

[0007] In particular, in solid tumors with limited presence of NK cells, CD16 shedding would be a great disadvantage for the ability of an effector cell to kill multiple tumor cell targets. Overexpression of Folate Receptor 1 (FOLR1) by a large number of solid tumors including ovarian, breast (particularly TNBC), renal, lung, colorectal (CRC), kidney (particularly ccRCC), pancreatic (particularly PDAC), endometrial and brain has been described in the art (Hartmann et al., *Int J Cancer* 2007, 121(5): 938-42; Yang et al., *Human Cancer Biology* 2007, 13(9): 2557-2567; Scaranti et al., *Nat Rev Clin Oncol.* 2020, 17(6): 349-359). Hence, due to its high expression in solid tumors, FOLR1 may be an attractive therapeutic target for the development of anti-cancer agents. A range of FOLR1-targeting approaches, including folic acid derivatives, folate drug-conjugates and small molecules, vaccines, terpolymer-based nanocapsules, T-cell therapies and monoclonal antibodies, have been developed for clinical application for both imaging and therapeutic purposes (Cheung et al., *Oncotarget* 2016, 7(32): 52553-52574, Bellotti et al., *biomedicines* 2021, 9, 1275).

[0008] However, there is still a need in the art for the provision of highly efficient therapeutic approaches for treating FOLR1 expressing cancer disease, such as anti-CD16A bispecific antibody constructs for use in immuno-oncology therapies to induce immune effector cell activation by binding to CD16A, thereby allowing for high cytokine production and long-lasting target cell killing by the activated NK cells. The present invention addresses this need as indicated herein.

Summary

[0009] The present disclosure is based at least partly on the surprising finding that a bispecific antibody construct comprising a high-affinity anti-CD16A first binding domain and a second binding domain for an antigen on the surface of a target cell, wherein said target antigen is FOLR1, can efficiently activate and redirect immune effector cells for ADCC, thereby avoiding CD16A shedding and immediate inactivation of the engaged effector cells. Specifically, the present inventors surprisingly observed that the high-affinity anti-CD16A binding domain comprised by the antibody construct of the present disclosure strongly stabilizes CD16A expression on NK effector cells after activation when compared to low-affinity anti-CD16A binding domains, despite the presence of target cells. In this respect, immune effector cells activated by the bispecific antibody construct of the present disclosure show high cytotoxic activity and induce target cell lysis without activation-induced CD16A shedding. This is particularly beneficial for the treatment of solid tumors with limited presence of NK cells, since CD16 shedding would be a great

disadvantage for the ability of an effector cell to kill multiple tumor cell targets. As shown in Examples 1, 2 and 12, the bispecific antibodies of the present disclosure comprising a specific CD16A binding domain (also named CD16a1 anti-CD16A effector domain or CD16a1 domain herein) show a higher affinity to human CD16A when compared to other CD16A binding domains (see **Figures 1, 2 and 16A-16C** and **Tables 3 and 15**). Moreover, as shown in Examples 5, the bispecific antibodies of the present disclosure comprising a specific CD16A binding domain show a significant CD16 shedding inhibition effect on stimulated NK cells when compared to shedding inhibition induced by other CD16A binding domains (see **Figures 5A-5C and 6A-6C**). Nonetheless, as demonstrated in Example 4, the bispecific antibodies of the present disclosure comprising a specific CD16A binding domain also show high lysis potential with EC₅₀ values in the low picomolar concentration range against target cells (see **Figures 4 and 17**) and show a low target cell-independent activation (see **Figures 7 and 18A-18B**). In sum, antibody constructs comprising the high-affinity anti-CD16A binding domain of the disclosure have surprisingly a high cytotoxic activity although these constructs prevent CD16A shedding upon NK cell activation.

[0010] The antibody constructs of the present disclosure can thus be useful for tumor therapy, in particular solid tumors, because they are not only capable of activating NK cells via high-affinity binding of CD16A receptor, but also achieve long-lasting activation of NK cells without loss of CD16A. Thus, the bispecific antibody constructs of the present disclosure lead to a high affinity binding of effector NK cells via CD16A and effective killing of target cells by cell mediated cytotoxicity, thereby allowing for sustained effector cell activation. The antibody constructs of the present disclosure can thus be useful for efficiently targeting various cancer diseases, in particular solid tumors overexpressing FOLR1 on the cell surface, and must be considered superior to antibody constructs comprising low-affinity CD16A binding domains (e.g. the CD16a2 or CD16a4 anti-CD16A effector domain disclosed herein). The antibody constructs of the present disclosure can thus be useful for treating solid tumors such as ovarian, breast (particularly TNBC), renal, lung, colorectal (CRC), kidney (particularly ccRCC), pancreatic (particularly PDAC), endometrial and brain tumors.

[0011] In one aspect, the present disclosure refers to a bispecific antibody construct comprising (a) a first binding domain (A), which is capable of specifically binding to a first target (A') that is CD16A on the surface of an immune effector cell, wherein the first binding domain comprises: (i) a VL region comprising CDR-L1 as depicted in SEQ ID NO: 4, a CDR-L2 as depicted in SEQ ID NO: 5, and a CDR-L3 as depicted in SEQ ID NO: 6; and (ii) a VH region as depicted in SEQ ID NO: 7 or SEQ ID NO: 94; and (b) a second binding domain (B), which is capable of specifically

binding to a second target (B') that is an antigen on the surface of a target cell, wherein the second target (B') is FOLR1.

[0012] In another aspect, the present disclosure refers to a nucleic acid molecule comprising a sequence encoding an antibody construct as described herein.

[0013] In yet another aspect, the present disclosure refers to a vector comprising a nucleic acid molecule as described herein.

[0014] In another aspect, the present disclosure refers to a host cell comprising a nucleic acid molecule as described herein or a vector as described herein.

[0015] In another aspect, the present disclosure refers to a method of producing an antibody construct as described herein, said method comprising culturing a host cell as described herein under conditions allowing the expression of the antibody construct as described herein and recovering the produced antibody construct from the culture.

[0016] In another aspect, the present disclosure refers to a pharmaceutical composition comprising an antibody construct as described herein, or produced by the method as described herein.

[0017] In another aspect, the present disclosure refers to an antibody construct as described herein for use in therapy.

[0018] The present disclosure also relates to a method of treatment or amelioration of a proliferative disease, a tumorous disease, a viral disease or an immunological disorder, comprising the step of administering to a subject in need thereof the antibody construct of the disclosure, or produced by the method of the disclosure.

[0018a] In yet another aspect, the present disclosure refers to a use of the antibody construct as described herein, or produced by the method as described herein, in the manufacture of a pharmaceutical composition for the prevention, treatment or amelioration of a disease selected from a proliferative disease, a tumorous disease, a viral disease or an immunological disorder, wherein said tumorous disease is preferably a solid tumor, preferably a malignant solid tumor, wherein said solid tumor is preferably selected from the group consisting of ovarian (optionally high grade serous ovarian cancer or epithelial ovarian cancer), breast (optionally TNBC), renal, lung (optionally non-small cell lung cancer (NSCLC) or mesotheliomas), colorectal (optionally CRC), kidney (optionally ccRCC), pancreatic (optionally PDAC), endometrial (optionally non-malignant endometrial cancer) and brain cancer.

[0019] In another aspect, the present disclosure refers to a kit comprising an antibody construct as described herein, or produced by the method as described herein, a nucleic acid molecule as described herein, a vector as described herein, and/or a host cell as described herein.

Brief description of the drawings

[0020] Figure 1: Detection of CD16A interaction with CD16A binding domains. CD123xCD16A ICE binding to human CD16A^{158V}, CD16A^{158F} and cynomolgus CD16 was measured by SPR using a multivalent multi-cycle kinetic set up at 37°C (n=3; ²) n=1) with biotin captured recombinant CD16A^{158V}, CD16A^{158F} and cynomolgus CD16 (ligand) and scFv-IgAb_268 (CD16a1xCD123-1), scFv-IgAb_148 (CD16a2xCD123-1), scFv-IgAb_264 (CD16a1xCD123-2) (analyte). Affinity and kinetic parameters were evaluated for interaction with human CD16A and cynomolgus CD16 using a 1:1 Binding model. All molecules show high interaction to human

Claims

1. A bispecific antibody construct comprising (a) a first binding domain (A), which is capable of specifically binding to a first target (A') that is CD16A on the surface of an immune effector cell, wherein the first binding domain comprises: (i) a VL region comprising CDR-L1 as depicted in SEQ ID NO: 4, a CDR-L2 as depicted in SEQ ID NO: 5, and a CDR-L3 as depicted in SEQ ID NO: 6; and (ii) a VH region as depicted in SEQ ID NO: 7 or SEQ ID NO: 94; and (b) a second binding domain (B), which is capable of specifically binding to a second target (B') that is an antigen on the surface of a target cell, wherein the second target (B') is FOLR1.
2. The antibody construct of claim 1, wherein the first binding domain (A) comprises a VL region as depicted in SEQ ID NO: 8 or SEQ ID NO: 95 and a VH region as depicted in SEQ ID NO: 7 or SEQ ID NO: 94.
3. The antibody construct of claim 1 or 2, wherein the first binding domain (A) is a variable domain (Fv), a single chain Fv (scFv), a Fab, a single chain diabody (scDb), a diabody (Db) or a double Fab, preferably a scFv.
4. The antibody construct of any one of claims 1 to 3, wherein the second binding domain (B) comprises a VH and a VL domain of an antibody.
5. The antibody construct of any one of claims 1 to 4, wherein the second binding domain (B) is a variable domain (Fv), a single chain Fv (scFv), a Fab, a single chain diabody (scDb), a diabody (Db) or a double Fab, preferably a double Fab.
6. The antibody construct of any one of claims 1 to 5, wherein the antibody construct binds to a target cell and an immune effector cell simultaneously.
7. The antibody construct of any one of claims 1 to 6, wherein the first binding domain binds to an epitope on CD16A which is C-terminal to the physiological Fcγ receptor binding domain, said epitope preferably comprising Y158 of SEQ ID NO: 50.

8. The antibody construct of any one of claims 1 to 7, further comprising a third domain (C) comprising a half-life extension domain.
9. The antibody construct of any one of claims 1 to 8, wherein said half-life extension domain comprises a CH2 domain, wherein the Fcγ receptor binding domain is silenced.
10. The antibody construct of any one of claims 1 to 8, wherein said half-life extension domain comprises a CH3 domain.
11. The antibody construct of any one of claims 1 to 10, wherein the antibody construct comprises at least one hinge domain and a CH3 domain fused to a CH2 domain in an amino to carboxyl order in the order hinge domain – CH2 domain – CH3 domain, preferably wherein the antibody construct comprises at least two of the hinge domain – CH2 domain – CH3 domain elements.
12. The antibody construct of any one of claims 1 to 11, wherein the first binding domain (A) is fused to the C terminus of a CH3 domain, and the second binding domain (B) is fused to the N terminus of a hinge region.
13. The antibody construct of any one of claims 1 to 12, wherein the antibody construct is monovalent for the first binding domain (A) and monovalent for the second binding domain (B).
14. The antibody construct of any one of claims 1 to 13, wherein the antibody construct is bivalent for the first binding domain (A) and bivalent for the second binding domain (B).
15. The antibody construct of any one of claims 1 to 12 or 14, wherein (a) the first binding domain (A), which is capable of specifically binding to a first target (A') that is CD16A on the surface of an immune effector cell comprises: (i) a VL region comprising CDR-L1 as depicted in SEQ ID NO: 4, a CDR-L2 as depicted in SEQ ID NO: 5, and a CDR-L3 as depicted in SEQ ID NO: 6, and (ii) a VH region as depicted in SEQ ID NO: 7 or SEQ ID NO: 94, wherein said first binding domain is a scFv; (b) the second binding domain which is capable of specifically binding to a second target (B') that is an antigen on the surface of a target cell that is FOLR1,

comprises: (i) a VL region comprising a CDR-L1 as depicted in SEQ ID NO: 117, a CDR-L2 as depicted in SEQ ID NO: 118, a CDR-L3 as depicted in SEQ ID NO: 119, and (ii) a VH region comprising a CDR-H1 as depicted in SEQ ID NO: 114, a CDR-H2 as depicted in SEQ ID NO: 115, a CDR-H3 as depicted in SEQ ID NO: 116, wherein said second binding domain is a Fab; and (c) the third binding domain comprises two of the hinge domain – CH2 domain – CH3 domain elements, preferably as depicted in SEQ ID NOs: 53 and 67; wherein the first binding domain (A) is fused to the C terminus of a CH3 domain of the third domain and the second binding domain (B) is fused to the N terminus of a hinge region of the third domain.

16. The antibody construct of any one of claims 1 to 12 or 14, wherein (a) the first binding domain (A), which is capable of specifically binding to a first target (A') that is CD16A on the surface of an immune effector cell comprises: (i) a VL region comprising CDR-L1 as depicted in SEQ ID NO: 4, a CDR-L2 as depicted in SEQ ID NO: 5, and a CDR-L3 as depicted in SEQ ID NO: 6, and (ii) a VH region as depicted in SEQ ID NO: 7 or SEQ ID NO: 94, wherein said first binding domain is a scFv; (b) the second binding domain which is capable of specifically binding to a second target (B') that is an antigen on the surface of a target cell that is FOLR1, comprises: (i) a VL region comprising a CDR-L1 as depicted in SEQ ID NO: 127, a CDR-L2 as depicted in SEQ ID NO: 128, and a CDR-L3 as depicted in SEQ ID NO: 129, and (ii) a VH region comprising a CDR-H1 as depicted in SEQ ID NO: 124, a CDR-H2 as depicted in SEQ ID NO: 125, a CDR-H3 as depicted in SEQ ID NO: 126, wherein said second binding domain is a Fab; and (c) the third binding domain comprises two of the hinge domain – CH2 domain – CH3 domain elements, preferably as depicted in SEQ ID NOs: 53 and 67; wherein the first binding domain (A) is fused to the C terminus of a CH3 domain of the third domain and the second binding domain (B) is fused to the N terminus of a hinge region of the third domain.

17. The antibody construct of any one of claims 1 to 12, 14 or 15, wherein (a) the first binding domain (A), which is capable of specifically binding to a first target (A') that is CD16A on the surface of an immune effector cell comprises (i) a VL region as depicted in SEQ ID NO: 8 or SEQ ID NO: 95, and (ii) a VH region as depicted in SEQ ID NO: 7 or SEQ ID NO: 94, wherein said first binding domain is a scFv; (b) the second binding domain which is capable of specifically binding to a second target (B') that is FOLR1 on the surface of a target cell comprises: (i) a VL region as depicted in SEQ ID NO: 121 and (ii) a VH region as depicted in SEQ ID NO: 120, wherein said second binding domain is a Fab; and (c) the third binding

domain comprises two of the hinge domain – CH2 domain – CH3 domain elements, preferably as depicted in SEQ ID NOs: 53 and 67; wherein the first binding domain (A) is fused to the C terminus of a CH3 domain of the third domain and the second binding domain (B) is fused to the N terminus of a hinge region of the third domain.

18. The antibody construct of any one of claims 1 to 12, 14 or 16, wherein (a) the first binding domain (A), which is capable of specifically binding to a first target (A') that is CD16A on the surface of an immune effector cell comprises (i) a VL region as depicted in SEQ ID NO: 8 or SEQ ID NO: 95, and (ii) a VH region as depicted in SEQ ID NO: 7 or SEQ ID NO: 94, wherein said first binding domain is a scFv; (b) the second binding domain which is capable of specifically binding to a second target (B') that is FOLR1 on the surface of a target cell comprises: (i) a VL region as depicted in SEQ ID NO: 131 and (ii) a VH region as depicted in SEQ ID NO: 130, wherein said second binding domain is a Fab; and (c) the third binding domain comprises two of the hinge domain – CH2 domain – CH3 domain elements, preferably as depicted in SEQ ID NOs: 53 and 67; wherein the first binding domain (A) is fused to the C terminus of a CH3 domain of the third domain and the second binding domain (B) is fused to the N terminus of a hinge region of the third domain.

19. The antibody construct of any one of claims 1 to 16, wherein the second binding domain (B) specific for FOLR1 comprises a pair of VH- and VL-chains having a sequence as depicted in the pair of sequences having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity each to SEQ ID NOs: 120 and 121, respectively, or having at least 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% identity each to SEQ ID NOs: 130 and 131, respectively.

20. The antibody construct of any one of claims 1 to 12, 14, 15 or 17, having an amino acid sequence selected from the group consisting of SEQ ID NOs: 142-143 and 138-139, wherein SEQ ID NOs: 142-143 are preferred.

21. The antibody construct of any one of claims 1 to 19, wherein the antibody construct induces less CD16A shedding as compared to a control construct having an amino acid sequence selected from the group consisting of SEQ ID NOs: 134-135, 136-137 and 140-142.

22. A nucleic acid molecule comprising a sequence encoding an antibody construct of any one of claims 1 to 21.
23. A vector comprising a nucleic acid molecule of claim 22.
24. A host cell comprising a nucleic acid molecule of claim 22 or a vector of claim 23.
25. A method of producing an antibody construct of any one of claims 1 to 21, said method comprising culturing a host cell of claim 24 under conditions allowing the expression of the antibody construct of any one of claims 1 to 21 and recovering the produced antibody construct from the culture.
26. A pharmaceutical composition comprising an antibody construct of any one of claims 1 to 21, or produced by the method of claim 25.
27. The antibody construct of any one of claims 1 to 21 for use in therapy.
28. Use of the antibody construct of any one of claims 1 to 21, or produced by the method of claim 25, in the manufacture of a pharmaceutical composition for the prevention, treatment or amelioration of a disease selected from a proliferative disease, a tumorous disease, a viral disease or an immunological disorder, wherein said tumorous disease is preferably a solid tumor, preferably a malignant solid tumor, wherein said solid tumor is preferably selected from the group consisting of ovarian (optionally high grade serous ovarian cancer or epithelial ovarian cancer), breast (optionally TNBC), renal, lung (optionally non-small cell lung cancer (NSCLC) or mesotheliomas), colorectal (optionally CRC), kidney (optionally ccRCC), pancreatic (optionally PDAC), endometrial (optionally non-malignant endometrial cancer) and brain cancer.
29. A kit comprising an antibody construct of any one of claims 1 to 21, or produced by the method of claim 25, a nucleic acid of claim 22, a vector of claim 23, and/or a host cell of claim 24.