

CLAIMS

1. An antibody, comprising:
an anti-claudin 18.2 (CLDN18.2) antibody or antigen-binding fragment~~unit~~ having binding specificity to a CLDN18.2 protein; and
an anti-4-1BB antibody or antigen-binding fragment~~unit~~ having binding specificity to a 4-1BB protein,
wherein the anti-4-1BB antibody or antigen-binding fragment comprises a heavy chain variable region (VH) comprising a CDRH1, a CDRH2, and a CDRH3, and a light chain variable region (VL) comprising a CDRL1, a CDRL2 and a CDRL3, wherein:
the CDRH1 comprises the amino acid sequence of SEQ ID NO:1;
the CDRH2 comprises the amino acid sequence of SEQ ID NO:2;
the CDRH3 comprises the amino acid sequence of SEQ ID NO:3, 56, 57, 58, or 59;
the CDRL1 comprises the amino acid sequence of SEQ ID NO: 4;
the CDRL2 comprises the amino acid sequence of SEQ ID NO: 4; and
the CDRL3 comprises the amino acid sequence of SEQ ID NO:6~~wherein the anti-4-1BB unit is incapable of activating 4-1BB signaling upon binding to a 4-1BB protein, in the absence of the anti-CLDN18.2 unit binding to a CLDN18.2 protein.~~

2. The antibody of claim 1, wherein the binding of the anti-4-1BB antibody or antigen-binding fragment~~unit~~ to 4-1BB proteins on a cell does not result in clustering of the 4-1BB proteins, in the absence of the anti-CLDN18.2 antibody or antigen-binding fragment~~unit~~ binding to a CLDN18.2 protein.

3. The antibody of claim 1 or 2, further comprising a Fc fragment that has reduced effector function.

4. The antibody of claim 3, wherein the effector function is antibody-dependent cell-mediated cytotoxicity (ADCC), complement-dependent cytotoxicity (CDC), or antibody-dependent cellular phagocytosis (ADCP).
5. The antibody of claim 4, wherein the Fc fragment has reduced binding or no binding to FcγR or C1q.
6. The antibody of claim 5, wherein the Fc fragment has one or more mutations to reduce or eliminate FcγR or C1q binding.
7. The antibody of claim 6, wherein the Fc fragment is selected from the group consisting of:
 - an IgG1 Fc fragment with a L235E mutation,
 - an IgG1 Fc fragment with a L234A and/or L235A mutation,
 - an IgG1 Fc fragment with a P329G or P329A mutation,
 - an IgG4 Fc fragment with a F234A and/or L235A or L235E mutation,
 - an IgG2 Fc fragment with a H268Q, V309L, A330S, and/or P331S mutation, and
 - an IgG2 Fc fragment with a V234A, G237A, P238S, H268A, V309L, A330S, and/or P331S mutation (EU numbering).
8. The antibody of claim 3, wherein the Fc fragment is aglycosylated.
9. The antibody of claim 8, wherein the Fc fragment comprises a mutation that eliminates glycosylation.
10. The antibody of claim 9, wherein the mutation is at N297, optionally N297A, N297G, or N297Q.

11. The antibody of claim 3, wherein the Fc fragment comprises a mutation of N297A or a combination of mutations of L234A and L235A.

12. The antibody of any one of claims 1-11, wherein the anti-CLDN18.2 antibody or antigen-binding fragment~~unit~~ comprises a Fab fragment.

13. The antibody of claim 12, wherein the anti-CLDN18.2 antibody or antigen-binding fragment~~unit~~ comprises a pair of Fab fragment.

14. The antibody of any one of claims 1-13, wherein the anti-4-1BB antibody or antigen-binding fragment~~unit~~ comprises a Fab fragment or a single chain fragment (scFv).

15. The antibody of claim 14, wherein the anti-4-1BB antibody or antigen-binding fragment~~unit~~ comprises a pair of the scFv.

16. The antibody of claim 14 or 15, wherein the scFv is at the C-terminal side of the Fc fragment of the anti-CLDN18.2 antibody or antigen-binding fragment~~unit~~.

~~17. The antibody of any one of claims 1-16, wherein the anti-4-1BB unit comprises a heavy chain variable region (VH) comprising a CDRH1, a CDRH2, and a CDRH3, and a light chain variable region (VL) comprising a CDRL1, a CDRL2 and a CDRL3, wherein:~~
~~the CDRH1 comprises the amino acid sequence of SEQ ID NO:1, or an amino acid sequence having one or two amino acid substitution from SEQ ID NO:1;~~
~~the CDRH2 comprises the amino acid sequence of SEQ ID NO:2, or an amino acid sequence having one or two amino acid substitution from SEQ ID NO:2;~~
~~the CDRH3 comprises the amino acid sequence of SEQ ID NO:3, 56, 57, 58, or 59, or an amino acid sequence having one or two amino acid substitution from SEQ ID NO: 3, 56, 57, 58, or 59;~~

the CDRL1 comprises the amino acid sequence of SEQ ID NO:4 or 60, or an amino acid sequence having one or two amino acid substitution from SEQ ID NO:4 or 60;

the CDRL2 comprises the amino acid sequence of SEQ ID NO:5 or 61, or an amino acid sequence having one or two amino acid substitution from SEQ ID NO:5 or 61; and

the CDRL3 comprises the amino acid sequence of SEQ ID NO:6 or 62, or an amino acid sequence having one or two amino acid substitution from SEQ ID NO:6 or 62.

18. The antibody of any one of claims 1-16, wherein the anti-4-1BB unit comprises a heavy chain variable region (VH) comprising a CDRH1, a CDRH2, and a CDRH3, and a light chain variable region (VL) comprising a CDRL1, a CDRL2 and a CDRL3, wherein:

the CDRH1 comprises the amino acid sequence of SEQ ID NO:1;

the CDRH2 comprises the amino acid sequence of SEQ ID NO:2;

the CDRH3 comprises the amino acid sequence of SEQ ID NO:3, 56, 57, 58, or 59;

the CDRL1 comprises the amino acid sequence of SEQ ID NO:4 or 60;

the CDRL2 comprises the amino acid sequence of SEQ ID NO:5 or 61; and

the CDRL3 comprises the amino acid sequence of SEQ ID NO:6 or 62.

17. The antibody of any one of claims 1-16, wherein the anti-4-1BB antibody or antigen-binding fragment comprises:

(a) a heavy chain variable region (VH) comprising an the amino acid sequence selected from the group consisting of SEQ ID NO:24, 46-51 and 63-68 and a light chain variable region (VL) comprising an the amino acid sequence selected from the group consisting of SEQ ID NO:25, 52-53 and 69-74;

(b) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 46 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 52;

(c) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 47 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 52;

(d) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 48 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 52;

(e) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 49 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 53;

(f) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 50 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 53;

(g) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 51 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 52;

(h) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 63 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 69;

(i) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 64 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 70;

(j) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 65 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 71;

(k) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 66 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 72;

(l) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 67 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 73; or

(m) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 68 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 74.

~~2018.~~ The antibody of any one of claims 1-~~19~~17, wherein the anti-CLDN18.2 antibody or antigen-binding fragment~~unit~~ comprises a heavy chain variable region (VH) comprising a CDRH1, a CDRH2, and a CDRH3, and a light chain variable region (VL) comprising a CDRL1, a CDRL2 and a CDRL3, wherein:

- (a) the CDRH1 comprises the amino acid sequence of SEQ ID NO:7;
the CDRH2 comprises the amino acid sequence of SEQ ID NO:8;
the CDRH3 comprises the amino acid sequence of SEQ ID NO:9;
the CDRL1 comprises the amino acid sequence of SEQ ID NO:10;
the CDRL2 comprises the amino acid sequence of SEQ ID NO:11; and
the CDRL3 comprises the amino acid sequence of SEQ ID NO:12,
- (b) the CDRH1 comprises the amino acid sequence of SEQ ID NO:13;
the CDRH2 comprises the amino acid sequence of SEQ ID NO:14;
the CDRH3 comprises the amino acid sequence of SEQ ID NO:15;
the CDRL1 comprises the amino acid sequence of SEQ ID NO:16;
the CDRL2 comprises the amino acid sequence of SEQ ID NO:17; and
the CDRL3 comprises the amino acid sequence of SEQ ID NO:18, or
- (c) the CDRH1 comprises the amino acid sequence of SEQ ID NO:19;
the CDRH2 comprises the amino acid sequence of SEQ ID NO:20;

the CDRH3 comprises the amino acid sequence of SEQ ID NO:21;
the CDRL1 comprises the amino acid sequence of SEQ ID NO:22;
the CDRL2 comprises the amino acid sequence of SEQ ID NO:11; and
the CDRL3 comprises the amino acid sequence of SEQ ID NO:23.

~~24~~19. The antibody of claim ~~20~~18, wherein the VH of the anti-CLDN18.2 antibody or antigen-binding fragment ~~unit~~ comprises an amino acid sequence selected from the group consisting of SEQ ID NO:26, 28 and 30, and the VL of the anti-CLDN18.2 antibody or antigen-binding fragment ~~unit~~ comprises an amino acid sequence selected from the group consisting of SEQ ID NO:27, 29 and 31.

~~22~~20. An antibody comprising two first polypeptides and two second polypeptides, wherein each first polypeptide comprises, from N- to C-terminus, a heavy chain variable region (VH), a CH1, a CH2, a CH3, and a single chain fragment (scFv) wherein the scFv has~~having~~ specificity to a 4-1BB protein and wherein the CH2 and CH3 constitutes a Fc fragment;

wherein each second polypeptide comprises a light chain variable region (VL) and a CL;

wherein ~~each~~ the VH of each first polypeptide is paired with one of the VL of one of the second polypeptides, and the VH/VL pair provides an anti-CLDN18.2 antibody or antigen-binding fragment and has ~~and have~~ specificity to a claudin 18.2 (CLDN18.2) protein; and

wherein the scFv comprises a heavy chain variable region (VH) comprising a CDRH1, a CDRH2, and a CDRH3, and a light chain variable region (VL) comprising a CDRL1, a CDRL2 and a CDRL3, wherein:

the CDRH1 comprises the amino acid sequence of SEQ ID NO:1;

the CDRH2 comprises the amino acid sequence of SEQ ID NO:2;

the CDRH3 comprises the amino acid sequence of SEQ ID NO:3, 56, 57, 58, or 59;

the CDRL1 comprises the amino acid sequence of SEQ ID NO:4;
the CDRL2 comprises the amino acid sequence of SEQ ID NO:5; and
the CDRL3 comprises the amino acid sequence of SEQ ID NO:6, wherein the scFv is
incapable of activating 4-1BB signaling upon binding to a 4-1BB protein, in
the absence of the VH/VL pairs binding to a CLDN18.2 protein.

~~2321.~~ The antibody of claim ~~2220~~, wherein the ~~CH2 and CH3~~ constitute an Fc fragment that has reduced effector function.

~~2422.~~ The antibody of claim ~~2321~~, wherein the Fc fragment is selected from the group consisting of:

an IgG1 Fc fragment with a L235E mutation,
an IgG1 Fc fragment with a L234A and/or L235A mutation,
an IgG1 Fc fragment with a P329G or P329A mutation,
an IgG4 Fc fragment with a F234A and/or L235A or L235E mutation,
an IgG2 Fc fragment with a H268Q, V309L, A330S, and/or P331S mutation, and
an IgG2 Fc fragment with a V234A, G237A, P238S, H268A, V309L, A330S, and/or P331S mutation (EU numbering).

~~2523.~~ The antibody of claim ~~2321~~, wherein the Fc fragment comprises a mutation at N297, optionally N297A, N297G, or N297Q.

~~26.~~ The antibody of any one of claims 22-25, wherein the scFv comprises a heavy chain variable region (VH) comprising a CDRH1, a CDRH2, and a CDRH3, and a light chain variable region (VL) comprising a CDRL1, a CDRL2 and a CDRL3, wherein:

~~the CDRH1 comprises the amino acid sequence of SEQ ID NO:1;~~
~~the CDRH2 comprises the amino acid sequence of SEQ ID NO:2;~~
~~the CDRH3 comprises the amino acid sequence of SEQ ID NO: 3, 56, 57, 58, or 59;~~

~~the CDRL1 comprises the amino acid sequence of SEQ ID NO:4 or 60;~~
~~the CDRL2 comprises the amino acid sequence of SEQ ID NO:5 or 61; and~~
~~the CDRL3 comprises the amino acid sequence of SEQ ID NO:6 or 62.~~

~~27~~24. The antibody of any one of claims ~~22~~20-26~~23~~, wherein the scFv comprises:

~~(a) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 63 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 69;~~

~~(b) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 64 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO:70;~~

~~(c) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 65 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 71;~~

~~(d) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 66 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 72;~~

~~(e) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 67 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 73; or~~

~~(f) a heavy chain variable region (VH) comprising the amino acid sequence of SEQ ID NO: 68 and a light chain variable region (VL) comprising the amino acid sequence of SEQ ID NO: 74, a heavy chain variable region (VH) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:24, 46-51 and 63-68 and a light chain variable region (VL) comprising an amino acid sequence selected from the group consisting of SEQ ID NO:25, 52-53 and 69-74.~~

~~28~~25. The antibody of any one of claims ~~22~~20-27~~24~~, wherein the VH of the VH/VL pair comprises a CDRH1, a CDRH2, and a CDRH3, and VL of the VH/VL pair comprises a CDRL1, a CDRL2 and a CDRL3, wherein:

- (a) the CDRH1 comprises the amino acid sequence of SEQ ID NO:7;
the CDRH2 comprises the amino acid sequence of SEQ ID NO:8;
the CDRH3 comprises the amino acid sequence of SEQ ID NO:9;
the CDRL1 comprises the amino acid sequence of SEQ ID NO:10;
the CDRL2 comprises the amino acid sequence of SEQ ID NO:11; and
the CDRL3 comprises the amino acid sequence of SEQ ID NO:12,
- (b) the CDRH1 comprises the amino acid sequence of SEQ ID NO:13;
the CDRH2 comprises the amino acid sequence of SEQ ID NO:14;
the CDRH3 comprises the amino acid sequence of SEQ ID NO:15;
the CDRL1 comprises the amino acid sequence of SEQ ID NO:16;
the CDRL2 comprises the amino acid sequence of SEQ ID NO:17; and
the CDRL3 comprises the amino acid sequence of SEQ ID NO:18, or
- (c) the CDRH1 comprises the amino acid sequence of SEQ ID NO:19;
the CDRH2 comprises the amino acid sequence of SEQ ID NO:20;
the CDRH3 comprises the amino acid sequence of SEQ ID NO:21;
the CDRL1 comprises the amino acid sequence of SEQ ID NO:22;
the CDRL2 comprises the amino acid sequence of SEQ ID NO:11; and
the CDRL3 comprises the amino acid sequence of SEQ ID NO:23.

~~29~~26. The antibody of claim ~~27~~25, wherein the VH of the VH/VL pair comprises an amino acid sequence selected from the group consisting of SEQ ID NO:26, 28 and 30, and the VL of the VH/VL pair comprises an amino acid sequence selected from the group consisting of SEQ ID NO:27, 29 and 31.

~~30~~27. The antibody of claim ~~22~~20, wherein each of the first polypeptides comprises the amino acid sequence of SEQ ID NO:40 and each of the second polypeptides comprises the amino acid sequence of SEQ ID NO:41.

~~31~~28. The antibody of claim ~~22~~20, wherein each of the first polypeptides comprises the amino acid sequence of SEQ ID NO:42 and each of the second polypeptides comprises the amino acid sequence of SEQ ID NO:43.

~~32~~29. The antibody of claim ~~22~~20, wherein each of the first polypeptides comprises the amino acid sequence of SEQ ID NO:44 and each of the second polypeptides comprises the amino acid sequence of SEQ ID NO:45.

~~33~~30. ~~One or more~~A polynucleotide encoding the antibody of any one of claims 1-~~32~~29.

~~34~~31. A composition comprising the antibody of any one of claims 1-~~32~~29 and a pharmaceutically acceptable carrier.

~~35~~32. Use of the antibody of any one of claims 1-~~32~~29 for the preparation of a medicament for the treatment of cancer.

~~36~~. ~~—A method for treating cancer in a patient in need thereof, comprising administering to the patient the antibody of any one of claims 1-32.~~

~~37~~33. The use of claim ~~35~~32~~or the method of claim 36~~, wherein the cancer is characterized by a cancer cell that overexpresses CLDN18.2 as compared to a counterpart normal cell.

~~38~~34. The use of claim ~~35~~32 or ~~37~~33, ~~or the method of claim 36 or 37~~, wherein the cancer is an epithelial tumor, or 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.

~~39~~35. The use of claim ~~35~~32 or ~~37~~33, ~~or the method of claim 36 or 37~~, wherein the cancer is selected from the group consisting of gastric cancer, pancreatic cancer, esophageal cancer, lung cancer and ovarian cancer.