

128-139). WO 2006/125668 and Reusch et al, MABS, 2014, 6:3:728-739 describe an antigen-binding protein – a bispecific tandem diabody - for engagement of CD16A and its use for natural killer (NK) cell therapy. The cytotoxic activity of NK cells can be enhanced by increasing the avidity through multivalent binding to CD16A, e.g. using constructs with bivalent binding to CD16A (WO2019/198051 Affimed GmbH).

[0004] Activation-induced down-regulation/shedding of CD16 on activated NK cells is caused by proteolytic cleavage of its extracellular portion by A disintegrin and metalloproteinase (ADAM17) (Romee et al., Blood, 2013, 121 (18):3599–3608), or membrane type 6 matrix metalloproteinase (MMP25) (Peruzzi et al., J. Immunol., 2013, 191:955-957). However, CD16 shedding does not immediately recover, suggesting that once NK cells are activated and CD16 is down-regulated, their capacity for ADCC is impaired for several days (Goodier et al., Front. Immunol., 2016, 7:384). Moreover, ADAM17 mediated CD16 shedding is also described to limit the efficacy of rituximab or trastuzumab antibody therapies that involve ADCC (Romee et al., Blood, 2013, 121 (18):3599–3608). Hence, down-regulation of CD16 expression on activated immune effector cells may limit or regulate their activity and ADCC-mediated cytotoxicity. On the other side, the usage of CD16 inhibitors and NK cells transfected to express a non-cleavable form of CD16 revealed that CD16 shedding upon NK cell activation may be considered important for the detachment of NK cells from opsonized target cells, thereby sustaining NK cell survival and increasing serial engagement of target cells (Srpan et al., J. Cell. Biol., 2018, 217(9):3267-3283).

[0005] In sum, there is still a need in the art for the provision of highly efficient 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

[0006] 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 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 can be beneficial for the treatment of hematological cancer diseases where CD16A shedding on circulating NK cells before these cells may be able to reach their intended tumor targets, which also sit in the bone marrow, would be a great disadvantage. Furthermore, 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. 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 16** 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 5 and 6**). 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 18**). 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.

[0007] The antibody constructs of the present disclosure can thus be useful for tumor therapy, in particular hematological 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 hematological tumors, 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).

[0008] The antibody constructs of the present disclosure can thus also be useful for tumor therapy, in particular solid tumors such as ovarian, breast, renal, lung, colorectal, and brain

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 such as ovarian, breast, renal, lung, colorectal, and brain tumors, 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).

[0009] In particular, the present disclosure relates 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: 134; 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.

[0009a] 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 a pair of VH- and VL-chains having sequences as depicted in the pair of sequences of SEQ ID NOs: 7 and 8; 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.

[0010] In another aspect, the present disclosure refers to a nucleic acid molecule comprising a sequence encoding an antibody construct as described herein.

[0011] In another aspect, the present disclosure refers to a vector comprising a nucleic acid molecule as described herein.

[0012] 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.

[0013] In yet 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.

[0014] 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.

[0015] The present disclosure also relates to an antibody construct of the disclosure for use in therapy.

[0016] 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.

[0016a] In 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, a hematological disease or disorder, preferably a hematological tumor disease, acute myelogenous leukemia (AML) or myelodysplastic syndrome (MDS), or a solid tumor.

[0017] In yet 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

[0018] 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-IgAb₂₆₈ (CD16a1xCD123-1), scFv-IgAb₁₄₈ (CD16a2xCD123-1), scFv-IgAb₂₆₄ (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 CD16A as well as to cynomolgus CD16 with apparent affinities in the range of K_D 0.195 nM – 2.48 nM.

[0019] Figure 2: Binding of CD123xCD16A constructs to cell lines expressing human CD16A.

Binding of antibody constructs to huCD16A-transfected CHO cells measured by flow cytometry, depicting the median fluorescent intensity (MFI) of titrated scFv-IgAb₂₆₈ (CD123-1xCD16a1, black dot), scFv-IgAb₁₄₈ (CD123-1xCD16a2, black triangle) and a negative control molecule (scFv-IgAb₁₃₉, CD123xRSV, grey dot) relative to overall CD16 expression as detected by the anti-human CD16 antibody clone 3G8. Exp. No. RHU 066

[0020] Figure 3: Cell surface retention of anti-CD123 antibodies on NK cells.

Enriched primary human NK cells were preloaded with 100 µg/mL scFv-IgAb₂₆₈ (CD123-1xCD16a1), Fc-enhanced anti-CD123 IgG1 (IgAb₃₃₈), or scFv-IgAb₁₄₈ (CD123-

1xCD16a2) on ice, washed, and then incubated at 37°C for the indicated time periods in an excess volume of complete RPMI 1640 medium to allow dissociation and to prevent re-association. Residual antibodies at each time point were determined by flow cytometry, and median fluorescence intensity (MFI) values at time-point 0 were taken to be 100%, and the percentages of remaining antibody were analysed and plotted by non-linear regression using GraphPad Prism.

[0021] Figure 4: ADCC against CD123+ EOL-1 cells by anti-CD123 antibodies.
Concentration-dependent induction of tumor cell lysis by bispecific antibody constructs scFv-

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 a pair of VH- and VL-chains having sequences as depicted in the pair of sequences of SEQ ID NOs: 7 and 8; 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.
2. The antibody construct of claim 1, 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.
3. The antibody construct of claim 1 or 2, wherein the second target (B') is selected from the group consisting of CD19, CD20, CD22, CD30, CD33, CD52, CD70, CD74, CD79b, CD123, CLL1, BCMA, FCRH5, EGFR, EGFRvIII, HER2, and GD2, preferably selected from the group consisting of CD19, CD20, CD30, CD33, and CD123, preferably CD123.
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 9, 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, wherein the antibody construct preferably 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 12, 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) is a scFv; (b) the second binding domain is capable of specifically binding to CD123 and comprises (i) a VL region comprising CDR-L1 as depicted in SEQ ID NO: 24, a CDR-L2 as depicted in SEQ ID NO: 25, and a CDR-L3 as depicted in SEQ ID NO: 26, and (ii) a VH region comprising CDR-H1 as depicted in SEQ ID NO: 21, a CDR-H2 as depicted in SEQ ID NO: 22, and a CDR-H3 as depicted in SEQ ID NO: 23, wherein said second binding domain is a Fab; and (c) the third 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) is a scFv; (b) the second binding domain is capable of specifically binding to CD123 and comprises: (i) a VL region as depicted in SEQ ID NO: 28 and (ii) a VH region as depicted in SEQ ID NO: 27, wherein said second binding domain is a Fab; and (c) the third 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 16, having an amino acid sequence selected from the group consisting of SEQ ID NOs: 86-87, and 88-89, wherein SEQ ID NOs: 88-89 are preferred.
18. The antibody construct of any one of claims 1 to 17, 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: 92-93, 82-83, and 84-85.
19. A nucleic acid molecule comprising a sequence encoding an antibody construct of any one of claims 1 to 18.
20. A vector comprising a nucleic acid molecule of claim 19.
21. A host cell comprising a nucleic acid molecule of claim 19 or a vector of claim 20.
22. A method of producing an antibody construct of any one of claims 1 to 18, said method comprising culturing a host cell of claim 21 under conditions allowing the expression of the antibody construct of any one of claims 1 to 18 and recovering the produced antibody construct from the culture.
23. A pharmaceutical composition comprising an antibody construct of any one of claims 1 to 18, or produced by the method of claim 22.
24. The antibody construct of any one of claims 1 to 18 for use in therapy.
25. Use of the antibody construct of any one of claims 1 to 18, or produced by the method of claim 22, 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, a hematological disease or disorder, preferably a hematological tumor disease, acute myelogenous leukemia (AML) or myelodysplastic syndrome (MDS), or a solid tumor.
26. A kit comprising an antibody construct of any one of claims 1 to 18, or produced by the method of claim 22, a nucleic acid of claim 19, a vector of claim 20, and/or a host cell of claim 21.