

CD16A is an activating receptor triggering the cytotoxic activity of NK cells. The affinity of antibodies for CD16A directly correlates with their ability to trigger NK cell activation, thus reducing the antibody dose required for activation.

The cytotoxic activity of NK cells can be increased by increasing the avidity through multivalent binding to CD16A, e.g., bivalent binding to CD16A.

However, bivalency and multivalency for CD16A may result in antibody-mediated cross-linking of CD16A-expressing cells, including NK cells, $\gamma\delta$ T cells and subsets of monocytes, macrophages and dendritic cells. Such interactions are expected to result in undesired cellular stimulation via CD16A. For example, cross-linking of NK cells with CD16A-expressing immune cells results in NK cell activation, cytokine release and induction of cell-directed cytotoxicity, which reduces NK cell activity and accelerates NK cell exhaustion that is independent of the desired anti-tumor effect. Hence, NK cell cross-linking with CD16A-expressing immune cells is expected to reduce therapeutic efficacy of NK cell-engagement. Most importantly, cross-linking of two or more NK cells through bivalent or multivalent interaction with CD16A can cause NK cell activation and induction of fratricide (NK-NK cell lysis) ultimately resulting in efficient NK cell depletion *in vivo*, as previously described using a CD16-directed murine IgG antibody (3G8) that is bivalent for CD16A in rhesus macaques and tamarins (Choi *et al.*, Immunology 2008;124:215-222; Yoshida *et al.*, Frontier in Microbiology (2010);1:128). Hence, induction of NK lysis reduces the number of effector cells available to mediate ADCC and impairs therapeutic antibody efficacy.

Thus, there is need for antibodies capable for enhanced NK cell engagement which is not diminished by fratricide (NK-NK cell lysis).

SUMMARY

The disclosure provides a CD16A-engaging antibody capable of at least bivalent interaction with CD16A on NK cells and, hence, with increased binding affinity and cytotoxic potency but, preferably, incapable of inducing NK-NK cell lysis.

In aspect, provided herein is a multispecific antigen-binding protein comprising:

a) two B cell maturation antigen (BCMA) antigen-binding moieties each comprising a Fab fragment and an Fc portion and wherein each Fab fragment comprises: a heavy chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:67, a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:68, and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:69; and a light chain variable region comprising a CDR1 consisting of the amino acid sequence set

forth in SEQ ID NO:70, a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:71, and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:72, and

b) two CD16A antigen-binding moieties each in the format of a single chain variable fragment (scFv) comprising: a heavy chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:73, a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:74, and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:75; and a light chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:76, a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:77, and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:78, wherein the variable region at the N-terminus of each of the two scFvs is the light chain variable region,

wherein the N-terminus of the first of the two scFvs is fused to the C-terminus of the Fc portion of the first of the two BCMA binding moieties and the N-terminus of the second of the two scFvs is fused to the C-terminus of the Fc portion of the second of the two BCMA binding moieties.

In another aspect, provided herein is a pharmaceutical composition comprising a multispecific antigen-binding protein comprising two CD16A antigen-binding moieties, wherein each CD16A antigen-binding moiety is in the format of a single chain variable fragment (scFv), and two B cell maturation antigen (BCMA) antigen-binding moieties, wherein each BCMA antigen-binding moiety comprises an Fab fragment and an Fc portion, wherein:

(i) the two CD16A antigen-binding moieties each comprise:

(a) a heavy chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:73, a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:74, and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:75; and

(b) a light chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:76, a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:77, and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:78; wherein the variable region at the N-terminus of each of the two scFvs is the light chain variable region;

(c) wherein the N-terminus of the first of the two scFvs is fused to the C-terminus of the Fc portion of the first of the two BCMA binding moieties and the N-

terminus of the second of the two scFvs is fused to the C-terminus of the Fc portion of the second of the two BCMA binding moieties; and

(ii) the two BCMA antigen-binding moieties each comprise:

(a) a heavy chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:67, a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:68, and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:69; and

(b) a light chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:70, a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:71, and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:72;

and a pharmaceutically acceptable carrier.

In another aspect, provided herein is a bispecific antigen-binding protein comprising two CD16A antigen-binding moieties each in the format of a single chain variable fragment (scFv) and two B cell maturation antigen (BCMA)-targeting moieties each comprising an Fab fragment and an Fc portion, wherein the N-terminus of the first of the two scFvs is fused to the C-terminus of the first of the two BCMA-targeting moieties, and the N-terminus of the second of the two scFvs is fused to the C-terminus of the second of the two BCMA-targeting moieties wherein:

(i) the two CD16A antigen-binding moieties each comprise:

(a) a heavy chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:73; a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:74; and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:75, and

(b) a light chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:76; a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:77; and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:78; wherein the variable region at the N-terminus of each of the two scFvs is the light chain variable region; and

(ii) the two BCMA antigen-binding moieties each comprise:

(a) a heavy chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:67; a CDR2 consisting of the amino acid

sequence set forth in SEQ ID NO:68; and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:69, and

(ii) a light chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:70; a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:71; and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:72.

In another aspect, provided herein is a use of the multispecific antigen-binding protein as described herein, the pharmaceutical composition as described herein, or the bispecific antigen-binding protein as described herein for use in therapy.

In another aspect, provided herein is a use of the multispecific antigen-binding protein as described herein, the pharmaceutical composition as described herein, or the bispecific antigen-binding protein as described herein in the manufacture of a medicament for treating a subject having a depleted or reduced NK cell population.

In another aspect, the present disclosure refers to a use of the multispecific antigen-binding protein as described herein, the pharmaceutical composition as described herein, or the bispecific antigen-binding protein as described herein in the manufacture of a medicament for treating cancer, wherein the medicament is to be administered to a subject in need thereof

The disclosure refers to:

A multivalent and multispecific antigen-binding protein comprising at least one target antigen-binding moiety and at least two CD16A antigen-binding moieties, wherein the two CD16A antigen-binding moieties are fused to a constant domain, for example a Fab fragment or a Fc portion. Preferably, the target antigen-binding moiety is also fused to the Fab fragment or the Fc portion.

For example, each of the antigen-binding moieties may be selected from the group consisting of a single-chain diabody (scDb), a diabody (Db), a single chain Fv (scFv) or a Fab fragment.

In some examples the light chain and heavy chain variable regions of the CD16A antigen-binding moiety are linked one after another in a polypeptide such that the variable region at the N-terminus of the polypeptide chain is the light chain variable region, thereby preventing induction of fratricide (NK-NK cell lysis).

A multitude of protein architectures is available by fusing the antigen-binding moieties N-terminally or C-terminally to various units comprising constant domains, such as F(ab)′, F(ab)₂′, CH₂-CH₃, Hinge-CH₂-CH₃, Fc, CH₁-Hinge-CH₂-CH₃ or IgG.

In some examples the antigen-binding protein comprises a silenced Fc portion that does not bind to the Fc-gammaR, but retains binding to FcRn and, optionally, at least one further Fc-functionality.

In some examples the antigen-binding protein comprises a Fc portion which on the one hand is silenced so that it does not bind to the Fc-γR and which on the other hand may include further mutation to increase or decrease its ability to bind to FcRn which does correlate with different in vivo pharmacokinetics based on different abilities for transcytosis and recycling.

In certain examples the antigen-binding protein comprises at least an HSA antigen-binding moiety. For example, the antigen-binding protein may comprise 2, 3 or 4 HSA antigen-binding moieties.

In certain examples the antigen-binding protein comprises at least two different target antigen-binding moieties, i.e. a first and a second target antigen-binding moiety.

In certain examples CD16A antigen-binding moieties with increased or decreased affinity to CD16A are applied.

Among others the disclosure is characterized by:

1. An enhanced NK cell engaging functionality, because of

- Two CD16A antigen-binding moieties fused to a Fab fragment or a Fc portion, thereby increasing the cytotoxic potency due to the bivalent binding to CD16A.
- Utilizing anti-CD16A antigen-binding moieties with high affinity binding to CD16A.

What is claimed is:

1. A multispecific antigen-binding protein comprising:
 - a) two B cell maturation antigen (BCMA) antigen-binding moieties each comprising a Fab fragment and an Fc portion and wherein each Fab fragment comprises: a heavy chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:67, a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:68, and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:69; and a light chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:70, a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:71, and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:72, and
 - b) two CD16A antigen-binding moieties each in the format of a single chain variable fragment (scFv) comprising: a heavy chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:73, a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:74, and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:75; and a light chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:76, a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:77, and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:78, wherein the variable region at the N-terminus of each of the two scFvs is the light chain variable region,

wherein the N-terminus of the first of the two scFvs is fused to the C-terminus of the Fc portion of the first of the two BCMA binding moieties and the N-terminus of the second of the two scFvs is fused to the C-terminus of the Fc portion of the second of the two BCMA binding moieties.
2. The multispecific antigen-binding protein of claim 1, comprising an Fc portion mutation selected from the group consisting of (a) C220S, C229S, E233P, L234A, L234V, L234F, L235A, L235E, P238S, D265A, N297A, N297Q, and P331S, or (b) L234A, L234V, L234F, L235A, L235E, P238S, and D265A, according to the Kabat EU numbering.
3. The multispecific antigen-binding protein of claim 1, wherein:

the heavy chain variable region of each of the two CD16A antigen-binding moieties comprises the amino acid sequence set forth in SEQ ID NO: 3; and/or the light chain

variable region of each of the two CD 16A antigen-binding moieties comprises the amino acid sequence set forth in SEQ ID NO: 2.

4. The multispecific antigen-binding protein of claim 1, wherein the protein is a tetramer comprising a first polypeptide chain consisting of the amino acid sequence set forth in SEQ ID NO: 61, and a second polypeptide chain consisting of the amino acid sequence set forth in SEQ ID NO: 62.

5. The multispecific antigen-binding protein of claim 1, wherein the heavy chain variable region of each of the two BCMA antigen-binding moieties comprises the amino acid sequence set forth in SEQ ID NO: 65 and/or the light chain variable region of each of the two BCMA antigen-binding moieties comprises the amino acid sequence set forth in SEQ ID NO: 66.

6. A pharmaceutical composition comprising a multispecific antigen-binding protein comprising two CD16A antigen-binding moieties, wherein each CD16A antigen-binding moiety is in the format of a single chain variable fragment (scFv), and two B cell maturation antigen (BCMA) antigen-binding moieties, wherein each BCMA antigen-binding moiety comprises an Fab fragment and an Fc portion, wherein:

(i) the two CD16A antigen-binding moieties each comprise:

(a) a heavy chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:73, a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:74, and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:75; and

(b) a light chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:76, a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:77, and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:78; wherein the variable region at the N-terminus of each of the two scFvs is the light chain variable region;

(c) wherein the N-terminus of the first of the two scFvs is fused to the C-terminus of the Fc portion of the first of the two BCMA binding moieties and the N-terminus of the second of the two scFvs is fused to the C-terminus of the Fc portion of the second of the two BCMA binding moieties; and

(ii) the two BCMA antigen-binding moieties each comprise:

(a) a heavy chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:67, a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:68, and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:69; and

(b) a light chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:70, a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:71, and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:72;

and a pharmaceutically acceptable carrier.

7. A bispecific antigen-binding protein comprising two CD16A antigen-binding moieties each in the format of a single chain variable fragment (scFv) and two B cell maturation antigen (BCMA)-targeting moieties each comprising an Fab fragment and an Fc portion, wherein the N-terminus of the first of the two scFvs is fused to the C-terminus of the first of the two BCMA-targeting moieties, and the N-terminus of the second of the two scFvs is fused to the C-terminus of the second of the two BCMA-targeting moieties wherein:

(i) the two CD16A antigen-binding moieties each comprise:

(a) a heavy chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:73; a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:74; and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:75, and

(b) a light chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:76; a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:77; and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:78; wherein the variable region at the N-terminus of each of the two scFvs is the light chain variable region; and

(ii) the two BCMA antigen-binding moieties each comprise:

(a) a heavy chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:67; a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:68; and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:69, and

(ii) a light chain variable region comprising a CDR1 consisting of the amino acid sequence set forth in SEQ ID NO:70; a CDR2 consisting of the amino acid sequence set forth in SEQ ID NO:71; and a CDR3 consisting of the amino acid sequence set forth in SEQ ID NO:72.

8. The bispecific antigen-binding protein of claim 7, wherein:

(i) the two CD16A antigen-binding moieties each comprise a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 3;

(ii) the two CD16A antigen-binding moieties each comprise a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 2;

(iii) the two BCMA antigen-binding moieties each comprise a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 65;

(iv) the two BCMA antigen-binding moieties each comprise a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 66;

(v) the two CD16A antigen-binding moieties each comprise a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 3 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 2;

(vi) the two BCMA antigen-binding moieties each comprise a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 65 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 66; and/or

(vii) the two CD16A antigen-binding moieties each comprise a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 3 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 2; and the two BCMA antigen-binding moieties each comprise a heavy chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 65 and a light chain variable region comprising the amino acid sequence set forth in SEQ ID NO: 66.

9. The pharmaceutical composition of claim 6, wherein the bispecific antigen-binding protein comprises an Fc portion mutation selected from the group consisting of (a) C220S, C229S, E233P, L234A, L234V, L234F, L235A, L235E, P238S, D265A, N297A, N297Q, and P331S, or (b) L234A, L234V, L234F, L235A, L235E, P238S, and D265A, according to the Kabat EU numbering.

10. The pharmaceutical composition of claim 6, wherein the heavy chain variable region of each of each of the two CD 16A antigen-binding moieties comprises the amino acid sequence set forth in SEQ ID NO: 3; and/or the light chain variable region of each of the two CD 16A antigen-binding moieties comprises the amino acid sequence set forth in SEQ ID NO: 2.
11. The pharmaceutical composition of claim 6, wherein the heavy chain variable region of each of the two BCMA antigen-binding moieties comprises the amino acid sequence set forth in SEQ ID NO: 65 and/or the light chain variable region of each of the two BCMA antigen-binding moieties comprises the amino acid sequence set forth in SEQ ID NO: 66.
12. The pharmaceutical composition of claim 6, wherein the bispecific antigen-binding protein is a tetramer comprising a first polypeptide chain consisting of the amino acid sequence set forth in SEQ ID NO: 61, and a second polypeptide chain consisting of the amino acid sequence set forth in SEQ ID NO: 62.
13. The bispecific antigen-binding protein of claim 7, comprising an Fc portion mutation selected from the group consisting of (a) C220S, C229S, E233P, L234A, L234V, L234F, L235A, L235E, P238S, D265A, N297A, N297Q, and P331S, or (b) L234A, L234V, L234F, L235A, L235E, P238S, and D265A, according to the Kabat EU numbering.
14. The bispecific antigen-binding protein of claim 7, wherein the bispecific antigen-binding protein is a tetramer comprising a first polypeptide chain consisting of the amino acid sequence set forth in SEQ ID NO: 61, and a second polypeptide chain consisting of the amino acid sequence set forth in SEQ ID NO: 62.
15. The multispecific antigen-binding protein of any one of claims 1-5, the pharmaceutical composition of any one of claims 6 or 9-12, or the bispecific antigen-binding protein of any one of claims 7, 8, 13 or 14 for use in therapy.
16. Use of the multispecific antigen-binding protein of any one of claims 1-5, the pharmaceutical composition of any one of claims 6 or 9-12, or the bispecific antigen-binding protein of any one of claims 7, 8, 13 or 14 in the manufacture of a medicament for treating cancer, wherein the medicament is to be administered to a subject in need thereof.
17. The use of claim 16, wherein the cancer is a hematologic cancer.

18. The use of claim 16 or 17, wherein the cancer is multiple myeloma.
19. The use of any one of claims 16-18, wherein the subject is a cancer patient.
20. The use of any one of claims 16-19, wherein the subject is a hematological cancer patient.
21. The use of any one of claims 16-20, wherein the subject is a multiple myeloma patient.
22. The use of any one of claims 16-21, wherein the subject is a relapsed/refractory multiple myeloma patient.
23. The use of any one of claims 16-22, wherein the subject has received an anti-CD38 therapy.
24. The use of any one of claims 16-23, wherein the subject has received daratumumab.
25. The use of any one of claims 16-24, wherein the subject is daratumumab naïve, daratumumab resistant, daratumumab refractory, or daratumumab relapsed.
26. The use of claim 25, wherein the subject is daratumumab resistant, daratumumab refractory, or daratumumab relapsed.
27. The use of any one of claims 16-26, wherein the subject expresses a CD16A polymorphism.
28. The use of claim 27, wherein the CD16A polymorphism is a CD16A-158V/F polymorphism.
29. Use of the multispecific antigen-binding protein of any one of claims 1-5, the pharmaceutical composition of any one of claims 6 or 9-12, or the bispecific antigen-binding protein of any one of claims 7, 8, 13, or 14 in the manufacture of a medicament for treating a subject having a depleted or reduced NK cell population.
30. The use of claim 29, wherein the subject has been previously treated with an anti-CD38 therapy.

31. The use of claim 30, wherein the anti-CD38 therapy is daratumumab therapy.
32. The use of any one of claims 29-31, wherein the medicament is to be administered intravenously.
33. The use of any one of claims 29-31, wherein the medicament is to be administered subcutaneously.
34. The use of any one of claims 29-33, wherein the medicament is to be further administered with a compound.
35. The use of claim 34, wherein the compound is selected from the group consisting of an anti-PD-1 antibody, an anti-PD-L1 antibodies, a CD3 bispecific antibody, an anti-TIGIT antibody, an anti-VEGF antibody, and an anti-FcRH5 antibody .
36. The use of claim 35, wherein the compound is a cytokine or any derivative or modification thereof.
37. The use of claim 36, wherein the cytokine is selected from the group consisting of IL-15, IL-2, IL-12, IL-21, and IL-6.
38. The use of claim 37, wherein the cytokine comprises IL-2.
39. The use of claim 37, wherein the cytokine comprises IL-15.