

CLAIMS

What is claimed is:

1. A pharmaceutical composition comprising:
 - a. at least one single-stranded RNA comprising one or more coding regions that encode an antibody agent that binds preferentially to a Claudin-18.2 (CLDN-18.2) polypeptide relative to a Claudin-18.1 polypeptide; and
 - b. lipid nanoparticles;wherein the at least one single-stranded RNA is encapsulated within at least one of the lipid nanoparticles.
2. The pharmaceutical composition of claim 1, wherein the antibody agent specifically binds to a first extracellular domain (ECD1) of a CLDN-18.2 polypeptide.
3. The pharmaceutical composition of claim 2, wherein the antibody agent specifically binds to an epitope of ECD1 that is exposed in cancer cells.
4. The pharmaceutical composition of any one of claims 1-3, wherein the antibody agent is or comprises an antibody or an antigen binding fragment thereof.
5. The pharmaceutical composition of any one of claims 1-4, wherein the at least one single-stranded RNA encodes both of: a variable heavy chain (V_H) domain of the antibody agent; and a variable light chain (V_L) domain of the antibody agent.
6. The pharmaceutical composition of claim 5, wherein the at least one single-stranded RNA is a first single-stranded RNA comprising a heavy chain-coding region that encodes at least a V_H domain of the antibody agent; and
 - a. wherein the first single-stranded RNA further comprises a light chain-coding region that encodes at least a V_L domain of the antibody agent; or
 - b. wherein the pharmaceutical composition further comprises a second single-stranded RNA comprising a light chain-coding region that encodes at least a V_L domain of the antibody agent.
7. The pharmaceutical composition of claim 6, wherein the heavy chain-coding region further encodes a constant heavy chain (C_H) domain; and/or the light chain-coding region further encodes a constant light chain (C_L) domain.
8. The pharmaceutical composition of claim 6, wherein the heavy chain-coding region encodes a V_H domain, a C_{H1} domain, a C_{H2} domain, and a C_{H3} domain of the antibody agent in an

immunoglobulin G (IgG) form; and/or the light chain-coding region encodes a V_L domain and a C_L domain of the antibody agent in an IgG form.

9. The pharmaceutical composition of claim 8, wherein the IgG is IgG1.
10. The pharmaceutical composition of any one of claims 6-9, wherein the heavy chain-coding region consists of or comprises a nucleotide sequence that encodes a full-length heavy chain of Zolbetuximab or Claudiximab.
11. The pharmaceutical composition of any one of claims 6-9, wherein the light chain-coding region consists of or comprises a nucleotide sequence that encodes a full-length light chain of Zolbetuximab or Claudiximab.
12. The pharmaceutical composition of any one of claims 6-11, wherein the first single-stranded and/or the second single-stranded RNA each independently comprise a secretion signal-encoding region.
13. The pharmaceutical composition of any one of claims 6-12, wherein the first single-stranded and/or the second single-stranded RNA each independently comprise at least one non-coding sequence element (*e.g.*, to enhance RNA stability and/or translation efficiency).
14. The pharmaceutical composition of claim 13, wherein the at least one non-coding sequence element comprises a 3' untranslated region (UTR), a 5' UTR, a cap structure for co-transcriptional capping of mRNA, and/or a poly adenine (polyA) tail.
15. The pharmaceutical composition of any one of claims 6-14, wherein the first single-stranded RNA comprises, in a 5' to 3' direction:
 - a. a 5'UTR-coding region;
 - b. a secretion signal-coding region;
 - c. the heavy chain-coding region;
 - d. a 3' UTR-coding region; and
 - e. a polyA tail-coding region.
16. The pharmaceutical composition of any one of claims 6-15, wherein the second single-stranded RNA comprises, in a 5' to 3' direction:
 - a. a 5'UTR-coding region;
 - b. a secretion signal-coding region;
 - c. the light chain-coding region;
 - d. a 3' UTR-coding region; and

- e. a polyA tail-coding region.
17. The pharmaceutical composition of claim 14 or 15, wherein the polyA tail is or comprises a modified polyA sequence.
 18. The pharmaceutical composition of any one of claims 6-16, wherein the first single-stranded and/or the second single-stranded RNA comprises a 5' cap.
 19. The pharmaceutical composition of any one of claims 6-18, wherein the first single-stranded and/or the second single-stranded RNA comprises at least one modified ribonucleotide.
 20. The pharmaceutical composition of claim 19, wherein the modified ribonucleotide comprises pseudouridine.
 21. The pharmaceutical composition of any one of claims 6-20, wherein the at least one single-stranded RNA comprises the first single-stranded RNA and the second single-stranded RNA.
 22. The pharmaceutical composition of any one of claims 6-21, wherein the first single-stranded RNA and the second single-stranded RNA are present in a weight ratio of 3:1 to 1:1.
 23. The pharmaceutical composition of any one of claims 1-22, wherein the lipid nanoparticles are liver-targeting lipid nanoparticles.
 24. The pharmaceutical composition of any one of claims 1-23, wherein the lipid nanoparticles are cationic lipid nanoparticles.
 25. The pharmaceutical composition of claim 24, wherein lipids that form the lipid nanoparticles comprise:
 - a polymer-conjugated lipid;
 - a cationic lipid; and
 - a neutral lipid.
 26. The pharmaceutical composition of claim 25, wherein:
 - a. the polymer-conjugated lipid is present in about 1-2.5 mol% of the total lipids;
 - b. the cationic lipid is present in 35-65 mol% of the total lipids; and
 - c. the neutral lipid is present in 35-65 mol% of the total lipids.
 27. The pharmaceutical composition of claim 25 or 26, wherein the polymer-conjugated lipid is a PEG-conjugated lipid (*e.g.*, 2-[(polyethylene glycol)-2000]-N,N-ditetradecylacetamide).
 28. The pharmaceutical composition of any one of claims 25-27, wherein the cationic lipid is ((3-hydroxypropyl)azanediyl)bis(nonane-9,1-diyl) bis(2-butyloctanoate).

29. The pharmaceutical composition of any one of claims 25-28, wherein the neutral lipid comprises 1,2-Distearoyl-sn-glycero-3-phosphocholine (DPSC) and/or cholesterol.
30. The pharmaceutical composition of any one of claims 1-29, wherein the lipid nanoparticles have an average size of about 50-150 nm.
31. The pharmaceutical composition of any one of claims 1-30, further comprising a cryoprotectant (*e.g.*, sucrose).
32. The pharmaceutical composition of any one of claims 1-31, comprising an aqueous buffered solution.
33. The pharmaceutical composition of claim 32, wherein the aqueous buffered solution includes sodium ions.
34. The pharmaceutical composition of any one of claims 1-33, further comprising a chemotherapeutic agent.
35. The pharmaceutical composition of claim 34, wherein the chemotherapeutic agent is a chemotherapeutic agent indicated for treatment of pancreatic cancer.
36. The pharmaceutical composition of any one of claims 1-35, wherein the at least one single-stranded RNA is present at a concentration of 0.5 mg/mL to 1.5 mg/mL.
37. A method comprising administering a pharmaceutical composition of any one of claim 1-36 to a subject suffering from a CLDN-18.2-positive solid tumor.
38. The method of claim 37, wherein the CLDN-18.2-positive tumor is a pancreatic tumor.
39. The method of claim 37, wherein the CLDN-18.2-positive tumor is a gastric tumor.
40. The method of claim 37, wherein the CLDN-18.2-positive tumor is a biliary tract tumor.
41. The method of any one of claims 37-40, wherein the CLDN-18.2-positive solid tumor is locally advanced, unresectable, or metastatic.
42. The method of any one of claims 37-41, wherein the subject has received a pre-treatment sufficient to increase CLDN-18.2 level such that a solid tumor from which the subject is suffering is characterized as a CLDN-18.2-positive solid tumor.
43. The method of any one of claims 37-42, wherein the CLDN-18.2-positive tumor is characterized in that $\geq 50\%$ of tumor cells show $\geq 2+$ CLDN-18.2 protein staining intensity as assessed by an immunohistochemistry assay in formalin-fixed, paraffin-embedded neoplastic tissue from the subject.

44. The method of any one of claims 37-43, wherein the pharmaceutical composition is administered as monotherapy.
45. The method of any one of claims 37-44, wherein the pharmaceutical composition is administered as part of combination therapy comprising the pharmaceutical composition and a chemotherapeutic agent.
46. The method of any one of claims 37-45, wherein the subject has received the chemotherapeutic agent.
47. The method of claim 45, further comprising administering to the subject the chemotherapeutic agent such that the subject is receiving the combination therapy.
48. The method of claim 47, wherein the chemotherapeutic agent is administered at least four hours after the administration of the pharmaceutical composition.
49. The method of any one of claims 45-48, wherein the chemotherapeutic agent is or comprises gemcitabine and/or paclitaxel (*e.g.*, nab-paclitaxel) for a subject suffering from a CLDN-18.2-positive pancreatic tumor.
50. The method of any one of claims 45-48, wherein the chemotherapeutic agent is or comprises FOLFIRINOX for a subject suffering from a CLDN-18.2-positive pancreatic tumor.
51. The method of any one of claims 45-48, wherein the chemotherapeutic agent is or comprises gemcitabine and/or cisplatin for a subject suffering from a CLDN-18.2-positive biliary tract cancer.
52. The method of any one of claims 37-51, wherein the subject is an adult subject.
53. The method of any one of claims 37-52, wherein the administering is performed by intravenous injection.
54. The method of any one of claims 37-53, wherein the pharmaceutical composition is administered in at least one, at least two, at least three or more dosing cycles.
55. The method of claim 54, wherein the pharmaceutical composition is administered as one or more doses per dosing cycle.
56. The method of claim 55, wherein each dosing cycle is a three-week dosing cycle.
57. The method of claim 55 or 56, wherein the one or more doses comprise the at least one single-stranded RNA within a range of 0.1 mg/kg to 5 mg/kg body weight of the subject.

58. In a method of delivering a CLDN-18.2-targeting antibody for cancer treatment in a subject, the improvement comprising administering to the subject the pharmaceutical composition of any one of claims 1-36.
59. A method of producing a CLDN-18.2-targeting antibody comprising administering to cells the pharmaceutical composition of any one of claims 1-35 so that the cells express and secrete the CLDN-18.2-targeting antibody encoded by the at least one single-stranded RNA of the pharmaceutical composition.
60. The method of claim 59, wherein the cells are liver cells.
61. The method of claim 59 or 60, wherein the cells are in a subject.
62. The method of claim 61, wherein the CLDN-18.2-targeting antibody is produced at a therapeutically relevant plasma concentration.
63. The method of claim 62, wherein the therapeutically relevant plasma concentration is sufficient to mediate cancer cell death through antibody-dependent cellular cytotoxicity (ADCC).
64. The method of claim 63, wherein the therapeutically relevant plasma concentration is 0.3–28 µg/mL.
65. A method comprising a step of:
determining one or more features of an antibody agent expressed from at least one mRNA introduced into cells, wherein the at least one mRNA comprises one or more of features of at least one or more single-stranded RNA comprising a coding region that encodes an antibody agent that binds preferentially to a Claudin-18.2 (CLDN-18.2) polypeptide relative to a Claudin-18.1 polypeptide, wherein the one or more features comprises: (i) protein expression level of the antibody agent; (ii) binding specificity of the antibody agent to CLDN-18.2; (iii) efficacy of the antibody agent to mediate target cell death through ADCC; and (iv) efficacy of the antibody agent to mediate target cell death through complement dependent cytotoxicity (CDC).
66. A method of characterizing a pharmaceutical composition targeting CLDN-18.2, the method comprising steps of:
contacting cells with at least one pharmaceutical composition as set forth in any one of claims 1-35; and
detecting the antibody agent produced by the cells.

67. The method of claim 66, further comprising determining one or more features of the antibody agent, wherein the one or more features comprises: (i) protein expression level of the antibody agent; (ii) binding specificity of the antibody agent to a CLDN-18.2 polypeptide; (iii) efficacy of the antibody agent to mediate target cell death through ADCC; and (iv) efficacy of the antibody agent to mediate target cell death through complement dependent cytotoxicity (CDC).
68. The method of any one of claims 65-67, wherein the cells are liver cells.
69. The method of claim 65 or 67, wherein the step of determining comprises comparing the one or more features of the antibody agent with that of a reference CLDN-18.2-targeting antibody.
70. The method of any one of claims 65 and 67-69, wherein the step of determining comprises assessing the protein expression level of the antibody agent above a threshold level.
71. The method of claim 70, wherein the threshold level is a level that is sufficient to induce ADCC.
72. The method of any one of claims 65 and 67-71, wherein the step of determining comprises assessing binding of the antibody agent to a CLDN-18.2 polypeptide.
73. The method of claim 72, wherein the assessing comprises determining binding of the antibody agent to a CLDN-18.2 polypeptide relative to its binding to a CLDN18.1 polypeptide.
74. The method of claim 72 or 73, wherein the assessing comprises determining a binding preference profile of the antibody agent at least comparable to that of a reference CLDN-18.2-targeting antibody.
75. The method of claim 69 or 74, wherein the reference CLDN-18.2-targeting antibody is Zolbetuximab or Claudiximab.
76. The method of any one of claims 65-75, further characterizing the antibody agent as a CLDN-18.2-targeting antibody agent if the antibody agent comprises the following features:
- a. protein level of the antibody agent expressed by the cells above a threshold level that is sufficient to induce ADCC;
 - b. preferential binding of the antibody agent to CLDN-18.2 relative to CLDN18.1; and
 - c. killing of at least 50% target cells mediated by ADCC and/or CDC.

77. The method of claim 76, further characterizing the antibody agent as a Zolbetuximab or Claudiximab-equivalent antibody if the features of the antibody are at least comparable to that of Zolbetuximab or Claudiximab.
78. The method of any one of claims 65-77, wherein the target cells are cancer cells.
79. The method of any one of claims 65 and 66-78, wherein the step of determining comprises determining whether, when assessed 48 hours after the contacting, the cells express an anti-CLDN18-2 antibody agent encoded by the at least one single-stranded RNA.
80. The method of any one of claims 65 and 66-79, wherein the step of determining comprises determining one or more of the following features:
- whether the antibody agent expressed by the cells binds preferentially to a CLDN-18.2 polypeptide relative to a CLDN18.1 polypeptide;
 - whether the antibody agent expressed by the cells exhibit comparable target specificity to CLDN-18.2 as observed in a flow cytometric binding assay with a reference CLDN-18.2-targeting monoclonal antibody;
 - whether, when assessed 48 hours after incubating immune effector cells (*e.g.*, PBMC cells) and CLDN-18.2 positive cells or CLDN-18.2 negative control cells in the presence of the antibody agent, the CLDN-18.2 positive cells, not the control cells, were lysed;
 - whether the antibody agent expressed by the cells exhibit at least comparable ADCC profile of targeted CLDN-18.2 positive cells as observed with a reference CLDN-18.2-targeting monoclonal antibody in the same concentration; and
 - whether, when assessed 2 hours after incubating CLDN-18.2 positive cells or CLDN-18.2 negative control cells with human serum in the presence of the antibody agent, the CLDN-18.2 positive cells, not the control cells, were lysed.
81. The method of any one of claims 66-80, wherein the cells are present in a subject (*e.g.*, a mouse or monkey subject).
82. The method of claim 81, wherein the one or more features include antibody level in one or more tissues in the subject.
83. The method of any one of claims 66-82, further comprising:
- administering the pharmaceutical composition to a group of animal subjects each bearing a human CLDN-18.2 positive xenograft tumor to determine anti-tumor activity if the pharmaceutical composition is characterized as CLDN-18.2-targeting.

84. A method of manufacture, the method comprising steps of:

(A) determining one or more features of a single stranded RNA (ssRNA) or composition thereof, which ssRNA encodes part or all of an antibody agent, which one or more features are selected from the group consisting of:

- (i) length and/or sequence of the ssRNA;
- (ii) integrity of the ssRNA;
- (iii) presence and/or location of one or more chemical moieties of the ssRNA;
- (iv) extent of expression of the antibody agent when the ssRNA is introduced into a cell;
- (v) stability of the ssRNA or composition thereof;
- (vi) level of antibody agent in a biological sample from an organism into which the ssRNA has been introduced;
- (vii) binding specificity of the antibody agent expressed from the ssRNA, optionally to CLDN-18.2 and optionally relative to CLDN18.1;
- (viii) efficacy of the antibody agent to mediate target cell death through ADCC;
- (ix) efficacy of the antibody agent to mediate target cell death through complement dependent cytotoxicity (CDC);
- (x) lipid identity and amount/concentration within the composition;
- (xi) size of lipid nanoparticles within the composition;
- (xii) polydispersity of lipid nanoparticles within the composition;
- (xiii) amount/concentration of the ssRNA within the composition;
- (xiv) extent of encapsulation of the ssRNA within lipid nanoparticles; and
- (xv) combinations thereof;

(B) comparing the one or more features of the ssRNA or composition thereof with that of an appropriate reference standard; and

(C) (i) designating the ssRNA or composition thereof for one or more further steps of manufacturing and/or distribution if the comparison demonstrates that the ssRNA or composition thereof meets or exceeds the reference standard; or

(ii) taking an alternative action if the comparison demonstrates that the ssRNA or composition thereof does not meet or exceed the reference standard.

85. The method of claim 84, wherein the ssRNA is assessed and the one or more further steps of step (C)(i) are or comprise at least formulation of the ssRNA.
86. The method of claim 84 or 85, wherein the composition is assessed and the composition comprises lipid nanoparticles and the one or more further steps of step (C)(i) are or comprise include release and distribution of the composition.
87. The method of claim 85, further comprising administering the formulation to a group of animal subjects each bearing a human CLDN-18.2 positive xenograft tumor to determine anti-tumor activity.
88. A method of determining a dosing regimen of a pharmaceutical composition targeting CLDN-18.2-targeting, the method comprising steps of:
- (A) administering a pharmaceutical composition set forth in any one of claims 1-35 to a group of animal subjects each bearing a human CLDN-18.2 positive xenograft tumor under a pre-determined dosing regimen;
 - (B) measuring tumor size of the animal subjects periodically;
 - (C) (i) increasing the dose and/or dosage frequency if reduction in tumor size after the administration of the pharmaceutical composition is not therapeutically relevant; or
 - (ii) decreasing the dose and/or dosage frequency if reduction in tumor size after the administration of the pharmaceutical composition is therapeutically relevant, and toxicity effect is shown in at least 30% of the animal subjects; or
 - (iii) making no changes to the dosage regimen if reduction in tumor size after the administration of the pharmaceutical composition is therapeutically relevant, and no toxicity effect is shown in the animal subjects.