MULTISPECIFIC BINDING PROTEINS TARGETING NKG2D, CD16, AND ~~CAIX, ANO1, MESOTHELIN,~~ TROP2~~, CEA, OR CLAUDIN-18.2~~

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. application number 62/464,341 filed February 27, 2017, U.S. application number 62/464,344 filed February 27, 2017, U.S. application number 62/464,347 filed February 27, 2017, U.S. application number 62/467,557 filed March 06, 2017, U.S. application number 62/473,652 filed March 20, 2017, and U.S. application number 62/473,659 filed March 20, 2017, the entire contents of each of which are hereby incorporated by reference in their entireties for all purposes.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on February 27, 2018, is named DFY-012WO_ST25.txt and is 212,480 bytes in size.

FIELD OF THE INVENTION

[0003] The invention relates to multi-specific binding proteins that bind to the NKG2D receptor, CD16, and a tumor-associated antigen selected from carbonic anhydrase 9 (CAIX), anoctamin-1 (ANO1), mesothelin, trophoblast cell-surface antigen (TROP2), carcinoembryonic antigen (CEA) and Claudin-18.2.

BACKGROUND

[0004] Cancer continues to be a significant health problem despite the substantial research efforts and scientific advances reported in the literature for treating this disease. Some of the most frequently diagnosed cancers include prostate cancer, breast cancer, and lung cancer. Prostate cancer is the most common form of cancer in men. Breast cancer remains a leading cause of death in women. Small cell lung cancer remains among the most deadly of malignancies. Current treatment options for these cancers are not effective for all patients and/or can have substantial adverse side effects. Other types of cancer also remain challenging to treat using existing therapeutic options.

[0005] Cancer immunotherapies are desirable because they are highly specific and can facilitate destruction of cancer cells using the patient's own immune system. Fusion proteins

with esophageal cancer, non-small cell lung carcinoma, ovarian cancer, colon cancer, and several forms of biliary ductal carcinoma.

SUMMARY

[0014] The disclosure provides multi-specific binding proteins that bind to CAIX, ANO1, mesothelin, TROP2, CEA or Claudin-18.2 on a cancer cell and to the NKG2D receptor and CD16 receptor on natural killer cells. Such proteins can engage more than one kind of NK activating receptor, and may block the binding of natural ligands to NKG2D. In certain examples, the proteins can agonize NK cells in humans, and in other species such as rodents and cynomolgus monkeys. Various aspects and examples of the disclosure are described in further detail below.

[0015] In one aspect, the present disclosure refers to a protein comprising: (a) a first antigen-binding site that binds NKG2D; (b) a second antigen-binding site that ~~binds CAIX, ANO1, mesothelin,~~ TROP2~~, or Claudin-18.2~~; and (c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16. In another aspect, the present disclosure refers to a formulation comprising a protein as described herein and a pharmaceutically acceptable carrier. In yet another aspect, the present disclosure refers to a cell comprising one or more nucleic acids encoding a protein as described herein. In yet another aspect, the present disclosure refers to an *in vitro* method of directly and/or indirectly enhancing tumor cell death, the method comprising exposing a tumor and natural killer cells to ~~a~~ the protein as described herein. In still another aspect, the present disclosure refers to use of a protein as described herein or ~~a~~ the formulation as described herein in the manufacture of a medicament for treating cancer.

[0015a] Accordingly, one example of the disclosure provides a protein that incorporates a first antigen-binding site that binds NKG2D; a second antigen-binding site that binds CAIX, ANO1, mesothelin, TROP2, CEA or Claudin-18.2; and an antibody Fc domain, a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16. The antigen-binding sites may each incorporate an antibody heavy chain variable domain and an antibody light chain variable domain (*e.g.* arranged as in an antibody, or fused together to from an scFv), or one or more of the antigen-binding sites may be a single domain antibody, such as a V_HH antibody like a camelid antibody or a V_{NAR} antibody like those found in cartilaginous fish.

[0016] In one example, the first antigen-binding site, which binds to NKG2D, incorporates a heavy chain variable domain related to SEQ ID NO:1, such as by having an amino acid sequence at least 90% (*e.g.*, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or 100%) identical

to SEQ ID NO:1, and/or incorporating amino acid sequences identical to the CDR1 (SEQ ID NO:51), CDR2 (SEQ ID NO:52), and CDR3 (SEQ ID NO:53) sequences of SEQ ID NO:1. The heavy chain variable domain related to SEQ ID NO:1 can be coupled a variety of light chain

CLAIMS

1. A protein comprising:

(a) a first antigen-binding site that binds NKG2D;

(b) a second antigen-binding site that binds ~~CAIX, ANO1, mesothelin,~~ TROP2~~, or Claudin-18.2~~; and

(c) an antibody Fc domain or a portion thereof sufficient to bind CD16, or a third antigen-binding site that binds CD16.

2. The protein of claim 1, wherein the first antigen-binding site binds to NKG2D in humans and non-human primates.

3. The protein of claim 1 or 2, wherein the first antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.

4. The protein according to claim 3, wherein the heavy chain variable domain and the light chain variable domain are present on the same polypeptide.

5. The protein according to claim~~s~~ 3 or 4, wherein the second antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.

6. The protein according to claim 5, wherein the heavy chain variable domain and the light chain variable domain of the second antigen-binding site are present on the same polypeptide.

7. The protein according to claim 5 or 6, wherein the light chain variable domain of the first antigen-binding site has an amino acid sequence identical to the amino acid sequence of the light chain variable domain of the second antigen-binding site.

8. The protein according to any one of claims 1-7, wherein the first antigen binding site comprises:

(a) a heavy chain complementarity determining region 1 (CDR1) amino acid sequence of SEQ ID NO:51;

a heavy chain complementarity determining region 2 (CDR2) amino acid sequence of SEQ ID NO:52; and

a heavy chain complementarity determining region 3 (CDR3) amino acid sequence of SEQ ID NO:53;

(b) a heavy chain CDR1 amino acid sequence of SEQ ID NO:54;

a heavy chain CDR2 amino acid sequence of SEQ ID NO:55;

a heavy chain CDR3 amino acid sequence of SEQ ID NO:56;

a light chain CDR1 amino acid sequence of SEQ ID NO:57;

a light chain CDR2 amino acid sequence of SEQ ID NO:58; and

a light chain CDR3 amino acid sequence of SEQ ID NO:59;

(c) a heavy chain CDR1 amino acid sequence of SEQ ID NO:60;

a heavy chain CDR2 amino acid sequence of SEQ ID NO:61;

a heavy chain CDR3 amino acid sequence of SEQ ID NO:62;

a light chain CDR1 amino acid sequence of SEQ ID NO:63;

a light chain CDR2 amino acid sequence of SEQ ID NO:64; and

a light chain CDR3 amino acid sequence of SEQ ID NO:65; or

(d) a heavy chain CDR1 amino acid sequence of SEQ ID NO:66;

a heavy chain CDR2 amino acid sequence of SEQ ID NO:67;

a heavy chain CDR3 amino acid sequence of SEQ ID NO:68;

a light chain CDR1 amino acid sequence of SEQ ID NO:69;

a light chain CDR2 amino acid sequence of SEQ ID NO:70; and

a light chain CDR3 amino acid sequence of SEQ ID NO:71.

~~8~~9. A protein according to any one of ~~the preceding~~ claims 1-7, wherein the first antigen-binding site comprises:

(a) a heavy chain variable domain at least 90% identical to SEQ ID NO:1~~.~~;

~~9. The protein according to any one of claims 1-7, wherein the first antigen-binding site comprises~~ (b) a heavy chain variable domain at least 90% identical to SEQ ID NO:41 and a light chain variable domain at least 90% identical to SEQ ID NO:42~~.~~;

~~10. The protein according to any one of claims 1-7, wherein the first antigen-binding site comprises~~ (c) a heavy chain variable domain at least 90% identical to SEQ ID NO:43 and a light chain variable domain at least 90% identical to SEQ ID NO:44~~.~~;

~~11. The protein according to any one of claims 1-7, wherein the first antigen-binding site comprises~~ (d) a heavy chain variable domain at least 90% identical to SEQ ID NO:45 and a light chain variable domain at least 90% identical to SEQ ID NO:46~~.~~;

~~12. The protein according to any one of claims 1-7, wherein the first antigen-binding site comprises~~ (e) a heavy chain variable domain at least 90% identical to SEQ ID NO:47 and a light chain variable domain at least 90% identical to SEQ ID NO:48~~.~~; or

~~13. The protein according to any one of claims 1-7, wherein the first antigen-binding site comprises~~ (f) a heavy chain variable domain at least 90% identical to SEQ ID NO:49 and a light chain variable domain at least 90% identical to SEQ ID NO:50.

~~14. The protein according to any one of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:132 and a light chain variable domain at least 90% identical to SEQ ID NO:133.~~

~~15. The protein according to any one of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:140 and a light chain variable domain at least 90% identical to SEQ ID NO:141.~~

~~16. The protein according to any one of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:148 and a light chain variable domain at least 90% identical to SEQ ID NO:149.~~

~~17. The protein according to any one of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:156 and a light chain variable domain at least 90% identical to SEQ ID NO:157.~~

~~18. The protein according to any one of claims 1-7, wherein the first antigen-binding site comprises a heavy chain variable domain at least 90% identical to SEQ ID NO:164 and a light chain variable domain at least 90% identical to SEQ ID NO:165.~~

~~19~~10. The protein of claim 1 or 2, wherein the first antigen-binding site is a single-domain antibody.

~~20~~11. The protein of claim ~~19~~10, wherein the single-domain antibody is a V_HH fragment or a V_{NAR} fragment.

~~21~~12. The protein according to any one of claims 1-2 or ~~19~~10-~~20~~11, wherein the second antigen-binding site comprises a heavy chain variable domain and a light chain variable domain.

~~22~~13. The protein according to claim ~~21~~12, wherein the heavy chain variable domain and the light chain variable domain of the second antigen-binding site are present on the same polypeptide.

14. The protein according to any one of claims 1-13, wherein the second antigen binding site comprises:

(a) a heavy chain CDR1 amino acid sequence of SEQ ID NO:174;
a heavy chain CDR2 amino acid sequence of SEQ ID NO:175;
a heavy chain CDR3 amino acid sequence of SEQ ID NO:176;
a light chain CDR1 amino acid sequence of SEQ ID NO:177;
a light chain CDR2 amino acid sequence of SEQ ID NO:178; and
a light chain CDR3 amino acid sequence of SEQ ID NO:179; or

(b) a heavy chain CDR1 amino acid sequence of SEQ ID NO:182;
a heavy chain CDR2 amino acid sequence of SEQ ID NO:183;
a heavy chain CDR3 amino acid sequence of SEQ ID NO:184;
a light chain CDR1 amino acid sequence of SEQ ID NO:185;
a light chain CDR2 amino acid sequence of SEQ ID NO:186; and
a light chain CDR3 amino acid sequence of SEQ ID NO:187.

~~23. The protein according to any of the preceding claims, wherein the second antigen-binding site binds CAIX and comprises a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:72 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:73.~~

~~24. The protein according to any of the preceding claims, wherein the second antigen-binding site binds CAIX and comprises:~~

~~a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:74;~~

~~a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:75; and~~

~~a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:76.~~

~~25. The protein according to claim 24, wherein the second antigen-binding site comprises:~~

~~a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:77;~~

~~a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:78; and~~

~~a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:79.~~

~~26. The protein according to any one of claims 1-22, wherein the second antigen-binding site binds CAIX and comprises a heavy chain variable domain of comprising an amino acid sequence at least 90% identical to SEQ ID NO:80 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:81.~~

~~27. The protein according to any one of claims 1-22 or 26, wherein the second antigen-binding site binds CAIX and comprises:~~

~~a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:82;~~

~~a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:83; and~~

~~a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:84.~~

28. The protein according to claim 27, wherein the second antigen-binding site comprises:

a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:85;

a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:86; and

a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:87.

29. The protein according to any one of claims 1-22, wherein the second antigen-binding site binds CAIX and comprises a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:88 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:89.

30. The protein according to any one of claims 1-22 or 29, wherein the second antigen-binding site binds CAIX and comprises:

a heavy chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:90;

a heavy chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:91; and

a heavy chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:92.

31. The protein according to claim 30, wherein the second antigen-binding site comprises:

a light chain CDR1 sequence identical to the amino acid sequence of SEQ ID NO:93;

a light chain CDR2 sequence identical to the amino acid sequence of SEQ ID NO:94; and

a light chain CDR3 sequence identical to the amino acid sequence of SEQ ID NO:95.

32. The protein according to any one of claims 1-22, wherein the second antigen-binding site binds ANO1 and comprises a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:97 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:98.

33. The protein according to any one of claims 1-22, wherein the second antigen-binding site binds mesothelin and comprises a heavy chain variable domain comprising an amino acid

~~sequence at least 90% identical to SEQ ID NO:106 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:107.~~

~~34. The protein according to any one of claims 1-22, wherein the second antigen-binding site binds mesothelin and comprises a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:114 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:115.~~

~~35. The protein according to any one of claims 1-22, wherein the second antigen-binding site binds mesothelin and comprises a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:122 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:123.~~

~~36~~15. The protein according to any one of claims 1-~~22~~13, wherein the second antigen-binding site ~~binds TROP2 and~~ comprises:

(a) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:172 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:173;

~~37. The protein according to any one of claims 1-22, wherein the second antigen-binding site binds TROP2 and comprises~~ (b) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:180 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:181~~.~~;

~~38. The protein according to any one of claims 1-22, wherein the second antigen-binding site binds TROP2 and comprises~~ (c) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:188 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:189~~.~~;

~~39. The protein according to any one of claims 1-22, wherein the second antigen-binding site binds TROP2 and comprises~~ (d) a heavy chain variable domain comprising

an amino acid sequence at least 90% identical to SEQ ID NO:190 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:191;. or

40. The protein according to any one of claims 1-22, wherein the second antigen-binding site binds TROP2 and comprises (e) a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:192 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:193.

41. The protein according to any one of claims 1-22, wherein the second antigen-binding site binds claudin-18.2 and comprises a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:220 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:221.

42. The protein according to any one of claims 1-22, wherein the second antigen-binding site binds claudin-18.2 and comprises a heavy chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:228 and a light chain variable domain comprising an amino acid sequence at least 90% identical to SEQ ID NO:229.

4316. A protein according to any one of claims 1-4 or 8-2011, wherein the second antigen-binding site is a single-domain antibody.

4417. The protein of claim 4316, wherein the second antigen-binding site is a V_HH fragment or a V_{NAR} fragment.

4518. A protein according to any one of the preceding claims 1-17, wherein the protein comprises a portion of an antibody Fc domain sufficient to bind CD16, wherein the antibody Fc domain comprises hinge and CH2 domains.

4619. A protein according to claim 4518, wherein the antibody Fc domain comprises hinge and CH2 domains of a human IgG1 antibody.

4720. A protein according to claim 45-18 or 4619, wherein the Fc domain comprises an amino acid sequence at least 90% identical to amino acids 234-332 of a human IgG1 antibody.

~~48~~21. A protein according to any one of claims ~~45~~18-~~47~~20, wherein the Fc domain comprises an amino acid sequence at least 90% identical to the Fc domain of human IgG1 and differs at one or more positions selected from the group consisting of Q347, Y349, L351, S354, E356, E357, K360, Q362, S364, T366, L368, K370, N390, K392, T394, D399, S400, D401, F405, Y407, K409, T411, and K439.

~~49~~22. A formulation comprising a protein according to any one of ~~the preceding~~ claims 1-21 and a pharmaceutically acceptable carrier.

~~50~~23. A cell comprising one or more nucleic acids encoding a protein according to any one of claims 1-~~48~~21.

~~51~~24. An *in vitro* method of directly and/or indirectly enhancing tumor cell death, the method comprising exposing a tumor and natural killer cells to ~~a~~ the protein according to any one of claims 1-~~48~~21.

25. The protein according to any one of claims 1-21 or the formulation of claim 22 for use in therapy.

~~52~~26. Use of a protein according to any one of claims 1-~~48~~ 21 or ~~a~~ the formulation according to claim ~~49~~ 22 in the manufacture of a medicament for treating cancer.

~~53. The use of claim 52, wherein the second antigen binding site of the protein binds CAIX, and the cancer to be treated is selected from the group consisting of renal cell carcinoma, breast cancer, glioblastoma, head and neck cancer, gastric cancers, bladder cancer, ovarian cancer, tumors of the esophagus, lung, colon, kidney, cervix and non-small cell lung carcinoma.~~

~~54. The use of claim 52, wherein the second antigen binding site of the protein binds mesothelin, and the cancer to be treated is selected from the group consisting of mesothelioma, ovarian cancer, pancreatic cancer, non-small cell lung cancer, breast cancer, cholangiocarcinoma, gastric cancer, uterine serous carcinoma, thymic carcinoma, and acute myeloid leukemia.~~

~~55. The use of claim 52, wherein the second antigen binding site of the protein binds ANO1, and the cancer to be treated is selected from the group consisting of esophageal squamous cell~~

~~cancer (ESCC), gastrointestinal stromal tumor (GIST), head and neck squamous cell carcinoma (HNSCC), pancreatic cancer, breast cancer, prostate cancer, and sarcoma.~~

~~56~~27. The use of claim ~~52~~26, wherein ~~the second antigen binding site of the protein binds TROP2, and~~ the cancer to be treated is selected from the group consisting of breast, lung, gastric, colorectal, pancreatic, prostatic, cervical, head-and-neck cancer, nasopharyngeal carcinoma, and ovarian carcinoma.

~~57. The use of claim 52, wherein the second antigen binding site of the protein binds claudin-18.2, and the cancer to be treated is selected from the group consisting of esophageal cancer, non-small cell lung carcinoma, ovarian cancer, colon cancer, and several forms of biliary ductal carcinoma.~~