

CLAIMS

What is claimed is:

1. An isolated antibody or an antigen binding fragment thereof that specifically bind to CLDN18.2, wherein the antibody or antigen binding fragment thereof exhibits one or more of the following characteristics:
 - a) having no cross-reactivity with human CLDN 18.1;
 - b) having no cross-reactivity with non-cancerous cells except stomach epithelial cells as measured by Immunohistochemistry assay (IHC);
 - c) having no cross-reactivity with non-cancerous human lung tissue as measured by IHC;
 - d) capable of specifically binding to CLDN18.2-expressing cells, optionally the CLDN18.2-expressing cells are pre-treated such that CLDN18.2 is denatured or otherwise no longer in its native conformation;
 - e) capable of binding to a fusion polypeptide comprising first extracellular loop of human CLDN18.2 at a K_d value of no more than 10nM as measured by Surface Plasmon resonance (SPR), or at an EC50 value of no more than 20 ng/ml as measured by enzyme-linked immunosorbent assays (ELISA);
 - f) showing no detectable binding to cell surface human CLDN 18.2 as measured by flow cytometry (FACS) assay;
 - g) capable of specifically binding to an epitope within the amino acid sequence of DQWSTQDLYN (SEQ ID NO: 19) as measured by ELISA; and/or
 - h) having no cross-reactivity to human CLDN18.1 in a formalin-fixed paraffin-embedded (FFPE) sample at an antibody concentration of 1nM as measured by ELISA or at an antibody concentration of 0.5ug/ml as measured by IHC.

2. An isolated antibody or an antigen binding fragment thereof that specifically bind to CLDN18.2, comprising:
 - a) a heavy chain CDR1 comprising the amino acid sequence of **X₁X₂YX₃H** (SEQ ID NO: 8), a heavy chain CDR2 comprising the amino acid sequence of **WIYPX₄GX₅X₆X₇X₈YX₉EKFKG** (SEQ ID NO: 12), and a heavy chain CDR3 comprising the amino acid sequence of **NYX₁₀STFGY** (SEQ ID NO: 24); and/or
 - b) a light chain CDR1 comprising the amino acid sequence of **RSSQNIVHSNGNTYLE** (SEQ ID NO: 2), a light chain CDR2 comprising the amino acid sequence of **KX₁₁SNRFS** (SEQ ID NO: 25), and a light chain CDR3 comprising the amino acid sequence of **FQGSHVPFT** (SEQ ID NO: 6);
 wherein X₁ is R or T, X₂ is N or Y, X₃ is F or I, X₄ is G or R, X₅ is F or G, X₆ is D or N, X₇ is I or T, X₈ is E or V, X₉ is S or N, X₁₀ is G or R, and X₁₁ is V or I.

3. The isolated antibody or antigen binding fragment there of claim 2, comprising:

- a) a heavy chain CDR1 comprising the amino acid sequence selected from: SEQ ID NO: 1 and SEQ ID NO: 7, and/or
 - b) a heavy chain CDR2 comprising the amino acid sequence selected from: SEQ ID NO: 3 and SEQ ID NO: 9, and/or
 - c) a heavy chain CDR3 comprising the amino acid sequence selected from: SEQ ID NO: 5 and SEQ ID NO: 11; and/or
 - d) a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 2, and/or
 - e) a light chain CDR2 comprising the amino acid sequence selected from: SEQ ID NO: 4 and SEQ ID NO: 10, and/or
 - f) a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 6.
4. An isolated antibody or an antigen binding fragment thereof that specifically bind to CLDN18.2, comprising:
- g) a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 1, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 5; or
 - h) a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 7, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 9, and a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 11.
5. The antibody or an antigen binding fragment thereof of claim 4, further comprising:
- a) a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 2, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 6; or
 - b) a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 2, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 6.
6. The antibody or an antigen binding fragment thereof of any one of the preceding claims, comprising:
- a) a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 1, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 3, a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 5, a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 2, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 4, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 6; or
 - b) a heavy chain CDR1 comprising the amino acid sequence of SEQ ID NO: 7, a heavy chain CDR2 comprising the amino acid sequence of SEQ ID NO: 9, a heavy chain CDR3 comprising the amino acid sequence of SEQ ID NO: 11, a light chain CDR1 comprising the amino acid sequence of SEQ ID NO: 2, a light chain CDR2 comprising the amino acid sequence of SEQ ID NO: 10, and a light chain CDR3 comprising the amino acid sequence of SEQ ID NO: 6.

7. The antibody or an antigen binding fragment thereof of any one of the preceding claims, comprising:
 - a) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 13, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 14; or
 - b) a heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 15, and a light chain variable region comprising the amino acid sequence of SEQ ID NO: 16.
8. The antibody or antigen-binding fragment thereof of any of claims 4-7, further comprising one or more amino acid residue mutations yet retaining binding specificity to human CLDN 18.2.
9. The antibody or antigen-binding fragment thereof of claim 8, at least one of the mutations are conservative substitutions, or all of the mutations are conservative substitutions.
10. The antibody or antigen-binding fragment thereof of claim 8 or 9, wherein at least one of the mutations is in one or more of the CDR sequences, and/or in one or more of the non-CDR sequences of the heavy chain variable region or light chain variable region.
11. The antibody or antigen-binding fragment thereof of any one of claims 8-10, comprising:
 - a) a heavy chain CDR1 (HCDR1) sequence having at least 80% (e.g. at least 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) sequence identity to SEQ ID NO: 1 or SEQ ID NO: 7, and/or
 - b) a heavy chain CDR2 (HCDR2) sequence having at least 80% (e.g. at least 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) sequence identity to SEQ ID NO: 3 or SEQ ID NO: 9, and/or
 - c) a heavy chain CDR3 (HCDR3) sequence having at least 80% (e.g. at least 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) sequence identity to SEQ ID NO: 5 or SEQ ID NO: 11, and/or

d) a light chain CDR1 (LCDR1) sequence having at least 80% (e.g. at least 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) sequence identity to SEQ ID NO: 2, and/or

e) a light chain CDR2 (LCDR2) sequence having at least 80% (e.g. at least 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) sequence identity to SEQ ID NO: 4 or SEQ ID NO: 10, and/or

f) a light chain CDR3 (LCDR3) sequence having at least 80% (e.g. at least 85%, 88%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%) sequence identity to SEQ ID NO: 6, and

in the meantime retain the binding specificity to CLDN18.2, optionally having binding affinity at a level similar to or even higher than its parent antibody.

12. The antibody or antigen-binding fragment thereof of any one of claims 8-11, comprises an HCDR1 having no more than 3, 2, or 1 amino acid mutations in SEQ ID NO: 1 or SEQ ID NO: 7, an HCDR2 having no more than 6, 5, 4, 3, 2, or 1 amino acid mutations in SEQ ID NO: 3 or SEQ ID NO: 9, an HCDR3 having no more than 6, 5, 4, 3, 2, or 1 amino acid mutations in SEQ ID NO: 5 or SEQ ID NO: 11, a LCDR1 having no more than 2 or 1 amino acid mutations in SEQ ID NO: 2, a LCDR2 having no more than 3, 2, or 1 amino acid mutations in SEQ ID NO: 4 or SEQ ID NO: 10, and/or a LCDR3 having no more than 3, 2, or 1 amino acid mutations in SEQ ID NO: 6, and in the meantime retain the binding specificity to CLDN18.2, and optionally having binding affinity at a level similar to or even higher than its parent antibody.
13. The antibody or antigen-binding fragment thereof of any of claims 8-12, wherein the heavy chain variable region comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 13 or SEQ ID NO: 15, and/or the light chain variable region comprises an amino acid sequence having at least 80% sequence identity to SEQ ID NO: 14 or SEQ ID NO: 16.
14. The antibody or antigen-binding fragment thereof of any one of the preceding claims, further comprising an immunoglobulin constant region, optionally comprising a heavy chain constant region of IgG, and/or a light chain constant region.
15. The antibody or antigen-binding fragment thereof of claim 14, wherein the constant region comprises a mouse constant region, a rabbit constant region, or a human

constant region.

16. The antibody or antigen-binding fragment thereof of claim 15, wherein the heavy chain constant region comprises an amino acid sequence of SEQ ID NO: 17 or a sequence having at least 80% sequence identity thereof, and/or the light chain constant region comprises an amino acid sequence of SEQ ID NO: 18 or a sequence having at least 80% sequence identity thereof.
17. The antibody or an antigen-binding fragment thereof of any one of the preceding claims, which is a monoclonal antibody, a bispecific antibody, a multi-specific antibody, a recombinant antibody, a chimeric antibody, a humanized antibody, a labeled antibody, a bivalent antibody, an anti-idiotypic antibody, a fusion protein, a dimerized or polymerized antibody, or a modified antibody (e.g. glycosylated antibody).
18. The antibody or antigen-binding fragment thereof of any one of the preceding claims, which is a diabody, a Fab, a Fab', a F(ab')₂, a Fd, an Fv fragment, a disulfide stabilized Fv fragment (dsFv), a (dsFv)₂, a bispecific dsFv (dsFv-dsFv'), a disulfide stabilized diabody (ds diabody), a single-chain antibody molecule (scFv), an scFv dimer (bivalent diabody), a multispecific antibody, a camelized single domain antibody, a nanobody, a domain antibody, or a bivalent domain antibody.
19. The antibody or antigen-binding fragment thereof of any one of the preceding claims, which is linked to one or more moieties.
20. The antibody or antigen-binding fragment thereof of claim 19, wherein the moiety comprises a radioactive isotope, a lanthanide, a chemluminescent label, a chromophoric moiety, colloidal gold particles, a fluorescent label, an enzyme-substrate label, a digoxigenin label, biotin/avidin, a hapten, a DNA molecule for detection or particle labels.
21. The antibody or antigen-binding fragment thereof of claim 20, wherein the moiety comprises a biotin or a hapten.
22. A monoclonal antibody or an antigen-binding fragment thereof, which competes for binding to CLDN18.2 with the antibody or antigen-binding fragment thereof of any one of the preceding claims.

23. An isolated polynucleotide encoding the antibody or an antigen-binding fragment thereof of any one of the preceding claims.
24. A vector comprising the isolated polynucleotide of claim 23.
25. A host cell comprising the vector of claim 24.
26. A method of expressing the antibody or antigen-binding fragment thereof of any of claims 1-22, comprising culturing the host cell of claim 25 under the condition at which the vector of claim 24 is expressed.
27. A method of detecting presence or expression level of CLDN18.2 in a sample, comprising contacting the sample with the antibody or antigen-binding fragment thereof of any of claims 1-22 under conditions that allow specific binding of the antibody or antigen-binding fragment thereof to human CLDN18.2, and determining the presence or expression level of CLDN18.2 in the sample.
28. A method for diagnosing a CLDN18.2-associated disease or condition (e.g. cancer) in a subject, comprising:
- a) contacting a sample obtained from the subject with the antibody or antigen-binding fragment thereof of any of claims 1-22 under conditions that allow specific binding of the antibody or antigen-binding fragment thereof to CLDN18.2; and
 - b) determining presence or expression level of CLDN18.2 in the sample;
- wherein the subject is diagnosed as having a CLDN18.2-associated disease or condition (e.g. cancer) when the presence of CLDN18.2 is found or when the expression level of CLDN18.2 reaches a threshold level.
29. A method for determining the eligibility of a subject having or at risk of a CLDN18.2-associated disease or condition for treatment with a CLDN18.2-targeting agent, comprising:
- a) contacting a sample obtained from the subject with the antibody or antigen-binding fragment thereof of any of claims 1-22 under conditions that allow specific binding of the antibody or antigen-binding fragment thereof to CLDN18.2;
 - b) determining presence or expression level of CLDN18.2 in the sample;

wherein the subject is determined as eligible for treatment with a CLDN18.2-targeting agent when the presence of CLDN18.2 is found or when the expression level of CLDN18.2 reaches a threshold level, or

wherein the subject is determined as not eligible for treatment with a CLDN18.2-targeting agent when the presence of CLDN18.2 is not found or when the expression level of CLDN18.2 is below a threshold level.

30. A method of predicting therapeutic effectiveness of a CLDN18.2-targeting agent in treating a CLDN18.2-associated disease or condition in a subject, comprising:

- a) contacting a sample obtained from the subject with the antibody or antigen-binding fragment thereof of any of claims 1-22 under condition that allow specific binding of the antibody or antigen-binding fragment thereof to CLDN18.2;
- b) determining presence or expression level of human CLDN18.2 in the sample;
- c) predicting the therapeutic effectiveness of the CLDN18.2-targeting agent, wherein the CLDN18.2-targeting agent is predicted to be effective in treating the subject when the presence of CLDN18.2 is found or when the expression level of CLDN18.2 reaches a threshold level, or

wherein the CLDN18.2-targeting agent is predicted to be not effective in treating the subject when the presence of CLDN18.2 is not found or when the expression level of CLDN18.2 is below the threshold level.

31. A method of treating a subject having or at risk of a CLDN18.2-associated disease or condition, comprising:

- a) selecting a subject that is suitable for the treatment, comprising:
 - i) contacting a sample obtained from the subject with the antibody or antigen-binding fragment thereof of any of claims 1-22 under conditions that allow specific binding of the antibody or antigen-binding fragment thereof to CLDN18.2;
 - ii) determining the presence or expression level of human CLDN18.2 in the sample;
 - iii) selecting the subject as suitable for the treatment of the CLDN18.2-associated disease or condition when the presence of CLDN18.2 is found or when the expression level of CLDN18.2 in the sample reaches a threshold level;
- b) administering a therapeutically effective amount of CLDN18.2-targeting agent to the selected subject.

32. A method of treating a subject having or at risk of cancer, comprising:
- a) selecting a subject comprising:
 - i) contacting a sample obtained from the subject with the antibody or antigen-binding fragment thereof of any of claims 1-22 under conditions that allow specific binding of the antibody or antigen-binding fragment thereof to CLDN18.2;
 - ii) determining the presence or expression level of CLDN18.2 in the sample;
 - iii) selecting the subject as not suitable for the treatment with a CLDN18.2-targeting agent when the presence of CLDN18.2 is not found or when the expression level of CLDN18.2 in the sample is below a threshold level;
 - b) administering to the selected subject a Standard-of-Care Therapeutic other than a CLDN18.2-targeting agent.
33. The method of any one of claims 27-32, wherein the sample is a cell sample or a tissue sample.
34. The method of claim 33, wherein the sample is a fixed tissue sample, optionally a formalin-fixed paraffin-embedded (FFPE) tissue sample.
35. The method of any one of claims 27-34, wherein the CLDN18.2 is cell surface or membrane-bound CLDN18.2.
36. The method of any one of claims 27-35, wherein the presence or expression level of CLDN18.2 is determined by immunohistochemistry (IHC), Immunocytochemistry (ICC), immunofluorescence (IF), enzyme immunoassay (EIA), Enzyme-Linked Immunosorbant Assay (ELISA), or immunoblotting.
37. The method of any one of claims 27-36, wherein the expression level is quantified based on percentage of positively-stained cells in the sample.
38. The method of any one of claims 27-36, wherein the expression level is quantified based on staining intensity for CLDN18.2 in the sample.
39. The method of any one of claims 28-38, wherein the CLDN18.2-associated disease or condition is cancer.
40. The method of claim 39, wherein the sample comprises a tumor sample.

41. The method of claim 40, wherein the tumor sample comprises a tumor tissue or a circulating tumor cell.
42. The method of claim 41, wherein the cancer is primary cancer or metastatic cancer.
43. The method of any one of claims 39-42, wherein the cancer is gastric cancer, lung cancer (non-small cell lung cancer or small cell lung cancer), bronchial cancer, bone cancer, liver and bile duct cancer, pancreatic cancer, breast cancer, liver cancer, ovarian cancer, testicle cancer, kidney cancer, bladder cancer, head and neck cancer, spine cancer, brain cancer, cervix cancer, uterine cancer, endometrial cancer, colon cancer, colorectal cancer, rectal cancer, anal cancer, esophageal cancer, gastrointestinal cancer, skin cancer, prostate cancer, pituitary cancer, stomach cancer, vagina cancer, thyroid cancer, glioblastoma, astrocytoma, melanoma, myelodysplastic syndrome, sarcoma, teratoma, cholangiocarcinoma, and/or adenocarcinoma.
44. The method of claim 43, wherein the cancer is gastric cancer, pancreatic cancer, cholangiocarcinoma, or lung cancer (e.g. non-small cell lung cancer or small cell lung cancer).
45. The method of any of claims 29-44, wherein the CLDN18.2-targeting agent is capable of inducing cytotoxicity to CLDN18.2-expressing cells.
46. The method of any of claims 29-45, wherein the CLDN18.2-targeting agent is a therapeutic anti-CLDN18.2 antibody or an CLDN18.2-binding molecule (e.g. anti-CLDN18.2 antibody conjugated to a cytotoxic agent, or a bispecific antibody), a CLDN18.2-targeting cell therapy (e.g. a CAR-T, TCR-T or CAR-NK cell expressing a CLDN18.2-binding CAR), a chemical compound targeting CLDN18.2, or a therapeutic nucleic acid targeting CLDN18.2.
47. The method of any one of claims 27-46, wherein the subject is receiving or has received anti-cancer-therapy, or suffers from cancer recurrence.
48. A kit comprising the isolated antibody or antigen-binding fragment thereof of any one of claims 1-22.
49. The kit of claim 48, further comprising a set of reagents for detecting a complex of the antibody or antigen-binding fragment thereof bound to CLDN18.2.

50. The kit of claim 49, wherein the set of reagents comprises a secondary antibody that binds to the antibody or antigen-binding fragment thereof of any one of claims 1-22, optionally the secondary antibody is detectably labeled.
51. The kit of claim 48, wherein the antibody or the antigen binding fragment thereof is detectably labeled.
52. The kit of claim 48, wherein the antibody or the antigen binding fragment thereof is conjugated with an indirectly detectable moiety.
53. The kit of claim 52, wherein the indirectly detectable moiety comprises biotin.
54. The kit of claim 53, wherein the set of reagents comprises a detectably labeled avidin or streptavidin.