TUMOR NECROSIS FACTOR RECEPTOR AGONISTIC MBS

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Patent Application No. 63/433,477 (filed on December 18, 2022), the disclosure of which is incorporated by reference herein in their entirety for all purposes.

SEQUENCE LISTING

[0002] The present specification makes reference to a Sequence listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. Said XML file, created on December 13, 2023, is named RBT-054WO_SL.xml, and is 530 kb in size.

BACKGROUND

[0003] The tumor necrosis factor (TNF) receptor superfamily includes various receptors that become activated upon ligand binding and multimerization of the receptors at the cell surface is critical for the initiation of downstream signaling. Depending on the type of TNF receptor, activation may induce a downstream signal involved in a variety of cellular processes. When activated, certain members of the TNF receptor family can provide co-stimulatory signals for T-cells such as tumor-antigen specific T-cells. Thus, these TNF receptor family members have been attractive targets for immunotherapies. However, therapies using this strategy face a trade-off between efficacy and safety. For example, urelumab, an antibody that binds to the TNF receptor family member 4-1BB, exhibits clinical efficacy and strong agonistic activity at 4-1BB. However, this agonistic activity is dependent on Fc crosslinking and is associated with liver toxicity. By contrast, utomilumab, another 4-1BB antibody, exhibits a clean liver profile but much weaker agonism which is also dependent on Fc crosslinking.

[0004] Thus, there is a need for improved therapeutics that exhibit both therapeutic efficacy and a desirable safety profile.

SUMMARY

[0005] The present disclosure addresses this need with the provision of self-assembled polypeptide complexes displaying TNF receptor-binding moieties. These complexes exhibit robust agonism of TNF receptors, independent of Fc crosslinking or Fc effector functions. Thus, these complexes may exhibit improve safety profiles without sacrificing efficacy.

Additionally, these complexes may provide a therapeutic modality for treating immunologically cold tumors, which have low levels or no infiltration of Fcγ receptor-expressing cells. Also disclosed are related fusion proteins, complexes, compositions, and methods.

[0006] In one aspect, described are fusion polypeptides comprising: (1) a tumor necrosis factor (TNF) receptor-binding moiety and (2) a nanocage monomer or subunit thereof, wherein the TNF receptor-binding moiety is not capable of binding to death receptor 4 (DR4) or death receptor 5 (DR5).

[0007] In some examples, the TNF receptor-binding moiety comprises an antibody or antigen-binding fragment thereof.

[0008] In some examples, the antibody or antigen-binding fragment thereof comprises an immunoglobulin heavy chain variable region (V_H) and an immunoglobulin light chain variable region (V_L or V_K).

[0009] In some examples, the antibody or antigen-binding fragment thereof comprises a Fab fragment.

[0010] In some examples, the Fab fragment is a single-chain Fab fragment (scFab).

[0011] In some examples, the TNF receptor-binding moiety is not capable of binding to a TNF receptor which comprises a cytoplasmic tail which comprises a death domain.

[0012] In some examples, the TNF-receptor-binding moiety is capable of binding to a TNF receptor that is expressed on a hematopoietic cell.

[0013] In some examples, the TNF receptor is selected from the group consisting of 4-1BB (also known as CD137), OX40 (also known as CD134), CD40, herpes-virus entry mediator (HVEM), CD30, CD27 (also known as TNFRSF7), GITR (also known as TNFRSF18), and TNFR2.

[0014] In some examples, the TNF receptor is 4-1BB or OX40.

[0015] In some examples, the TNF receptor-binding moiety comprises an antibody fragment comprising a heavy chain variable region (V_H) and light chain variable region (V_L or V_K), wherein the CDRs of the V_H and the V_L or V_K (CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3) have sequences which each differ by at most two amino acids from the CDRs of a 4-1BB or OX40 antibody.

[0016] In some examples, the CDRs of the V_H and the V_L or V_K have sequences which are identical to those of the heavy and light chain CDRs of a 4-1BB or OX40 antibody, except for one or two amino acid substitutions total across all six CDRs.

[0017] In some examples, the CDRs of the V_H and the V_L or V_K are identical to those of the heavy and light chain CDRs of a 4-1BB or OX40 antibody.

[0018] In some examples, the TNF receptor-binding moiety comprises an antibody fragment comprising heavy and light chain variable regions with sequences that have at least 85% identity to those of the heavy and light chain variable regions of a 4-1BB or OX40 antibody.

[0019] In some examples, the nanocage monomer is a ferritin monomer or a subunit thereof.

[0020] In some examples, the ferritin monomer is a human ferritin monomer.

[0021] In some examples, the ferritin monomer is a ferritin light chain.

[0022] In some examples, the TNF receptor-binding moiety is linked via an amino acid linker to the nanocage monomer or subunit thereof.

[0023] In some examples, the TNF receptor-binding moiety is linked via the N-terminus of the nanocage monomer or subunit thereof.

[0024] In one aspect, described are self-assembled polypeptide complexes comprising: (a) a plurality of TNF receptor-binding fusion polypeptides, each TNF receptor-binding fusion polypeptide being a fusion polypeptide of as disclosed herein; and (b) a plurality of Fc fusion polypeptides, each Fc fusion polypeptide comprising (1) an Fc polypeptide and (2) a nanocage monomer or subunit thereof.

[0025] In some examples, the self-assembled polypeptide complex further comprises (c) a plurality of tumor-binding fusion polypeptides, each tumor-binding fusion polypeptide comprising (1) tumor-binding moiety and (2) a nanocage monomer or subunit thereof.

[0026] In some examples, within each Fc fusion polypeptide, the Fc polypeptide is linked via an amino acid linker to the nanocage monomer or subunit thereof.

[0027] In some examples, the Fc polypeptide is linked via the N-terminus of the nanocage monomer or subunit thereof.

[0028] In some examples, the Fc polypeptide is linked via the C-terminus of the nanocage monomer or subunit thereof.

[0029] In some examples, with each tumor-binding fusion polypeptide, the tumor-binding moiety is linked via an amino acid linker to the nanocage monomer or subunit thereof.

[0030] In some examples, the tumor-binding moiety is linked via the N-terminus of the nanocage monomer or subunit thereof.

[0031] In some examples, the tumor-binding moiety comprises an antibody or antigen-binding fragment thereof.

[0032] In some examples, the antibody or antigen-binding fragment thereof comprises an immunoglobulin heavy chain variable region (V_H) and an immunoglobulin light chain variable region (V_L or V_K).

[0033] In some examples, the antibody or antigen-binding fragment thereof comprises a Fab fragment.

[0034] In some examples, the Fab fragment is a single-chain Fab fragment (scFab).

[0035] In some examples, the tumor-binding moiety is a PD-L1-binding moiety, an EGFR-binding moiety, or a B7-H3-binding moiety.

[0036] In some examples, the tumor-binding moiety comprises an antibody fragment comprising a heavy chain variable region (V_H) and light chain variable region (V_L or V_K), wherein the CDRs of the V_H and the V_L or V_K (CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2, and CDR-L3) have sequences which each differ by at most two amino acids from the CDRs of a PD-L1 binding moiety, an EGFR-binding moiety, or a B7-H3-binding moiety antibody.

[0037] In some examples, the CDRs of the V_H and the V_L or V_K have sequences which are identical to those of the heavy and light chain CDRs of a PD-L1, an EGFR-binding moiety, or a B7-H3-binding moiety antibody, except for one or two amino acid substitutions total across all six CDRs.

[0038] In some examples, the CDRs of the V_H and the V_L or V_K are identical to those of the heavy and light chain CDRs of a PD-L1, EGFR, or B7-H3 antibody.

[0039] In some examples, the tumor-binding moiety comprises an antibody fragment comprising heavy and light chain variable regions with sequences that have at least 85% identity to those of the heavy and light chain variable regions of a PD-L1, EGFR, or B7-H3 antibody.

[0040] In some examples, the nanocage monomers within the Fc fusion polypeptide and within TNF receptor-binding fusion polypeptide and/or the tumor-binding fusion polypeptide are each a ferritin monomer or a subunit thereof.

[0041] In some examples, the ferritin monomer is a human ferritin monomer.

[0042] In some examples, the ferritin monomer is a ferritin light chain.

[0043] In some examples, the self-assembled polypeptide complex does not comprise any ferritin heavy chains or subunits of ferritin heavy chains.

[0044] In some examples, the self-assembled polypeptide complex does not comprise any iron-binding moieties.

[0045] In one aspect, described are pharmaceutical compositions comprising a self-assembled polypeptide complex as disclosed herein and a pharmaceutically acceptable excipient.

[0046] In one aspect, described are uses of a self-assembled polypeptide complex or a pharmaceutical composition as disclosed herein in the manufacture of a medicament for treating, ameliorating, or preventing a disease or condition in a subject.

[0047] In one example, described are methods of treating, ameliorating, or preventing a disease or condition, the method comprising administering to a subject a self-assembled polypeptide complex or a pharmaceutical composition as disclosed herein.

[0048] In some examples, the subject is a mammal, e.g., a human.

[0049] In some examples, the disease or condition is cancer.

[0050] In some examples, the subject has an immunologically cold tumor.

[0051] In some examples, the subject has a tumor, and said step of administering results in regression of the tumor. In some examples, the step of administering results in complete regression of the tumor.

BRIEF DESCRIPTION OF THE DRAWINGS

[0052] The patent or application file contains at least one drawing executed in color. Copies of this patent or patent application publication with color drawing(s) will be provided by the Office upon request and payment of the necessary fee.

[0053] **FIG. 1A** is a diagrammatic representation of human ferritin light chain (hFTL) and example N-half ferritin (N-hFTL) and C-half ferritin (C-hFTL) molecules.

[0054] **FIGs. 1B** and **1C** are diagrammatic representations of sets of fusion polypeptides that together may form exemplary Multabodies (MBs) of the disclosure.

[0055] **FIG. 2** is a schematic depicting the various extracellular cysteine-rich domains (CRDs) of 4-1BB and their respective distances from the cell membrane.

[0056] **FIGs. 3A** and **3B** are schematics that depict an *in vitro* 4-1BB reporter assay. Luciferase expression is induced by downstream signals upon activation (clustering) of 4-1BB at the surface of effector cells. The assay can be performed without (**FIG. 3A**) or with (**FIG. 3B**) FcγRIIb CHO-K1 cells to assess Fc-independent or Fc-dependent crosslinking, respectively.

[0057] **FIGs. 4A** and **4B** are graphs showing the fold induction (based on luciferase signal) of 4-1BB activation (y-axis) plotted against concentration of urelumab (x-axis). **FIG. 4A** depicts the results from assays conducted without FcγRIIb CHO-K1 cells (and therefore

CLAIMS

1. A fusion polypeptide comprising: (1) a tumor necrosis factor (TNF) receptor-binding moiety and (2) a nanocage monomer or subunit thereof, wherein the TNF receptor-binding moiety is not capable of binding to death receptor 4 (DR4) or death receptor 5 (DR5).

2. The fusion polypeptide of claim 1, wherein the TNF receptor-binding moiety comprises an antibody or antigen-binding fragment thereof.

3. The fusion polypeptide of claim 1 or 2, wherein the TNF receptor-binding moiety is not capable of binding to a TNF receptor which comprises a cytoplasmic tail which comprises a death domain.

4. The fusion polypeptide of any one of claims 1-3, wherein the TNF-receptor-binding moiety is capable of binding to a TNF receptor that is expressed on a hematopoietic cell.

5. The fusion polypeptide of claim 4, wherein the TNF receptor is selected from the group consisting of 4-1BB (also known as CD137), OX40 (also known as CD134), CD40, herpes-virus entry mediator (HVEM), CD30, CD27 (also known as TNFRSF7), GITR (also known as TNFRSF18), and TNFR2.

6. The fusion polypeptide of claim 5, wherein the TNF receptor is 4-1BB or OX40.

7. The fusion polypeptide of any one of claims 1-6, wherein the nanocage monomer is a ferritin monomer or a subunit thereof.

8. The fusion polypeptide of claim 7, wherein the ferritin monomer is a human ferritin monomer.

9. The fusion polypeptide of claim 7 or 8, wherein the ferritin monomer is a ferritin light chain.

10. A self-assembled polypeptide complex comprising:

(a) a plurality of TNF receptor-binding fusion polypeptides, each TNF receptor-binding fusion polypeptide being a fusion polypeptide of any one of claims 1-9; and

(b) a plurality of Fc fusion polypeptides, each Fc fusion polypeptide comprising (1) an Fc polypeptide and (2) a nanocage monomer or subunit thereof.

11. The self-assembled polypeptide complex of claim 10, further comprising

(c) a plurality of tumor-binding fusion polypeptides, each tumor-binding fusion polypeptide comprising (1) tumor-binding moiety and (2) a nanocage monomer or subunit thereof.

12. The self-assembled polypeptide complex of claim 11, wherein the tumor-binding moiety comprises an antibody or antigen-binding fragment thereof.

13. The self-assembled polypeptide complex of claim 12, wherein the tumor-binding moiety is a PD-L1-binding moiety, an EGFR-binding moiety, or a B7-H3-binding moiety.

14. The self-assembled polypeptide complex of any one of claims 10-13, wherein the nanocage monomers within the Fc fusion polypeptide and within TNF receptor-binding fusion polypeptide and/or the tumor-binding fusion polypeptide are each a ferritin monomer or a subunit thereof.

15. The self-assembled polypeptide complex of claim 14, wherein the ferritin monomer is a human ferritin monomer.

16. The self-assembled polypeptide complex of claim 14 or 15, wherein the ferritin monomer is a ferritin light chain.

17. The self-assembled polypeptide complex of claim 16, which does not comprise any ferritin heavy chains or subunits of ferritin heavy chains.

18. The self-assembled polypeptide complex of any one of claims 14-17, which does not comprise any iron-binding moieties.

19. A pharmaceutical composition comprising a self-assembled polypeptide complex of any one of claims 10-18 and a pharmaceutically acceptable excipient.

20. Use of the self-assembled polypeptide complex of any one of claims 10-18 or the pharmaceutical composition of claim 19 in the manufacture of a medicament for treating, ameliorating, or preventing a disease or condition in a subject.

21. The use of claim 20, wherein the subject is a mammal.

22. The use of claim 21, wherein the subject is human.

23. The use of any one of claims 20-22, wherein the disease or condition is cancer.

24. The use of any one of claims 20-22, wherein the disease or condition is an autoimmune disease or condition.