins of the present disclosure were inserted into the pTT-5 vector between the HindIII and BamHI restriction sites. The three vectors (made as endotoxin-free midipreps) were used to cotransfect EXPI-293F cells (Life Technologies) in the presence of PEI (37°C, 5% CO₂, 150 rpm). The cells were used to seed culture flasks at a density of 1 x 10⁶ cells per ml (EXPI293 medium, Gibco). As a reference, for the NKp46-CD123_F25 binding protein, we used a DNA ratio of 0.1 µg/ml (polypeptide chain I), 0.4 µg/ml (polypeptide chain II), or 0.8 µg/ml (polypeptide chain III). Valproic Acid (final concentration 0.5 mM), glucose (4 g/L) and tryptone N1 (0.5%) were added. The supernatant was harvested after six days after and passed through a Stericup filter with 0.22 µm pores.

A.2. Purification of NKCEs.

The NKp46-CD123_F25 binding proteins of the present disclosure were purified from the supernatant following harvesting using rProtein A Sepharose Fast Flow (GE Healthcare, reference 17-1279-03). A Cation Ion Exchange Chromatography (CIEX) purification was then performed after dialysis of the sample in a Na₂HPO₄/KH₂PO₄ 50mM pH 6.2 phosphate buffer. Prior to injection to the two « in series » column HiTrap SP-HP 1mL from GE Healthcare (ref 17-1151-01), the sample was filtered on a 0.22 µm device. The starting and the elution buffers were respectively Na₂HPO₄/KH₂PO₄ 50mM pH 6.2 and Na₂HPO₄/KH₂PO₄ 25mM pH 6.2; 1M NaCl. The elution was performed using a linear gradient from 0% to 50% (elution buffer) on 100 CV. The peak of interest is finally dialyzed against PBS1X, overnight at 4°C under agitation.

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A.3. Biological samples

Healthy Human buffy coats were provided by the Etablissement Français du Sang (EFS, Marseille; AC-2019-3428). Peripheral mononuclear cells (PBMC) were isolated from buffy coats by using Ficoll density gradient centrifugation. Human NK cells were purified from PBMC by using beads-based negative selection kit from StemCell or Miltenyi.

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- 5 Acute myeloid leukemia (AML) samples from patients were provided by Institut Paoli-Calmettes (Marseille, SA-IPH-MImAbs Contract).

A.4. Cell lines

CD123 expressing Acute myeloid leukemia (AML) cell line: MOLM-13 and THP-1
10 were purchased at ATCC. Cells were cultured in complete RPMI medium (RPMI-1640 containing 10 % FBS, 2 mM L-Glutamine, 1 mM Sodium pyruvate and non-essential amino-acids 1X). 25mM HEPES were added in the culture medium for THP-1 cells.

THP-1 CD64KO and THP-1 CD32KO cells were generated with CRISPR/Cas endonucleases. THP-1 cells were cultured in RPMI-1640, 10%SVF, 2mM L-Glu, 1mM
15 sodium pyruvate, 0.1mM non-essential amino acid. To generate CD64 deficient THP-1 cells, 2,5.10⁶ cells were nucleofected (Neon Transfection System, 100ul tip, 1700V, 20ms, 1 pulse) with two sgRNAs (CD64.1: CUUGAGGUGUCAUGCGUGGA (SEQ ID NO: 80); CD64.2: AAGCAUCGCUACACAUCAGC (SEQ ID NO: 81; Synthego) at a CAS9:sgRNA ratio of 1:9 (Alt-R™ S.p. Cas9 Nuclease 3NLS, Integrated DNA
20 Technology). Lack of CD64 expression was monitored by flow cytometry and cells were either sorted or sub-cloned.

To generate CD32-knockout (KO) THP-1 cells, 2.5.10⁶ cells were nucleofected (Neon Transfection System, 100 µL tip, 1700 V, 20 ms, 1 pulse) with a couple of sgRNAs (CD32A: AUGUAUGUCCCAGAAACCUG ; CD32B: AAGCAUAUGACCCCAAGGCU
25 (Integrated DNA Technologies)) at a CAS9:sgRNA ratio of 1:9 (Alt-R™ S.p. Cas9 Nuclease 3NLS, Integrated DNA Technology). The THP-1 CD32KO cells were cell sorted only. Following cell sorting, absence of CD32 expression was monitored by flow cytometry.

A.5. NK cell-based cytotoxic assay

30 Target cells were loaded with ⁵¹Cr (for MOLM-13, THP-1 or THP-1 CD64KO, THP-1 CD32KO cells) or with CalceinAM (Life technologies, ref: C3100MP or equivalent) for AML blasts from patient samples. Tested antibodies, labelled target cells and fresh or overnight-rested human NK cells from healthy donors were successively added in each well of round bottom 96-well plates to obtain a 10:1 (E:T) ratio. After 4h of co-incubation, the
35 supernatant was transferred into a Lumaplate (for ⁵¹Cr) or into a flat bottom culture plate (for CalceinAM).

5 For ^{51}Cr -based cytotoxic assay, the ^{51}Cr released from dead target cells was dosed using a TopCount NXT™ (Microplate Scintillation and Luminescence Counter; Perkin Elmer). Radioactivity was measured by counting γ -emission during 60s for each well and these results was expressed in cpm = count per minute.

For Calcein-based cytotoxic assay, the CalceinAM released from dead target cells was
10 dosed by measuring relative fluorescence units (RFU) with a Luminoter (EnSpire®Multimode Plate Reader (Perkinelmer): fluorescence emission at $\lambda = 516$ nm after an excitation at $\lambda = 495$ nm).

For the analysis, the percent specific lysis was calculated using the following formula:

$$\text{Specific lysis (\%)} = \frac{ER (\text{cpm or RFU}) - SR (\text{cpm or RFU})}{MR (\text{cpm or RFU}) - SR (\text{cpm or RFU})} \times 100$$

15 with **ER** = experimental release, **SR** = spontaneous release and **MR** = maximal release

EC₅₀ of each antibody are determined with drawing appropriate non-linear regression curve (choice of “log(agonist) vs. response – Variable slope (four parameters)” model) by using
20 Graphpad Prism Software.

Phenotyping AML cells

The expression of CD32, CD64 and CD123 on AML samples derived from patient blood and on AML cell lines was controlled by flow cytometry using Anti-human CD33-
25 BB515 (BD Biosciences 564588 Clone WM53), Anti-human CD45-Viogreen (Milenyi 130-096-906 Clone 5B1), Anti-human CD123-AF647 (BD Biosciences 563599 Clone 9F5), Anti-human CD32-PE (Beckman Coulter IM1935 Clone 2E1), Anti-human CD64-PE (Beckman Coulter IM3601U Clone 22), Anti-human CD123-PE (Biolegend 306006 Clone 6H6), and cognate isotype control antibodies mIgG1-PE (IC-1 ; BD Biosciences 555749
30 Clone MOPC-21), and mIgG2a-PE (IC-2a ; Beckman Coulter A09142 Clone 7T4-1F5). Target cells were saturated with normal mouse serum diluted at 1/10e in staining buffer (SB) and then mix with antibodies coupled to dyes. Cells were fixed in BD Cellfix diluted at 1/10e in H2O during 30 min after staining and analyzed by flow cytometry with a FACS Canto II. FSC-A, FSC-H, SSC-A, SSC-H, FL2-A, FL4-A and FL7-A or FSC-A, FSC-H, SSC-A,
35 SSC-H, FL1-A, FL2-A, FL3-A, FL5-A and FL8-A (for AML samples derived from patient

- 5 blood) parameters were recorded and the analyses were done with FlowJo software. The phenotyping results are shown in **Figure 6A**, **Figure 6B**, and **Figure 14B**.

A.6. NK cell degranulation assay with AML samples

In order to test NK cell activation as in **Figure 7**, tested antibodies, AML blast and
10 autologous NK cells derived from AML patients were successively added in each well of round bottom 96-well plates. After overnight co-incubation with the NKp46-CD123_F25 binding proteins of the present disclosure, anti-human CD107a and CD107b antibodies were added in each well for 4 hours. Cells were then washed and stained with the following mix:
15 markers of viability, APC-coupled anti-human CD45, BB515-coupled anti-human CD33, PeCy7-coupled anti-human CD56, BV510-coupled anti-human CD3 antibodies. Cells were then washed, fixed and analyzed by flow cytometry. Obtained data were analyzed by using Flowjo Software to analyze NK cell degranulation through the expression of CD107 on NK cells identified as alive CD45⁺CD33⁻CD56⁺CD3⁻ cells.

In order to test NK cell activation and cytokine/chemokine production towards
20 MOLM-13 cells via NKp46-CD123_F25 binding proteins as in **Figure 17**, flow cytometric analysis was performed using the following antibody markers: CD69, CD107a/b, IFN α , TNF α , MIP1 β .

First, purified primary human NK cells from three separate donors were co-incubated with or without MOLM-13 cells at a 1:1 ratio (seeded at 50,000 cells/well; U bottom 96-well plate) for 4 h at 37°C, in the presence of increasing concentrations of NKp46-
25 CD123_F25 or controls (NKp46-IC_F25 and no antibody). Concomitantly, BD GolgiSTOPTM was added to both experimental and control samples at a final dilution of 1/6000 in each well. A positive control of NK cell activation was performed by using 125 ng/mL final of PMA and 1 μ g/mL final of IONO added on 50,000 resting NK cells by well
30 (data not shown).

After the 4 hr incubation, cells were washed in staining buffer (PBS with 0.2% BSA, 2 mM EDTA, and 0.02% sodium azide and stained with the following extracellular antibody mixture: anti-human CD3-Pacific Blue, anti-human CD56-Pe-Vio770, anti-human CD69-FITC, anti-human CD107a (LAMP-1)-APC, anti-human CD107b-APC according to
35 manufacturer's recommended incubation and dilution ratios. After a fixation and permeabilization step, intracellular staining was performed using the following intracellular

5 antibody mixture: anti-human IFN γ -BV605, anti-human TNF α -BUV395, and anti-human MIP1 β -PE. To eliminate aggregates, antibody mixtures were centrifuged at 16,000 g for 10 min at 4°C, washed, and resuspended in sample buffer.

Flow cytometry was performed on a LSR Fortessa™ X-20, equipped with BD FACSDiva acquisition software measuring FSC-A, FSC-H, SSC-A, SSC-H, FL-1, FL-3,
10 FL-6, FL-7, FL-9, FL-13 and FL-16 parameters. All data were analyzed with FlowJo software.

Marker percent of NK cell sample was done using GraphPad prism. Top of activation values corresponded to the observed maximum activation. Half maximal effective concentration (EC₅₀) values were calculated using 4 parameter logistics non-linear
15 regression model corresponding to the following equation:

NK cell activation (%)

$$= \text{calculated bottom} + \frac{\text{calculated top} - \text{calculated bottom}}{1 + 10^{(\log(\text{EC}_{50}) - \log(\text{concentration}))^{\text{slope}}}}$$

20 Calculated bottom of activation, calculated top of activation, slope and 95% confidence interval (CI) values were calculated using the same model as EC₅₀.

These parameters were calculated for each activation marker (CD69, CD107a/b), cytokine (IFN α , TNF α) and chemokine (MIP1 β).

25 **A.7. Human recombinant protein, cloning, production and purification (SPR)**

The sequence encoding the Extracellular Domain (ECD) of human NKp46 (Gln22-Asn255, NCBI Reference: NM_004829.5) was inserted into the SLX192 vector (Selexis) between the HindIII and XbaI restriction sites. A C-terminal 6xHis tag was added for purification. The following primers were used for PCR on human PBMCs: 5'
30 TACGACTCACAAGCTTGCCGCCACCATGTCTTCCACACTCCCTGC 3' and 5' CCGCCCCGACTCTAGATCAATGGTGATGGTGGTGATGATTCTGGGCAGTGTGA TCCC 3'. The sequence of the amplicon was checked. The vector was then used to transfect a CHO cell line and a clone producing the protein was selected. The protein was purified from the culture supernatant with Ni-NTA beads (Qiagen, #1018244) and S200 size
35 exclusion chromatography was performed to ensure the elimination of aggregates prior to

5 characterization of binding kinetics with surface plasmon resonance (SPR). The recombinant human NKp46-derived polypeptide sequence is reported herein as **SEQ ID NO: 84**; which includes part of the extracellular domain of NKp46.

The sequence encoding the ECD of Cynomolgus NKp46 (Gln17-Asn254, NP_001271509.1) was cloned into the SLX192 vector between the HindIII and XbaI
10 restriction sites. A C-terminal Flag-M2 tag was added for purification. The primers used to amplify the expected sequence from cynomolgus PBMC were: 5' TACGACTCACAAAGCTTGCCGCCACCATGTCTTCCACACTCCGTGC 3' and 5' CCGCCCCGACTCTAGATCACTTGTCATCGTCATCTTTGTAATCATTCTGGGCAG TGTGGTCC 3'. After sequence validation, the vector was used to transfect the CHO-K1SV
15 cell line and a producing cell clone was selected. The recombinant Cynomolgus NKp46-FlagM2 protein sequence is reported herein as **SEQ ID NO: 85**; which includes part of the extracellular domain of NKp46 (GenBank number: CAC41080.1). The first three batches (150602CCe batch 1, 150618CCe batch 2 and 150715CCe batch 3) were purified by M2 affinity chromatography. The beads were incubated with the supernatant containing the
20 recombinant protein overnight. The beads were then washed with PBS1X and the elution is performed with elution peptide at 150 ng/μl in PBS1X. The proteins are then dialyzed against PBS1X. The next batches (161003CDe batch 1 and 161116CDe batch 2) were purified by affinity chromatography by coupling the anti-NKp46 antibody HUX1-M-H46-17E1 to the AminoLink Coupling Resin according to the manufacturer's instructions (GE Healthcare,
25 #20381, batch QB213815). The beads were then incubated with the supernatant containing the recombinant protein overnight. The beads were then washed with PBS1X and the elution is performed using Glycine 0.1M pH2.5. The proteins are then dialyzed against TBS buffer pH7.5 and concentrated to perform a preparative size exclusion chromatography on a Superdex 200 Increase 10/300 GL column.

30

A recombinant human CD123 from ACRO Biosystems (catalog no. ILA-H52H6), recombinant Human Fc gamma RIIIA/CD16a (V176) (Biotechne, catalog no. 4325-FC), and recombinant Human Fc gamma RIIIA/CD16a (V176F) (Biotechne, catalog no. 8894-FC) were further used.

35

5 **A.8. Analytical procedure for determination of the antigen binding properties of multispecific binding proteins by surface plasmon resonance.**

A Biacore T200 instrument (Cytiva, Uppsala, Catalog No. 28975001) was used with a Series S CM5 sensor chip (Cytiva, Uppsala, Catalog No. 29149603).

For binding kinetics measurements with NKp46 and CD123, HBS-EP+ buffer (Cytiva, Uppsala, Catalog No. BR1006-69) was prepared by mixing 100 mL 10x HBS-EP+ buffer with 900 mL of purified water. Affinity capture of the bispecific Ab sample was achieved using the human antibody capture kit (Cytiva, Uppsala, Catalog No. BR1008-39). The anti-Fc capture antibody was diluted in running buffer 1:20 and coupled to the CM5 chip (Cytiva, Uppsala, Catalog No. 29149603) using standard amine coupling to yield approximately 8000 response units (RU) using the amine coupling kit (Cytiva, Uppsala, Catalog No. BR-100-50). Seven serial 1:1 dilutions of either human NKp46 (Innate Pharma) or human CD123 (ACRO Biosystems) in HBS-EP+ assay buffer were prepared to concentrations of 1.56 nmol/L, 3.13 nmol/L, 6.25 nmol/L, 12.5 nmol/L, 25 nmol/L, 50 nmol/L and 100 nmol/L. The bispecific antibody was diluted with HBS-EP+ buffer to a concentration of 0.06 µg/mL and used at this concentration in the experiments. The antibody was captured at a flow rate of 10 µL/min for 90 sec to yield maximal response (R_{max}) values of approximately 30 RU. Measurements were performed in multicycle kinetics experiments for both antigens. In each multicycle experiment the antibody was captured via an anti-human Fc antibody immobilized on a series S CM5 sensor chip (human antibody capture kit, Cytiva, Uppsala, Catalog No. BR1008-39). Human and cynomolgus NKp46 (Innate Pharma) or human CD123 (ACRO Biosystems), diluted into HBS-EP+ buffer, were injected in a 1:1 dilution series from 1.56 nmol/L to 100 nmol/L for 240 sec at a flow rate of 30 µL/min followed by a dissociation phase of 1200 sec. All analyte concentrations were run in duplicate together with multiple buffer blanks for double referencing. Regeneration of the capture surface was performed with regeneration solution (3 mol/L MgCl₂) for 60 sec at 30 µL/min. Binding kinetics data were evaluated with the Biacore T200 Evaluation Software version 3.0 (Cytiva, Uppsala) for all other antibodies using a 1:1 binding model with mass transport limitation.

For binding affinity measurements with CD16a, HBS-EP+ buffer (Cytiva, Uppsala, Catalog No. BR1006-69) was prepared by mixing 100 mL 10x HBS-EP+ buffer with 900 mL of purified water. Affinity capture of the human CD16a proteins was achieved using the His capture kit (Cytiva, Uppsala, Catalog No. 28995056). The anti-His capture antibody was

5 diluted in running buffer 1:20 and coupled to the CM5 chip (Cytiva, Uppsala, Catalog No. 29149603) using standard amine coupling to yield approximately 8000 response units (RU) using the amine coupling kit (Cytiva, Uppsala, Catalog No. BR-100-50). Ten serial 1:1 dilutions of the bispecific antibody in HBS-EP+ assay buffer were prepared to concentrations of 5.8 nmol/L, 11.7 nmol/L, 23.4 nmol/L, 46.8 nmol/L, 93.75 nmol/L, 187.5
10 nmol/L, 375 nmol/L, 750 nmol/L, 1500 nmol/L and 3000 nmol/L. The CD16a (V/F) proteins were diluted with HBS-EP+ buffer to a concentration of 0.1 ng/mL and used at this concentration in the experiments. CD16a (V176) and CD16a (V176F) were captured at a flow rate of 10 μ L/min for 30 sec on flow cells 2 and 4, respectively to yield maximal response (R_{max}) values of approximately 30 RU. Measurements were performed in
15 multicycle kinetics experiments. In each multicycle experiment CD16a was captured via an anti-His antibody immobilized on a series S CM5 sensor chip (human antibody capture kit, Cytiva, Uppsala, Catalog No. BR1008-39). The bispecific antibody diluted into HBS-EP+ buffer, was injected in a 1:1 dilution series from 5.8 nmol/L to 3000 nmol/L for 120 sec at a flow rate of 30 μ L/min followed by a dissociation phase of 120 sec. All analyte
20 concentrations were run in duplicate together with multiple buffer blanks for double referencing. Regeneration of the capture surface was performed with two consecutive injects of regeneration solution (10 mmol/L Glycine pH 1.5) for 30 sec at 30 μ L/min. Binding affinities (K_D values) of the bispecific antibody to human CD16a were evaluated with the Biacore T200 Evaluation Software version 3.0 (Cytiva, Uppsala) using a steady state fit of
25 the SPR response for the measured antibody concentrations.

A.9. Anti-tumor activity against MOLM-13 human AML injected in SCID mice.

The efficacy of muNKp46-huCD123_F25 a murine surrogate version of the NKp46-CD123_F25 was evaluated in severe-combined-immunodeficient (SCID) mice engrafted
30 with disseminated human MOLM-13 cells. This surrogate is different from NKp46-CD123_F25 for the arm targeting the NKp46 protein (as it targets the murine protein instead of the human one) and is similar to NKp46-CD123_F25 for the other arms (human CD123 binding arm and human IgG1competent Fc domain able to bind to all activating murine Fc γ Rs, to recruit murine effector cells and to induce ADCC with murine NK cells).

35 The muNKp46-huCD123_F25 activity was compared to an anti-CD123 ADCC-enhanced antibody (Reference-1) able to bind murine Fc γ Rs and to recruit murine effector

5 cells. The muNKP46-huCD123_F25 activity was also compared to an isotype control binding of muNKP46 and murine FcγRs but not to bind to huCD123 (muNKP46-IC).

Mice were intravenously inoculated with tumor cells (5×10^6) on day 0. Treatments were administered by intraperitoneal route on day 1 post tumor implantation.

10 In the first experiment (**Figure 8**), mice were randomized in 4 groups (n = 10 mice in treated groups and 20 mice in control group) on day 1 post tumor implantation. muNKP46-huCD123_F25 was administered at 0.5, 0.25 and 0.05 mg/kg following intra-parenteral administrations on day 1.

15 In the second experiment (**Figure 18**), muNKP46-IC was administered at 0.5 mg/kg. muNKP46-huCD123_F25 and Reference-1 were administered at 5, 0.5, 0.25 and 0.05 mg/kg. The control group was left untreated.

20 In the third experiment (**Figure 19**), mice were randomized into the 4 groups (untreated control group; untreated control group + anti-asialoGM1; NKCE control; or NKCE + anti-asialoGM1). A day before tumor implantation (day -1) as well as at day 5 post-tumor implantation the experimental groups received NK cell depletion antibody, anti-asialo GM1. Treatments (Vehicle or NKCE) were administered intraperitoneally on day 1 post tumor implantation at a single dose of 0.5 mg/kg. NKCE included Nkp46-CD123_F25, muNKP46-IC, an isotype control antibody binding huCD123 and murine FcγRs but not murine NKP46 (IC-huCD123).

25 Mice were checked and adverse clinical reactions noted. Individual mice were weighed daily until the end of the experiment (day 70). Mice were euthanized when turning moribund according to predefined criteria in order to avoid animal suffering. Clinical signs related to the pathology, considered as critical are limb paralysis, ascites, palpable internal tumor mass, morbidity or weight loss superior or equal to 20%.

30 The primary efficacy endpoints were the Median Survival Time (MST) in day, the percent Increased Lifespan (%ILS), and the long-term survivor rate.

Individual days of death (if any) of each mouse was reported. MST was determined for each group and the ratio ILS was calculated and expressed as percentage:

$$\%ILS = 100 \times (T - C) / C$$

Where T = MST of treated group and C = MST of control group.

35 For the purpose of this example, a dose was considered as therapeutically active when %ILS is superior to 25% and highly active when %ILS is superior to 50% (Johnson JJ,

- 5 Decker S, Zaharevitz D, Rubinstein LV, Venditti JM, Schepartz S, Kalyandrug S, et al. Relationships between drug activity in NCI preclinical in vitro and in vivo models and early clinical trials. Br. J. Cancer. 2001 May;84(10):1424-31).

Long term survivor rate is defined as the number of mice with survival duration superior or equal to 2 times the MST of control group on the total number of mice in the
10 group expressed in percentage.

A.10. Anti-tumor activity in non-human primate (NHP)

A qualified flow cytometry panel used for evaluating in monkey cynomolgus blood samples, the phenotyping and count of Basophils and total CD123 immune cells. The panel
15 was composed by the antibodies against the antigen CD45 (Clone D058-1283), CD14 (clone REA599), CD203c (clone NP4D6) , CD193 (clone 5E8) , IgE (clone REA1049), CD123 (clone CD123), CD33 (clone AC104.3E3) and the viability marker Zombie Nir (Biolegend 423106). 100µL of whole blood sample collected into a 3K-EDTA anticoagulated air-vacuum sampling were incubated with a lysis solution (Biocytex CP025) during 10 min
20 following by a centrifugation step at 300 g at room temperature during 5 min with DPBS (Sigma D8537). The resuspended cells were stained during 10 min at room temperature with the antibodies and viability marker. A third step of centrifugation were done in order to eliminate non-fixed antibodies. Cells were resuspended into 250 µL of fixative solution (Biocytex CP026) and were incubated during one hour at room temperature. 100 µL of Flow
25 count beads (Beckman A91346) were added into cells tube and were acquired on a Gallios Beckman coulter instrument equipped by 3 lasers and 10 colors.

A.11. In vitro effect on CD123+ normal blood cells and associated cytokine release in human peripheral blood mononuclear cell (PBMC)

30 PBMCs from human healthy donors (N=10) were seeded in 96-well U-bottom plates (Ultra low binding Costar ref#CLS7007) in 190 µL complete culture medium (500,000 cells per well). and incubated at 37°C in the presence of 5% CO2 for 20 hours with serial dilutions of CD123-NKCE, IC-NKCE control and CD123-TCE molecules. The basophil population, defined as TCRαβ negative, CD14 negative and IgE receptor positive viable cells, was
35 analyzed by flow cytometry and cytokine absolute concentrations released in the supernatant were analyzed by meso scale discovery (MSD) assay.

5

Flow cytometry- assay

Cell pellets were suspended in cold 50 μ L Stain Buffer (AutoMACS Running Buffer Miltenyi Ref#130-091-221) completed with 1 μ L FcR Blocking reagent, human (Miltenyi Ref#130-059-901). A mix of PBMC subsets specific labelling antibodies and the viability reagent were added into the PBMC suspension following supplier recommendations. As Fluorescence Minus One (FMO) control, further points were performed by labelling PBMC with the same mix in which each labelling antibody was replaced by its corresponding isotype control Cells with mixed antibodies were incubated 1 hour at 4°C in the dark. Then, cell suspensions were centrifuged twice at 300 g during 5 minutes at 4°C, with the supernatant discarded and 200 μ L of Stain buffer added between each centrifugation. Cells were analyzed using the MACSQuant® Analyser, Miltenyi flow cytometer. Analysis of raw data (fcs-files) exported from the flow cytometer was performed using the VenturiOne® software (AppliedCytometry inc.). The populations were gated from forward scatter/sideward scatter dotplot, single cells and further viable cells were gated on Iodure Propidium viable negative gate. Gates were set according to FMO controls.

Cytokine MSD assay

Cell supernatant was collected and diluted in MSD buffer following purchaser recommendations. Diluted samples or pre-diluted multi-analyte calibrators samples are added in pre-coated plate supplied in the kit. After adding a solution of detection antibodies conjugated with electrochemiluminescent labels (MSD SULFO-TAG), the plates were incubated at room temperature for 2 hours. Then, MSD buffer creating the appropriate chemical environment for electrochemiluminescence (ECL) was added and the plates loaded into an MSD instrument where a voltage applied to the plate electrodes causes the captured labels to emit light. The instrument measures the intensity of emitted light and provide a quantitative measure of each analyte in the samples.

Analysis of raw data exported from the MSD instrument was performed using Excel software. Concentrations of IL-6, IL-1b, IFN γ and TNF α are determined from ECL signals by back-fitting to a calibration curve established with a 4-parameter logistic model with 1/Y² weighting.

5 **A.12. Cytokine release determination in non-human primate (NHP) plasma**

 An ECLIA (Electrochemoluminescence Assay) method using Mesoscale (MSD) Proinflammatory Panel1 (NHP) kit (ref. K15056D) was developed and validated to quantify IL-2, IFN- γ , IL-6, and IL-10 in monkey K₃-EDTA plasma. It is a quantitative sandwich enzyme immunoassay using anti-human IL-2, IL-6, IL10 and IFN- γ antibodies immobilized
10 on the working electrode surface and a Ruthenium anti-human IL-2, IL-6, IL-10 and IFN- γ antibodies. 50.0 μ L of diluted samples were dispensed into the 96 microplate wells coated with the human anti-human IL-2, IL-6, IL-10 and IFN- γ antibodies. After an overnight period of incubation at room temperature and 3 steps of washing, 25.0 μ L of sulfotag conjugated anti-human IL-2, IL-6, IL-10 and IFN- γ antibodies were added. After 3 steps of
15 washing, 150 μ L of read buffer (2X) were added that creates the appropriate chemical environment for the electrochemiluminescence. The instrument measures the intensity of emitted light to provide a quantitative measure of analytes in the sample. Analyses were performed in duplicate.

20 Pharmacokinetic and pharmacodynamics study in non-human primate

 CD123-NKCE solutions for administration were prepared extemporaneously by dilution of the stock solution in the vehicle and were kept at room temperature before and during administration. To avoid adsorption, PolyPropylene, PolyCarbonate or PETG containers were used for dilutions and these containers were coated with a solution of NaCl
25 0.9% containing 100 ppm of PS80 before use. Tubing used for each intravenous dosing (syringe/winged needle) were coated by successive flushes with a solution of NaCl 0.9% containing 100 ppm of PS80.

 Animals were identified as M1 and M2 for males dosed at 0.1 mg/kg/administration, F3 and F4 for females dosed at 0.1 mg/kg/administration, M5 and M6 for males dosed at 3
30 mg/kg/administration, and F7 and F8 for females dosed at 0.1 mg/kg/administration, respectively. Dosing was performed on Days 1, 8, 15 and 22. The potential delayed onset toxicity and/or the reversibility of potential toxicity was assessed one week (Day 29) and up to 4 weeks (Day 50) after the last (4th) administration. M2, F4, M6 and F8 were euthanized and necropsied on Day 29.

35

Parameters were evaluated for each treated animal:

- 5 - in blood
 on pretest (predose)
 Day 1 at 1.5, 5, 24, 72 hours after the start of infusion
 Day 8 at 24 hours after the start of infusion
 Day 15 at 1.5 and 24 hours after the start of infusion
 10 Day 22 at 24 hours after the start of infusion
 Day 29 (1 week after the last administration; all animals)
 Day 50 (4 weeks after the last administration; recovery animals).
 - in bone marrow
 Pretest (predose)
 15 Day 9
 Day 29 (1 week after the last administration; all animals)
 Day 50 (4 weeks after the last administration; recovery animals)

Blood samples (serial sampling) were withdrawn from brachial or saphenous vein
 20 into K3-EDTA polypropylene tubes. Blood samples were placed on wet ice and centrifuged.
 Plasma samples obtained were frozen at -80°C pending their analyses. CD123-NKCE
 concentrations were determined in plasma using a dedicated immunoassay method where
 CD123-NKCE were captured by biotin-coupled CD123 recombinant proteins and revealed
 by a monkey absorbed alexa-goat anti-human IgG, with a Lower Limit of Quantification
 25 (LLOQ) value of 0.250 ng/mL.

RESULTS

B.1. NKp46-CD123_F25 binding protein

The F25 format, or its variants, is illustrated in **Figures 1 and 2**, and comprises
 30 three polypeptide chains. The NKp46-CD123_F25 binding protein comprises three
 polypeptide chains including a human CD123 binding domain and a human NKp46 binding
 domain, respectively including hypervariable regions comprising polypeptide sequences
SEQ ID NO: 1, 2, 3, 7, 8, 9 and SEQ ID NO: 13, 14, 15, 27, 28, 29.

Each polypeptide chain (I, II, and III) is expressed with a signal (or “leader”)
 35 sequence that is cleaved intracellularly before assembly.

The first polypeptide chain (or “polypeptide chain (I)” or “Fragment I” or
 “Fragment 1”) comprises from N-term to C-term, the V_L (CD123-binding) domain

5 corresponding to the amino sequence of **SEQ ID NO:43**, a native C_K (or C_K) domain derived from human IgG1, a modified human IgG1 hinge region (“DKTHTCPPCP”) wherein residue D (position according to EU numbering) is connected to the C-terminal cysteine of the human C_K domain. The Fc region or variant thereof is further derived from a native human IgG1 antibody comprising a C_{H2}-C_{H3} domain. Disulfide bridges are potentially
10 formed extracellularly with the second polypeptide chain (“chain II”) with native cysteines.

The second polypeptide chain (“polypeptide chain (II)” or “Fragment II” or “Fragment 2”) comprises, from N-term to C-term, the V_H (CD123-binding) domain corresponding to the amino sequence of **SEQ ID NO:41**, a native C_{H1} domain derived from human IgG1, an unmodified human IgG1 hinge region (“EPKSCDKTHTCPPCP”), and a
15 Fc region or variant thereof derived from a human IgG1 including a C_{H2}-C_{H3} domain wherein the last residue of the C_{H3} domain is removed and replaced by a small four amino-acid “STGS” linker, a V_H (NKp46-binding) domain corresponding to the amino sequence of **SEQ ID NO:45**, a second native C_{H1} domain which is identical to the C_{H1} domain of the first polypeptide chain, and a C-terminal hinge sequence from human IgG1.

20 The third polypeptide chain (“polypeptide chain (III)” or “Fragment III” or “Fragment 3”) comprises a V_L (NKp46-binding) domain corresponding to the amino sequence of **SEQ ID NO: 53** and a C_K domain terminated with a Cysteine.

The C_{H2} domains of the Fc part of the NKp46-CD123_F25 binding protein of the present disclosure are glycosylated at position N297 to ensure binding to CD16 (FcγR).

25 Overall, the NKp46-CD123_F25 binding protein comprises four predicted interchain disulfide bridges:

- (i) one disulfide bridge connecting the C-term C_K cysteine of the first polypeptide chain to the first hinge cysteine of the second polypeptide chain;
- (ii) two disulfide bridges formed with two cysteines of the hinge region of the
30 first and second polypeptide chains;
- (iii) one disulfide bridge connecting the last C-term cysteine of the second polypeptide chain to the C-terminal C_{H1} domain of the second polypeptide chain.

The results related to binding, *in-vitro*, *ex-vivo* and *in-vivo* activity and safety
35 profile showed in section B3 to B11 were obtained with NKp46-CD123_F25 binding protein of the present disclosure comprising polypeptide (I), (II) and (III); wherein the polypeptide

- 5 (I) consists of an amino acid sequence of **SEQ ID NO: 64**, the polypeptide (II) consists of an amino acid sequence of **SEQ ID NO: 65**, and the polypeptide (III) consists of an amino acid sequence of **SEQ ID NO: 66**.

10 **B.3. Characterization of the NKp46-CD123_F25 binding protein construct binding to human Fc- γ receptors by SPR**

The NKp46-CD123_F25 binding protein (NKp46-CD123_F25) was tested by SPR in order to confirm its affinity toward a set of human Fc γ receptors, including CD64 and two variants of the CD16a receptor.

Kd (nM)	hFc γ R1 hCD64*	hFc γ R3a-V hCD16a-V	hFc γ R3a-F hCD16a-F
NKp46-CD123_F25	6.9	462	2606

15

The human CD16a-V receptor, or CD16a^V, refers to a polypeptide construct comprising a fragment of the CD16 human receptor binding to a Fc region of a natural antibody, mediating antibody-dependent cellular cytotoxicity and bearing a Valine (V) on position 158, which is also reported in the literature as allotype CD16a V158.

20

The human CD16a-F receptor, or CD16a^F, refers to a polypeptide construct comprising a fragment of the CD16 human receptor binding to a Fc region of a natural antibody, mediating antibody-dependent cellular cytotoxicity and bearing a Phenylalanine (F) on position 158, which is also reported in the literature as allotype CD16a F158.

- 25 The conclusion of this experiment is that the constant regions which compose NKp46-CD123_F25 retains their affinity toward a plurality of human Fc- γ receptors, including human CD16 and human CD64.

B.4. Characterization of the NKp46-CD123_F25 binding protein binding to NKp46 and CD123 by SPR

30

The same experiment was performed with human and monkey versions of NKp46. The results are summarized in the two tables hereafter (Table 1 and Table 2).

5 **Table 1**

NKp46	HUMAN (hNKp46)			MONKEY (CynoNKp46)		
	k_a (1/Ms)	k_d (1/S)	K_D (nM)	k_a (1/Ms)	k_d (1/S)	K_D (nM)
NKp46-CD123_F25	5.38E+04	9.43E-04	17.5	3.64E+04	2.47E-03	67.9

Table 2

CD123	HUMAN (hCD123)			MONKEY (CynoCD123)		
	k_a (1/Ms)	k_d (1/S)	K_D (nM)	k_a (1/Ms)	k_d (1/S)	K_D (nM)
NKp46-CD123_F25	1.19E+05	2.85E-05	0.24	1.83E+05	5.29E-05	0.29

10 The conclusion of this experiment is that the NKp46-CD123_F25 binding protein related to the present disclosure (NKp46-CD123_F25) retains affinity for NKp46 and CD123 targets, which applies both to human and monkey isoforms.

B.5. NKp46-CD123_F25 binding protein induces AML cell cytotoxicity

15 **Figure 4** reports *in vitro* cytotoxicity against MOLM-13 AML cells (**Figure 4A**). The same experiment is reproduced against *ex vivo* patient blast samples as target cells (**Figure 4B**). Cytotoxicity is assessed as a function of the tested NKp46-CD123_F25 binding protein (NKp46-CD123_F25) concentration in the experiment.

Overall, the experiments show that NKp46-CD123_F25 is responsible for dose-dependent cytotoxicity in both *in vitro* and *ex vivo* tested samples. For a similar concentration, the observed cytotoxicity is also higher than the one observed with an anti-CD123 antibody ADCC-enhanced with no specificity for NKp46 (Reference-1). Conversely, a negative control variant of format F25 binding NKp46 only (NKp46-IC_F25) shows little cytotoxicity under the tested conditions. Hence, the experiments support a synergistic effect of the dual binding toward both CD123 and NKp46 in a Fc-competent construct (F25) to lead to cytotoxicity against CD123-positive tumor cells.

25

Figure 5 provides data based on EC_{50} on a MOLM-13 cell line. Improvement of *in vitro* cytotoxicity (which translates into a decreased EC_{50}) is observed with the NKp46-CD123_F25 binding protein (NKp46-CD123_F25). In contrast, the **F6** control (NKp46-CD123_F6), which lacks N-glycosylation on residue 297, provides a decreased cytotoxicity since it activates NK cells by engaging NKp46 only and not CD16a. Hence, this second

30

5 experiment provides evidence of the synergistic effect observed through binding and activation of NKp46 and CD16a NK cell markers.

Accordingly, specific lysis was illustrated with NKp46-CD123_F25, and with an anti-CD123 antibody ADCC-enhanced with no specificity for NKp46 (Reference-1) in the presence of human NK cells against CD123-positive MOLM-13 AML cells. The EC₅₀ value is established based on the variation of cell lysis over the concentration of binders. The results are shown hereafter.

Molecules	EC ₅₀ (pM)
	Mean +/- sem (4 donors)
NKp46-CD123_F25	80.3 ± 44.9
Reference-1	85.3 ± 41.3

15 Hence, it is shown that the NKp46-CD123_F25 binding protein (NKp46-CD123_F25) exhibits a cytotoxic activity which is at least equal or superior to the anti-CD123 ADCC-enhanced antibody (Reference-1).

Reference-1 is a fully-humanized monoclonal antibody indicated for the treatment of AML, which targets the alpha chain of the interleukin 3 receptor (IL3R α ; also known as CD123) and is optimized for enhanced activation of antibody-dependent cell-mediated cytotoxicity (ADCC) via natural killer cells.

B.6. CD64 expression on AML does not impact NKCE cytotoxic activity while it negatively impacts REFERENCE-1 activity

25 To further document and compare the activity of NKp46-CD123_F25 and Reference-1 antibody, cytotoxicity experiments were performed using different AML cell lines expressing CD123 as targets. Surprisingly, even though THP-1 and MOLM-13 cells express comparable level of CD123 at the cell surface, Reference-1 antibody efficiently killed MOLM-13 cells but was not active against THP-1 cells (**Figure 6A**-upper panels). Contrary to Reference-1 antibody, NKp46-CD123_F25 demonstrated comparable killing activity on both AML cell lines (**Figure 6A**-upper panels). **Figure 6A**-lower panels show that MOLM-13 and THP-1 cells differ for the expression of CD32a/b and CD64 Fc γ Rs at

5 the cell surface as analyzed by flow cytometry. MOLM-13 cells had much lower CD64 levels, and also lower levels of CD32a/b, than THP-1 cells (**Figure 6A**- lower panels). CD64 (FcγRI) is a high affinity receptor for human IgG expressed on healthy monocytes and macrophages and found expressed on AML blasts in about one third of patients which may be considered as CD64-positive Acute Myeloid Leukemia. To investigate the role of CD64
10 expression on cytotoxicity for the NK Cell engager (NKCE), compared to the humanized monoclonal antibody Reference-1, the expression of CD32a/b and CD64 was selectively knocked down in THP-1 cells.

Killing experiments performed on THP-1 sub-clones expressing CD32a/b but not CD64 or expressing CD64 but not CD32a/b (**Figure 6B**) demonstrated that CD64
15 expression on THP-1 was responsible for the inhibition of Reference-1 ADCC activity as killing of this antibody was restored only on sub-clones inactivated for CD64 expression. Accordingly, these results indicate that cis capture of antibody FC by FcγR, CD64, at the surface of AML cells interfere with ADCC probably by competing with the binding of CD16a to NK cells.

20

Interestingly, NKp46-CD123_F25 demonstrated consistent killing activity on all AML cell lines and all THP-1 sub-clones emphasizing that the NKp46-CD123_F25 binding proteins related to the present disclosure are more efficient for inducing NK cell-mediated cytotoxicity of AML blasts, as compared to Reference-1, whatever the CD64 expression
25 status.

Figure 14A-B confirm that Reference-1 activity is negatively impacted by expression of CD64 on AML cells.

Figure 14A reports the NKp46-CD123_F25 and Reference-1-mediated
30 cytotoxicity of primary malignant AML blasts from four representative patients (AML#1, #2, #5 and #6 ; N=8) which were evaluated ex vivo using healthy donor NK cells as effectors. As observed with MOLM-13 and THP-1 cell lines, Reference-1 antibody mediated the killing of CD64-negative patient samples (AML#1 and #2; **Figure 14A**) but was barely active against blasts from CD64-positive AML patient samples (AML#5 and #6; **Figure**
35 **14A**). Accordingly, AML#5- AML#6, (Reference-1-non-responders) have higher staining

- 5 levels of CD32 and CD64 than AML#1 and AML#2 (Reference-1 responders)(**Figure 14B**, right panels, compare peak shifts in CD64 and CD32 from controls in both groups).

In contrast, trifunctional NKp46-NKCEs targeting CD123 had strong anti-tumor effects on both CD64-positive and CD64-negative AML patient samples (**Figure 14**).

- 10 **Figures 6 and 14** demonstrate that CD123-NKp46_F25 was equally potent against the parental THP-1 cell line, THP-1 subclones, and MOLM-13 cells, regardless of FcγR expression status, and, more specifically, CD64 expression on target cells. Moreover, trifunctional NKCE molecules also display killing activity against all primary malignant AML cells, promoting significant antitumor activity in CD64-positive AML patient samples
15 (AML#5 and 6) against which Reference-1 was completely inactive (**Figure 14A**).

- Regarding experiments using MOLM-13 cells, trifunctional molecules (CD123-NKp46_F25) were more potent than the bispecific reagents activating NKp46 (CD123-NKp46_F6) or CD16a (CD123-IC_F25) separately (**Figure 15A**), demonstrating potent
20 killing activity (geometric mean EC₅₀ of 4.2 [95% CI: 2.7, 6.3] pM, a mean observed maximum specific lysis of 71 ± 5%) and good consistency between healthy NK cell donors (**Figure 15B**).

B.7. NK cell activation in autologous primary AML samples by NK cell engagers

- 25 The properties of NKp46-CD123_F25 binding proteins of the present disclosure (NKp46-CD123_F25) to induce NK degranulation against primary CD64(+) or CD64(-) AML blasts were established in **figure 7** through measurement of the percentage of CD107-positive NK cells.

- Overall, this experiment provides evidence that the NKp46-CD123_F25 binding
30 proteins of the present disclosure are able to activate NK cells in primary samples from AML patients in an autologous assay, e.g., with primary blasts and NK cells from the same patient.

- These results were further consolidated in a dedicated autologous NK-cell activation assay with two additional AML patient samples without (sample 10) and with (sample 8 and 9) CD64 expression (**Figure 16A**). Again, NKp46-CD123-NKCEs mediated
35 the autologous activation (see shift in CD107 staining) of the patients' NK cells against their

- own malignant cells regardless of CD64 expression status on blasts, whereas Reference-1 was active only against the CD64-negative sample (sample 10) (**Figure 16B**).

Accordingly, this experiment supports the capacity of the NKp46-CD123_F25 binding protein of the present disclosure to activate NK cells *ex vivo*, both with respect to CD64(+) and CD64(-) AML cells.

Besides, in this autologous assay, NKp46-CD123_F25 binding protein of the present disclosure are able to engage NK cells in the presence of CD64(+) cells at a much lower concentration than what is observed in the presence of Reference-1.

- In addition, mean EC₅₀ were also quantified for NKp46-CD123_F25 and Reference-1, in a blast killing assay using six NK healthy donors against four AML samples (2 CD64(+) and 2 CD64(-), and the results are reproduced here below.

	Mean EC ₅₀ CD64(-) AML blasts +/- SD (pM)	Mean EC ₅₀ CD64(+) AML blasts +/- SD (pM)
NKp46-CD123_F25	27.9 +/- 14.1	18.6 +/- 1.9
Reference-1	48.0 +/- 36.3	inactive

- Overall, these experiments again demonstrate that the blast killing activity of the NKp46-CD123_F25 binding proteins related to the present disclosure is superior to the Reference-1 antibody, even for CD64(-) AML samples, with Reference-1 being inactive against CD64(+) AML blasts.

B.8. NKp46-CD123 NK cell engagers induce anti-tumor activity against MOLM-13 human AML injected in a SCID mouse model

- Figure 8** reports dose-dependent anti-tumor activity with a muNKp46-huCD123_F25 binding protein (muNKp46-huCD123_F25) inducing 50% mice survival at 0.5 mg/kg, 70 days after tumor implantation, using a SCID mouse model. More specifically, the control group treated showed an MST of 27.5 days and 5% of long-term survivors.

- X-axis marks the number of days after tumor implantation, consisting of an intravenous injection of human MOLM-13, including a single compound administration on

5 day one by the intraperitoneal (i.p.) route. Y-axis marks the percentage of survival based on ten mice for the treated group and twenty mice for the control group. *** marks a p value < 0.001 vs. control group.

The group treated with muNkp46-huCD123_F25 at 0.5 mg/kg showed a MST of 66 days, an increased lifespan of 140% and 50% of long term survivors, muNkp46-
 10 huCD123_F25 at 0.5 mg/kg was statistically significantly active as compared to the control group (p < 0.0001). For the dose of 0.25 mg/kg, the group showed an MST of 36 days, an increased lifespan of 31% and 10% of long-term survivors, muNkp46-huCD123_F25 inducing at 0.25 mg/kg was not statistically different to the control group. For the dose of 0.05 mg/kg, the group showed an MST of 33 days, an increased lifespan of 20% and no
 15 long-term survivors, muNkp46-huCD123_F25 at 0.05 mg/kg was not statistically different to the control group.

The NKp46-CD123_F25 binding protein related to the present disclosure showed a dose-dependent anti-tumor *in-vivo* activity with a robust activity at 0.5 mg/kg. Those results are summarized in the following Table 3:

20 **Table 3**

Group	Long term survivors	Median Survival Time in days	Increase of lifespan
F25 at 0.5 mg/kg	50%	66	140%
F25 at 0.25 mg/kg	10%	36	31%
F25 at 0.05 mg/kg	0%	33	20%
Control	5%	27.5	-

Hence, this confirms that the NK cell engagers are efficient for the treatment of proliferative disorders *in-vivo* in an animal model.

25 Additionally, for further evaluation of efficacy, the experiment described above and reported in **Figure 8** was repeated but including a 5 mg/kg of muNkp46-huCD123_F25 NKCE or control as well as with an additional group administered that was Reference-1. The results are presented in **Figure 18**.

Consistent with the Figure 8 study, in the **Figure 18** study the surrogate muNkp46-huCD123_F25 induced a statistically significant activity at the doses of 5, 0.5,
 30 0.25 and 0.05 mg/kg in human MOLM-13 disseminated model, with an ILS compared to

- 5 control of 100% and 60% of long-term survivors for the doses of 5, 0.5 and 0.25 mg/kg and an ILS of 30% and 10% of long-term survivors for the dose of 0.05 mg/kg.

Reference-1 induced a statistically significant activity at the dose of 5 mg/kg in human MOLM-13 disseminated model, with an ILS of 70% and 40% of long-term survivors. It was not active at the doses of 0.5, 0.25 and 0.05 mg/kg.

- 10 The muNKP46-huCD123_F25 was statistically significantly more active than Reference-1 at the doses of 0.5 and 0.25 mg/kg.

Tabular results for **Figure 18** are summarized as follows:

Group	Long term survivors	Median Survival Time in days	Increase of lifespan
muNKP46-huCD123_F25 at 5 mg/kg	60%	> 70	> 97%
muNKP46-huCD123_F25 at 0.5 mg/kg	60%	> 70	> 97%
muNKP46-huCD123_F25 at 0.25 mg/kg	60%	> 70	> 97%
muNKP46-huCD123_F25 at 0.05 mg/kg	10%	46	30%
Reference-1 at 5 mg/kg	40%	60.5	70%
Reference 1 at 0.5 mg/kg	0%	32	0%
Reference 1 at 0.25 mg/kg	10%	37	4%
Reference 1 at 0.05 mg/kg	20%	40.5	14%
Control	0%	35.5	-

- 15 In conclusion, muNKP46-huCD123_F25 surrogate showed a dose-dependent activity with a robust activity from 0.25 mg/kg. These data demonstrated the benefit of co-engaging NK cells with NKp46/FcγRs leading to an improved in vivo efficacy relative to an anti-CD123 antibody (Reference-1).

5

B.9. NKp46-CD123 NK cell engagers induce anti-tumor activity *in vivo* in non-human primates

The absence of pro-inflammatory cytokine release of NKCE in human PBMCs *in vitro* was further confirmed in two dedicated pharmacokinetic, pharmacodynamic and safety studies performed on NHPs. Cynomolgus monkeys were selected as a relevant species for preclinical pharmacokinetics, pharmacodynamics and toxicological studies on (1) the basis of their tissue distributions of NKp46 and CD123, which are similar to those in humans (Walzer et al. PNAS; 2007; 104:3384-3398 and ChiChili et al. Sci Transl Med; 2015; 7:289) and (2) because the antibodies and Fc fragment constituting the CD123-NKCE molecule bind to cynomolgus antigens and FcγRs with affinities similar to those for human molecules as shown in the **Table 4** below. Specifically, CD16a (FcγRIIIA) has a monovalent K_D of $0.46 \pm 0.01 \mu M$ and $2.61 \pm 0.09 \mu M$ for its 158V and 158F isoforms, respectively, and anti-NKp46 and anti-CD123 antibody moieties bind to human NKp46 and human CD123 with monovalent K_D of $16.3 \pm 2.9 nM$ and $0.40 \pm 0.04 nM$, respectively.

Table 4

	K_D (nM) Mean +/- SD (n=3)	
	NKp46-CD123_F25	Human IgG1 control
Human molecules		
CD123	0.40 ± 0.02	NA
NKp46	16.6 ± 1.1	NA
Human FcRn	109 +/- 28	94 +/- 25
Human FcγRI	0.2 +/- 0.0	0.2 +/- 0.0
Human FcγRIIa	1222 +/- 99	1574 +/- 108
Human FcγRIIb	3196 +/- 375	4232 +/- 483
Human FcγRIIIaF	2606 +/- 91	2820 +/- 58
Human FcγRIIIaV	462 +/- 12	575 +/- 16
Human FcγRIIIb	6688 +/- 413	7541 +/- 838
Cynomolgus molecules		
CD123	1.2317 ± 0.132	NA
NKp46	29.038.9 ± 1.74	NA
Cynomolgus FcRn	282 +/- 41	237 +/- 22
Cynomolgus FcγRI	12 +/- 1	18 +/- 2
Cynomolgus FcγRIIa	5177 +/- 290	6336 +/- 377
Cynomolgus FcγRIIb	1891 +/- 130	2320 +/- 165
Cynomolgus FcγRIII	466 +/- 36	580 +/- 56

Displayed results in table 4 were performed at 25°C pH5.6 ; default condition : 25°C pH7.4. NA : not applicable. SD : standard deviation. K_D : Dissociation constant.

5 **Figure 9** further shows a complete and sustained CD123-positive basophil depletion at 3 µg/kg for up to 20 days after the injection of the NKp46-CD123_F25 binding protein related to the present disclosure (NKp46-CD123_F25), and up to 5 hours at 0.5 µg/kg for non-human primates.

 Accordingly, this result demonstrates that the *in vitro*, *ex vivo* and *in vivo* results
10 previously obtained, including those observed in the SCID mouse model, can be extrapolated to non-human primates for NK cell engagers.

 Overall, CD123-positive immune cell depletion is a hallmark of *in vivo* activity of the NKp46-CD123_F25 binding proteins in non-human primate. It is found that rapid and sustained depletion of CD123-positive basophils is observed, which occurs at about 1.5
15 hours from start of infusion, and is maintained up to Day 7, with cell number returning to baseline on Day 28.

B.10. Comparison of Fc-competent formats, respectively F5 vs. 25 formats in a cytotoxicity assay

20 **Figures 10A and 10B** illustrate that NKp46-CD123_F25 and NKp46-CD123_F5 binding proteins show the same cytotoxic activity against both MOLM-13 and THP-1 AML cell lines, by engaging NK cells from healthy donors (D648).

 The main difference between MOLM-13 and THP-1 cells relates to their level of expression of the CD64 marker. MOLM-13 cells do not express CD64(-), whereas THP-
25 1 cells express high level of CD64(+). Both cells express CD32a.

 Thus, this example reports the variation of specific lysis for three distinct binding proteins : (i) a NKp46-CD123_F5 binding protein in the **F5** format (NKp46-CD123_F5), (ii) a NKp46-CD123_F25 binding protein in the **F25** format (NKp46-CD123_F25), and (iii) a negative isotype control variant of format F5 binding CD123 only (CD123-IC_F5).

30 Overall, those results demonstrate that cytotoxic activity is maintained with two Fc competent NKp46-CD123 binding proteins in F5 and F25 formats. In contrast, the negative controls do not lead to detectable cytotoxic activity.

5 **B.11. NKp46-CD123 NKCEs-induced CD123-positive basophil depletion is associated to low cytokine release when compared to T-cells engager tool**

10 Potent cytotoxicity may be associated with toxicity in patients. To investigate the cytokine release from human PBMCs induced by CD123-NKCE *in vitro*, as a predictive assay of potential cytokine release syndrome (CRS) in patients, the following experiment was performed.

Human PBMCs ($N=10$ samples) were cultured for 20 hours in the presence of a NKp46-CD123_F25 (CD123-NKCE; dose of 0.1, 1, or 10 $\mu\text{g/mL}$; 0.68-68 nM), a negative isotype control variant of format F25 binding NKp46 only (NKp46-IC_F25; dose of 0.1, 1 or 10 $\mu\text{g/mL}$; 0.68-68 nM), or with an anti-CD123 T-cell engager antibody tool with specificity for CD3 and no specificity for NKp46 (CD123-TCE, Reference-1; 0.1 $\mu\text{g/mL}$; 1.6 nM) over a concentration gradient (10^{-3} to 10^1 $\mu\text{g/mL}$).

20 Amongst human PBMCs, CD123 is constitutively expressed on a subset of circulating basophils and plasmacytoid dendritic cells (pDC). Given that basophils have higher CD123⁺ expression than pDCs, the percent depletion of basophils was monitored for each treatment group indicated above. **Figure 11** shows the treatment of human PBMCs with CD123-NKCE promoted a dose-dependent partial depletion of CD123⁺ basophils with a median maximum depletion of 37% [31; 50], and a geometric mean EC₅₀ value of 38 pM (95% CI [12.9; 401]), calculated with six of 10 donor samples. In contrast, basophil depletion did not occur in a considerable amount in the presence of a F25 binding molecule lacking a CD123 binding site (NKp46-IC_F25). From the human PBMCs treatment groups indicated above, the supernatant was collected to quantify the amount of cytokine release. **Figure 12** demonstrates that the *in vitro* IL-6, and IL-1 β pro-inflammatory cytokine and TNF- α and IFN- γ cytokine release associated to the administration of the NKp46-CD123_F25 binding protein related to the present disclosure (NKp46-CD123_F25) was much lower than the corresponding IL-6 release associated with the administration of a positive control known to induce cytokine release syndrome (CRS), Reference-1, at a 100-fold lower dose (0.1 $\mu\text{g/mL}$).

30 CD123-NKCE induced much lower levels of cytokine release than CD123-TCE, even at concentrations 42-times higher.

35 **Figure 11 and 12** demonstrate that the treatment of PBMCs with CD123-NKCEs promoted a depletion of CD123⁺ basophils but induced much lower levels of IL-6, IL-1 β , TNF- α and IFN- γ release than treatment with CD3-CD123 antibody molecule.

5 In conclusion, the NKp46-CD123_F25 binding protein related to the present disclosure (NKp46-CD123_F25) has shown its ability to engage primary NK cells to target and kill CD123+ primary normal mononuclear blood cells associated with minor cytokine release and may have a better benefit/risk profile than TCEs for the treatment of AML.

10 Regardless of the dose level and up to a high dose of 3 mg/kg, **Figure 13A – Figure 13F** show that very low cytokine release (IL6 and IL10) was observed in all treated animals (male Cynomolgus monkeys) after the start of the injection of NKp46-CD123_F25 binding protein related to the present disclosure (NKp46-CD123_F25), without any associated clinical signs. No IL-2 nor IFN γ cytokine release were detected. More
15 particularly, transient IL6 and IL10 peaks are detected in non-human primates after a single intravenous injection of F25 constructs at 3 mg/kg. This transient peak occurs from 1 hour to 5 hours and returns to baseline within 1 or 2 days.

Also, those very low levels of IL-6 and IL-10 cytokine release are not associated with clinical signs up to 3 mg/kg dose. This indicates that such NK cell engagers possess a
20 good safety profile in non-human primates.

B.12. NKp46-CD123 NK cell engagers promote NK-cell activation *in vitro* that is commensurate with cytokine/chemokine production

Flow cytometric analysis corresponding to **Figure 17** demonstrate that NKp46-
25 CD123_F25 binding proteins promoted NK-cell activation only when CD123 expressing target cells are present.

Primary donor NK cells (N=3) incubated in the presence of NKp46-CD123_F25 displayed higher expression levels of NK cell activation markers, CD107 and CD69, as well as cytokines, TNF- α , IFN- γ , and chemokine, MIP-1 β , in a dose-dependent manner, when
30 MOLM-13 target cells were present (**Figure 17**, comparing NK alone vs NK + MOLM-13 condition).

Overall, this experiment provides evidence that the NKp46-CD123_F25 binding proteins of the present disclosure activate and commensurately promote cytokine/chemokine production in primary NK cells towards CD123+ AML cells with no off-target activation of
35 NK cells.

5 **B. 13. NK cells are the effector lymphocyte subset responsible for the antitumor activity of NKp46-CD123_F25**

To test whether the efficacy of the NKp46-CD123_F25 depended on NK cells anti-tumor activity against MOLM-13 human AML injected in a SCID mouse model, mice underwent an NK cell depletion regimen during the experimental set-up outlined in B.8.

10 The results are presented on **Figure 18** and **Figure 19** and corresponding tabular results are shown in table 5 below.

Table 5

Group	Long term survivors	Median Survival Time in days	Increase of lifespan
muNKp46-IC	0%	31	7%
muNKp46-IC + anti-aGM1	10%	23.5	≤ 0%
IC-huCD123	20%	37	28%
IC-huCD123 + anti-aGM1	0%	25	≤ 0%
muNKp46-huCD123_F25	40%	53.5	84%
muNKp46-huCD123_F25 + anti-aGM1	0%	33	14%
Control	0%	29	-

15 The control group treated showed a median survival time (MST) of 29 days and no long-term survivors.

The muNKp46-IC isotype control did not show activity with an ILS of 7% and no long-term survivors. No impact of the NK depletion is observed on the group treated with muNKp46-IC isotype control with no increased lifespan and 10% of long-term survivors. The IC-huCD123 isotype control was statistically significantly active with an ILS of 28% and 20% of long-term survivors. A statistically significant impact of the depletion was

5 observed on the group treated with IC-huCD123 isotype control with no increased lifespan and no long-term survivors.

The muNKp46-huCD123_F25 was statistically significantly active with an ILS of 84% and 40% of long-term survivors. A statistically significant impact of the depletion was observed on the group treated with muNKp46-huCD123_F25 with an ILS of 14% and
10 no long-term survivors.

In conclusion, the NK depletion impacted the anti-tumoral activity of muNKp46-huCD123_F25 confirming the NK involvement as effector cells in muNKp46-huCD123_F25 NKCE in vivo efficacy.

15 **B. 14. NKp46-CD123 NK cell engagers are safe and efficient in NHPs**

To confirm the safety profile of the Nkp46-CD123 cell engagers in the NHP study conducted in **Figure 9** and **Figure 13** interrogating the pharmacokinetics and pharmacodynamics of CD123-NKCEs administered by a single i.v. injection of a high (3 mg/kg) or low (3 µg/kg and 0.5 µg/kg) doses in male cynomolgus monkeys (2 animals each
20 for the 3 mg/kg and 3 µg/kg doses and 1 animal for the 0.5 µg/kg dose).

Treatment with CD123-NKCE promoted a sustained and complete depletion of CD123⁺ cells in the blood of all monkeys, for more than 10 days, at both the 3 mg/kg and 3 µg/kg doses (as exemplified for CD123⁺ basophils and total CD123⁺ cells in **Figure 20A** and **Figure 20B**), with only very small amounts (< 50 pg/mL) of the pro-inflammatory
25 cytokines IL-6 and IL-10 released (**Figure 20C**) without any associated clinical signs.

A transient and partial depletion of CD123⁺ cells was observed in the monkey treated at the lowest dose (0.5 µg/kg, data not shown), but 3 µg/kg was considered to be the lowest effective dose in this species. The PK profiles of the two monkeys treated at the highest dose (3 mg/kg) were marked by an anti-drug antibody (ADA) response (data not shown) occurring
30 12-14 days after treatment (**Figure 20D**) and associated with the recovery of CD123⁺ cells from the blood at later timepoints.

The preclinical safety profile of CD123-NKCE was further interrogated through an exploratory repeat-dose toxicity study in which four monkeys (2/sex/dose) were treated weekly, for four weeks, at a dose of 3 mg/kg/administration or 0.1 mg/kg/administration,
35 administered by intravenous infusion for one hour (**Figure 21**). In all monkeys (except one of the monkeys, monkey M5, male No. 5; **Figure 21**), exposure to CD123-NKCEs lasted for

- 5 at least two weeks, at both tested doses, with the presence of Anti-Drug Antibody (ADA) detected (data not shown) from the third administration (Day 15) (Table 6).

Table 6 below displays individual CD123-NKCE plasma concentration values after a weekly repeat 1-hour intravenous infusion at 0.1 and 3 mg/kg/administration for 4 weeks (on Days 1, 8, 15 and 22) to cynomolgus monkeys.

10

Table 6

Day	Sampling	Concentration in plasma (ng/mL)							
		0.1 mg/kg/administration				3 mg/kg/administration			
		M1♂	M2♂	F3♀	F4♀	M5♂	M6♂	F7♀	F8♀
1	Predose	<LLO Q	<LLO Q	<LLO Q	<LLO Q	2.27	<LLO Q	<LLO Q	<LLO Q
1	1h	1750	1200	1550	1870	37000	68700	100000	46200#
1	1.5h	1100	1520	1390	1750	78600	67000	98600	48300#
1	5h	736	1350	986	469	64100	72200	76300	47500#
2	24h	160	381	250	448	221000#	30800	55200	41500#
4	72h	1.66	9.17	7.83	14.8	31600	31100	27500	29100#
8	168h/Predose	<LLO Q	0.484	0.698	1.18	16000	17300	20100	37800
8	1h	1340	3.32	1980	1610	92000	91900	137000	87500
8	1.5h	1250	29.6	1680	1460	89900	88400	110000	82500
8	5h	892	132	1280	1210	38600	72900	74800	88300
9	24h	239	78.2	554	512	64500	61200	46100	65200
11	72h	1.17	35.7	43.8	20.8	46300	47100	23000	30500
15	168h/Predose	<LLO Q	<LLO Q	2.45	<LLO Q	45000	51.8	<LLO Q	<LLO Q
15	1h	19.3	205	256	5.61	124000	21600	2310	11900
15	1.5h	5.53	105	190	3.30	75900	33600	497	9800
15	5h	<LLO Q	22.2	157	<LLO Q	85400	10600	246	1170
16	24h	<LLO Q	0.333	35.1	<LLO Q	51700	300	<LLO Q	37.9
18	72h	US	US	US	US	US	US	US	US
22	168h/Predose	<LLO Q	<LLO Q	<LLO Q	<LLO Q	9120	<LLO Q	<LLO Q	1.09
22	1h	0.998	<LLO Q	<LLO Q	<LLO Q	61600	365	<LLO Q	149
22	1.5h	<LLO Q	<LLO Q	<LLO Q	<LLO Q	55500	228	<LLO Q	53.7

22	5h	<LLO Q	<LLO Q	<LLO Q	<LLO Q	44000	26.3	<LLO Q	<LLOQ
23	24h	<LLO Q	<LLO Q	<LLO Q	<LLO Q	26100	<LLO Q	<LLO Q	<LLOQ
25	72h	US	US	US	US	US	US	US	US
29	168h	<LLO Q	<LLO Q	<LLO Q	<LLO Q	110	<LLO Q	<LLO Q	0.525 #

5 Values are rounded to 3 significant figures.

LLOQ (Lower Limit Of Quantification) 0.250 ng/mL; # aberrant value excluded for TK analysis; US :unscheduled sampling; ## Given as indicative due to a technical issue during 1-hour infusion on Day 1 (i.e., 50% of the dose received subcutaneously)

10 Transient minimal increases in IL-6 concentration were observed after each weekly administration, for both doses (**Table 7** maximum levels of 25 and 160 pg/mL for doses of 0.1 and 3 mg/kg/administration, respectively). **Table 7** displays individual IL-6 plasma concentration values after a weekly repeat 1-hour intravenous infusion of CD123-NKCE at 0.1 and 3 mg/kg/administration for 4 weeks (on Days 1, 8, 15 and 22) to cynomolgus monkeys.

15 **Table 7**

Day	Sampling Time pre or post dose	IL-6 concentration in plasma (pg/mL)							
		0.1 mg/kg/administration				3 mg/kg/administration			
		M1♂	M2♂	F3♀	F4♀	M5♂	M6♂	F7♀	F8♀
1	Predose	<LLOQ	<LLOQ	0.84	0.54	<LLOQ	<LLOQ	<LLOQ	2.09
1	1h	1.52	1.92	3.12	4.65	1.49	1.61	2.63	7.99
1	1.5h	2.99	4.54	5.09	6.79	2.17	2.98	5.61	14.37
1	5h	1.83	3.00	3.17	3.55	1.57	8.92	11.41	15.86
2	24h	<LLOQ	<LLOQ	0.66	1.98	0.79	1.96	2.15	1.61
8	168h/Predose	<LLOQ	<LLOQ	<LLOQ	<LLOQ	0.73	1.63	1.52	0.57
8	1h	1.42	2.12	5.04	2.60	1.65	2.35	2.74	4.48
8	1.5h	2.97	18.84	9.85	2.46	1.90	2.5	3.92	8.50
8	5h	2.15	18.89	7.69	1.39	1.53	38.04	2.34	2.71
9	24h	0.92	4.23	1.18	1.56	0.68	3.98	11.58	1.03
15	168h/Predose	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	<LLOQ	0.62	1.58
15	1h	1.20	1.98	2.93	1.63	2.17	3.14	7.67	68.51
15	1.5h	1.47	1.88	5.72	1.65	2.35	5.02	12.19	85.36
15	5h	2.33	1.33	1.57	1.33	5.53	2.01	4.09	7.11
16	24h	<LLOQ	<LLOQ	1.48	6.47	2.76	<LLOQ	1.40	0.71
22	168h/Predose	<LLOQ	<LLOQ	<LLOQ	<LLOQ	1.16	<LLOQ	1.34	1.11

22	1h	2.22	2.79	1.35	4.07	3.12	123.36	27.39	150.97
22	1.5h	3.54	3.52	2.23	5.08	2.83	121.88	51.43	159.71
22	5h	2.68	4.42	2.89	22.72	2.56	4.07	6.35	22.23
23	24h	<LLOQ	1.53	<LLOQ	0.81	4.52	<LLOQ	1.10	1.29
29	168h	0.79	<LLOQ	<LLOQ	<LLOQ	<LLOQ	0.74	<LLOQ	0.69

5 LLOQ (Lower Limit Of Quantification): 0.53 pg/mL

In particular, no significant IL-6 release was observed in monkey M5 treated at the high dose (3 mg/kg/administration) which did not exhibit ADA response and was exposed to CD123-NKCE throughout the study (**Figure 21A** and **Figure 21B**), whereas strong PD effects of CD123⁺ cell depletion were observed in both blood and bone marrow of this monkey (**Table 8** and **Figure 21C**).

Table 8 displays the individual absolute counts of basophils and total CD123-positive cells in blood and bone marrow after a weekly repeat 1-hour intravenous infusion of CD123-NKCE at 0.1 and 3 mg/kg/administration for 4 weeks to cynomolgus monkeys.

Day	Sampling Time	0.1 mg/kg/administration				3 mg/kg/administration			
		M1♂	M2♂	F3♀	F4♀	M5♂	M6♂	F7♀	F8♀
		Absolute count of CD123-positive basophils in blood (cells/μL)							
	Predose	1.16	2.79	8.60	2.25	5.65	11.6	15.1	<DL
1	1.5h	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
1	5h	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
2		<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
4		<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
9		<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
15	1.5h	<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
16		1.90	<DL	<DL	<DL	<DL	<DL	1.87	<DL
23		1.56	<DL	<DL	7.61	<DL	10.4	34.7	9.58
29		11.8	3.30	<DL	34.5	<DL	24.9	62.3	17.0
50		4.19	nd	6.30	nd	13.1	nd	12.9	nd
		Absolute count of total CD123-positive cells in blood (cells/μL)							
	Predose	6.26	6.32	21.9	4.02	9.72	22.8	19.3	1.59
1	1.5h	5.90	1.83	3.01	3.74	1.50	<DL	1.18	1.24
1	5h	4.06	1.40	<DL	1.90	<DL	<DL	<DL	<DL
2		3.79	2.00	<DL	2.47	<DL	2.08	2.30	1.48
4		10.3	1.95	2.47	2.43	1.54	1.32	1.84	2.80
9		1.44	<DL	<DL	<DL	<DL	<DL	<DL	<DL
15	1.5h	5.77	2.16	2.40	3.25	1.15	8.14	12.9	12.3
16		17.7	3.77	2.97	6.42	1.43	7.16	10.2	8.91
23		18.5	8.33	9.52	12.2	1.28	58.5	49.7	32.4
29		33.9	18.2	29.5	47.0	4.15	82.9	72.1	38.1

50		8.81	nd	14.5	nd	16.8	nd	17.3	nd
Absolute count of CD123-positive basophils in bone marrow (cells/ μ L)									
	Predose	439	11.5	30.2	30.0	21.4	14.7	155	21.3
9		<DL	<DL	<DL	<DL	<DL	<DL	<DL	<DL
29		74.5	25.6	50.2	89.2	<DL	50.0	235.6	131
50		54.0	nd	185	nd	29.6	nd	43.9	nd
Absolute count of total CD123-positive cells in bone marrow (cells/ μ L)									
	Predose	914	19.8	45.9	51.1	61.6	28.9	218	41.6
9		5.85	<DL	<DL	<DL	<DL	<DL	<DL	<DL
29		140	49.6	192	142	68.4	98.1	311	200.8
50		112	nd	402	nd	50.9	nd	71.6	nd

5 DL (detection limit) : 1.15 cells/ μ L (blood) or 2.10 cells/ μ L (bone marrow); nd : not done

In all the other animals, a sustained depletion of CD123-expressing cells was observed in the blood 1.5 hours after the first administration and up to 24 hours after the 3rd administration, with a rebound (above baseline) observed on days 22 to 29 (**Table 8**).
 10 Moreover, all the treated monkeys presented a complete depletion of CD123-positive cells from the bone marrow on day 9 (24 hours after the second administration), for both doses (**Table 8**; values below the detection limit on day 9 for most of animals), with a restoration of CD123-positive populations at day 29, one week after the last administration.

No clinical signs, changes in body weight or body temperature, and no effects on
 15 ECG potentially attributable to treatment with CD123-NKCE were observed, whatever the dose. No compound-related adverse effects on hematological, coagulation, clinical chemistry, or urinary parameters were observed either. Microscopic examination of the tissues sampled revealed no evidence of organ targeting, and all observations noted were considered to lie within the background range of variation and to be unrelated to the
 20 administration of the CD123-NKCE.

Overall, these results thus constitute proof-of-principle for the efficacy of CD123-NKCE *in vivo*, with no signs of toxicity.

B. 15. NKp46-CD123 NK cell engager tumor cell killing with healthy donor NK cells

25 NKp46-CD123_F25 and its isotype control IC-CD123_F6 were tested in an *in vitro* tumor cell killing assay with NK cells taken from 2 different healthy donors (HD).

NK cells purification and AML cell line

Human peripheral blood mononuclear cells (PBMC) from anonymous healthy donors (HD) were isolated by Ficoll density gradient centrifugation. NK cells were purified

5 from these PBMCs with MACSxpress® Whole Blood NK cells isolation kit (Miltenyi Biotec). The NK cells were rested overnight in RPMI1640 (Gibco) supplemented with 10 % SVF (BioWest) and 1 % L-Glutamine (Gibco).

THP1 cells (CD123+, CD64+) were chosen for this study based on their expression of CD123. Before the experiment, THP1 cells were infected with Incucyte® Nuclight Green
10 Lentivirus (Sartorius) to express the Green Fluorescent Protein (GFP).

NK functional assay in presence of NKp46-CD123_F25 over time

NK cells and THP1 GFP target cells were incubated in presence of NKp46-CD123_F25 or its isotype control IC-CD123_F6 at 0.1, 1, 10 and 100 ng/mL at 37°C. The ratio effector:target cells was 1:1. The medium used was the same as for NK cell culture.
15 The target cells were monitored by florescence imaging over 74h using Incucyte Live Cell Analysis system (EssenBioscience). The number of live target cells was quantified using IncucyteS3 software (2020B version).

Conclusion

20 As shown in **Figure 22**, NKp46-CD123_F25 at different concentrations (1, 10 and 100 ng/mL) enhances the cytotoxic activity of HD NK cells against THP1 GFP AML cells over time at the effector:target cells ratio of 1:1.

5

CLAIMS

1. A binding protein comprising a first and a second antigen binding domain (ABD) and all or part of an immunoglobulin Fc region or variant thereof, wherein each of said ABD comprises an immunoglobulin heavy chain variable domain (VH) and an immunoglobulin light chain variable domain (VL), wherein each VH and VL comprises three complementary determining regions (CDR-1 to CDR-3); and wherein:
- 10 (i) the first ABD binds specifically to human CD123 and comprises:
- a VH1 comprising a CDR-H1, H2 and H3 corresponding to the amino acid sequences of **SEQ ID NO: 1 to 3** respectively or corresponding to the amino acid sequences of **SEQ ID NO: 4 to 6** respectively, and
 - 15 - a VL1 comprising a CDR-L1, L2 and L3 corresponding to the amino acid sequences of **SEQ ID NO: 7 to 9** respectively or corresponding to the amino acid sequences of **SEQ ID NO: 10 to 12** respectively;
- (ii) the second ABD binds specifically to human NKp46 and comprises:
- a VH2 comprising a CDR-H1, 2 and 3 corresponding to:
 - 20 the amino acid sequences of **SEQ ID NO: 13 to 15** respectively;
 - the amino acid sequences of **SEQ ID NO: 16 to 18** respectively;
 - the amino acid sequences of **SEQ ID NO: 19 to 21** respectively;
 - the amino acid sequences of **SEQ ID NO: 22 to 24** respectively; or
 - the amino acid sequences of **SEQ ID NO: 16, 25 and 26** respectively;
 - 25 and
 - a VL2 comprising a CDR-L1, 2 and 3 corresponding to:
 - the amino acid sequences of **SEQ ID NO: 27 to 29** respectively;
 - the amino acid sequences of **SEQ ID NO: 30 to 32** respectively;
 - the amino acid sequences of **SEQ ID NO: 33 to 35** respectively;
 - 30 the amino acid sequences of **SEQ ID NO: 36 to 38** respectively; or
 - the amino acid sequences **SEQ ID NO: 39, 31 and 40** respectively;
 - and wherein all or part of the immunoglobulin Fc region or variant thereof binds to a human Fc- γ receptor.

- 5 2. The binding protein according to claim 1, comprising three polypeptide chains (I), (II) and (III) that form two ABDs, as defined below:



10 wherein :

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

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

C_{1A} and C_{1B} form a pair C_1 (C_{H1}/C_L) and C_{2A} and C_{2B} form a pair C_2 (C_{H1}/C_L) wherein C_{H1} is an immunoglobulin heavy chain constant domain 1 and C_L is an immunoglobulin light chain constant domain;

15 Hinge₁, Hinge₂ and Hinge₃ are identical or different and correspond to all or part of an immunoglobulin hinge region;

($C_{H2}-C_{H3}$)_A and ($C_{H2}-C_{H3}$)_B are identical or different, and comprise an immunoglobulin heavy chain constant domain 2 (C_{H2}) and an immunoglobulin heavy chain constant domain 3 (C_{H3});

20 L₁ is an amino acid linker.

3. The binding protein according to claim 2 wherein:

C_{1B} is an immunoglobulin heavy chain constant domain 1 (C_{H1});

25 C_{2A} is an immunoglobulin heavy chain constant domain 1 (C_{H1});

C_L corresponds to an immunoglobulin kappa light chain constant domain (C_K);

($C_{H2}-C_{H3}$)_A corresponds to the amino acid sequence of **SEQ ID NO: 69** ;

($C_{H2}-C_{H3}$)_B corresponds to the amino acid sequence of **SEQ ID NO: 70**;

Hinge₁ corresponds to the amino acid sequence of **SEQ ID NO:74**;

30 Hinge₂ corresponds to the amino acid sequence of **SEQ ID NO:75**;

Hinge₃ corresponds to the amino acid sequence of **SEQ ID NO: 77**;

L₁ corresponds to the amino acid sequence of **SEQ ID NO: 76**.

4. The binding protein according to any one of the preceding claims, wherein the
35 residue N297 of the Fc region or variant thereof according to EU numbering comprises a N-linked glycosylation.

5

5. The binding protein according to any one of the preceding claims, wherein the all or part of the Fc region or variant thereof binds to a human CD16A (FcγRIII) polypeptide.

6. The binding protein according to anyone of claims 2 to 5, comprising at least two
10 polypeptide chains linked by at least one disulfide bridge.

7. The binding protein according to claim 6, wherein the polypeptide chains (I) and (II) are linked by at least one disulfide bridge between C_{1A} and Hinge₂ and/or wherein the polypeptide chains (II) and (III) are linked by at least one disulfide bridge between Hinge₃
15 and C_{2B}.

8. The binding protein according to anyone of claims 2 to 7, wherein V_{1A} is V_{L1} and V_{1B} is V_{H1}.

9. The binding protein according to anyone of claims 2 to 8, wherein V_{2A} is V_{H2} and V_{2B} is V_{L2}.
20

10. The binding protein according to any one of the preceding claims, wherein:

(a) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 1**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 2**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 3**; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 7**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 8**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 9**; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 13**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 14**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 15**; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 27**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 28**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 29**;
25
30

(b) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 1**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 2**; a CDR-H3 comprising
35

5 the amino acid sequence of **SEQ ID NO: 3**; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 7**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 8**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 9**; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 16**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 17**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 18**; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 30**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 31**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 32**;

(c) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 1**;
15 a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 2**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 3**; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 7**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 8**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 9**; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 19**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 20**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 21**; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 33**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 34**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 35**;

25 (d) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 1**;
a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 2**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 3**; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 7**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 8**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 9**; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 22**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 23**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 24**; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 36**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 37**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 38**;

35

5 (e) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 1**;
a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 2**; a CDR-H3 comprising
the amino acid sequence of **SEQ ID NO: 3**; V_{L1} comprises a CDR-L1 comprising the amino
acid sequence of **SEQ ID NO: 7**; a CDR-L2 comprising the amino acid sequence of **SEQ**
ID NO: 8; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 9**; V_{H2} comprises
10 a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 16**; a CDR-H2 comprising
the amino acid sequence of **SEQ ID NO: 25**; a CDR-H3 comprising the amino acid sequence
of **SEQ ID NO: 26**; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of **SEQ**
ID NO: 39; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 31**; a CDR-L3
comprising the amino acid sequence of **SEQ ID NO: 40**;

15

(f) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 4**;
a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 5**; a CDR-H3 comprising
the amino acid sequence of **SEQ ID NO: 6**; V_{L1} comprises a CDR-L1 comprising the amino
acid sequence of **SEQ ID NO: 10**; a CDR-L2 comprising the amino acid sequence of **SEQ**
20 **ID NO: 11**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 12**; V_{H2}
comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 13**; a CDR-H2
comprising the amino acid sequence of **SEQ ID NO: 14**; a CDR-H3 comprising the amino
acid sequence of **SEQ ID NO: 15**; V_{L2} comprises a CDR-L1 comprising the amino acid
sequence of **SEQ ID NO: 27**; a CDR-L2 comprising the amino acid sequence of **SEQ ID**
25 **NO: 28**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 29**;

(g) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 4**;
a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 5**; a CDR-H3 comprising
the amino acid sequence of **SEQ ID NO: 6**; V_{L1} comprises a CDR-L1 comprising the amino
acid sequence of **SEQ ID NO: 10**; a CDR-L2 comprising the amino acid sequence of **SEQ**
30 **ID NO: 11**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 12**; V_{H2}
comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 16**; a CDR-H2
comprising the amino acid sequence of **SEQ ID NO: 17**; a CDR-H3 comprising the amino
acid sequence of **SEQ ID NO: 18**; V_{L2} comprises a CDR-L1 comprising the amino acid
sequence of **SEQ ID NO: 30**; a CDR-L2 comprising the amino acid sequence of **SEQ ID**
35 **NO: 31**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 32**;

5

(h) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 4**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 5**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 6**; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 10**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 11**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 12**; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 19**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 20**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 21**; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 33**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 34**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 35**;

(i) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 4**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 5**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 6**; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 10**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 11**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 12**; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 22**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 23**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 24**; V_{L2} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 36**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 37**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 38**; or

(j) V_{H1} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 4**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 5**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 6**; V_{L1} comprises a CDR-L1 comprising the amino acid sequence of **SEQ ID NO: 10**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 11**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 12**; V_{H2} comprises a CDR-H1 comprising the amino acid sequence of **SEQ ID NO: 16**; a CDR-H2 comprising the amino acid sequence of **SEQ ID NO: 25**; a CDR-H3 comprising the amino acid sequence of **SEQ ID NO: 26**; V_{L2} comprises a CDR-L1 comprising the amino acid

5 sequence of **SEQ ID NO: 39**; a CDR-L2 comprising the amino acid sequence of **SEQ ID NO: 31**; a CDR-L3 comprising the amino acid sequence of **SEQ ID NO: 40**.

11. The binding protein according to any one of the preceding claims, wherein:

(a) V_{H1} and V_{L1} corresponds to the amino acid sequences of **SEQ ID NO: 41** and **43**
10 respectively or corresponds to the amino acid sequences of **SEQ ID NO: 42** and **44** respectively;
and/or

(b) V_{H2} and V_{L2} corresponds to
the amino acid sequences of **SEQ ID NO: 45** and **53** respectively;
15 the amino acid sequences of **SEQ ID NO: 46** and **54** respectively;
the amino acid sequences of **SEQ ID NO: 47** and **55** respectively;
the amino acid sequences of **SEQ ID NO: 48** and **56** respectively;
the amino acid sequences of **SEQ ID NO: 49** and **57** respectively;
the amino acid sequences of **SEQ ID NO: 50** and **58** respectively;
20 the amino acid sequences of **SEQ ID NO: 51** and **59** respectively; or
the amino acid sequences of **SEQ ID NO: 52** and **60** respectively.

12. The binding protein according to claim 11, wherein:

(q) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 41**; V_{L1} comprises the
25 amino acid sequence of **SEQ ID NO: 43**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 45**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 53**;

(r) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 41**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 43**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 46**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 54**;

30 (s) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 41**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 43**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 47**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 55**;

(t) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 41**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 43**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 48**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 56**;

- 5 (u) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 41**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 43**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 49**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 57**;
- (v) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 41**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 43**; V_{H2} comprises the amino acid sequence of **SEQ**
- 10 **ID NO: 50**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 58**;
- (w) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 41**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 43**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 51**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 59**;
- (x) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 41**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 43**; V_{H2} comprises the amino acid sequence of **SEQ**
- 15 **ID NO: 52**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 60**;
- (y) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 42**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 44**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 45**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 53**;
- 20 (z) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 42**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 44**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 46**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 54**;
- (aa) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 42**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 44**; V_{H2} comprises the amino acid sequence of **SEQ**
- 25 **ID NO: 47**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 55**;
- (bb) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 42**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 44**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 48**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 56**;
- (cc) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 42**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 44**; V_{H2} comprises the amino acid sequence of **SEQ**
- 30 **ID NO: 49**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 57**;
- (dd) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 42**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 44**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 50**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 58**.

5 (ee) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 42**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 44**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 51**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 59**;

(ff) V_{H1} comprises the amino acid sequence of **SEQ ID NO: 42**; V_{L1} comprises the amino acid sequence of **SEQ ID NO: 44**; V_{H2} comprises the amino acid sequence of **SEQ ID NO: 52**; V_{L2} comprises the amino acid sequence of **SEQ ID NO: 60**.

10

13. The binding protein according to any one of claims 2 to 12, wherein:

- polypeptide (I) consists of an amino acid sequence of **SEQ ID NO: 64**;
- polypeptide (II) consists of an amino acid sequence of **SEQ ID NO: 65**; and
- 15 - polypeptide (III) consists of an amino acid sequence of **SEQ ID NO: 66**.

14. The binding protein according to any one of the preceding claims, for use as a medicament.

20 15. The binding protein according to any one of the preceding claims, for use in a method for the treatment or prevention of blood cancer.

16. The binding protein according to any one of the preceding claims, for use in a method for the treatment or prevention of a myelodysplastic syndrome (MDS) or of a lymphoproliferative disorder.

25

17. The binding protein according to any one of the preceding claims, for use in a method for the treatment or prevention of Acute Myeloid Leukemia (AML).

30 18. The binding protein according to any one of the preceding claims, for use in a method for the treatment or prevention of CD64-positive and CD64-negative Acute Myeloid Leukemia (AML).

19. A pharmaceutical composition comprising the binding protein according to any one of claims 1 to 13 and a pharmaceutically acceptable carrier.

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- 5 20. An isolated nucleic acid molecule comprising a nucleotide sequence that encodes the binding protein according to any one of claims 1 to 13.
21. An expression vector comprising the nucleic acid molecule of claim 20.
- 10 22. An isolated cell comprising the nucleic acid molecule of claim 20.
23. An isolated cell comprising the expression vector of claim 21.
24. The isolated cell of claim 23, wherein the host cell is a mammalian cell.
- 15 25. A method for making the binding protein according to any one of claims 1 to 13, comprising a step of:
- (a) culturing host cell(s) under conditions suitable for expressing **a plurality** of recombinant polypeptides, said plurality comprising (i) a polypeptide comprising an amino acid sequence of **SEQ ID NO: 64, and** (ii) a polypeptide comprising an amino acid sequence of **SEQ ID NO: 65, and** (iii) a polypeptide comprising an amino acid sequence of **SEQ ID NO: 66;**
- 20 (b) optionally recovering the expressed recombinant polypeptides.
- 25 26. A method of treating or preventing a blood cancer, the method comprising administering to a subject in need of said treatment or prevention the pharmaceutical composition of claim 19.
27. A method of treating or preventing a myelodysplastic syndrome (MDS) or a lymphoproliferative disorder, the method comprising administering to a subject in need of said treatment or prevention the pharmaceutical composition of claim 19.
- 30 28. A method of treating or preventing an Acute Myeloid Leukemia (AML), the method comprising administering to a subject in need of said treatment or prevention the pharmaceutical composition of claim 19.
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5 29. A method of treating or preventing a CD64-positive and CD64-negative Acute
Myeloid Leukemia (AML), the method comprising administering to a subject in need of said
treatment or prevention the pharmaceutical composition of claim 19.

 30. A method of treating or preventing a CD64-positive Acute Myeloid Leukemia
10 (AML), the method comprising administering to a subject in need of said treatment or
prevention a binding protein comprising a first and a second antigen binding domain (ABD)
and all or part of an immunoglobulin Fc region or variant thereof, wherein the first ABD
binds specifically to human CD123, the second ABD binds specifically to human NKp46,
and wherein all or part of the immunoglobulin Fc region or variant thereof binds to a human
15 Fc- γ receptor.