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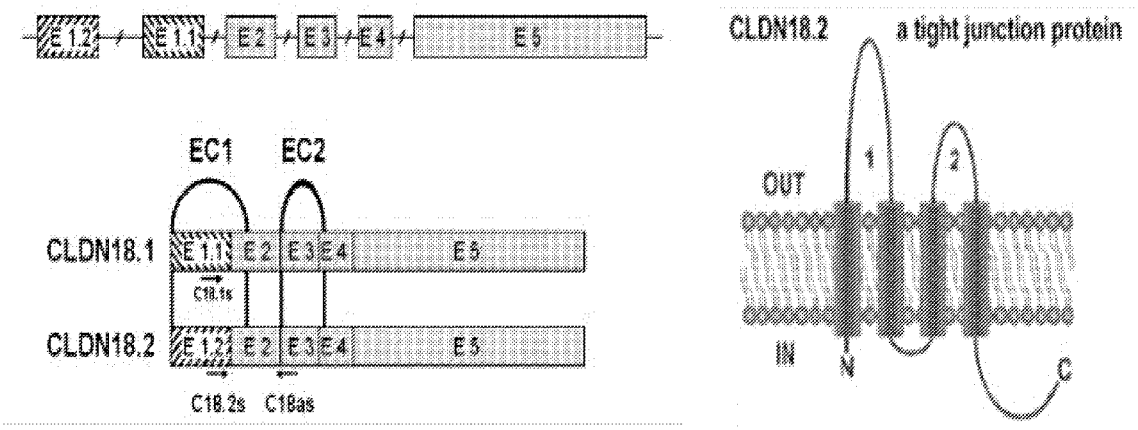

Figure 1

(57) Abstract: The present disclosure relates generally to immunoglobulin-related compositions (e.g., antibodies or antigen binding fragments thereof) that can bind to the Claudin 18.2 protein. The antibodies of the present technology are useful in methods for detecting and treating a Claudin 18.2-associated cancer in a subject in need thereof.

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ANTI-CLAUDIN 18.2 ANTIBODIES AND USES THEREOF

CROSS-REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to US Provisional Appl. No. 63/061,895, filed August 6, 2020, US Provisional Appl. No. 63/074,582, filed September 4, 2020, and US Provisional Appl. No. 63/144,657, filed February 2, 2021, the disclosures of which are incorporated by reference herein in their entireties.

TECHNICAL FIELD

[0002] The present technology relates generally to the preparation of immunoglobulin-related compositions (*e.g.*, antibodies or antigen binding fragments thereof) that specifically bind Claudin 18.2 protein and uses of the same. In particular, the present technology relates to the preparation of Claudin 18.2 binding antibodies and their use in detecting and treating cancer.

BACKGROUND

[0003] The following description of the background of the present technology is provided simply as an aid in understanding the present technology and is not admitted to describe or constitute prior art to the present technology.

[0004] Claudins are integral membrane proteins that form tight junctions. Tight junctions serve as a physical barrier to prevent solutes and water from passing freely through the intercellular space between epithelial or endothelial cell sheets (Markov, A.G., *et al.*, *IUBMB Life* 67: 29–35 (2015); Furuse, M., *et al.*, *J Cell Biol* 141: 1539–1550 (1998); Nitta, T., *et al.*, *J Cell Biol* 161: 653–660 (2003); Deli, M.A., *Biochim Biophys Acta* 1788: 892–910 (2009)). Additionally, tight junctions also play critical roles in maintaining cell polarity and signal transduction. Disruption of the cellular polarity of the epithelium is an early event in malignant transformation (Martin, T.A. And Jiang, W.G., *Biochim Biophys Acta* 1788: 872–891(2009)). Claudin 18.2 is abundant in a significant proportion of primary gastric cancers and its metastases, and plays an important role in their malignant transformation. For example, frequent ectopic activation of claudin 18.2 was found in pancreatic, esophageal, ovarian, and lung tumors (Niimi et al., (2001) *Mol Cell Biol* 21(21): 7380-7390; Tanaka et al. (2011) J *Histochem Cytochem* 59(10): 942-952; Micke et al., (2014) *Int J Cancer* 135(9): 2206-2214; Shimobaba et al. (2016) *Biochim Biophys*

Acta 1863(6 Pt A): 1170-1178; Singh et al., (2017) *J Hematol Oncol* 10(1): 105; Tokumitsu et al., (2017) *Cytopathology* 28(2): 116-121).

[0005] Accordingly, there is an urgent need for novel anti-Claudin 18.2 immunoglobulin-related compositions that are effective in treating Claudin 18.2-associated malignancies.

SUMMARY OF THE PRESENT TECHNOLOGY

[0006] In one aspect, the present disclosure provides an antibody or an antigen binding fragment thereof, comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein (a) the V_H comprises a V_H-CDR1 sequence selected from the group consisting of SEQ ID NOs: 6, 12, 18, 24 and 30, a V_H-CDR2 sequence selected from the group consisting of SEQ ID NOs: 7, 13, 19, 25, and 31, and a V_H-CDR3 sequence selected from the group consisting of SEQ ID NOs: 8, 14, 20, 26, and 32, and/or (b) the V_L comprises a V_L-CDR1 sequence selected from the group consisting of SEQ ID NOs: 9, 15, 21, 27, and 33, a V_L-CDR2 sequence selected from the group consisting of SEQ ID NOs: 10, 16, 22, 28, 34, 157, and 158, and a V_L-CDR3 sequence selected from the group consisting of SEQ ID NOs: 11, 17, 23, 29, and 35.

[0007] In one aspect, the present disclosure provides an antibody or an antigen binding fragment thereof, comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein (a) the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 6, a V_H-CDR2 sequence of SEQ ID NO: 7, and a V_H-CDR3 sequence of SEQ ID NO: 8, and/or the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 9, a V_L-CDR2 sequence of SEQ ID NO: 10 or SEQ ID NO: 157, and a V_L-CDR3 sequence of SEQ ID NO: 11; (b) the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 12, a V_H-CDR2 sequence of SEQ ID NO: 13, and a V_H-CDR3 sequence of SEQ ID NO: 14, and/or the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 15, a V_L-CDR2 sequence of SEQ ID NO: 16 or SEQ ID NO: 158, and a V_L-CDR3 sequence of SEQ ID NO: 17; (c) the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 18, a V_H-CDR2 sequence of SEQ ID NO: 19, and a V_H-CDR3 sequence of SEQ ID NO: 20, and/or the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 21, a V_L-CDR2 sequence of SEQ ID NO: 22, and a V_L-CDR3 sequence of SEQ ID NO: 23; (d) the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 24, a V_H-CDR2 sequence of SEQ ID NO: 25, and a V_H-CDR3 sequence of SEQ ID NO: 26, and/or the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 27, a V_L-CDR2 sequence of SEQ ID NO: 28, and a V_L-CDR3 sequence of SEQ ID NO: 29; or (e) the V_H comprises a

V_H-CDR1 sequence of SEQ ID NO: 30, a V_H-CDR2 sequence of SEQ ID NO: 31, and a V_H-CDR3 sequence of SEQ ID NO: 32, and/or the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 33, a V_L-CDR2 sequence of SEQ ID NO: 34, and a V_L-CDR3 sequence of SEQ ID NO: 35. In some embodiments, the V_H comprises an amino acid sequence selected from any one of SEQ ID NOs: 36, 38, 40, 42, 44, 46-49, or 54-57; and/or (b) the V_L comprises an amino acid sequence selected from any one of SEQ ID NOs: 37, 39, 41, 43, 45, 50-53, or 58-61. Additionally or alternatively, in some embodiments, the antigen binding fragment may be selected from the group consisting of Fab, $F(ab')_2$, Fab', scF_v, and F_v.

[0008] Additionally or alternatively, in some embodiments, the antibody or antigen binding fragment further comprises a Fc domain of an isotype selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM, IgD, and IgE. In certain embodiments, the antibody or antigen binding fragment comprises an IgG1 constant region comprising one or more amino acid substitutions selected from the group consisting of N297A, K322A, L234A and L235A. In other embodiments, the antibody or antigen binding fragment comprises an IgG4 constant region comprising a S228P mutation.

[0009] In another aspect, the present disclosure provides an antibody comprising (a) a light chain immunoglobulin variable domain sequence that is at least 95% identical to the light chain immunoglobulin variable domain sequence of any one of SEQ ID NOs: 37, 39, 41, 43, 45, 50-53, or 58-61; and/or (b) a heavy chain immunoglobulin variable domain sequence that is at least 95% identical to the heavy chain immunoglobulin variable domain sequence of any one of SEQ ID NOs: 36, 38, 40, 42, 44, 46-49, or 54-57.

[0010] In one aspect, the present disclosure provides an antibody comprising a heavy chain (HC) amino acid sequence comprising SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, or a variant thereof having one or more conservative amino acid substitutions, and/or a light chain (LC) amino acid sequence comprising SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, or a variant thereof having one or more conservative amino acid substitutions. In some embodiments, the antibody comprises a HC amino acid sequence and a LC amino acid sequence selected from the group consisting of: SEQ ID NO: 62 and SEQ ID NO: 63, SEQ ID NO: 64 and SEQ ID NO: 65, SEQ ID NO: 66 and SEQ ID NO: 67, and SEQ ID NO: 68 and SEQ ID NO: 69, respectively. In another aspect, the present disclosure provides an antibody comprising: (a) a LC sequence that is at

least 95% identical to the LC sequence present in SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, or SEQ ID NO: 69; and/or (b) a HC sequence that is at least 95% identical to the HC sequence present in SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, or SEQ ID NO: 68. In certain embodiments, the immunoglobulin-related compositions contain an IgG1 constant region comprising one or more amino acid substitutions selected from the group consisting of N297A, K322A, L234A and L235A. Additionally or alternatively, in some embodiments, the immunoglobulin-related compositions contain an IgG4 constant region comprising a S228P mutation. In certain embodiments, the antibody or antigen binding fragment comprises an IgG1 constant region comprising one or more amino acid substitutions selected from the group consisting of N297A, K322A, L234A and L235A.

[0011] In any and all embodiments of the antibody or antigen binding fragment disclosed herein, the antibody or antigen binding fragment binds to a CLDN18.2 polypeptide comprising an extracellular loop 1 (EL1) sequence. The extracellular loop 1 (EL1) sequence may comprise the amino acid sequence of SEQ ID NO: 2, or the CLDN18.2 polypeptide may comprise the amino acid sequence of SEQ ID NO: 4. Additionally or alternatively, in some embodiments, the antibody of the present technology is a monoclonal antibody, a chimeric antibody, or a humanized antibody, and/or lacks α-1,6-fucose modifications.

[0012] In one aspect, the present disclosure provides a recombinant nucleic acid sequence encoding any of the antibodies or antigen binding fragments described herein. In another aspect, the present disclosure provides a host cell or vector comprising any of the recombinant nucleic acid sequences disclosed herein.

[0013] In yet another aspect, the present disclosure provides a pharmaceutical composition comprising any of the antibodies or antigen binding fragments described herein and a pharmaceutically-acceptable carrier. In some embodiments, the pharmaceutical composition further comprises an agent selected from the group consisting of isotopes, dyes, chromagens, contrast agents, drugs, toxins, cytokines, enzymes, enzyme inhibitors, hormones, hormone antagonists, growth factors, radionuclides, metals, liposomes, nanoparticles, RNA, DNA or any combination thereof. In another aspect, the present disclosure provides a composition comprising any of the antibodies or antigen binding fragments of the present technology and a pharmaceutically-acceptable carrier, wherein the antibody or antigen binding fragment is optionally conjugated to an agent selected from the

group consisting of isotopes, dyes, chromagens, contrast agents, drugs, toxins, cytokines, enzymes, enzyme inhibitors, hormones, hormone antagonists, growth factors, radionuclides, metals, liposomes, nanoparticles, RNA, DNA or any combination thereof.

[0014] In one aspect, the present disclosure provides a method for treating cancer in a subject in need thereof, comprising administering to the subject an effective amount of any of the antibodies or antigen binding fragments described herein, or any of the pharmaceutical compositions disclosed herein, wherein the antibody or antigen binding fragment specifically binds to CLDN18.2. In some embodiments, the cancer is a solid tumor. Examples of cancer include, but are not limited to, gastric cancer, esophageal cancer, pancreatic cancer, lung cancer, non small cell lung cancer (NSCLC), ovarian cancer, colon cancer, hepatic cancer, head-neck cancer, and gallbladder cancer. In some embodiments of the method, the antibody or antigen binding fragment is administered to the subject separately, sequentially or simultaneously with an additional therapeutic agent. Examples of additional therapeutic agents include one or more of alkylating agents, platinum agents, taxanes, vinca agents, anti-estrogen drugs, aromatase inhibitors, ovarian suppression agents, VEGF/VEGFR inhibitors, EGF/EGFR inhibitors, PARP inhibitors, cytostatic alkaloids, cytotoxic antibiotics, antimetabolites, endocrine/hormonal agents, bisphosphonate therapy agents, and immuno-modulating/stimulating antibodies (*e.g.*, an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-CTLA-4 antibody, an anti-TIM3 antibody, an anti-4-1BB antibody, an anti-CD73 antibody, an anti-GITR antibody, or an anti-LAG-3 antibody).

[0015] In another aspect, the present disclosure provides a method for detecting cancer in a subject *in vivo* comprising (a) administering to the subject an effective amount of an antibody or antigen binding fragment of the present technology, wherein the antibody or antigen binding fragment is configured to localize to a cancer cell expressing CLDN18.2 and is labeled with a radioisotope; and (b) detecting the presence of a tumor in the subject by detecting radioactive levels emitted by the antibody or antigen binding fragment that are higher than a reference value. In certain embodiments, the cancer is a solid tumor. In some embodiments, the subject is diagnosed with or is suspected of having cancer (*e.g.*, gastric cancer, esophageal cancer, pancreatic cancer, lung cancer, non small cell lung cancer (NSCLC), ovarian cancer, colon cancer, hepatic cancer, head-neck cancer, and gallbladder cancer). Radioactive levels emitted by the antibody or antigen binding fragment may be detected using positron emission tomography or single photon emission computed

tomography. Additionally or alternatively, in some embodiments, the method further comprises administering to the subject an effective amount of an immunoconjugate comprising an antibody or antigen binding fragment of the present technology conjugated to a radionuclide. In some embodiments, the radionuclide is an alpha particle-emitting isotope, a beta particle-emitting isotope, an Auger-emitter, or any combination thereof. Examples of beta particle-emitting isotopes include ^{86}Y, ^{90}Y, ^{89}Sr, ^{165}Dy, ^{186}Re, ^{188}Re, ^{177}Lu, and ^{67}Cu.

[0016] In any and all embodiments of the methods disclosed herein, the subject is human.

[0017] In yet another aspect, the present disclosure provides a method for detecting CLDN18.2 protein expression levels in a biological sample comprising contacting the biological sample with any of the antibodies or antigen binding fragments disclosed herein, and detecting binding to CLDN18.2 protein in the biological sample.

[0018] Also disclosed herein are kits for the detection and/or treatment of CLDN18.2-associated cancers comprising at least one immunoglobulin-related composition of the present technology (*e.g.*, any antibody or antigen binding fragment described herein), or a functional variant (*e.g.*, substitutional variant) thereof and instructions for use. In certain embodiments, the immunoglobulin-related composition is coupled to one or more detectable labels. In one embodiment, the one or more detectable labels comprise a radioactive label, a fluorescent label, or a chromogenic label. Additionally or alternatively, in some embodiments, the kit further comprises a secondary antibody that specifically binds to an anti- CLDN18.2 immunoglobulin-related composition described herein. In some embodiments, the secondary antibody is coupled to at least one detectable label selected from the group consisting of a radioactive label, a fluorescent label, or a chromogenic label.

[0019] In one aspect, the present disclosure provides a chimeric antigen receptor (CAR) comprising any and all embodiments of the antibody or antigen-binding fragment of the present technology. In another aspect, the present disclosure provides an engineered immune cell comprising the CAR disclosed herein, optionally wherein the engineered immune cell is a B cell, a T cell, or a natural killer (NK) cell. In yet another aspect, the present disclosure provides a method for treating cancer in a subject in need thereof, comprising administering to the subject an effective amount of any and all embodiments of the engineered immune cell described herein. Examples of cancer include, but are not limited to solid tumors, gastric cancer, esophageal cancer, pancreatic cancer, lung cancer,

non small cell lung cancer (NSCLC), ovarian cancer, colon cancer, hepatic cancer, head-neck cancer, and gallbladder cancer.

BRIEF DESCRIPTION OF THE DRAWINGS

[0020] Figure 1 shows splicing variants and the schematic protein structure of Claudin 18.2 (adapted from Markov, A.G. *et al.*, *IUBMB Life* 67: 29–35 (2015)).

[0021] Figure 2 shows an amino acid sequence alignment of hCLDN18.1-EL1 (SEQ ID NO: 1), hCLDN18.2-EL1 (SEQ ID NO: 2) and mCLDN18.2-EL1 (SEQ ID NO: 2), and an amino acid sequence alignment of hCLDN18.1-EL2 (SEQ ID NO: 3) and hCLDN18.2-EL2 (SEQ ID NO: 3). The amino acid sequences of hCLDN18.2-EL1 and mCLDN18.2-EL1 are identical. The amino acid sequences of hCLDN18.1-EL2 and hCLDN18.2-EL2 are identical.

[0022] Figure 3 shows RNA and protein expression of CLDN18 in normal human tissues (adapted from Human Protein Atlas data: www.proteinatlas.org/ENSG00000066405-CLDN18/tissue).

[0023] Figure 4 shows expression of CLDN18 in human cancer tissues (adapted from Sahin U., *et al.*, *Clin Cancer Res* 14:7624–7634 (2008)).

[0024] Figure 5 shows cell lines (*e.g.*, CHO, 3T3 and HEK293) that stably express human CLDN18.2 (analyzed using the benchmark antibody IMAB362, produced according to sequences from imgt.org/3Dstructure-DB/cgi/details.cgi?pdbcode=10473).

[0025] Figure 6 shows Virus-like-particles (VLPs) that express human CLDN18.2 EL1 (analyzed using the benchmark antibody IMAB362, produced according to sequences from imgt.org/3Dstructure-DB/cgi/details.cgi?pdbcode=10473). More than 90% of the purified VLPs (Right) were expressing hCLDN18.2 EL1 compared to control (Left).

[0026] Figure 7 shows binding of 5 select clones (32G4, 47D10, 29G4, 31A6 and 15B10) that exhibited specific binding to human CLDN18.2 as determined by FACS analysis. Top panel: Binding of mouse chimeric antibody clones to CLDN18.1 expressed at the cell surface. Bottom panel: Binding of mouse chimeric antibody clones to CLDN18.2 expressed at the cell surface.

[0027] Figure 8 shows the binding affinity of the murine anti-CLDN18.2 chimeric antibody clones 32G4, 47D10, 29G4, 31A6 and 15B10.

[0028] Figure 9A shows the binding affinity of exemplary humanized 32G4 antibody variants compared to the mouse 32G4 chimeric control antibodies.

[0029] Figure 9B shows the binding affinity of exemplary humanized 47D10 antibody variants compared to the mouse 47D10 chimeric control antibodies.

[0030] Figure 10 shows the amino acid sequence of human CLDN18.2 protein (SEQ ID NO: 4).

[0031] Figure 11 shows the amino acid sequence of human CLDN18.1 protein (SEQ ID NO: 5).

[0032] Figure 12 shows the V_H CDR1, V_H CDR2, V_H CDR3, V_L CDR1, V_L CDR2, and V_L CDR3 sequences of murine clones 32G4 (SEQ ID NOs: 6-11, respectively), 47D10 (SEQ ID NOs: 12-17, respectively), 29G4 (SEQ ID NOs: 18-23, respectively), 31A6 (SEQ ID NOs: 24-29, respectively), and 15B10 (SEQ ID NOs: 30-35, respectively). SEQ ID NO: 157 corresponds to the 32G4-huVL4 CDR2 sequence and SEQ ID NO: 158 corresponds to the 47D10-huVL4 CDR2 sequence.

[0033] Figure 13 shows the amino acid sequences of the variable heavy immunoglobulin domain (V_H) and the variable light immunoglobulin domain (V_L) of murine clones 32G4 (SEQ ID NO: 36 and SEQ ID NO: 37, respectively), 47D10 (SEQ ID NO: 38 and SEQ ID NO: 39, respectively), 29G4 (SEQ ID NO: 40 and SEQ ID NO: 41, respectively), 31A6 (SEQ ID NO: 42 and SEQ ID NO: 43, respectively) and 15B10 (SEQ ID NO: 44 and SEQ ID NO: 45, respectively). The V_H CDR 1-3 and V_L CDR 1-3 amino acid sequences are underlined.

[0034] Figure 14 shows the amino acid sequences of four humanized V_H variants (SEQ ID NOs: 46-49) and four humanized V_L variants (SEQ ID NOs: 50-53) for clone 32G4. The V_H CDR 1-3 and V_L CDR 1-3 amino acid sequences are underlined.

[0035] Figure 15 shows the amino acid sequences of four humanized V_H variants (SEQ ID NOs: 54-57) and four humanized V_L variants (SEQ ID NOs: 58-61) for clone 47D10. The V_H CDR 1-3 and V_L CDR 1-3 amino acid sequences are underlined.

[0036] Figure 16 shows the heavy chain (HC) and light chain (LC) amino acid sequences of 32G4-huIgG1-V8 (SEQ ID NO: 62 and SEQ ID NO: 63) and 32G4-huIgG1-V9 (SEQ ID NO: 64 and SEQ ID NO: 65). The V_H CDR 1-3 and V_L CDR 1-3 amino acid sequences are underlined, and the V_H and V_L amino acid sequences are italicized.

[0037] Figure 17 shows the heavy chain (HC) and light chain (LC) amino acid sequences of 47D10-huIgG1-V6 (SEQ ID NO: 66 and SEQ ID NO: 67) and 47D10-huIgG1-V7 (SEQ ID NO: 68 and SEQ ID NO: 69). The V_H CDR 1-3 and V_L CDR 1-3 amino acid sequences are underlined, and the V_H and V_L amino acid sequences are italicized.

[0038] Figure 18 shows the exemplary antibody-dependent cellular cytotoxicity (ADCC) assay data of 32G4 and 47D10 clones, in comparison to the IMAB362 benchmark antibody and the negative isotype control.

[0039] Figure 19 shows the cross species binding of exemplary humanized 32G4 and 47D10 antibody variants to cynomolgus monkey and mouse claudin 18.2 target protein on cell surface relative to the IMAB362 benchmark antibody and the negative isotype control.

DETAILED DESCRIPTION

[0040] It is to be appreciated that certain aspects, modes, embodiments, variations and features of the present methods are described below in various levels of detail in order to provide a substantial understanding of the present technology.

[0041] The present disclosure generally provides immunoglobulin-related compositions (*e.g.*, antibodies or antigen binding fragments thereof), which can specifically bind to Claudin 18.2 polypeptides. The immunoglobulin-related compositions of the present technology are useful in methods for detecting or treating Claudin 18.2-associated cancers in a subject in need thereof. Accordingly, the various aspects of the present methods relate to the preparation, characterization, and manipulation of anti-Claudin 18.2 antibodies. The immunoglobulin-related compositions of the present technology are useful alone or in combination with additional therapeutic agents for treating cancer. In some embodiments, the immunoglobulin-related composition is a monoclonal antibody, a humanized antibody, or a chimeric antibody.

[0042] In practicing the present methods, many conventional techniques in molecular biology, protein biochemistry, cell biology, immunology, microbiology and recombinant DNA are used. *See, e.g.*, Sambrook and Russell eds. (2001) *Molecular Cloning: A Laboratory Manual*, 3rd edition; the series Ausubel *et al.* eds. (2007) *Current Protocols in Molecular Biology*; the series *Methods in Enzymology* (Academic Press, Inc., N.Y.); MacPherson *et al.* (1991) *PCR 1: A Practical Approach* (IRL Press at Oxford University Press); MacPherson *et al.* (1995) *PCR 2: A Practical Approach*; Harlow and Lane eds.

(1999) *Antibodies, A Laboratory Manual*; Freshney (2005) *Culture of Animal Cells: A Manual of Basic Technique*, 5th edition; Gait ed. (1984) *Oligonucleotide Synthesis*; U.S. Patent No. 4,683,195; Hames and Higgins eds. (1984) *Nucleic Acid Hybridization*; Anderson (1999) *Nucleic Acid Hybridization*; Hames and Higgins eds. (1984) *Transcription and Translation; Immobilized Cells and Enzymes* (IRL Press (1986)); Perbal (1984) *A Practical Guide to Molecular Cloning*; Miller and Calos eds. (1987) *Gene Transfer Vectors for Mammalian Cells* (Cold Spring Harbor Laboratory); Makrides ed. (2003) *Gene Transfer and Expression in Mammalian Cells*; Mayer and Walker eds. (1987) *Immunochemical Methods in Cell and Molecular Biology* (Academic Press, London); and Herzenberg *et al.* eds (1996) *Weir's Handbook of Experimental Immunology*. Methods to detect and measure levels of polypeptide gene expression products (*i.e.*, gene translation level) are well-known in the art and include the use of polypeptide detection methods such as antibody detection and quantification techniques. (*See also*, Strachan & Read, *Human Molecular Genetics*, Second Edition. (John Wiley and Sons, Inc., NY, 1999)).

Definitions

[0043] Unless defined otherwise, all technical and scientific terms used herein generally have the same meaning as commonly understood by one of ordinary skill in the art to which this technology belongs. As used in this specification and the appended claims, the singular forms "a", "an" and "the" include plural referents unless the content clearly dictates otherwise. For example, reference to "a cell" includes a combination of two or more cells, and the like. Generally, the nomenclature used herein and the laboratory procedures in cell culture, molecular genetics, organic chemistry, analytical chemistry and nucleic acid chemistry and hybridization described below are those well-known and commonly employed in the art.

[0044] As used herein, the term "about" in reference to a number is generally taken to include numbers that fall within a range of 1%, 5%, or 10% in either direction (greater than or less than) of the number unless otherwise stated or otherwise evident from the context (except where such number would be less than 0% or exceed 100% of a possible value).

[0045] As used herein, the "administration" of an agent or drug to a subject includes any route of introducing or delivering to a subject a compound to perform its intended function. Administration can be carried out by any suitable route, including but not limited to, orally, intranasally, parenterally (intravenously, intramuscularly, intraperitoneally, or

subcutaneously), rectally, intrathecally, intratumorally or topically. Administration includes self-administration and the administration by another.

[0046] An "adjuvant" refers to one or more substances that cause stimulation of the immune system. In this context, an adjuvant is used to enhance an immune response to one or more vaccine antigens or antibodies. An adjuvant may be administered to a subject before, in combination with, or after administration of the vaccine. Examples of chemical compounds used as adjuvants include aluminum compounds, oils, block polymers, immune stimulating complexes, vitamins and minerals (*e.g.*, vitamin E, vitamin A, selenium, and vitamin B12), Quil A (saponins), bacterial and fungal cell wall components (*e.g.*, lipopolysaccarides, lipoproteins, and glycoproteins), hormones, cytokines, and co-stimulatory factors.

[0047] As used herein, the term "antibody" collectively refers to immunoglobulins or immunoglobulin-like molecules including by way of example and without limitation, IgA, IgD, IgE, IgG and IgM, combinations thereof, and similar molecules produced during an immune response in any vertebrate, for example, in mammals such as humans, goats, rabbits and mice, as well as non-mammalian species, such as shark immunoglobulins. As used herein, "antibodies" (includes intact immunoglobulins) and "antigen binding fragments" specifically bind to a molecule of interest (or a group of highly similar molecules of interest) to the substantial exclusion of binding to other molecules (for example, antibodies and antibody fragments that have a binding constant for the molecule of interest that is at least 10^3 M^{-1} greater, at least 10^4 M^{-1} greater or at least 10^5 M^{-1} greater than a binding constant for other molecules in a biological sample). The term "antibody" also includes genetically engineered forms such as chimeric antibodies (for example, humanized murine antibodies), heteroconjugate antibodies (such as, bispecific antibodies). See also, Pierce Catalog and Handbook, 1994-1995 (Pierce Chemical Co., Rockford, Ill.); Kuby, J., *Immunology*, 3rd Ed., W.H. Freeman & Co., New York, 1997.

[0048] More particularly, antibody refers to a polypeptide ligand comprising at least a light chain immunoglobulin variable region or heavy chain immunoglobulin variable region which specifically recognizes and binds an epitope of an antigen. Antibodies are composed of a heavy and a light chain, each of which has a variable region, termed the variable heavy (V_H) region and the variable light (V_L) region. Together, the V_H region and the V_L region are responsible for binding the antigen recognized by the antibody. Typically, an

immunoglobulin has heavy (H) chains and light (L) chains interconnected by disulfide bonds. There are two types of light chain, lambda (λ) and kappa (κ). There are five main heavy chain classes (or isotypes) which determine the functional activity of an antibody molecule: IgM, IgD, IgG, IgA and IgE. Each heavy and light chain contains a constant region and a variable region, (the regions are also known as "domains"). In combination, the heavy and the light chain variable regions specifically bind the antigen. Light and heavy chain variable regions contain a "framework" region interrupted by three hypervariable regions, also called "complementarity-determining regions" or "CDRs". The extent of the framework region and CDRs have been defined (see, Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, U.S. Department of Health and Human Services, 1991, which is hereby incorporated by reference). The Kabat database is now maintained online. The sequences of the framework regions of different light or heavy chains are relatively conserved within a species. The framework region of an antibody, that is the combined framework regions of the constituent light and heavy chains, largely adopt a β-sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β-sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions.

[0049] The CDRs are primarily responsible for binding to an epitope of an antigen. The CDRs of each chain are typically referred to as CDR1, CDR2, and CDR3, numbered sequentially starting from the N-terminus, and are also typically identified by the chain in which the particular CDR is located. Thus, a V_H CDR3 is located in the variable domain of the heavy chain of the antibody in which it is found, whereas a V_L CDR1 is the CDR1 from the variable domain of the light chain of the antibody in which it is found. An antibody that binds Claudin 18.2 protein will have a specific V_H region and the V_L region sequence, and thus specific CDR sequences. Antibodies with different specificities (*i.e.* different combining sites for different antigens) have different CDRs. Although it is the CDRs that vary from antibody to antibody, only a limited number of amino acid positions within the CDRs are directly involved in antigen binding. These positions within the CDRs are called specificity determining residues (SDRs). "Immunoglobulin-related compositions" as used herein, refers to antibodies (including monoclonal antibodies, polyclonal antibodies, humanized antibodies, chimeric antibodies, recombinant antibodies, multi-specific antibodies, bispecific antibodies, *etc.*,) as well as antibody fragments. An antibody or antigen binding fragment thereof specifically binds to an antigen.

[0050] As used herein, the term "antibody-related polypeptide" means antigen-binding antibody fragments, including single-chain antibodies, that can comprise the variable region(s) alone, or in combination, with all or part of the following polypeptide elements: hinge region, CH_1, CH_2, and CH_3 domains of an antibody molecule. Also included in the technology are any combinations of variable region(s) and hinge region, CH_1, CH_2, and CH_3 domains. Antibody-related molecules useful in the present methods, *e.g.*, but are not limited to, Fab, Fab′ and $F(ab')_2$, Fd, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv) and fragments comprising either a V_L or V_H domain. Examples include: (i) a Fab fragment, a monovalent fragment consisting of the V_L, V_H, C_L and CH_1 domains; (ii) a $F(ab')_2$ fragment, a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) a Fd fragment consisting of the V_H and CH_1 domains; (iv) a Fv fragment consisting of the V_L and V_H domains of a single arm of an antibody, (v) a dAb fragment (Ward *et al.*, *Nature* 341: 544-546, 1989), which consists of a V_H domain; and (vi) an isolated complementarity determining region (CDR). As such "antibody fragments" or "antigen binding fragments" can comprise a portion of a full length antibody, generally the antigen binding or variable region thereof. Examples of antibody fragments or antigen binding fragments include Fab, Fab', $F(ab')_2$, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multi-specific antibodies formed from antibody fragments.

[0051] As used herein, the term "antibody-dependent cell-mediated cytotoxicity" or "ADCC", refers to a mechanism of cell-mediated immune defense whereby an effector cell of the immune system actively lyses a target cell, such as a tumor cell, whose membrane-surface antigens have been bound by antibodies, such as anti-CLDN18.2 antibodies.

[0052] As used herein, an "antigen" refers to a molecule to which an antibody (or antigen binding fragment thereof) can selectively bind. The target antigen may be a protein, carbohydrate, nucleic acid, lipid, hapten, or other naturally occurring or synthetic compound. In some embodiments, the target antigen may be a polypeptide (*e.g.*, a CLDN18.2 polypeptide). An antigen may also be administered to an animal to generate an immune response in the animal.

[0053] The term "antigen binding fragment" refers to a fragment of the whole immunoglobulin structure which possesses a part of a polypeptide responsible for binding to antigen. Examples of the antigen binding fragment useful in the present technology include

scFv, $(scFv)_2$, scFvFc, Fab, Fab′ and $F(ab')_2$, but are not limited thereto. Any of the above-noted antibody fragments are obtained using conventional techniques known to those of skill in the art, and the fragments are screened for binding specificity and neutralization activity in the same manner as are intact antibodies.

[0054] As used herein, "binding affinity" means the strength of the total noncovalent interactions between a single binding site of a molecule (*e.g.*, an antibody) and its binding partner (*e.g.*, an antigen or antigenic peptide). The affinity of a molecule X for its partner Y can generally be represented by the dissociation constant (K_D). Affinity can be measured by standard methods known in the art, including those described herein. A low-affinity complex contains an antibody that generally tends to dissociate readily from the antigen, whereas a high-affinity complex contains an antibody that generally tends to remain bound to the antigen for a longer duration.

[0055] As used herein, the term "biological sample" means sample material derived from living cells. Biological samples may include tissues, cells, protein or membrane extracts of cells, and biological fluids (*e.g.*, ascites fluid or cerebrospinal fluid (CSF)) isolated from a subject, as well as tissues, cells and fluids present within a subject. Biological samples of the present technology include, but are not limited to, samples taken from breast tissue, renal tissue, the uterine cervix, the endometrium, the head or neck, the gallbladder, parotid tissue, the prostate, the brain, the pituitary gland, kidney tissue, muscle, the esophagus, the stomach, the small intestine, the colon, the liver, the spleen, the pancreas, thyroid tissue, heart tissue, lung tissue, the bladder, adipose tissue, lymph node tissue, the uterus, ovarian tissue, adrenal tissue, testis tissue, the tonsils, thymus, blood, hair, buccal, skin, serum, plasma, CSF, semen, prostate fluid, seminal fluid, urine, feces, sweat, saliva, sputum, mucus, bone marrow, lymph, and tears. Biological samples can also be obtained from biopsies of internal organs or from cancers. Biological samples can be obtained from subjects for diagnosis or research or can be obtained from non-diseased individuals, as controls or for basic research. Samples may be obtained by standard methods including, *e.g.*, venous puncture and surgical biopsy. In certain embodiments, the biological sample is a tissue sample obtained by needle biopsy.

[0056] As used herein, the term "CDR grafting" means replacing at least one CDR of an "acceptor" antibody by a CDR "graft" from a "donor" antibody possessing a desirable antigen specificity.

[0057] As used herein, the term "chimeric antibody" means an antibody in which the Fc constant region of a monoclonal antibody from one species (*e.g.*, a mouse Fc constant region) is replaced, using recombinant DNA techniques, with an Fc constant region from an antibody of another species (*e.g.*, a human Fc constant region). *See generally*, Robinson *et al.*, PCT/US86/02269; Akira *et al.*, European Patent Application 184,187; Taniguchi, European Patent Application 171,496; Morrison *et al.*, European Patent Application 173,494; Neuberger *et al.*, WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.*, European Patent Application 0125,023; Better *et al.*, *Science* 240: 1041-1043, 1988; Liu *et al.*, *Proc. Natl. Acad. Sci. USA* 84: 3439-3443, 1987; Liu *et al.*, *J. Immunol* 139: 3521-3526, 1987; Sun *et al.*, *Proc. Natl. Acad. Sci. USA* 84: 214-218, 1987; Nishimura *et al.*, *Cancer Res* 47: 999-1005, 1987; Wood *et al.*, Nature 314: 446-449, 1885; and Shaw *et al.*, *J. Natl. Cancer Inst.* 80: 1553-1559, 1988.

[0058] As used herein, the term "complement-dependent cytotoxicity" or "CDC" generally refers to an effector function of IgG and IgM antibodies, which trigger classical complement pathway when bound to a surface antigen, inducing formation of a membrane attack complex and target cell lysis.

[0059] As used herein, the term "conjugated" refers to the association of two molecules by any method known to those in the art. Suitable types of associations include chemical bonds and physical bonds. Chemical bonds include, for example, covalent bonds and coordinate bonds. Physical bonds include, for instance, hydrogen bonds, dipolar interactions, van der Waal forces, electrostatic interactions, hydrophobic interactions and aromatic stacking.

[0060] As used herein, the term "consensus FR" means a framework (FR) antibody region in a consensus immunoglobulin sequence. The FR regions of an antibody do not contact the antigen.

[0061] As used herein, a "control" is an alternative sample used in an experiment for comparison purpose. A control can be "positive" or "negative." For example, where the purpose of the experiment is to determine a correlation of the efficacy of a therapeutic agent for the treatment for a particular type of disease, a positive control (a compound or composition known to exhibit the desired therapeutic effect) and a negative control (a subject or a sample that does not receive the therapy or receives a placebo) are typically employed.

[0062] As used herein, the term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments comprise a heavy-chain variable domain (V_H) connected to a light-chain variable domain (V_L) in the same polypeptide chain (V_H V_L). By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen binding sites. Diabodies are described more fully in, *e.g.*, EP 404,097; WO 93/11161; and Hollinger *et al.*, *Proc Natl Acad Sci USA*, 90: 6444-6448 (1993).

[0063] As used herein, the term "EC50", known as half maximal effective concentration, refers to the concentration of an antibody which induces a response halfway between the baseline and maximum after a specified exposure time.

[0064] As used herein, the term "effective amount" refers to a quantity sufficient to achieve a desired therapeutic and/or prophylactic effect, *e.g.*, an amount which results in the prevention of, or a decrease in a disease or condition described herein or one or more signs or symptoms associated with a disease or condition described herein. In the context of therapeutic or prophylactic applications, the amount of a composition administered to the subject will vary depending on the composition, the degree, type, and severity of the disease and on the characteristics of the individual, such as general health, age, sex, body weight and tolerance to drugs. The skilled artisan will be able to determine appropriate dosages depending on these and other factors. The compositions can also be administered in combination with one or more additional therapeutic compounds. In the methods described herein, the therapeutic compositions may be administered to a subject having one or more signs or symptoms of a disease or condition described herein. As used herein, a "therapeutically effective amount" of a composition refers to composition levels in which the physiological effects of a disease or condition are ameliorated or eliminated. A therapeutically effective amount can be given in one or more administrations.

[0065] As used herein, the term "effector cell" means an immune cell which is involved in the effector phase of an immune response, as opposed to the cognitive and activation phases of an immune response. Exemplary immune cells include a cell of a myeloid or lymphoid origin, *e.g.*, lymphocytes (*e.g.*, B cells and T cells including cytolytic T cells (CTLs)), killer cells, natural killer cells, macrophages, monocytes, eosinophils, neutrophils, polymorphonuclear cells, granulocytes, mast cells, and basophils. Effector cells express specific Fc receptors and carry out specific immune functions. An effector cell can induce

antibody-dependent cell-mediated cytotoxicity (ADCC), *e.g.*, a neutrophil capable of inducing ADCC. For example, monocytes, macrophages, neutrophils, eosinophils, and lymphocytes which express FcαR are involved in specific killing of target cells and presenting antigens to other components of the immune system, or binding to cells that present antigens.

[0066] As used herein, the term "epitope" means a protein determinant capable of specific binding to an antibody. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents. In some embodiments, an "epitope" of the CLDN18.2 protein is a region of the protein to which the anti-CLDN18.2 antibodies of the present technology specifically bind. In some embodiments, the epitope is a conformational epitope or a non-conformational epitope. To screen for anti-CLDN18.2 antibodies which bind to an epitope, a routine cross-blocking assay such as that described in Antibodies, *A Laboratory Manual*, Cold Spring Harbor Laboratory, Ed Harlow and David Lane (1988), can be performed. This assay can be used to determine if an anti-CLDN18.2 antibody binds the same site or epitope as an anti-CLDN18.2 antibody of the present technology. Alternatively, or additionally, epitope mapping can be performed by methods known in the art. For example, the antibody sequence can be mutagenized such as by alanine scanning, to identify contact residues. In a different method, peptides corresponding to different regions of CLDN18.2 protein can be used in competition assays with the test antibodies or with a test antibody and an antibody with a characterized or known epitope.

[0067] As used herein, "expression" includes one or more of the following: transcription of the gene into precursor mRNA; splicing and other processing of the precursor mRNA to produce mature mRNA; mRNA stability; translation of the mature mRNA into protein (including codon usage and tRNA availability); and glycosylation and/or other modifications of the translation product, if required for proper expression and function.

[0068] As used herein, the term "gene" means a segment of DNA that contains all the information for the regulated biosynthesis of an RNA product, including promoters, exons, introns, and other untranslated regions that control expression.

[0069] As used herein, "homology" or "identity" or "similarity" refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, at least 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 98% or 99%) of "sequence identity" to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences. This alignment and the percent homology or sequence identity can be determined using software programs known in the art. In some embodiments, default parameters are used for alignment. One alignment program is BLAST, using default parameters. In particular, programs are BLASTN and BLASTP, using the following default parameters: Genetic code=standard; filter=none; strand=both; cutoff=60; expect=10; Matrix=BLOSUM62; Descriptions=50 sequences; sort by =HIGH SCORE; Databases=non-redundant, GenBank+EMBL+DDBJ+PDB+GenBank CDS translations+SwissProtein+SPupdate+PIR. Details of these programs can be found at the National Center for Biotechnology Information. Biologically equivalent polynucleotides are those having the specified percent homology and encoding a polypeptide having the same or similar biological activity. Two sequences are deemed "unrelated" or "non-homologous" if they share less than 40% identity, or less than 25% identity, with each other.

[0070] As used herein, "humanized" forms of non-human (*e.g.*, murine) antibodies are chimeric antibodies which contain minimal sequence derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins in which hypervariable region residues of the recipient are replaced by hypervariable region residues from a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. In some embodiments, Fv framework region (FR) residues of the human immunoglobulin are replaced by corresponding non-human residues. Furthermore, humanized antibodies may comprise residues which are not found in the recipient antibody or in the donor antibody. These modifications are made to further refine antibody performance such as binding

affinity. Generally, the humanized antibody will comprise substantially all of at least one, and typically two, variable domains (*e.g.*, Fab, Fab', $F(ab')_2$, or Fv), in which all or substantially all of the hypervariable loops correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus FR sequence although the FR regions may include one or more amino acid substitutions that improve binding affinity. The number of these amino acid substitutions in the FR are typically no more than 6 in the H chain, and in the L chain, no more than 3. The humanized antibody optionally may also comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin. For further details, see Jones *et al.*, *Nature* 321:522-525 (1986); Reichmann *et al.*, *Nature* 332:323-329 (1988); and Presta, *Curr. Op. Struct. Biol.* 2:593-596 (1992). See *e.g.*, Ahmed & Cheung, *FEBS Letters* 588(2):288-297 (2014).

[0071] As used herein, the term "hypervariable region" refers to the amino acid residues of an antibody which are responsible for antigen-binding. The hypervariable region generally comprises amino acid residues from a "complementarity determining region" or "CDR" (*e.g.*, around about residues 24-34 (L1), 50-56 (L2) and 89-97 (L3) in the V_L, and around about 31-35B (H1), 50-65 (H2) and 95-102 (H3) in the V_H (Kabat *et al.*, *Sequences of Proteins of Immunological Interest*, 5th Ed. Public Health Service, National Institutes of Health, Bethesda, MD. (1991)) and/or those residues from a "hypervariable loop" (*e.g.*, residues 26-32 (L1), 50-52 (L2) and 91-96 (L3) in the V_L, and 26-32 (H1), 52A-55 (H2) and 96-101 (H3) in the V_H (Chothia and Lesk *J. Mol. Biol.* 196:901-917 (1987)).

[0072] As used herein, the terms "identical" or percent "identity", when used in the context of two or more nucleic acids or polypeptide sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same (*i.e.*, about 60%, 65%, 70%, 75%, 80%, 85%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, 99%, or higher identity over a specified region (*e.g.*, nucleotide sequence encoding an antibody described herein or amino acid sequence of an antibody described herein)), when compared and aligned for maximum correspondence over a comparison window or designated region as measured using a BLAST or BLAST 2.0 sequence comparison algorithms with default parameters described below, or by manual alignment and visual inspection (*e.g.*, NCBI web site). Such sequences are then said to be "substantially identical." This term also refers to, or can be applied to, the complement of a test sequence. The term also includes sequences that have deletions and/or additions, as

well as those that have substitutions. In some embodiments, identity exists over a region that is at least about 25 amino acids or nucleotides in length, or 50-100 amino acids or nucleotides in length.

[0073] As used herein, "immunogen" refers to any antigen that is capable of inducing humoral and/or cell-mediated immune response rather than immunological tolerance.

[0074] As used herein, the term "intact antibody" or "intact immunoglobulin" means an antibody that has at least two heavy (H) chain polypeptides and two light (L) chain polypeptides interconnected by disulfide bonds. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as HCVR or V_H) and a heavy chain constant region. The heavy chain constant region is comprised of three domains, CH_1, CH_2 and CH_3. Each light chain is comprised of a light chain variable region (abbreviated herein as LCVR or V_L) and a light chain constant region. The light chain constant region is comprised of one domain, C_L. The V_H and V_L regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each V_H and V_L is composed of three CDRs and four FRs, arranged from amino-terminus to carboxyl-terminus in the following order: FR_1, CDR_1, FR_2, CDR_2, FR_3, CDR_3, FR_4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies can mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (*e.g.*, effector cells) and the first component (Clq) of the classical complement system.

[0075] As used herein, the terms "individual", "patient", or "subject" can be an individual organism, a vertebrate, a mammal, or a human. In some embodiments, the individual, patient or subject is a human.

[0076] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous antibodies, *i.e.*, the individual antibodies comprising the population are identical except for possible naturally occurring mutations that may be present in minor amounts. For example, a monoclonal antibody can be an antibody that is derived from a single clone, including any eukaryotic, prokaryotic, or phage clone, and not the method by which it is produced. A monoclonal antibody composition displays a single binding specificity and affinity for a particular epitope. Monoclonal

antibodies are highly specific, being directed against a single antigenic site. Furthermore, in contrast to conventional (polyclonal) antibody preparations which typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen. The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. Monoclonal antibodies can be prepared using a wide variety of techniques known in the art including, *e.g.*, but not limited to, hybridoma, recombinant, and phage display technologies. For example, the monoclonal antibodies to be used in accordance with the present methods may be made by the hybridoma method first described by Kohler *et al., Nature* 256:495 (1975), or may be made by recombinant DNA methods (*See*, *e.g.*, U.S. Patent No. 4,816,567). The "monoclonal antibodies" may also be isolated from phage antibody libraries using the techniques described in Clackson *et al., Nature* 352:624-628 (1991) and Marks *et al., J. Mol. Biol.* 222:581-597 (1991), for example.

[0077] As used herein, the term "nucleic acid" or "polynucleotide" means any RNA or DNA, which may be unmodified or modified RNA or DNA. Polynucleotides include, without limitation, single- and double-stranded DNA, DNA that is a mixture of single- and double-stranded regions, single- and double-stranded RNA, RNA that is mixture of single- and double-stranded regions, and hybrid molecules comprising DNA and RNA that may be single-stranded or, more typically, double-stranded or a mixture of single- and double-stranded regions. In addition, polynucleotide refers to triple-stranded regions comprising RNA or DNA or both RNA and DNA. The term polynucleotide also includes DNAs or RNAs containing one or more modified bases and DNAs or RNAs with backbones modified for stability or for other reasons.

[0078] As used herein, the term "pharmaceutically acceptable carrier" is intended to include any and all solvents, dispersion media, coatings, antibacterial and antifungal compounds, isotonic and absorption delaying compounds, and the like, compatible with pharmaceutical administration. Pharmaceutically-acceptable carriers and their formulations are known to one skilled in the art and are described, for example, in Remington's Pharmaceutical Sciences (20th edition, ed. A. Gennaro, 2000, Lippincott, Williams & Wilkins, Philadelphia, Pa.).

[0079] As used herein, the term "polyclonal antibody" means a preparation of antibodies derived from at least two (2) different antibody-producing cell lines. The use of this term includes preparations of at least two (2) antibodies that contain antibodies that specifically bind to different epitopes or regions of an antigen.

[0080] As used herein, the terms "polypeptide," "peptide" and "protein" are used interchangeably herein to mean a polymer comprising two or more amino acids joined to each other by peptide bonds or modified peptide bonds, *i.e.*, peptide isosteres. Polypeptide refers to both short chains, commonly referred to as peptides, glycopeptides or oligomers, and to longer chains, generally referred to as proteins. Polypeptides may contain amino acids other than the 20 gene-encoded amino acids. Polypeptides include amino acid sequences modified either by natural processes, such as post-translational processing, or by chemical modification techniques that are well known in the art. Such modifications are well described in basic texts and in more detailed monographs, as well as in a voluminous research literature.

[0081] As used herein, the term "recombinant" when used with reference, *e.g.*, to a cell, or nucleic acid, protein, or vector, indicates that the cell, nucleic acid, protein or vector, has been modified by the introduction of a heterologous nucleic acid or protein or the alteration of a native nucleic acid or protein, or that the material is derived from a cell so modified. Thus, for example, recombinant cells express genes that are not found within the native (non-recombinant) form of the cell or express native genes that are otherwise abnormally expressed, under expressed or not expressed at all.

[0082] As used herein, the term "separate" therapeutic use refers to an administration of at least two active ingredients at the same time or at substantially the same time by different routes.

[0083] As used herein, the term "sequential" therapeutic use refers to administration of at least two active ingredients at different times, the administration route being identical or different. More particularly, sequential use refers to the whole administration of one of the active ingredients before administration of the other or others commences. It is thus possible to administer one of the active ingredients over several minutes, hours, or days before administering the other active ingredient or ingredients. There is no simultaneous treatment in this case.

[0084] As used herein, the term "simultaneous" therapeutic use refers to the administration of at least two active ingredients by the same route and at the same time or at substantially the same time.

[0085] As used herein, the terms "single-chain antibodies" or "single-chain Fv (scFv)" refer to an antibody fusion molecule of the two domains of the Fv fragment, V_L and V_H. Single-chain antibody molecules may comprise a polymer with a number of individual molecules, for example, dimer, trimer or other polymers. Furthermore, although the two domains of the F_v fragment, V_L and V_H, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the V_L and V_H regions pair to form monovalent molecules (known as single-chain F_v (scF_v)). Bird *et al.* (1988) *Science* 242:423-426 and Huston *et al.* (1988) *Proc Natl Acad Sci* 85:5879-5883. Such single-chain antibodies can be prepared by recombinant techniques or enzymatic or chemical cleavage of intact antibodies.

[0086] As used herein, "specifically binds" refers to a molecule (*e.g.*, an antibody or antigen binding fragment thereof) which recognizes and binds another molecule (*e.g.*, an antigen), but that does not substantially recognize and bind other molecules. The terms "specific binding," "specifically binds to," or is "specific for" a particular molecule (*e.g.*, a polypeptide, or an epitope on a polypeptide), as used herein, can be exhibited, for example, by a molecule having a K_D for the molecule to which it binds to of about 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, or 10^{-12} M. The term "specifically binds" may also refer to binding where a molecule (*e.g.*, an antibody or antigen binding fragment thereof) binds to a particular polypeptide (*e.g.*, a CLDN18.2 polypeptide), or an epitope on a particular polypeptide, without substantially binding to any other polypeptide, or polypeptide epitope.

[0087] As used herein, "sequence liabilities" refer to any feature in nucleic acid or amino acid sequences that can affect the heterogeneity of the immunoglobulin-related compositions of the present disclosure. Such sequence liabilities include but not limited to, any sequence motifs that are prone to deamidation, isomerization, cleavage, oxidation, and glycosylation.

[0088] As used herein, the terms "subject", "patient", or "individual" can be an individual organism, a vertebrate, a mammal, or a human. In some embodiments, the subject, patient or individual is a human.

[0089] As used herein, the term "therapeutic agent" is intended to mean a compound that, when present in an effective amount, produces a desired therapeutic effect on a subject in need thereof.

[0090] "Treating" or "treatment" as used herein covers the treatment of a disease or disorder described herein, in a subject, such as a human, and includes: (i) inhibiting a disease or disorder, *i.e.*, arresting its development; (ii) relieving a disease or disorder, *i.e.*, causing regression of the disorder; (iii) slowing progression of the disorder; and/or (iv) inhibiting, relieving, or slowing progression of one or more symptoms of the disease or disorder. In some embodiments, treatment means that the symptoms associated with the disease are, *e.g.*, alleviated, reduced, cured, or placed in a state of remission.

[0091] It is also to be appreciated that the various modes of treatment of disorders as described herein are intended to mean "substantial," which includes total but also less than total treatment, and wherein some biologically or medically relevant result is achieved. The treatment may be a continuous prolonged treatment for a chronic disease or a single, or few time administrations for the treatment of an acute condition.

[0092] Amino acid sequence modification(s) of the anti-CLDN18.2 antibodies described herein are contemplated. Such modifications can be performed to improve the binding affinity and/or other biological properties of the antibody, for examples, to render the encoded amino acid glycosylated, or to destroy the antibody's ability to bind to C1q, Fc receptor, or to activate the complement system. Amino acid sequence variants of an anti-CLDN18.2 antibody are prepared by introducing appropriate nucleotide changes into the antibody nucleic acid, by peptide synthesis, or by chemical modifications. Such modifications include, for example, deletions from, and/or insertions into and/or substitutions of, residues within the amino acid sequences of the antibody. Any combination of deletion, insertion, and substitution is made to obtain the antibody of interest, as long as the obtained antibody possesses the desired properties. The modification also includes the change of the pattern of glycosylation of the protein. The sites of greatest interest for substitutional mutagenesis include the hypervariable regions, but FR alterations are also contemplated.

[0093] Conservative amino acid substitutions are amino acid substitutions that change a given amino acid to a different amino acid with similar biochemical properties (*e.g.*, charge, hydrophobicity and size). "Conservative substitutions" are shown in the Table below.

Table 1. Amino Acid Substitutions		
Original Residue	**Exemplary Substitutions**	**Conservative Substitutions**
Ala (A)	val; leu; ile	val
Arg (R)	lys; gln; asn	lys
Asn (N)	gln; his; asp, lys; arg	gln
Asp (D)	glu; asn	glu
Cys (C)	ser; ala	ser
Gln (Q)	asn; glu	asn
Glu (E)	asp; gln	asp
Gly (G)	ala	ala
His (H)	asn; gln; lys; arg	arg
Ile (I)	leu; val; met; ala; phe; norleucine	leu
Leu (L)	norleucine; ile; val; met; ala; phe	ile
Lys (K)	arg; gln; asn	arg
Met (M)	leu; phe; ile	leu
Phe (F)	leu; val; ile; ala; tyr	tyr
Pro (P)	ala	ala
Ser (S)	thr	thr
Thr (T)	ser	ser
Trp (W)	tyr; phe	tyr
Tyr (Y)	trp; phe; thr; ser	phe
Val (V)	ile; leu; met; phe; ala; norleucine	leu

[0094] One type of substitutional variant involves substituting one or more hypervariable region residues of a parent antibody. A convenient way for generating such substitutional variants involves affinity maturation using phage display. Specifically, several hypervariable region sites (*e.g.*, 6-7 sites) are mutated to generate all possible amino acid substitutions at each site. The antibody variants thus generated are displayed in a

monovalent fashion from filamentous phage particles as fusions to the gene III product of M13 packaged within each particle. The phage-displayed variants are then screened for their biological activity (*e.g.*, binding affinity) as herein disclosed. In order to identify candidate hypervariable region sites for modification, alanine scanning mutagenesis can be performed to identify hypervariable region residues contributing significantly to antigen binding. Alternatively, or additionally, it may be beneficial to analyze a crystal structure of the antigen-antibody complex to identify contact points between the antibody and the antigen. Such contact residues and neighboring residues are candidates for substitution according to the techniques elaborated herein. Once such variants are generated, the panel of variants is subjected to screening as described herein and antibodies with similar or superior properties in one or more relevant assays may be selected for further development.

Claudins

[0095] In human, 27 family members of claudin have been described, including claudin 18. All claudins have four transmembrane domains and two extracellular loops, with the N-terminus and the C-terminus in the cytoplasm (Markov, A.G., *et al.*, *IUBMB Life* 67: 29–35 (2015); Furuse, M., *et al.*, *J Cell Biol* 141: 1539–1550 (1998); Turksen, K. And Troy, T.C., *Biochim Biophys Acta* 1816: 73–79 (2011)).

[0096] The claudin family member 18 gene is composed of 5 exons. There are two splicing variants, Claudin 18.1 (CLDN18.1) and Claudin 18.2 (CLDN18.2). The two variants are the products of alternative splicing that utilize alternative DNA sequences in exon 1, which encode the N-terminal portion of the protein including the first extracellular loop (EL1) (**Figure 1**) (Mineta, K., *et al.*, *FEBS Lett* 585: 606–612 (2011); Suzuki, H., *et al.*, *Science* 344: 304–307 (2014)). CLDN18.1 and CLDN18.2 have different EL1 sequences, but share an identical EL2 sequence (**Figure 2**). The homology of CLDN18.2 is extremely high in species such as human, cynomolgus, and mice, as they all possess an identical EL1 amino acid sequence.

[0097] The expression of Claudin 18 in normal human tissues is highly restricted, with CLDN18.1 found predominately in lung and CLDN18.2 in stomach (**Figure 3**) (Sahin U., *et al.*, *Clin Cancer Res* 14:7624–7634 (2008)). Cancerous expression of CLDN18.2 has been reported in gastric, pancreatic, and other cancers (**Figure 4**) (Sahin U., *et al.*, Clin Cancer Res 14:7624–7634 (2008); Karanjawala, Z.E., *et al.*, *Am J Surg Pathol* 32: 188–196 (2008)). One study reported that 70% of gastric cancers, 50% of pancreatic cancers, 30% of

esophageal cancers, and 25% of NSCLC express CLDN18.2 (Sahin U., *et al.*, *Clin Cancer Res* 14:7624–7634 (2008)). CLDN18.2 has been regarded as a specific gastric tumor associated antigen (TAA).

[0098] The malignancy associated expression of CLDN18.2 and its tissue restricted expression makes it an ideal target for antibody-based therapy (Sahin U., *et al.*, *Clin Cancer Res* 14:7624–7634 (2008)). While there is no open access to the normal tight junction forming CLDN18.2 in the gastric mucosa, CLDN18.2 epitopes become exposed on the cell surface upon malignant transformation, thereby making them accessible to therapeutic antibodies.

Immunoglobulin-related Compositions of the Present Technology

[0099] The present technology describes methods and compositions for the generation and use of anti-CLDN18.2 immunoglobulin-related compositions (*e.g.*, anti-CLDN18.2 antibodies or antigen binding fragments thereof). The antibodies and antigen binding fragments of the present technology selectively bind to CLDN18.2 polypeptides (**Figure 10**) instead of CLDN18.1 polypeptides (**Figure 11**). The anti-CLDN18.2 immunoglobulin-related compositions of the present disclosure may be useful in the diagnosis, or treatment of CLDN18.2-associated cancers. Anti-CLDN18.2 immunoglobulin-related compositions within the scope of the present technology include, *e.g.*, but are not limited to, monoclonal, chimeric, humanized antibodies and diabodies that specifically bind the target polypeptide, a homolog, derivative or a fragment thereof. The present disclosure also provides antigen binding fragments of any of the anti-CLDN18.2 antibodies disclosed herein, wherein the antigen binding fragment is selected from the group consisting of Fab, F(ab)'2, Fab', scF_v, and F_v. The amino acid sequences of the anti-CLDN18.2 immunoglobulin-related compositions of the present technology are described in **Figures 12-17**.

[00100] In one aspect, the present disclosure provides an antibody or an antigen binding fragment thereof, comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein (a) the V_H comprises a V_H-CDR1 sequence selected from the group consisting of SEQ ID NOs: 6, 12, 18, 24 and 30, a V_H-CDR2 sequence selected from the group consisting of SEQ ID NOs: 7, 13, 19, 25, and 31, and a V_H-CDR3 sequence selected from the group consisting of SEQ ID NOs: 8, 14, 20, 26, and 32, and/or (b) the V_L comprises a V_L-CDR1 sequence selected from the group consisting of SEQ ID NOs: 9, 15, 21, 27, and 33, a V_L-CDR2 sequence selected from the

group consisting of SEQ ID NOs: 10, 16, 22, 28, 34, 157, and 158, and a V_L-CDR3 sequence selected from the group consisting of SEQ ID NOs: 11, 17, 23, 29, and 35.

[00101] In one aspect, the present disclosure provides an antibody or an antigen binding fragment thereof, comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein (a) the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 6, a V_H-CDR2 sequence of SEQ ID NO: 7, and a V_H-CDR3 sequence of SEQ ID NO: 8, and/or the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 9, a V_L-CDR2 sequence of SEQ ID NO: 10 or SEQ ID NO: 157, and a V_L-CDR3 sequence of SEQ ID NO: 11; (b) the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 12, a V_H-CDR2 sequence of SEQ ID NO: 13, and a V_H-CDR3 sequence of SEQ ID NO: 14, and/or the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 15, a V_L-CDR2 sequence of SEQ ID NO: 16 or SEQ ID NO: 158, and a V_L-CDR3 sequence of SEQ ID NO: 17; (c) the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 18, a V_H-CDR2 sequence of SEQ ID NO: 19, and a V_H-CDR3 sequence of SEQ ID NO: 20, and/or the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 21, a V_L-CDR2 sequence of SEQ ID NO: 22, and a V_L-CDR3 sequence of SEQ ID NO: 23; (d) the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 24, a V_H-CDR2 sequence of SEQ ID NO: 25, and a V_H-CDR3 sequence of SEQ ID NO: 26, and/or the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 27, a V_L-CDR2 sequence of SEQ ID NO: 28, and a V_L-CDR3 sequence of SEQ ID NO: 29; or (e) the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 30, a V_H-CDR2 sequence of SEQ ID NO: 31, and a V_H-CDR3 sequence of SEQ ID NO: 32, and/or the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 33, a V_L-CDR2 sequence of SEQ ID NO: 34, and a V_L-CDR3 sequence of SEQ ID NO: 35.

[00102] In one aspect, the present disclosure provides an antibody or antigen binding fragment thereof comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein: (a) the V_H comprises an amino acid sequence selected from any one of SEQ ID NOs: 36, 38, 40, 42, 44, 46-49, or 54-57; and/or (b) the V_L comprises an amino acid sequence selected from any one of SEQ ID NOs: 37, 39, 41, 43, 45, 50-53, or 58-61.

[00103] In any of the above embodiments, the antibody further comprises a Fc domain of any isotype, *e.g.*, but are not limited to, IgG (including IgG1, IgG2, IgG3, and IgG4), IgA

(including IgA_1 and IgA_2), IgD, IgE, or IgM, and IgY. Non-limiting examples of constant region sequences include:

[00104] Human IgD constant region, Uniprot: P01880 (SEQ ID NO: 70)

APTKAPDVFPIISGCRHPKDNSPVVLACLITGYHPTSVTVTWYMGTQSQPQRTFPEI
QRRDSYYMTSSQLSTPLQQWRQGEYKCVVQHTASKSKKEIFRWPESPKAQASSVP
TAQPQAEGSLAKATTAPATTRNTGRGGEEKKKEKEKEEQEERETKTPECPSHTQPL
GVYLLTPAVQDLWLRDKATFTCFVVGSDLKDAHLTWEVAGKVPTGGVEEGLLER
HSNGSQSQHSRLTLPRSLWNAGTSVTCTLNHPSLPPQRLMALREPAAQAPVKLSLN
LLASSDPPEAASWLLCEVSGFSPPNILLMWLEDQREVNTSGFAPARPPPQPGSTTFW
AWSVLRVPAPPSPQPATYTCVVSHEDSRTLLNASRSLEVSYVTDHGPMK

[00105] Human IgG1 constant region, Uniprot: P01857 (SEQ ID NO: 71)

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPA
PELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNA
KTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ
PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLD
SDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00106] Human IgG2 constant region, Uniprot: P01859 (SEQ ID NO: 72)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSNFGTQTYTCNVDHKPSNTKVDKTVERKCCVECPPCPAPP
VAGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFNWYVDGVEVHNAKT
KPREEQFNSTFRVVSVLTVVHQDWLNGKEYKCKVSNKGLPAPIEKTISKTKGQPRE
PQVYTLPPSREEMTKNQVSLTCLVKGFYPSDISVEWESNGQPENNYKTTPPMLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00107] Human IgG3 constant region, Uniprot: P01860 (SEQ ID NO: 73)

ASTKGPSVFPLAPCSRSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAV
LQSSGLYSLSSVVTVPSSSLGTQTYTCNVNHKPSNTKVDKRVELKTPLGDTTHTCPR
CPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPEPKSCDTPPPCPRCPAPELLGGPSVFL
FPPKPKDTLMISRTPEVTCVVVDVSHEDPEVQFKWYVDGVEVHNAKTKPREEQYN
STFRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKTKGQPREPQVYTLPPS
REEMTKNQVSLTCLVKGFYPSDIAVEWESSGQPENNYNTTPPMLDSDGSFFLYSKL
TVDKSRWQQGNIFSCSVMHEALHNRFTQKSLSLSPGK

[00108] Human IgM constant region, Uniprot: P01871 (SEQ ID NO: 74)

GSASAPTLFPLVSCENSPSDTSSVAVGCLAQDFLPDSITLSWKYKNNSDISSTRGFPS
VLRGGKYAATSQVLLPSKDVMQGTDEHVVCKVQHPNGNKEKNVPLPVIAELPPKV
SVFVPPRDGFFGNPRKSKLICQATGFSPRQIQVSWLREGKQVGSGVTTDQVQAEAK
ESGPTTYKVTSTLTIKESDWLGQSMFTCRVDHRGLTFQQNASSMCVPDQDTAIRVF
AIPPSFASIFLTKSTKLTCLVTDLTTYDSVTISWTRQNGEAVKTHTNISESHPNATFSA
VGEASICEDDWNSGERFTCTVTHTDLPSPLKQTISRPKGVALHRPDVYLLPPAREQL
NLRESATITCLVTGFSPADVFVQWMQRGQPLSPEKYVTSAPMPEPQAPGRYFAHSIL
TVSEEEWNTGETYTCVAHEALPNRVTERTVDKSTGKPTLYNVSLVMSDTAGTCY

[00109] Human IgG4 constant region, Uniprot: P01861 (SEQ ID NO: 75)

ASTKGPSVFPLAPCSRSTSESTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVL
QSSGLYSLSSVVTVPSSSLGTKTYTCNVDHKPSNTKVDKRVESKYGPPCPSCPAPEF
LGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSQEDPEVQFNWYVDGVEVHNAKT
KPREEQFNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKGLPSSIEKTISKAKGQPRE
PQVYTLPPSQEEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSRLTVDKSRWQEGNVFSCSVMHEALHNHYTQKSLSLSLGK

[00110] Human IgA1 constant region, Uniprot: P01876 (SEQ ID NO: 76)

ASPTSPKVFPLSLCSTQPDGNVVIACLVQGFFPQEPLSVTWSESGQGVTARNFPPSQ
DASGDLYTTSSQLTLPATQCLAGKSVTCHVKHYTNPSQDVTVPCPVPSTPPTPSPST
PPTPSPSCCHPRLSLHRPALEDLLLGSEANLTCTLTGLRDASGVTFTWTPSSGKSAV
QGPPERDLCGCYSVSSVLPGCAEPWNHGKTFTCTAAYPESKTPLTATLSKSGNTFRP
EVHLLPPPSEELALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQE
PSQGTTTFAVTSILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRLAGKPTHVN
VSVVMAEVDGTCY

[00111] Human IgA2 constant region, Uniprot: P01877 (SEQ ID NO: 77)

ASPTSPKVFPLSLDSTPQDGNVVVACLVQGFFPQEPLSVTWSESGQNVTARNFPPSQ
DASGDLYTTSSQLTLPATQCPDGKSVTCHVKHYTNPSQDVTVPCPVPPPPPCCHPRL
SLHRPALEDLLLGSEANLTCTLTGLRDASGATFTWTPSSGKSAVQGPPERDLCGCY
SVSSVLPGCAQPWNHGETFTCTAAHPELKTPLTANITKSGNTFRPEVHLLPPPSEEL
ALNELVTLTCLARGFSPKDVLVRWLQGSQELPREKYLTWASRQEPSQGTTTFAVTS
ILRVAAEDWKKGDTFSCMVGHEALPLAFTQKTIDRMAGKPTHVNVSVVMAEVDG
TCY

[00112] Human Ig kappa constant region, Uniprot: P01834 (SEQ ID NO: 78)

TVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVT
EQDSKDSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

[00113] In some embodiments, the immunoglobulin-related compositions of the present technology comprise a heavy chain constant region that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or is 100% identical to SEQ ID NOs: 70-77. Additionally or alternatively, in some embodiments, the immunoglobulin-related compositions of the present technology comprise a light chain constant region that is at least 80%, at least 85%, at least 90%, at least 95%, at least 99%, or is 100% identical to SEQ ID NO: .78

[00114] Additionally or alternatively, in some embodiments, the antibody or antigen binding fragment binds to the first extracellular loop of a CLDN18.2 polypeptide. In some embodiments, the CLDN18.2 polypeptide has the amino acid sequence of SEQ ID NO: 4. In certain embodiments, the first extracellular loop comprises the amino acid sequence of SEQ ID NO: 2 (see **Figure 2**). In certain embodiments, the epitope is a conformational epitope or non-conformational epitope.

[00115] In another aspect, the present disclosure provides an isolated immunoglobulin-related composition (*e.g.*, an antibody or antigen binding fragment thereof) comprising a heavy chain (HC) amino acid sequence comprising SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, or a variant thereof having one or more conservative amino acid substitutions. Additionally or alternatively, in some embodiments, the immunoglobulin-related compositions of the present technology comprise a light chain (LC) amino acid sequence comprising SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, or a variant thereof having one or more conservative amino acid substitutions. In some embodiments, the immunoglobulin-related compositions of the present technology comprise a HC amino acid sequence and a LC amino acid sequence selected from the group consisting of: SEQ ID NO: 62 and SEQ ID NO: 63, SEQ ID NO: 64 and SEQ ID NO: 65, SEQ ID NO: 66 and SEQ ID NO: 67, and SEQ ID NO: 68 and SEQ ID NO: 69, respectively.

[00116] In any of the above embodiments of the immunoglobulin-related compositions, the HC and LC immunoglobulin variable domain sequences form an antigen binding site that binds to the first extracellular loop of a CLDN18.2 polypeptide. In certain embodiments, the first extracellular loop comprises the amino acid sequence of SEQ ID NO: 2. In some embodiments, the epitope is a conformational epitope or a non-conformational epitope.

[00117] In some embodiments, the HC and LC immunoglobulin variable domain sequences are components of the same polypeptide chain. In other embodiments, the HC and LC immunoglobulin variable domain sequences are components of different polypeptide chains. In certain embodiments, the antibody is a full-length antibody.

[00118] In some embodiments, the immunoglobulin-related compositions of the present technology bind specifically to at least one CLDN18.2 polypeptide. In some embodiments, the immunoglobulin-related compositions of the present technology bind at least one CLDN18.2 polypeptide with a dissociation constant (K_D) of about 10^{-3} M, 10^{-4} M, 10^{-5} M, 10^{-6} M, 10^{-7} M, 10^{-8} M, 10^{-9} M, 10^{-10} M, 10^{-11} M, or 10^{-12} M. In certain embodiments, the immunoglobulin-related compositions are monoclonal antibodies, chimeric antibodies, or humanized antibodies. In some embodiments, the antibodies comprise a human antibody framework region.

[00119] In certain embodiments, the immunoglobulin-related composition includes one or more of the following characteristics: (a) a light chain immunoglobulin variable domain sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the light chain immunoglobulin variable domain sequence of any one of SEQ ID NOs: 37, 39, 41, 43, 45, 50-53, or 58-61; and/or (b) a heavy chain immunoglobulin variable domain sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the heavy chain immunoglobulin variable domain sequence of any one of SEQ ID NOs: 36, 38, 40, 42, 44, 46-49, or 54-57. In another aspect, one or more amino acid residues in the immunoglobulin-related compositions provided herein are substituted with another amino acid. The substitution may be a "conservative substitution" as defined herein.

[00120] In another aspect, the present disclosure provides an antibody comprising (a) a LC sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the LC sequence present in SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, or SEQ ID NO: 69; and/or (b) a HC sequence that is at least 80%, at least 85%, at least 90%, at least 95%, or at least 99% identical to the HC sequence present in SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, or SEQ ID NO: 68.

[00121] In certain embodiments, the immunoglobulin-related compositions contain an IgG1 constant region comprising one or more amino acid substitutions selected from the group consisting of N297A, K322A, L234A and L235A. Additionally or alternatively, in some embodiments, the immunoglobulin-related compositions contain an IgG4 constant region comprising a S228P mutation.

[00122] In some aspects, the anti-CLDN18.2 immunoglobulin-related compositions described herein contain structural modifications to facilitate rapid binding and cell uptake and/or slow release. In some aspects, the anti-CLDN18.2 immunoglobulin-related composition of the present technology (*e.g.*, an antibody) may contain a deletion in the CH2 constant heavy chain region to facilitate rapid binding and cell uptake and/or slow release. In some aspects, a Fab fragment is used to facilitate rapid binding and cell uptake and/or slow release. In some aspects, a $F(ab)'_2$ fragment is used to facilitate rapid binding and cell uptake and/or slow release.

[00123] In one aspect, the present technology provides a nucleic acid sequence encoding any of the immunoglobulin-related compositions described herein. Also disclosed herein are recombinant nucleic acid sequences encoding any of the antibodies described herein.

[00124] In another aspect, the present technology provides a host cell expressing any nucleic acid sequence encoding any of the immunoglobulin-related compositions described herein.

[00125] The immunoglobulin-related compositions of the present technology (*e.g.*, an anti-CLDN18.2 antibody) can be specifically bind an epitope on one or more CLDN18.2 polypeptides as well as for heterologous compositions including the CLDN18.2 polypeptide, such as a heterologous polypeptide or solid support material. In some embodiments, the immunoglobulin-related compositions are chimeric. In certain embodiments, the immunoglobulin-related compositions are humanized.

[00126] The immunoglobulin-related compositions of the present technology can further be recombinantly fused to a heterologous polypeptide at the N- or C-terminus or chemically conjugated (including covalently and non-covalently conjugations) to polypeptides or other compositions. For example, the immunoglobulin-related compositions of the present technology can be recombinantly fused or conjugated to molecules useful as labels in detection assays and effector molecules such as heterologous polypeptides, drugs, or toxins. *See*, *e.g.*, WO 92/08495; WO 91/14438; WO 89/12624; U.S. Pat. No. 5,314,995; and EP 0 396 387.

[00127] In any of the above embodiments of the immunoglobulin-related compositions of the present technology, the antibody or antigen binding fragment may be optionally conjugated to an agent selected from the group consisting of isotopes, dyes, chromagens, contrast agents, drugs, toxins, cytokines, enzymes, enzyme inhibitors, hormones, hormone antagonists, growth factors, radionuclides, metals, liposomes, nanoparticles, RNA, DNA or any combination thereof. For a chemical bond or physical bond, a functional group on the immunoglobulin-related composition typically associates with a functional group on the agent. Alternatively, a functional group on the agent associates with a functional group on the immunoglobulin-related composition.

[00128] The functional groups on the agent and immunoglobulin-related composition can associate directly. For example, a functional group (*e.g.*, a sulfhydryl group) on an agent can associate with a functional group (*e.g.*, sulfhydryl group) on an immunoglobulin-related

composition to form a disulfide. Alternatively, the functional groups can associate through a cross-linking agent (*i.e.*, linker). Some examples of cross-linking agents are described below. The cross-linker can be attached to either the agent or the immunoglobulin-related composition. The number of agents or immunoglobulin-related compositions in a conjugate is also limited by the number of functional groups present on the other. For example, the maximum number of agents associated with a conjugate depends on the number of functional groups present on the immunoglobulin-related composition. Alternatively, the maximum number of immunoglobulin-related compositions associated with an agent depends on the number of functional groups present on the agent.

[00129] In yet another embodiment, the conjugate comprises one immunoglobulin-related composition associated to one agent. In one embodiment, a conjugate comprises at least one agent chemically bonded (*e.g.*, conjugated) to at least one immunoglobulin-related composition. The agent can be chemically bonded to an immunoglobulin-related composition by any method known to those in the art. For example, a functional group on the agent may be directly attached to a functional group on the immunoglobulin-related composition. Some examples of suitable functional groups include, for example, amino, carboxyl, sulfhydryl, maleimide, isocyanate, isothiocyanate and hydroxyl.

[00130] The agent may also be chemically bonded to the immunoglobulin-related composition by means of cross-linking agents, such as dialdehydes, carbodiimides, dimaleimides, and the like. Cross-linking agents can, for example, be obtained from Pierce Biotechnology, Inc., Rockford, Ill. The Pierce Biotechnology, Inc. web-site can provide assistance. Additional cross-linking agents include the platinum cross-linking agents described in U.S. Pat. Nos. 5,580,990; 5,985,566; and 6,133,038 of Kreatech Biotechnology, B.V., Amsterdam, The Netherlands.

[00131] Alternatively, the functional group on the agent and immunoglobulin-related composition can be the same. Homobifunctional cross-linkers are typically used to cross-link identical functional groups. Examples of homobifunctional cross-linkers include EGS (*i.e.*, ethylene glycol bis[succinimidylsuccinate]), DSS (*i.e.*, disuccinimidyl suberate), DMA (*i.e.*, dimethyl adipimidate.2HCl), DTSSP (*i.e.*, 3,3'-dithiobis[sulfosuccinimidylpropionate])), DPDPB (*i.e.*, 1,4-di-[3'-(2'-pyridyldithio)-propionamido]butane), and BMH (*i.e.*, bis-maleimidohexane). Such homobifunctional cross-linkers are also available from Pierce Biotechnology, Inc.

[00132] In other instances, it may be beneficial to cleave the agent from the immunoglobulin-related composition. The web-site of Pierce Biotechnology, Inc. described above can also provide assistance to one skilled in the art in choosing suitable cross-linkers which can be cleaved by, for example, enzymes in the cell. Thus the agent can be separated from the immunoglobulin-related composition. Examples of cleavable linkers include SMPT (*i.e.*, 4-succinimidyloxycarbonyl-methyl-a-[2-pyridyldithio]toluene), Sulfo-LC-SPDP (*i.e.*, sulfosuccinimidyl 6-(3-[2-pyridyldithio]-propionamido)hexanoate), LC-SPDP (*i.e.*, succinimidyl 6-(3-[2-pyridyldithio]-propionamido)hexanoate), Sulfo-LC-SPDP (*i.e.*, sulfosuccinimidyl 6-(3-[2-pyridyldithio]-propionamido)hexanoate), SPDP (*i.e.*, N-succinimidyl 3-[2-pyridyldithio]-propionamidohexanoate), and AEDP (*i.e.*, 3-[(2-aminoethyl)dithio]propionic acid HCl).

[00133] In another embodiment, a conjugate comprises at least one agent physically bonded with at least one immunoglobulin-related composition. Any method known to those in the art can be employed to physically bond the agents with the immunoglobulin-related compositions. For example, the immunoglobulin-related compositions and agents can be mixed together by any method known to those in the art. The order of mixing is not important. For instance, agents can be physically mixed with immunoglobulin-related compositions by any method known to those in the art. For example, the immunoglobulin-related compositions and agents can be placed in a container and agitated, by for example, shaking the container, to mix the immunoglobulin-related compositions and agents.

[00134] The immunoglobulin-related compositions can be modified by any method known to those in the art. For instance, the immunoglobulin-related composition may be modified by means of cross-linking agents or functional groups, as described above.

A. Methods of Preparing Anti-CLDN18.2 Antibodies of the Present Technology

[00135] *General Overview*. Initially, a target polypeptide is chosen to which an antibody of the present technology can be raised. For example, an antibody may be raised against the full-length CLDN18.2 protein, or to a portion of the first extracellular loop of the CLDN18.2 protein. Techniques for generating antibodies directed to such target polypeptides are well known to those skilled in the art. Examples of such techniques include, for example, but are not limited to, those involving display libraries, xeno or human mice, hybridomas, and the like. Target polypeptides within the scope of the present

technology include any polypeptide derived from CLDN18.2 protein containing the first extracellular loop which is capable of eliciting an immune response.

[00136] It should be understood that recombinantly engineered antibodies and antibody fragments, *e.g.*, antibody-related polypeptides, which are directed to CLDN18.2 protein and fragments thereof are suitable for use in accordance with the present disclosure.

[00137] Anti-CLDN18.2 antibodies that can be subjected to the techniques set forth herein include monoclonal and polyclonal antibodies, and antibody fragments such as Fab, Fab′, $F(ab')_2$, Fd, scFv, diabodies, antibody light chains, antibody heavy chains and/or antibody fragments. Methods useful for the high yield production of antibody Fv-containing polypeptides, *e.g.*, Fab′ and $F(ab')_2$ antibody fragments have been described. *See* U.S. Pat. No. 5,648,237.

[00138] Generally, an antibody is obtained from an originating species. More particularly, the nucleic acid or amino acid sequence of the variable portion of the light chain, heavy chain or both, of an originating species antibody having specificity for a target polypeptide antigen is obtained. An originating species is any species which was useful to generate the antibody of the present technology or library of antibodies, *e.g.*, rat, mouse, rabbit, chicken, monkey, human, and the like.

[00139] Phage or phagemid display technologies are useful techniques to derive the antibodies of the present technology. Techniques for generating and cloning monoclonal antibodies are well known to those skilled in the art. Expression of sequences encoding antibodies of the present technology, can be carried out in *E. coli*.

[00140] Due to the degeneracy of nucleic acid coding sequences, other sequences which encode substantially the same amino acid sequences as those of the naturally occurring proteins may be used in the practice of the present technology These include, but are not limited to, nucleic acid sequences including all or portions of the nucleic acid sequences encoding the above polypeptides, which are altered by the substitution of different codons that encode a functionally equivalent amino acid residue within the sequence, thus producing a silent change. It is appreciated that the nucleotide sequence of an immunoglobulin according to the present technology tolerates sequence homology variations of up to 25% as calculated by standard methods ("Current Methods in Sequence Comparison and Analysis," *Macromolecule Sequencing and Synthesis, Selected Methods and Applications*, pp. 127-149, 1998, Alan R. Liss, Inc.) so long as such a variant forms an

operative antibody which recognizes CLDN18.2 proteins. For example, one or more amino acid residues within a polypeptide sequence can be substituted by another amino acid of a similar polarity which acts as a functional equivalent, resulting in a silent alteration. Substitutes for an amino acid within the sequence may be selected from other members of the class to which the amino acid belongs. For example, the nonpolar (hydrophobic) amino acids include alanine, leucine, isoleucine, valine, proline, phenylalanine, tryptophan and methionine. The polar neutral amino acids include glycine, serine, threonine, cysteine, tyrosine, asparagine, and glutamine. The positively charged (basic) amino acids include arginine, lysine and histidine. The negatively charged (acidic) amino acids include aspartic acid and glutamic acid. Also included within the scope of the present technology are proteins or fragments or derivatives thereof which are differentially modified during or after translation, *e.g.*, by glycosylation, proteolytic cleavage, linkage to an antibody molecule or other cellular ligands, *etc*. Additionally, an immunoglobulin encoding nucleic acid sequence can be mutated *in vitro* or *in vivo* to create and/or destroy translation, initiation, and/or termination sequences or to create variations in coding regions and/or form new restriction endonuclease sites or destroy pre-existing ones, to facilitate further *in vitro* modification. Any technique for mutagenesis known in the art can be used, including but not limited to *in vitro* site directed mutagenesis, *J. Biol. Chem.* 253:6551, use of Tab linkers (Pharmacia), and the like.

[00141] *Preparation of Polyclonal Antisera and Immunogens.* Methods of generating antibodies or antibody fragments of the present technology typically include immunizing a subject (generally a non-human subject such as a mouse or rabbit) with a purified CLDN18.2 protein or fragment thereof, a nucleic acid encoding CLDN18.2 protein or fragment thereof, or with a cell expressing the CLDN18.2 protein or fragment thereof. An appropriate immunogenic preparation can contain, *e.g.*, a recombinantly-expressed CLDN18.2 protein or a chemically-synthesized CLDN18.2 peptide. The first extracellular loop of the CLDN18.2 protein, or a portion or fragment thereof, can be used as an immunogen to generate an anti-CLDN18.2 antibody that binds to the CLDN18.2 protein, or a portion or fragment thereof using standard techniques for polyclonal and monoclonal antibody preparation. In some embodiments, the antigenic CLDN18.2 peptide comprises at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, or at least 100 amino acid residues. Longer antigenic peptides are sometimes desirable over shorter antigenic peptides, depending on use and according to methods well

known to those skilled in the art. Multimers of a given epitope are sometimes more effective than a monomer.

[00142] By way of example, but not by way of limitation, an immunogenic preparation may comprise, *e.g.*, a recombinantly-expressed CLDN18.2 protein or a chemically-synthesized CLDN18.2 peptide comprising the amino acid sequence of SEQ ID NO: 4. The first extracellular loop of the CLDN18.2 protein, or a portion or fragment thereof, *e.g.*, a CLDN18.2-EL1 having amino acid sequence of SEQ ID NO: 2, may be used as an immunogen to generate an anti-CLDN18.2 antibody that binds to the EL1 portion of the CLDN18.2 protein.

[00143] If needed, the immunogenicity of the CLDN18.2 protein (or fragment thereof) can be increased by fusion or conjugation to a carrier protein such as keyhole limpet hemocyanin (KLH) or ovalbumin (OVA). Many such carrier proteins are known in the art. One can also combine the CLDN18.2 protein with a conventional adjuvant such as Freund's complete or incomplete adjuvant to increase the subject's immune reaction to the polypeptide. Various adjuvants used to increase the immunological response include, but are not limited to, Freund's (complete and incomplete), mineral gels (*e.g.*, aluminum hydroxide), surface active substances (*e.g.*, lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, dinitrophenol, *etc.*), human adjuvants such as Bacille Calmette-Guerin and *Corynebacterium parvum*, or similar immunostimulatory compounds. These techniques are standard in the art.

[00144] Alternatively, nanoparticles, for example, virus-like particles (VLPs), can be used to present antigens, *e.g.*, CLDN18.2-EL1, to a host animal. Virus-like particles are multiprotein structures that mimic the organization and conformation of authentic native viruses without being infectious, since they do not carry any viral genetic material (Urakami A, *et al*, *Clin Vaccine Immunol* 24: e00090-17 (2017)). When introduced to a host immune system, VLPs can evoke effective immune responses, making them attractive carriers of foreign antigens. An important advantage of a VLP-based antigen presenting platform is that it can display antigens in a dense, repetitive manner. Thus, antigen-bearing VLPs are able to induce strong B-cell responses by effectively enabling the cross-linking of B cell receptors (BCRs). VLPs may be genetically manipulated to fine their properties, *e.g.*, immunogenicity. These techniques are standard in the art.

[00145] The isolation of sufficient purified protein or polypeptide to which an antibody is to be raised may be time consuming and sometimes technically challenging. Additional challenges associated with conventional protein-based immunization include concerns over safety, stability, scalability and consistency of the protein antigen. Nucleic acid (DNA and RNA) based immunizations have emerged as promising alternatives. DNA vaccines are usually based on bacterial plasmids that encode the polypeptide sequence of candidate antigen, *e.g.*, CLDN18.2. With a robust eukaryotic promoter, the encoded antigen can be expressed to yield enough levels of transgene expression once the host is inoculated with the plasmids (Galvin T.A., *et al.*, *Vaccine* 2000, 18:2566-2583). Modern DNA vaccine generation relies on DNA synthesis, possibly one-step cloning into the plasmid vector and subsequent isolation of the plasmid, which takes significantly less time and cost to manufacture. The resulting plasmid DNA is also highly stable at room temperature, avoiding cold transportation and leading to substantially extended shelf-life. These techniques are standard in the art.

[00146] Alternatively, nucleic acid sequences encoding the antigen of interest, *e.g.*, CLDN18.2, can be synthetically introduced into a mRNA molecule. The mRNA is then delivered into a host animal, whose cells would recognize and translate the mRNA sequence to the polypeptide sequence of the candidate antigen, *e.g.*, CLDN18.2, thus triggering the immune response to the foreign antigen. An attractive feature of mRNA antigen or mRNA vaccine is that mRNA is a non-infectious, non-integrating platform. There is no potential risk of infection or insertional mutagenesis associated with DNA vaccines. In addition, mRNA is degraded by normal cellular processes and has a controllable *in vivo* half-life through modification of design and delivery methods (Kariko, K., *et al.*, *Mol Ther* 16: 1833–1840 (2008); Kauffman, K. J., *et al.*, *J Control Release* 240, 227–234 (2016); Guan, S. & Rosenecker, J., *Gene Ther* 24, 133–143 (2017); Thess, A., *et al.*, *Mol Ther* 23, 1456–1464 (2015)). These techniques are standard in the art.

[00147] In describing the present technology, immune responses may be described as either "primary" or "secondary" immune responses. A primary immune response, which is also described as a "protective" immune response, refers to an immune response produced in an individual as a result of some initial exposure (*e.g.*, the initial "immunization" or "priming") to a particular antigen, *e.g.*, CLDN18.2 protein. In some embodiments, the immunization can occur as a result of vaccinating the individual with a vaccine containing the antigen. For example, the vaccine can be a CLDN18.2 vaccine comprising one or more

CLDN18.2 protein-derived antigens. A primary immune response can become weakened or attenuated over time and can even disappear or at least become so attenuated that it cannot be detected. Accordingly, the present technology also relates to a "secondary" immune response, which is also described here as a "memory immune response." The term secondary immune response refers to an immune response elicited in an individual after a primary immune response has already been produced.

[00148] Thus, a secondary immune response can be elicited, *e.g.,* to enhance an existing immune response that has become weakened or attenuated (*e.g.*, boosting), or to recreate a previous immune response that has either disappeared or can no longer be detected. The secondary or memory immune response can be either a humoral (antibody) response or a cellular response. A secondary or memory humoral response occurs upon stimulation of memory B cells that were generated at the first presentation of the antigen. Delayed type hypersensitivity (DTH) reactions are a type of cellular secondary or memory immune response that are mediated by $CD4^+$ T cells. A first exposure to an antigen primes the immune system and additional exposure(s) results in a DTH.

[00149] Following appropriate immunization, the anti-CLDN18.2 antibody can be prepared from the subject's serum. If desired, the antibody molecules directed against the CLDN18.2 protein can be isolated from the mammal (*e.g.*, from the blood) and further purified by well-known techniques, such as polypeptide A chromatography to obtain the IgG fraction.

[00150] *Monoclonal Antibody*. In one embodiment of the present technology, the antibody is an anti-CLDN18.2 monoclonal antibody. For example, in some embodiments, the anti-CLDN18.2 monoclonal antibody may be a human or a mouse anti-CLDN18.2 monoclonal antibody. For preparation of monoclonal antibodies directed towards the CLDN18.2 protein, or derivatives, fragments, analogs or homologs thereof, any technique that provides for the production of antibody molecules by continuous cell line culture can be utilized. Such techniques include, but are not limited to, the hybridoma technique (*See*, *e.g.*, Kohler & Milstein, 1975. *Nature* 256: 495-497); the trioma technique; the human B-cell hybridoma technique (*See*, *e.g.*, Kozbor, *et al.*, 1983. *Immunol. Today* 4: 72) and the EBV hybridoma technique to produce human monoclonal antibodies (*See*, *e.g.*, Cole, *et al.*, 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). Human monoclonal antibodies can be utilized in the practice of the present

technology and can be produced by using human hybridomas (*See*, *e.g.*, Cote, *et al.*, 1983. *Proc. Natl. Acad. Sci.* USA 80: 2026-2030) or by transforming human B-cells with Epstein Barr Virus *in vitro* (*See*, *e.g.*, Cole, *et al.*, 1985. In: MONOCLONAL ANTIBODIES AND CANCER THERAPY, Alan R. Liss, Inc., pp. 77-96). For example, a population of nucleic acids that encode regions of antibodies can be isolated. PCR utilizing primers derived from sequences encoding conserved regions of antibodies is used to amplify sequences encoding portions of antibodies from the population and then DNAs encoding antibodies or fragments thereof, such as variable domains, are reconstructed from the amplified sequences. Such amplified sequences also can be fused to DNAs encoding other proteins – *e.g.*, a bacteriophage coat, or a bacterial cell surface protein – for expression and display of the fusion polypeptides on phage or bacteria. Amplified sequences can then be expressed and further selected or isolated based, *e.g.*, on the affinity of the expressed antibody or fragment thereof for an antigen or epitope present on the CLDN18.2 protein. Alternatively, hybridomas expressing anti-CLDN18.2 monoclonal antibodies can be prepared by immunizing a subject and then isolating hybridomas from the subject's spleen using routine methods. *See, e.g.*, Milstein *et al.,* (Galfre and Milstein, *Methods Enzymol* (1981) 73: 3-46). Screening the hybridomas using standard methods will produce monoclonal antibodies of varying specificity (*i.e.*, for different epitopes) and affinity. A selected monoclonal antibody with the desired properties, *e.g.*, CLDN18.2 binding, can be used as expressed by the hybridoma, it can be bound to a molecule such as polyethylene glycol (PEG) to alter its properties, or a cDNA encoding it can be isolated, sequenced and manipulated in various ways. Synthetic dendromeric trees can be added to reactive amino acid side chains, *e.g.*, lysine, to enhance the immunogenic properties of CLDN18.2 protein. Also, CPG-dinucleotide techniques can be used to enhance the immunogenic properties of the CLDN18.2 protein. Other manipulations include substituting or deleting particular amino acyl residues that contribute to instability of the antibody during storage or after administration to a subject, and affinity maturation techniques to improve affinity of the antibody of the CLDN18.2 protein.

[00151] *Hybridoma Technique*. In some embodiments, the antibody of the present technology is an anti-CLDN18.2 monoclonal antibody produced by a hybridoma which includes a B cell obtained from a transgenic non-human animal, *e.g.*, a transgenic mouse, having a genome comprising a human heavy chain transgene and a light chain transgene fused to an immortalized cell. Hybridoma techniques include those known in the art and

taught in Harlow *et al.*, *Antibodies: A Laboratory Manual* Cold Spring Harbor Laboratory, Cold Spring Harbor, NY, 349 (1988); Hammerling *et al.*, *Monoclonal Antibodies And T-Cell Hybridomas,* 563-681 (1981). Other methods for producing hybridomas and monoclonal antibodies are well known to those of skill in the art.

[00152] *Phage Display Technique.* As noted above, the antibodies of the present technology can be produced through the application of recombinant DNA and phage display technology. For example, anti-CLDN18.2 antibodies, can be prepared using various phage display methods known in the art. In phage display methods, functional antibody domains are displayed on the surface of a phage particle which carries polynucleotide sequences encoding them. Phages with a desired binding property are selected from a repertoire or combinatorial antibody library (*e.g.,* human or murine) by selecting directly with an antigen, typically an antigen bound or captured to a solid surface or bead. Phages used in these methods are typically filamentous phage including fd and M13 with Fab, Fv or disulfide stabilized Fv antibody domains that are recombinantly fused to either the phage gene III or gene VIII protein. In addition, methods can be adapted for the construction of Fab expression libraries (*See, e.g.*, Huse, *et al.*, *Science* 246: 1275-1281, 1989) to allow rapid and effective identification of monoclonal Fab fragments with the desired specificity for a CLDN18.2 polypeptide, *e.g.*, a polypeptide or derivatives, fragments, analogs or homologs thereof. Other examples of phage display methods that can be used to make the antibodies of the present technology include those disclosed in Huston *et al.*, *Proc. Natl. Acad. Sci U.S.A.*, 85: 5879-5883, 1988; Chaudhary *et al.*, *Proc. Natl. Acad. Sci U.S.A.,* 87: 1066-1070, 1990; Brinkman *et al.*, *J. Immunol. Methods* 182: 41-50, 1995; Ames *et al.*, *J. Immunol. Methods* 184: 177-186, 1995; Kettleborough *et al.*, *Eur. J. Immunol.* 24: 952-958, 1994; Persic *et al.*, *Gene* 187: 9-18, 1997; Burton *et al.*, *Advances in Immunology* 57: 191-280, 1994; PCT/GB91/01134; WO 90/02809; WO 91/10737; WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; WO 96/06213; WO 92/01047 (Medical Research Council *et al.*); WO 97/08320 (Morphosys); WO 92/01047 (CAT/MRC); WO 91/17271 (Affymax); and U.S. Pat. Nos. 5,698,426, 5,223,409, 5,403,484, 5,580,717, 5,427,908, 5,750,753, 5,821,047, 5,571,698, 5,427,908, 5,516,637, 5,780,225, 5,658,727 and 5,733,743. Methods useful for displaying polypeptides on the surface of bacteriophage particles by attaching the polypeptides *via* disulfide bonds have been described by Lohning, U.S. Pat. No. 6,753,136. As described in the above references, after phage selection, the antibody coding regions from the phage can be isolated and used to generate whole

antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host including mammalian cells, insect cells, plant cells, yeast, and bacteria. For example, techniques to recombinantly produce Fab, Fab′ and $F(ab')_2$ fragments can also be employed using methods known in the art such as those disclosed in WO 92/22324; Mullinax *et al.*, *BioTechniques* 12: 864-869, 1992; and Sawai *et al.*, *AJRI* 34: 26-34, 1995; and Better *et al.*, *Science* 240: 1041-1043, 1988.

[00153] Generally, hybrid antibodies or hybrid antibody fragments that are cloned into a display vector can be selected against the appropriate antigen in order to identify variants that maintain good binding activity, because the antibody or antibody fragment will be present on the surface of the phage or phagemid particle. *See, e.g.,* Barbas III *et al.*, *Phage Display, A Laboratory Manual* (Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 2001). However, other vector formats could be used for this process, such as cloning the antibody fragment library into a lytic phage vector (modified T7 or Lambda Zap systems) for selection and/or screening.

[00154] *Expression of Recombinant Anti-CLDN18.2 Antibodies.* As noted above, the antibodies of the present technology can be produced through the application of recombinant DNA technology. Recombinant polynucleotide constructs encoding an anti-CLDN18.2 antibody of the present technology typically include an expression control sequence operably-linked to the coding sequences of anti-CLDN18.2 antibody chains, including naturally-associated or heterologous promoter regions. As such, another aspect of the technology includes vectors containing one or more nucleic acid sequences encoding an anti-CLDN18.2 antibody of the present technology. For recombinant expression of one or more of the polypeptides of the present technology, the nucleic acid containing all or a portion of the nucleotide sequence encoding the anti-CLDN18.2 antibody is inserted into an appropriate cloning vector, or an expression vector (*i.e.*, a vector that contains the necessary elements for the transcription and translation of the inserted polypeptide coding sequence) by recombinant DNA techniques well known in the art and as detailed below. Methods for producing diverse populations of vectors have been described by Lerner *et al.*, U.S. Pat. Nos. 6,291,160 and 6,680,192.

[00155] In general, expression vectors useful in recombinant DNA techniques are often in the form of plasmids. In the present disclosure, "plasmid" and "vector" can be used interchangeably as the plasmid is the most commonly used form of vector. However, the

present technology is intended to include such other forms of expression vectors that are not technically plasmids, such as viral vectors (*e.g.*, replication defective retroviruses, adenoviruses and adeno-associated viruses), which serve equivalent functions. Such viral vectors permit infection of a subject and expression of a construct in that subject. In some embodiments, the expression control sequences are eukaryotic promoter systems in vectors capable of transforming or transfecting eukaryotic host cells. Once the vector has been incorporated into the appropriate host, the host is maintained under conditions suitable for high level expression of the nucleotide sequences encoding the anti-CLDN18.2 antibody, and the collection and purification of the anti-CLDN18.2 antibody, *e.g.*, cross-reacting anti-CLDN18.2 antibodies. *See generally*, U.S. 2002/0199213. These expression vectors are typically replicable in the host organisms either as episomes or as an integral part of the host chromosomal DNA. Commonly, expression vectors contain selection markers, *e.g.*, ampicillin-resistance or hygromycin-resistance, to permit detection of those cells transformed with the desired DNA sequences. Vectors can also encode signal peptide, *e.g.*, pectate lyase, useful to direct the secretion of extracellular antibody fragments. *See* U.S. Pat. No. 5,576,195.

[00156] The recombinant expression vectors of the present technology comprise a nucleic acid encoding a protein with CLDN18.2 binding properties in a form suitable for expression of the nucleic acid in a host cell, which means that the recombinant expression vectors include one or more regulatory sequences, selected on the basis of the host cells to be used for expression that is operably-linked to the nucleic acid sequence to be expressed. Within a recombinant expression vector, "operably-linked" is intended to mean that the nucleotide sequence of interest is linked to the regulatory sequence(s) in a manner that allows for expression of the nucleotide sequence (*e.g.*, in an *in vitro* transcription/translation system or in a host cell when the vector is introduced into the host cell). The term "regulatory sequence" is intended to include promoters, enhancers and other expression control elements (*e.g.*, polyadenylation signals). Such regulatory sequences are described, *e.g.*, in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Regulatory sequences include those that direct constitutive expression of a nucleotide sequence in many types of host cell and those that direct expression of the nucleotide sequence only in certain host cells (*e.g.*, tissue-specific regulatory sequences). It will be appreciated by those skilled in the art that the design of the expression vector can depend on such factors as the choice of the host cell to

be transformed, the level of expression of polypeptide desired, *etc.* Typical regulatory sequences useful as promoters of recombinant polypeptide expression (*e.g.*, anti-CLDN18.2 antibody), include, *e.g.*, but are not limited to, promoters of 3-phosphoglycerate kinase and other glycolytic enzymes. Inducible yeast promoters include, among others, promoters from alcohol dehydrogenase, isocytochrome C, and enzymes responsible for maltose and galactose utilization. In one embodiment, a polynucleotide encoding an anti-CLDN18.2 antibody of the present technology is operably-linked to an *ara B* promoter and expressible in a host cell. *See* U.S. Pat. 5,028,530. The expression vectors of the present technology can be introduced into host cells to thereby produce polypeptides or peptides, including fusion polypeptides, encoded by nucleic acids as described herein (*e.g.*, anti-CLDN18.2 antibody, *etc.*).

[00157] Another aspect of the present technology pertains to anti-CLDN18.2 antibody-expressing host cells, which contain a nucleic acid encoding one or more anti-CLDN18.2 antibodies. The recombinant expression vectors of the present technology can be designed for expression of an anti-CLDN18.2 antibody in prokaryotic or eukaryotic cells. For example, an anti-CLDN18.2 antibody can be expressed in bacterial cells such as *Escherichia coli*, insect cells (using baculovirus expression vectors), fungal cells, *e.g.*, yeast, yeast cells or mammalian cells. Suitable host cells are discussed further in Goeddel, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990). Alternatively, the recombinant expression vector can be transcribed and translated *in vitro*, *e.g.,* using T7 promoter regulatory sequences and T7 polymerase. Methods useful for the preparation and screening of polypeptides having a predetermined property, *e.g.*, anti-CLDN18.2 antibody, *via* expression of stochastically generated polynucleotide sequences has been previously described. *See* U.S. Pat. Nos. 5,763,192; 5,723,323; 5,814,476; 5,817,483; 5,824,514; 5,976,862; 6,492,107; 6,569,641.

[00158] Expression of polypeptides in prokaryotes is most often carried out in *E. coli* with vectors containing constitutive or inducible promoters directing the expression of either fusion or non-fusion polypeptides. Fusion vectors add a number of amino acids to a polypeptide encoded therein, usually to the amino terminus of the recombinant polypeptide. Such fusion vectors typically serve three purposes: (i) to increase expression of recombinant polypeptide; (ii) to increase the solubility of the recombinant polypeptide; and (iii) to aid in the purification of the recombinant polypeptide by acting as a ligand in affinity purification. Often, in fusion expression vectors, a proteolytic cleavage site is introduced at the junction

of the fusion moiety and the recombinant polypeptide to enable separation of the recombinant polypeptide from the fusion moiety subsequent to purification of the fusion polypeptide. Such enzymes, and their cognate recognition sequences, include Factor Xa, thrombin and enterokinase. Typical fusion expression vectors include pGEX (Pharmacia Biotech Inc; Smith and Johnson, 1988. *Gene* 67: 31-40), pMAL (New England Biolabs, Beverly, Mass.) and pRIT5 (Pharmacia, Piscataway, N.J.) that fuse glutathione S-transferase (GST), maltose E binding polypeptide, or polypeptide A, respectively, to the target recombinant polypeptide.

[00159] Examples of suitable inducible non-fusion *E. coli* expression vectors include pTrc (Amrann *et al.*, (1988) *Gene* 69: 301-315) and pET 11d (Studier *et al.*, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 60-89). Methods for targeted assembly of distinct active peptide or protein domains to yield multifunctional polypeptides *via* polypeptide fusion has been described by Pack *et al.*, U.S. Pat. Nos. 6,294,353; 6,692,935. One strategy to maximize recombinant polypeptide expression, *e.g.*, an anti-CLDN18.2 antibody, in *E. coli* is to express the polypeptide in host bacteria with an impaired capacity to proteolytically cleave the recombinant polypeptide. *See*, *e.g.*, Gottesman, GENE EXPRESSION TECHNOLOGY: METHODS IN ENZYMOLOGY 185, Academic Press, San Diego, Calif. (1990) 119-128. Another strategy is to alter the nucleic acid sequence of the nucleic acid to be inserted into an expression vector so that the individual codons for each amino acid are those preferentially utilized in the expression host, *e.g.*, *E. coli* (*See*, *e.g.*, Wada, *et al.*, 1992. *Nucl. Acids Res.* 20: 2111-2118). Such alteration of nucleic acid sequences of the present technology can be carried out by standard DNA synthesis techniques.

[00160] In another embodiment, the anti-CLDN18.2 antibody expression vector is a yeast expression vector. Examples of vectors for expression in yeast *Saccharomyces cerevisiae* include pYepSec1 (Baldari, *et al.*, 1987. *EMBO J.* 6: 229-234), pMFa (Kurjan and Herskowitz, *Cell* 30: 933-943, 1982), pJRY88 (Schultz *et al.*, *Gene* 54: 113-123, 1987), pYES2 (Invitrogen Corporation, San Diego, Calif.), and picZ (Invitrogen Corp, San Diego, Calif.). Alternatively, an anti-CLDN18.2 antibody can be expressed in insect cells using baculovirus expression vectors. Baculovirus vectors available for expression of polypeptides, *e.g.*, anti-CLDN18.2 antibody, in cultured insect cells (*e.g.*, SF9 cells) include the pAc series (Smith, *et al.*, *Mol. Cell. Biol.* 3: 2156-2165, 1983) and the pVL series (Lucklow and Summers, 1989. *Virology* 170: 31-39).

[00161] In yet another embodiment, a nucleic acid encoding an anti-CLDN18.2 antibody of the present technology is expressed in mammalian cells using a mammalian expression vector. Examples of mammalian expression vectors include, *e.g.*, but are not limited to, pCDM8 (Seed, *Nature* 329: 840, 1987) and pMT2PC (Kaufman, *et al.*, *EMBO J.* 6: 187-195, 1987). When used in mammalian cells, the expression vector's control functions are often provided by viral regulatory elements. For example, commonly used promoters are derived from polyoma, adenovirus 2, cytomegalovirus, and simian virus 40. For other suitable expression systems for both prokaryotic and eukaryotic cells that are useful for expression of the anti-CLDN18.2 antibody of the present technology, see, *e.g.*, Chapters 16 and 17 of Sambrook, *et al.*, MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989.

[00162] In another embodiment, the recombinant mammalian expression vector is capable of directing expression of the nucleic acid in a particular cell type (*e.g.*, tissue-specific regulatory elements). Tissue-specific regulatory elements are known in the art. Non-limiting examples of suitable tissue-specific promoters include the albumin promoter (liver-specific; Pinkert, *et al.*, *Genes Dev.* 1: 268-277, 1987), lymphoid-specific promoters (Calame and Eaton, *Adv. Immunol.* 43: 235-275, 1988), promoters of T cell receptors (Winoto and Baltimore, *EMBO J.* 8: 729-733, 1989) and immunoglobulins (Banerji, *et al.*, 1983. *Cell* 33: 729-740; Queen and Baltimore, *Cell* 33: 741-748, 1983.), neuron-specific promoters (*e.g.*, the neurofilament promoter; Byrne and Ruddle, *Proc. Natl. Acad. Sci. USA* 86: 5473-5477, 1989), pancreas-specific promoters (Edlund, *et al.*, 1985. Science 230: 912-916), and mammary gland-specific promoters (*e.g.*, milk whey promoter; U.S. Pat. No. 4,873,316 and European Application Publication No. 264,166). Developmentally-regulated promoters are also encompassed, *e.g.*, the murine hox promoters (Kessel and Gruss, *Science* 249: 374-379, 1990) and the α-fetoprotein promoter (Campes and Tilghman, *Genes Dev.* 3: 537-546, 1989).

[00163] Another aspect of the present methods pertains to host cells into which a recombinant expression vector of the present technology has been introduced. The terms "host cell" and "recombinant host cell" are used interchangeably herein. It is understood that such terms refer not only to the particular subject cell but also to the progeny or potential progeny of such a cell. Because certain modifications may occur in succeeding generations due to either mutation or environmental influences, such progeny may not, in

fact, be identical to the parent cell, but are still included within the scope of the term as used herein.

[00164] A host cell can be any prokaryotic or eukaryotic cell. For example, an anti-CLDN18.2 antibody can be expressed in bacterial cells such as *E. coli*, insect cells, yeast or mammalian cells. Mammalian cells are a suitable host for expressing nucleotide segments encoding immunoglobulins or fragments thereof. *See* Winnacker, *From Genes To Clones*, (VCH Publishers, NY, 1987). A number of suitable host cell lines capable of secreting intact heterologous proteins have been developed in the art, and include Chinese hamster ovary (CHO) cell lines, various COS cell lines, HeLa cells, L cells and myeloma cell lines. In some embodiments, the cells are non-human. Expression vectors for these cells can include expression control sequences, such as an origin of replication, a promoter, an enhancer, and necessary processing information sites, such as ribosome binding sites, RNA splice sites, polyadenylation sites, and transcriptional terminator sequences. Queen *et al.*, *Immunol. Rev.* 89: 49, 1986. Illustrative expression control sequences are promoters derived from endogenous genes, cytomegalovirus, SV40, adenovirus, bovine papillomavirus, and the like. Co *et al.*, *J Immunol.* 148: 1149, 1992. Other suitable host cells are known to those skilled in the art.

[00165] Vector DNA can be introduced into prokaryotic or eukaryotic cells *via* conventional transformation or transfection techniques. As used herein, the terms "transformation" and "transfection" are intended to refer to a variety of art-recognized techniques for introducing foreign nucleic acid (*e.g.*, DNA) into a host cell, including calcium phosphate or calcium chloride co-precipitation, DEAE-dextran-mediated transfection, lipofection, electroporation, biolistics or viral-based transfection. Other methods used to transform mammalian cells include the use of polybrene, protoplast fusion, liposomes, electroporation, and microinjection (*See generally*, Sambrook *et al.*, *Molecular Cloning*). Suitable methods for transforming or transfecting host cells can be found in Sambrook, *et al.* (MOLECULAR CLONING: A LABORATORY MANUAL. 2nd ed., Cold Spring Harbor Laboratory, Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y., 1989), and other laboratory manuals. The vectors containing the DNA segments of interest can be transferred into the host cell by well-known methods, depending on the type of cellular host.

[00166] Non-limiting examples of suitable vectors include those designed for propagation and expansion, or for expression or both. For example, a cloning vector can be selected from the group consisting of the pUC series, the pBluescript series (Stratagene, LaJolla, Calif.), the pET series (Novagen, Madison, Wis.), the pGEX series (Pharmacia Biotech, Uppsala, Sweden), and the pEX series (Clontech, Palo Alto, Calif.). Bacteriophage vectors, such as lamda-GT10, lamda-GT11, lamda-ZapII (Stratagene), lamda-EMBL4, and lamda-NM1149, can also be used. Non-limiting examples of plant expression vectors include pBI110, pBI101.2, pBI101.3, pBI121 and pBIN19 (Clontech). Non-limiting examples of animal expression vectors include pEUK-C1, pMAM and pMAMneo (Clontech). The TOPO cloning system (Invitrogen, Calsbad, CA) can also be used in accordance with the manufacturer's recommendations.

[00167] In certain embodiments, the vector is a mammalian vector. In certain embodiments, the mammalian vector contains at least one promoter element, which mediates the initiation of transcription of mRNA, the antibody-coding sequence, and signals required for the termination of transcription and polyadenylation of the transcript. In certain embodiments, the mammalian vector contains additional elements, such as, for example, enhancers, Kozak sequences and intervening sequences flanked by donor and acceptor sites for RNA splicing. In certain embodiments, highly efficient transcription can be achieved with, for example, the early and late promoters from SV40, the long terminal repeats (LTRS) from retroviruses, for example, RSV, HTLVI, HIVI and the early promoter of the cytomegalovirus (CMV). Cellular elements can also be used (*e.g.*, the human actin promoter). Non-limiting examples of mammalian expression vectors include, vectors such as pIRESlneo, pRetro-Off, pRetro-On, PLXSN, or pLNCX (Clonetech Labs, Palo Alto, Calif.), pcDNA3.1 (+/-), pcDNA/Zeo (+/-) or pcDNA3.1/Hygro (+/-) (Invitrogen, Calsbad, CA), PSVL and PMSG (Pharmacia, Uppsala, Sweden), pRSVcat (ATCC 37152), pSV2dhfr (ATCC 37146) and pBC12MI (ATCC 67109). Non-limiting examples of mammalian host cells that can be used in combination with such mammalian vectors include human Hela 293, HEK 293, H9 and Jurkat cells, mouse 3T3, NIH3T3 and C127 cells, Cos 1, Cos 7 and CV 1, quail QC1-3 cells, mouse L cells and Chinese hamster ovary (CHO) cells.

[00168] In certain embodiments, the vector is a viral vector, for example, retroviral vectors, parvovirus-based vectors, *e.g.*, adeno-associated virus (AAV)-based vectors, AAV-adenoviral chimeric vectors, and adenovirus-based vectors, and lentiviral vectors, such as Herpes simplex (HSV)-based vectors. In certain embodiments, the viral vector is

manipulated to render the virus replication deficient. In certain embodiments, the viral vector is manipulated to eliminate toxicity to the host. These viral vectors can be prepared using standard recombinant DNA techniques described in, for example, Sambrook *et al.*, Molecular Cloning, a Laboratory Manual, 2d edition, Cold Spring Harbor Press, Cold Spring Harbor, N.Y. (1989); and Ausubel *et al.*, Current Protocols in Molecular Biology, Greene Publishing Associates and John Wiley & Sons, New York, N.Y. (1994).

[00169] In certain embodiments, a vector or polynucleotide described herein can be transferred to a cell (*e.g.*, an *ex vivo* cell) by conventional techniques and the resulting cell can be cultured by conventional techniques to produce an anti-CLDN18.2 antibody or antigen binding fragment described herein. Accordingly, provided herein are cells comprising a polynucleotide encoding an anti-CLDN18.2 antibody or antigen binding fragment thereof operably linked to a regulatory expression element (*e.g.*, promoter) for expression of such sequences in the host cell. In certain embodiments, a vector encoding the heavy chain operably linked to a promoter and a vector encoding the light chain operably linked to a promoter can be co-expressed in the cell for expression of the entire anti-CLDN18.2 antibody or antigen binding fragment. In certain embodiments, a cell comprises a vector comprising a polynucleotide encoding both the heavy chain and the light chain of an anti-CLDN18.2 antibody or antigen binding fragment described herein that are operably linked to a promoter. In certain embodiments, a cell comprises two different vectors, a first vector comprising a polynucleotide encoding a heavy chain operably linked to a promoter, and a second vector comprising a polynucleotide encoding a light chain operably linked to a promoter. In certain embodiments, a first cell comprises a first vector comprising a polynucleotide encoding a heavy chain of an anti-CLDN18.2 antibody or antigen binding fragment described herein, and a second cell comprises a second vector comprising a polynucleotide encoding a light chain of an anti-CLDN18.2 antibody or antigen binding fragment described herein. In certain embodiments, provided herein is a mixture of cells comprising said first cell and said second cell. Examples of cells include, but are not limited to, a human cell, a human cell line, *E. coli* (*e.g.*, E. coli TB-1, TG-2, DH5a, XL-Blue MRF' (Stratagene), SA2821 and Y1090), *B. subtilis, P. aerugenosa, S. cerevisiae, N. crassa*, insect cells (*e.g.*, Sf9, Ea4) and the like.

[00170] For stable transfection of mammalian cells, it is known that, depending upon the expression vector and transfection technique used, only a small fraction of cells may integrate the foreign DNA into their genome. In order to identify and select these

integrants, a gene that encodes a selectable marker (*e.g.*, resistance to antibiotics) is generally introduced into the host cells along with the gene of interest. Various selectable markers include those that confer resistance to drugs, such as G418, hygromycin and methotrexate. Nucleic acid encoding a selectable marker can be introduced into a host cell on the same vector as that encoding the anti-CLDN18.2 antibody or can be introduced on a separate vector. Cells stably transfected with the introduced nucleic acid can be identified by drug selection (*e.g.*, cells that have incorporated the selectable marker gene will survive, while the other cells die).

[00171] A host cell that includes an anti-CLDN18.2 antibody of the present technology, such as a prokaryotic or eukaryotic host cell in culture, can be used to produce (*i.e.*, express) recombinant anti-CLDN18.2 antibody. In one embodiment, the method comprises culturing the host cell (into which a recombinant expression vector encoding the anti-CLDN18.2 antibody has been introduced) in a suitable medium such that the anti-CLDN18.2 antibody is produced. In another embodiment, the method further comprises the step of isolating the anti-CLDN18.2 antibody from the medium or the host cell. Once expressed, collections of the anti-CLDN18.2 antibody, *e.g.*, the anti-CLDN18.2 antibodies or the anti-CLDN18.2 antibody-related polypeptides are purified from culture media and host cells. The anti-CLDN18.2 antibody can be purified according to standard procedures of the art, including HPLC purification, column chromatography, gel electrophoresis and the like. In one embodiment, the anti-CLDN18.2 antibody is produced in a host organism by the method of Boss *et al.*, U.S. Pat. No. 4,816,397. Usually, anti-CLDN18.2 antibody chains are expressed with signal sequences and are thus released to the culture media. However, if the anti-CLDN18.2 antibody chains are not naturally secreted by host cells, the anti-CLDN18.2 antibody chains can be released by treatment with mild detergent. Purification of recombinant polypeptides is well known in the art and includes ammonium sulfate precipitation, affinity chromatography purification technique, column chromatography, ion exchange purification technique, gel electrophoresis and the like (*See generally* Scopes, Protein Purification (Springer-Verlag, N.Y., 1982).

[00172] Polynucleotides encoding anti-CLDN18.2 antibodies, *e.g.*, the anti-CLDN18.2 antibody coding sequences, can be incorporated in transgenes for introduction into the genome of a transgenic animal and subsequent expression in the milk of the transgenic animal. *See*, *e.g.*, U.S. Pat. Nos. 5,741,957, 5,304,489, and 5,849,992. Suitable transgenes include coding sequences for light and/or heavy chains in operable linkage with a promoter

and enhancer from a mammary gland specific gene, such as casein or β-lactoglobulin. For production of transgenic animals, transgenes can be microinjected into fertilized oocytes, or can be incorporated into the genome of embryonic stem cells, and the nuclei of such cells transferred into enucleated oocytes.

[00173] *Single-Chain Antibodies.* In one embodiment, the anti-CLDN18.2 antibody of the present technology is a single-chain anti-CLDN18.2 antibody. According to the present technology, techniques can be adapted for the production of single-chain antibodies specific to a CLDN18.2 protein (*See*, *e.g.*, U.S. Pat. No. 4,946,778). Examples of techniques which can be used to produce single-chain Fvs and antibodies of the present technology include those described in U.S. Pat. Nos. 4,946,778 and 5,258,498; Huston *et al.*, *Methods in Enzymology*, 203: 46-88, 1991; Shu, L. *et al.*, *Proc. Natl. Acad. Sci. USA,* 90: 7995-7999, 1993; and Skerra *et al.*, *Science* 240: 1038-1040, 1988.

[00174] *Chimeric and Humanized Antibodies.* In one embodiment, the anti-CLDN18.2 antibody of the present technology is a chimeric anti-CLDN18.2 antibody. In one embodiment, the anti-CLDN18.2 antibody of the present technology is a humanized anti-CLDN18.2 antibody. In one embodiment of the present technology, the donor and acceptor antibodies are monoclonal antibodies from different species. For example, the acceptor antibody is a human antibody (to minimize its antigenicity in a human), in which case the resulting CDR-grafted antibody is termed a "humanized" antibody.

[00175] Recombinant anti-CLDN18.2 antibodies, such as chimeric and humanized monoclonal antibodies, comprising both human and non-human portions, can be made using standard recombinant DNA techniques, and are within the scope of the present technology. For some uses, including *in vivo* use of the anti-CLDN18.2 antibody of the present technology in humans as well as use of these agents in *in vitro* detection assays, it is possible to use chimeric or humanized anti-CLDN18.2 antibodies. Such chimeric and humanized monoclonal antibodies can be produced by recombinant DNA techniques known in the art. Such useful methods include, *e.g.*, but are not limited to, methods described in International Application No. PCT/US86/02269; U.S. Pat. No. 5,225,539; European Patent No. 184187; European Patent No. 171496; European Patent No. 173494; PCT International Publication No. WO 86/01533; U.S. Pat. Nos. 4,816,567; 5,225,539; European Patent No. 125023; Better, *et al.*, 1988. *Science* 240: 1041-1043; Liu, *et al.*, 1987. *Proc. Natl. Acad. Sci.* USA 84: 3439-3443; Liu, *et al.*, 1987. *J. Immunol.* 139: 3521-3526; Sun, *et al.*, 1987.

Proc. Natl. Acad. Sci. USA 84: 214-218; Nishimura, *et al.*, 1987. *Cancer Res.* 47: 999-1005; Wood, *et al.*, 1985. *Nature* 314: 446-449; Shaw, *et al.*, 1988. *J. Natl. Cancer Inst.* 80: 1553-1559; Morrison (1985) *Science* 229: 1202-1207; Oi, *et al.* (1986) *BioTechniques* 4: 214; Jones, *et al.*, 1986. *Nature* 321: 552-525; Verhoeyan, *et al.*, 1988. *Science* 239: 1534; Morrison, *Science* 229: 1202, 1985; Oi *et al.*, *BioTechniques* 4: 214, 1986; Gillies *et al.*, *J. Immunol. Methods,* 125: 191-202, 1989; U.S. Pat. No. 5,807,715; and Beidler, *et al.*, 1988. *J. Immunol.* 141: 4053-4060. For example, antibodies can be humanized using a variety of techniques including CDR-grafting (EP 0 239 400; WO 91/09967; U.S. Pat. No. 5,530,101; 5,585,089; 5,859,205; 6,248,516; EP460167), veneering or resurfacing (EP 0 592 106; EP 0 519 596; Padlan E. A., *Molecular Immunology*, 28: 489-498, 1991; Studnicka *et al.*, *Protein Engineering* 7: 805-814, 1994; Roguska *et al.*, *PNAS* 91: 969-973, 1994), and chain shuffling (U.S. Pat. No. 5,565,332). In one embodiment, a cDNA encoding a murine anti-CLDN18.2 monoclonal antibody is digested with a restriction enzyme selected specifically to remove the sequence encoding the Fc constant region, and the equivalent portion of a cDNA encoding a human Fc constant region is substituted (*See* Robinson *et al.*, PCT/US86/02269; Akira *et al.*, European Patent Application 184,187; Taniguchi, European Patent Application 171,496; Morrison *et al.*, European Patent Application 173,494; Neuberger *et al.*, WO 86/01533; Cabilly *et al.* U.S. Patent No. 4,816,567; Cabilly *et al.*, European Patent Application 125,023; Better *et al.* (1988) *Science* 240: 1041-1043; Liu *et al.* (1987) *Proc. Natl. Acad. Sci.* USA 84: 3439-3443; Liu *et al.* (1987) *J Immunol* 139: 3521-3526; Sun *et al.* (1987) *Proc. Natl. Acad. Sci. USA* 84: 214-218; Nishimura *et al.* (1987) *Cancer Res* 47: 999-1005; Wood *et al.* (1985) *Nature* 314: 446-449; and Shaw *et al.* (1988) *J. Natl. Cancer Inst.* 80: 1553-1559; U.S. Pat. No. 6,180,370; U.S. Pat. Nos. 6,300,064; 6,696,248; 6,706,484; 6,828,422.

[00176] In one embodiment, the present technology provides the construction of humanized anti-CLDN18.2 antibodies that are unlikely to induce a human anti-mouse antibody (hereinafter referred to as "HAMA") response, while still having an effective antibody effector function. As used herein, the terms "human" and "humanized", in relation to antibodies, relate to any antibody which is expected to elicit a therapeutically tolerable weak immunogenic response in a human subject. In one embodiment, the present technology provides for a humanized anti-CLDN18.2 antibodies, heavy and light chain immunoglobulins.

[00177] *CDR Antibodies*. In some embodiments, the anti-CLDN18.2 antibody of the present technology is an anti-CLDN18.2 CDR antibody. Generally the donor and acceptor antibodies used to generate the anti-CLDN18.2 CDR antibody are monoclonal antibodies from different species; typically the acceptor antibody is a human antibody (to minimize its antigenicity in a human), in which case the resulting CDR-grafted antibody is termed a "humanized" antibody. The graft may be of a single CDR (or even a portion of a single CDR) within a single V_H or V_L of the acceptor antibody, or can be of multiple CDRs (or portions thereof) within one or both of the V_H and V_L. Frequently, all three CDRs in all variable domains of the acceptor antibody will be replaced with the corresponding donor CDRs, though one needs to replace only as many as necessary to permit adequate binding of the resulting CDR-grafted antibody to CLDN18.2 protein. Methods for generating CDR-grafted and humanized antibodies are taught by Queen *et al.* U.S. Pat. No. 5,585,089; U.S. Pat. No. 5,693,761; U.S. Pat. No. 5,693,762; and Winter U.S. 5,225,539; and EP 0682040. Methods useful to prepare V_H and V_L polypeptides are taught by Winter *et al.*, U.S. Pat. Nos. 4,816,397; 6,291,158; 6,291,159; 6,291,161; 6,545,142; EP 0368684; EP0451216; and EP0120694.

[00178] After selecting suitable framework region candidates from the same family and/or the same family member, either or both the heavy and light chain variable regions are produced by grafting the CDRs from the originating species into the hybrid framework regions. Assembly of hybrid antibodies or hybrid antibody fragments having hybrid variable chain regions with regard to either of the above aspects can be accomplished using conventional methods known to those skilled in the art. For example, DNA sequences encoding the hybrid variable domains described herein (*i.e.*, frameworks based on the target species and CDRs from the originating species) can be produced by oligonucleotide synthesis and/or PCR. The nucleic acid encoding CDR regions can also be isolated from the originating species antibodies using suitable restriction enzymes and ligated into the target species framework by ligating with suitable ligation enzymes. Alternatively, the framework regions of the variable chains of the originating species antibody can be changed by site-directed mutagenesis.

[00179] Since the hybrids are constructed from choices among multiple candidates corresponding to each framework region, there exist many combinations of sequences which are amenable to construction in accordance with the principles described herein. Accordingly, libraries of hybrids can be assembled having members with different

combinations of individual framework regions. Such libraries can be electronic database collections of sequences or physical collections of hybrids.

[00180] This process typically does not alter the acceptor antibody's FRs flanking the grafted CDRs. However, one skilled in the art can sometimes improve antigen binding affinity of the resulting anti-CLDN18.2 CDR-grafted antibody by replacing certain residues of a given FR to make the FR more similar to the corresponding FR of the donor antibody. Suitable locations of the substitutions include amino acid residues adjacent to the CDR, or which are capable of interacting with a CDR (*See*, *e.g.*, US 5,585,089, especially columns 12-16). Or one skilled in the art can start with the donor FR and modify it to be more similar to the acceptor FR or a human consensus FR. Techniques for making these modifications are known in the art. Particularly if the resulting FR fits a human consensus FR for that position, or is at least 90% or more identical to such a consensus FR, doing so may not increase the antigenicity of the resulting modified anti-CLDN18.2 CDR-grafted antibody significantly compared to the same antibody with a fully human FR.

[00181] *Fc Modifications*. In some embodiments, the anti-CLDN18.2 antibodies of the present technology comprise a variant Fc region, wherein said variant Fc region comprises at least one amino acid modification relative to a wild-type Fc region (or the parental Fc region), such that said molecule has an altered affinity for an Fc receptor (*e.g.*, an FcγR), provided that said variant Fc region does not have a substitution at positions that make a direct contact with Fc receptor based on crystallographic and structural analysis of Fc-Fc receptor interactions such as those disclosed by Sondermann *et al.*, *Nature*, 406:267-273 (2000). Examples of positions within the Fc region that make a direct contact with an Fc receptor such as an FcγR, include amino acids 234-239 (hinge region), amino acids 265-269 (B/C loop), amino acids 297-299 (C7E loop), and amino acids 327-332 (F/G) loop.

[00182] In some embodiments, an anti-CLDN18.2 antibody of the present technology has an altered affinity for activating and/or inhibitory receptors, having a variant Fc region with one or more amino acid modifications, wherein said one or more amino acid modification is a N297 substitution with alanine, or a K322 substitution with alanine. Additionally or alternatively, in some embodiments, the Fc regions of the CLDN18.2 antibodies disclosed herein comprise two amino acid substitutions, Leu234Ala and Leu235Ala (so called LALA mutations) to eliminate FcγRIIa binding. The LALA

mutations are commonly used to alleviate the cytokine induction from T cells, thus reducing toxicity of the antibodies (Wines BD, *et al.*, *J Immunol* 164:5313–5318 (2000)).

[00183] *Glycosylation Modifications*. In some embodiments, anti-CLDN18.2 antibodies of the present technology have an Fc region with variant glycosylation as compared to a parent Fc region. In some embodiments, variant glycosylation includes the absence of fucose; in some embodiments, variant glycosylation results from expression in GnT1-deficient CHO cells.

[00184] In some embodiments, the antibodies of the present technology, may have a modified glycosylation site relative to an appropriate reference antibody that binds to an antigen of interest (*e.g.,* CLDN18.2), without altering the functionality of the antibody, *e.g.*, binding activity to the antigen. As used herein, "glycosylation sites" include any specific amino acid sequence in an antibody to which an oligosaccharide (*i.e.*, carbohydrates containing two or more simple sugars linked together) will specifically and covalently attach.

[00185] Oligosaccharide side chains are typically linked to the backbone of an antibody *via* either N-or O-linkages. N-linked glycosylation refers to the attachment of an oligosaccharide moiety to the side chain of an asparagine residue. O-linked glycosylation refers to the attachment of an oligosaccharide moiety to a hydroxyamino acid, *e.g.*, serine, threonine. For example, an Fc-glycoform (hCLDN18.2-IgGln) that lacks certain oligosaccharides including fucose and terminal N- acetylglucosamine may be produced in special CHO cells and exhibit enhanced ADCC effector function.

[00186] In some embodiments, the carbohydrate content of an immunoglobulin-related composition disclosed herein is modified by adding or deleting a glycosylation site. Methods for modifying the carbohydrate content of antibodies are well known in the art and are included within the present technology, see, *e.g.*, U.S. Patent No. 6,218,149; EP 0359096B1; U.S. Patent Publication No. US 2002/0028486; International Patent Application Publication WO 03/035835; U.S. Patent Publication No. 2003/0115614; U.S. Patent No. 6,218,149; U.S. Patent No. 6,472,511; all of which are incorporated herein by reference in their entirety. In some embodiments, the carbohydrate content of an antibody (or relevant portion or component thereof) is modified by deleting one or more endogenous carbohydrate moieties of the antibody. In some certain embodiments, the present

technology includes deleting the glycosylation site of the Fc region of an antibody, by modifying position 297 from asparagine to alanine.

[00187] Engineered glycoforms may be useful for a variety of purposes, including but not limited to enhancing or reducing effector function. Engineered glycoforms may be generated by any method known to one skilled in the art, for example by using engineered or variant expression strains, by co-expression with one or more enzymes, for example N-acetylglucosaminyltransferase III (GnTIII), by expressing a molecule comprising an Fc region in various organisms or cell lines from various organisms, or by modifying carbohydrate(s) after the molecule comprising Fc region has been expressed. Methods for generating engineered glycoforms are known in the art, and include but are not limited to those described in Umana *et al.*, 1999, *Nat. Biotechnol.* 17: 176-180; Davies *et al.*, 2001, *Biotechnol. Bioeng.* 74:288-294; Shields *et al.*, 2002, *J. Biol. Chem.* 277:26733-26740; Shinkawa *et al.*, 2003, *J. Biol. Chem.* 278:3466-3473; U.S. Patent No. 6,602,684; U.S. Patent Application Serial No. 10/277,370; U.S. Patent Application Serial No. 10/113,929; International Patent Application Publications WO 00/61739A1 ; WO 01/292246A1; WO 02/311140A1; WO 02/30954A1; POTILLEGENT™ technology (Biowa, Inc. Princeton, N.J.); GLYCOMAB™ glycosylation engineering technology (GLYCART biotechnology AG, Zurich, Switzerland); each of which is incorporated herein by reference in its entirety. See, *e.g.*, International Patent Application Publication WO 00/061739; U.S. Patent Application Publication No. 2003/0115614; Okazaki *et al.*, 2004, *JMB*, 336: 1239-49.

[00188] *Fusion Proteins*. In one embodiment, the anti-CLDN18.2 antibody of the present technology is a fusion protein. The anti-CLDN18.2 antibodies of the present technology, when fused to a second protein, can be used as an antigenic tag. Examples of domains that can be fused to polypeptides include not only heterologous signal sequences, but also other heterologous functional regions. The fusion does not necessarily need to be direct, but can occur through linker sequences. Moreover, fusion proteins of the present technology can also be engineered to improve characteristics of the anti-CLDN18.2 antibodies. For instance, a region of additional amino acids, particularly charged amino acids, can be added to the N-terminus of the anti-CLDN18.2 antibody to improve stability and persistence during purification from the host cell or subsequent handling and storage. Also, peptide moieties can be added to an anti-CLDN18.2 antibody to facilitate purification. Such regions can be removed prior to final preparation of the anti-CLDN18.2 antibody. The addition of peptide moieties to facilitate handling of polypeptides are familiar and

routine techniques in the art. The anti-CLDN18.2 antibody of the present technology can be fused to marker sequences, such as a peptide which facilitates purification of the fused polypeptide. In select embodiments, the marker amino acid sequence is a hexa-histidine peptide, such as the tag provided in a pQE vector (QIAGEN, Inc., Chatsworth, Calif), among others, many of which are commercially available. As described in Gentz *et al.*, *Proc. Natl. Acad. Sci. USA* 86: 821-824, 1989, for instance, hexa-histidine provides for convenient purification of the fusion protein. Another peptide tag useful for purification, the "HA" tag, corresponds to an epitope derived from the influenza hemagglutinin protein. Wilson *et al.*, *Cell* 37: 767, 1984.

[00189] Thus, any of these above fusion proteins can be engineered using the polynucleotides or the polypeptides of the present technology. Also, in some embodiments, the fusion proteins described herein show an increased half-life *in vivo*.

[00190] Fusion proteins having disulfide-linked dimeric structures (due to the IgG) can be more efficient in binding and neutralizing other molecules compared to the monomeric secreted protein or protein fragment alone. Fountoulakis *et al.*, *J. Biochem.* 270: 3958-3964, 1995.

[00191] Similarly, EP-A-O 464 533 (Canadian counterpart 2045869) discloses fusion proteins comprising various portions of constant region of immunoglobulin molecules together with another human protein or a fragment thereof. In many cases, the Fc part in a fusion protein is beneficial in therapy and diagnosis, and thus can result in, *e.g.*, improved pharmacokinetic properties. *See* EP-A 0232 262. Alternatively, deleting or modifying the Fc part after the fusion protein has been expressed, detected, and purified, may be desired. For example, the Fc portion can hinder therapy and diagnosis if the fusion protein is used as an antigen for immunizations. In drug discovery, *e.g.*, human proteins, such as hIL-5, have been fused with Fc portions for the purpose of high-throughput screening assays to identify antagonists of hIL-5. Bennett *et al.*, *J. Molecular Recognition* 8: 52-58, 1995; Johanson *et al.*, *J. Biol. Chem.*, 270: 9459-9471, 1995.

[00192] *Labeled Anti-CLDN18.2 antibodies*. In one embodiment, the anti-CLDN18.2 antibody of the present technology is coupled with a label moiety, *i.e.*, detectable group. The particular label or detectable group conjugated to the anti-CLDN18.2 antibody is not a critical aspect of the technology, so long as it does not significantly interfere with the specific binding of the anti-CLDN18.2 antibody of the present technology to the CLDN18.2

protein. The detectable group can be any material having a detectable physical or chemical property. Such detectable labels have been well-developed in the field of immunoassays and imaging. In general, almost any label useful in such methods can be applied to the present technology. Thus, a label is any composition detectable by spectroscopic, photochemical, biochemical, immunochemical, electrical, optical or chemical means. Labels useful in the practice of the present technology include magnetic beads (*e.g.*, Dynabeads™), fluorescent dyes (*e.g.*, fluorescein isothiocyanate, Texas red, rhodamine, and the like), radiolabels (*e.g.*, ^{3}H, ^{14}C, ^{35}S, ^{125}I, ^{121}I, ^{131}I, ^{112}In, ^{99}mTc), other imaging agents such as microbubbles (for ultrasound imaging), ^{18}F, ^{11}C, ^{15}O, ^{89}Zr (for Positron emission tomography), ^{99m}TC, ^{111}In (for Single photon emission tomography), enzymes (*e.g.*, horse radish peroxidase, alkaline phosphatase and others commonly used in an ELISA), and calorimetric labels such as colloidal gold or colored glass or plastic (*e.g.*, polystyrene, polypropylene, latex, and the like) beads. Patents that describe the use of such labels include U.S. Pat. Nos. 3,817,837; 3,850,752; 3,939,350; 3,996,345; 4,277,437; 4,275,149; and 4,366,241, each incorporated herein by reference in their entirety and for all purposes. *See also* Handbook of Fluorescent Probes and Research Chemicals (6^{th} Ed., Molecular Probes, Inc., Eugene OR.).

[00193] The label can be coupled directly or indirectly to the desired component of an assay according to methods well known in the art. As indicated above, a wide variety of labels can be used, with the choice of label depending on factors such as required sensitivity, ease of conjugation with the compound, stability requirements, available instrumentation, and disposal provisions.

[00194] Non-radioactive labels are often attached by indirect means. Generally, a ligand molecule (*e.g.*, biotin) is covalently bound to the molecule. The ligand then binds to an anti-ligand (*e.g.*, streptavidin) molecule which is either inherently detectable or covalently bound to a signal system, such as a detectable enzyme, a fluorescent compound, or a chemiluminescent compound. A number of ligands and anti-ligands can be used. Where a ligand has a natural anti-ligand, *e.g.*, biotin, thyroxine, and cortisol, it can be used in conjunction with the labeled, naturally-occurring anti-ligands. Alternatively, any haptenic or antigenic compound can be used in combination with an antibody, *e.g.*, an anti-CLDN18.2 antibody.

[00195] The molecules can also be conjugated directly to signal generating compounds, *e.g.*, by conjugation with an enzyme or fluorophore. Enzymes of interest as labels will primarily be hydrolases, particularly phosphatases, esterases and glycosidases, or oxidoreductases, particularly peroxidases. Fluorescent compounds useful as labeling moieties, include, but are not limited to, *e.g.*, fluorescein and its derivatives, rhodamine and its derivatives, dansyl, umbelliferone, and the like. Chemiluminescent compounds useful as labeling moieties, include, but are not limited to, *e.g.*, luciferin, and 2,3-dihydrophthalazinediones, *e.g.*, luminol. For a review of various labeling or signal-producing systems which can be used, see U.S. Pat. No. 4,391,904.

[00196] Means of detecting labels are well known to those of skill in the art. Thus, for example, where the label is a radioactive label, means for detection include a scintillation counter or photographic film as in autoradiography. Where the label is a fluorescent label, it can be detected by exciting the fluorochrome with the appropriate wavelength of light and detecting the resulting fluorescence. The fluorescence can be detected visually, by means of photographic film, by the use of electronic detectors such as charge coupled devices (CCDs) or photomultipliers and the like. Similarly, enzymatic labels can be detected by providing the appropriate substrates for the enzyme and detecting the resulting reaction product. Finally, simple colorimetric labels can be detected simply by observing the color associated with the label. Thus, in various dipstick assays, conjugated gold often appears pink, while various conjugated beads appear the color of the bead.

[00197] Some assay formats do not require the use of labeled components. For instance, agglutination assays can be used to detect the presence of the target antibodies, *e.g.*, the anti-CLDN18.2 antibodies. In this case, antigen-coated particles are agglutinated by samples comprising the target antibodies. In this format, none of the components need be labeled and the presence of the target antibody is detected by simple visual inspection.

B. Identifying and Characterizing the Anti-CLDN18.2 Antibodies of the Present Technology

[00198] *Methods for identifying and/or screening the anti-CLDN18.2 antibodies of the present technology.* Methods useful to identify and screen antibodies against CLDN18.2 polypeptides for those that possess the desired specificity to CLDN18.2 protein (*e.g.*, those that bind to the first extracellular loop of CLDN18.2 protein, such as polypeptides comprising the amino acid sequence of SEQ ID NO: 2) include any immunologically-mediated techniques known within the art. Components of an immune response can be

detected *in vitro* by various methods that are well known to those of ordinary skill in the art. For example, (1) cytotoxic T lymphocytes can be incubated with radioactively labeled target cells and the lysis of these target cells detected by the release of radioactivity; (2) helper T lymphocytes can be incubated with antigens and antigen presenting cells and the synthesis and secretion of cytokines measured by standard methods (Windhagen A *et al.*, *Immunity,* 2: 373-80, 1995); (3) antigen presenting cells can be incubated with whole protein antigen and the presentation of that antigen on MHC detected by either T lymphocyte activation assays or biophysical methods (Harding *et al.*, *Proc. Natl. Acad. Sci.*, 86: 4230-4, 1989); (4) mast cells can be incubated with reagents that cross-link their Fc-epsilon receptors and histamine release measured by enzyme immunoassay (Siraganian *et al.*, *TIPS,* 4: 432-437, 1983); and (5) enzyme-linked immunosorbent assay (ELISA).

[00199] Similarly, products of an immune response in either a model organism (*e.g.*, mouse) or a human subject can also be detected by various methods that are well known to those of ordinary skill in the art. For example, (1) the production of antibodies in response to vaccination can be readily detected by standard methods currently used in clinical laboratories, *e.g.*, an ELISA; (2) the migration of immune cells to sites of inflammation can be detected by scratching the surface of skin and placing a sterile container to capture the migrating cells over scratch site (Peters *et al.*, *Blood,* 72: 1310-5, 1988); (3) the proliferation of peripheral blood mononuclear cells (PBMCs) in response to mitogens or mixed lymphocyte reaction can be measured using ^{3}H-thymidine; (4) the phagocytic capacity of granulocytes, macrophages, and other phagocytes in PBMCs can be measured by placing PBMCs in wells together with labeled particles (Peters *et al.*, *Blood,* 72: 1310-5, 1988); and (5) the differentiation of immune system cells can be measured by labeling PBMCs with antibodies to CD molecules such as CD4 and CD8 and measuring the fraction of the PBMCs expressing these markers.

[00200] In one embodiment, anti-CLDN18.2 antibodies of the present technology are selected using display of CLDN18.2 peptides on the surface of replicable genetic packages. *See*, *e.g.*, U.S. Pat. Nos. 5,514,548; 5,837,500; 5,871,907; 5,885,793; 5,969,108; 6,225,447; 6,291,650; 6,492,160; EP 585 287; EP 605522; EP 616640; EP 1024191; EP 589 877; EP 774 511; EP 844 306. Methods useful for producing/selecting a filamentous bacteriophage particle containing a phagemid genome encoding for a binding molecule with a desired specificity has been described. *See*, *e.g.*, EP 774 511; US 5871907; US 5969108; US 6225447; US 6291650; US 6492160.

[00201] In some embodiments, anti-CLDN18.2 antibodies of the present technology are selected using display of CLDN18.2 peptides on the surface of a yeast host cell. Methods useful for the isolation of scFv polypeptides by yeast surface display have been described by Kieke *et al.*, *Protein Eng.* 1997 Nov; 10(11): 1303-10.

[00202] In some embodiments, anti-CLDN18.2 antibodies of the present technology are selected using ribosome display. Methods useful for identifying ligands in peptide libraries using ribosome display have been described by Mattheakis *et al.*, *Proc. Natl. Acad. Sci. USA* 91: 9022-26, 1994; and Hanes *et al.*, *Proc. Natl. Acad. Sci. USA* 94: 4937-42, 1997.

[00203] In certain embodiments, anti-CLDN18.2 antibodies of the present technology are selected using tRNA display of CLDN18.2 peptides. Methods useful for *in vitro* selection of ligands using tRNA display have been described by Merryman *et al.*, *Chem. Biol.*, 9: 741-46, 2002.

[00204] In one embodiment, anti-CLDN18.2 antibodies of the present technology are selected using RNA display. Methods useful for selecting peptides and proteins using RNA display libraries have been described by Roberts *et al. Proc. Natl. Acad. Sci. USA*, 94: 12297-302, 1997; and Nemoto *et al.*, *FEBS Lett.*, 414: 405-8, 1997. Methods useful for selecting peptides and proteins using unnatural RNA display libraries have been described by Frankel *et al.*, *Curr. Opin. Struct. Biol.*, 13: 506-12, 2003.

[00205] In some embodiments, anti-CLDN18.2 antibodies of the present technology are expressed in the periplasm of gram negative bacteria and mixed with labeled CLDN18.2 protein. *See* WO 02/34886. In clones expressing recombinant polypeptides with affinity for CLDN18.2 protein, the concentration of the labeled CLDN18.2 protein bound to the anti-CLDN18.2 antibodies is increased and allows the cells to be isolated from the rest of the library as described in Harvey *et al.*, *Proc. Natl. Acad. Sci.* 22: 9193-98 2004 and U.S. Pat. Publication No. 2004/0058403.

[00206] After selection of the desired anti-CLDN18.2 antibodies, it is contemplated that said antibodies can be produced in large volume by any technique known to those skilled in the art, *e.g.*, prokaryotic or eukaryotic cell expression and the like. The anti-CLDN18.2 antibodies which are, *e.g.*, but not limited to, anti-CLDN18.2 hybrid antibodies or fragments can be produced by using conventional techniques to construct an expression vector that encodes an antibody heavy chain in which the CDRs and, if necessary, a minimal portion of the variable region framework, that are required to retain original species antibody binding

specificity (as engineered according to the techniques described herein) are derived from the originating species antibody and the remainder of the antibody is derived from a target species immunoglobulin which can be manipulated as described herein, thereby producing a vector for the expression of a hybrid antibody heavy chain.

[00207] *Measurement of CLDN18.2 Binding.* In some embodiments, a CLDN18.2 binding assay refers to an assay format wherein CLDN18.2 protein and an anti-CLDN18.2 antibody are mixed under conditions suitable for binding between the CLDN18.2 protein and the anti-CLDN18.2 antibody and assessing the amount of binding between the CLDN18.2 protein and the anti-CLDN18.2 antibody. The amount of binding is compared with a suitable control, which can be the amount of binding in the absence of the CLDN18.2 protein, the amount of the binding in the presence of a non-specific immunoglobulin composition, or both. The amount of binding can be assessed by any suitable method. Binding assay methods include, *e.g.*, ELISA, radioimmunoassays, scintillation proximity assays, fluorescence energy transfer assays, liquid chromatography, membrane filtration assays, and the like. Biophysical assays for the direct measurement of CLDN18.2 protein binding to anti-CLDN18.2 antibody are, *e.g.*, nuclear magnetic resonance, fluorescence, fluorescence polarization, surface plasmon resonance (BIACORE chips) and the like. Specific binding is determined by standard assays known in the art, *e.g.*, radioligand binding assays, ELISA, FRET, immunoprecipitation, SPR, NMR (2D-NMR), mass spectroscopy and the like. If the specific binding of a candidate anti-CLDN18.2 antibody is at least 1 percent greater than the binding observed in the absence of the candidate anti-CLDN18.2 antibody, the candidate anti-CLDN18.2 antibody is useful as an anti-CLDN18.2 antibody of the present technology.

Uses of the Anti-CLDN18.2 Antibodies of the Present Technology

[00208] *General.* The anti-CLDN18.2 antibodies of the present technology are useful in methods known in the art relating to the localization and/or quantitation of CLDN18.2 protein (*e.g.*, for use in measuring levels of the CLDN18.2 protein within appropriate physiological samples, for use in diagnostic methods, for use in imaging the polypeptide, and the like). Antibodies of the present technology are useful to isolate a CLDN18.2 protein by standard techniques, such as affinity chromatography or immunoprecipitation. An anti-CLDN18.2 antibody of the present technology can facilitate the purification of natural immunoreactive CLDN18.2 proteins from biological samples, *e.g.*, mammalian sera

or cells as well as recombinantly-produced immunoreactive CLDN18.2 proteins expressed in a host system. Moreover, anti-CLDN18.2 antibodies can be used to detect an immunoreactive CLDN18.2 protein (*e.g.*, in plasma, a cellular lysate or cell supernatant) in order to evaluate the abundance and pattern of expression of the immunoreactive polypeptide. The anti-CLDN18.2 antibodies of the present technology can be used diagnostically to monitor immunoreactive CLDN18.2 protein levels in tissue as part of a clinical testing procedure, *e.g.*, to determine the efficacy of a given treatment regimen. As noted above, the detection can be facilitated by coupling (*i.e.*, physically linking) the anti-CLDN18.2 antibodies of the present technology to a detectable substance.

[00209] *Detection of CLDN18.2 protein.* An exemplary method for detecting the presence or absence of an immunoreactive CLDN18.2 protein in a biological sample involves obtaining a biological sample from a test subject and contacting the biological sample with an anti-CLDN18.2 antibody of the present technology capable of detecting an immunoreactive CLDN18.2 protein such that the presence of an immunoreactive CLDN18.2 protein is detected in the biological sample. Detection may be accomplished by means of a detectable label attached to the antibody.

[00210] The term "labeled" with regard to the anti-CLDN18.2 antibody is intended to encompass direct labeling of the antibody by coupling (*i.e.*, physically linking) a detectable substance to the antibody, as well as indirect labeling of the antibody by reactivity with another compound that is directly labeled, such as a secondary antibody. Examples of indirect labeling include detection of a primary antibody using a fluorescently-labeled secondary antibody and end-labeling of a DNA probe with biotin such that it can be detected with fluorescently-labeled streptavidin.

[00211] In some embodiments, the anti-CLDN18.2 antibodies disclosed herein are conjugated to one or more detectable labels. For such uses, anti-CLDN18.2 antibodies may be detectably labeled by covalent or non-covalent attachment of a chromogenic, enzymatic, radioisotopic, isotopic, fluorescent, toxic, chemiluminescent, nuclear magnetic resonance contrast agent or other label.

[00212] Examples of suitable chromogenic labels include diaminobenzidine and 4-hydroxyazo-benzene-2-carboxylic acid. Examples of suitable enzyme labels include malate dehydrogenase, staphylococcal nuclease, Δ-5-steroid isomerase, yeast-alcohol dehydrogenase, α-glycerol phosphate dehydrogenase, triose phosphate isomerase,

peroxidase, alkaline phosphatase, asparaginase, glucose oxidase, β-galactosidase, ribonuclease, urease, catalase, glucose-6-phosphate dehydrogenase, glucoamylase, and acetylcholine esterase.

[00213] Examples of suitable radioisotopic labels include ^{3}H, ^{111}In, ^{125}I, ^{131}I, ^{32}P, ^{35}S, ^{14}C, ^{51}Cr, ^{57}To, ^{58}Co, ^{59}Fe, ^{75}Se, ^{152}Eu, ^{90}Y, ^{67}Cu, ^{217}Ci, ^{211}At, ^{212}Pb, ^{47}Sc, ^{109}Pd, *etc.* ^{111}In is an exemplary isotope where *in vivo* imaging is used since its avoids the problem of dehalogenation of the ^{125}I or ^{131}I-labeled CLDN18.2-binding antibodies by the liver. In addition, this isotope has a more favorable gamma emission energy for imaging (Perkins *et al*, *Eur. J. Nucl. Med.* 70:296-301 (1985); Carasquillo *et al.*, *J. Nucl. Med.* 25:281-287 (1987)). For example, ^{111}In coupled to monoclonal antibodies with 1-(P-isothiocyanatobenzyl)-DPTA exhibits little uptake in non-tumorous tissues, particularly the liver, and enhances specificity of tumor localization (Esteban *et al.*, *J. Nucl. Med.* 28:861-870 (1987)). Examples of suitable non-radioactive isotopic labels include ^{157}Gd, ^{55}Mn, ^{162}Dy, ^{52}Tr, and ^{56}Fe.

[00214] Examples of suitable fluorescent labels include an ^{152}Eu label, a fluorescein label, an isothiocyanate label, a rhodamine label, a phycoerythrin label, a phycocyanin label, an allophycocyanin label, a Green Fluorescent Protein (GFP) label, an o-phthaldehyde label, and a fluorescamine label. Examples of suitable toxin labels include diphtheria toxin, ricin, and cholera toxin.

[00215] Examples of chemiluminescent labels include a luminol label, an isoluminol label, an aromatic acridinium ester label, an imidazole label, an acridinium salt label, an oxalate ester label, a luciferin label, a luciferase label, and an aequorin label. Examples of nuclear magnetic resonance contrasting agents include heavy metal nuclei such as Gd, Mn, and iron.

[00216] The detection method of the present technology can be used to detect an immunoreactive CLDN18.2 protein in a biological sample *in vitro* as well as *in vivo*. *In vitro* techniques for detection of an immunoreactive CLDN18.2 protein include enzyme linked immunosorbent assays (ELISAs), Western blots, immunoprecipitations, radioimmunoassay, and immunofluorescence. Furthermore, *in vivo* techniques for detection of an immunoreactive CLDN18.2 protein include introducing into a subject a labeled anti-CLDN18.2 antibody. For example, the anti-CLDN18.2 antibody can be labeled with a radioactive marker whose presence and location in a subject can be detected by standard

imaging techniques. In one embodiment, the biological sample contains CLDN18.2 protein molecules from the test subject.

[00217] *Immunoassay and Imaging.* An anti-CLDN18.2 antibody of the present technology can be used to assay immunoreactive CLDN18.2 protein levels in a biological sample (*e.g.*, human plasma) using antibody-based techniques. For example, protein expression in tissues can be studied with classical immunohistological methods. Jalkanen, M. *et al.*, *J. Cell. Biol.* 101: 976-985, 1985; Jalkanen, M. *et al.*, *J. Cell. Biol.* 105: 3087-3096, 1987. Other antibody-based methods useful for detecting protein gene expression include immunoassays, such as the enzyme linked immunosorbent assay (ELISA) and the radioimmunoassay (RIA). Suitable antibody assay labels are known in the art and include enzyme labels, such as, glucose oxidase, and radioisotopes or other radioactive agent, such as iodine (^{125}I, ^{121}I, ^{131}I), carbon (^{14}C), sulfur (^{35}S), tritium (^{3}H), indium (^{112}In), and technetium (^{99}mTc), and fluorescent labels, such as fluorescein, rhodamine, and green fluorescent protein (GFP), as well as biotin.

[00218] In addition to assaying immunoreactive CLDN18.2 protein levels in a biological sample, anti-CLDN18.2 antibodies of the present technology may be used for *in vivo* imaging of CLDN18.2. Antibodies useful for this method include those detectable by X-radiography, NMR or ESR. For X-radiography, suitable labels include radioisotopes such as barium or cesium, which emit detectable radiation but are not overtly harmful to the subject. Suitable markers for NMR and ESR include those with a detectable characteristic spin, such as deuterium, which can be incorporated into the anti-CLDN18.2 antibodies by labeling of nutrients for the relevant scFv clone.

[00219] An anti-CLDN18.2 antibody which has been labeled with an appropriate detectable imaging moiety, such as a radioisotope (*e.g.*, ^{131}I, ^{112}In, ^{99}mTc), a radio-opaque substance, or a material detectable by nuclear magnetic resonance, is introduced (*e.g.*, parenterally, subcutaneously, or intraperitoneally) into the subject. It will be understood in the art that the size of the subject and the imaging system used will determine the quantity of imaging moiety needed to produce diagnostic images. In the case of a radioisotope moiety, for a human subject, the quantity of radioactivity injected will normally range from about 5 to 20 millicuries of ^{99}mTc. The labeled anti-CLDN18.2 antibody will then accumulate at the location of cells which contain the specific target polypeptide. For

example, labeled anti-CLDN18.2 antibodies of the present technology will accumulate within the subject in cells and tissues in which the CLDN18.2 protein has localized.

[00220] Thus, the present technology provides a diagnostic method of a medical condition, which involves: (a) assaying the expression of immunoreactive CLDN18.2 protein by measuring binding of an anti-CLDN18.2 antibody of the present technology in cells or body fluid of an individual; (b) comparing the amount of immunoreactive CLDN18.2 protein present in the sample with a standard reference, wherein an increase or decrease in immunoreactive CLDN18.2 protein levels compared to the standard is indicative of a medical condition.

[00221] *Affinity Purification.* The anti-CLDN18.2 antibodies of the present technology may be used to purify immunoreactive CLDN18.2 protein from a sample. In some embodiments, the antibodies are immobilized on a solid support. Examples of such solid supports include plastics such as polycarbonate, complex carbohydrates such as agarose and sepharose, acrylic resins and such as polyacrylamide and latex beads. Techniques for coupling antibodies to such solid supports are well known in the art (Weir *et al.*, "Handbook of Experimental Immunology" 4th Ed., Blackwell Scientific Publications, Oxford, England, Chapter 10 (1986); Jacoby *et al.*, *Meth. Enzym.* 34 Academic Press, N.Y. (1974)).

[00222] The simplest method to bind the antigen to the antibody-support matrix is to collect the beads in a column and pass the antigen solution down the column. The efficiency of this method depends on the contact time between the immobilized antibody and the antigen, which can be extended by using low flow rates. The immobilized antibody captures the antigen as it flows past. Alternatively, an antigen can be contacted with the antibody-support matrix by mixing the antigen solution with the support (*e.g.*, beads) and rotating or rocking the slurry, allowing maximum contact between the antigen and the immobilized antibody. After the binding reaction has been completed, the slurry is passed into a column for collection of the beads. The beads are washed using a suitable washing buffer and then the pure or substantially pure antigen is eluted.

[00223] An antibody or polypeptide of interest can be conjugated to a solid support, such as a bead. In addition, a first solid support such as a bead can also be conjugated, if desired, to a second solid support, which can be a second bead or other support, by any suitable means, including those disclosed herein for conjugation of a polypeptide to a support. Accordingly, any of the conjugation methods and means disclosed herein with reference to

conjugation of a polypeptide to a solid support can also be applied for conjugation of a first support to a second support, where the first and second solid support can be the same or different.

[00224] Appropriate linkers, which can be cross-linking agents, for use for conjugating a polypeptide to a solid support include a variety of agents that can react with a functional group present on a surface of the support, or with the polypeptide, or both. Reagents useful as cross-linking agents include homo-bi-functional and, in particular, hetero-bi-functional reagents. Useful bi-functional cross-linking agents include, but are not limited to, *N*-SIAB, dimaleimide, DTNB, N-SATA, N-SPDP, SMCC and 6-HYNIC. A cross-linking agent can be selected to provide a selectively cleavable bond between a polypeptide and the solid support. For example, a photolabile cross-linker, such as 3-amino-(2-nitrophenyl)propionic acid can be employed as a means for cleaving a polypeptide from a solid support. (Brown *et al., Mol. Divers*, pp, 4-12 (1995); Rothschild *et al., Nucl. Acids Res.,* 24:351-66 (1996); and US. Pat. No. 5,643,722). Other cross-linking reagents are well-known in the art. (*See*, *e.g.*, Wong (1991), *supra*; and Hermanson (1996), *supra*).

[00225] An antibody or polypeptide can be immobilized on a solid support, such as a bead, through a covalent amide bond formed between a carboxyl group functionalized bead and the amino terminus of the polypeptide or, conversely, through a covalent amide bond formed between an amino group functionalized bead and the carboxyl terminus of the polypeptide. In addition, a bi-functional trityl linker can be attached to the support, *e.g.*, to the 4-nitrophenyl active ester on a resin, such as a Wang resin, through an amino group or a carboxyl group on the resin *via* an amino resin. Using a bi-functional trityl approach, the solid support can require treatment with a volatile acid, such as formic acid or trifluoroacetic acid to ensure that the polypeptide is cleaved and can be removed. In such a case, the polypeptide can be deposited as a beadless patch at the bottom of a well of a solid support or on the flat surface of a solid support. After addition of a matrix solution, the polypeptide can be desorbed into a MS.

[00226] Hydrophobic trityl linkers can also be exploited as acid-labile linkers by using a volatile acid or an appropriate matrix solution, *e.g.*, a matrix solution containing 3-HPA, to cleave an amino linked trityl group from the polypeptide. Acid lability can also be changed. For example, trityl, monomethoxytrityl, dimethoxytrityl or trimethoxytrityl can be changed to the appropriate *p*-substituted, or more acid-labile tritylamine derivatives, of the

polypeptide, *i.e.*, trityl ether and tritylamine bonds can be made to the polypeptide. Accordingly, a polypeptide can be removed from a hydrophobic linker, *e.g.*, by disrupting the hydrophobic attraction or by cleaving tritylether or tritylamine bonds under acidic conditions, including, if desired, under typical MS conditions, where a matrix, such as 3-HPA acts as an acid.

[00227] Orthogonally cleavable linkers can also be useful for binding a first solid support, *e.g.*, a bead to a second solid support, or for binding a polypeptide of interest to a solid support. Using such linkers, a first solid support, *e.g.*, a bead, can be selectively cleaved from a second solid support, without cleaving the polypeptide from the support; the polypeptide then can be cleaved from the bead at a later time. For example, a disulfide linker, which can be cleaved using a reducing agent, such as DTT, can be employed to bind a bead to a second solid support, and an acid cleavable bi-functional trityl group could be used to immobilize a polypeptide to the support. As desired, the linkage of the polypeptide to the solid support can be cleaved first, *e.g.*, leaving the linkage between the first and second support intact. Trityl linkers can provide a covalent or hydrophobic conjugation and, regardless of the nature of the conjugation, the trityl group is readily cleaved in acidic conditions.

[00228] For example, a bead can be bound to a second support through a linking group which can be selected to have a length and a chemical nature such that high density binding of the beads to the solid support, or high density binding of the polypeptides to the beads, is promoted. Such a linking group can have, *e.g.*, "tree-like" structure, thereby providing a multiplicity of functional groups per attachment site on a solid support. Examples of such linking group; include polylysine, polyglutamic acid, penta-erythrole and *tris*-hydroxy-aminomethane.

[00229] *Noncovalent Binding Association.* An antibody or polypeptide can be conjugated to a solid support, or a first solid support can also be conjugated to a second solid support, through a noncovalent interaction. For example, a magnetic bead made of a ferromagnetic material, which is capable of being magnetized, can be attracted to a magnetic solid support, and can be released from the support by removal of the magnetic field. Alternatively, the solid support can be provided with an ionic or hydrophobic moiety, which can allow the interaction of an ionic or hydrophobic moiety, respectively, with a

polypeptide, *e.g.*, a polypeptide containing an attached trityl group or with a second solid support having hydrophobic character.

[00230] A solid support can also be provided with a member of a specific binding pair and, therefore, can be conjugated to a polypeptide or a second solid support containing a complementary binding moiety. For example, a bead coated with avidin or with streptavidin can be bound to a polypeptide having a biotin moiety incorporated therein, or to a second solid support coated with biotin or derivative of biotin, such as iminobiotin.

[00231] It should be recognized that any of the binding members disclosed herein or otherwise known in the art can be reversed. Thus, biotin, *e.g.*, can be incorporated into either a polypeptide or a solid support and, conversely, avidin or other biotin binding moiety would be incorporated into the support or the polypeptide, respectively. Other specific binding pairs contemplated for use herein include, but are not limited to, hormones and their receptors, enzyme, and their substrates, a nucleotide sequence and its complementary sequence, an antibody and the antigen to which it interacts specifically, and other such pairs knows to those skilled in the art.

A. Diagnostic Uses of Anti-CLDN18.2 Antibodies of the Present Technology

[00232] *General.* The anti-CLDN18.2 antibodies of the present technology are useful in diagnostic methods. As such, the present technology provides methods using the antibodies in the diagnosis of CLDN18.2 activity in a subject. Anti-CLDN18.2 antibodies of the present technology may be selected such that they have any level of epitope binding specificity and very high binding affinity to a CLDN18.2 protein. In general, the higher the binding affinity of an antibody the more stringent wash conditions can be performed in an immunoassay to remove nonspecifically bound material without removing target polypeptide. Accordingly, anti-CLDN18.2 antibodies of the present technology useful in diagnostic assays usually have binding affinities of about 10^8 M^{-1}, 10^9 M^{-1}, 10^{10} M^{-1}, 10^{11} M^{-1} or 10^{12} M^{-1}. Further, it is desirable that anti-CLDN18.2 antibodies used as diagnostic reagents have a sufficient kinetic on-rate to reach equilibrium under standard conditions in at least 12 h, at least five (5) h, or at least one (1) hour.

[00233] Anti-CLDN18.2 antibodies can be used to detect an immunoreactive CLDN18.2 protein in a variety of standard assay formats. Such formats include immunoprecipitation, Western blotting, ELISA, radioimmunoassay, and immunometric assays. *See* Harlow & Lane, *Antibodies, A Laboratory Manual* (Cold Spring Harbor Publications, New York,

1988); U.S. Pat. Nos. 3,791,932; 3,839,153; 3,850,752; 3,879,262; 4,034,074, 3,791,932; 3,817,837; 3,839,153; 3,850,752; 3,850,578; 3,853,987; 3,867,517; 3,879,262; 3,901,654; 3,935,074; 3,984,533; 3,996,345; 4,034,074; and 4,098,876. Biological samples can be obtained from any tissue or body fluid of a subject. In certain embodiments, the subject is at an early stage of cancer. In one embodiment, the early stage of cancer is determined by the level or expression pattern of CLDN18.2 protein in a sample obtained from the subject. In certain embodiments, the sample is selected from the group consisting of urine, blood, serum, plasma, saliva, amniotic fluid, cerebrospinal fluid (CSF), and biopsied body tissue.

[00234] Immunometric or sandwich assays are one format for the diagnostic methods of the present technology. *See* U.S. Pat. No. 4,376,110, 4,486,530, 5,914,241, and 5,965,375. Such assays use one antibody, *e.g.*, an anti-CLDN18.2 antibody or a population of anti-CLDN18.2 antibodies immobilized to a solid phase, and another anti-CLDN18.2 antibody or a population of anti-CLDN18.2 antibodies in solution. Typically, the solution anti-CLDN18.2 antibody or population of anti-CLDN18.2 antibodies is labeled. If an antibody population is used, the population can contain antibodies binding to different epitope specificities within the target polypeptide. Accordingly, the same population can be used for both solid phase and solution antibody. If anti-CLDN18.2 monoclonal antibodies are used, first and second CLDN18.2 monoclonal antibodies having different binding specificities are used for the solid and solution phase. Solid phase (also referred to as "capture") and solution (also referred to as "detection") antibodies can be contacted with target antigen in either order or simultaneously. If the solid phase antibody is contacted first, the assay is referred to as being a forward assay. Conversely, if the solution antibody is contacted first, the assay is referred to as being a reverse assay. If the target is contacted with both antibodies simultaneously, the assay is referred to as a simultaneous assay. After contacting the CLDN18.2 protein with the anti-CLDN18.2 antibody, a sample is incubated for a period that usually varies from about 10 min to about 24 hr and is usually about 1 hr. A wash step is then performed to remove components of the sample not specifically bound to the anti-CLDN18.2 antibody being used as a diagnostic reagent. When solid phase and solution antibodies are bound in separate steps, a wash can be performed after either or both binding steps. After washing, binding is quantified, typically by detecting a label linked to the solid phase through binding of labeled solution antibody. Usually for a given pair of antibodies or populations of antibodies and given reaction conditions, a calibration curve is prepared from samples containing known concentrations of target antigen. Concentrations

of the immunoreactive CLDN18.2 protein in samples being tested are then read by interpolation from the calibration curve (*i.e.*, standard curve). Analyte can be measured either from the amount of labeled solution antibody bound at equilibrium or by kinetic measurements of bound labeled solution antibody at a series of time points before equilibrium is reached. The slope of such a curve is a measure of the concentration of the CLDN18.2 protein in a sample.

[00235] Suitable supports for use in the above methods include, *e.g.*, nitrocellulose membranes, nylon membranes, and derivatized nylon membranes, and also particles, such as agarose, a dextran-based gel, dipsticks, particulates, microspheres, magnetic particles, test tubes, microtiter wells, SEPHADEX™ (Amersham Pharmacia Biotech, Piscataway N.J.), and the like. Immobilization can be by absorption or by covalent attachment. Optionally, anti-CLDN18.2 antibodies can be joined to a linker molecule, such as biotin for attachment to a surface bound linker, such as avidin.

[00236] In some embodiments, the present disclosure provides an anti-CLDN18.2 antibody of the present technology conjugated to a diagnostic agent. The diagnostic agent may comprise a radioactive or non-radioactive label, a contrast agent (such as for magnetic resonance imaging, computed tomography or ultrasound), and the radioactive label can be a gamma-, beta-, alpha-, Auger electron-, or positron-emitting isotope. A diagnostic agent is a molecule which is administered conjugated to an antibody moiety, *i.e.*, antibody or antibody fragment, or subfragment, and is useful in diagnosing or detecting a disease by locating the cells containing the antigen.

[00237] Useful diagnostic agents include, but are not limited to, radioisotopes, dyes (such as with the biotin-streptavidin complex), contrast agents, fluorescent compounds or molecules and enhancing agents (*e.g.*, paramagnetic ions) for magnetic resonance imaging (MRI). U.S. Pat. No. 6,331,175 describes MRI technique and the preparation of antibodies conjugated to a MRI enhancing agent and is incorporated in its entirety by reference. In some embodiments, the diagnostic agents are selected from the group consisting of radioisotopes, enhancing agents for use in magnetic resonance imaging, and fluorescent compounds. In order to load an antibody component with radioactive metals or paramagnetic ions, it may be necessary to react it with a reagent having a long tail to which are attached a multiplicity of chelating groups for binding the ions. Such a tail can be a polymer such as a polylysine, polysaccharide, or other derivatized or derivatizable chain

having pendant groups to which can be bound chelating groups such as, *e.g.*, ethylenediaminetetraacetic acid (EDTA), diethylenetriaminepentaacetic acid (DTPA), porphyrins, polyamines, crown ethers, bis-thiosemicarbazones, polyoximes, and like groups known to be useful for this purpose. Chelates may be coupled to the antibodies of the present technology using standard chemistries. The chelate is normally linked to the antibody by a group which enables formation of a bond to the molecule with minimal loss of immunoreactivity and minimal aggregation and/or internal cross-linking. Other methods and reagents for conjugating chelates to antibodies are disclosed in U.S. Pat. No. 4,824,659. Particularly useful metal-chelate combinations include 2-benzyl-DTPA and its monomethyl and cyclohexyl analogs, used with diagnostic isotopes for radio-imaging. The same chelates, when complexed with non-radioactive metals, such as manganese, iron and gadolinium are useful for MRI, when used along with the CLDN18.2 antibodies of the present technology. Macrocyclic chelates such as NOTA (1,4,7-triaza-cyclononane-N,N′,N″-triacetic acid), DOTA, and TETA (p-bromoacetamido-benzyl-tetraethylaminetetraacetic acid) are of use with a variety of metals and radiometals, such as radionuclides of gallium, yttrium and copper, respectively. Such metal-chelate complexes can be stabilized by tailoring the ring size to the metal of interest. Examples of other DOTA chelates include (i) DOTA-Phe-Lys(HSG)-D-Tyr-Lys(HSG)-NH_2; (ii) Ac-Lys(HSG)D-Tyr-Lys(HSG)-Lys(Tscg-Cys)-NH_2; (iii) DOTA-D-Asp-D-Lys(HSG)-D-Asp-D-Lys(HSG)-NH_2; (iv) DOTA-D-Glu-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH_2; (v) DOTA-D-Tyr-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH_2; (vi) DOTA-D-Ala-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH_2; (vii) DOTA-D-Phe-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-NH_2; (viii) Ac-D-Phe-D-Lys(DOTA)-D-Tyr-D-Lys(DOTA)-NH_2; (ix) Ac-D-Phe-D-Lys(DTPA)-D-Tyr-D-Lys(DTPA)-NH_2; (x) Ac-D-Phe-D-Lys(Bz-DTPA)-D-Tyr-D-Lys(Bz-DTPA)-NH_2; (xi) Ac-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-D-Lys(Tscg-Cys)-NH_2; (xii) DOTA-D-Phe-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-D-Lys(Tscg-Cys)-NH_2; (xiii) (Tscg-Cys)-D-Phe-D-Lys(HSG)-D-Tyr-D-Lys(HSG)-D-Lys(DOTA)-NH_2; (xiv) Tscg-D-Cys-D-Glu-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH_2; (xv) (Tscg-Cys)-D-Glu-D-Lys(HSG)-D-Glu-D-Lys(HSG)-NH_2; (xvi) Ac-D-Cys-D-Lys(DOTA)-D-Tyr-D-Ala-D-Lys(DOTA)-D-Cys-NH_2; (xvii) Ac-D-Cys-D-Lys(DTPA)-D-Tyr-D-Lys(DTPA)-NH_2; (xviii) Ac-D-Lys(DTPA)-D-Tyr-D-Lys(DTPA)-D-Lys(Tscg-Cys)-NH_2; and (xix) Ac-D-Lys(DOTA)-D-Tyr-D-Lys(DOTA)-D-Lys(Tscg-Cys)-NH_2.

[00238] Other ring-type chelates such as macrocyclic polyethers, which are of interest for stably binding nuclides, such as ^{223}Ra for RAIT are also contemplated.

B. Therapeutic Use of Anti-CLDN18.2 Antibodies of the Present Technology

[00239] In one aspect, the immunoglobulin-related compositions (*e.g.*, antibodies or antigen binding fragments thereof) of the present technology are useful for the treatment of CLDN18.2-associated cancers, such as gastric cancer, esophageal cancer, pancreatic cancer, lung cancer such as non small cell lung cancer (NSCLC), ovarian cancer, colon cancer, hepatic cancer, head-neck cancer, and cancers of the gallbladder, or any other neoplastic tissue that expresses CLDN18.2. In some embodiments, the CLDN18.2-associated cancer is a solid tumor. Such treatment can be used in patients identified as having pathologically high levels of the CLDN18.2 (*e.g.*, those diagnosed by the methods described herein) or in patients diagnosed with a disease known to be associated with such pathological levels.

[00240] The compositions of the present technology may be employed in conjunction with other therapeutic agents useful in the treatment of CLDN18.2-associated cancers. For example, the antibodies or antigen binding fragments of the present technology may be separately, sequentially or simultaneously administered with at least one additional therapeutic agent-selected from the group consisting of alkylating agents, platinum agents, taxanes, vinca agents, anti-estrogen drugs, aromatase inhibitors, ovarian suppression agents, VEGF/VEGFR inhibitors, EGF/EGFR inhibitors, PARP inhibitors, cytostatic alkaloids, cytotoxic antibiotics, antimetabolites, endocrine/hormonal agents, bisphosphonate therapy agents and targeted biological therapy agents (*e.g.*, therapeutic peptides described in US 6306832, WO 2012007137, WO 2005000889, WO 2010096603 *etc.*). In some embodiments, the at least one additional therapeutic agent is a chemotherapeutic agent. Specific chemotherapeutic agents include, but are not limited to, cyclophosphamide, fluorouracil (or 5-fluorouracil or 5-FU), methotrexate, edatrexate (10-ethyl-10-deaza-aminopterin), thiotepa, carboplatin, cisplatin, taxanes, paclitaxel, protein-bound paclitaxel, docetaxel, vinorelbine, tamoxifen, raloxifene, toremifene, fulvestrant, gemcitabine, irinotecan, ixabepilone, temozolmide, topotecan, vincristine, vinblastine, eribulin, mutamycin, capecitabine, anastrozole, exemestane, letrozole, leuprolide, abarelix, buserlin, goserelin, megestrol acetate, risedronate, pamidronate, ibandronate, alendronate, denosumab, zoledronate, trastuzumab, tykerb, anthracyclines (*e.g.*, daunorubicin and doxorubicin), bevacizumab, oxaliplatin, melphalan, etoposide, mechlorethamine, bleomycin, microtubule poisons, annonaceous acetogenins, or combinations thereof.

[00241] Additionally or alternatively, in some embodiments, the antibodies or antigen binding fragments of the present technology may be separately, sequentially or simultaneously administered with at least one additional immuno-modulating/stimulating antibody including but not limited to anti-PD-1 antibody, anti-PD-L1 antibody, anti-PD-L2 antibody, anti-CTLA-4 antibody, anti-TIM3 antibody, anti-4-1BB antibody, anti-CD73 antibody, anti-GITR antibody, and anti-LAG-3 antibody.

[00242] The compositions of the present technology may optionally be administered as a single bolus to a subject in need thereof. Alternatively, the dosing regimen may comprise multiple administrations performed at various times after the appearance of tumors.

[00243] Administration can be carried out by any suitable route, including orally, intranasally, parenterally (intravenously, intramuscularly, intraperitoneally, or subcutaneously), rectally, intracranially, intratumorally, intrathecally, or topically. Administration includes self-administration and the administration by another. It is also to be appreciated that the various modes of treatment of medical conditions as described are intended to mean "substantial", which includes total but also less than total treatment, and wherein some biologically or medically relevant result is achieved.

[00244] In some embodiments, the antibodies of the present technology comprise pharmaceutical formulations which may be administered to subjects in need thereof in one or more doses. Dosage regimens can be adjusted to provide the desired response (*e.g.*, a therapeutic response).

[00245] Typically, an effective amount of the antibody compositions of the present technology, sufficient for achieving a therapeutic effect, range from about 0.000001 mg per kilogram body weight per day to about 10,000 mg per kilogram body weight per day. Typically, the dosage ranges are from about 0.0001 mg per kilogram body weight per day to about 100 mg per kilogram body weight per day. For administration of anti-CLDN18.2 antibodies, the dosage ranges from about 0.0001 to 100 mg/kg, and more usually 0.01 to 5 mg/kg every week, every two weeks or every three weeks, of the subject body weight. For example, dosages can be 1 mg/kg body weight or 10 mg/kg body weight every week, every two weeks or every three weeks or within the range of 1-10 mg/kg every week, every two weeks or every three weeks. In one embodiment, a single dosage of antibody ranges from 0.1-10,000 micrograms per kg body weight. In one embodiment, antibody concentrations in a carrier range from 0.2 to 2000 micrograms per delivered milliliter. An exemplary

treatment regime entails administration once per every two weeks or once a month or once every 3 to 6 months. Anti-CLDN18.2 antibodies may be administered on multiple occasions. Intervals between single dosages can be hourly, daily, weekly, monthly or yearly. Intervals can also be irregular as indicated by measuring blood levels of the antibody in the subject. In some methods, dosage is adjusted to achieve a serum antibody concentration in the subject of from about 75 μg/mL to about 125 μg/mL, 100 μg/mL to about 150 μg/mL, from about 125 μg/mL to about 175 μg/mL, or from about 150 μg/mL to about 200 μg/mL. Alternatively, anti-CLDN18.2 antibodies can be administered as a sustained release formulation, in which case less frequent administration is required. Dosage and frequency vary depending on the half-life of the antibody in the subject. The dosage and frequency of administration can vary depending on whether the treatment is prophylactic or therapeutic. In prophylactic applications, a relatively low dosage is administered at relatively infrequent intervals over a long period of time. In therapeutic applications, a relatively high dosage at relatively short intervals is sometimes required until progression of the disease is reduced or terminated, or until the subject shows partial or complete amelioration of symptoms of disease. Thereafter, the patient can be administered a prophylactic regime.

[00246] In another aspect, the present disclosure provides a method for detecting cancer in a subject *in vivo* comprising (a) administering to the subject an effective amount of an antibody (or antigen binding fragment thereof) of the present technology, wherein the antibody is configured to localize to a cancer cell expressing CLDN18.2 and is labeled with a radioisotope; and (b) detecting the presence of a tumor in the subject by detecting radioactive levels emitted by the antibody that are higher than a reference value. In some embodiments, the reference value is expressed as injected dose per gram (%ID/g). The reference value may be calculated by measuring the radioactive levels present in non-tumor (normal) tissues, and computing the average radioactive levels present in non-tumor (normal) tissues ± standard deviation. In some embodiments, the ratio of radioactive levels between a tumor and normal tissue is about 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 15:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, 55:1, 60:1, 65:1, 70:1, 75:1, 80:1, 85:1, 90:1, 95:1 or 100:1.

[00247] In some embodiments, the subject is diagnosed with or is suspected of having cancer. Radioactive levels emitted by the antibody may be detected using positron emission tomography or single photon emission computed tomography.

[00248] Additionally or alternatively, in some embodiments, the method further comprises administering to the subject an effective amount of an immunoconjugate comprising an antibody of the present technology conjugated to a radionuclide. In some embodiments, the radionuclide is an alpha particle-emitting isotope, a beta particle-emitting isotope, an Auger-emitter, or any combination thereof. Examples of beta particle-emitting isotopes include ^{86}Y, ^{90}Y, ^{89}Sr, ^{165}Dy, ^{186}Re, ^{188}Re, ^{177}Lu, and ^{67}Cu. Examples of alpha particle-emitting isotopes include ^{213}Bi, ^{211}At, ^{225}Ac, ^{152}Dy, ^{212}Bi, ^{223}Ra, ^{219}Rn, ^{215}Po, ^{211}Bi, ^{221}Fr, ^{217}At, and ^{255}Fm. Examples of Auger-emitters include ^{111}In, ^{67}Ga, ^{51}Cr, ^{58}Co, ^{99m}Tc, ^{103m}Rh, ^{195m}Pt, ^{119}Sb, ^{161}Ho, ^{189m}Os, ^{192}Ir, ^{201}Tl, and ^{203}Pb. In some embodiments of the method, nonspecific FcR-dependent binding in normal tissues is eliminated or reduced (*e.g.*, *via* N297A mutation in Fc region, which results in aglycosylation). The therapeutic effectiveness of such an immunoconjugate may be determined by computing the area under the curve (AUC) tumor: AUC normal tissue ratio. In some embodiments, the immunoconjugate has a AUC tumor: AUC normal tissue ratio of about 2:1, 3:1, 4:1, 5:1, 6:1, 7:1, 8:1, 9:1, 10:1, 15:1, 20:1, 25:1, 30:1, 35:1, 40:1, 45:1, 50:1, 55:1, 60:1, 65:1, 70:1, 75:1, 80:1, 85:1, 90:1, 95:1 or 100:1.

[00249] *Toxicity*. Optimally, an effective amount (*e.g.*, dose) of an anti-CLDN18.2 antibody described herein will provide therapeutic benefit without causing substantial toxicity to the subject. Toxicity of the anti-CLDN18.2 antibody described herein can be determined by standard pharmaceutical procedures in cell cultures or experimental animals, *e.g.*, by determining the LD_{50} (the dose lethal to 50% of the population) or the LD_{100} (the dose lethal to 100% of the population). The dose ratio between toxic and therapeutic effect is the therapeutic index. The data obtained from these cell culture assays and animal studies can be used in formulating a dosage range that is not toxic for use in human. The dosage of the anti-CLDN18.2 antibody described herein lies within a range of circulating concentrations that include the effective dose with little or no toxicity. The dosage can vary within this range depending upon the dosage form employed and the route of administration utilized. The exact formulation, route of administration and dosage can be chosen by the individual physician in view of the subject's condition. *See*, *e.g.*, Fingl *et al.*, In: *The Pharmacological Basis of Therapeutics*, Ch. 1 (1975).

[00250] *Formulations of Pharmaceutical Compositions.* According to the methods of the present technology, the anti-CLDN18.2 antibody can be incorporated into pharmaceutical compositions suitable for administration. The pharmaceutical compositions

generally comprise recombinant or substantially purified antibody and a pharmaceutically-acceptable carrier in a form suitable for administration to a subject. Pharmaceutically-acceptable carriers are determined in part by the particular composition being administered, as well as by the particular method used to administer the composition. Accordingly, there is a wide variety of suitable formulations of pharmaceutical compositions for administering the antibody compositions (*See, e.g.*, *Remington's Pharmaceutical Sciences*, Mack Publishing Co., Easton, PA 18th ed., 1990). The pharmaceutical compositions are generally formulated as sterile, substantially isotonic and in full compliance with all Good Manufacturing Practice (GMP) regulations of the U.S. Food and Drug Administration. The pharmaceutical composition may further comprise an agent selected from the group consisting of isotopes, dyes, chromagens, contrast agents, drugs, toxins, cytokines, enzymes, enzyme inhibitors, hormones, hormone antagonists, growth factors, radionuclides, metals, liposomes, nanoparticles, RNA, DNA or any combination thereof.

[00251] The terms "pharmaceutically-acceptable," "physiologically-tolerable," and grammatical variations thereof, as they refer to compositions, carriers, diluents and reagents, are used interchangeably and represent that the materials are capable of administration to or upon a subject without the production of undesirable physiological effects to a degree that would prohibit administration of the composition. For example, "pharmaceutically-acceptable excipient" means an excipient that is useful in preparing a pharmaceutical composition that is generally safe, non-toxic, and desirable, and includes excipients that are acceptable for veterinary use as well as for human pharmaceutical use. Such excipients can be solid, liquid, semisolid, or, in the case of an aerosol composition, gaseous. "Pharmaceutically-acceptable salts and esters" means salts and esters that are pharmaceutically-acceptable and have the desired pharmacological properties. Such salts include salts that can be formed where acidic protons present in the composition are capable of reacting with inorganic or organic bases. Suitable inorganic salts include those formed with the alkali metals, *e.g.,* sodium and potassium, magnesium, calcium, and aluminum. Suitable organic salts include those formed with organic bases such as the amine bases, *e.g.,* ethanolamine, diethanolamine, triethanolamine, tromethamine, N-methylglucamine, and the like. Such salts also include acid addition salts formed with inorganic acids (*e.g.,* hydrochloric and hydrobromic acids) and organic acids (*e.g.,* acetic acid, citric acid, maleic acid, and the alkane- and arene-sulfonic acids such as methanesulfonic acid and benzenesulfonic acid). Pharmaceutically-acceptable esters include esters formed from

carboxy, sulfonyloxy, and phosphonoxy groups present in the anti-CLDN18.2 antibody, *e.g.*, C_{1-6} alkyl esters. When there are two acidic groups present, a pharmaceutically-acceptable salt or ester can be a mono-acid-mono-salt or ester or a di-salt or ester; and similarly where there are more than two acidic groups present, some or all of such groups can be salified or esterified. An anti-CLDN18.2 antibody named in this technology can be present in unsalified or unesterified form, or in salified and/or esterified form, and the naming of such anti-CLDN18.2 antibody is intended to include both the original (unsalified and unesterified) compound and its pharmaceutically-acceptable salts and esters. Also, certain embodiments of the present technology can be present in more than one stereoisomeric form, and the naming of such anti-CLDN18.2 antibody is intended to include all single stereoisomers and all mixtures (whether racemic or otherwise) of such stereoisomers. A person of ordinary skill in the art, would have no difficulty determining the appropriate timing, sequence and dosages of administration for particular drugs and compositions of the present technology.

[00252] Examples of such carriers or diluents include, but are not limited to, water, saline, Ringer's solutions, dextrose solution, and 5% human serum albumin. Liposomes and non-aqueous vehicles such as fixed oils may also be used. The use of such media and compounds for pharmaceutically active substances is well known in the art. Except insofar as any conventional media or compound is incompatible with the anti-CLDN18.2 antibody, use thereof in the compositions is contemplated. Supplementary active compounds can also be incorporated into the compositions.

[00253] A pharmaceutical composition of the present technology is formulated to be compatible with its intended route of administration. The anti-CLDN18.2 antibody compositions of the present technology can be administered by parenteral, topical, intravenous, oral, subcutaneous, intraarterial, intradermal, transdermal, rectal, intracranial, intrathecal, intraperitoneal, intranasal; or intramuscular routes, or as inhalants. The anti-CLDN18.2 antibody can optionally be administered in combination with other agents that are at least partly effective in treating various CLDN18.2-associated cancers.

[00254] Solutions or suspensions used for parenteral, intradermal, or subcutaneous application can include the following components: a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerine, propylene glycol or other synthetic solvents; antibacterial compounds such as benzyl alcohol or methyl

parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating compounds such as ethylenediaminetetraacetic acid (EDTA); buffers such as acetates, citrates or phosphates, and compounds for the adjustment of tonicity such as sodium chloride or dextrose. The pH can be adjusted with acids or bases, such as hydrochloric acid or sodium hydroxide. The parenteral preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[00255] Pharmaceutical compositions suitable for injectable use include sterile aqueous solutions (where water soluble) or dispersions and sterile powders for the extemporaneous preparation of sterile injectable solutions or dispersion. For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor EL™ (BASF, Parsippany, N.J.) or phosphate buffered saline (PBS). In all cases, the composition must be sterile and should be fluid to the extent that easy syringeability exists. It must be stable under the conditions of manufacture and storage and must be preserved against the contaminating action of microorganisms such as bacteria and fungi. The carrier can be a solvent or dispersion medium containing, *e.g.*, water, ethanol, polyol (*e.g.*, glycerol, propylene glycol, and liquid polyethylene glycol, and the like), and suitable mixtures thereof. The proper fluidity can be maintained, *e.g.*, by the use of a coating such as lecithin, by the maintenance of the required particle size in the case of dispersion and by the use of surfactants. Prevention of the action of microorganisms can be achieved by various antibacterial and antifungal compounds, *e.g.*, parabens, chlorobutanol, phenol, ascorbic acid, thimerosal, and the like. In many cases, it will be desirable to include isotonic compounds, *e.g.*, sugars, polyalcohols such as manitol, sorbitol, sodium chloride in the composition. Prolonged absorption of the injectable compositions can be brought about by including in the composition a compound which delays absorption, *e.g.*, aluminum monostearate and gelatin.

[00256] Sterile injectable solutions can be prepared by incorporating an anti-CLDN18.2 antibody of the present technology in the required amount in an appropriate solvent with one or a combination of ingredients enumerated above, as required, followed by filtered sterilization. Generally, dispersions are prepared by incorporating the anti-CLDN18.2 antibody into a sterile vehicle that contains a basic dispersion medium and the required other ingredients from those enumerated above. In the case of sterile powders for the preparation of sterile injectable solutions, methods of preparation are vacuum drying and freeze-drying that yields a powder of the active ingredient plus any additional desired

ingredient from a previously sterile-filtered solution thereof. The antibodies of the present technology can be administered in the form of a depot injection or implant preparation which can be formulated in such a manner as to permit a sustained or pulsatile release of the active ingredient.

[00257] Oral compositions generally include an inert diluent or an edible carrier. They can be enclosed in gelatin capsules or compressed into tablets. For the purpose of oral therapeutic administration, the anti-CLDN18.2 antibody can be incorporated with excipients and used in the form of tablets, troches, or capsules. Oral compositions can also be prepared using a fluid carrier for use as a mouthwash, wherein the compound in the fluid carrier is applied orally and swished and expectorated or swallowed. Pharmaceutically compatible binding compounds, and/or adjuvant materials can be included as part of the composition. The tablets, pills, capsules, troches and the like can contain any of the following ingredients, or compounds of a similar nature: a binder such as microcrystalline cellulose, gum tragacanth or gelatin; an excipient such as starch or lactose, a disintegrating compound such as alginic acid, Primogel, or corn starch; a lubricant such as magnesium stearate or Sterotes; a glidant such as colloidal silicon dioxide; a sweetening compound such as sucrose or saccharin; or a flavoring compound such as peppermint, methyl salicylate, or orange flavoring.

[00258] For administration by inhalation, the anti-CLDN18.2 antibody is delivered in the form of an aerosol spray from pressured container or dispenser which contains a suitable propellant, *e.g.*, a gas such as carbon dioxide, or a nebulizer.

[00259] Systemic administration can also be by transmucosal or transdermal means. For transmucosal or transdermal administration, penetrants appropriate to the barrier to be permeated are used in the formulation. Such penetrants are generally known in the art, and include, *e.g.*, for transmucosal administration, detergents, bile salts, and fusidic acid derivatives. Transmucosal administration can be accomplished through the use of nasal sprays or suppositories. For transdermal administration, the anti-CLDN18.2 antibody is formulated into ointments, salves, gels, or creams as generally known in the art.

[00260] The anti-CLDN18.2 antibody can also be prepared as pharmaceutical compositions in the form of suppositories (*e.g.*, with conventional suppository bases such as cocoa butter and other glycerides) or retention enemas for rectal delivery.

[00261] In one embodiment, the anti-CLDN18.2 antibody is prepared with carriers that will protect the anti-CLDN18.2 antibody against rapid elimination from the body, such as a controlled release formulation, including implants and microencapsulated delivery systems. Biodegradable, biocompatible polymers can be used, such as ethylene vinyl acetate, polyanhydrides, polyglycolic acid, collagen, polyorthoesters, and polylactic acid. Methods for preparation of such formulations will be apparent to those skilled in the art. The materials can also be obtained commercially from Alza Corporation and Nova Pharmaceuticals, Inc. Liposomal suspensions (including liposomes targeted to infected cells with monoclonal antibodies to viral antigens) can also be used as pharmaceutically-acceptable carriers. These can be prepared according to methods known to those skilled in the art, *e.g.*, as described in U.S. Pat. No. 4,522,811.

C. Kits

[00262] The present technology provides kits for the detection and/or treatment of CLDN18.2-associated cancers, comprising at least one immunoglobulin-related composition of the present technology (*e.g.*, any antibody or antigen binding fragment described herein), or a functional variant (*e.g.*, substitutional variant) thereof, or the engineered immune cells described herein. Optionally, the above described components of the kits of the present technology are packed in suitable containers and labeled for diagnosis and/or treatment of CLDN18.2-associated cancers. The above-mentioned components may be stored in unit or multi-dose containers, for example, sealed ampoules, vials, bottles, syringes, and test tubes, as an aqueous, preferably sterile, solution or as a lyophilized, preferably sterile, formulation for reconstitution. The kit may further comprise a second container which holds a diluent suitable for diluting the pharmaceutical composition towards a higher volume. Suitable diluents include, but are not limited to, the pharmaceutically acceptable excipient of the pharmaceutical composition and a saline solution. Furthermore, the kit may comprise instructions for diluting the pharmaceutical composition and/or instructions for administering the pharmaceutical composition, whether diluted or not. The containers may be formed from a variety of materials such as glass or plastic and may have a sterile access port (for example, the container may be an intravenous solution bag or a vial having a stopper which may be pierced by a hypodermic injection needle). The kit may further comprise more containers comprising a pharmaceutically acceptable buffer, such as phosphate-buffered saline, Ringer's solution and dextrose solution. It may further include other materials desirable from a commercial and user

standpoint, including other buffers, diluents, filters, needles, syringes, culture medium for one or more of the suitable hosts. The kits may optionally include instructions customarily included in commercial packages of therapeutic or diagnostic products, that contain information about, for example, the indications, usage, dosage, manufacture, administration, contraindications and/or warnings concerning the use of such therapeutic or diagnostic products.

[00263] The kits are useful for detecting the presence of an immunoreactive CLDN18.2 protein in a biological sample, *e.g.*, any body fluid including, but not limited to, *e.g.*, serum, plasma, lymph, cystic fluid, urine, stool, cerebrospinal fluid, ascitic fluid or blood and including biopsy samples of body tissue. For example, the kit can comprise: one or more humanized, or chimeric anti-CLDN18.2 antibodies of the present technology (or antigen binding fragments thereof) capable of binding a CLDN18.2 protein in a biological sample; means for determining the amount of the CLDN18.2 protein in the sample; and means for comparing the amount of the immunoreactive CLDN18.2 protein in the sample with a standard. One or more of the anti-CLDN18.2 antibodies may be labeled. The kit components, (*e.g.,* reagents) can be packaged in a suitable container. The kit can further comprise instructions for using the kit to detect the immunoreactive CLDN18.2 protein.

[00264] For antibody-based kits, the kit can comprise, *e.g.*, 1) a first antibody, *e.g.* a humanized, or chimeric CLDN18.2 antibody of the present technology (or an antigen binding fragment thereof), attached to a solid support, which binds to a CLDN18.2 protein; and, optionally; 2) a second, different antibody which binds to either the CLDN18.2 protein or to the first antibody, and is conjugated to a detectable label.

[00265] The kit can also comprise, *e.g.*, a buffering agent, a preservative or a protein-stabilizing agent. The kit can further comprise components necessary for detecting the detectable-label, *e.g.*, an enzyme or a substrate. The kit can also contain a control sample or a series of control samples, which can be assayed and compared to the test sample. Each component of the kit can be enclosed within an individual container and all of the various containers can be within a single package, along with instructions for interpreting the results of the assays performed using the kit. The kits of the present technology may contain a written product on or in the kit container. The written product describes how to use the reagents contained in the kit, *e.g.*, for detection of a CLDN18.2 protein *in vitro* or *in vivo*, or for treatment of CLDN18.2-associated cancers in a subject in need thereof. In certain

embodiments, the use of the reagents can be according to the methods of the present technology.

Chimeric Antigen Receptors

[00266] In one aspect, the present disclosure provides engineered immune cells that express at least one chimeric antigen receptor (CAR) that targets CLDN18.2 expressing cells. CARs are engineered receptors, which graft or confer a specificity of interest onto an immune effector cell. For example, CARs can be used to graft the specificity of a monoclonal antibody onto an immune cell, such as a T cell. In some embodiments, transfer of the coding sequence of the CAR is facilitated by nucleic acid vector, such as a retroviral vector.

[00267] There are currently three generations of CARs. In some embodiments, the engineered immune cells provided herein express a "first generation" CAR. "First generation" CARs are typically composed of an extracellular antigen binding domain (*e.g.*, a single-chain variable fragment (scFv)) fused to a transmembrane domain fused to cytoplasmic/intracellular domain of the T cell receptor (TCR) chain. "First generation" CARs typically have the intracellular domain from the CD3ζ chain, which is the primary transmitter of signals from endogenous TCRs. "First generation" CARs can provide *de novo* antigen recognition and cause activation of both $CD4^+$ and $CD8^+$ T cells through their CD3ζ chain signaling domain in a single fusion molecule, independent of HLA-mediated antigen presentation.

[00268] In some embodiments, the engineered immune cells provided herein express a "second generation" CAR. "Second generation" CARs add intracellular domains from various co-stimulatory molecules (*e.g.*, CD28, 4-1BB, ICOS, OX40) to the cytoplasmic tail of the CAR to provide additional signals to the T cell. "Second generation" CARs comprise those that provide both co-stimulation (*e.g.*, CD28 or 4-1BB) and activation (*e.g.*, CD3ζ). Preclinical studies have indicated that "Second Generation" CARs can improve the antitumor activity of T cells.

[00269] In some embodiments, the engineered immune cells provided herein express a "third generation" CAR. "Third generation" CARs comprise those that provide multiple co-stimulation (*e.g.*, CD28 and 4-1BB) and activation (*e.g.*, CD3ζ).

[00270] In accordance with the presently disclosed subject matter, the CARs of the engineered immune cells provided herein comprise an extracellular antigen-binding domain, a transmembrane domain and an intracellular domain.

[00271] *Extracellular Antigen-Binding Domain of a CAR.* In certain embodiments, the extracellular antigen-binding domain of a CAR specifically binds a CLDN18.2 antigen. In certain embodiments, the extracellular antigen-binding domain is derived from a monoclonal antibody (mAb) that binds to a CLDN18.2 antigen. In some embodiments, the extracellular antigen-binding domain comprises an scFv. In some embodiments, the extracellular antigen-binding domain comprises a Fab, which is optionally crosslinked. In some embodiments, the extracellular binding domain comprises a $F(ab)_2$. In some embodiments, any of the foregoing molecules are included in a fusion protein with a heterologous sequence to form the extracellular antigen-binding domain. In certain embodiments, the extracellular antigen-binding domain comprises a human scFv that binds specifically to a CLDN18.2 antigen. In certain embodiments, the scFv is identified by screening scFv phage library with a CLDN18.2 antigen-Fc fusion protein.

[00272] In certain embodiments, the extracellular antigen-binding domain of a presently disclosed CAR has a high binding specificity and high binding affinity to a CLDN18.2 antigen. For example, in some embodiments, the extracellular antigen-binding domain of the CAR (embodied, for example, in a human scFv or an analog thereof) binds to a particular CLDN18.2 antigen with a dissociation constant (K_d) of about 1×10^{-5} M or less. In certain embodiments, the K_d is about 5×10^{-6} M or less, about 1×10^{-6} M or less, about 5×10^{-7} M or less, about 1×10^{-7} M or less, about 5×10^{-8} M or less, about 1×10^{-8} M or less, about 5×10^{-9} or less, about 4×10^{-9} or less, about 3×10^{-9} or less, about 2×10^{-9} or less, or about 1×10^{-9} M or less. In certain non-limiting embodiments, the K_d is from about 3×10^{-9} M or less. In certain non-limiting embodiments, the K_d is from about 3×10^{-9} to about 2×10^{-7}.

[00273] Binding of the extracellular antigen-binding domain (embodiment, for example, in an scFv or an analog thereof) of a presently disclosed CLDN18.2-specific CAR can be confirmed by, for example, enzyme-linked immunosorbent assay (ELISA), radioimmunoassay (RIA), FACS analysis, bioassay (*e.g.*, growth inhibition), or Western Blot assay. Each of these assays generally detect the presence of protein-antibody complexes of particular interest by employing a labeled reagent (*e.g.*, an antibody, or an

scFv) specific for the complex of interest. For example, the scFv can be radioactively labeled and used in a radioimmunoassay (RIA) (see, for example, Weintraub, B., *Principles of Radioimmunoassays*, Seventh Training Course on Radioligand Assay Techniques, The Endocrine Society, March, 1986, which is incorporated by reference herein). The radioactive isotope can be detected by such means as the use of a γ counter or a scintillation counter or by autoradiography. In certain embodiments, the extracellular antigen-binding domain of the CLDN18.2-specific CAR is labeled with a fluorescent marker. Non-limiting examples of fluorescent markers include green fluorescent protein (GFP), blue fluorescent protein (*e.g.*, EBFP, EBFP2, Azurite, and mKalamal), cyan fluorescent protein (*e.g.*, ECFP, Cerulean, and CyPet), and yellow fluorescent protein (*e.g.*, YFP, Citrine, Venus, and YPet). In certain embodiments, the scFv of a presently disclosed CLDN18.2-specific CAR is labeled with GFP.

[00274] In some embodiments, the extracellular antigen-binding domain of the expressed CAR binds to a CLDN18.2 antigen that is expressed by a tumor cell. In some embodiments, the extracellular antigen-binding domain of the expressed CAR binds to a CLDN18.2 antigen that is expressed on the surface of a tumor cell. In some embodiments, the extracellular antigen-binding domain of the expressed CAR binds to a CLDN18.2 antigen that is expressed on the surface of a tumor cell in combination with an MHC protein. In some embodiments, the MHC protein is a MHC class I protein. In some embodiments, the MHC Class I protein is an HLA-A, HLA-B, or HLA-C molecules. In some embodiments, the extracellular antigen-binding domain of the expressed CAR binds to a CLDN18.2 antigen that is expressed on the surface of a tumor cell not in combination with an MHC protein.

[00275] In some embodiments, the extracellular antigen-binding domain of the expressed CAR binds to a CLDN18.2 antigen. In some embodiments, the extracellular antigen-binding domain of the expressed CAR binds to a CLDN18.2 antigen presented in the context of an MHC molecule. In some embodiments, the extracellular antigen-binding domain of the expressed CAR binds to a CLDN18.2 antigen presented in the context of an HLA-A2 molecule.

[00276] In certain embodiments, the extracellular antigen-binding domain (*e.g.*, human scFv) comprises a heavy chain variable (V_H) region and a light chain variable (V_L) region, optionally linked with a linker sequence, for example a linker peptide (*e.g.*,

GGGGSGGGGSGGGGS (SEQ ID NO: 79)), between the heavy chain variable (V_H) region and the light chain variable (V_L) region. In certain embodiments, the extracellular antigen-binding domain is a human scFv-Fc fusion protein or full length human IgG with V_H and V_L regions.

[00277] In certain non-limiting embodiments, an extracellular antigen-binding domain of the presently disclosed CAR can comprise a linker connecting the heavy chain variable (V_H) region and light chain variable (V_L) region of the extracellular antigen-binding domain. As used herein, the term "linker" refers to a functional group (*e.g.*, chemical or polypeptide) that covalently attaches two or more polypeptides or nucleic acids so that they are connected to one another. As used herein, a "peptide linker" refers to one or more amino acids used to couple two proteins together (*e.g.*, to couple V_H and V_L domains). In certain embodiments, the linker comprises amino acids having the sequence set forth in SEQ ID NO: 79. In certain embodiments, the nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 79 is set forth in SEQ ID NO: 80, which is provided below: GGCGGCGGCGGATCTGGAGGTGGTGGCTCAGGTGGCGGAGGCTCC (SEQ ID NO: 80).

[00278] Additionally or alternatively, in some embodiments, the extracellular antigen-binding domain can comprise a leader or a signal peptide sequence that directs the nascent protein into the endoplasmic reticulum. The signal peptide or leader can be essential if the CAR is to be glycosylated and anchored in the cell membrane. The signal sequence or leader sequence can be a peptide sequence (about 5, about 10, about 15, about 20, about 25, or about 30 amino acids long) present at the N-terminus of the newly synthesized proteins that direct their entry to the secretory pathway.

[00279] In certain embodiments, the signal peptide is covalently joined to the N-terminus of the extracellular antigen-binding domain. In certain embodiments, the signal peptide comprises a human CD8 signal polypeptide comprising amino acids having the sequence set forth in SEQ ID NO: 81 as provided below: MALPVTALLLPLALLLHAARP (SEQ ID NO: 81). The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 81 is set forth in SEQ ID NO: 82, which is provided below: ATGGCCCTGCCAGTAACGGCTCTGCTGCTGCCACTTGCTCTGCTCCTCCATGCAG CCAGGCCT (SEQ ID NO: 82). In certain embodiments, the signal peptide comprises a human CD8 signal polypeptide comprising amino acids having the sequence set forth in

SEQ ID NO: 83 as provided below: MALPVTALLLPLALLLHA (SEQ ID NO: 83). The nucleotide sequence encoding the amino acid sequence of SEQ ID NO: 83 is set forth in SEQ ID NO: 84, which is provided below: ATGGCTCTCCCAGTGACTGCCCTACTGCTTCCCCTAGCGCTTCTCCTGCATGCA (SEQ ID NO: 84).

[00280] *Transmembrane Domain of a CAR.* In certain non-limiting embodiments, the transmembrane domain of the CAR comprises a hydrophobic alpha helix that spans at least a portion of the membrane. Different transmembrane domains result in different receptor stability. After antigen recognition, receptors cluster and a signal is transmitted to the cell. In accordance with the presently disclosed subject matter, the transmembrane domain of the CAR can comprise a CD8 polypeptide, a CD28 polypeptide, a CD3ζ polypeptide, a CD4 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, an ICOS polypeptide, a CTLA-4 polypeptide, a PD-1 polypeptide, a LAG-3 polypeptide, a 2B4 polypeptide, a BTLA polypeptide, a synthetic peptide (*e.g.*, a transmembrane peptide not based on a protein associated with the immune response), or a combination thereof.

[00281] In certain embodiments, the transmembrane domain of a presently disclosed CAR comprises a CD28 polypeptide. The CD28 polypeptide can have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or 100% homologous to the sequence having a UniProtKB Reference No: P10747 or NCBI Reference No: NP006130 (SEQ ID NO: 85), or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. In certain embodiments, the CD28 polypeptide can have an amino acid sequence that is a consecutive portion of SEQ ID NO: 85 which is at least 20, or at least 30, or at least 40, or at least 50, and up to 220 amino acids in length. Additionally or alternatively, in non- limiting various embodiments, the CD28 polypeptide has an amino acid sequence of amino acids 1 to 220, 1 to 50, 50 to 100, 100 to 150, 114 to 220, 150 to 200, or 200 to 220 of SEQ ID NO: 85. In certain embodiments, the CAR of the present disclosure comprises a transmembrane domain comprising a CD28 polypeptide, and optionally an intracellular domain comprising a co-stimulatory signaling region that comprises a CD28 polypeptide. In certain embodiments, the CD28 polypeptide comprised in the transmembrane domain and the intracellular domain has an amino acid sequence of amino acids 114 to 220 of SEQ ID NO: 85. In certain embodiments, the CD28 polypeptide

comprised in the transmembrane domain has an amino acid sequence of amino acids 153 to 179 of SEQ ID NO: 85.

SEQ ID NO: 85 is provided below:
MLRLLLALNLFPSIQVTGNKILVKQSPMLVAYDNAVNLSCKYSYNLFSREFRASLH
KGLDSAVEVCVVYGNYSQQLQVYSKTGFNCDGKLGNESVTFYLQNLYVNQTDIYF
CKIEVMYPPPYLDNEKSNGTIIHVKGKHLCPSPLFPGPSKPFWVLVVVGGVLACYSL
LVTVAFIIFWVRSKRSRLLHSDYMNMTPRRPGPTRKHYQPYAPPRDFAAYRS (SEQ ID NO: 85)

[00282] In accordance with the presently disclosed subject matter, a "CD28 nucleic acid molecule" refers to a polynucleotide encoding a CD28 polypeptide. In certain embodiments, the CD28 nucleic acid molecule encoding the CD28 polypeptide comprised in the transmembrane domain (and optionally the intracellular domain (*e.g.*, the co-stimulatory signaling region)) of the presently disclosed CAR (*e.g.*, amino acids 114 to 220 of SEQ ID NO: 85 or amino acids 153 to 179 of SEQ ID NO: 85) comprises at least a portion of the sequence set forth in SEQ ID NO: 86 as provided below.
ATTGAAGTTATGTATCCTCCTCCTTACCTAGACAATGAGAAGAGCAATGGAACC
ATTATCCATGTGAAAGGGAAACACCTTTGTCCAAGTCCCCTATTTCCCGGACCT
TCTAAGCCCTTTTGGGTGCTGGTGGTGGTTGGTGGAGTCCTGGCTTGCTATAGCT
TGCTAGTAACAGTGGCCTTTATTATTTTCTGGGTGAGGAGTAAGAGGAGCAGGC
TCCTGCACAGTGACTACATGAACATGACTCCCCGCCGCCCCGGGCCCACCCGCA
AGCATTACCAGCCCTATGCCCCACCACGCGACTTCGCAGCCTATCGCTCC (SEQ ID NO: 86)

[00283] In certain embodiments, the transmembrane domain comprises a CD8 polypeptide. The CD8 polypeptide can have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100%) homologous to SEQ ID NO: 87 (homology herein may be determined using standard software such as BLAST or FASTA) as provided below, or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions. In certain embodiments, the CD8 polypeptide can have an amino acid sequence that is a consecutive portion of SEQ ID NO: 87 which is at least 20, or at least 30, or at least 40, or at least 50, and up to 235 amino acids in length. Additionally or alternatively, in various embodiments, the CD8 polypeptide has an amino acid sequence of

amino acids 1 to 235, 1 to 50, 50 to 100, 100 to 150, 150 to 200, or 200 to 235 of SEQ ID NO: 87.

[00284] MALPVTALLLPLALLLHAARPSQFRVSPLDRTWNLGETVELKCQVLLSN PTSGCSWLFQPRGAAASPTFLLYLSQNKPKAAEGLDTQRFSGKRLGDTFVLTLSDF RRENEGYYFCSALSNSIMYFSHFVPVFLPAKPTTTPAPRPPTPAPTIASQPLSLRPEAC RPAAGGAVHTRGLDFACDIYIWAPLAGTCGVLLLSLVITLYCNHRNRRRVCKCPRP VVKSGDKPSLSARYV (SEQ ID NO: 87)

[00285] In certain embodiments, the transmembrane domain comprises a CD8 polypeptide comprising amino acids having the sequence set forth in SEQ ID NO: 88 as provided below:

[00286] PTTTPAPRPPTPAPTIASQPLSLRPEACRPAAGGAVHTRGLDFACDIYIWA PLAGTCGVLLLSLVITLYCN (SEQ ID NO: 88)

[00287] In accordance with the presently disclosed subject matter, a "CD8 nucleic acid molecule" refers to a polynucleotide encoding a CD8 polypeptide. In certain embodiments, the CD8 nucleic acid molecule encoding the CD8 polypeptide comprised in the transmembrane domain of the presently disclosed CAR (SEQ ID NO: 88) comprises nucleic acids having the sequence set forth in SEQ ID NO: 89 as provided below.

[00288] CCCACCACGACGCCAGCGCCGCGACCACCAACCCCGGCGCCCACGAT CGCGTCGCAGCCCCTGTCCCTGCGCCCAGAGGCGTGCCGGCCAGCGGCGGGGG GCGCAGTGCACACGAGGGGGCTGGACTTCGCCTGTGATATCTACATCTGGGCGC CCCTGGCCGGGACTTGTGGGGTCCTTCTCCTGTCACTGGTTATCACCCTTTACTG CAAC (SEQ ID NO: 89)

[00289] In certain non-limiting embodiments, a CAR can also comprise a spacer region that links the extracellular antigen-binding domain to the transmembrane domain. The spacer region can be flexible enough to allow the antigen-binding domain to orient in different directions to facilitate antigen recognition while preserving the activating activity of the CAR. In certain non-limiting embodiments, the spacer region can be the hinge region from IgGl, the CH_2CH_3 region of immunoglobulin and portions of CD3, a portion of a CD28 polypeptide (*e.g.*, SEQ ID NO: 85), a portion of a CD8 polypeptide (*e.g.*, SEQ ID NO: 87), a variation of any of the foregoing which is at least about 80%, at least about 85%, at least about 90%, or at least about 95% homologous thereto, or a synthetic spacer

sequence. In certain non-limiting embodiments, the spacer region may have a length between about 1-50 (*e.g.*, 5-25, 10-30, or 30-50) amino acids.

[00290] *Intracellular Domain of a CAR.* In certain non-limiting embodiments, an intracellular domain of the CAR can comprise a CD3ζ polypeptide, which can activate or stimulate a cell (*e.g.*, a cell of the lymphoid lineage, *e.g.*, a T cell). CD3ζ comprises 3 ITAMs, and transmits an activation signal to the cell (*e.g.*, a cell of the lymphoid lineage, *e.g.*, a T cell) after antigen is bound. The CD3ζ polypeptide can have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to the sequence having a NCBI Reference No: NP_932170 (SEQ ID NO: 90), or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

[00291] In certain embodiments, the CD3ζ polypeptide can have an amino acid sequence that is a consecutive portion of SEQ ID NO: 91 which is at least 20, or at least 30, or at least 40, or at least 50, and up to 164 amino acids in length. Additionally or alternatively, in various embodiments, the CD3ζ polypeptide has an amino acid sequence of amino acids 1 to 164, 1 to 50, 50 to 100, 100 to 150, or 150 to 164 of SEQ ID NO: 91. In certain embodiments, the CD3ζ polypeptide has an amino acid sequence of amino acids 52 to 164 of SEQ ID NO: 91.

[00292] SEQ ID NO: 91 is provided below:
MKWKALFTAAILQAQLPITEAQSFGLLDPKLCYLLDGILFIYGVILTALFLRVKFSRS ADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPQRRKNPQEGLYN ELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQALPPR (SEQ ID NO: 91)

[00293] In certain embodiments, the CD3ζ polypeptide has the amino acid sequence set forth in SEQ ID NO: 92, which is provided below:
RVKFSRSAEPPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQ ALPPR (SEQ ID NO: 92)

[00294] In certain embodiments, the CD3ζ polypeptide has the amino acid sequence set forth in SEQ ID NO: 93, which is provided below:
RVKFSRSADAPAYQQGQNQLYNELNLGRREEYDVLDKRRGRDPEMGGKPRRKNP

QEGLYNELQKDKMAEAYSEIGMKGERRRGKGHDGLYQGLSTATKDTYDALHMQ ALPPR (SEQ ID NO: 93)

[00295] In accordance with the presently disclosed subject matter, a "CD3ζ nucleic acid molecule" refers to a polynucleotide encoding a CD3ζ polypeptide. In certain embodiments, the CD3ζ nucleic acid molecule encoding the CD3ζ polypeptide (SEQ ID NO: 92) comprised in the intracellular domain of the presently disclosed CAR comprises a nucleotide sequence as set forth in SEQ ID NO: 94 as provided below.

AGAGTGAAGTTCAGCAGGAGCGCAGAGCCCCCCGCGTACCAGCAGGGCCAGAA CCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGG ACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAA CCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCT ACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGG CCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACAT GCAGGCCCTGCCCCCTCGCG (SEQ ID NO: 94)

[00296] In certain embodiments, the CD3ζ nucleic acid molecule encoding the CD3ζ polypeptide (SEQ ID NO: 93) comprised in the intracellular domain of the presently disclosed CAR comprises a nucleotide sequence as set forth in SEQ ID NO: 95 as provided below.

AGAGTGAAGTTCAGCAGGAGCGCAGACGCCCCCGCGTACCAGCAGGGCCAGAA CCAGCTCTATAACGAGCTCAATCTAGGACGAAGAGAGGAGTACGATGTTTTGG ACAAGAGACGTGGCCGGGACCCTGAGATGGGGGGAAAGCCGAGAAGGAAGAA CCCTCAGGAAGGCCTGTACAATGAACTGCAGAAAGATAAGATGGCGGAGGCCT ACAGTGAGATTGGGATGAAAGGCGAGCGCCGGAGGGGCAAGGGGCACGATGG CCTTTACCAGGGTCTCAGTACAGCCACCAAGGACACCTACGACGCCCTTCACAT GCAGGCCCTGCCCCCTCGCTAA (SEQ ID NO: 95)

[00297] In certain non-limiting embodiments, an intracellular domain of the CAR further comprises at least one signaling region. The at least one signaling region can include a CD28 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, an ICOS polypeptide, a DAP- 10 polypeptide, a PD-1 polypeptide, a CTLA-4 polypeptide, a LAG-3 polypeptide, a 2B4 polypeptide, a BTLA polypeptide, a synthetic peptide (not based on a protein associated with the immune response), or a combination thereof.

[00298] In certain embodiments, the signaling region is a co-stimulatory signaling region.

[00299] In certain embodiments, the co-stimulatory signaling region comprises at least one co-stimulatory molecule, which can provide optimal lymphocyte activation. As used herein, "co-stimulatory molecules" refer to cell surface molecules other than antigen receptors or their ligands that are required for an efficient response of lymphocytes to antigen. The at least one co-stimulatory signaling region can include a CD28 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, an ICOS polypeptide, a DAP-10 polypeptide, or a combination thereof. The co-stimulatory molecule can bind to a co-stimulatory ligand, which is a protein expressed on cell surface that upon binding to its receptor produces a co-stimulatory response, *i.e.,* an intracellular response that effects the stimulation provided when an antigen binds to its CAR molecule. Co-stimulatory ligands, include, but are not limited to CD80, CD86, CD70, OX40L, 4-1BBL, CD48, TNFRSF14, and PD- Ll. As one example, a 4-1BB ligand (*i.e.,* 4-1BBL) may bind to 4-1BB (also known as "CD 137") for providing an intracellular signal that in combination with a CAR signal induces an effector cell function of the CAR^+ T cell. CARs comprising an intracellular domain that comprises a co-stimulatory signaling region comprising 4-1BB, ICOS or DAP-10 are disclosed in U.S. 7,446,190, which is herein incorporated by reference in its entirety. In certain embodiments, the intracellular domain of the CAR comprises a co-stimulatory signaling region that comprises a CD28 polypeptide. In certain embodiments, the intracellular domain of the CAR comprises a co-stimulatory signaling region that comprises two co-stimulatory molecules: CD28 and 4-1BB or CD28 and OX40.

[00300] 4-1BB can act as a tumor necrosis factor (TNF) ligand and have stimulatory activity. The 4-1BB polypeptide can have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or 100% homologous to the sequence having a UniProtKB Reference No: P41273 or NCBI Reference No: NP_001552 (SEQ ID NO: 96) or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

[00301] SEQ ID NO: 96 is provided below:

MGNSCYNIVATLLLVLNFERTRSLQDPCSNCPAGTFCDNNRNQICSPCPPNSFSSAG
GQRTCDICRQCKGVFRTRKECSSTSNAECDCTPGFHCLGAGCSMCEQDCKQGQEL
TKKGCKDCCFGTFNDQKRGICRPWTNCSLDGKSVLVNGTKERDVVCGPSPADLSP

GASSVTPPAPAREPGHSPQIISFFLALTSTALLFLLFFLTLRFSVVKRGRKKLLYIFKQ PFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO: 96)

[00302] In certain embodiments, the 4-1BB co-stimulatory domain has the amino acid sequence set forth in SEQ ID NO: 97, which is provided below:

KRGRKKLLYIFKQPFMRPVQTTQEEDGCSCRFPEEEEGGCEL (SEQ ID NO: 97)

[00303] In accordance with the presently disclosed subject matter, a "4-1BB nucleic acid molecule" refers to a polynucleotide encoding a 4-1BB polypeptide. In certain embodiments, the 4-1BB nucleic acid molecule encoding the 4-1BB polypeptide (SEQ ID NO: 97) comprised in the intracellular domain of the presently disclosed CAR comprises a nucleotide sequence as set forth in SEQ ID NO: 98 as provided below.

AAACGGGGCAGAAAGAAGCTCCTGTATATATTCAAACAACCATTTATGAGACC AGTACAAACTACTCAAGAGGAAGATGGCTGTAGCTGCCGATTTCCAGAAGAAG AAGAAGGAGGATGTGAACTG (SEQ ID NO: 98)

[00304] An OX40 polypeptide can have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or 100% homologous to the sequence having a UniProtKB Reference No: P43489 or NCBI Reference No: NP_003318 (SEQ ID NO: 99), or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

[00305] SEQ ID NO: 99 is provided below:

MCVGARRLGRGPCAALLLLGLGLSTVTGLHCVGDTYPSNDRCCHECRPGNGMVS RCSRSQNTVCRPCGPGFYNDVVSSKPCKPCTWCNLRSGSERKQLCTATQDTVCRC RAGTQPLDSYKPGVDCAPCPPGHFSPGDNQACKPWTNCTLAGKHTLQPASNSSDAI CEDRDPPATQPQETQGPPARPITVQPTEAWPRTSQGPSTRPVEVPGGRAVAAILGLG LVLGLLGPLAILLALYLLRRDQRLPPDAHKPPGGGSFRTPIQEEQADAHSTLAKI (SEQ ID NO: 99)

[00306] In accordance with the presently disclosed subject matter, an "OX40 nucleic acid molecule" refers to a polynucleotide encoding an OX40 polypeptide.

[00307] An ICOS polypeptide can have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or 100% homologous to the sequence having a NCBI Reference No: NP_036224 (SEQ ID NO: 100)

or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

[00308] SEQ ID NO: 100 is provided below:
MKSGLWYFFLFCLRIKVLTGEINGSANYEMFIFHNGGVQILCKYPDIVQQFKMQLL
KGGQILCDLTKTKGSGNTVSIKSLKFCHSQLSNNSVSFFLYNLDHSHANYYFCNLSI
FDPPPFKVTLTGGYLHIYESQLCCQLKFWLPIGCAAFVVVCILGCILICWLTKKKYSS
SVHDPNGEYMFMRAVNTAKKSRLTDVTL (SEQ ID NO: 100)

[00309] In accordance with the presently disclosed subject matter, an "ICOS nucleic acid molecule" refers to a polynucleotide encoding an ICOS polypeptide.

[00310] CTLA-4 is an inhibitory receptor expressed by activated T cells, which when engaged by its corresponding ligands (CD80 and CD86; B7-1 and B7-2, respectively), mediates activated T cell inhibition or anergy. In both preclinical and clinical studies, CTLA-4 blockade by systemic antibody infusion, enhanced the endogenous anti-tumor response albeit, in the clinical setting, with significant unforeseen toxicities.

[00311] CTLA-4 contains an extracellular V domain, a transmembrane domain, and a cytoplasmic tail. Alternate splice variants, encoding different isoforms, have been characterized. The membrane-bound isoform functions as a homodimer interconnected by a disulfide bond, while the soluble isoform functions as a monomer. The intracellular domain is similar to that of CD28, in that it has no intrinsic catalytic activity and contains one YVKM motif able to bind PI3K, PP2A and SHP-2 and one proline-rich motif able to bind SH3 containing proteins. One role of CTLA-4 in inhibiting T cell responses seem to be directly via SHP-2 and PP2A dephosphorylation of TCR-proximal signaling proteins such as CD3 and LAT. CTLA-4 can also affect signaling indirectly via competing with CD28 for CD80/86 binding. CTLA-4 has also been shown to bind and/or interact with PI3K, CD80, AP2M1, and PPP2R5A.

[00312] In accordance with the presently disclosed subject matter, a CTLA-4 polypeptide can have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to UniProtKB/Swiss-Prot Ref. No.: P16410.3 (SEQ ID NO: 101) (homology herein may be determined using standard software such as BLAST or FASTA) or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

[00313] SEQ ID NO: 101 is provided below:
MACLGFQRHKAQLNLATRTWPCTLLFFLLFIPVFCKAMHVAQPAVVLASSRGIASF
VCEYASPGKATEVRVTVLRQADSQVTEVCAATYMMGNELTFLDDSICTGTSSGNQ
VNLTIQGLRAMDTGLYICKVELMYPPPYYLGIGNGTQIYVIDPEPCPDSDFLLWILA
AVSSGLFFYSFLLTAVSLSKMLKKRSPLTTGVYVKMPPTEPECEKQFQPYFIPIN
(SEQ ID NO: 101)

[00314] In accordance with the presently disclosed subject matter, a "CTLA-4 nucleic acid molecule" refers to a polynucleotide encoding a CTLA-4 polypeptide.

[00315] PD-1 is a negative immune regulator of activated T cells upon engagement with its corresponding ligands PD-L1 and PD-L2 expressed on endogenous macrophages and dendritic cells. PD-1 is a type I membrane protein of 268 amino acids. PD-1 has two ligands, PD-L1 and PD-L2, which are members of the B7 family. The protein's structure comprises an extracellular IgV domain followed by a transmembrane region and an intracellular tail. The intracellular tail contains two phosphorylation sites located in an immunoreceptor tyrosine-based inhibitory motif and an immunoreceptor tyrosine- based switch motif, that PD-1 negatively regulates TCR signals. SHP- I and SHP-2 phosphatases bind to the cytoplasmic tail of PD-1 upon ligand binding. Upregulation of PD-L1 is one mechanism tumor cells may evade the host immune system. In pre-clinical and clinical trials, PD-1 blockade by antagonistic antibodies induced anti -tumor responses mediated through the host endogenous immune system. In accordance with the presently disclosed subject matter, a PD-1 polypeptide can have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to NCBI Reference No: NP_005009.2 (SEQ ID NO: 102) or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

[00316] SEQ ID NO: 102 is provided below:
MQIPQAPWPVVWAVLQLGWRPGWFLDSPDRPWNPPTFSPALLVVTEGDNATFTCS
FSNTSESFVLNWYRMSPSNQTDKLAAFPEDRSQPGQDCRFRVTQLPNGRDFHMSV
VRARRNDSGTYLCGAISLAPKAQIKESLRAELRVTERRAEVPTAHPSPSPRPAGQFQ
TLVVGVVGGLLGSLVLLVWVLAVICSRAARGTIGARRTGQPLKEDPSAVPVFSVDY
GELDFQWREKTPEPPVPCVPEQTEYATIVFPSGMGTSSPARRGSADGPRSAQPLRPE
DGHCSWPL (SEQ ID NO: 102)

[00317] In accordance with the presently disclosed subject matter, a "PD-1 nucleic acid molecule" refers to a polynucleotide encoding a PD-1 polypeptide.

[00318] Lymphocyte-activation protein 3 (LAG-3) is a negative immune regulator of immune cells. LAG-3 belongs to the immunoglobulin (Ig) superfamily and contains 4 extracellular Ig-like domains. The LAG3 gene contains 8 exons. The sequence data, exon/intron organization, and chromosomal localization all indicate a close relationship of LAG3 to CD4. LAG3 has also been designated CD223 (cluster of differentiation 223).

[00319] In accordance with the presently disclosed subject matter, a LAG-3 polypeptide can have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to UniProtKB/Swiss-Prot Ref. No.: P18627.5 (SEQ ID NO: 103) or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

[00320] SEQ ID NO: 103 is provided below:
MWEAQFLGLLFLQPLWVAPVKPLQPGAEVPVVWAQEGAPAQLPCSPTIPLQDLSLL
RRAGVTWQHQPDSGPPAAAPGHPLAPGPHPAAPSSWGPRPRRYTVLSVGPGGLRS
GRLPLQPRVQLDERGRQRGDFSLWLRPARRADAGEYRAAVHLRDRALSCRLRLRL
GQASMTASPPGSLRASDWVILNCSFSRPDRPASVHWFRNRGQGRVPVRESPHHHLA
ESFLFLPQVSPMDSGPWGCILTYRDGFNVSIMYNLTVLGLEPPTPLTVYAGAGSRVG
LPCRLPAGVGTRSFLTAKWTPPGGGPDLLVTGDNGDFTLRLEDVSQAQAGTYTCHI
HLQEQQLNATVTLAIITVTPKSFGSPGSLGKLLCEVTPVSGQERFVWSSLDTPSQRSF
SGPWLEAQEAQLLSQPWQCQLYQGERLLGAAVYFTELSSPGAQRSGRAPGALPAG
HLLLFLILGVLSLLLLVTGAFGFHLWRRQWRPRRFSALEQGIHPPQAQSKIEELEQEP
EPEPEPEPEPEPEPEPEQL (SEQ ID NO: 103)

[00321] In accordance with the presently disclosed subject matter, a "LAG-3 nucleic acid molecule" refers to a polynucleotide encoding a LAG-3 polypeptide.

[00322] Natural Killer Cell Receptor 2B4 (2B4) mediates non-MHC restricted cell killing on NK cells and subsets of T cells. To date, the function of 2B4 is still under investigation, with the 2B4-S isoform believed to be an activating receptor, and the 2B4-L isoform believed to be a negative immune regulator of immune cells. 2B4 becomes engaged upon binding its high-affinity ligand, CD48. 2B4 contains a tyrosine-based switch motif, a molecular switch that allows the protein to associate with various phosphatases. 2B4 has also been designated CD244 (cluster of differentiation 244).

[00323] In accordance with the presently disclosed subject matter, a 2B4 polypeptide can have an amino acid sequence that is at least about 85%, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to UniProtKB/Swiss-Prot Ref. No.: Q9BZW8.2 (SEQ ID NO: 104) or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

[00324] SEQ ID NO: 104 is provided below:
MLGQVVTLILLLLLKVYQGKGCQGSADHVVSISGVPLQLQPNSIQTKVDSIAWKKL
LPSQNGFHHILKWENGSLPSNTSNDRFSFIVKNLSLLIKAAQQQDSGLYCLEVTSISG
KVQTATFQVFVFESLLPDKVEKPRLQGQGKILDRGRCQVALSCLVSRDGNVSYAW
YRGSKLIQTAGNLTYLDEEVDINGTHTYTCNVSNPVSWESHTLNLTQDCQNAHQEF
RFWPFLVIIVILSALFLGTLACFCVWRRKRKEKQSETSPKEFLTIYEDVKDLKTRRNH
EQEQTFPGGGSTIYSMIQSQSSAPTSQEPAYTLYSLIQPSRKSGSRKRNHSPSFNSTIY
EVIGKSQPKAQNPARLSRKELENFDVYS (SEQ ID NO: 104)

[00325] In accordance with the presently disclosed subject matter, a "2B4 nucleic acid molecule" refers to a polynucleotide encoding a 2B4 polypeptide.

[00326] B- and T-lymphocyte attenuator (BTLA) expression is induced during activation of T cells, and BTLA remains expressed on Thl cells but not Th2 cells. Like PD1 and CTLA4, BTLA interacts with a B7 homolog, B7H4. However, unlike PD-1 and CTLA-4, BTLA displays T-Cell inhibition via interaction with tumor necrosis family receptors (TNF-R), not just the B7 family of cell surface receptors. BTLA is a ligand for tumor necrosis factor (receptor) superfamily, member 14 (TNFRSF14), also known as herpes virus entry mediator (HVEM). BTLA-HVEM complexes negatively regulate T-cell immune responses. BTLA activation has been shown to inhibit the function of human $CD8^+$ cancer-specific T cells. BTLA has also been designated as CD272 (cluster of differentiation 272).

[00327] In accordance with the presently disclosed subject matter, a BTLA polypeptide can have an amino acid sequence that is at least about 85%>, about 90%, about 95%, about 96%, about 97%, about 98%, about 99% or about 100% homologous to UniProtKB/Swiss-Prot Ref. No.: Q7Z6A9.3 (SEQ ID NO: 105) or fragments thereof, and/or may optionally comprise up to one or up to two or up to three conservative amino acid substitutions.

[00328] SEQ ID NO: 105 is provided below:
MKTLPAMLGTGKLFWVFFLIPYLDIWNIHGKESCDVQLYIKRQSEHSILAGDPFELE
CPVKYCANRPHVTWCKLNGTTCVKLEDRQTSWKEEKNISFFILHFEPVLPNDNGSY

RCSANFQSNLIESHSTTLYVTDVKSASERPSKDEMASRPWLLYRLLPLGGLPLLITTC FCLFCCLRRHQGKQNELSDTAGREINLVDAHLKSEQTEASTRQNSQVLLSETGIYD NDPDLCFRMQEGSEVYSNPCLEENKPGIVYASLNHSVIGPNSRLARNVKEAPTEYA SICVRS (SEQ ID NO: 105)

[00329] In accordance with the presently disclosed subject matter, a "BTLA nucleic acid molecule" refers to a polynucleotide encoding a BTLA polypeptide.

Engineered Immune Cells of the Present Technology

[00330] As described herein, immune cells can be engineered to constitutively or conditionally express an anti-CLDN18.2 antigen binding fragment that binds to a CLDN18.2 antigen present on the cell surface of target cells. The engineered immune cells of the present technology express a chimeric antigen receptor comprising an anti-CLDN18.2 antigen binding fragment (*e.g.*, scFv) that permits delivery of the immune cell to the target cells. In some embodiments, the engineered immune cells provided herein express a T-cell receptor (TCR) or other cell-surface ligand that binds to a CLDN18.2 antigen. In some embodiments, the T cell receptor is a chimeric T-cell receptor (CAR).

[00331] In exemplary embodiments provided herein, the engineered immune cells provided herein express a T-cell receptor (TCR) (*e.g.*, a CAR) or other cell-surface ligand that binds to a CLDN18.2 antigen. In some embodiments, the engineered immune cells provided herein express a T-cell receptor (TCR) (*e.g.*, a CAR) or other cell-surface ligand that binds to a CLDN18.2 antigen presented in the context of an MHC molecule. In some embodiments, the engineered immune cells provided herein express a T-cell receptor (TCR) (*e.g.*, a CAR) or other cell-surface ligand that binds to a CLDN18.2 antigen presented in the context of an HLA-A2 molecule.

[00332] The engineered immune cells (*e.g.*, CAR T cells) provided herein that express a CLDN18.2-specific antigen receptor, *e.g.*, a chimeric antigen receptor, are useful in methods for treating or ameliorating the effects of CLDN18.2-associated cancers, such as gastric cancer, esophageal cancer, pancreatic cancer, lung cancer, non small cell lung cancer (NSCLC), ovarian cancer, colon cancer, hepatic cancer, head-neck cancer, and gallbladder cancer.

[00333] In certain embodiments, the engineered immune cells will proliferate extensively (*e.g.*, 100 times or more) when it encounters a CLDN18.2 antigen at a tissue site, thus

significantly increasing production of the chimeric antigen receptor comprising the anti-CLDN18.2 antigen binding fragment. The engineered immune cells (*e.g.*, CAR T cells) can be generated by *in vitro* transduction of immune cells with a nucleic acid encoding the chimeric antigen receptor comprising the anti-CLDN18.2 antigen binding fragment. Further, the activity of the engineered immune cells (*e.g.*, CAR T cells) can be adjusted by selection of co-stimulatory molecules included in the chimeric antigen receptor.

[00334] In some embodiments, the chimeric antigen receptor comprises a CLDN18.2 antigen binding fragment (*e.g.*, scFv) comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein: (a) the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 6, a V_H-CDR2 sequence of SEQ ID NO: 7, and a V_H-CDR3 sequence of SEQ ID NO: 8, and/or the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 9, a V_L-CDR2 sequence of SEQ ID NO: 10 or SEQ ID NO: 157, and a V_L-CDR3 sequence of SEQ ID NO: 11; (b) the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 12, a V_H-CDR2 sequence of SEQ ID NO: 13, and a V_H-CDR3 sequence of SEQ ID NO: 14, and/or the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 15, a V_L-CDR2 sequence of SEQ ID NO: 16 or SEQ ID NO: 158, and a V_L-CDR3 sequence of SEQ ID NO: 17; (c) the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 18, a V_H-CDR2 sequence of SEQ ID NO: 19, and a V_H-CDR3 sequence of SEQ ID NO: 20, and/or the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 21, a V_L-CDR2 sequence of SEQ ID NO: 22, and a V_L-CDR3 sequence of SEQ ID NO: 23; (d) the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 24, a V_H-CDR2 sequence of SEQ ID NO: 25, and a V_H-CDR3 sequence of SEQ ID NO: 26, and/or the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 27, a V_L-CDR2 sequence of SEQ ID NO: 28, and a V_L-CDR3 sequence of SEQ ID NO: 29; or (e) the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 30, a V_H-CDR2 sequence of SEQ ID NO: 31, and a V_H-CDR3 sequence of SEQ ID NO: 32, and/or the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 33, a V_L-CDR2 sequence of SEQ ID NO: 34, and a V_L-CDR3 sequence of SEQ ID NO: 35.

[00335] Additionally or alternatively, in some embodiments, the amino acid sequence of the V_H of the anti-CLDN18.2 antigen binding fragment (*e.g.*, scFv) is selected from any one of SEQ ID NOs: 36, 38, 40, 42, 44, 46-49, or 54-57.

[00336] Additionally or alternatively, in some embodiments, the amino acid sequence of the V_L of the anti-CLDN18.2 antigen binding fragment (*e.g.*, scFv) is selected from any one of SEQ ID NOs: 37, 39, 41, 43, 45, 50-53, or 58-61.

[00337] In some embodiments, the anti-CLDN18.2 antigen binding fragment is an scFv, a Fab, or a $(Fab)_2$.

[00338] In certain embodiments, the CLDN18.2-specific CAR of the present technology and a reporter or selection marker (*e.g.*, GFP, LNGFR) are expressed as a single polypeptide linked by a self-cleaving linker, such as a P2A linker. In certain embodiments, the CAR and a reporter or selection marker (*e.g.*, GFP, LNGFR) are expressed as two separate polypeptides.

[00339] In certain embodiments, the CAR comprises an extracellular antigen binding fragment (*e.g.*, scFv) that specifically binds to a CLDN18.2 antigen, a transmembrane domain comprising a CD28 polypeptide and/or a CD8 polypeptide, and an intracellular domain comprising a CD3ζ polypeptide and optionally a co-stimulatory signaling region disclosed herein. The CAR may also comprise a signal peptide or a leader sequence covalently joined to the N-terminus of the extracellular antigen binding fragment. The signal peptide comprises amino acids having the sequence set forth in SEQ ID NO: 81, or SEQ ID NO: 83.

[00340] In some embodiments, the nucleic acid encoding the CAR of the present technology is operably linked to an inducible promoter. In some embodiments, the nucleic acid encoding the CAR of the present technology is operably linked to a constitutive promoter.

[00341] In some embodiments, the inducible promoter is a synthetic Notch promoter that is activatable in a CAR T cell, where the intracellular domain of the CAR contains a transcriptional regulator that is released from the membrane when engagement of the CAR with the CLDN18.2 antigen induces intramembrane proteolysis (*see*, *e.g.*, Morsut *et al.*, *Cell* 164(4): 780–791 (2016). Accordingly, further transcription of the CLDN18.2-specific CAR is induced upon binding of the engineered immune cell with the CLDN18.2 antigen.

[00342] The presently disclosed subject matter also provides isolated nucleic acid molecules encoding the CAR constructs described herein or a functional portion thereof. In certain embodiments, the isolated nucleic acid molecule encodes an anti- CLDN18.2-

targeted CAR comprising (a) an antigen binding fragment (*e.g.*, an scFv) that specifically binds to a CLDN18.2 antigen, (b) a transmembrane domain comprising a CD8 polypeptide or CD28 polypeptide, and (c) an intracellular domain comprising a CD3ζ polypeptide, and optionally one or more of a co-stimulatory signaling region disclosed herein, a P2A self-cleaving peptide, and/or a reporter or selection marker (*e.g.*, GFP, LNGFR) provided herein. The at least one co-stimulatory signaling region can include a CD28 polypeptide, a 4-1BB polypeptide, an OX40 polypeptide, an ICOS polypeptide, a DAP- 10 polypeptide, a PD-1 polypeptide, a CTLA-4 polypeptide, a LAG-3 polypeptide, a 2B4 polypeptide, a BTLA polypeptide, a synthetic peptide (not based on a protein associated with the immune response), or a combination thereof.

[00343] In certain embodiments, the isolated nucleic acid molecule encodes an anti-CLDN18.2-targeted CAR comprising an antigen binding fragment (*e.g.*, an scFv) that specifically binds to a CLDN18.2 antigen, fused to a synthetic Notch transmembrane domain and an intracellular cleavable transcription factor. In certain embodiments, the present disclosure provides an isolated nucleic acid molecule encoding a CLDN18.2-specific CAR that is inducible by release of the transcription factor of a synthetic Notch system.

[00344] In certain embodiments, the isolated nucleic acid molecule encodes a functional portion of a presently disclosed CAR constructs. As used herein, the term "functional portion" refers to any portion, part or fragment of a CAR, which portion, part or fragment retains the biological activity of the parent CAR. For example, functional portions encompass the portions, parts or fragments of a CLDN18.2-specific CAR that retains the ability to recognize a target cell, to treat cancer, to a similar, same, or even a higher extent as the parent CAR. In certain embodiments, an isolated nucleic acid molecule encoding a functional portion of a CLDN18.2-specific CAR can encode a protein comprising, *e.g.*, about 10%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, and about 95%, or more of the parent CAR.

[00345] The presently disclosed subject matter provides engineered immune cells expressing a CLDN18.2-specific T-cell receptor (*e.g.*, a CAR) or other ligand that comprises an extracellular antigen-binding domain, a transmembrane domain and an intracellular domain, where the extracellular antigen-binding domain specifically binds a

CLDN18.2 antigen. In certain embodiments immune cells can be transduced with a presently disclosed CAR constructs such that the cells express the CAR. The presently disclosed subject matter also provides methods of using such cells for the treatment of cancer.

[00346] The engineered immune cells of the presently disclosed subject matter can be cells of the lymphoid lineage or myeloid lineage. The lymphoid lineage, comprising B, T, and natural killer (NK) cells, provides for the production of antibodies, regulation of the cellular immune system, detection of foreign agents in the blood, detection of cells foreign to the host, and the like. Non-limiting examples of immune cells of the lymphoid lineage include T cells, Natural Killer (NK) cells, embryonic stem cells, and pluripotent stem cells (*e.g.*, those from which lymphoid cells may be differentiated). T cells can be lymphocytes that mature in the thymus and are chiefly responsible for cell-mediated immunity. T cells are involved in the adaptive immune system. The T cells of the presently disclosed subject matter can be any type of T cells, including, but not limited to, T helper cells, cytotoxic T cells, memory T cells (including central memory T cells, stem-cell-like memory T cells (or stem-like memory T cells), and two types of effector memory T cells: *e.g.*, T_{EM} cells and TEMRA cells, Regulatory T cells (also known as suppressor T cells), Natural killer T cells, Mucosal associated invariant T cells, and γδ T cells. Cytotoxic T cells (CTL or killer T cells) are a subset of T lymphocytes capable of inducing the death of infected somatic or tumor cells. In certain embodiments, the CAR-expressing T cells express Foxp3 to achieve and maintain a T regulatory phenotype.

[00347] Natural killer (NK) cells can be lymphocytes that are part of cell-mediated immunity and act during the innate immune response. NK cells do not require prior activation in order to perform their cytotoxic effect on target cells.

[00348] The engineered immune cells of the presently disclosed subject matter can express an extracellular antigen-binding domain (*e.g.*, an scFv, a Fab that is optionally crosslinked, or a $F(ab)_2$) that specifically binds to a CLDN18.2 antigen, for the treatment of cancer. Such engineered immune cells can be administered to a subject (*e.g.*, a human subject) in need thereof for the treatment of cancer. In some embodiments, the immune cell is a lymphocyte, such as a T cell, a B cell or a natural killer (NK) cell. In certain embodiments, the engineered immune cell is a T cell. The T cell can be a $CD4^+$ T cell or a

$CD8^+$ T cell. In certain embodiments, the T cell is a $CD4^+$ T cell. In certain embodiments, the T cell is a $CD8^+$ T cell.

[00349] The engineered immune cells of the present disclosure can further include at least one recombinant or exogenous co-stimulatory ligand. For example, the engineered immune cells of the present disclosure can be further transduced with at least one co-stimulatory ligand, such that the engineered immune cells co-expresses or is induced to co-express the CLDN18.2-specific CAR and the at least one co-stimulatory ligand. The interaction between the CLDN18.2-specific CAR and the at least one co-stimulatory ligand provides a non-antigen-specific signal important for full activation of an immune cell (*e.g.*, T cell). Co-stimulatory ligands include, but are not limited to, members of the tumor necrosis factor (TNF) superfamily, and immunoglobulin (Ig) superfamily ligands. TNF is a cytokine involved in systemic inflammation and stimulates the acute phase reaction. Its primary role is in the regulation of immune cells. Members of TNF superfamily share a number of common features. The majority of TNF superfamily members are synthesized as type II transmembrane proteins (extracellular C-terminus) containing a short cytoplasmic segment and a relatively long extracellular region. TNF superfamily members include, without limitation, nerve growth factor (NGF), CD40L (CD40L)/CD 154, CD137L/4-1BBL, TNF-α, CD134L/OX40L/CD252, CD27L/CD70, Fas ligand (FasL), CD30L/CD153, tumor necrosis factor beta (TNFP)/lymphotoxin-alpha (LT-α), lymphotoxin-beta (LT-β), CD257/B cell-activating factor (BAFF)/BLYS/THANK/TALL-1, glucocorticoid-induced TNF Receptor ligand (GITRL), TNF-related apoptosis-inducing ligand (TRAIL), and LIGHT (TNFSF14). The immunoglobulin (Ig) superfamily is a large group of cell surface and soluble proteins that are involved in the recognition, binding, or adhesion processes of cells. These proteins share structural features with immunoglobulins — they possess an immunoglobulin domain (fold). Immunoglobulin superfamily ligands include, but are not limited to, CD80 and CD86, both ligands for CD28, or PD-L1/(B7-H1) that are ligands for PD-1. In certain embodiments, the at least one co-stimulatory ligand is selected from the group consisting of 4-1BBL, CD80, CD86, CD70, OX40L, CD48, TNFRSF14, PD-L1, and combinations thereof. In certain embodiments, the engineered immune cell comprises one recombinant co-stimulatory ligand (*e.g.*, 4-1BBL). In certain embodiments, the engineered immune cell comprises two recombinant co-stimulatory ligands (*e.g.*, 4-1BBL and CD80). CARs comprising at least one co-stimulatory ligand are described in U.S. Patent No. 8,389,282, which is incorporated by reference in its entirety.

[00350] Furthermore, the engineered immune cells of the present disclosure can further comprise at least one exogenous cytokine. For example, a presently disclosed engineered immune cell can be further transduced with at least one cytokine, such that the engineered immune cell secretes the at least one cytokine as well as expresses the CLDN18.2-specific CAR. In certain embodiments, the at least one cytokine is selected from the group consisting of IL-2, IL- 3, IL-6, IL-7, IL-11, IL-12, IL-15, IL-17, and IL-21.

[00351] The engineered immune cells can be generated from peripheral donor lymphocytes, *e.g.*, those disclosed in Sadelain, M., *et al., Nat Rev Cancer* 3 :35-45 (2003) (disclosing peripheral donor lymphocytes genetically modified to express CARs), in Morgan, R.A. *et al., Science* 314: 126-129 (2006) (disclosing peripheral donor lymphocytes genetically modified to express a full-length tumor antigen-recognizing T cell receptor complex comprising the α and β heterodimer), in Panelli *et al., J Immunol* 164:495-504 (2000); Panelli *et al., J Immunol* 164:4382-4392 (2000) (disclosing lymphocyte cultures derived from tumor infiltrating lymphocytes (TILs) in tumor biopsies), and in Dupont *et al., Cancer Res* 65:5417-5427 (2005); Papanicolaou *et al., Blood* 102:2498-2505 (2003) (disclosing selectively inv/Yro-expanded antigen-specific peripheral blood leukocytes employing artificial antigen-presenting cells (AAPCs) or pulsed dendritic cells). The engineered immune cells (*e.g.*, T cells) can be autologous, non-autologous (*e.g.*, allogeneic), or derived *in vitro* from engineered progenitor or stem cells.

[00352] In certain embodiments, the engineered immune cells of the present disclosure (*e.g.*, T cells) express from about 1 to about 5, from about 1 to about 4, from about 2 to about 5, from about 2 to about 4, from about 3 to about 5, from about 3 to about 4, from about 4 to about 5, from about 1 to about 2, from about 2 to about 3, from about 3 to about 4, or from about 4 to about 5 vector copy numbers per cell of a presently disclosed CLDN18.2-specific CAR.

[00353] For example, the higher the CAR expression level in an engineered immune cell, the greater cytotoxicity and cytokine production the engineered immune cell exhibits. An engineered immune cell (*e.g.*, T cell) having a high CLDN18.2-specific CAR expression level can induce antigen-specific cytokine production or secretion and/or exhibit cytotoxicity to a tissue or a cell having a low expression level of CLDN18.2-specific CAR, *e.g.*, about 2,000 or less, about 1,000 or less, about 900 or less, about 800 or less, about 700 or less, about 600 or less, about 500 or less, about 400 or less, about 300 or less, about 200

or less, about 100 or less of CLDN18.2 antigen binding sites/cell. Additionally or alternatively, the cytotoxicity and cytokine production of a presently disclosed engineered immune cell (*e.g.*, T cell) are proportional to the expression level of CLDN18.2 antigen in a target tissue or a target cell. For example, the higher the expression level of CLDN18.2 antigen in the target, the greater cytotoxicity and cytokine production the engineered immune cell exhibits.

[00354] The unpurified source of immune cells may be any source known in the art, such as the bone marrow, fetal, neonate or adult or other hematopoietic cell source, *e.g.*, fetal liver, peripheral blood or umbilical cord blood. Various techniques can be employed to separate the cells. For instance, negative selection methods can remove non-immune cells initially. Monoclonal antibodies are particularly useful for identifying markers associated with particular cell lineages and/or stages of differentiation for both positive and negative selections.

[00355] A large proportion of terminally differentiated cells can be initially removed by a relatively crude separation. For example, magnetic bead separations can be used initially to remove large numbers of irrelevant cells. Suitably, at least about 80%, usually at least 70% of the total hematopoietic cells will be removed prior to cell isolation.

[00356] Procedures for separation include, but are not limited to, density gradient centrifugation; resetting; coupling to particles that modify cell density; magnetic separation with antibody-coated magnetic beads; affinity chromatography; cytotoxic agents joined to or used in conjunction with a mAb, including, but not limited to, complement and cytotoxins; and panning with antibody attached to a solid matrix, *e.g.,* plate, chip, elutriation or any other convenient technique.

[00357] Techniques for separation and analysis include, but are not limited to, flow cytometry, which can have varying degrees of sophistication, *e.g.*, a plurality of color channels, low angle and obtuse light scattering detecting channels, impedance channels.

[00358] The cells can be selected against dead cells, by employing dyes associated with dead cells such as propidium iodide (PI). Usually, the cells are collected in a medium comprising 2% fetal calf serum (FCS) or 0.2% bovine serum albumin (BSA) or any other suitable (*e.g.*, sterile), isotonic medium.

[00359] In some embodiments, the engineered immune cells comprise one or more additional modifications. For example, in some embodiments, the engineered immune cells comprise and express (are transduced to express) an antigen recognizing receptor that binds to a second antigen that is different than the first CLDN18.2 antigen. The inclusion of an antigen recognizing receptor in addition to a presently disclosed CAR on the engineered immune cell can increase the avidity of the CAR (or the engineered immune cell comprising the same) on a target cell, especially, the CAR is one that has a low binding affinity to a particular CLDN18.2 antigen, *e.g.*, a K_d of about 2×10^{-8} M or more, about 5×10^{-8} M or more, about 8×10^{-8} M or more, about 9×10^{-8} M or more, about 1×10^{-7} M or more, about 2×10^{-7} M or more, or about 5×10^{-7} M or more.

[00360] In certain embodiments, the antigen recognizing receptor is a chimeric co-stimulatory receptor (CCR). CCR is described in Krause, *et al., J. Exp. Med.* 188(4):619-626(1998), and US20020018783, the contents of which are incorporated by reference in their entireties. CCRs mimic co-stimulatory signals, but unlike, CARs, do not provide a T-cell activation signal, *e.g.*, CCRs lack a CD3ζ polypeptide. CCRs provide co-stimulation, *e.g.*, a CD28-like signal, in the absence of the natural co-stimulatory ligand on the antigen-presenting cell. A combinatorial antigen recognition, *i.e.,* use of a CCR in combination with a CAR, can augment T-cell reactivity against the dual-antigen expressing cells, thereby improving selective targeting. Kloss *et al.,* describe a strategy that integrates combinatorial antigen recognition, split signaling, and, critically, balanced strength of T-cell activation and costimulation to generate T cells that eliminate target cells that express a combination of antigens while sparing cells that express each antigen individually (Kloss *et al., Nature Biotechnology* 31(l):71-75 (2013)). With this approach, T-cell activation requires CAR-mediated recognition of one antigen, whereas costimulation is independently mediated by a CCR specific for a second antigen. To achieve tumor selectivity, the combinatorial antigen recognition approach diminishes the efficiency of T-cell activation to a level where it is ineffective without rescue provided by simultaneous CCR recognition of the second antigen. In certain embodiments, the CCR comprises (a) an extracellular antigen-binding domain that binds to an antigen different than the first CLDN18.2 antigen, (b) a transmembrane domain, and (c) a co-stimulatory signaling region that comprises at least one co-stimulatory molecule, including, but not limited to, CD28, 4-1BB, OX40, ICOS, PD-1, CTLA-4, LAG-3, 2B4, and BTLA. In certain embodiments, the co-stimulatory signaling region of the CCR comprises one co-stimulatory signaling molecule. In certain

embodiments, the one co-stimulatory signaling molecule is CD28. In certain embodiments, the one co-stimulatory signaling molecule is 4-1BB. In certain embodiments, the co-stimulatory signaling region of the CCR comprises two co-stimulatory signaling molecules. In certain embodiments, the two co-stimulatory signaling molecules are CD28 and 4-1BB. A second antigen is selected so that expression of both the first CLDN18.2 antigen and the second antigen is restricted to the targeted cells (*e.g.*, cancerous cells). Similar to a CAR, the extracellular antigen-binding domain can be an scFv, a Fab, a $F(ab)_2$; or a fusion protein with a heterologous sequence to form the extracellular antigen-binding domain. In certain embodiments, the CCR comprises an scFv that binds to CD138, transmembrane domain comprising a CD28 polypeptide, and a co-stimulatory signaling region comprising two co-stimulatory signaling molecules that are CD28 and 4-1BB.

[00361] In certain embodiments, the antigen recognizing receptor is a truncated CAR. A "truncated CAR" is different from a CAR by lacking an intracellular signaling domain. For example, a truncated CAR comprises an extracellular antigen-binding domain and a transmembrane domain, and lacks an intracellular signaling domain. In accordance with the presently disclosed subject matter, the truncated CAR has a high binding affinity to a second antigen expressed on the targeted cells. The truncated CAR functions as an adhesion molecule that enhances the avidity of a presently disclosed CAR, especially, one that has a low binding affinity to a CLDN18.2 antigen, thereby improving the efficacy of the presently disclosed CAR or engineered immune cell (*e.g.*, T cell) comprising the same. In certain embodiments, the truncated CAR comprises an extracellular antigen-binding domain that binds to CD138, a transmembrane domain comprising a CD8 polypeptide. A presently disclosed T cell comprises or is transduced to express a presently disclosed CAR targeting CLDN18.2 antigen and a truncated CAR targeting CD138. In certain embodiments, the targeted cells are solid tumor cells. In some embodiments, the engineered immune cells are further modified to suppress expression of one or more genes. In some embodiments, the engineered immune cells are further modified *via* genome editing. Various methods and compositions for targeted cleavage of genomic DNA have been described. Such targeted cleavage events can be used, for example, to induce targeted mutagenesis, induce targeted deletions of cellular DNA sequences, and facilitate targeted recombination at a predetermined chromosomal locus. *See*, for example, U.S. Patent Nos. 7,888,121 ; 7,972,854; 7,914,796; 7,951,925; 8,110,379; 8,409,861 ; 8,586,526; U.S. Patent Publications 20030232410; 20050208489; 20050026157; 20050064474; 20060063231 ;

201000218264; 20120017290; 20110265198; 20130137104; 20130122591; 20130177983 and 20130177960, the disclosures of which are incorporated by reference in their entireties. These methods often involve the use of engineered cleavage systems to induce a double strand break (DSB) or a nick in a target DNA sequence such that repair of the break by an error born process such as non-homologous end joining (NHEJ) or repair using a repair template (homology directed repair or HDR) can result in the knock out of a gene or the insertion of a sequence of interest (targeted integration). Cleavage can occur through the use of specific nucleases such as engineered zinc finger nucleases (ZFN), transcription-activator like effector nucleases (TALENs), or using the CRISPR/Cas system with an engineered crRNA/tracr RNA ('single guide RNA') to guide specific cleavage. In some embodiments, the engineered immune cells are modified to disrupt or reduce expression of an endogenous T-cell receptor gene (*see*, *e.g.,* WO 2014153470, which is incorporated by reference in its entirety). In some embodiments, the engineered immune cells are modified to result in disruption or inhibition of PD1, PDL-1 or CTLA-4 (see, *e.g.,* U.S. Patent Publication 20140120622), or other immunosuppressive factors known in the art (Wu *et al.* (2015) *Oncoimmunology* 4(7): e1016700, Mahoney *et al.* (2015) *Nature Reviews Drug Discovery* 14, 561–584).

EXAMPLES

[00362] The present technology is further illustrated by the following Examples, which should not be construed as limiting in any way. The following Examples demonstrate the preparation, characterization, and use of illustrative anti-CLDN18.2 antibodies of the present technology.

Example 1: Materials and Methods

[00363] *Construction of hCLDN18.2 and hCLDN18.1 Gene Expression Vectors.* Human cDNA encoding CLDN18.2 protein (SEQ ID NO: 4, shown in **Figure 10**) was cloned into a pCMV3 expression vector (Sino Biological US Inc., Chesterbrook, PA), and was used for stable cell line generation and as DNA immunogen for mouse immunization. Similarly, human CLDN18.1 cDNA (SEQ ID NO: 5, shown in **Figure 11**) was cloned into a pCMV3 expression vector and used for stable cell line generation. The human CLDN18.1 cell lines were used in a counter screen for selectivity.

[00364] *Generation of cell lines expressing hCLDN18.2 and hCLDN18.1.* The constructed pCMV3-hCLDN18.2 and pCMV3-hCLDN18.1 expression plasmids were used

to transfect cells for the development of the following stable or transient cell lines: 1) 3T3-hCLDN18.2, a mouse embryo fibroblast cell line, which was used for boosting mouse immunizations; 2) CHO-hCLDN18.2, which was used for antibody screening by ELISA and FACS; 3) HEK293-hCLDN18.2, which was used for antibody screening by ELISA and FACS; 4) HEK293-hCLDN18.1, which was used for antibody counter-screening. For the stable cell lines, all the final selected clones exhibited high expression levels of the target proteins. As shown in **Figure 5**, all the 3 cells lines transfected with pCMV3-hCLDN18.2 exhibited at least 100 times greater expression of hCLDN18.2 than the parental control cell line.

[00365] *Expression of hCLDN18.2-EL1 in virus-like-particles (VLPs).* To raise an anti-hCLDN18.2 specific antibody, the EL1 region was targeted because CLDN18.2 and 18.1 share identical EL2 sequences. To drive an immune response towards the EL1 region, a vector was constructed to express the hCLDN18.2 EL1 region in virus-like particles (VLPs). pEF6-CLDN18.2EL1 and a pEF6-vector (Thermo Fisher Scientific, Waltham MA) carrying a chimeric gene of CLDN18.2EL1 with CD81-cytosolic domains (pEF6-CLDN18.2EL1-CD81cd) were transfected to Expi293 cells using the following protocol: Expi293 cells were co-transfected with pEF6-CLDN18.2EL1 or pEF6-CLDN18.2EL1-CD81cd and VLP-core coding vector with 180 µl-Epifectamine in 4mL of OptimMEM for 24 hours at 4°C with rotation. After 24 hours of transfection, cell suspension was added to 26mL of Expi293 expression medium and cultured at 37°C with shaking at 125 rpm. At 24 hours in the shaking culture, enhancers 1 and 2 of the EXPI293™ MembranePro Expression System (Thermo Fisher Scientific, Waltham MA) were added at 150 µl and 1.5 mL, respectively, and further cultured for another 24 hours. Cells were subsequently centrifuged for FACS and the supernatant was collected for VLP precipitation. Cells were probed with mouse anti-CEA Ab followed by anti-mouse-PE conjugate. Total mouse IgG was used as an isotype control. FACS analysis showed that more than 90% of the purified VLPs were expressing hCLDN18.2 EL1 (**Figure 6**). The purified VLPs were used for boost immunizations in mice.

[00366] *Human CLDN18.2- and CLDN18.1-expressing cancer cell lines.* To facilitate antibody characterization, *in vitro* cell killing assay development, and animal xenograft model development, the following cancer cell lines were purchased from ATCC, Manassas, VA: 1) Gastric cancer cell lines Kato III, NCI-N87, NUGC4 and SNU-16, all of which express CLDN18.2; and 2) Lung cancer cell line A529, which expresses CLDN18.1.

[00367] *Mouse immunization*. Balb/C mice were immunized with eukaryotic expression vectors encoding CLDN18.2. Briefly, 70 μg pCMV3-hCLDN18.2 plasmid was injected intramuscularly using HELIOS® Gene Gun System (Bio-rad, Hercules CA) every two weeks for up to four times, and a final boost with 10^7 3T3-hCLDN18.2 cells and 10 μg VLPs expressing hCLDN18.2-EL1 was co-administered. Serum titers were monitored using CHO-hCLDN18.2 cell-based ELISA assays during the immunization course using the benchmark IMAB362 antibody as a positive control.

[00368] *Hybridoma fusion, screening, and subcloning*. After the final boost immunization, three mice that had high serum titer against the benchmark IMAB362 antibody were chosen for hybridoma fusion experiments. Three days after the final boost, freshly harvested mouse B-cells from lymph nodes and spleen were co-pelleted with mouse NS0 myeloma cells by centrifugation and fused by electroporation. The fused cells were resuspended in HAT selection medium and distributed into 96-well microtiter plates (60 plates for each fusion). Hybridomas were grown to at least 50% confluence (10-14 days post fusion) and then screened for production of CLDN18.2-specific antibody using CHO-hCLDN18.2 cell-based ELISA with IMAB362 as a positive control. Positive clones were then confirmed by FACS analysis with CLDN18.2- and CLDN18.1-expressing cells. Only those clones with specific and stronger binding signals than the benchmark IMAB362 antibody were advanced for subcloning, and 2-3 rounds of limiting dilution cloning were performed to confirm clonality.

[00369] *FACS cell binding assays.* Cells were incubated with 5 μg/mL of the primary anti-claudin 18.2 antibodies for thirty minutes at 4°C in PBS, and then a secondary phycoerythrin-labeled antibody specific for human Fc was added after washing off excessive primary antibody. Cells were fixed with 1% paraformaldehyde (PFA) prior to analysis on FACSCalibur cytometer (BD biosciences, Franklin Lakes, New Jersey, U.S.). Controls were cells with secondary antibody only, for which the mean fluorescent intensity (MFI) was set to 5.

[00370] *Antibody purification and characterization.* After screening about 4000 hybridoma clones, 5 clones that showed higher binding signal than the benchmark IMAB362 antibody were selected for subcloning. The cells of the 5 final subcloned hybridomas were expanded to 50-100 ml culture in a density about 10^6 cells/ml, and the secreted antibodies were purified using standard protein A or protein G columns. The

purified antibodies were subjected to characterization to further confirm their binding specificity and affinity with recombinant and endogenous cell lines.

[00371] *Antibody gene sequencing.* The heavy and light chain variable genes of the five selected lead murine antibodies were amplified by PCR using degenerated primers (targeting the leader sequence region) disclosed in Table 2 and the PCR products were used directly for sequencing as a first pass. To have clean readouts, a TA cloning/sequencing step was added as a final confirmation. The V_H and V_L sequences were cloned into human IgG1 constant regions to form the chimeric antibodies. The plasmids expressing the respective heavy and light chain of a chosen anti-CLDN18.2 antibody was transiently co-expressed in HEK293 cells. Co-transfection was performed with polyethyleneimine (PEI) as the transfection reagent. The supernatant was collected 6–8 days after transfection. Antibodies were purified by protein A chromatography. The amino acid sequences of the heavy and light chain variable regions of the five mouse clones (SEQ ID NOs: 36-45) are shown in **Figure 13**.

Table 2

Primer Names	Primer Sequences	SEQ ID NO
VL-F1	TGGGTATCTGGTACCTGTGGG	SEQ ID NO: 106
VL-F2	AGGCTGTTGGTGCTGATGTTCTGGATT	SEQ ID NO: 107
VL-F3	CTCTTGGTGCTTCTGTTGTTCTGGATTCCT	SEQ ID NO: 108
VL-F4	CTGTTAGTGCTCTGGATTCGGGAAACC	SEQ ID NO: 109
VL-F5	GGGCTGCTTKTGYTCTGKATCYCT	SEQ ID NO: 110
VL-F6	CTAGGGSTGCTKVTRCTCTGGATCCCWGGW	SEQ ID NO: 111
VL-F7	CTGYTATGGGTRCTGCKGCTCTGG	SEQ ID NO: 112
VL-F8	TTSYTGCTAATCAGTGYCWCAGTYRYAATG	SEQ ID NO: 113
VL-F9	GTCACAGTCATAGTGTCTAATGGA	SEQ ID NO: 114
VL-F10	GGKCTCYTGTTGCTCTGKYTTCMWGGT	SEQ ID NO: 115
VL-F11	GGMWTCTTGTTGCTCTGGTTTCCAGGT	SEQ ID NO: 116
VL-F12	CAGGTCCTGGSGTTGCTGCTGCTG	SEQ ID NO: 117
VL-F13	TTTCTACTGCTCTGTGTGTCTGGT	SEQ ID NO: 118
VL-F14	YTKCTSTGGTTRTMTGGWGYTGAWGGR	SEQ ID NO: 119
VL-F15	CAGKTYYTBGKRYTYYTKCTKYTCTGG	SEQ ID NO: 120
VL-F16	CTGCTAATCAGTGCCTCAGTCATAATATCC	SEQ ID NO: 121
VL-F17	TTCAGCTTCCTGCTAATCAGTGCYTCA	SEQ ID NO: 122
VL-F18	CTRTKGGTGCTGMTGYTCTGGRTTCCW	SEQ ID NO: 123
VL-F19	TTGCTCTKKTTTCMAGGTAYCARATGT	SEQ ID NO: 124
VL-F20	CAGTTCCTGTTTCTGTTAGTGCTCTGG	SEQ ID NO: 125
VL-F21	CAGGTCYTKGYRTTSSTGYTKCTSTGG	SEQ ID NO: 126

VL-F22	GCCACCATGGRYWTHMRRRTG	SEQ ID NO: 127
mVL-R	ACTGGATGGTGGGAAGAT	SEQ ID NO: 128
mVH-R	AGGGGCCAGTGGATAGAC	SEQ ID NO: 129
mIgG1-R	ATAGACAGATGGGGGTGTCGTTTTGGC	SEQ ID NO: 130
mIgG2a-R	CTTGACCAGGCATCCTAGAGTCA	SEQ ID NO: 131
mIgG2b-R	AGGGGCCAGTGGATAGACTGATGG	SEQ ID NO: 132
mIgG3-R	AGGGACCAAGGGATAGACAGATGG	SEQ ID NO: 133
mH1	SARGTNMAGCTGSAGSAGTC	SEQ ID NO: 134
mH2	SARGTNMAGCTGSAGSAGTCWGG	SEQ ID NO: 135
mK-F	GAYATTGTGMTSACMCARWCTMCA	SEQ ID NO: 136
mKL-R	GGATGGTGGGAAGATGGATACAGTTGGTGC	SEQ ID NO: 137
VH-F1	ATGATGGTGTTAAGTCTTCTGTACCTG	SEQ ID NO: 138
VH-F2	CTGTTSACAGYCYTTCCKGGTATCCTG	SEQ ID NO: 139
VH-F3	RCATTYCCAAGCTGTRTCCTDTCC	SEQ ID NO: 140
VH-F4	CTGCTGMYTGTCCCTGCATATGTC	SEQ ID NO: 141
VH-F5	CTCYTGTCAGDAACTGCAGGYGTC	SEQ ID NO: 142
VH-F6	ATGGGATGGAGCYGKATCWTBCTCTTY	SEQ ID NO: 143
VH-F7	CACTGGATCTTTCTCTCCCTG	SEQ ID NO: 144
VH-F8	ATGGGATGGAGCTATATCWTBCTCTTY	SEQ ID NO: 145
VH-F9	GCAACAGCYWYMGGTGTCCACTCC	SEQ ID NO: 146
VH-F10	CTGATGGCAGTGGTTAYAGGGGTC	SEQ ID NO: 147
VH-F11	KCADYARCTACAGGTGYYCACTCC	SEQ ID NO: 148
VH-F12	CTTTTAMAWGGTRTCCAGTGT	SEQ ID NO: 149
VH-F13	GCTCTTTTAAAAGGGGTCCAG	SEQ ID NO: 150
VH-F14	CTGAGCTGTGYWTTYATTRTT	SEQ ID NO: 151
VH-F15	CTTGTCSTTVTTTTAAAAGGTGTC	SEQ ID NO: 152
VH-F16	ATGCTGTTAGGGCTGGTTTTC	SEQ ID NO: 153
VH-F17	TTCCTGATGGCAGCTGCCCAAAGT	SEQ ID NO: 154
VH-F18	CTGTTSACAGCCWTTCCTGGT	SEQ ID NO: 155
VH-F19	CTGGCATTACTCTTCTGCCTG	SEQ ID NO: 156

R=AG, Y=CT, M=AC, K=GT, S=CG, W=AT, H=ACT, B=CGT, V=ACG, D= AGT, N=ACGT

[00372] *Humanization of 32G4 and 47D10 clones.* The variable regions of mouse clones 32G4 and 47D10, including V_H and V_L, were humanized using germline CDR grafting. Briefly, the original murine sequences were aligned to all human germline sequences. The original mouse and closest matching germline sequences were analyzed for sequence liabilities and the most appropriate germline frameworks were selected. Complementarity determining regions (CDRs) from the parent mouse anti-CLDN18.2 antibodies were grafted onto the human frameworks and back mutations introduced as necessary. For both 32G4 and 47D10, four humanized V_H and four humanized V_L sequences were generated. The four V_H and V_L sequence variants from each clone may be combined to generate 16

humanized antibody variants for 32G4 or 47D10. The amino acid sequences of the four humanized V_H and V_L variants of 32G4 and 47D10 are shown in **Figure 14** and **Figure 15**, respectively.

[00373] *Construction of humanized full IgG1 32G4 and 47D10 expression constructs.* Two humanized full IgG1 antibody variants for 32G4 and 47D10 were constructed and used for further characterization. The amino acid sequences of the two full humanized IgG1 antibodies from 32G4 and 47D10 are shown in **Figure 16** and **Figure 17,** respectively.

Example 2: Characterization of Anti-CLDN18.2 Antibodies of the Present Technology

[00374] Five clones (32G4, 47D10, 29G4, 31A6 and 15B10) were selected based on their binding affinity and selective binding to human CLDN18.2 protein. FACS data display the MFI value in the upper right panel of each plot.

[00375] As shown in **Figure 7**, the binding of murine clones 32G4, 47D10, 29G4, 31A6 and 15B10 to CLDN18.2 is at least 1000 times stronger than their respective binding to CLDN18.1 as determined by FACS analysis. The binding affinity of the five murine clones to human CLDN18.2 was further evaluated using FACS cell surface binding analysis. As shown in **Figure 8**, the EC50 of the binding of 32G4, 47D10, 29G4, 31A6 and 15B10 to human CLDN18.2 was 0.502 nM, 1.973 nM, 1.260 nM, 10.903 nM and 2.196 nM, respectively. The EC50 of the binding of the 32G4-huIgG1-V8, 32G4-huIgG1-V9, 47D10-huIgG1-V6, and 47D10-huIgG1-V7 to human CLDN18.2 was 0.147 nM, 0.129 nM, 0.22 nM and 0.361 nM, respectively. See **Figures 9A-9B**. As shown in **Figure 19**, humanized 32G4 and 47D10 antibody variants showed elevated binding to cynomolgus monkey and mouse claudin 18.2 target proteins compared to the IMAB362 positive control antibody.

[00376] *ADCC assays.* Antibody-dependent cellular cytotoxicity (ADCC) assays were performed using a bioluminescent reporter assay (Promega Cat# 7015, Madison WI) in which engineered Jurkat cells with NFAT-luc and Fc-RIIIa are used as effector cells and NUGC4 gastric cancer cells as target cells. Briefly, PBMCs were cultured in complete RPMI1640 medium containing 50 ng/ml IL-2, overnight (18 hours). ADCC assay was performed according to manufacturer's instructions. Briefly, 2×10^4 cells of NUGC4 (target cells) were seeded into each well of a 96 well plate and cultured in 100µl/well of complete growth medium overnight. 50 µl of the anti-CLDN18.2 antibodies were added into each well (concentration at 0, 0.001, 0.01, 0.1, 1 µg/ml). 6×10^4 Jurkat effector cells in 50 µl medium were added into each well. The cells were gently mixed. 21 hours later, the plate

was centrifuged at 1000 rpm for 5 min. Then, 50 µl cell culture medium from each well was collected and assayed for lactate dehydrogenase (LDH) release as a measure of cell toxicity. As shown in **Figure 18**, the 32G4 and 47D10 monoclonal antibodies of the present technology exhibited superior antibody-dependent cellular cytotoxicity (ADCC) compared to the IMAB362 positive control antibody.

[00377] These results demonstrate that the anti-CLDN18.2 immunoglobulin-related compositions of the present technology are useful in methods for detecting CLDN18.2 polypeptides in a biological sample.

Example 3: Characterization of In Vivo and In Vitro Cytotoxic Activities of the Anti-CLDN18.2 Antibodies of the Present Technology

[00378] *In vivo mouse xenograft model development.* Gastric cancer cell line xenograft (CDX) models using Kato-III and NUGC4, in the BRG (Balb/c $Rag2^{-/-}$, $IL2R\gamma^{-/-}$) mouse will be developed. This mouse strain lacks adaptive immune cells and NK cells and will be used for cancer cell engraftment. Twenty-five million cancer cells are implanted subcutaneously, and the tumor volume is measured twice weekly by caliper. Once the tumor reaches around 1500 mm^3, animals are randomized for efficacy studies. Animals are divided into the following three groups. Group 1: 5 experimental mice (experimental group with anti-CLDN18.2; dose concentration: 2.5 mg/KG; injection schedule: every other day; route: IV); Group 2: 5 control mice (PBS/saline; injection schedule: every other day; route: IV); Group 3: Benchmark antibody (IMAB362, 2.5 mg/KG, injection schedule: every other day; route: IV). Tumor measurements are performed twice weekly up to 40 days post graft.

[00379] It is anticipated that the anti-CLDN18.2 immunoglobulin-related compositions of the present technology will exhibit potent *in vitro* and/or *in vivo* cytotoxic activity against one or more CLDN18.2-associated cancers. Accordingly, the immunoglobulin-related compositions of the present technology are useful to treat a Claudin 18.2-associated cancer in a subject in need thereof.

EQUIVALENTS

[00380] The present technology is not to be limited in terms of the particular embodiments described in this application, which are intended as single illustrations of individual aspects of the present technology. Many modifications and variations of this present technology can be made without departing from its spirit and scope, as will be apparent to those skilled in the art. Functionally equivalent methods and apparatuses within

the scope of the present technology, in addition to those enumerated herein, will be apparent to those skilled in the art from the foregoing descriptions. Such modifications and variations are intended to fall within the scope of the present technology. It is to be understood that this present technology is not limited to particular methods, reagents, compounds compositions or biological systems, which can, of course, vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to be limiting.

[00381] In addition, where features or aspects of the disclosure are described in terms of Markush groups, those skilled in the art will recognize that the disclosure is also thereby described in terms of any individual member or subgroup of members of the Markush group.

[00382] As will be understood by one skilled in the art, for any and all purposes, particularly in terms of providing a written description, all ranges disclosed herein also encompass any and all possible subranges and combinations of subranges thereof. Any listed range can be easily recognized as sufficiently describing and enabling the same range being broken down into at least equal halves, thirds, quarters, fifths, tenths, *etc.* As a non-limiting example, each range discussed herein can be readily broken down into a lower third, middle third and upper third, *etc.* As will also be understood by one skilled in the art all language such as "up to," "at least," "greater than," "less than," and the like, include the number recited and refer to ranges which can be subsequently broken down into subranges as discussed above. Finally, as will be understood by one skilled in the art, a range includes each individual member. Thus, for example, a group having 1-3 cells refers to groups having 1, 2, or 3 cells. Similarly, a group having 1-5 cells refers to groups having 1, 2, 3, 4, or 5 cells, and so forth.

[00383] All patents, patent applications, provisional applications, and publications referred to or cited herein are incorporated by reference in their entirety, including all figures and tables, to the extent they are not inconsistent with the explicit teachings of this specification.

Claims

1. An antibody or antigen binding fragment thereof comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein:

(a) the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 6, a V_H-CDR2 sequence of SEQ ID NO: 7, and a V_H-CDR3 sequence of SEQ ID NO: 8, and/or the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 9, a V_L-CDR2 sequence of SEQ ID NO: 10 or SEQ ID NO: 157, and a V_L-CDR3 sequence of SEQ ID NO: 11;

(b) the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 12, a V_H-CDR2 sequence of SEQ ID NO: 13, and a V_H-CDR3 sequence of SEQ ID NO: 14, and/or the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 15, a V_L-CDR2 sequence of SEQ ID NO: 16 or SEQ ID NO: 158, and a V_L-CDR3 sequence of SEQ ID NO: 17;

(c) the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 18, a V_H-CDR2 sequence of SEQ ID NO: 19, and a V_H-CDR3 sequence of SEQ ID NO: 20, and/or the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 21, a V_L-CDR2 sequence of SEQ ID NO: 22, and a V_L-CDR3 sequence of SEQ ID NO: 23;

(d) the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 24, a V_H-CDR2 sequence of SEQ ID NO: 25, and a V_H-CDR3 sequence of SEQ ID NO: 26, and/or the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 27, a V_L-CDR2 sequence of SEQ ID NO: 28, and a V_L-CDR3 sequence of SEQ ID NO: 29; or

(e) the V_H comprises a V_H-CDR1 sequence of SEQ ID NO: 30, a V_H-CDR2 sequence of SEQ ID NO: 31, and a V_H-CDR3 sequence of SEQ ID NO: 32, and/or the V_L comprises a V_L-CDR1 sequence of SEQ ID NO: 33, a V_L-CDR2 sequence of SEQ ID NO: 34, and a V_L-CDR3 sequence of SEQ ID NO: 35.

2. An antibody or antigen binding fragment thereof comprising a heavy chain immunoglobulin variable domain (V_H) and a light chain immunoglobulin variable domain (V_L), wherein the V_H comprises an amino acid sequence selected from any one of SEQ ID NOs: 36, 38, 40, 42, 44, 46-49, or 54-57; and/or (b) the V_L comprises an amino acid sequence selected from any one of SEQ ID NOs: 37, 39, 41, 43, 45, 50-53, or 58-61.

3. The antibody or antigen binding fragment of claim 1 or 2, further comprising a Fc domain of an isotype selected from the group consisting of IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgM, IgD, and IgE.

4. The antibody of claim 3, comprising an IgG1 constant region comprising one or more amino acid substitutions selected from the group consisting of N297A, K322A, L234A and L235A.

5. The antibody of claim 3, comprising an IgG4 constant region comprising a S228P mutation.

6. The antigen binding fragment of claim 1 or 2, wherein the antigen binding fragment is selected from the group consisting of Fab, $F(ab')_2$, Fab', scF_v, and F_v.

7. An antibody comprising a heavy chain (HC) amino acid sequence comprising SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, SEQ ID NO: 68, or a variant thereof having one or more conservative amino acid substitutions, and/or a light chain (LC) amino acid sequence comprising SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, SEQ ID NO: 69, or a variant thereof having one or more conservative amino acid substitutions.

8. The antibody of claim 7, comprising a HC amino acid sequence and a LC amino acid sequence selected from the group consisting of: SEQ ID NO: 62 and SEQ ID NO: 63, SEQ ID NO: 64 and SEQ ID NO: 65, SEQ ID NO: 66 and SEQ ID NO: 67, and SEQ ID NO: 68 and SEQ ID NO: 69, respectively.

9. An antibody comprising (a) a light chain immunoglobulin variable domain sequence that is at least 95% identical to the light chain immunoglobulin variable domain sequence of any one of SEQ ID NOs: 37, 39, 41, 43, 45, 50-53, or 58-61; and/or (b) a heavy chain immunoglobulin variable domain sequence that is at least 95% identical to the heavy chain immunoglobulin variable domain sequence of any one of SEQ ID NOs: 36, 38, 40, 42, 44, 46-49, or 54-57.

10. An antibody comprising:

(a) a LC sequence that is at least 95% identical to the LC sequence present in SEQ ID NO: 63, SEQ ID NO: 65, SEQ ID NO: 67, or SEQ ID NO: 69; and/or

(b) a HC sequence that is at least 95% identical to the HC sequence present in SEQ ID NO: 62, SEQ ID NO: 64, SEQ ID NO: 66, or SEQ ID NO: 68.

11. The antibody of any one of claims 7-10, wherein the antibody comprises an IgG1 constant region comprising one or more amino acid substitutions selected from the group consisting of N297A, K322A, L234A and L235A.

12. The antibody or antigen binding fragment of any one of claims 1-11, wherein the antibody or antigen binding fragment binds to a CLDN18.2 polypeptide comprising an extracellular loop 1 (EL1) sequence.

13. The antibody or antigen binding fragment of claim 12, wherein the extracellular loop 1 (EL1) sequence comprises the amino acid sequence of SEQ ID NO: 2 or the CLDN18.2 polypeptide comprises the amino acid sequence of SEQ ID NO: 4.

14. The antibody or antigen binding fragment of any one of claims 1-13, wherein the antibody is a monoclonal antibody, a chimeric antibody, or a humanized antibody

15. The antibody of any one of claims 1-5, or 7-14, wherein the antibody lacks α-1,6-fucose modifications.

16. A recombinant nucleic acid sequence encoding the antibody or antigen binding fragment of any one of claims 1-15.

17. A host cell or vector comprising the recombinant nucleic acid sequence of claim 16.

18. A pharmaceutical composition comprising the antibody or antigen binding fragment of any one of claims 1-15 and a pharmaceutically-acceptable carrier, wherein the antibody or antigen binding fragment is optionally conjugated to an agent selected from the group consisting of isotopes, dyes, chromagens, contrast agents, drugs, toxins, cytokines, enzymes, enzyme inhibitors, hormones, hormone antagonists, growth factors, radionuclides, metals, liposomes, nanoparticles, RNA, DNA or any combination thereof.

19. The pharmaceutical composition of claim 18, wherein the pharmaceutical composition further comprises an agent selected from the group consisting of isotopes, dyes, chromagens, contrast agents, drugs, toxins, cytokines, enzymes, enzyme inhibitors, hormones, hormone antagonists, growth factors, radionuclides, metals, liposomes, nanoparticles, RNA, DNA or any combination thereof.

20. A method for treating cancer in a subject in need thereof, comprising administering to the subject an effective amount of the antibody or antigen binding

fragment of any one of claims 1-15 or the pharmaceutical composition of any one of claims 18-19, wherein the antibody or antigen binding fragment specifically binds to CLDN18.2.

21. The method of claim 20, wherein the cancer is a solid tumor.

22. The method of claim 20 or 21, wherein the cancer is selected from the group consisting of gastric cancer, esophageal cancer, pancreatic cancer, lung cancer, non small cell lung cancer (NSCLC), ovarian cancer, colon cancer, hepatic cancer, head-neck cancer, and gallbladder cancer.

23. The method of any one of claims 20-22, wherein the antibody or antigen binding fragment is administered to the subject separately, sequentially or simultaneously with an additional therapeutic agent.

24. The method of claim 23, wherein the additional therapeutic agent is one or more of alkylating agents, platinum agents, taxanes, vinca agents, anti-estrogen drugs, aromatase inhibitors, ovarian suppression agents, VEGF/VEGFR inhibitors, EGF/EGFR inhibitors, PARP inhibitors, cytostatic alkaloids, cytotoxic antibiotics, antimetabolites, endocrine/hormonal agents, and bisphosphonate therapy agents.

25. The method of claim 23, wherein the additional therapeutic agent is an immuno-modulating/stimulating antibody.

26. The method of claim 25, wherein the immuno-modulating/stimulating antibody is an anti-PD-1 antibody, an anti-PD-L1 antibody, an anti-PD-L2 antibody, an anti-CTLA-4 antibody, an anti-TIM3 antibody, an anti-4-1BB antibody, an anti-CD73 antibody, an anti-GITR antibody, or an anti-LAG-3 antibody.

27. A method for detecting cancer in a subject *in vivo* comprising

(a) administering to the subject an effective amount of the antibody or antigen binding fragment of any one of claims 1-15, wherein the antibody or antigen binding fragment is configured to localize to a cancer cell expressing CLDN18.2 and is labeled with a radioisotope; and

(b) detecting the presence of a tumor in the subject by detecting radioactive levels emitted by the antibody or antigen binding fragment that are higher than a reference value.

28. The method of claim 27, wherein the subject is diagnosed with or is suspected of having cancer.

29. The method of claim 27 or 28, wherein the radioactive levels emitted by the antibody or antigen binding fragment are detected using positron emission tomography or single photon emission computed tomography.

30. The method of any one of claims 27-29, further comprising administering to the subject an effective amount of an immunoconjugate comprising the antibody or antigen binding fragment of any one of claims 1-15 conjugated to a radionuclide.

31. The method of any one of claims 27-30, wherein the cancer is a solid tumor.

32. The method of any one of claims 27-31, wherein the cancer is selected from the group consisting of gastric cancer, esophageal cancer, pancreatic cancer, lung cancer, non small cell lung cancer (NSCLC), ovarian cancer, colon cancer, hepatic cancer, head-neck cancer, and gallbladder cancer.

33. The method of any one of claims 20-32, wherein the subject is human.

34. A kit comprising the antibody or antigen binding fragment of any one of claims 1-15 and instructions for use.

35. The kit of claim 34, wherein the antibody or antigen binding fragment is coupled to at least one detectable label selected from the group consisting of a radioactive label, a fluorescent label, and a chromogenic label.

36. The kit of claim 34 or 35, further comprising a secondary antibody that specifically binds to the antibody or antigen binding fragment of any one of claims 1-15.

37. A method for detecting CLDN18.2 protein expression levels in a biological sample comprising contacting the biological sample with the antibody or antigen binding fragment of any one of claims 1-15, and detecting binding to CLDN18.2 protein in the biological sample.

38. A chimeric antigen receptor (CAR) comprising the antibody or antigen-binding fragment of any one of claims 1-15.

39. An engineered immune cell comprising the CAR of claim 38, optionally wherein the engineered immune cell is a B cell, a T cell, or a natural killer (NK) cell.

40. A method for treating cancer in a subject in need thereof, comprising administering to the subject an effective amount of the engineered immune cell of claim 39.

41. The method of claim 40, wherein the cancer is a solid tumor.

42. The method of claim 40 or 41, wherein the cancer is selected from the group consisting of gastric cancer, esophageal cancer, pancreatic cancer, lung cancer, non small cell lung cancer (NSCLC), ovarian cancer, colon cancer, hepatic cancer, head-neck cancer, and gallbladder cancer.

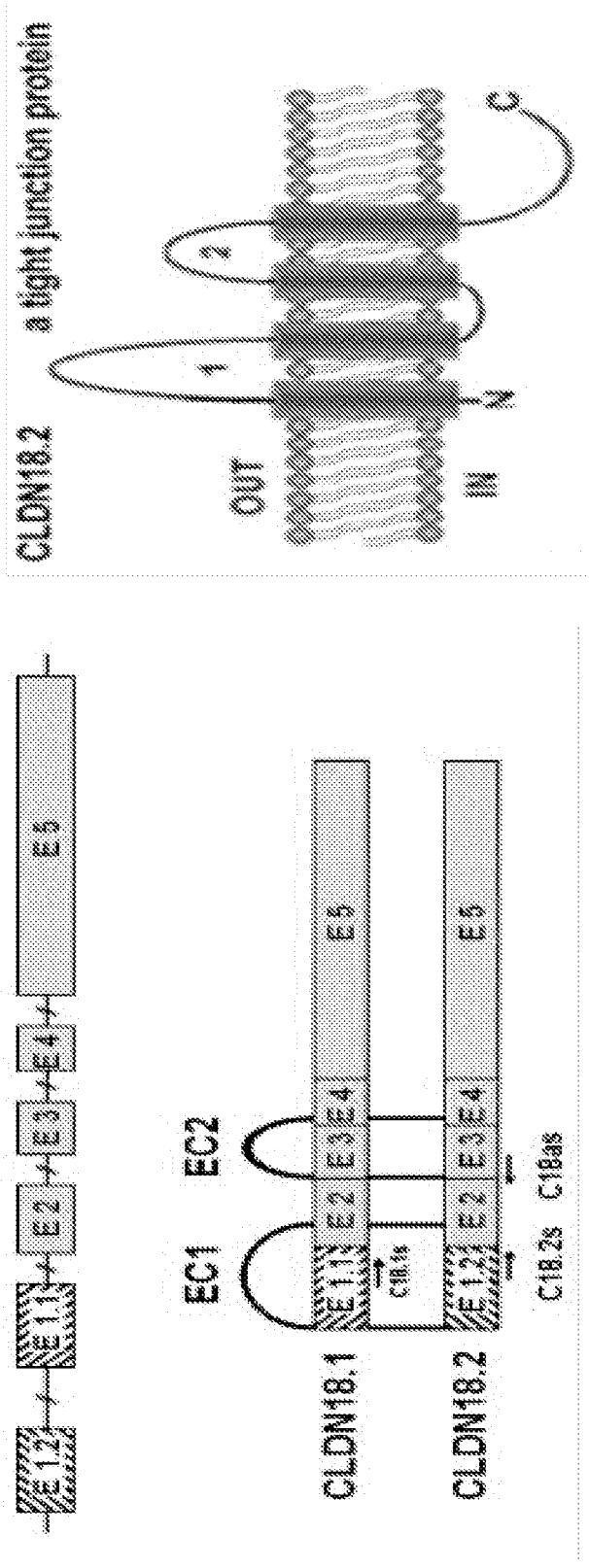

a tight junction protein
CLDN18.2
OUT
IN
1
2
N
C
E 1.2
E 1.1
E 2
E 3
E 4
E 5
EC1
EC2
CLDN18.1
CLDN18.2
C18.1s
C18.2s
C18as

Figure 1

Figure 2

h18.1-EL1 (SEQ ID NO: 1): h18.1-EL1: DWWSTQDLYDNPVTSVFQYEGLWRSCVRQSSGFTECRPYFTILGLPAMLQAVRA
h18.2-EL1 (SEQ ID NO: 2): h18.2-EL1: DQWSTQDLYNNPVTAVFNYQGLWRSCVRESSGFTECRGYFTLLGLPAMLQAVRA
m18.2-EL1 (SEQ ID NO: 2): m18.2-EL1: DQWSTQDLYNNPVTAVFNYQGLWRSCVRESSGFTECRGYFTLLGLPAMLQAVRA

h18.1-EL2 (SEQ ID NO: 3): h18.1-EL2: VTNFTMSTAMVTGMGGMVQTVQTRYTFGAA
h18.2-EL2 (SEQ ID NO: 3): h18.2-EL2: VTNFTMSTAMVTGMGGMVQTVQTRYTFGAA

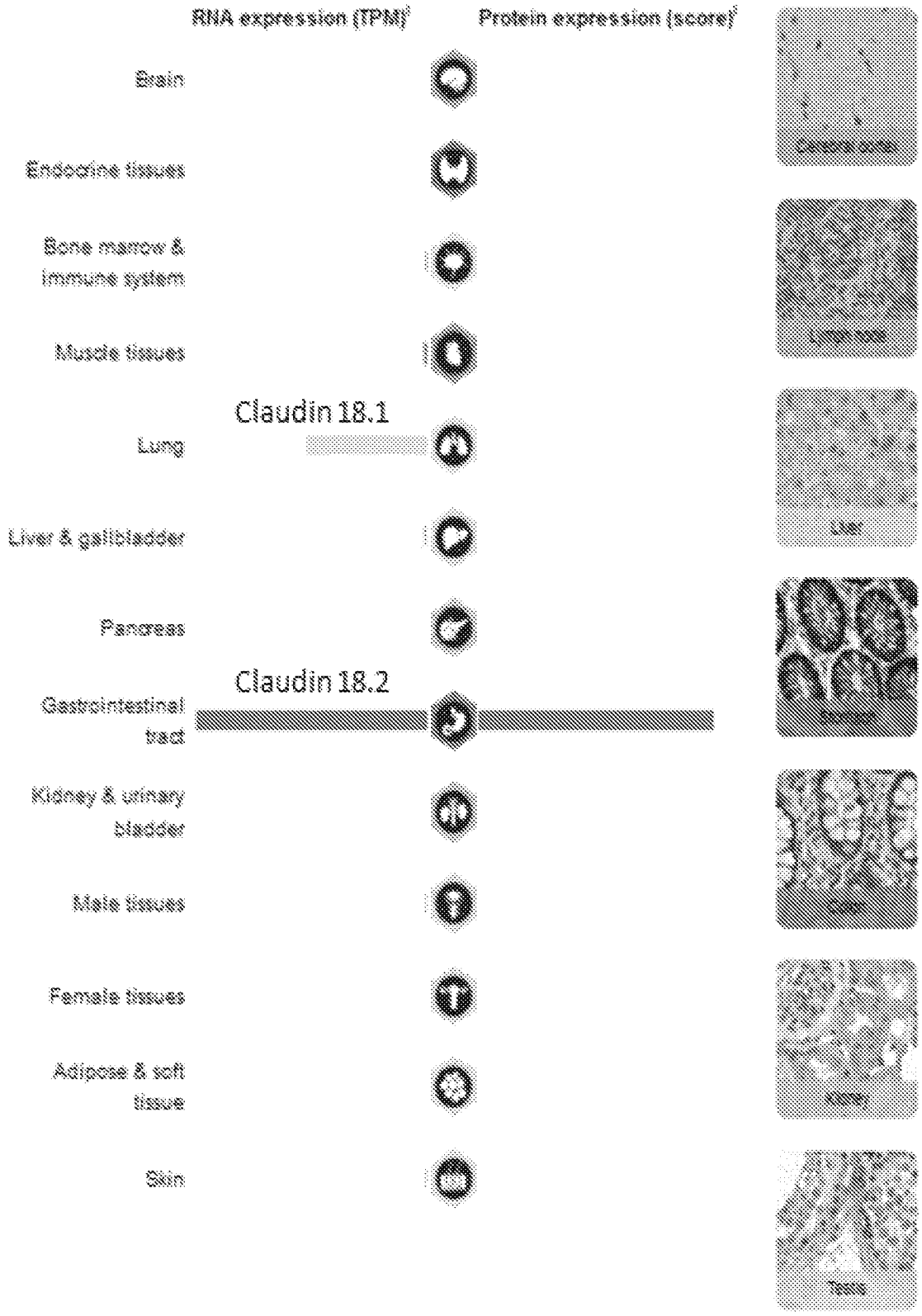

RNA expression (TPM)
Protein expression (score)
Brain
Endocrine tissues
Bone marrow & immune system
Muscle tissues
Claudin 18.1
Lung
Liver & gallbladder
Pancreas
Claudin 18.2
Gastrointestinal tract
Kidney & urinary bladder
Male tissues
Female tissues
Adipose & soft tissue
Skin
Cerebral cortex
Lung
Liver
Pancreas
Kidney
Testis

Figure 3

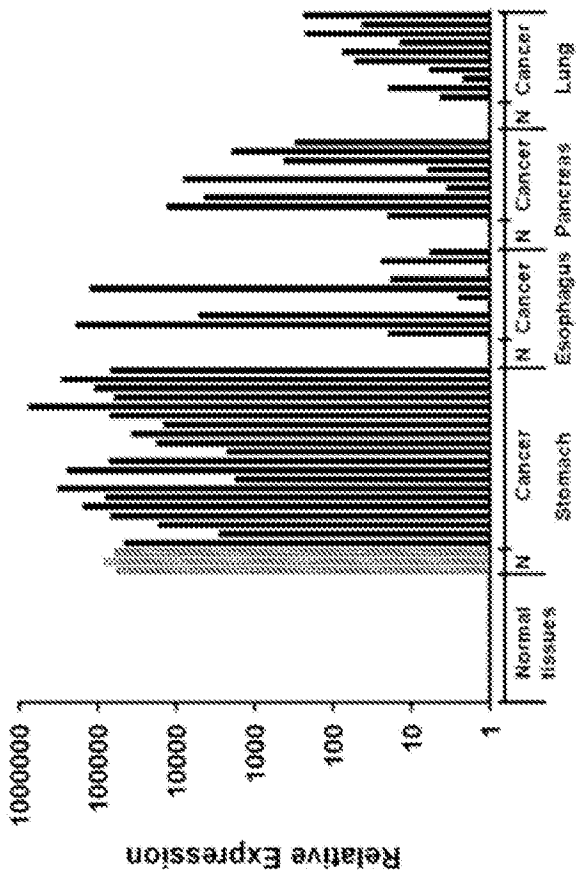

Relative Expression
1000000
100000
10000
1000
100
10
1
Normal tissues
N
Cancer
Stomach
N
Cancer
Esophagus
N
Cancer
Pancreas
N
Cancer
Lung

Figure 4

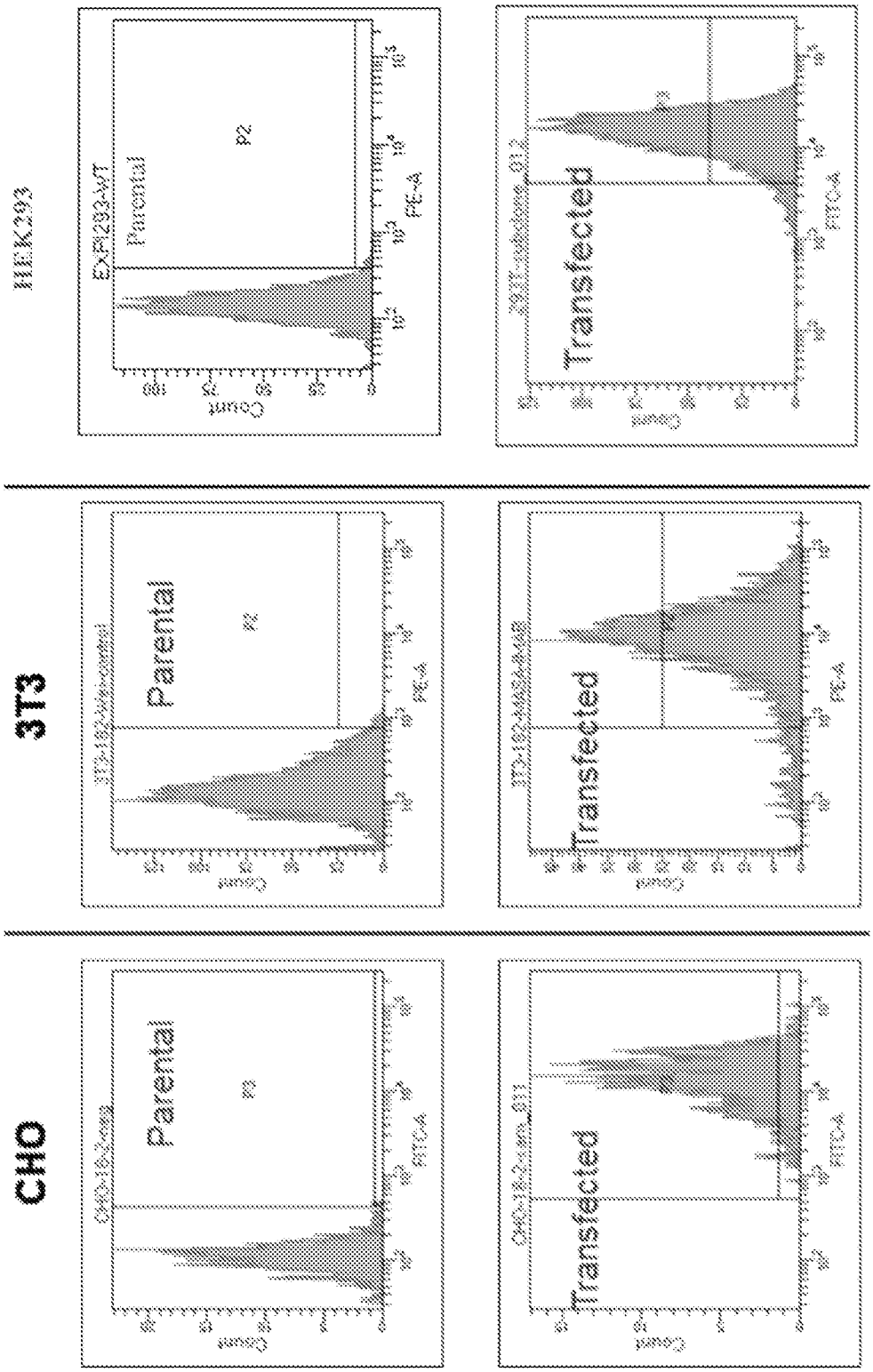

HEK293
EXPI293-WT
Parental
P2
Count
FITC-A
Transfected
3T3
Parental
Transfected
CHO
Parental
Transfected

Figure 5

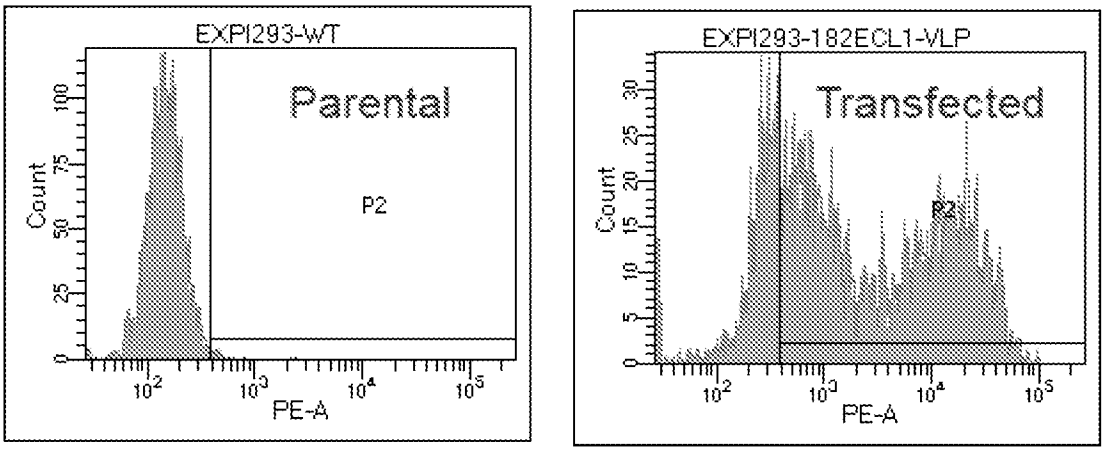

EXPI293-WT
Parental
P2
Count
PE-A
EXPI293-182ECL1-VLP
Transfected
P2
Count
PE-A

Figure 6

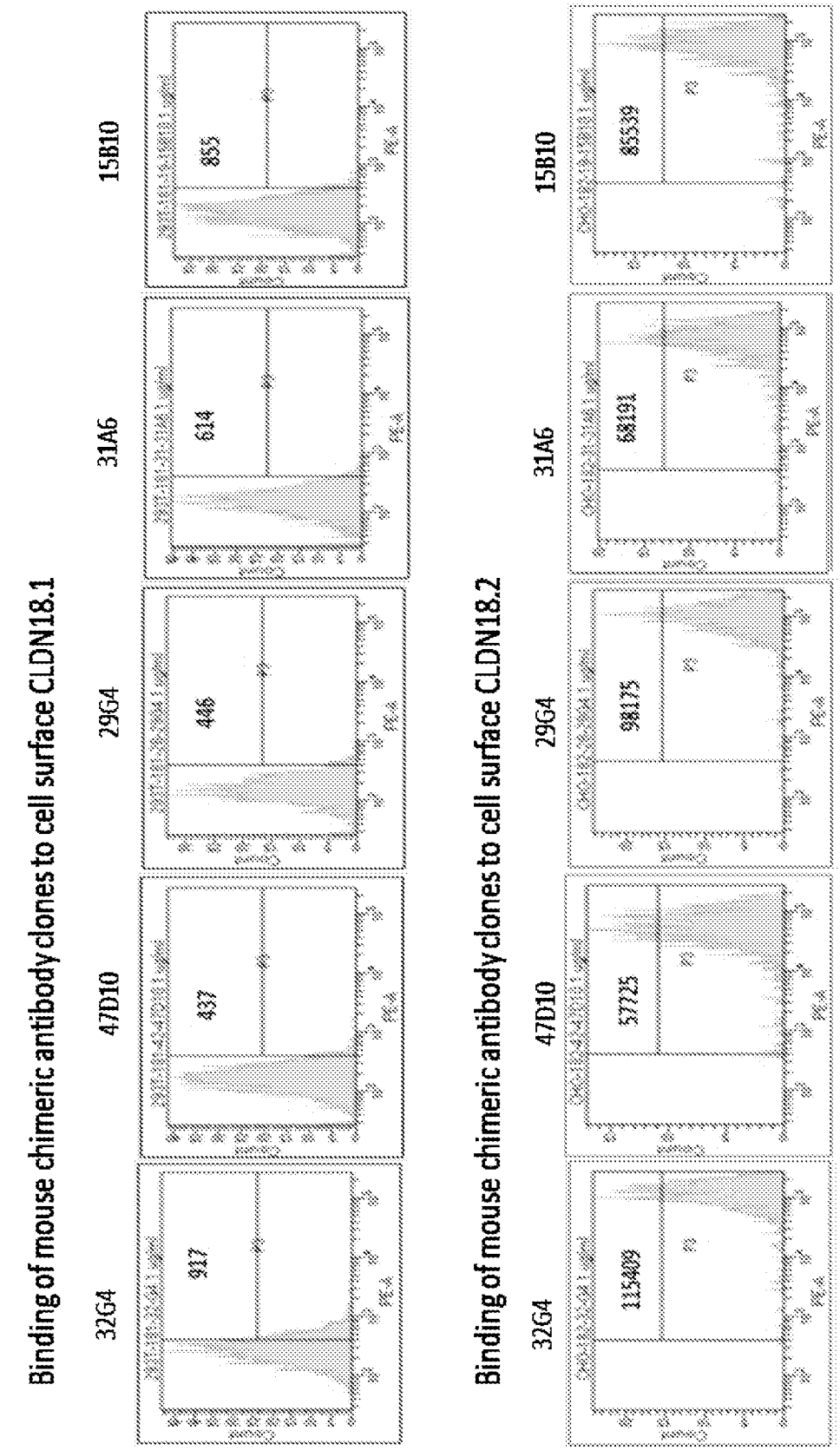
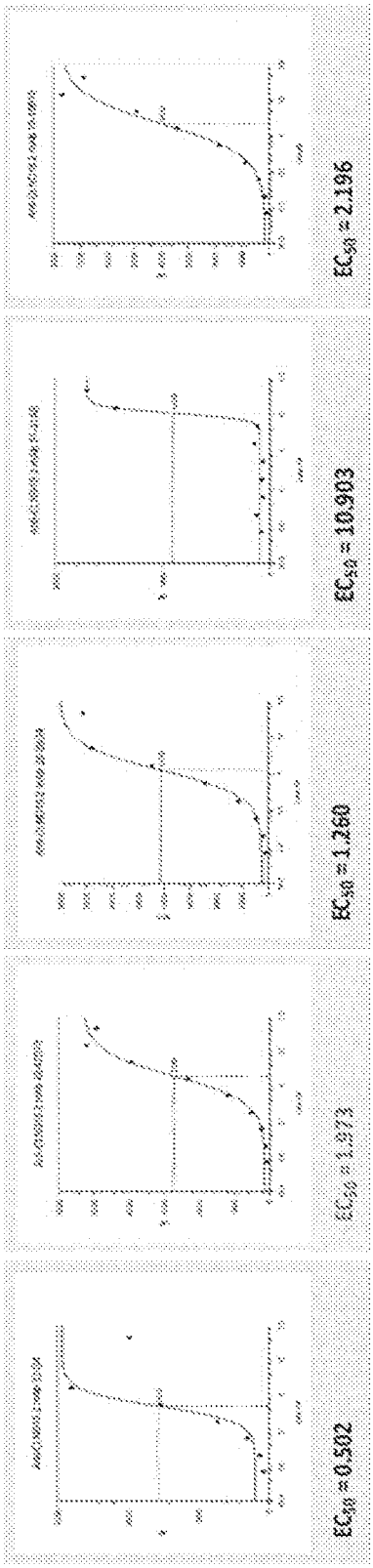

Binding of mouse chimeric antibody clones to cell surface CLDN18.1
32G4
47D10
29G4
31A6
15B10
917
437
446
614
855
Binding of mouse chimeric antibody clones to cell surface CLDN18.2
32G4
47D10
29G4
31A6
15B10
115409
57725
98175
68191
85539

Figure 7



Clone ID	32G4	47D10	29G4	31A6	15B10
EC50 (nM)	0.502	1.973	1.260	10.903	2.196

Figure 8

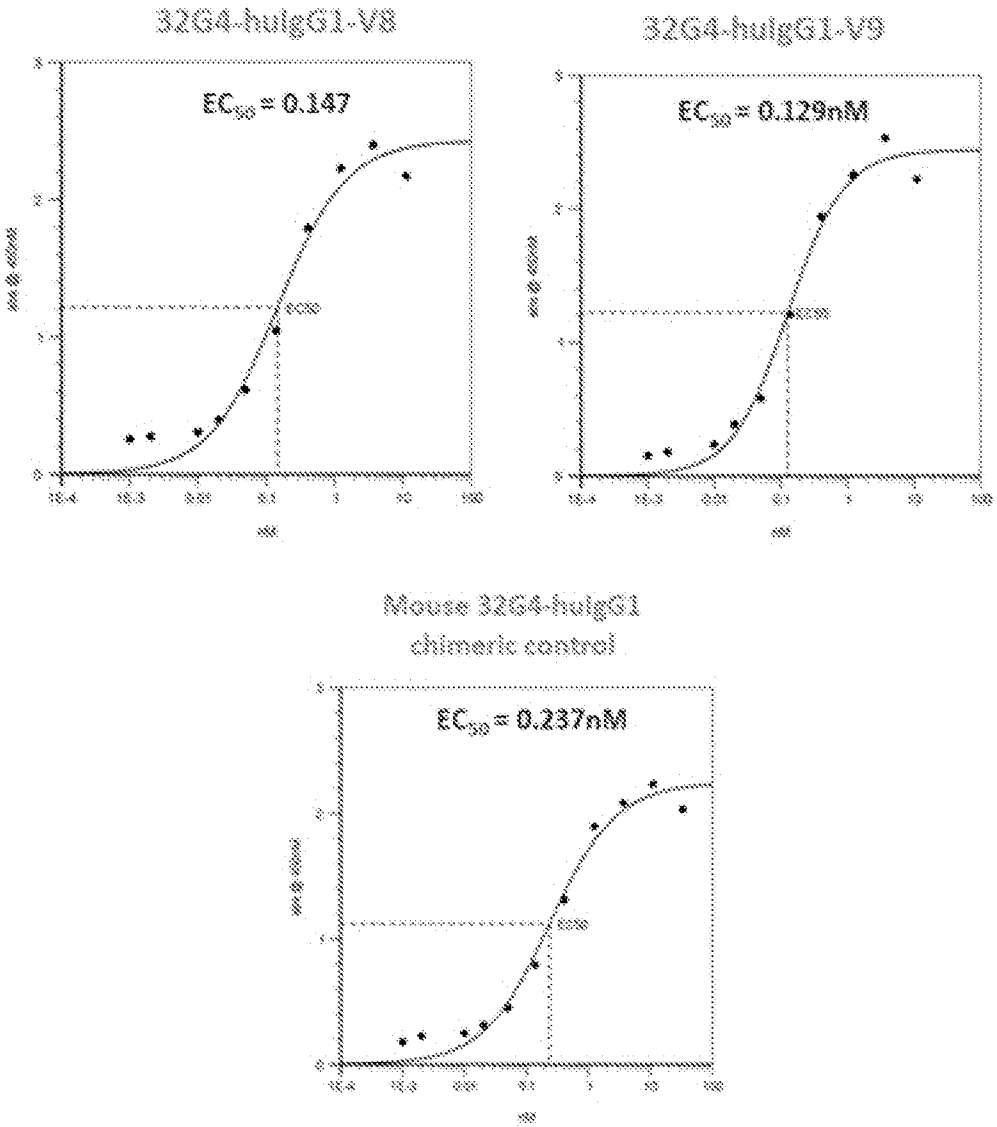

32G4-hulgG1-V8
EC50 = 0.147
32G4-hulgG1-V9
EC50 = 0.129nM
Mouse 32G4-hulgG1
chimeric control
EC50 = 0.237nM

Figure 9A

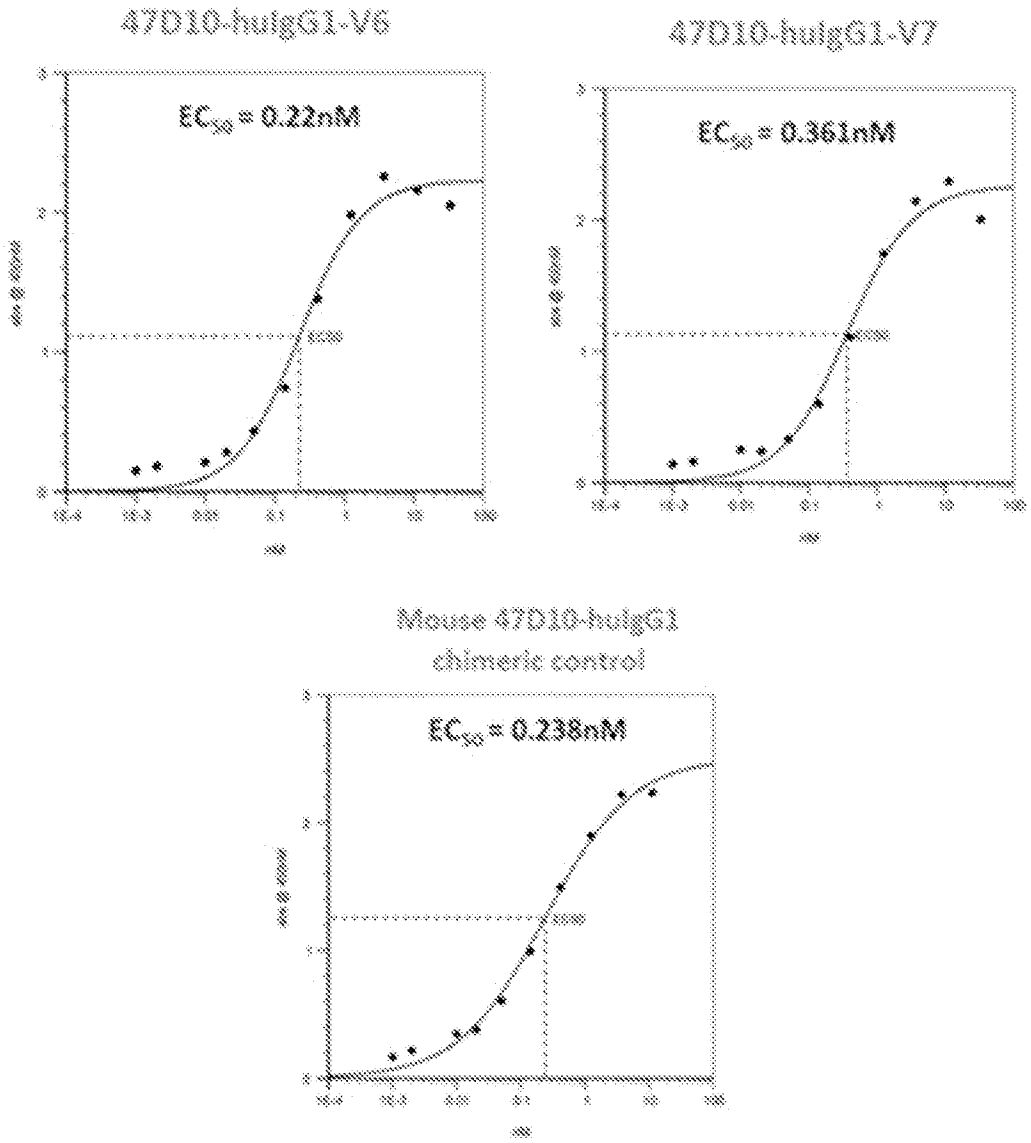

47D10-huIgG1-V6
EC50 = 0.22nM
47D10-huIgG1-V7
EC50 = 0.361nM
Mouse 47D10-huIgG1
chimeric control
EC50 = 0.238nM

Figure 9B

hCLDN18.2 (SEQ ID NO: 4):

```
        10          20          30          40          50
MAVTACQGLG FVVSLIGLAG IIAATCMDQW STQDLYNNPV TAVFNYQGLW
        60          70          80          90          100
RSCVRESSGF TECRGYFTLL GLPAMLQAVR ALMIVGIVLG AIGLLVSIFA
        110         120         130         140         150
LKCIRIGSME DSAKANMTLT SGIMFIVSGL CAIAGVSVFA NMLVTNFWMS
        160         170         180         190         200
TANMYTGMGG MVQTVQTRYT FGAALFVGWV AGGLTLIGGV MMCIACRGLA
        210         220         230         240         250
PEETNYKAVS YHASGHSVAY KPGGFKASTG FGSNTKNKKI YDGGARTEDE
        260
VQSYPSKHDY V
```

Figure 10

hCLDN18.1 (SEQ ID NO: 5):

```
        10          20          30          40          50
MSTTTCQVVA FLLSILGLAG CIAATGMDMW STQDLYDNPV TSVFQYEGLW
        60          70          80          90          100
RSCVRQSSGF TECRPYFTIL GLPAMLQAVR ALMIVGIVLG AIGLLVSIFA
        110         120         130         140         150
LKCIRIGSME DSAKANMTLT SGIMFIVSGL CAIAGVSVFA NMLVTNFWMS
        160         170         180         190         200
TANMYTGMGG MVQTVQTRYT FGAALFVGWV AGGLTLIGGV MMCIACRGLA
        210         220         230         240         250
PEETNYKAVS YHASGHSVAY KPGGFKASTG FGSNTKNKKI YDGGARTEDE
        260
VQSYPSKHDY V
```

Figure 11

32G4-VH CDR1 (SEQ ID NO: 6): GYSSTGYN
32G4-VH CDR2 (SEQ ID NO: 7): INPYYGGI
32G4-VH CDR3 (SEQ ID NO: 8): ARDYYGNSFAY
32G4-VL CDR1 (SEQ ID NO: 9): QSLLNSGNQKNY
32G4-VL CDR2 (SEQ ID NO: 10): WASTRES
32G4-huVL4 CDR2 (SEQ ID NO: 157): WASTLES
32G4-VL CDR3 (SEQ ID NO: 11): QNNYYFPYT

47D10-VH CDR1 (SEQ ID NO: 12): GFAFSSSA
47D10-VH CDR2 (SEQ ID NO: 13): MTVGYIYT
47D10-VH CDR3 (SEQ ID NO: 14): ARQGYGNAMDY
47D10-VL CDR1 (SEQ ID NO: 15): QSLLNSGNQKNY
47D10-VL CDR2 (SEQ ID NO: 16): WASTRES
47D10-huVL4 CDR2 (SEQ ID NO: 158): WASTRET
47D10-VL CDR3 (SEQ ID NO: 17): QNNYIYPLT

29G4-VH CDR1 (SEQ ID NO: 18): GFSLTNYG
29G4-VH CDR2 (SEQ ID NO: 19): IWAGGNT
29G4-VH CDR3 (SEQ ID NO: 20): ARGGYGNSCDY
29G4-VL CDR1 (SEQ ID NO: 21): QSLLNSGNQKNY
29G4-VL CDR2 (SEQ ID NO: 22): WASTRGS
29G4-VL CDR3 (SEQ ID NO: 23): QNDYFYPLT

31A6-VH CDR1 (SEQ ID NO: 24): GYTFASYN
31A6-VH CDR2 (SEQ ID NO: 25): IYPGKDGS
31A6-VH CDR3 (SEQ ID NO: 26): ARGGYYGNSFDY
31A6-VL CDR1 (SEQ ID NO: 27): QSLLNSGNLKNY
31A6-VL CDR2 (SEQ ID NO: 28): WASIRES
31A6-VL CDR3 (SEQ ID NO: 29): QNAYTFPFT

15B10-VH CDR1 (SEQ ID NO: 30): GYAFSNYL
15B10-VH CDR2 (SEQ ID NO: 31): IHPGSGT
15B10-VH CDR3 (SEQ ID NO: 32): SRRWYGNSLIY
15B10-VL CDR1 (SEQ ID NO: 33): QSLLNSGNQRNY
15B10-VL CDR2 (SEQ ID NO: 34): WASTRES
15B10-VL CDR3 (SEQ ID NO: 35): QNDYFYPFT

Figure 12

32G4-VH (SEQ ID NO: 36):
EVQLQQSGPELENPGASVKIFCKASGYSSTGYNINWVKQSSGKSLEWIGNINPYYGGI
TYNQKFKGKATLTVDKSSSTAYVQLKSLTSEDSAVYYCARDYYGNSFAYWGQGTL
VTVST
32G4-VL (SEQ ID NO: 37):
DIVMTQSPSSLTVTAGEKVTMSCKSSQSLLNSGNQKNYLTWYQQNPGQPPKLLIFW
ASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNNYYFPYTFGGGTKLEIR

47D10-VH sequence (SEQ ID NO: 38):
QVMLVESGGGLVKPGGSLKLSCAASGFAFSSSAMSWIRQTPEKRLEWVATMTVGYI
YTYYLDTVKGRFIISRDNANNTLYLQMSSLRSEDSAMYYCARQGYGNAMDYWGQG
TSVTVSS
47D10-VL sequence (SEQ ID NO: 39):
DIVMTQSPSSLTVTAGEKVTMSCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIYW
ASTRESGVPYRFTGSGSGTDFTLTISSVEAEDLAVYYCQNNYIYPLTFGAGTKLELK

29G4-VH sequence (SEQ ID NO: 40):
QVQLKESGPGLVAPSQTLSITCTVSGFSLTNYGVQWVRQPPGKGLEWLGVIWAGGN
TNYNSALLSRLSISKDTSKSQVFLKMNSLKTDDTAMYYCARGGYGNSCDYWGQGT
LVTVSA
29G4-VL sequence (SEQ ID NO: 41):
DIVMTQSPSSLTVTAGEKVTMNCKSSQSLLNSGNQKNYLAWYRQKPGQPPKLLIYW
ASTRGSGVPDRFTGSGSGTDFALTISSVQAEDLAVYYCQNDYFYPLTFGAGTKLDLK

31A6-VH sequence (SEQ ID NO: 42):
QTYLQQSGAELVRSGASVKMSCKASGYTFASYNIHWVKQTPGQGLEWIGYIYPGKD
GSHYNQKFRDKATLTADTSSSTAYMQISSLTSEDSAVYFCARGGYYGNSFDYWGQG
TLVTVSA
31A6-VL sequence (SEQ ID NO: 43):
DIVMSQSPSSLTVTTGEKVTMTCKSSQSLLNSGNLKNYLAWYQQKPGQPPKLLIYW
ASIRESGVPDRFTGSGSGTDFTLTIISVQAEDLAIYYCQNAYTFPFTLGGGTKLEIK

15B10-VH sequence (SEQ ID NO: 44):
QVQLQQSGAELVRPGTSVKVSCKASGYAFSNYLIDWVKQRPGQGLEWIGVIHPGSG
TNYNENFKGKATLTADKSSSTAYMQLSSLTSDDSAVYFCSRRWYGNSLIYWGQGTL
VTVSA
15B10-VL sequence (SEQ ID NO: 45):
DIVMTQSPSSLTVTAGEKVTMTCKSSQSLLNSGNQRNYLTWYQQKPGQPPKLLIYW
ASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDYFYPFTFGSGTKLEIK

Figure 13

32G4-huVH1 (SEQ ID NO: 46):
EVQLVQSGAEVKKPGASVKVSCKASGYSSTGYNINWVRQAPGQGLEWIGNINPYYG
GITYNQKFQGRVTLTVDKSSSTAYMELSRLRSDDTVVYYCARDYYGNSFAYWGQG
TLVTVSS

32G4-huVH2 (SEQ ID NO: 47):
QVQLVQSGAEVKKPGASVKVSCKASGYSSTGYNINWVRQAPGQGLEWMGNINPYY
GGITYNQKFQGRVTMTVDKSISTAYMELSRLRSDDTVVYYCARDYYGNSFAYWGQ
GTLVTVSS

32G4-huVH3 (SEQ ID NO: 48):
EVQLVQSGSELKKPGASVKVSCKASGYSSTGYNINWVRQAPGQGLEWIGNINPYYG
GITYNQGFTGRFVLSVDKSSSTAYLQISSLKAEDTAVYYCARDYYGNSFAYWGQGT
LVTVSS

32G4-huVH4 (SEQ ID NO: 49):
QVQLVQSGSELKKPGASVKVSCKASGYSSTGYNINWVRQAPGQGLEWMGNINPYY
GGITYNQGFTGRFVFSVDKSVSTAYLQISSLKAEDTAVYYCARDYYGNSFAYWGQG
TLVTVSS

32G4-huVL1 (SEQ ID NO: 50):
DIVMTQSPDSLAVSLGERATINCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIFWA
STRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNNYYFPYTFGQGTKLEIK

32G4-huVL2 (SEQ ID NO: 51):
DIVMTQSPLSLPVTPGEPASISCRSSQSLLNSGNQKNYLTWYLQKPGQSPQLLIFWAS
TRESGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCQNNYYFPYTFGQGTKLEIK

32G4-huVL3 (SEQ ID NO: 52):
DIQMTQSPSSLSASVGDRVTITCRSSQSLLNSGNQKNYLTWYQQKPGKAPKLLIFWA
STRESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNNYYFPYTFGQGTKLEIK

32G4-huVL4 (SEQ ID NO: 53):
DIQMTQSPSSLSASVGDRVTITCRSSQSLLNSGNQKNYLTWYQQKPGKAPKLLIFWA
STLESGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNNYYFPYTFGQGTKLEIK

Figure 14

47D10-huVH1 (SEQ ID NO: 54):
QVMLVESGGGLVKPGGSLRLSCAASGFAFSSSAMSWIRQAPGKGLEWVATMTVGYI
YTYYLDSVKGRFTISRDNANNSLYLQMNSLRAEDTAVYYCARQGYGNAMDYWGQ
GTLVTVSS

47D10-huVH2 (SEQ ID NO: 55):
QVQLVESGGGLVKPGGSLRLSCAASGFAFSSSAMSWIRQAPGKGLEWVATMTVGYI
YTYYLDSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARQGYGNAMDYWGQ
GTLVTVSS

47D10-huVH3 (SEQ ID NO: 56):
QVMLVQSGAEVKKPGASVKVSCKASGFAFSSSAMSWIRQAPGQRLEWVATMTVGY
IYTYYLDKFQGRVTITRDNANNTLYMELSSLRSEDTAVYYCARQGYGNAMDYWGQ
GTLVTVSS

47D10-huVH4 (SEQ ID NO: 57):
QVQLVQSGAEVKKPGASVKVSCKASGFAFSSSAMSWVRQAPGQRLEWMATMTVG
YIYTYYLDKFQGRVTITRDNSANTLYMELSSLRSEDTAVYYCARQGYGNAMDYWG
QGTLVTVSS

47D10-huVL1 (SEQ ID NO: 58):
DIVMTQSPDSLAVSLGERATINCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIYWA
STRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNNYIYPLTFGQGTKLEIK

47D10-huVL2 (SEQ ID NO: 59):
DIVMTQSPLSLPVTPGEPASISCRSSQSLLNSGNQKNYLTWYLQKPGQSPQLLIYWAS
TRESGVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCQNNYIYPLTFGQGTKLEIK

47D10-huVL3 (SEQ ID NO: 60):
DIVMTQSPGTLSLSPGERATLSCRSSQSLLNSGNQKNYLTWYQQKPGQAPRLLIYWA
STRESGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQNNYIYPLTFGQGTKLEIK

47D10-huVL4 (SEQ ID NO: 61):
EIVMTQSPGTLSLSPGERATLSCRSSQSLLNSGNQKNYLTWYQQKPGQAPRLLIYWA
STRETGIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQNNYIYPLTFGQGTKLEIK

Figure 15

32G4-huIgG1-V8

32G4-huIgG1-V8 heavy chain (SEQ ID NO: 62):
QVQLVQSGAEVKKPGASVKVSCKASGYSSTGYNINWVRQAPGQGLEWMGNINPYYGGITY
*NQKFQGRVTMTVDKSISTAYMELSRLRSDDTVVYYCARDYYGNSFAYWGQGTLVTVSS*AS
TKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

32G4-huIgG1-V8 light chain (SEQ ID NO: 63):
DIQMTQSPSSLSASVGDRVTITCRSSQSLLNSGNQKNYLTWYQQKPGKAPKLLIFWASTLES
*GVPSRFSGSGSGTDFTLTISSLQPEDFATYYCQNNYYFPYTFGQGTKLEIK*RTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

32G4-huIgG1-V9

32G4-huIgG1-V9 heavy chain (SEQ ID NO: 64):
EVQLVQSGSELKKPGASVKVSCKASGYSSTGYNINWVRQAPGQGLEWIGNINPYYGGITYN
*QGFTGRFVLSVDKSSSTAYLQISSLKAEDTAVYYCARDYYGNSFAYWGQGTLVTVSS*ASTK
GPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGG
PSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE
QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYT
LPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYS
KLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

32G4-huIgG1-V9 light chain (SEQ ID NO: 65):
DIVMTQSPDSLAVSLGERATINCKSSQSLLNSGNQKNYLTWYQQKPGQPPKLLIFWASTRE
*SGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNNYYFPYTFGQGTKLEIK*RTVAAPSVF
IFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 16

47D10-huIgG1-V6

47D10-huIgG1-V6 heavy chain (SEQ ID NO: 66):
QVQLVESGGGLVKPGGSLRLSCAASGFAFSSSAMSWIRQAPGKGLEWVATMTVGYIYTYYL
*DSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARQGYGNAMDYWGQGTLVTVSS*AST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

47D10-huIgG1-V6 light chain (SEQ ID NO: 67):
DIVMTQSPLSLPVTPGEPASISCRSSQSLLNSGNQKNYLTWYLQKPGQSPQLLIYWASTRES
*GVPDRFSGSGSGTDFTLKISRVEAEDVGVYYCQNNYIYPLTFGQGTKLEIK*RTVAAPSVFI
FPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTY
SLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

47D10-huIgG1-V7

47D10-huIgG1-V7 heavy chain (SEQ ID NO: 68):
QVQLVESGGGLVKPGGSLRLSCAASGFAFSSSAMSWIRQAPGKGLEWVATMTVGYIYTYYL
*DSVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARQGYGNAMDYWGQGTLVTVSS*AST
KGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSS
GLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELL
GGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKP
REEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQ
VYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSF
FLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

47D10-huIgG1-V7 light chain (SEQ ID NO: 69):
DIVMTQSPGTLSLSPGERATLSCRSSQSLLNSGNQKNYLTWYQQKPGQAPRLLIYWASTRES
*GIPDRFSGSGSGTDFTLTISRLEPEDFAVYYCQNNYIYPLTFGQGTKLEIK*RTVAAPSVFIF
PPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYS
LSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC

Figure 17

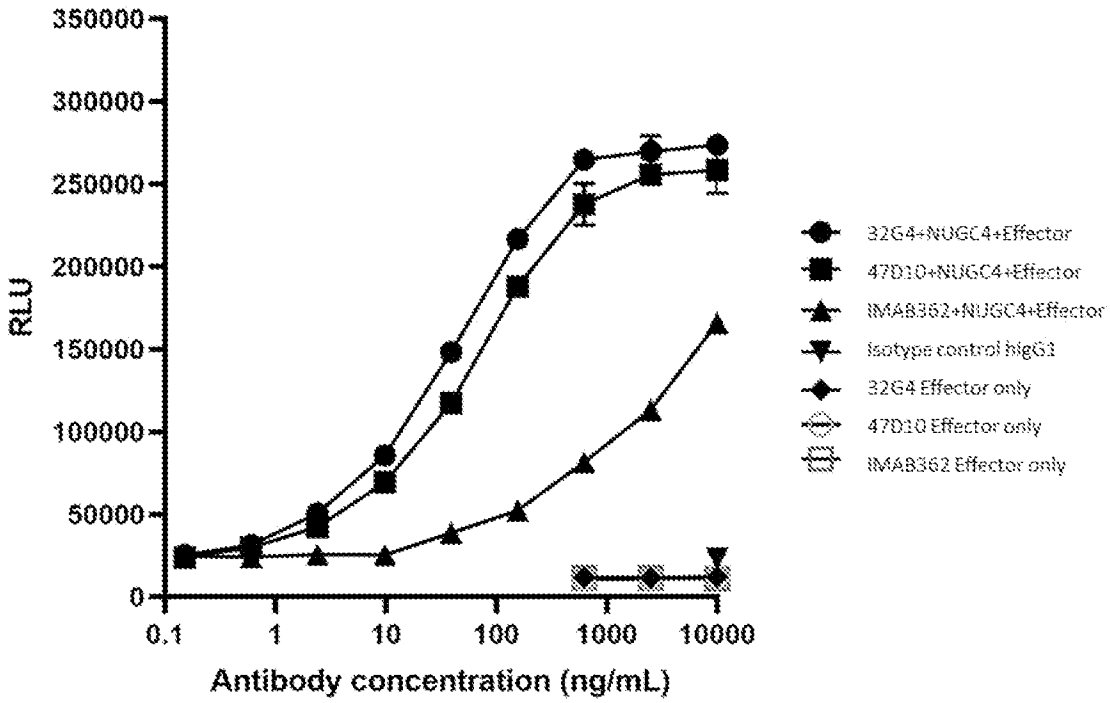

350000
300000
250000
200000
150000
100000
50000
0
RLU
0.1
1
10
100
1000
10000
Antibody concentration (ng/mL)
32G4+NUGC4+Effector
47D10+NUGC4+Effector
IMAB362+NUGC4+Effector
Isotype control hIgG1
32G4 Effector only
47D10 Effector only
IMAB362 Effector only

Figure 18

Binding to mouse and cyno claudin 18.2

	ECS0 Cyno 18.2	ECS0 Mouse 18.2
32G4-V8	0.087nM	0.189nM
32G4-V9	0.115nM	0.231nM
47D10-V6	0.163nM	0.518nM
47D10-V7	0.195nM	0.443nM
IMAB362	144.643nM	183.537nM

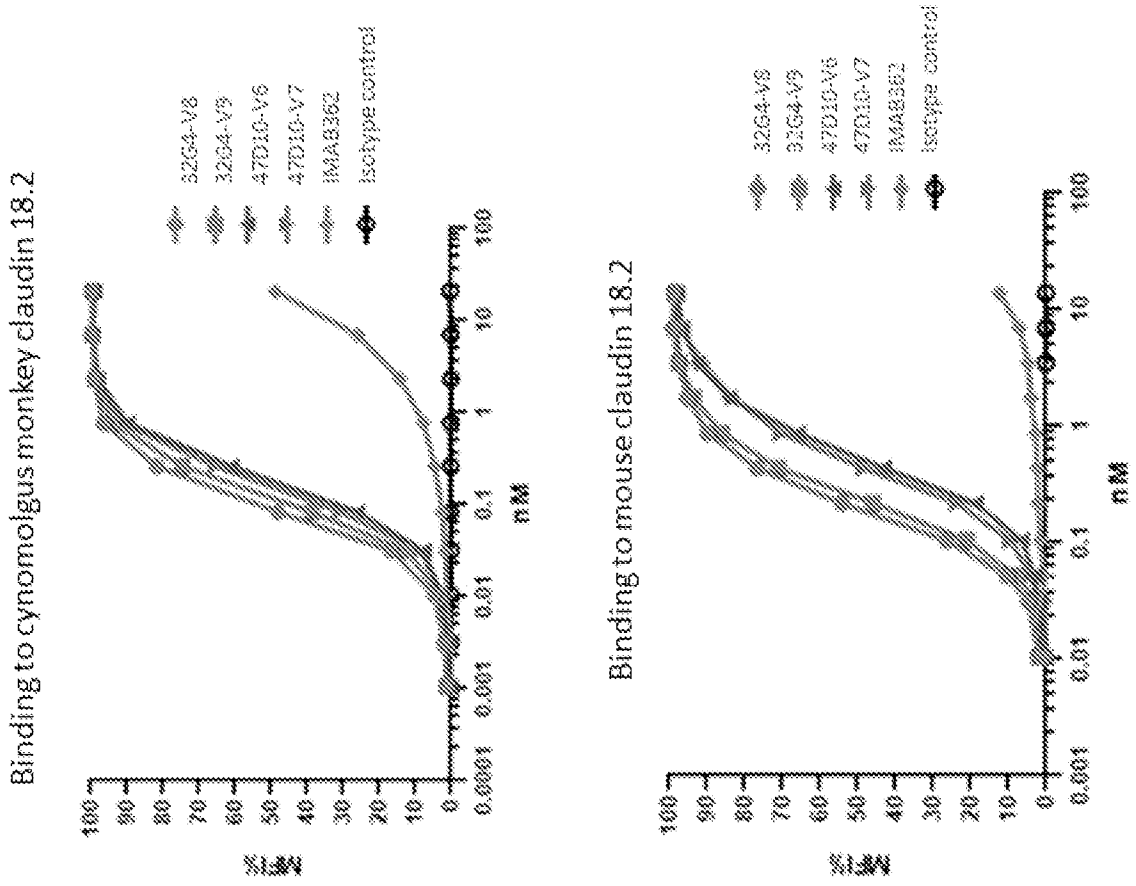


Figure 19