SUMMARY

[0007] The present disclosure provides bispecific antibodies having binding specificities to both VEGF and PD-1 proteins. As shown in the experimental examples, these bispecific antibodies exhibited high binding affinity to both proteins and were effective in inhibiting VEGF enzymatic activities and blocking PD-1 to PD-L1 binding, resulting in T-cell activation.

[0008] In one aspect, the present disclosure provides a bispecific antibody comprising: (i)an anti-VEGF antibody or an antigen binding fragment thereof comprising: (a) a Fab fragment, wherein the Fab fragment binds to human VEGF protein and comprises a heavy chain variable region (VH) comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO:9, a CDRH2 comprising the amino acid sequence of SEQ ID NO:10, and a CDRH3 comprising the amino acid sequence of SEQ ID NO:11, and a light chain variable region (VL) comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO:12, a CDRL2 comprising the amino acid sequence of SEQ ID NO:13, and a CDRL3 comprising the amino acid sequence of SEQ ID NO:14; and (b) an IgG Fc fragment; (ii) a first anti-PD-1 single domain antibody (sdAb), wherein the first anti-PD-1 sdAb binds to human PD-1 protein and comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:5, a CDR2 comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 comprising the amino acid sequence of SEQ ID NO:7; and (iii) a second anti-PD-1 sdAb, wherein the second anti-PD-1 sdAb binds to human PD-1 protein and comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:5, a CDR2 comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 comprising the amino acid sequence of SEQ ID NO:7, and wherein the first anti-PD-1 sdAb and the second anti-PD-1 sdAb each is fused to the anti-VEGF antibody or an antigen binding fragment thereof at the C-terminus of the IgG Fc fragment. One example of the present disclosure provides a bispecific antibody comprising an anti-VEGF portion and an anti-PD-1 portion, wherein the anti-VEGF portion comprises an anti-VEGF antibody or antigen binding fragment having binding specificity to a human VEGF protein, which comprises a heavy chain variable region (VH) comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO:9, a CDRH2 comprising the amino acid sequence of SEQ ID NO:10, and a CDRH3 comprising the amino acid sequence of SEQ ID NO:11, and a light chain variable region (VL) comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO:12, a CDRL2 comprising the amino acid sequence of SEQ ID

NO:13, and a CDRL3 comprising the amino acid sequence of SEQ ID NO:14, and wherein the anti-PD-1 portion comprises an anti-PD-1 single domain antibody having binding specificity to a human PD-1 protein, which comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:5, a CDR2 comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 comprising the amino acid sequence of SEQ ID NO:7 or 8.

[0008a] In another aspect, the present disclosure provides a bispecific antibody comprising: (i) an anti-VEGF antibody or an antigen binding fragment thereof comprising: (a) a Fab fragment, wherein the Fab fragment binds to human VEGF protein and comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:3 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:4; and (b) an IgG Fc fragment; (ii) a first anti-PD-1 sdAb, wherein the first anti-PD-1 sdAb binds to human PD-1 protein and comprises the amino acid sequence of SEQ ID NO:1; and (iii) a second anti-PD-1 sdAb, wherein the second anti-PD-1 sdAb binds to human PD-1 protein and comprises the amino acid sequence of SEQ ID NO:1, wherein the first anti-PD-1 sdAb and the second anti-PD-1 sdAb each is fused to the anti-VEGF antibody or an antigen binding fragment thereof at the C-terminus of the IgG Fc fragment.

[0008b] In yet another aspect, the present disclosure provides a bispecific antibody comprising: (i) an anti-VEGF antibody or an antigen binding fragment thereof comprising: (a) a Fab fragment, wherein the Fab fragment binds to human VEGF protein and comprises a heavy chain variable region (VH) comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO:9, a CDRH2 comprising the amino acid sequence of SEQ ID NO:10, and a CDRH3 comprising the amino acid sequence of SEQ ID NO:11, and a light chain variable region (VL) comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO:12, a CDRL2 comprising the amino acid sequence of SEQ ID NO:13, and a CDRL3 comprising the amino acid sequence of SEQ ID NO:14; (b) an IgG Fc fragment; (ii) a first anti-PD-1 sdAb, wherein the first anti-PD-1 sdAb binds to human PD-1 protein and comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:5, a CDR2 comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 comprising the amino acid sequence of SEQ ID NO:7; (iii) a second anti-PD-1 sdAb, wherein the second anti-PD-1 sdAb binds to human PD-1 protein and comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:5, a CDR2 comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 comprising the

amino acid sequence of SEQ ID NO:7; (iv) a third anti-PD-1 sdAb, wherein the third anti-PD-1 sdAb binds to human PD-1 protein and comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:5, a CDR2 comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 comprising the amino acid sequence of SEQ ID NO:7, and (v) a fourth anti-PD-1 sdAb, wherein the fourth anti-PD-1 sdAb binds to human PD-1 protein and comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:5, a CDR2 comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 comprising the amino acid sequence of SEQ ID NO:7, and wherein the first anti-PD-1 sdAb and the second anti-PD-1 sdAb each is fused to the anti-VEGF antibody or an antigen binding fragment thereof at the C-terminus of the IgG Fc fragment, and wherein the third anti-PD-1 sdAb is fused to the first anti-PD-1 sdAb at the C-terminus of the first anti-PD-1 sdAb, and wherein the fourth anti-PD-1 sdAb is fused to the second anti-PD-1 sdAb at the C-terminus of the second anti-PD-1 sdAb.

[0008c] In yet another aspect, the present disclosure provides a bispecific antibody comprising: (i)an anti-VEGF antibody or an antigen binding fragment thereof comprising: (a) a Fab fragment, wherein the Fab fragment binds to human VEGF protein and comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:3 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:4; (b) an IgG Fc fragment; (ii) a first anti-PD-1 sdAb, wherein the first anti-PD-1 sdAb binds to human PD-1 protein and comprises the amino acid sequence of SEQ ID NO:1; (iii) a second anti-PD-1 sdAb, wherein the second anti-PD-1 sdAb binds to human PD-1 protein and comprises the amino acid sequence of SEQ ID NO:1; (iv) a third anti-PD-1 sdAb, wherein the third anti-PD-1 sdAb binds to human PD-1 protein and comprises the amino acid sequence of SEQ ID NO:1, and (v) a fourth anti-PD-1 sdAb, wherein the fourth anti-PD-1 sdAb binds to human PD-1 protein and comprises the amino acid sequence of SEQ ID NO:1, and wherein the first anti-PD-1 sdAb and the second anti-PD-1 sdAb each is fused to the anti-VEGF antibody or an antigen binding fragment thereof at the C-terminus of the IgG Fc fragment, and wherein the third anti-PD-1 sdAb is fused to the first anti-PD-1 sdAb at the C-terminus of the first anti-PD-1 sdAb, and wherein the fourth anti-PD-1 sdAb is fused to the second anti-PD-1 sdAb at the C-terminus of the second anti-PD-1 sdAb.

[0009] In some examples, In some examples, the VH of the anti-VEGF antibody or antigen binding fragment comprises the amino acid sequence of SEQ ID NO:3, and the VL of the anti-VEGF antibody or antigen binding fragment comprises the amino acid sequence of SEQ ID NO:4.

[0010] In some examples, the anti-VEGF antibody or antigen binding fragment is a full size Fab antibody. In some examples, the full size Fab antibody comprises an IgG Fc fragment. In some examples, the IgG Fc fragment comprises LALA mutations.

[0011] In some examples, the anti-PD-1 single domain antibody comprises the amino acid sequence of SEQ ID NO:1 or 2.

[0012] In some examples, the anti-PD-1 single domain antibody is deposited at the C-terminal side of the anti-VEGF antibody or antigen binding fragment thereof. In some

examples, the anti-PD-1 single domain antibody is fused, through a (G4S)4 (SEQ ID NO:15) linker, to the C-terminus of the anti-VEGF antibody. In some examples, the bispecific antibody comprises a single anti-PD-1 single domain antibody on each peptide chain of the bispecific antibody. In some examples, the bispecific antibody comprises two anti-PD-1 single domain antibodies on each peptide chain of the bispecific antibody. In some examples, the two anti-PD-1 single domain antibodies on each peptide chain is linked through a (G4S)4 (SEQ ID NO:15) linker.

[0013] In one aspect, the present disclosure provides a bispecific antibody comprising an anti-VEGF portion and an anti-PD-1 portion, wherein the bispecific antibody comprises the amino acid sequences of SEQ ID NO:18 and 17. In another aspect, the present disclosure provides a bispecific antibody comprising an anti-VEGF portion and an anti-PD-1 portion, wherein the bispecific antibody comprises the amino acid sequences of SEQ ID NO:16 and 17. In one example, provided is a bispecific antibody that comprises the amino acid sequences of SEQ ID NO:16 and 17. In one example, provided is a bispecific antibody that comprises the amino acid sequences of SEQ ID NO:18 and 17. In one example, provided is a bispecific antibody that comprises the amino acid sequences of SEQ ID NO:19 and 17.

[0013a] In a further aspect, the present disclosure provides a bispecific antibody comprising: (i) a first polypeptide comprising the amino acid sequence of SEQ ID NO:17; (ii) a second polypeptide comprising the amino acid sequence of SEQ ID NO:18; (iii) a third polypeptide comprising the amino acid sequence of SEQ ID NO:18; and (iv) a fourth polypeptide comprising the amino acid sequence of SEQ ID NO:17. In yet another aspect, the present disclosure provides a bispecific antibody comprising: (i) a first polypeptide comprising the amino acid sequence of SEQ ID NO:17; (ii) a second polypeptide comprising the amino acid sequence of SEQ ID NO:16; (iii) a third polypeptide comprising the amino acid sequence of SEQ ID NO:16; and (iv) a fourth polypeptide comprising the amino acid sequence of SEQ ID NO:17.

[0014] In one aspect, the present disclosure provides a composition comprising the bispecific antibody as described herein and a pharmaceutically acceptable carrier. In another aspect, the present disclosure provides a polynucleotide encoding (i) a heavy chain of the bispecific

antibody as described herein or (ii) a heavy chain and a light chain of the bispecific antibody as described herein. Also provided, in one example, is a composition comprising the bispecific antibody of the present disclosure and a pharmaceutically acceptable carrier. Further provided is one or more polynucleotide encoding a heavy chain of the bispecific antibody of the present disclosure. In one example, the one or more polynucleotide encodes the bispecific antibody.

[0014a] In one aspect, the present disclosure provides a method of making the bispecific antibody of any one of claims 1-14, comprising expressing a polynucleotide encoding a heavy chain of the bispecific antibody and a polynucleotide encoding a light chain of the bispecific antibody in a CHO cell. In another aspect, the present disclosure provides a bispecific antibody made by the method as disclosed herein.

[0015] In a further aspect, the present disclosure provides a cell comprising a polynucleotide encoding a heavy chain of the bispecific antibody as described herein and a polynucleotide encoding a light chain of the bispecific antibody as described herein, optionally wherein the cell is a CHO-K1 cell. In yet another aspect, the present disclosure provides a use of the bispecific antibody as described herein in the manufacture of a medicament for treating cancer in a patient in need thereof. Yet another example provides a cell comprising the one or more polynucleotide of the present disclosure. Another example provides a method of treating cancer in a patient in need thereof, comprising administering to the patient the bispecific antibody. In some examples, the cancer is selected from the group consisting of bladder cancer, breast cancer, colorectal cancer, endometrial cancer, esophageal cancer, head and neck cancer, kidney cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, pancreatic cancer, prostate cancer, and thyroid cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0016] FIG. 1 shows the schematic of anti-PD1-VEGF bispecific antibodies.

[0017] FIG. 2 shows that all the test anti-PD1-VEGF bispecific antibodies bound to human VEGF protein in a concentration-dependent manner.

CLAIMS

What is claimed is:

1. A bispecific antibody comprising:

(i) an anti-VEGF antibody or an antigen binding fragment thereof comprising:

(a) a Fab fragment, wherein the Fab fragment binds to human VEGF protein and comprises a heavy chain variable region (VH) comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO:9, a CDRH2 comprising the amino acid sequence of SEQ ID NO:10, and a CDRH3 comprising the amino acid sequence of SEQ ID NO:11, and a light chain variable region (VL) comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO:12, a CDRL2 comprising the amino acid sequence of SEQ ID NO:13, and a CDRL3 comprising the amino acid sequence of SEQ ID NO:14; and

(b) an IgG Fc fragment;

(ii) a first anti-PD-1 single domain antibody (sdAb), wherein the first anti-PD-1 sdAb binds to human PD-1 protein and comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:5, a CDR2 comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 comprising the amino acid sequence of SEQ ID NO:7; and

(iii) a second anti-PD-1 sdAb, wherein the second anti-PD-1 sdAb binds to human PD-1 protein and comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:5, a CDR2 comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 comprising the amino acid sequence of SEQ ID NO:7, and

wherein the first anti-PD-1 sdAb and the second anti-PD-1 sdAb each is fused to the anti-VEGF antibody or an antigen binding fragment thereof at the C-terminus of the IgG Fc fragment.

2. A bispecific antibody comprising:

(i) an anti-VEGF antibody or an antigen binding fragment thereof comprising:

(a) a Fab fragment, wherein the Fab fragment binds to human VEGF protein and comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ

ID NO:3 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:4; and

(b) an IgG Fc fragment;

(ii) a first anti-PD-1 sdAb, wherein the first anti-PD-1 sdAb binds to human PD-1 protein and comprises the amino acid sequence of SEQ ID NO:1; and

(iii) a second anti-PD-1 sdAb, wherein the second anti-PD-1 sdAb binds to human PD-1 protein and comprises the amino acid sequence of SEQ ID NO:1,

wherein the first anti-PD-1 sdAb and the second anti-PD-1 sdAb each is fused to the anti-VEGF antibody or an antigen binding fragment thereof at the C-terminus of the IgG Fc fragment.

3. A bispecific antibody comprising:

(i) an anti-VEGF antibody or an antigen binding fragment thereof comprising:

(a) a Fab fragment, wherein the Fab fragment binds to human VEGF protein and comprises a heavy chain variable region (VH) comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO:9, a CDRH2 comprising the amino acid sequence of SEQ ID NO:10, and a CDRH3 comprising the amino acid sequence of SEQ ID NO:11, and a light chain variable region (VL) comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO:12, a CDRL2 comprising the amino acid sequence of SEQ ID NO:13, and a CDRL3 comprising the amino acid sequence of SEQ ID NO:14;

(b) an IgG Fc fragment;

(ii) a first anti-PD-1 sdAb, wherein the first anti-PD-1 sdAb binds to human PD-1 protein and comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:5, a CDR2 comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 comprising the amino acid sequence of SEQ ID NO:7;

(iii) a second anti-PD-1 sdAb, wherein the second anti-PD-1 sdAb binds to human PD-1 protein and comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:5, a

CDR2 comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 comprising the amino acid sequence of SEQ ID NO:7;

(iv) a third anti-PD-1 sdAb, wherein the third anti-PD-1 sdAb binds to human PD-1 protein and comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:5, a CDR2 comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 comprising the amino acid sequence of SEQ ID NO:7, and

(v) a fourth anti-PD-1 sdAb, wherein the fourth anti-PD-1 sdAb binds to human PD-1 protein and comprises a CDR1 comprising the amino acid sequence of SEQ ID NO:5, a CDR2 comprising the amino acid sequence of SEQ ID NO:6, and a CDR3 comprising the amino acid sequence of SEQ ID NO:7, and

wherein the first anti-PD-1 sdAb and the second anti-PD-1 sdAb each is fused to the anti-VEGF antibody or an antigen binding fragment thereof at the C-terminus of the IgG Fc fragment, and

wherein the third anti-PD-1 sdAb is fused to the first anti-PD-1 sdAb at the C-terminus of the first anti-PD-1 sdAb, and

wherein the fourth anti-PD-1 sdAb is fused to the second anti-PD-1 sdAb at the C-terminus of the second anti-PD-1 sdAb.

4. A bispecific antibody comprising:

(i) an anti-VEGF antibody or an antigen binding fragment thereof comprising:

(a) a Fab fragment, wherein the Fab fragment binds to human VEGF protein and comprises a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO:3 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:4;

(b) an IgG Fc fragment;

(ii) a first anti-PD-1 sdAb, wherein the first anti-PD-1 sdAb binds to human PD-1 protein and comprises the amino acid sequence of SEQ ID NO:1;

(iii) a second anti-PD-1 sdAb, wherein the second anti-PD-1 sdAb binds to human PD-1 protein and comprises the amino acid sequence of SEQ ID NO:1;

(iv) a third anti-PD-1 sdAb, wherein the third anti-PD-1 sdAb binds to human PD-1 protein and comprises the amino acid sequence of SEQ ID NO:1, and

(v) a fourth anti-PD-1 sdAb, wherein the fourth anti-PD-1 sdAb binds to human PD-1 protein and comprises the amino acid sequence of SEQ ID NO:1, and

wherein the first anti-PD-1 sdAb and the second anti-PD-1 sdAb each is fused to the anti-VEGF antibody or an antigen binding fragment thereof at the C-terminus of the IgG Fc fragment, and

wherein the third anti-PD-1 sdAb is fused to the first anti-PD-1 sdAb at the C-terminus of the first anti-PD-1 sdAb, and

wherein the fourth anti-PD-1 sdAb is fused to the second anti-PD-1 sdAb at the C-terminus of the second anti-PD-1 sdAb.

5. The bispecific antibody of claim 1 or 3, wherein the VH of the Fab fragment of the anti-VEGF antibody comprises the amino acid sequence of SEQ ID NO:3, and the VL of the Fab fragment of the anti-VEGF antibody comprises the amino acid sequence of SEQ ID NO:4.

6. The bispecific antibody of claim 1 or 3, wherein the first and/or second anti-PD-1 sdAb comprises the amino acid sequence of SEQ ID NO:1.

7. The bispecific antibody of any one of claims 1-6, wherein the IgG Fc fragment comprises L234A and L235A amino acid substitutions according to EU numbering as compared to a wild-type IgG Fc fragment.

8. The bispecific antibody of any one of claims 1-7, wherein the first and/or second anti-PD-1 sdAb is fused, through a $(G4S)_4$ (SEQ ID NO:15) linker, to the C-terminus of the IgG Fc fragment.

9. The bispecific antibody of any one of claims 3-8, wherein the third anti-PD-1 sdAb is fused, through a (G4S)4 (SEQ ID NO:15) linker, to the first anti-PD-1 sdAb at the C-terminus of the first anti-PD-1 sdAb, and wherein the fourth anti-PD-1 sdAb is fused, through a (G4S)4 (SEQ ID NO:15) linker, to the second anti-PD-1 sdAb at the C-terminus of the second anti-PD-1 sdAb.

10. A bispecific antibody comprising an anti-VEGF portion and an anti-PD-1 portion, wherein the bispecific antibody comprises the amino acid sequences of SEQ ID NO:18 and 17.

11. A bispecific antibody comprising an anti-VEGF portion and an anti-PD-1 portion, wherein the bispecific antibody comprises the amino acid sequences of SEQ ID NO:16 and 17.

12. A bispecific antibody comprising:

(i) a first polypeptide comprising the amino acid sequence of SEQ ID NO:17;

(ii) a second polypeptide comprising the amino acid sequence of SEQ ID NO:18;

(iii) a third polypeptide comprising the amino acid sequence of SEQ ID NO:18; and

(iv) a fourth polypeptide comprising the amino acid sequence of SEQ ID NO:17.

13. A bispecific antibody comprising:

(i) a first polypeptide comprising the amino acid sequence of SEQ ID NO:17;

(ii) a second polypeptide comprising the amino acid sequence of SEQ ID NO:16;

(iii) a third polypeptide comprising the amino acid sequence of SEQ ID NO:16; and

(iv) a fourth polypeptide comprising the amino acid sequence of SEQ ID NO:17.

14. The bispecific antibody of claim 12 or 13, wherein the first polypeptide and the second polypeptide are linked to each other via a disulfide bond, wherein the second polypeptide and the third polypeptide are linked to each other linked via two disulfide bonds, and wherein the third polypeptide and the fourth polypeptide are linked to each other linked via a disulfide bond.

15. A composition comprising the bispecific antibody of any one of claims 1-14 and a pharmaceutically acceptable carrier.

16. A polynucleotide encoding (i) a heavy chain of the bispecific antibody of any one of claims 1-14 or (ii) a heavy chain and a light chain of the bispecific antibody of any one of claims 1-14.

17. A method of making the bispecific antibody of any one of claims 1-14, comprising expressing a polynucleotide encoding a heavy chain of the bispecific antibody and a polynucleotide encoding a light chain of the bispecific antibody in a CHO cell.

18. A bispecific antibody made by the method of claim 17.

19. A cell comprising a polynucleotide encoding a heavy chain of the bispecific antibody of any one of claims 1-14 and a polynucleotide encoding a light chain of the bispecific antibody of any one of claims 1-14, optionally wherein the cell is a CHO-K1 cell.

20. The bispecific antibody of any one of claims 1-14, wherein the bispecific antibody is produced in a CHO-K1 cell.

21. The bispecific antibody of any one of claims 1-14, wherein the bispecific antibody is produced by expressing a polynucleotide encoding a heavy chain of the bispecific antibody and a polynucleotide encoding a light chain of the bispecific antibody.

22. Use of the bispecific antibody of any one of claims 1-14, 18, and 20-21 in the manufacture of a medicament for treating cancer in a patient in need thereof.

23. The use of claim 22, wherein the cancer is selected from the group consisting of bladder cancer, breast cancer, colorectal cancer, endometrial cancer, esophageal cancer, head and neck cancer, kidney cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, pancreatic cancer, prostate cancer, and thyroid cancer.