

would be particularly useful.

SUMMARY

[0012] In one aspect, the present disclosure provides a bispecific antigen binding construct comprising a binding domain capable of binding to an HLA-G epitope and an additional binding domain capable of binding to a second epitope. In some examples, the second epitope comprises or consists of a CD3 ϵ epitope. In some examples, the CD3 ϵ epitope comprises or consists of an amino acid sequence set forth in SEQ ID NO: 629.

[0013] In some examples, the additional binding domain capable of binding to a CD3 ϵ epitope comprises or consists of a heavy chain variable region (VH) and a light chain variable region (VL), with VH and/or VL comprising 1, 2, 3, 4, 5, or 6 of the following:

- a) a VHCDR1 having the sequence set forth in any one of SEQ ID NOS: 346-349 or 354-357,
- b) a VHCDR2 having the sequence set forth in any one of SEQ ID NOS: 362-365 or 371-375,
- c) a VHCDR3 having the sequence set forth in any one of SEQ ID NOS: 379-382,
- d) a VLCDR1 having the sequence set forth in any one of SEQ ID NOS: 388-392,
- e) a VLCDR2 having the sequence set forth in any one of SEQ ID NOS: 396-400,
and
- f) a VLCDR3 having the sequence set forth in any one of SEQ ID NOS: 404-408.

[0014] In some examples, the additional binding domain comprises or consists of an NK cell engager, dendritic cell engager, monocyte, or macrophage engager. In some examples, the NK cell engager comprises or consists of an antibody for a CD16 epitope. In some examples, the NK cell engager comprises or consists of an antibody for NKp46. In some examples, the NK cell engager comprises or consists of an antibody for NKp30. In some examples, the monocyte or macrophage engager comprises or consists of an antibody for a CD16 epitope.

[0015] In some examples, the HLA-G epitope comprises or consists of an amino acid sequence set forth in SEQ ID NO: 342. In some embodiments, the binding domain capable of binding to an HLA-G epitope comprises or consists of a heavy chain variable region (VH) and a light chain variable region (VL), with VH and/or VL comprising 1, 2, 3, 4, 5, or 6 of the following:

- a) a VHCDR1 having the sequence set forth in any one of SEQ ID NOS: 1-14 or 18-34,
- b) a VHCDR2 having the sequence set forth in any one of SEQ ID NOS: 38-50 or 54-71,
- c) a VHCDR3 having the sequence set forth in any one of SEQ ID NOS: 76-101,
- d) a VLCDR1 having the sequence set forth in any one of SEQ ID NOS: 105-124,
- e) a VLCDR2 having the sequence set forth in any one of SEQ ID NOS: 128-145,
- and
- f) a VLCDR3 having the sequence set forth in any one of SEQ ID NOS: 149-166.

[0016] In some examples, the binding domain capable of binding to an HLA-G epitope comprises a heavy chain variable region (VH) and a light chain variable region (VL), with VH comprising, consisting of, or consisting essentially of a VH having the sequence set forth in SEQ ID NOS: 170-200 and with the VL comprising, consisting of, or consisting essentially of a VL having the sequence set forth in SEQ ID NO: 204-228 and the additional binding domain capable of binding to a CD3 ϵ epitope comprises a heavy chain variable region (VH) and a light chain variable region (VL), with VH comprising, consisting of, or consisting essentially of a VH having the sequence set forth in SEQ ID NOS: 413-418 and with the VL comprising, consisting of, or consisting essentially of a VL having the sequence set forth in SEQ ID NOS: 422-427.

[0017] In another aspect, the present disclosure provides a pharmaceutical composition comprising or consisting of any of the bispecific antigen binding constructs provided herein. In some examples, the pharmaceutical composition further comprises an effective amount of one or more of:

- a) an anti-PD-L1 antibody or small molecule inhibitor;

- r) a chemotherapy;
- s) ADCC capable therapies using effector competent antibodies such as anti-CD19, anti-CD20, anti-EGFR, anti-Her2, anti-SLAMF7, anti-CD52, anti-BCMA, anti-GD2, and/or anti-CCR4.

In some examples, the pharmaceutical composition further comprises one or both of:

- a) an antibody to an immune inhibitory receptor or ligand and/or
- b) an antibody to an immune stimulatory receptor or ligand.

[0018] In another aspect, the present disclosure provides one or more nucleic acids encoding any of the bispecific antigen binding constructs provided herein. In some examples, the one or more nucleic acids comprises one or more vectors. In another aspect, the present disclosure provides one or more vectors comprising the one or more nucleic acids as described herein. In another aspect, the present disclosure provides a host transformed with a vector as described herein.

[0019] In another aspect, the present disclosure provides a method for the production of one or more bispecific antigen binding construct comprising the steps of expressing any of the one or more nucleic acids provided herein in a prokaryotic or eukaryotic host cell and recovering the one or more bispecific antigen binding construct from the cell or the cell culture supernatant.

[0020] The present disclosure provides provides a method for treating a subject with cancer comprising administering a therapeutically effective amount of any of the bispecific antigen binding constructs or pharmaceutical compositions provided herein to the subject. In another aspect, the present disclosure provides the bispecific antigen binding construct as described herein or the pharmaceutical composition as described herein for use in therapy. In another aspect, the present disclosure provides use of the bispecific antigen binding construct as described herein or the pharmaceutical composition as described herein in the manufacture of a medicament for treating a subject with cancer.

[0021] In some examples, the cancer is a solid cancer. In some examples, the cancer is a hematological cancer. In some examples, the cancer is selected from the group consisting of a hematopoietic cancer, hepatocellular carcinoma, leukemia, colorectal cancer (CRC), breast cancer, gastric cancer, esophageal cancer, endometrial cancer, prostate cancer, bladder cancer, thyroid cancer, liver cancer, pancreatic cancer, triple negative breast cancer, cervical cancer, ovarian cancer, uterine cancer, vaginal cancer, vulvar cancer, lung cancer, head and neck cancer, melanoma, renal cell carcinoma, cutaneous squamous cell carcinoma, Hodgkin's lymphoma, a metastasis of the brain, a metastasis of the lung, a metastasis of the liver, and/or a

metastasis of the bone, or an unresectable or metastatic solid tumor with DNA mismatch repair deficiencies or a microsatellite instability-high state. In some examples, the cancer is a cancer that expresses HLA-G.

[0022] In some examples, the method further comprises one or more of the following:

- a) administering chemotherapy to the subject;
- b) administering radiation therapy to the subject; and/or
- c) administering one or more additional therapeutic agents to the subject.

In some examples, the one or more additional therapeutic agents comprise one or more immunomodulatory agents. For example, these agents could be ones that antagonize or block inhibitory signals and interactions. The agents could provide activation signals or costimulation to immune cells.

[0023] In some examples, the one or more immunomodulatory agents comprise an antagonist to an inhibitory receptor of an immune cell. In some examples, the inhibitory receptor is at least one of LILRB1, LILRB2, LILRB4, KIR2DL4, CTLA-4, PD-1, PD-L1, PD-L2, LAG-3, Tim3, TIGIT, B7-H3, B7-H4, neuritin, BTLA, CECAM-1, CECAM-5, VISTA, LAIR1, CD160, 2B4, TGF-B receptor, NKG2A, and/or a Killer-cell immunoglobulin-like receptor (KIR). In some examples, the one or more immunomodulatory agents comprise an agonist of a co-stimulatory receptor of an immune cell. In some examples, the co-stimulatory receptor is at least one of OX40, CD2, CD27, ICAM-1, LFA-1, ICOS (CD278), 4-1BB (CD137), GITR, CD28, CD30, CD40, BAFFR, HVEM, CD7, LIGHT, NKG2C, SLAMF7, NKp30, NKp46, NKp80, CD160, and/or CD83.

[0024] In some examples, the one or more immunomodulatory agents is one or more cytokines. In some examples, the one or more cytokines is at least one of G-CSF, GM-CSF, IFN-alpha, IFN-beta, IFN-gamma, FLt3 ligand, IL-1, IL-2, IL-5, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21, and/or IL-27.

[0025] In some examples, the one or more immunomodulatory agents is one or more oncolytic viruses. In some examples, the one or more oncolytic viruses is a Herpes simplex virus, a Vesicular stomatitis virus, an adenovirus, a Newcastle disease virus, a vaccinia virus,

Claims:

1. A bispecific antigen binding construct comprising a binding domain capable of binding to an HLA-G epitope and an additional binding domain capable of binding to a second epitope.
2. The bispecific antigen binding construct according to claim 1, wherein the second epitope comprises a CD3 ϵ epitope.
3. The bispecific antigen binding construct according to claim 2, wherein the CD3 ϵ epitope comprises or consists of an amino acid sequence set forth in SEQ ID NO: 629.
4. The bispecific antigen binding construct according to any one of claims 2-3, wherein the additional binding domain capable of binding to a CD3 ϵ epitope comprises or consists of a heavy chain variable region (VH) and a light chain variable region (VL), with VH and/or VL comprising 1, 2, 3, 4, 5, or 6 of the following:
 - a) a VHCDR1 having the sequence set forth in any one of SEQ ID NOS: 346-349 or 354-357,
 - b) a VHCDR2 having the sequence set forth in any one of SEQ ID NOS: 362-365 or 371-375,
 - c) a VHCDR3 having the sequence set forth in any one of SEQ ID NOS: 379-382,
 - d) a VLCDR1 having the sequence set forth in any one of SEQ ID NOS: 388-392,
 - e) a VLCDR2 having the sequence set forth in any one of SEQ ID NOS: 396-400,and
 - f) a VLCDR3 having the sequence set forth in any one of SEQ ID NOS: 404-408.

5. The bispecific antigen binding construct according to claim 1, wherein the additional binding domain comprises an NK cell engager, dendritic cell engager, monocyte, or macrophage engager.
6. The bispecific antigen binding construct according to claim 5, wherein the NK cell engager comprises an antibody for a CD16 epitope.
7. The bispecific antigen binding construct according to claim 5, wherein the NK cell engager comprises an antibody for NKp46.
8. The bispecific antigen binding construct according to claim 5, wherein the NK cell engager comprises an antibody for NKp30.
9. The bispecific antigen binding construct according to claim 5, wherein the monocyte or macrophage engager comprises an antibody for a CD16 epitope.
10. The bispecific antigen binding construct according to claim 1, wherein the HLA-G epitope comprises or consists of an amino acid sequence set forth in SEQ ID NO: 342.
11. The bispecific antigen binding construct according to any of claims 1-10, wherein the binding domain capable of binding to an HLA-G epitope comprises or consists of a heavy chain variable region (VH) and a light chain variable region (VL), with VH and/or VL comprising 1, 2, 3, 4, 5, or 6 of the following:
 - a) a VHCDR1 having the sequence set forth in any one of SEQ ID NOS: 1-14 or 18-34,

- b) a VHCDR2 having the sequence set forth in any one of SEQ ID NOS: 38-50 or 54-71,
- c) a VHCDR3 having the sequence set forth in any one of SEQ ID NOS: 76-101,
- d) a VLCDR1 having the sequence set forth in any one of SEQ ID NOS: 105-124,
- e) a VLCDR2 having the sequence set forth in any one of SEQ ID NOS: 128-145, and
- f) a VLCDR3 having the sequence set forth in any one of SEQ ID NOS: 149-166.

12. The bispecific antigen binding construct according to any of claims 1-4, wherein the binding domain capable of binding to an HLA-G epitope comprises a heavy chain variable region (VH) and a light chain variable region (VL), with VH comprising, consisting of, or consisting essentially of a VH having the sequence set forth in SEQ ID NOS: 170-200 and with the VL comprising, consisting of, or consisting essentially of a VL having the sequence set forth in SEQ ID NO: 204-228 and the additional binding domain capable of binding to a CD3 ϵ epitope comprises a heavy chain variable region (VH) and a light chain variable region (VL), with VH comprising, consisting of, or consisting essentially of a VH having the sequence set forth in SEQ ID NOS: 413-418 and with the VL comprising, consisting of, or consisting essentially of a VL having the sequence set forth in SEQ ID NOS: 422-427.

13. A pharmaceutical composition comprising or consisting of a bispecific antigen binding construct according to claims 1-12.

14. The pharmaceutical composition according to claim 13, further comprising an effective amount of one or more of:

- a) an anti-PD-L1 antibody or small molecule inhibitor;

- b) an anti-PD-1 antibody or small molecule inhibitor;
- c) an anti-CD38 antibody or small molecule inhibitor;
- d) an anti-CD39 antibody or small molecule inhibitor;
- e) an anti-CD73 antibody or small molecule inhibitor;
- f) an anti-A2A receptor antibody or small molecule inhibitor;
- g) an anti-A2B receptor antibody or small molecule inhibitor;
- h) an anti-A2A/A2B dual receptor antibody or small molecule inhibitor;
- i) an anti-CD47 antibody or small molecule inhibitor;
- j) an anti-CTLA-4 antibody or small molecule inhibitor;
- k) an anti-LAG-3 antibody or small molecule inhibitor;
- l) an anti-TIM-3 antibody or small molecule inhibitor;
- m) an anti-TIGIT antibody or small molecule inhibitor;
- n) an anti-VISTA antibody or small molecule inhibitor;
- o) an anti-CD94 antibody or small molecule inhibitor;
- p) a small molecule inhibitor;
- q) an oncolytic virus;

- r) a chemotherapy;
- s) an adoptive cell therapy; and/or
- t) ADCC capable therapies using effector competent antibodies such as anti-CD19, anti-CD20, anti-EGFR, anti-Her2, anti-SLAMF7, anti-CD52, anti-BCMA, anti-GD2, and/or anti-CCR4.

15. The pharmaceutical composition according to claim 13 or claim 14, further comprising one or both of:

- a) an antibody to an immune inhibitory receptor or ligand and/or
- b) an antibody to an immune stimulatory receptor or ligand.

16. One or more nucleic acids encoding the bispecific antigen binding constructs according to any one of claims 1-15.

17. One or more vectors comprising the one or more nucleic acids according to claim 16.

18. A host transformed with a vector according to claim 17.

19. A method for the production of one or more bispecific antigen binding construct comprising the steps of expressing one or more nucleic acids according to any of claims 16-18 in a prokaryotic or eukaryotic host cell and recovering the one or more bispecific antigen binding construct from the cell or the cell culture supernatant.

20. The bispecific antigen binding construct according to any one of claims 1 to 12 or the pharmaceutical composition according to any one of claims 13 to 15 for use in therapy.
21. Use of the bispecific antigen binding construct according to any one of claims 1 to 12 or the pharmaceutical composition according to any of claims 13 to 15 in the manufacture of a medicament for treating a subject with cancer.
22. The use according to claim 21, wherein the cancer is a solid cancer.
23. The use according to claim 21, wherein the cancer is a hematological cancer.
24. The use according to claim 21, wherein the cancer is selected from the group consisting of a hematopoietic cancer, hepatocellular carcinoma, leukemia, colorectal cancer (CRC), breast cancer, gastric cancer, esophageal cancer, endometrial cancer, prostate cancer, bladder cancer, thyroid cancer, liver cancer, pancreatic cancer, triple negative breast cancer, cervical cancer, ovarian cancer, uterine cancer, vaginal cancer, vulvar cancer, lung cancer, head and neck cancer, melanoma, renal cell carcinoma, cutaneous squamous cell carcinoma, Hodgkin's lymphoma, a metastasis of the brain, a metastasis of the lung, a metastasis of the liver, and/or a metastasis of the bone, or an unresectable or metastatic solid tumor with DNA mismatch repair deficiencies or a microsatellite instability-high state.
25. The use according to any of claims 21 to 24, wherein the use further comprises one or more of the following:
- a) chemotherapy is to be administered to the subject;
 - b) radiation therapy is to be administered to the subject; and/or

c) one or more additional therapeutic agents are to be administered to the subject.

26. The use according to claim 25, wherein the one or more additional therapeutic agents comprise one or more immunomodulatory agents.

27. The use according to claim 26, wherein the one or more immunomodulatory agents comprise an antagonist to an inhibitory receptor of an immune cell.

28. The use according to claim 27, wherein the inhibitory receptor is at least one of LILRB1, LILRB2, LILRB4, KIR2DL4, CTLA-4, PD-1, PD-L1, PD-L2, LAG-3, Tim3, TIGIT, B7-H3, B7-H4, neuritin, BTLA, CECAM-1, CECAM-5, VISTA, LAIR1, CD160, 2B4, TGF-B receptor, NKG2A, and/or a Killer-cell immunoglobulin-like receptor (KIR).

29. The use according to claim 27, wherein the one or more immunomodulatory agents comprise an agonist of a co-stimulatory receptor of an immune cell.

30. The use according to claim 29, wherein the co-stimulatory receptor is at least one of OX40, CD2, CD27, ICAM-1, LFA-1, ICOS (CD278), 4-1BB (CD137), GITR, CD28, CD30, CD40, BAFFR, HVEM, CD7, LIGHT, NKG2C, SLAMF7, NKp30, NKp46, NKp80, CD160, and/or CD83.

31. The use according to claim 29, wherein the one or more immunomodulatory agents is one or more cytokines.

32. The use according to claim 31, wherein the one or more cytokines is at least one of G-CSF, GM-CSF, IFN-alpha, IFN-beta, IFN-gamma, FLt3 ligand, IL-1, IL-2, IL-5, IL-7, IL-10, IL-12, IL-15, IL-18, IL-21, and/or IL-27.

33. The use according to claim 26, wherein the one or more immunomodulatory agents is one or more oncolytic viruses.

34. The use according to claim 33, wherein the one or more oncolytic viruses is a Herpes simplex virus, a Vesicular stomatitis virus, an adenovirus, a Newcastle disease virus, a vaccinia virus, and/or a maraba virus.

35. The use according to claim 26, wherein the one or more immunomodulatory agents is a chimeric antigen engineered T cell.