

[0065] In some examples, the heavy chain variable region sequence and the light chain variable region sequence in the first binding modules are attached by a glycine-serine linker.

[0066] In some examples, the glycine-serine linker is a 4xG4S linker (SEQ ID NO: 65).

[0067] In some examples, the heavy chain of the antibody scaffold module, the glycine-serine linker, and the first binding module comprise a sequence as set forth in SEQ ID NO: 14.

[0068] In some examples, the antibody scaffold module further comprises a constant region.

[0069] In some examples, the constant region comprises one or more Fc silencing mutations.

[0070] In some examples, the Fc silencing mutation can be L234A/L235A (LALA) alone or in combination with P329A mutation (LALAP) or N297A

[0071] In some examples, the constant region comprises SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69, or SEQ ID NO: 73.

[0072] Also provided herein is a bispecific binding protein that binds a tumor associated antigen and CD137 comprising: (a) an antibody scaffold module comprising a means for binding the tumor associated antigen via a first antigen-binding site and a second antigen-binding site; and (b) at least one first binding module comprising a means for binding CD137 via a third antigen-binding site.

[0073] The present disclosure also provides a pharmaceutical composition comprising the bispecific binding protein as disclosed herein and a pharmaceutically acceptable carrier.

[0074] The present disclosure also provides a method of treating or preventing cancer, the method comprising administering the bispecific binding protein as disclosed herein to a patient in need thereof.

[0075] Also provided herein is an isolated polynucleotide comprising a sequence encoding the bispecific binding protein as disclosed herein. The present disclosure also provides vectors or cells comprising the polynucleotide disclosed herein. Also provided herein is a method for the production of the bispecific binding protein as disclosed herein comprising culturing the cell disclosed herein.

[0075a] In one aspect, the present disclosure relates to a bispecific binding protein that binds Claudin 6 and CD137 comprising: (a) an antibody scaffold module comprising a first heavy chain comprising a first heavy chain variable region, a second heavy chain comprising a second heavy chain variable region, a first light chain comprising a first light chain variable region, and a second light chain comprising a second light chain variable region, wherein the first heavy chain variable region and the first light chain variable region form a first antigen binding site that binds Claudin 6 and the second chain heavy variable region and the second light chain variable region form a second antigen binding site that binds Claudin 6, wherein each of the first heavy chain variable region and the first light chain variable region and the second heavy chain variable region and the second light chain variable region are selected from:

(i) a heavy chain variable region comprising CDR1: SEQ ID NO: 45, CDR2: SEQ ID NO: 46, and CDR3: SEQ ID NO: 47; and a light chain variable region comprising CDR1: SEQ ID NO: 48, CDR2: SEQ ID NO: 49, and CDR3: SEQ ID NO: 50; or (ii) a heavy chain variable region comprising CDR1: SEQ ID NO: 51, CDR2: SEQ ID NO: 52, and CDR3: SEQ ID NO: 53; and a light chain variable region comprising CDR1: SEQ ID NO: 54, CDR2: SEQ ID NO: 55, and CDR3: SEQ ID NO: 56; and (b) at least one first binding module comprising a third antigen-binding site that binds CD137, wherein the third antigen-binding site comprises a third heavy chain variable region comprising CDR1: SEQ ID NO: 39, CDR2: SEQ ID NO: 40, and CDR3: SEQ ID NO: 41; and a third light chain variable region comprising CDR1: SEQ ID NO: 42, CDR2: SEQ ID NO: 43, and CDR3: SEQ ID NO: 44; wherein the first binding module is covalently attached to the C-terminus of one or both of the antibody scaffold module heavy chains, the C-terminus of one or both of the antibody scaffold module light chains, the N-terminus of one or both of the antibody scaffold module heavy chains, the N-terminus of one or both of the antibody scaffold module light chains, or combinations thereof, and wherein the first binding module and the antibody scaffold module are covalently attached to each other directly or through an interlinker.

[0075b] In another aspect, the present disclosure relates to a bispecific protein that binds Claudin 6 and CD137 comprising: a) a first monomer according to the amino acid sequence SEQ ID NO:72; b) a second monomer according to the amino acid sequence of SEQ ID NO:72; c) a first light chain according to the amino acid sequence of SEQ ID NO: 9; and d) a second light chain according to the amino acid sequence of SEQ ID NO:9.

[0075c] In another aspect, the present disclosure relates to a pharmaceutical composition comprising the bispecific binding protein as disclosed herein and a pharmaceutically acceptable carrier.

[0075d] In another aspect, the present disclosure relates to use of the bispecific binding protein as disclosed herein in the manufacture of a medicament for treating a Claudin 6-associated cancer.

[0075e] In another aspect, the present disclosure relates to an isolated polynucleotide comprising a sequence encoding the bispecific binding protein as disclosed herein.

[0075f] In another aspect, the present disclosure relates to a vector comprising the polynucleotide as disclosed herein.

[0075g] In another aspect, the present disclosure relates to a cell comprising a polynucleotide as disclosed herein, and/or a vector as disclosed herein.

[0075h] In another aspect, the present disclosure relates to a method for the production of the bispecific binding protein as disclosed herein, the method comprising culturing the cell as disclosed herein.

WHAT IS CLAIMED IS:

1. A bispecific binding protein that binds Claudin 6 and CD137 comprising:

(a) an antibody scaffold module comprising a first heavy chain comprising a first heavy chain variable region, a second heavy chain comprising a second heavy chain variable region, a first light chain comprising a first light chain variable region, and a second light chain comprising a second light chain variable region,

wherein the first heavy chain variable region and the first light chain variable region form a first antigen binding site that binds Claudin 6 and the second chain heavy variable region and the second light chain variable region form a second antigen binding site that binds Claudin 6,

wherein each of the first heavy chain variable region and the first light chain variable region and the second heavy chain variable region and the second light chain variable region are selected from:

(i) a heavy chain variable region comprising CDR1: SEQ ID NO: 45, CDR2: SEQ ID NO: 46, and CDR3: SEQ ID NO: 47; and a light chain variable region comprising CDR1: SEQ ID NO: 48, CDR2: SEQ ID NO: 49, and CDR3: SEQ ID NO: 50; or

(ii) a heavy chain variable region comprising CDR1: SEQ ID NO: 51, CDR2: SEQ ID NO: 52, and CDR3: SEQ ID NO: 53; and a light chain variable region comprising CDR1: SEQ ID NO: 54, CDR2: SEQ ID NO: 55, and CDR3: SEQ ID NO: 56; and

(b) at least one first binding module comprising a third antigen-binding site that binds CD137, wherein the third antigen-binding site comprises a third heavy chain variable region comprising CDR1: SEQ ID NO: 39, CDR2: SEQ ID NO: 40, and CDR3: SEQ ID NO: 41; and a third light chain variable region comprising CDR1: SEQ ID NO: 42, CDR2: SEQ ID NO: 43, and CDR3: SEQ ID NO: 44;

wherein the first binding module is covalently attached to the C-terminus of one or both of the antibody scaffold module heavy chains, the C-terminus of one or both of the antibody scaffold module light chains, the N-terminus of one or both of the antibody scaffold module heavy chains, the N-terminus of one or both of the antibody scaffold module light chains, or combinations thereof, and wherein the first binding module and the antibody scaffold module are covalently attached to each other directly or through an interlinker.

2. The bispecific binding protein of claim 1, wherein each of the first heavy chain variable region and the first light chain variable region and the second heavy chain variable region and the second light chain variable region are selected from:

(i) a heavy chain variable region comprising SEQ ID NO: 25 and a light chain variable region comprising SEQ ID NO: 26; or

(ii) a heavy chain variable region comprising SEQ ID NO: 27 and a light chain variable region comprising SEQ ID NO: 28.

3. The bispecific binding protein of claim 1 or 2, wherein the first binding module is an antibody fragment.

4. The bispecific binding protein of any one of claims 1-3, wherein the first binding module is an scFv.

5. The bispecific binding protein of any one of claims 1-4, wherein the first binding module and the antibody scaffold module are covalently attached to each other through an interlinker having a sequence as set forth in SEQ ID NO: 64 or SEQ ID NO: 65.

6. The bispecific binding protein of any one of claims 1-5, wherein the third heavy chain variable region comprises SEQ ID NO: 23; and the third light chain variable region comprises SEQ ID NO: 24.

7. The bispecific binding protein of any one of claims 1-5, wherein the bispecific binding protein comprises two first binding modules that bind CD137 and wherein:

the first heavy chain variable region and the second heavy chain variable region are each according to SEQ ID NOs: 25 or 27;

the first light chain variable region and the second light chain variable region are each according to SEQ ID NOs: 26 or 28;

the third heavy chain variable regions of the first binding modules are each according to SEQ ID NO: 23, and

the third light chain variable regions of the first binding modules are each according to SEQ ID NO: 24,

wherein the first binding modules are separately attached to the C-terminus of each heavy chain in the antibody scaffold module by a glycine-serine linker.

8. The bispecific binding protein of any one of claims **1-7**, wherein the first heavy chain and second heavy chain of the antibody scaffold module each further comprise a constant region.

9. The bispecific binding protein of claim **8**, wherein the constant region comprises SEQ ID NO: 66, SEQ ID NO: 67, SEQ ID NO: 68, SEQ ID NO: 69 or SEQ ID NO: 73.

10. A bispecific protein that binds Claudin 6 and CD137 comprising:

- a) a first monomer according to the amino acid sequence SEQ ID NO:72;
- b) a second monomer according to the amino acid sequence of SEQ ID NO:72;
- c) a first light chain according to the amino acid sequence of SEQ ID NO: 9; and
- d) a second light chain according to the amino acid sequence of SEQ ID NO:9.

11. A pharmaceutical composition comprising the bispecific binding protein of any one of claims **1-10** and a pharmaceutically acceptable carrier.

12. Use of the bispecific binding protein of any one of claims **1-10** in the manufacture of a medicament for treating a Claudin 6-associated cancer.

13. An isolated polynucleotide comprising a sequence encoding the bispecific binding protein of any one of claims **1-10**.

14. A vector comprising the polynucleotide of claim **13**.

15. A cell comprising a polynucleotide of claim **13**, and/or a vector of claim **14**.

16. A method for the production of the bispecific binding protein of any one of claims **1-10**, the method comprising culturing the cell of claim **15**.