MULTISPECIFIC CONSTRUCTS AND USES THEREOF

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to International Application No. PCT/CN2022/070870, filed January 9, 2022, the contents content of which are incorporated herein by reference in their entirety.

FIELD OF THE APPLICATION

[0002] The present application relates to multispecific molecules, *e.g.*, anti-CLDN6/ anti-4-1BB bispecific antibodies, and uses thereof including treating diseases or conditions.

BACKGROUND OF THE APPLICATION

[0003] Claudins are a family of proteins that form the important components of the tight cell junctions. Claudin-6 (CLDN6) is a tetraspan membrane protein involved in the formation of tight junctions. In the adult human normal tissues, CLDN6 mRNA and protein is absent. On the other hand, high CLDN6 transcript levels are frequently detected in various human solid cancers such as testicular, ovarian, uterine and lung adenocarcinoma. In line with mRNA level, CLDN6 proteins are reported to be high and homogenous in these human cancers.

[0004] 4-1BB (CD137, tumor necrosis factor receptor superfamily 9) is a member of TNF-receptor superfamily (TNFRSF) and is a costimulatory molecule which is expressed following the activation of immune cells, both innate and adaptive immune cells. 4-1BB plays an important role in modulating the activity of various immune cells. 4-1BB agonists enhance immune cell proliferation, survival, secretion of cytokines and cytolytic activity CD8 T cells. Many other studies showed that activation of 4-1BB enhances immune response to eliminate tumors in mice. Therefore, it was suggested that 4-1BB is a promising target molecule in cancer immunology.

[0005] The disclosures of all publications, patents, patent applications and published patent applications referred to herein are hereby incorporated herein by reference in their entirety.

SUMMARY

[0006] In one example, provided herein is a multispecific construct comprising: a first antibody moiety that specifically binds to a tumor antigen; and a second antibody moiety that specifically binds to 4-1BB, wherein binding of the first antibody moiety with the tumor antigen triggers the

second antibody moiety to activate 4-1BB. In some examples, the second antibody moiety specifically binds to CRD 3/4 region of 4-1BB.

[0006a] In one aspect, the present disclosure refers to a multispecific construct comprising: (1) a first antibody moiety that specifically binds to claudin-6 ("CLDN6"); and (2) a second antibody moiety that specifically binds to 4-1BB.

[0007] In some examples, provided herein is a multispecific construct comprising a first antibody moiety that specifically binds to a tumor antigen; and a second antibody moiety that specifically binds to 4-1BB, wherein binding of the first antibody moiety with the tumor antigen triggers the second antibody moiety to activate 4-1BB. In some examples, the activation of 4-1BB by the second antibody moiety is enhanced by at least about any one of 2-fold, 3-fold, 4-fold, 5-fold, 6-fold, 7-fold, 8-fold, 9-fold, 10-fold, 20-fold, 50-fold, 100-fold, 200-fold, 500-fold, or 1000-fold after binding of the first antibody moiety with the tumor antigen, including any range in between these values. In some examples, without binding to the tumor antigen, the multispecific construct does activate 4-1BB signaling. In some examples, without binding to the tumor antigen, the second moiety does not activate 4-1BB signaling.

[0008] In some examples, provided are multispecific constructs comprising a) a first antibody moiety that specifically binds to claudin-6 ("CLDN6"); and b) a second antibody moiety that specifically binds to 4-1BB. In some examples, the first antibody moiety comprises a full-length antibody comprising two heavy chains and two light chains.

[0009] Provided herein is a multispecific construct comprising: (1) a first antibody moiety that specifically binds to claudin-6 ("CLDN6"); and (2) a second antibody moiety that specifically binds to 4-1BB.

[0010] In some examples, the first antibody moiety is selected from the group consisting of a full-length antibody, Fab, Fab', $F(ab')_2$, scFv, and sdAb. In some examples, wherein the first antibody moiety comprises: (1) a HC-CDR1, a HC-CDR2, and a HC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a heavy variable region (VH) comprising the sequence set forth in SEQ ID No: 7; and (2) a LC-CDR1, a LC-CDR2, and a LC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a light chain variable region (VL) comprising the sequence set forth in SEQ ID No: 8. In some examples, the first antibody moiety comprises a heavy variable region (VH) and a light chain variable region (VL), wherein:(a) the VH comprises: (i) a HC-CDR1 comprising an amino acid sequence of SEQ ID NO: 1, (ii) a HC-CDR2 comprising an amino acid sequence of SEQ ID NO: 2, and (iii) a HC-CDR3 comprising an amino acid sequence of SEQ ID NO: 3, and (b) the VL comprises: (i) a LC-CDR1 comprising an amino acid

sequence of SEQ ID NO: 4, (ii) a LC-CDR2 comprising an amino acid sequence of SEQ ID NO: 5, and (iii) a LC-CDR3 comprising an amino acid sequence of SEQ ID NO: 6. In some examples, the first antibody moiety comprises a heavy variable region (VH) and a light chain variable region (VL), and wherein: (1) the VH comprises the amino acid sequence of SEQ ID NO: 7, or a variant thereof comprising at least about 80% sequence identity to SEQ ID NO: 7; and/or (2) the VL comprises the amino acid sequence of SEQ ID NO: 8, or a variant thereof comprising at least about 80% sequence identity to SEQ ID NO: 8. In some examples, the first antibody moiety comprises: (1) a HC-CDR1, a HC-CDR2, and a HC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a heavy variable region (VH) comprising the sequence set forth in SEQ ID No: 18; and (2) a LC-CDR1, a LC-CDR2, and a LC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a light chain variable region (VL) comprising the sequence set forth in SEQ ID N): 19. In some examples, the first antibody moiety comprises a heavy variable region (VH) and a light chain variable region (VL), wherein: (1) the VH comprises: (i) a HC-CDR1 comprising an amino acid sequence of SEQ ID NO: 12, (ii) a HC-CDR2 comprising an amino acid sequence of SEQ ID NO: 13, and (iii) a HC-CDR3 comprising an amino acid sequence of SEQ ID NO: 14, and; (2) the VL comprises: (i) a LC-CDR1 comprising an amino acid sequence of SEQ ID NO: 15, (ii) a LC-CDR2 comprising an amino acid sequence of SEQ ID NO: 16, and (iii) a LC-CDR3 comprising an amino acid sequence of SEQ ID NO: 17. In some examples, the first antibody moiety comprises a heavy variable region (VH) and a light chain variable region (VL), and wherein: (1) the VH comprises the amino acid sequence of SEQ ID NO: 18, or a variant thereof having at least about 80% sequence identity to SEQ ID NO: 18; and/or (2) the VL comprises the amino acid sequence of SEQ ID NO: 19, or a variant thereof having at least about 80% sequence identity to SEQ ID NO: 19. In some examples, binding of the first antibody moiety with CLDN6 triggers the second antibody moiety to activate 4-1BB.

[0011] In some examples, the second antibody moiety specifically binds to CRD 3/4 region of 4-1BB. In some examples, the second antibody moiety is selected from the group consisting of a full-length antibody, Fab, Fab', $F(ab')_2$, scFv, and sdAb. In some examples, the second antibody moiety is a sdAb. In some examples, the sdAb comprises a sdAb-CDR1, a sdAb-CDR2, and a sdAb-CDR3, respectively comprising the amino acid sequence of a CDR1, a CDR2, and a CDR3 within a single monomeric variable antibody domain comprising the

amino acid sequence set forth in SEQ ID NOs: 27. In some examples, the sdAb comprises: (1) a sdAb-CDR1 comprising an amino acid sequence of SEQ ID NO: 24; (2) a sdAb-CDR2 comprising an amino acid sequence of SEQ ID NO: 25; and (3) a sdAb-CDR3 comprising an amino acid sequence of SEQ ID NO: 26. In some examples, the second antibody moiety comprises the amino acid sequence of SEQ ID NO:27, or a variant thereof having at least about 80% sequence identity to SEQ ID NOs: 27.

[0012] In some examples, the multispecific construct is a bispecific antibody or a bispecific binding fragment. In some examples, (1) the first antibody moiety comprises: (a) a HC-CDR1, a HC-CDR2, and a HC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a heavy variable region (VH) comprising the sequence set forth in SEQ ID No: 7; (b) a LC-CDR1, a LC-CDR2, and a LC-CDR3, respectively comprising the amino acid sequences of a CDR1, a CDR2, and a CDR3 within a light chain variable region (VL) comprising the sequence set forth in SEQ ID No: 8, and (2) the second antibody moiety comprises a sdAb-CDR1, a sdAb-CDR2, and a sdAb CDR3, respectively comprising the amino acid sequence of a CDR1, a CDR2, and a CDR3 within a single monomeric variable antibody domain comprising the amino acid sequence set forth in SEQ ID NOs: 27.

[0013] In some examples, provided is a pharmaceutical composition comprising a multispecific construct described herein and a pharmaceutical acceptable carrier.

[0013a] In one aspect, the present disclosure refers to a pharmaceutical composition comprising the multispecific construct disclosed herein, and a pharmaceutical acceptable carrier.

[0014] In some examples, provided is a nucleic acid encoding a multispecific construct described herein . In some examples, provided is a vector comprising such nucleic acid. In some examples, provided us a host cell comprising such nucleic acid or such vector.

[0014a] In one aspect, the present disclosure refers to a nucleic acid encoding the multispecific construct disclosed herein.

[0014b] In one aspect, the present disclosure refers to a vector comprising the nucleic acid disclosed herein.

[0014c] In one aspect, the present disclosure refers to a host cell comprising the nucleic acid disclosed herein, or the vector disclosed herein.

[0015] Also provided herein is a method of treating a disease or condition in a subject in need thereof, comprising administering to the subject an effective amount of comprising a multispecific construct described herein or a pharmaceutical composition described herein. Also provided herein is a use of a multispecific construct described herein in the preparation of a medicament for treating a disease or a condition in a subject in need thereof. . In some examples, the disease or condition is cancer.

[0015a] In one aspect, the present disclosure refers to use of the multispecific construct disclosed herein, or the pharmaceutical composition disclosed herein, in the manufacture of a medicament for treating a disease or condition in a subject in need thereof.

[0016] In some examples, provided is a multispecific construct comprising: (1) a first antibody moiety that specifically binds to a tumor antigen; and (2) a second antibody moiety that specifically binds to 4-1BB, wherein binding of the first antibody moiety with the tumor antigen

triggers the second antibody moiety to activate 4-1BB. In some examples, the activation of 4-1BB by the second antibody moiety is enhanced by at least 10-fold after binding of the first antibody moiety with the tumor antigen. In some examples, the second antibody moiety specifically binds to CRD 3/4 region of 4-1BB. In some examples, the second antibody moiety is a sdAb. In some examples, the sdAb comprises a sdAb-CDR1, a sdAb-CDR2, and a sdAb-CDR3, respectively comprising the amino acid sequence of a CDR1, a CDR2, and a CDR3 within a single monomeric variable antibody domain comprising the amino acid sequence set forth in SEQ ID NOs: 27. In some examples, the sdAb comprises: (1) a sdAb-CDR1 comprising an amino acid sequence of SEQ ID NO: 24; (2) a sdAb-CDR2 comprising an amino acid sequence of SEQ ID NO: 25; and (3) a sdAb-CDR3 comprising an amino acid sequence of SEQ ID NO: 26. In some examples, the tumor antigen is CLDN6.

[0016a] In one aspect, the present disclosure refers to a multispecific construct comprising: (1) a first antibody moiety that specifically binds to a tumor antigen; and (2) a second antibody moiety that specifically binds to 4-1BB, wherein binding of the first antibody moiety with the tumor antigen triggers the second antibody moiety to activate 4-1BB.

[0017] Also provided herein is a multispecific construct, comprising two heavy chain components and two light chain components, wherein: (a) each heavy chain component comprises a sequence set forth in SEQ ID NO: 28 and/or SEQ ID NO: 44 and each light chain component comprises a sequence set forth in SEQ ID NO: 29; (b) each heavy chain component comprises a sequence set forth in SEQ ID NO: 30 and each light chain component comprises a sequence set forth in SEQ ID NO: 31; (c) each heavy chain component comprises a sequence set forth in SEQ ID NO: 32 and each light chain component comprises a sequence set forth in SEQ ID NO: 33; (d) each heavy chain component comprises a sequence set forth in SEQ ID NO: 34 and each light chain component comprises a sequence set forth in SEQ ID NO: 35; (e) each heavy chain component comprises a sequence set forth in SEQ ID NO: 36 and each light chain component comprises a sequence set forth in SEQ ID NO: 37; or (f) each heavy chain component comprises a sequence set forth in SEQ ID NO: 38 and each light chain component comprises a sequence set forth in SEQ ID NO: 39.

[0017a] In one aspect, the present disclosure refers to a multispecific construct, comprising two heavy chain components and two light chain components, wherein: (a) each heavy chain component comprises a sequence set forth in SEQ ID NO: 28 and each light chain component comprises a sequence set forth in SEQ ID NO: 29; (b) each heavy chain component comprises a sequence set forth in SEQ ID NO: 30 and each light chain component comprises a sequence set forth in SEQ ID NO: 31; (c) each heavy chain component comprises a sequence set forth in SEQ ID NO: 32 and each light chain component comprises a sequence set forth in SEQ ID NO: 33; (d) each heavy chain component comprises a sequence set forth in SEQ ID NO: 34 and each light chain component comprises a sequence set forth in SEQ ID NO: 35; (e) each heavy chain component comprises a sequence set forth in SEQ ID NO: 36 and each light chain component comprises a sequence set forth in SEQ ID NO: 37; or (f) each heavy chain component comprises a sequence set forth in SEQ ID NO: 38 and each light chain component comprises a sequence set forth in SEQ ID NO: 39.

[0018] The present application provides kits comprising a multispecific construct described herein and/or a pharmaceutical described herein and a package insert or instructions for using the multispecific construct (or pharmaceutical composition) for treating a disease or condition (e.g., cancer).

[0019] It is to be understood that one, some, or all of the properties of the various examples described herein may be combined to form other examples of the present disclosure. These and other examples of the disclosure will become apparent to one of skill in the

art. These and other examples of the disclosure are further described by the detailed description that follows.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] **FIG. 1A-1B** illustrate binding affinity of CLDN6 x 4-1BB bispecific antibodies (BsAbs) disclosed herein with CLDN6.

[0021] **FIG. 2A-2C** illustrate binding affinity of CLDN6 x 4-1BB BsAbs disclosed herein with CLDN6 expressing cells.

[0022] **FIG. 3A-3D** illustrate binding affinity of CLDN6 x 4-1BB BsAbs disclosed herein with 4-1BB.

[0023] **FIG. 4A-4B** illustrate binding of CLDN6 x 4-1BB BsAbs disclosed herein with soluble 4-1BB and cells expressing 4-1BB.

[0024] **FIG. 5A-5D** illustrate CLDN6-dependent 4-1BB activation of CLDN6 x 4-1BB BsAbs disclosed herein.

[0025] **FIG. 6A-6H** illustrate activation of PBMC by CLDN6 x 4-1BB BsAbs disclosed herein.

[0026] **FIG. 7A-7B** illustrate tumor inhibition in hu4-1BB mice bearing CT26 murine colorectal carcinoma tumors after treatment with CLDN6 x 4-1BB BsAbs disclosed herein.

[0027] **FIG. 8** illustrates liver function after treatment of the CLDN6X4-1BB BsAbs of the present application.

[0028] **FIG. 9A-9B** illustrate tumor inhibition in hu4-1BB mice bearing MC38 murine colon adenocarcinoma tumors after treatment with CLDN6 x 4-1BB BsAbs disclosed herein.

DETAILED DESCRIPTION OF THE APPLICATION

Definitions

[0029] The term “antibody” is used in its broadest sense and encompasses various antibody structures, including but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.*, bispecific antibodies), full-length antibodies and antigen-binding fragments thereof, so long as they exhibit the desired antigen-binding activity. The term “antibody moiety” refers to a full-length antibody or an antigen-binding fragment thereof.