

ANTIBODY-DRUG CONJUGATES TARGETING CLAUDIN 18.2

BACKGROUND

[0001] Claudins, such as claudin 18.2, are considered promising targets for cancer immunotherapy. Claudins are a family of proteins that form the important components of the tight cell junctions. They establish a paracellular barrier which controls the flow of molecules between the cells. The proteins have N-terminus and a C-terminus in the cytoplasm. Different claudins are expressed on different tissues, their altered function has linked to formation of cancers of respective tissues. Claudin-1 is expressed in colon cancer, claudin-18 is expressed in gastric cancer, and claudin-10 is expressed in hepatocellular carcinoma.

[0002] Claudin-18 has two isoforms, isoform 1 and isoform 2. Isoform 2 (Claudin 18.2 or CLDN18.2) is a highly selective cell lineage marker. Claudin 18.2's expression in normal tissues is strictly confined to differentiated epithelial cells of the gastric mucosa, but it was absent from the gastric stem cell zone. Claudin 18.2 was retained on malignant transformation and was expressed in a significant proportion of primary gastric cancers and its metastases. Frequently ectopic activation of claudin 18.2 was also found in pancreatic, esophageal, ovarian, and lung tumors. These data suggested that CLDN18.2 has highly restricted expression pattern in normal tissues, with frequent ectopic activation in a diversity of human cancers.

SUMMARY

[0003] Anti-claudin 18.2 antibodies are discovered herein that selectively bind to wild-type claudin 18.2 and a common mutant M149L, and do not bind to other claudin 18 isoforms, such as claudin 18.1. In a surprising and unexpected discovery, the present disclosure demonstrates that these antibodies are highly effective in inducing receptor-mediated antibody internalization, in particular when compared to IMAB362 (claudiximab), a lead anti-claudin 18.2 antibody under clinical development. Therefore, when conjugated to a drug moiety, these antibodies are capable of efficiently delivering the drug into target cells, such as cancer cells overexpressing the claudin 18.2 protein.

[0004] The greatly increased ability to induce receptor-mediated antibody internalization of the presently disclosed antibodies may be attributed to the way these antibodies bind to the claudin 18.2 protein. As demonstrated in **Example 14** and illustrated in **FIG. 20**, amino acid

residues on the claudin 18.2 protein that are important for the binding to the antibodies include those that are important for stabilizing the conformation of the extracellular loops (e.g., W30, L49, W50, C53, C63 and R80). More important, the residues that are involved in binding to the antibodies are contemplated to include N45, Y46, G48, V54, R55, E56, S58, F60, and E62, which are located between the β 3 and β 4 strands of the first extracellular loop, and Y169 and G172, which are in β 5 of the second extracellular loop. By contrast, it is believed that known anti-claudin 18.2 antibodies only bind to one of the extracellular loops.

[0005] In accordance with one embodiment of the present disclosure, provided is an antibody-drug conjugate, comprising a drug moiety covalently attached to an antibody or fragment thereof having binding specificity to a wild-type human claudin 18.2 (CLDN18.2) protein, wherein the antibody or the fragment thereof binds to the β 3- β 4 loop and the β 5 strand of CLDN18.2. The β 3- β 4 loop consists of residues 45-63 of SEQ ID NO:30 (NYQGLWRSCVRESSGFTEC), and the β 5 strand consists of residues 169-172 of SEQ ID NO:30 (YTFG).

[0006] In some embodiments, the ratio of the number of drug moieties to the number of antibody or fragment is 1:1 to 20:1. In some embodiments, the ratio is 2:1 to 10:1. In some embodiments, the ratio is 2:1 to 6:1. In some embodiments, the ratio is about 2:1, 2.5:1, 3:1, 3.5:1, 4:1, 4.5:1 or 5:1.

[0007] In some embodiments, the antibody or the fragment thereof does not bind to β 1 and β 2, or binds to β 1 or β 2 at an affinity that is at least 10 fold lower than to the β 3- β 4 loop or the β 5 strand. In some embodiments, the antibody or the fragment thereof does not bind to the CLDN18.1 protein or binds to CLDN18.1 at an affinity that is at least 10 fold lower than to CLDN18.2.

[0008] In some embodiments, the antibody or the fragment thereof binds to the CLDN18.2 M149L mutant at an affinity that is at least 1% of the affinity to the wild-type CLDN18.2 protein.

[0009] In some embodiments, the antibody or the fragment thereof binds to at least an amino acid residue selected from the group consisting of N45, Y46, G48, V54, R55, E56, S58, F60, and E62; and at least an amino acid residue selected from the group consisting of Y169 and G172, of SEQ ID NO:30.

[0010] The drug moiety may be a cytotoxic or cytostatic agent, an immunosuppressive agent, a radioisotope, a toxin, or the like. The drug moiety, once released in cancer cell, can inhibit the multiplication of the cancer cell, or causing apoptosis in the cancer cell. Examples of drug moieties are selected from the group consisting of DM1 (maytansine, N2'-deacetyl-N2'-(3-mercapto-1-oxopropyl)- or N2'-deacetyl-N2'-(3-mercapto-1-oxopropyl)-maytansine), mc-MMAD (6-maleimidocaproyl-monomethylauristatin-D or N-methyl-L-valyl-N-[(1S,2R)-2-methoxy-4-[(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[[[(1S)-2-phenyl-1-(2-thiazolyl)ethyl]amino]propyl]-1-pyrrolidinyl]-1-[(1S)-1-methylpropyl]-4-oxobutyl]-N-methyl-(9Cl)-L-valinamide), mc-MMAF (maleimidocaproyl-monomethylauristatin F or N-[[6-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-1-oxohexyl]-N-methyl-L-valyl-L-valyl-(3R,4S,5S)-3-methoxy-5-methyl-4-(methylamino)heptanoyl-(α R, β R,2S)- β -methoxy- α -methyl-2-pyrrolidinepropanoyl-L-phenylalanine) and mc-Val-Cit-PABA-MMAE (6-maleimidocaproyl-ValCit-(p-aminobenzyloxycarbonyl)-monomethylauristatin E or N-[[[4-[[N-[6-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-1-oxohexyl]-L-valyl-N5-(aminocarbonyl)-L-ornithyl]amino]phenyl]methoxy]carbonyl]-N-methyl-L-valyl-N-[(1S,2R)-4-[(2S)-2-[(1R,2R)-3-[(1R,2S)-2-hydroxy-1-methyl-2-phenylethyl]amino]-1-methoxy-2-methyl-3-oxopropyl]-1-pyrrolidinyl]-2-methoxy-1-[(1S)-1-methylpropyl]-4-oxobutyl]-N-methyl-L-valinamide). DM1 is a derivative of the tubulin inhibitor maytansine while MMAD, MMAE, and MMAF are auristatin derivatives.

[0011] Methods and uses for the treatment of diseases and conditions are also provided. In one embodiment, provided is a method of treating cancer in a patient in need thereof, comprising administering to the patient the antibody-drug conjugate of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0012] **FIG. 1** shows that the mouse sera from all mice after DNA immunisation have high titration reacted with HEK293 cells transfected with CLD 18A2 by flow cytometry, CLD 18A1 as negative control.

[0013] **FIG. 2** shows that the hybridoma supernatants can bind to HEK293 cells transfected with human CLD18A2 by cell ELISA or flow cytometry.

[0014] **FIG. 3** shows that the purified murine antibodies can bind to MKN45 cells transfected with human CLD18A2 by flow cytometry with high EC50, compared with positive reference antibody.

[0015] **FIG. 4** shows that the purified murine antibodies can bind to SU620 cells endogenously expressing human CLD18A2 bearing M149L mutation by flow cytometry with high EC50, while the reference antibody did not.

[0016] **FIG. 5** shows that the purified murine antibodies can bind to HEK293 cells transfected with mouse CLD18A2 by flow cytometry with high EC50.

[0017] **FIG. 6** shows that the purified murine antibodies can bind to HEK293 cells transfected with cyno CLD18A2 by flow cytometry with high EC50.

[0018] **FIG. 7** shows that the purified murine antibodies can bind to HEK293 cells transfected with human CLD18A2 by flow cytometry with high EC50.

[0019] **FIG. 8** shows that the chimeric antibodies can bind to MKN45 cells transfected with human CLD18A2 by flow cytometry with high EC50, compared with positive reference antibody.

[0020] **FIG. 9** shows that the chimeric antibodies can not bind to MKN45 cells transfected with human CLD18A1 by flow cytometry.

[0021] **FIG. 10** shows that humanized antibodies can bind to MKN45 cells transfected with human CLD18A2 by flow cytometry with high EC50, compared with positive reference antibody.

[0022] **FIG. 11** shows that the humanized antibodies cannot bind to MKN45 cells transfected with human CLD18A1 by flow cytometry.

[0023] **FIG. 12** shows that humanized antibodies with CDR mutation can bind to MKN45 cells transfected with human CLD18A2 by flow cytometry with high EC50, compared with positive reference antibody.

[0024] **FIG. 13** shows that the humanized antibodies with CDR mutation cannot bind to MKN45 cells transfected with human CLD18A1 by flow cytometry.

[0025] **FIG. 14** shows that the de-risked variants had potent binding to cell surface CLD18A2.

[0026] **FIG. 15** shows that certain mutations of CLD18A2 have significant effect on the indicated antibodies binding to HEK293 cells transfected these mutants, suggesting that these amino acid residues constitute at least part of the epitope.

[0027] **FIG. 16** shows that antibodies 4F11E2, 72C1B6A3 and 120B7B2 had superior binding in both claudin 18.2 high and low CHO-K1 cells, as compared to 175D10.

[0028] **FIG. 17** shows the potent ADCC testing results of 4F11E2, 72C1B6A3 and 120B7B2 using 175D10 antibody as a reference.

[0029] **FIG. 18** shows that the S239D/I332E versions of the 4F11E2, 72C1B6A3 and 120B7B2 outperformed the 175D10 counterpart in the ADCC assays.

[0030] **FIG. 19** shows that 4F11E2, 72C1B6A3 and 120B7B2 also had better ADCP effects than 175D10.

[0031] **FIG. 20** illustrates the 3D and motif structures of claudin proteins.

[0032] **FIG. 21** shows the internalization results of tested chimeric antibodies as compared to reference antibody IMAB362 on CHO cells expressing claudin 18.2.

[0033] **FIG. 22** shows the internalization results of tested humanized antibodies as compared to reference antibody IMAB362 on CHO cells expressing claudin 18.2.

[0034] **FIG. 23** shows the internalization results of tested humanized antibodies as compared to reference antibody IMAB362 on MKN45 cells expressing claudin 18.2.

[0035] **FIG. 24** shows the binding affinities of the antibodies and their drug conjugates.

[0036] **FIG. 25A-C** show the cell toxicity of the tested antibody-MMAE conjugates upon internalization in DAN-G, NUGC or SCG-7901 transfectants.

[0037] **FIG. 26** shows the cell toxicity of the tested antibody-MMAE conjugates upon internalization in SNU620 endogenously expressing human claudin 18.2.

[0038] FIG. 27 compares antibody-drug conjugate with antibody alone in reducing tumor growth in test animals.

[0039] FIG. 28 shows the average or individual tumor reduction effects of the antibody-drug conjugate.

DETAILED DESCRIPTION

Definitions

[0040] It is to be noted that the term “a” or “an” entity refers to one or more of that entity; for example, “an antibody,” is understood to represent one or more antibodies. As such, the terms “a” (or “an”), “one or more,” and “at least one” can be used interchangeably herein.

[0041] As used herein, the term “polypeptide” is intended to encompass a singular “polypeptide” as well as plural “polypeptides,” and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term “polypeptide” refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, “protein,” “amino acid chain,” or any other term used to refer to a chain or chains of two or more amino acids, are included within the definition of “polypeptide,” and the term “polypeptide” may be used instead of, or interchangeably with any of these terms. The term “polypeptide” is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non- naturally occurring amino acids. A polypeptide may be derived from a natural biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It may be generated in any manner, including by chemical synthesis.

[0042] The term “isolated” as used herein with respect to cells, nucleic acids, such as DNA or RNA, refers to molecules separated from other DNAs or RNAs, respectively, that are present in the natural source of the macromolecule. The term “isolated” as used herein also refers to a nucleic acid or peptide that is substantially free of cellular material, viral material, or culture medium when produced by recombinant DNA techniques, or chemical precursors or other chemicals when chemically synthesized. Moreover, an “isolated nucleic acid” is

meant to include nucleic acid fragments which are not naturally occurring as fragments and would not be found in the natural state. The term “isolated” is also used herein to refer to cells or polypeptides which are isolated from other cellular proteins or tissues. Isolated polypeptides is meant to encompass both purified and recombinant polypeptides.

[0043] As used herein, the term “recombinant” as it pertains to polypeptides or polynucleotides intends a form of the polypeptide or polynucleotide that does not exist naturally, a non-limiting example of which can be created by combining polynucleotides or polypeptides that would not normally occur together.

[0044] “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. An “unrelated” or “non-homologous” sequence shares less than 40% identity, though preferably less than 25% identity, with one of the sequences of the present disclosure.

[0045] A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, 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, for example those described in Ausubel *et al.* eds. (2007) Current Protocols in Molecular Biology. Preferably, 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. Biologically equivalent polynucleotides are those having the above-noted specified percent homology and encoding a polypeptide having the same or similar biological activity.

[0046] The term “an equivalent nucleic acid or polynucleotide” refers to a nucleic acid having a nucleotide sequence having a certain degree of homology, or sequence identity, with the nucleotide sequence of the nucleic acid or complement thereof. A homolog of a double stranded nucleic acid is intended to include nucleic acids having a nucleotide sequence which has a certain degree of homology with or with the complement thereof. In one aspect, homologs of nucleic acids are capable of hybridizing to the nucleic acid or complement thereof. Likewise, “an equivalent polypeptide” refers to a polypeptide having a certain degree of homology, or sequence identity, with the amino acid sequence of a reference polypeptide. In some aspects, the sequence identity is at least about 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%. In some aspects, the equivalent polypeptide or polynucleotide has one, two, three, four or five addition, deletion, substitution and their combinations thereof as compared to the reference polypeptide or polynucleotide. In some aspects, the equivalent sequence retains the activity (*e.g.*, epitope-binding) or structure (*e.g.*, salt-bridge) of the reference sequence.

[0047] Hybridization reactions can be performed under conditions of different “stringency”. In general, a low stringency hybridization reaction is carried out at about 40°C in about 10 x SSC or a solution of equivalent ionic strength/temperature. A moderate stringency hybridization is typically performed at about 50°C in about 6 x SSC, and a high stringency hybridization reaction is generally performed at about 60°C in about 1 x SSC. Hybridization reactions can also be performed under “physiological conditions” which is well known to one of skill in the art. A non-limiting example of a physiological condition is the temperature, ionic strength, pH and concentration of Mg^{2+} normally found in a cell.

[0048] A polynucleotide is composed of a specific sequence of four nucleotide bases: adenine (A); cytosine (C); guanine (G); thymine (T); and uracil (U) for thymine when the polynucleotide is RNA. Thus, the term “polynucleotide sequence” is the alphabetical representation of a polynucleotide molecule. This alphabetical representation can be input into databases in a computer having a central processing unit and used for bioinformatics applications such as functional genomics and homology searching. The term “polymorphism” refers to the coexistence of more than one form of a gene or portion thereof. A portion of a gene of which there are at least two different forms, *i.e.*, two different nucleotide sequences, is referred to as a “polymorphic region of a gene”. A polymorphic region can be a single nucleotide, the identity of which differs in different alleles.

[0049] The terms “polynucleotide” and “oligonucleotide” are used interchangeably and refer to a polymeric form of nucleotides of any length, either deoxyribonucleotides or ribonucleotides or analogs thereof. Polynucleotides can have any three-dimensional structure and may perform any function, known or unknown. The following are non-limiting examples of polynucleotides: a gene or gene fragment (for example, a probe, primer, EST or SAGE tag), exons, introns, messenger RNA (mRNA), transfer RNA, ribosomal RNA, ribozymes, cDNA, dsRNA, siRNA, miRNA, recombinant polynucleotides, branched polynucleotides, plasmids, vectors, isolated DNA of any sequence, isolated RNA of any sequence, nucleic acid probes and primers. A polynucleotide can comprise modified nucleotides, such as methylated nucleotides and nucleotide analogs. If present, modifications to the nucleotide structure can be imparted before or after assembly of the polynucleotide. The sequence of nucleotides can be interrupted by non-nucleotide components. A polynucleotide can be further modified after polymerization, such as by conjugation with a labeling component. The term also refers to both double- and single-stranded molecules. Unless otherwise specified or required, any embodiment of this disclosure that is a polynucleotide encompasses both the double-stranded form and each of two complementary single-stranded forms known or predicted to make up the double-stranded form.

[0050] The term “encode” as it is applied to polynucleotides refers to a polynucleotide which is said to “encode” a polypeptide if, in its native state or when manipulated by methods well known to those skilled in the art, it can be transcribed and/or translated to produce the mRNA for the polypeptide and/or a fragment thereof. The antisense strand is the complement of such a nucleic acid, and the encoding sequence can be deduced therefrom.

[0051] As used herein, an “antibody” or “antigen-binding polypeptide” refers to a polypeptide or a polypeptide complex that specifically recognizes and binds to an antigen. An antibody can be a whole antibody and any antigen binding fragment or a single chain thereof. Thus the term “antibody” includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule having biological activity of binding to the antigen. Examples of such include, but are not limited to a complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region, a heavy chain or light chain constant region, a framework (FR) region, or any portion thereof, or at least one portion of a binding protein.

[0052] The terms “antibody fragment” or “antigen-binding fragment”, as used herein, is a portion of an antibody such as $F(ab')_2$, $F(ab)_2$, Fab' , Fab , Fv , $scFv$ and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. The term “antibody fragment” includes aptamers, spiegelmers, and diabodies. The term “antibody fragment” also includes any synthetic or genetically engineered protein that acts like an antibody by binding to a specific antigen to form a complex.

[0053] A “single-chain variable fragment” or “ $scFv$ ” refers to a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of immunoglobulins. In some aspects, the regions are connected with a short linker peptide of ten to about 25 amino acids. The linker can be rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the V_H with the C-terminus of the V_L , or vice versa. This protein retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of the linker. $ScFv$ molecules are known in the art and are described, *e.g.*, in US patent 5,892,019.

[0054] The term antibody encompasses various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon (γ , μ , α , δ , ϵ) with some subclasses among them (*e.g.*, $\gamma 1$ - $\gamma 4$). It is the nature of this chain that determines the “class” of the antibody as IgG, IgM, IgA, IgG, or IgE, respectively. The immunoglobulin subclasses (isotypes) *e.g.*, IgG₁, IgG₂, IgG₃, IgG₄, IgG₅, etc. are well characterized and are known to confer functional specialization. Modified versions of each of these classes and isotypes are readily discernable to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of the instant disclosure. All immunoglobulin classes are clearly within the scope of the present disclosure, the following discussion will generally be directed to the IgG class of immunoglobulin molecules. With regard to IgG, a standard immunoglobulin molecule comprises two identical light chain polypeptides of molecular weight approximately 23,000 Daltons, and two identical heavy chain polypeptides of molecular weight 53,000-70,000. The four chains are typically joined by disulfide bonds in a “Y” configuration wherein the light chains bracket the heavy chains starting at the mouth of the “Y” and continuing through the variable region.

[0055] Antibodies, antigen-binding polypeptides, variants, or derivatives thereof of the disclosure include, but are not limited to, polyclonal, monoclonal, multispecific, human,

humanized, primatized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and F(ab')₂, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a VK or VH domain, fragments produced by a Fab expression library, and anti- idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to LIGHT antibodies disclosed herein). Immunoglobulin or antibody molecules of the disclosure can be of any type (*e.g.*, IgG, IgE, IgM, IgD, IgA, and IgY), class (*e.g.*, IgG1, IgG2, IgG3, IgG4, IgA1 and IgA2) or subclass of immunoglobulin molecule.

[0056] Light chains are classified as either kappa or lambda (K, λ). Each heavy chain class may be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the “tail” portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated either by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain.

[0057] Both the light and heavy chains are divided into regions of structural and functional homology. The terms “constant” and “variable” are used functionally. In this regard, it will be appreciated that the variable domains of both the light (VK) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CK) and the heavy chain (CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen-binding site or amino- terminus of the antibody. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 and CK domains actually comprise the carboxy-terminus of the heavy and light chain, respectively.

[0058] As indicated above, the variable region allows the antibody to selectively recognize and specifically bind epitopes on antigens. That is, the VK domain and VH domain, or subset of the complementarity determining regions (CDRs), of an antibody combine to form the variable region that defines a three dimensional antigen-binding site. This quaternary antibody structure forms the antigen-binding site present at the end of each arm of the Y. More specifically, the antigen-binding site is defined by three CDRs on each of the VH and

VK chains (*i.e.* CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3). In some instances, *e.g.*, certain immunoglobulin molecules derived from camelid species or engineered based on camelid immunoglobulins, a complete immunoglobulin molecule may consist of heavy chains only, with no light chains. *See, e.g.*, Hamers-Casterman *et al.*, *Nature* 363:446-448 (1993).

[0059] In naturally occurring antibodies, the six “complementarity determining regions” or “CDRs” present in each antigen-binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen-binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen-binding domains, referred to as “framework” regions, show less inter-molecular variability. The framework regions 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. The antigen-binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids comprising the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been precisely defined (*see* “Sequences of Proteins of Immunological Interest,” Kabat, E., *et al.*, U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, *J. Mol. Biol.*, 196:901-917 (1987)).

[0060] In the case where there are two or more definitions of a term which is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term “complementarity determining region” (“CDR”) to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. This particular region has been described by Kabat *et al.*, U.S. Dept. of Health and Human Services, “Sequences of Proteins of Immunological Interest” (1983) and by Chothia *et al.*, *J. Mol. Biol.* 196:901-917 (1987), which are incorporated herein by reference in their entireties. The CDR definitions according to Kabat and Chothia include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either

definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which encompass the CDRs as defined by each of the above cited references are set forth in the table below as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

	Kabat	Chothia
CDR-H1	31-35	26-32
CDR-H2	50-65	52-58
CDR-H3	95-102	95-102
CDR-L1	24-34	26-32
CDR-L2	50-56	50-52
CDR-L3	89-97	91-96

[0061] Kabat *et al.* also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of “Kabat numbering” to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, “Kabat numbering” refers to the numbering system set forth by Kabat *et al.*, U.S. Dept. of Health and Human Services, “Sequence of Proteins of Immunological Interest” (1983).

[0062] In addition to table above, the Kabat number system describes the CDR regions as follows: CDR-H1 begins at approximately amino acid 31 (*i.e.*, approximately 9 residues after the first cysteine residue), includes approximately 5-7 amino acids, and ends at the next tryptophan residue. CDR-H2 begins at the fifteenth residue after the end of CDR-H1, includes approximately 16-19 amino acids, and ends at the next arginine or lysine residue. CDR-H3 begins at approximately the thirty third amino acid residue after the end of CDR-H2; includes 3-25 amino acids; and ends at the sequence W-G-X-G, where X is any amino acid. CDR-L1 begins at approximately residue 24 (*i.e.*, following a cysteine residue); includes approximately 10-17 residues; and ends at the next tryptophan residue. CDR-L2 begins at approximately the sixteenth residue after the end of CDR-L1 and includes approximately 7 residues. CDR-L3 begins at approximately the thirty third residue after the end of CDR-L2 (*i.e.*, following a cysteine residue); includes approximately 7-11 residues and ends at the sequence F or W-G-X-G, where X is any amino acid.

[0063] Antibodies disclosed herein may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, donkey, rabbit, goat, guinea pig, camel, llama, horse, or chicken antibodies. In another embodiment, the variable region may be chondrichthoid in origin (*e.g.*, from sharks).

[0064] As used herein, the term “heavy chain constant region” includes amino acid sequences derived from an immunoglobulin heavy chain. A polypeptide comprising a heavy chain constant region comprises at least one of: a CH1 domain, a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, or a variant or fragment thereof. For example, an antigen-binding polypeptide for use in the disclosure may comprise a polypeptide chain comprising a CH1 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH2 domain; a polypeptide chain comprising a CH1 domain and a CH3 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH3 domain, or a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, a CH2 domain, and a CH3 domain. In another embodiment, a polypeptide of the disclosure comprises a polypeptide chain comprising a CH3 domain. Further, an antibody for use in the disclosure may lack at least a portion of a CH2 domain (*e.g.*, all or part of a CH2 domain). As set forth above, it will be understood by one of ordinary skill in the art that the heavy chain constant region may be modified such that they vary in amino acid sequence from the naturally occurring immunoglobulin molecule.

[0065] The heavy chain constant region of an antibody disclosed herein may be derived from different immunoglobulin molecules. For example, a heavy chain constant region of a polypeptide may comprise a CH1 domain derived from an IgG₁ molecule and a hinge region derived from an IgG₃ molecule. In another example, a heavy chain constant region can comprise a hinge region derived, in part, from an IgG₁ molecule and, in part, from an IgG₃ molecule. In another example, a heavy chain portion can comprise a chimeric hinge derived, in part, from an IgG₁ molecule and, in part, from an IgG₄ molecule.

[0066] As used herein, the term “light chain constant region” includes amino acid sequences derived from antibody light chain. Preferably, the light chain constant region comprises at least one of a constant kappa domain or constant lambda domain.

[0067] A “light chain-heavy chain pair” refers to the collection of a light chain and heavy chain that can form a dimer through a disulfide bond between the CL domain of the light chain and the CH1 domain of the heavy chain.

[0068] As previously indicated, the subunit structures and three dimensional configuration of the constant regions of the various immunoglobulin classes are well known. As used herein, the term “VH domain” includes the amino terminal variable domain of an immunoglobulin heavy chain and the term “CH1 domain” includes the first (most amino terminal) constant region domain of an immunoglobulin heavy chain. The CH1 domain is adjacent to the VH domain and is amino terminal to the hinge region of an immunoglobulin heavy chain molecule.

[0069] As used herein the term “CH2 domain” includes the portion of a heavy chain molecule that extends, *e.g.*, from about residue 244 to residue 360 of an antibody using conventional numbering schemes (residues 244 to 360, Kabat numbering system; and residues 231-340, EU numbering system; *see* Kabat *et al.*, U.S. Dept. of Health and Human Services, “Sequences of Proteins of Immunological Interest” (1983). The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule. It is also well documented that the CH3 domain extends from the CH2 domain to the C-terminal of the IgG molecule and comprises approximately 108 residues.

[0070] As used herein, the term “hinge region” includes the portion of a heavy chain molecule that joins the CH1 domain to the CH2 domain. This hinge region comprises approximately 25 residues and is flexible, thus allowing the two N-terminal antigen-binding regions to move independently. Hinge regions can be subdivided into three distinct domains: upper, middle, and lower hinge domains (Roux *et al.*, *J. Immunol* 161:4083 (1998)).

[0071] As used herein the term “disulfide bond” includes the covalent bond formed between two sulfur atoms. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group. In most naturally occurring IgG molecules, the CH1 and CK regions are linked by a disulfide bond and the two heavy chains are linked by two disulfide bonds at positions corresponding to 239 and 242 using the Kabat numbering system (position 226 or 229, EU numbering system).

[0072] As used herein, the term “chimeric antibody” will be held to mean any antibody wherein the immunoreactive region or site is obtained or derived from a first species and the constant region (which may be intact, partial or modified in accordance with the instant disclosure) is obtained from a second species. In certain embodiments the target binding region or site will be from a non-human source (*e.g.* mouse or primate) and the constant region is human.

[0073] As used herein, “percent humanization” is calculated by determining the number of framework amino acid differences (*i.e.*, non-CDR difference) between the humanized domain and the germline domain, subtracting that number from the total number of amino acids, and then dividing that by the total number of amino acids and multiplying by 100.

[0074] By “specifically binds” or “has specificity to,” it is generally meant that an antibody binds to an epitope via its antigen-binding domain, and that the binding entails some complementarity between the antigen-binding domain and the epitope. According to this definition, an antibody is said to “specifically bind” to an epitope when it binds to that epitope, via its antigen-binding domain more readily than it would bind to a random, unrelated epitope. The term “specificity” is used herein to qualify the relative affinity by which a certain antibody binds to a certain epitope. For example, antibody “A” may be deemed to have a higher specificity for a given epitope than antibody “B,” or antibody “A” may be said to bind to epitope “C” with a higher specificity than it has for related epitope “D.”

[0075] As used herein, the terms “treat” or “treatment” refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the progression of cancer. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. “Treatment” can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

[0076] By “subject” or “individual” or “animal” or “patient” or “mammal,” is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sport, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows, and so on.

[0077] As used herein, phrases such as “to a patient in need of treatment” or “a subject in need of treatment” includes subjects, such as mammalian subjects, that would benefit from administration of an antibody or composition of the present disclosure used, *e.g.*, for detection, for a diagnostic procedure and/or for treatment.

Anti-Claudin 18.2 Antibodies and Fragments

[0078] The present disclosure provides anti-claudin 18.2 antibodies with high affinity to both the wild-type claudin 18.2 and a common mutant, M149L (the SU620 cell endogenously expressing this mutation). To the best knowledge of the inventors, all currently known anti-claudin 18.2 proteins do not bind to this mutant. The antibodies of the present disclosure, therefore, have the unique advantage of being capable of targeting both the wild-type and the M149L mutant claudin 18.2 protein. This advantage is important because a significant portion of cancer patients harbor this common mutation. It is also worth noting that the antibodies of the present disclosure do not bind to the other claudin 18 isoform, claudin 18.1 (or binds claudin 18.1 at a much lower affinity).

[0079] The antibodies and fragments of the present disclosure exhibited superior properties even when using a clinical candidate as a reference. 175D10 (IMAB362; claudiximab) is currently undergoing phase III clinical trials for treating gastric and gastroesophageal junction adenocarcinoma. The instant antibodies and fragment not only showed stronger binding activities, they also exhibited higher ADCC and ADCP activities under various different conditions, as compared to 175D10.

[0080] Also important, the present disclosure demonstrates that these antibodies are highly effective in inducing receptor-mediated antibody internalization, even when compared to IMAB362. The greatly increased ability to induce receptor-mediated antibody internalization of the presently disclosed antibodies, it is contemplated, can be attributed to how these antibodies bind to the claudin 18.2 protein. As demonstrated in **Example 14** and illustrated in

FIG. 20, amino acid residues on the claudin 18.2 protein that are important for the binding to the antibodies include those that are important for stabilizing the conformation of the extracellular loops (e.g., W30, L49, W50, C53, C63 and R80). W30, L49 and W50 are part of the W-LW-C-C consensus motif that helps to stabilize the conformation of loop 1. C53 and C63 form an inter-beta-strand disulfide bond. R80 is likely important for maintaining the interaction between parallel claudin 18.2 molecules on the cell surface, or for stabilizing the conformation of loop 1.

[0081] Also important for the antibody binding are residues N45, Y46, G48, V54, R55, E56, S58, F60, E62, Y169 and G172. Among them, N45, Y46, G48, V54, R55, E56, S58, F60 and E62 are located within the $\beta 3$ strand, or through C63 in the $\beta 4$ strand. This region, consisting of residues 45-63 of SEQ ID NO:30 (NYQGLWRSCVRESSGFTEC), is hereby referred to as the “ $\beta 3$ to $\beta 4$ loop,” which is part of the first extracellular loop (loop 1) of claudin 18.2. Y169 and G172, by contrast, are part of the $\beta 5$ strand (residues 169-172 of SEQ ID NO:30; YTFG) of the second extracellular loop (loop 2).

[0082] The greatly increased activity by the presently disclosed antibodies to induce receptor-mediated antibody internalization, it is contemplated, is due to their ability to bind to residues in both the $\beta 3$ to $\beta 4$ loop and the $\beta 5$ strand. In this context, it is believed that known anti-claudin 18.2 antibodies only bind to one of the loops.

[0083] The experimental data also show that the presently disclosed antibodies have higher binding specificity and improved ADCC and ADCP as compared to known ones.

Human claudin 18.2 sequence

Name	Sequence (SEQ ID NO:30)
Human Claudin 18.2 (NP_001002026)	1 MAVTACQGLG FVVSLLIGIAG IIAATC MDQW ST Q DLYNNPV TAVF NYQGLW ----- 51 RS CVRESSGF TEC RGYFTLL GLPAMLQAVR ALMIVGIVLG AIGLLVSIFA ----- $\beta 3$ - $\beta 4$ loop 101 LKCIRIGSME DSAKANMTLT SGIMFIVSGL CAIAGVSVFA NMLVTNFWMS 151 TANMYTGMGG MVQTVQTR YT FG AALFVGWV AGGLTLIGGV MMCIACRGLA -- -- B5 201 PEETNYKA VS YHASGHSVAY KPGGFKASTG FGSNTKNKKI YDGGARTEDE

	251 VQSYPSKHDY V
--	------------------

[0084] In accordance with one embodiment of the present disclosure, provided is an antibody or fragment thereof having binding specificity to a wild-type human claudin 18.2 (CLDN18.2) protein, wherein the antibody or the fragment thereof binds to both the first extracellular loop and the second extracellular loop of CLDN18.2. In some embodiments, the antibody or the fragment thereof binds to both the β 3- β 4 loop and the β 5 strand of CLDN18.2.

[0085] In accordance with another embodiment of the present disclosure, provided is an antibody or fragment thereof having binding specificity to a wild-type human claudin 18.2 (CLDN18.2) protein, wherein the antibody or fragment further binds to a M149L mutant of the CLDN18.2 protein. In some embodiments, the antibody or fragment does not bind to a human wild-type claudin 18.1 (CLDN18.1) protein, or does not bind CLDN18.1 at an affinity that is greater than about 1% of the affinity to the wild-type CLDN18.2 protein.

[0086] The binding affinity of an antibody or fragment to a protein can be measured with many methods known in the art. For examples, it can be measured cell-free assays with standalone CLDN18.1 or CLDN18.2 proteins. Preferably, however, the measurement is done with the CLDN18.1 or CLDN18.2 protein on a cell surface mimicking the actual binding environment. Such binding assays are adequately exemplified in the experimental examples.

[0087] In some embodiments, the antibodies or fragments thereof have a binding affinity to the M149L mutant that is at least 1%, or alternatively at least 0.001%, 0.01%, 0.1%, 0.5%, 2%, 3%, 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 60%, 70%, 80%, 90%, 95%, 98%, or 99% of the affinity to the wild-type CLDN18.2 protein.

[0088] In some embodiments, the antibodies or fragments thereof do not bind human CLDN18.1. In some embodiments, the antibodies or fragments thereof, as compared to binding to CLDN18.2, have a much weaker binding to human CLDN18.1, e.g., not greater than 10%, 5%, 2%, 1%, 0.5%, 0.1%, 0.05%, 0.01%, 0.005%, or 0.001%, without limitation.

[0089] As described above, the antibodies and fragments thereof of the present disclosure bind to the claudin 18.2 protein at an epitope that is different from known antibodies (see FIG. 4; at least the reference antibody interacts with M149 whereas the presently disclosed ones do not). In one embodiment, therefore, provided is an antibody or fragment thereof

having binding specificity to a wild-type human claudin 18.2 (CLDN18.2) protein, wherein the binding between the antibody or fragment thereof and the wild-type CLDN18.2 protein involves amino acid residues comprising at least an amino acid residue selected from the group consisting of N45, Y46, G48, V54, R55, E56, S58, F60, and E62; and at least an amino acid residue selected from the group consisting of Y169 and G172, of the wild-type CLDN18.2 protein.

[0090] In some embodiments, the antibody or fragment thereof binds to N45 of CLDN18.2.

In some embodiments, the antibody or fragment thereof binds to Y46 of CLDN18.2. In some embodiments, the antibody or fragment thereof binds to G48 of CLDN18.2. In some embodiments, the antibody or fragment thereof binds to L49 of CLDN18.2. In some embodiments, the antibody or fragment thereof binds to W50 of CLDN18.2. In some embodiments, the antibody or fragment thereof binds to C53 of CLDN18.2. In some embodiments, the antibody or fragment thereof binds to V54 of CLDN18.2. In some embodiments, the antibody or fragment thereof binds to R55 of CLDN18.2. In some embodiments, the antibody or fragment thereof binds to E56 of CLDN18.2. In some embodiments, the antibody or fragment thereof binds to E58 of CLDN18.2. In some embodiments, the antibody or fragment thereof binds to F60 of CLDN18.2. In some embodiments, the antibody or fragment thereof binds to E62 of CLDN18.2. In some embodiments, the antibody or fragment thereof binds to C63 of CLDN18.2.

[0091] In some embodiments, the antibody or fragment thereof binds to at least two amino acid residues selected from N45, Y46, G48, V54, R55, E56, S58, F60, and E62. In some embodiments, the antibody or fragment thereof binds to at least three amino acid residues selected from N45, Y46, G48, V54, R55, E56, S58, F60, and E62. In some embodiments, the antibody or fragment thereof binds to at least four amino acid residues selected from N45, Y46, G48, V54, R55, E56, S58, F60, and E62. In some embodiments, the antibody or fragment thereof binds to at least five amino acid residues selected from N45, Y46, G48, V54, R55, E56, S58, F60, and E62.

[0092] In some embodiments, the antibody or fragment thereof binds to at least Y169 of CLDN18.2. In some embodiments, the antibody or fragment thereof binds to at least G172 of CLDN18.2. In some embodiments, the antibody or fragment thereof binds to at least two amino acid residues selected from Y169 and G172 of CLDN18.2.

[0093] In some embodiments, the binding involves amino acid residues comprising W30; two, three, four, five or more amino acid residues selected from the group consisting of N45, Y46, G48, V54, R55, E56, S58, F60, and E62; and at least an amino acid residue selected from the group consisting of Y169 and G172, of the wild-type CLDN18.2 protein. In some embodiments, the binding involves amino acid residues comprising W30, N45, Y46, G48, V54, R55, E56, S58, F60, E62 and Y169 of the wild-type CLDN18.2 protein.

[0094] The weaker bindings to these amino acids on CLDN18.2 may be as compared to the other amino acids, such as G48, L49, W50, C53, V54, R55, E56. In some embodiments, the comparison is to the binding at the same amino acid to 175D10. For instance, the binding of the antibody or fragment of the present disclosure is weaker than that of 175D10 (IMGT/2Dstructure-DB card No: 10473) to at least one, two, three, four, five or all of D28, Q33, N38, V43, G59 and V79.

[0095] In some embodiments, the antibody or fragment thereof does not bind M149L of the CLDN18.2 protein. In some embodiments, the antibody or fragment thereof binds to a M149L mutant of the CLDN18.2 protein.

[0096] In accordance with one embodiment of the present disclosure, provided is an antibody or fragment thereof that includes the heavy chain and light chain variable domains with the CDR regions as shown in the CDR combinations of **Table A**.

Table A. CDR combinations of tested antibodies (Kabat numbering)

Comb No.	Antibody	CDRL1 (SEQ ID NO:)	CDRL2 (SEQ ID NO:)	CDRL3 (SEQ ID NO:)	CDRH1 (SEQ ID NO:)	CDRH2 (SEQ ID NO:)	CDRH3 (SEQ ID NO:)
1	64G11B4	KSSQSLLLNSGNQRNYLT (208)	WASTRES (227)	QNDYFYPFT (42)	NYLLE (234)	EINFGNGGSGNYNEKFKG (255)	IYYGNSFAY (281)
2	65G888	KSSQSLLLNSGNLKNYLT (209)	WASTRES (227)	QNVYIYPFT (43)	SYGVS (235)	VIWGDGNTIYHSALKS (256)	QGLYGHAMDY (282)
3	56E8F10F4	KSSQSLLLNSGNQKNYLT (210)	WASTRES (227)	QNDYFYPFT (44)	SFGMN (236)	FISGGSNTIHYLDTVKG (257)	LALGNAMDY (283)
4	54A2C4	KSSQSLLLNSGNQKNYLA (211)	GASTRES (228)	QNDLYYPWT (45)	TNAMN (237)	RIRSKSNNYATYYADSVKD (258)	GAYYGNASKAFDY (284)
5	54A2C4'	KSSQSLLLNSGNQKNYLA (211)	GASTRES (228)	QNDLYYPWT (45)	NYLLE (234)	EINFGNGGSGNYNEKFKG (255)	IYYGNSFAY (281)
6	54A2C4''	KSSQSLLLNSGNQKNYLA (211)	GASTRES (228)	QNDLYYPWT (45)	TYSIH (238)	YINPSTIYTNYNQKFKY (259)	EGYGRGNAMDY (285)
7	44F6B11	KSNQSLLLNSGNQKKYLT (212)	WASTRES (227)	QNGYSYPFT (46)	NYGMS (239)	TFSYGDSDSHNYYSDSVKG (260)	FGRGNTMDY (286)
8	15C2B7	KSSQSLLLNSGNQKNYLT (210)	WASTRES (227)	QNNYFPLT (47)	NYGMN (240)	WINANTGEPTYAEFFKG (261)	LTRGNSFDY (287)
9	20F1E10	KSSQSLFNSGNQRNYLT (213)	WASTRES (227)	QNVYSYPLT (48)	KYGMN (241)	WISTNTGEPTYAEFFKG (262)	LVRGNSFDF (288)
10	72C1B6A3	KSSQSLLLNSGNQKNYLT (210)	RASSRES (229)	QNDYIYPYT (8)	TYPIE (242)	NFHPYNDDTKYNEKFKG (263)	RAYGYPYAMDY (289)
11	58G2C2	KSSQSLLLNSGNQKNYLT (210)	WASTRES (230)	QNSYSYPFT (49)	NYLIE (243)	VINPGRSGTNYNEKFKG (264)	TRYGGNAMDY (290)
12	101C4F12	KSSQSLLLNSGNQRNYLT (208)	WSSTRDS (231)	QNNFIYPLT (50)	SYGVH (244)	VIWAGGSTNYDSALMS (265)	SLYGNSLDS (291)
13	103A10B2	RSSMSLFNSGNQKSYSLS (214)	WASTRES (232)	HNDYIYPLT (51)	SFGVH (245)	VIWAGGSTNYNSALMS (266)	SLYGNISFDY (292)
14	78E8G9G6	RSIQSLLLNSGNQKNYLS (215)	WASTRES (227)	QNSYSYPFT (49)	SYGVH (244)	VIWAGGRTNYNSALMS (267)	DRYGGNSLDY (293)
15	4F11E2	RSSQSLLLNSGNQRNYLT (216)	WASTRES (227)	QWAYSYFPT (13)	TFGMH (246)	YITSGNSPIYFTDTVKG (268)	SSYGNNSMDY (294)
16	10G7G11	KSSQSLFNSGNQRNYLT (213)	WASTRES (227)	QWAYYFPT (19)	TYGVH (247)	VMLSDGNTVYNSLSKS (269)	HKAYGNAMDY (295)
17	12F1F4	KSSQSLFNSGNQRNYLT (213)	WSSTRDS (233)	QNNYFYPFT (52)	NYGVS (248)	VIWGDGNTNYQSALRS (270)	VGRGNAMDH (296)
18	78C10B6G4	KSSQSLLLNSGNQKNYLT (210)	RASSRES (229)	QNDYIYPYT (8)	NYGVS (248)	VIRGPDGNTNYQSALRS (271)	VGRGNAMDH (296)
19	119G11D9	RSTQSLFNSGNQKNYLT (217)	WASTRES (227)	QWAYYYPLT (53)	GFLMH (249)	YINPYNDGTKYSEKFKG (272)	LDYGNAMDY (297)
20	113G12F5E6	KPSQSLLLNSGNQKNYLA (218)	WASTRES (227)	QWAYFYPCT (54)	KYGVH (250)	VIWTGGNTDYNPALIP (273)	NGYYGNAMDY (298)
21	116A8B7	RSTQSLFNSGNQRNYLT (219)	WASTRES (227)	QWAYYYPLT (53)	GFLMH (249)	YINPYNDGTKYSEKFKG (272)	LDYGNAMDY (297)
22	105F7G12	KSSQSLLLNSGNQKNYLA (220)	WASTRES (227)	QWAYFYPCT (54)	KYGVH (250)	VIWTGGNTDYNPALIP (273)	NGYYGNAMDY (298)
23	84F9E12	KSSQSVFNSGNQRNYLT (221)	WASTRES (227)	QNDYFPLT (55)	SGYFW (251)	YISYDGSNNYNSPSLKN (274)	FRFFAY (299)
24	103F4D4	RSSQSLLLNSGNQKNYLT (222)	WASTRES (227)	QWAYFYPFT (56)	TYSIH (238)	YINPSTIYTNYNQKFKY (259)	EGYGRGNAMDY (285)
25	110C12B6	RSTQSLFNSGNQRNYLT (219)	WASTRES (227)	QWAYYYPLT (53)	GFLMH (249)	YINPYNDGTKYSEKFKG (272)	LDYGNAMDY (297)
26	85H12E8	KSSQSLLLNSGNQRNYLS (223)	WASTRES (227)	QWAYFYPFT (56)	NYGVS (248)	VIWAGGNTNYNSALMS (275)	HGYGKGNAMDN (300)
27	103H2B4	KSSQSLLLNSGNQKNYLT (210)	WASTRES (227)	QNNYFPLT (57)	NFLTH (252)	EINPTNGRTYYNEKFKR (276)	IYYGNNSMDY (301)

28	103F6D3	RSSQSLLLNGGNQKNYLT (222)	WASTRES (227)	QNAYFYFFT (56)	TYSIH (238)	YINENTIYTNYNQKFKY (277)	EGYGRGNAMDY (285)
29	113E12F7	KSSQSLFNSGNQKNYLT (224)	WASTRES (227)	QNNYIYPLA (58)	SYGVH (244)	VIWAGGSTNYDSALMS (265)	SLYGNSFDH (302)
30	12087B2	KSSQSLLLNSGNQKNYLT (210)	WASTRES (227)	QNGYFFFT (3)	GYIIQ (253)	FINPYNDGTYNEQFKG (278)	AYFGNSFAY (303)
31	111B12D11	RSSQSLFNSGNQKNYLT (225)	WASTRES (227)	QNNYIYPLA (58)	SYGVH (244)	VIWAGGSTNYDSTLMS (279)	SLYGNSFDH (302)
32	111E7E2	KSSQSLFNSGNQKNYLT (224)	WASTRES (227)	QNNYIYPLA (58)	SYGAH (254)	VIWAGGSTNYDSALMS (265)	SLYGNSFDH (302)
33	100F4G12	KSTQSLLLNSGNQKNYLT (226)	WASTRES (227)	QNAYYYFLT (53)	GFLMH (249)	YINPYNDGTYSERFKG (280)	LDYGNAMDY (297)

Table B. CDRs of 120B7B2 (Kabat numbering)

CDR	Sequence (SEQ ID NO:)	De-Risked Versions (SEQ ID NO:)
CDRL1	KSSQSLLNSGNQKNYLT (210)	KSSQSLLN <u>A</u> GNQKNYLT (304) KSSQSLL <u>E</u> SGNQKNYLT (305)
CDRL2	WASTRES (227)	
CDRL3	QNGYYFPFT (3)	QNA <u>A</u> YYFPFT (19) Q <u>E</u> GYYPFT (20)
CDRH1	GYIIQ (253)	
CDRH2	FINPYNDGTKYNEQFKG (278)	FINPYND <u>D</u> TKYNEQFKG (306)
CDRH3	AYFGNSFAY (303)	AYFGN <u>A</u> FAY (307)

Table C. CDRs of 72C1B6A3 (Kabat numbering)

CDR	Sequence (SEQ ID NO:)	De-Risked Versions (SEQ ID NO:)
CDRL1	KSSQSLLNSGNQKNYLT (210)	KSSQSLLN <u>A</u> GNQKNYLT (304) KSSQSLL <u>E</u> SGNQKNYLT (305)
CDRL2	RASSRES (229)	
CDRL3	QNDYIYPYT (8)	
CDRH1	TYPE (242)	
CDRH2	NFHPYNDDTKYNEKFKG (263)	
CDRH3	RAYGYPYAMDY (289)	

Table D. CDRs of 4F11E2 (Kabat numbering)

CDR	Sequence (SEQ ID NO:)	De-Risked Versions (SEQ ID NO:)
CDRL1	RSSQSLLNSGNRKNYLT (216)	RSSQSLL <u>E</u> SGNRKNYLT (308) RSSQSLLN <u>A</u> GNRKNYLT (309)
CDRL2	WASTRES (227)	
CDRL3	QNAYSYPFT (13)	
CDRH1	TFGMH (246)	
CDRH2	YITSGNSPIYFTDTVKG (268)	YITSG <u>Q</u> SPIYFTDTVKG (310) YITSG <u>E</u> SPIYFTDTVKG (311)
CDRH3	SSYYGNSMDY (294)	SSYYG <u>Q</u> SMDY (312) SSYYG <u>E</u> SMDY (313) SSYYGN <u>A</u> MDY (314)

[0097] The antibodies that contained these CDR regions, whether mouse, humanized or chimeric, had potent claudin 18.2 binding and inhibitory activities. As shown in Examples 11 and 12, certain residues within the CDR can be modified to retain or improve the property or reduce their potential to have post-translational modifications (PTMs). Such modified CDR can be referred to as affinity matured or de-risked CDRs.

[0098] Non-limiting examples of de-risked CDRs are provided in **Tables B-D**, in the third columns. Affinity matured ones can include those having one, two or three amino acid addition, deletion and/or substitutions. In some embodiments, the substitutions can be conservative substitutions.

[0099] A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a nonessential amino acid residue in an immunoglobulin polypeptide is preferably replaced with another amino acid residue from the same side chain family. In another embodiment, a string of amino acids can be replaced with a structurally similar string that differs in order and/or composition of side chain family members.

[0100] Non-limiting examples of conservative amino acid substitutions are provided in the table below, where a similarity score of 0 or higher indicates conservative substitution between the two amino acids.

Table E. Amino Acid Similarity Matrix

	C	G	P	S	A	T	D	E	N	Q	H	K	R	V	M	I	L	F	Y	W
W	-8	-7	-6	-2	-6	-5	-7	-7	-4	-5	-3	-3	2	-6	-4	-5	-2	0	0	17
Y	0	-5	-5	-3	-3	-3	-4	-4	-2	-4	0	-4	-5	-2	-2	-1	-1	7	10	
F	-4	-5	-5	-3	-4	-3	-6	-5	-4	-5	-2	-5	-4	-1	0	1	2	9		
L	-6	-4	-3	-3	-2	-2	-4	-3	-3	-2	-2	-3	-3	2	4	2	6			
I	-2	-3	-2	-1	-1	0	-2	-2	-2	-2	-2	-2	-2	4	2	5				
M	-5	-3	-2	-2	-1	-1	-3	-2	0	-1	-2	0	0	2	6					
V	-2	-1	-1	-1	0	0	-2	-2	-2	-2	-2	-2	-2	4						
R	-4	-3	0	0	-2	-1	-1	-1	0	1	2	3	6							
K	-5	-2	-1	0	-1	0	0	0	1	1	0	5								
H	-3	-2	0	-1	-1	-1	1	1	2	3	6									
Q	-5	-1	0	-1	0	-1	2	2	1	4										
N	-4	0	-1	1	0	0	2	1	2											
E	-5	0	-1	0	0	0	3	4												
D	-5	1	-1	0	0	0	4													
T	-2	0	0	1	1	3														
A	-2	1	1	1	2															
S	0	1	1	1																
P	-3	-1	6																	
G	-3	5																		
C	12																			

Table F. Conservative Amino Acid Substitutions

For Amino Acid	Substitution With
Alanine	D-Ala, Gly, Aib, β -Ala, L-Cys, D-Cys
Arginine	D-Arg, Lys, D-Lys, Orn D-Orn
Asparagine	D-Asn, Asp, D-Asp, Glu, D-Glu Gln, D-Gln
Aspartic Acid	D-Asp, D-Asn, Asn, Glu, D-Glu, Gln, D-Gln
Cysteine	D-Cys, S-Me-Cys, Met, D-Met, Thr, D-Thr, L-Ser, D-Ser
Glutamine	D-Gln, Asn, D-Asn, Glu, D-Glu, Asp, D-Asp
Glutamic Acid	D-Glu, D-Asp, Asp, Asn, D-Asn, Gln, D-Gln
Glycine	Ala, D-Ala, Pro, D-Pro, Aib, β -Ala
Isoleucine	D-Ile, Val, D-Val, Leu, D-Leu, Met, D-Met
Leucine	Val, D-Val, Met, D-Met, D-Ile, D-Leu, Ile
Lysine	D-Lys, Arg, D-Arg, Orn, D-Orn
Methionine	D-Met, S-Me-Cys, Ile, D-Ile, Leu, D-Leu, Val, D-Val
Phenylalanine	D-Phe, Tyr, D-Tyr, His, D-His, Trp, D-Trp
Proline	D-Pro
Serine	D-Ser, Thr, D-Thr, allo-Thr, L-Cys, D-Cys
Threonine	D-Thr, Ser, D-Ser, allo-Thr, Met, D-Met, Val, D-Val
Tyrosine	D-Tyr, Phe, D-Phe, His, D-His, Trp, D-Trp
Valine	D-Val, Leu, D-Leu, Ile, D-Ile, Met, D-Met

[0101] In one embodiment, therefore, provided is an antibody or fragment thereof having binding specificity to a wild-type human claudin 18.2 (CLDN18.2) protein, wherein the antibody or fragment thereof comprises a light chain variable region comprising light chain complementarity determining regions CDRL1, CDRL2, and CDRL3 and a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3, and wherein the CDRL1, CDRL2, CDRL3, CDRH1, CDRH2, and CDRH3 are selected from combinations 1-33 of **Table A** or each of the combinations 1-33 in which one or more of the CDRL1, CDRL2, CDRL3, CDRH1, CDRH2, and CDRH3 each includes one, two, or three amino acid addition, deletion, conservative amino acid substitution or the combinations thereof.

[0102] In some embodiments, an anti-CLDN18.2 antibody or fragment is provided that includes CDRL1, CDRL2, CDRL3, CDRH1, CDRH2, and CDRH3, each of which is selected from Table A or Tables B-D. For instance, provided is an antibody or fragment thereof having binding specificity to a wild-type human claudin 18.2 (CLDN18.2) protein, wherein the antibody or fragment thereof comprises a light chain variable region comprising light chain complementarity determining regions CDRL1, CDRL2, and CDRL3 and a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3, and wherein: the CDRL1 comprises an amino acid sequence selected from the group of SEQ ID NO:208-226, or comprises an amino acid sequence derived anyone of SEQ ID NO:208-226 by one, two, or three amino acid addition, deletion, amino acid substitution; the CDRL2 comprises an amino acid sequence selected from the group of SEQ ID NO:227-233, or comprises an amino acid sequence derived anyone of SEQ ID NO:227-233 by an amino acid addition, deletion, amino acid substitution; the CDRL3 comprises an amino acid sequence selected from the group of SEQ ID NO:3, 8, 13, 19 and 42-58, or comprises an amino acid sequence derived anyone of SEQ ID NO: 3, 8, 13, 19 and 42-58 by one, two, or three amino acid addition, deletion, amino acid substitution; the CDRH1 comprises an amino acid sequence selected from the group of SEQ ID NO:234-254, or comprises an amino acid sequence derived anyone of SEQ ID NO:234-254 by one, two, or three amino acid addition, deletion, amino acid substitution; the CDRH2 comprises an amino acid sequence selected from the group of SEQ ID NO:255-280, or comprises an amino acid sequence derived anyone of SEQ ID NO:255-280 by one, two, or three amino acid addition, deletion, amino acid substitution; and the CDRH3 comprises an amino acid sequence selected from the group of SEQ ID NO:281-303, or comprises an amino acid sequence derived

anyone of SEQ ID NO:281-303 by one, two, or three amino acid addition, deletion, amino acid substitution.

[0103] In some embodiments, the CDRL1 comprises an amino acid sequence selected from the group of SEQ ID NO:208-226, 304-305 and 308-309; the CDRL2 comprises an amino acid sequence selected from the group of SEQ ID NO:227-233; the CDRL3 comprises an amino acid sequence selected from the group of SEQ ID NO:3, 8, 13, 19, 20 and 42-58; the CDRH1 comprises an amino acid sequence selected from the group of SEQ ID NO:234-254; the CDRH2 comprises an amino acid sequence selected from the group of SEQ ID NO:255-280, 306, 310 and 311; and the CDRH3 comprises an amino acid sequence selected from the group of SEQ ID NO:281-303, 307, and 312-314.

[0104] The antibody 120B7B2 has been demonstrated to be potent inhibitor of claudin 18.2. Its CDR sequences, along with a few de-risked versions, are provided in Table B. In one embodiment, the present disclosure provides an antibody or fragment thereof having binding specificity to a wild-type human claudin 18.2 (CLDN18.2) protein, wherein the antibody or fragment thereof comprises a light chain variable region comprising light chain complementarity determining regions CDRL1, CDRL2, and CDRL3 and a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3, and wherein: the CDRL1 comprises the amino acid sequence of QSLNLSGNQKNY (SEQ ID NO:1), QSLNAGNQKNY (SEQ ID NO:17) or QSLLESGNQKNY (SEQ ID NO:18) or an amino acid sequence having one, two or three amino acid substitution from SEQ ID NO:1, 17 or 18, the CDRL2 comprises the amino acid sequence of WAS (SEQ ID NO:2) or an amino acid sequence having one or two amino acid substitution from SEQ ID NO:2, the CDRL3 comprises the amino acid sequence of CQNGYYFPFT (SEQ ID NO:3), QNAYYFPFT (SEQ ID NO:19) or QEGYYFPFT (SEQ ID NO:20) or an amino acid sequence having one, two or three amino acid substitution from SEQ ID NO:3, 19 or 20, the CDRH1 comprises the amino acid sequence of GYTFTGYI (SEQ ID NO:4) or an amino acid sequence having one, two or three amino acid substitution from SEQ ID NO:4, the CDRH2 comprises the amino acid sequence of INPYNDGT (SEQ ID NO:5) or INPYNDDT (SEQ ID NO:21) or an amino acid sequence having one, two or three amino acid substitution from SEQ ID NO:5 or 21, and the CDRH3 comprises the amino acid sequence of ARAYFGNSFAY (SEQ ID NO:6) or ARAYFGNAFAY (SEQ ID NO:22)

or an amino acid sequence having one, two or three amino acid substitution from SEQ ID NO:6 or 22.

[0105] It is interesting to note (see Table A) that the CDRs from different antibody share great homology. It is then contemplated that each corresponding CDR can be interchanged without greatly impacting the antibody or fragment's binding affinity or activity. Alternatively, each particular amino acid in a CDR can be substituted with another amino acid present in a corresponding CDR from a different antibody.

[0106] In some embodiments, an antibody or fragment thereof is provided having binding specificity to a wild-type human claudin 18.2 (CLDN18.2) protein. In some embodiments, the antibody or fragment thereof comprises a light chain variable region comprising light chain complementarity determining regions CDRL1, CDRL2, and CDRL3 and a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3, and wherein: the CDRL1 comprises the amino acid sequence of SEQ ID NO:210, 304 or 305 or an amino acid sequence having one, two or three amino acid substitution from SEQ ID NO:210, 304 or 305, the CDRL2 comprises the amino acid sequence of SEQ ID NO:227 or an amino acid sequence having one or two amino acid substitution from SEQ ID NO:227, the CDRL3 comprises the amino acid sequence of SEQ ID NO:3, 19 or 20 or an amino acid sequence having one, two or three amino acid substitution from SEQ ID NO:3, 19 or 20, the CDRH1 comprises the amino acid sequence of SEQ ID NO:253 or an amino acid sequence having one, two or three amino acid substitution from SEQ ID NO: 253, the CDRH2 comprises the amino acid sequence of SEQ ID NO:278 or 306 or an amino acid sequence having one, two or three amino acid substitution from SEQ ID NO:278 or 306, and the CDRH3 comprises the amino acid sequence of SEQ ID NO:303 or 307 or an amino acid sequence having one, two or three amino acid substitution from SEQ ID NO:303 or 307.

[0107] In some embodiments, the CDRL1 comprises the amino acid sequence of SEQ ID NO:210, 304 or 305, the CDRL2 comprises the amino acid sequence of SEQ ID NO:227, the CDRL3 comprises the amino acid sequence of SEQ ID NO:3, 19 or 20, the CDRH1 comprises the amino acid sequence of SEQ ID NO:253, the CDRH2 comprises the amino acid sequence of SEQ ID NO:278 or 306, and the CDRH3 comprises the amino acid sequence of SEQ ID NO:303 or 307.

[0108] Non-limiting examples of a light chain variable region includes an amino acid sequence selected from the group consisting of SEQ ID NO:141, 192-195 and 206-207, or a biological equivalent, such as a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:141, 192-195 and 206-207.

[0109] Non-limiting examples of a heavy chain variable region include an amino acid sequence selected from the group consisting of SEQ ID NO:171, 188-191 and 205, or a biological equivalent, such as a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:171, 188-191 and 205.

[0110] In some embodiments, the CDRL1 comprises the amino acid sequence of SEQ ID NO:304, the CDRL2 comprises the amino acid sequence of SEQ ID NO:227, the CDRL3 comprises the amino acid sequence of SEQ ID NO:19, the CDRH1 comprises the amino acid sequence of SEQ ID NO:253, the CDRH2 comprises the amino acid sequence of SEQ ID NO:306, and the CDRH3 comprises the amino acid sequence of SEQ ID NO:307. A non-limiting example of the antibody or fragment includes a light chain variable region comprising an amino acid sequence of SEQ ID NO:206 and a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:205.

[0111] Likewise, 72C1B6A3 has been shown to be a good antibody. In another embodiment, therefore, provided is an antibody or fragment thereof having binding specificity to a wild-type human claudin 18.2 (CLDN18.2) protein, wherein the antibody or fragment thereof comprises a light chain variable region comprising light chain complementarity determining regions CDRL1, CDRL2, and CDRL3 and a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3, and wherein: the CDRL1 comprises the amino acid sequence of SEQ ID NO:210, 304 or 305 or an amino acid sequence having one, two or three amino acid substitution from SEQ ID NO:210, 304 or 305, the CDRL2 comprises the amino acid sequence of SEQ ID NO:229 or an amino acid sequence having one or two amino acid substitution from SEQ ID NO:229, the CDRL3 comprises the amino acid sequence of SEQ ID NO:8 or an amino acid sequence having one, two or three amino acid substitution from SEQ ID NO:8, the CDRH1 comprises the amino acid sequence of SEQ ID NO:242 or an amino acid sequence having one, two or three amino acid substitution from SEQ ID NO:242, the CDRH2 comprises the amino acid sequence of SEQ ID NO:263 or an amino acid sequence having one, two or three amino acid substitution from SEQ ID NO:263, and the CDRH3 comprises the amino acid sequence of SEQ ID

NO:289 or an amino acid sequence having one, two or three amino acid substitution from SEQ ID NO:289.

[0112] In some embodiments, the CDRL1 comprises the amino acid sequence of SEQ ID NO:210, 304 or 305, the CDRL2 comprises the amino acid sequence of SEQ ID NO:229, the CDRL3 comprises the amino acid sequence of SEQ ID NO:8, the CDRH1 comprises the amino acid sequence of SEQ ID NO:242, the CDRH2 comprises the amino acid sequence of SEQ ID NO:263, and the CDRH3 comprises the amino acid sequence of SEQ ID NO:289.

[0113] Non-limiting examples of a light chain variable region include an amino acid sequence selected from the group consisting of SEQ ID NO:124, 185-187 and 203-204, or a biological equivalent, such as a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:124, 185-187 and 203-204.

[0114] Non-limiting examples of a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:153 and 181-184, or a biological equivalent, such as a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:153 and 181-184.

[0115] In some embodiments, the CDRL1 comprises the amino acid sequence of SEQ ID NO:304, the CDRL2 comprises the amino acid sequence of SEQ ID NO:229, the CDRL3 comprises the amino acid sequence of SEQ ID NO:8, the CDRH1 comprises the amino acid sequence of SEQ ID NO:242, the CDRH2 comprises the amino acid sequence of SEQ ID NO:263, and the CDRH3 comprises the amino acid sequence of SEQ ID NO:289. A non-limiting example antibody or fragment thereof includes a light chain variable region comprising an amino acid sequence of SEQ ID NO:203 and a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:181.

[0116] Also, 4F11E2 has been shown to be a good antibody. In another embodiment, therefore, provided is an antibody or fragment thereof having binding specificity to a wild-type human claudin 18.2 (CLDN18.2) protein, wherein the antibody or fragment thereof comprises a light chain variable region comprising light chain complementarity determining regions CDRL1, CDRL2, and CDRL3 and a heavy chain variable region comprising heavy chain complementarity determining regions CDRH1, CDRH2, and CDRH3, and wherein: the CDRL1 comprises the amino acid sequence of SEQ ID NO:216, 308 or 309 or an amino acid sequence having one, two or three amino acid substitution from SEQ ID NO:216, 308 or 309,

the CDRL2 comprises the amino acid sequence of SEQ ID NO:227 or an amino acid sequence having one or two amino acid substitution from SEQ ID NO:227, the CDRL3 comprises the amino acid sequence of SEQ ID NO:13 or an amino acid sequence having one, two or three amino acid substitution from SEQ ID NO:13, the CDRH1 comprises the amino acid sequence of SEQ ID NO:246 or an amino acid sequence having one, two or three amino acid substitution from SEQ ID NO:246, the CDRH2 comprises the amino acid sequence of SEQ ID NO:268, 310 or 311 or an amino acid sequence having one, two or three amino acid substitution from SEQ ID NO:268, 310 or 311, and the CDRH3 comprises the amino acid sequence of SEQ ID NO:294, 312, 313 or 314, or an amino acid sequence having one, two or three amino acid substitution from SEQ ID NO:294, 312, 313 or 314.

[0117] In some embodiments, the CDRL1 comprises the amino acid sequence of SEQ ID NO:216, 308 or 309, the CDRL2 comprises the amino acid sequence of SEQ ID NO:227, the CDRL3 comprises the amino acid sequence of SEQ ID NO:13, the CDRH1 comprises the amino acid sequence of SEQ ID NO:246, the CDRH2 comprises the amino acid sequence of SEQ ID NO:268, 310 or 311, and the CDRH3 comprises the amino acid sequence of SEQ ID NO:294, 312, 313 or 314.

[0118] Non-limiting examples of a light chain variable region include an amino acid sequence selected from the group consisting of SEQ ID NO:129, 178-180 and 201-202, or a biological equivalent, such as a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:129, 178-180 and 201-202.

[0119] Non-limiting examples of a heavy chain variable region comprising an amino acid sequence selected from the group consisting of SEQ ID NO:159, 175-177 and 196-200, or a biological equivalents, such as a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:159, 175-177 and 196-200.

[0120] In some embodiments, the CDRL1 comprises the amino acid sequence of SEQ ID NO:309, the CDRL2 comprises the amino acid sequence of SEQ ID NO:227, the CDRL3 comprises the amino acid sequence of SEQ ID NO:13, the CDRH1 comprises the amino acid sequence of SEQ ID NO:246, the CDRH2 comprises the amino acid sequence of SEQ ID NO:311, and the CDRH3 comprises the amino acid sequence of SEQ ID NO:294. A non-limiting example of the antibody or fragment includes a light chain variable region

comprising an amino acid sequence of SEQ ID NO:202 and a heavy chain variable region comprising an amino acid sequence of SEQ ID NO:197.

[0121] In some embodiments, the antibodies are humanized antibodies. Humanized antibodies, as shown in Example 9, can include one or more back mutations to the mouse counterpart. Examples of such back mutations are shown in Table 3. In some embodiments, the antibody or fragment can include one, two, three, four, five or more of the back mutations.

[0122] In some embodiments, the anti-claudin 18.2 antibody of the present disclosure includes a VL of any one of SEQ ID NO: 117-144, 178-180, 185-187, 192-195, 201-202, 203-204 or 206-207, and a VH of any one of SEQ ID NO: 145-174, 175-177, 181-184, 188-191, 196-200, or 205 or their respective biological equivalents. A biological equivalent of a VH or VL is a sequence that includes the designated amino acids while having an overall 80%, 85%, 90%, 95%, 98% or 99% sequence identity. A biological equivalent of SEQ ID NO:145, therefore, can be a VH that has an overall 80%, 85%, 90%, 95%, 98% or 99% sequence identity to SEQ ID NO:145 but retains the CDRs, and optionally retains one or more, or all of the back-mutations.

[0123] It will also be understood by one of ordinary skill in the art that antibodies as disclosed herein may be modified such that they vary in amino acid sequence from the naturally occurring binding polypeptide from which they were derived. For example, a polypeptide or amino acid sequence derived from a designated protein may be similar, *e.g.*, have a certain percent identity to the starting sequence, *e.g.*, it may be 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the starting sequence.

[0124] In certain embodiments, the antibody comprises an amino acid sequence or one or more moieties not normally associated with an antibody. Exemplary modifications are described in more detail below. For example, an antibody of the disclosure may comprise a flexible linker sequence, or may be modified to add a functional moiety (*e.g.*, PEG, a drug, a toxin, or a label).

[0125] Antibodies, variants, or derivatives thereof of the disclosure include derivatives that are modified, *i.e.*, by the covalent attachment of any type of molecule to the antibody such that covalent attachment does not prevent the antibody from binding to the epitope. For example, but not by way of limitation, the antibodies can be modified, *e.g.*, by glycosylation,

acetylation, pegylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, linkage to a cellular ligand or other protein, etc. Any of numerous chemical modifications may be carried out by known techniques, including, but not limited to specific chemical cleavage, acetylation, formylation, metabolic synthesis of tunicamycin, etc. Additionally, the antibodies may contain one or more non-classical amino acids.

Antibody-Drug Conjugates

[0126] In some embodiments, the antibodies or fragments may be conjugated to therapeutic agents, prodrugs, peptides, proteins, enzymes, viruses, lipids, biological response modifiers, pharmaceutical agents, or PEG.

[0127] In one embodiment, the antibodies or fragments of the disclosure are covalently attached to a drug moiety. The drug moiety may be, or be modified to include, a group reactive with a conjugation point on the antibody. For example, a drug moiety can be attached by alkylation (e.g., at the epsilon-amino group lysines or the N-terminus of antibodies), reductive amination of oxidized carbohydrate, transesterification between hydroxyl and carboxyl groups, amidation at amino groups or carboxyl groups, and conjugation to thiols.

[0128] In some embodiments, the number of drug moieties, p , conjugated per antibody molecule ranges from an average of 1 to 8; 1 to 7, 1 to 6, 1 to 5, 1 to 4, 1 to 3, or 1 to 2. In some embodiments, p ranges from an average of 2 to 8, 2 to 7, 2 to 6, 2 to 5, 2 to 4 or 2 to 3. In other embodiments, p is an average of 1, 2, 3, 4, 5, 6, 7 or 8. In some embodiments, p ranges from an average of about 1 to about 20, about 1 to about 10, about 2 to about 10, about 2 to about 9, about 1 to about 8, about 1 to about 7, about 1 to about 6, about 1 to about 5, about 1 to about 4, about 1 to about 3, or about 1 to about 2. In some embodiments, p ranges from about 2 to about 8, about 2 to about 7, about 2 to about 6, about 2 to about 5, about 2 to about 4 or about 2 to about 3.

[0129] For example, when chemical activation of the protein results in formation of free thiol groups, the protein may be conjugated with a sulfhydryl reactive agent. In one aspect, the agent is one which is substantially specific for free thiol groups. Such agents include, for example, maleimide, haloacetamides (e.g., iodo, bromo or chloro), haloesters (e.g., iodo,

bromo or chloro), halomethyl ketones (e.g., iodo, bromo or chloro), benzylic halides (e.g., iodide, bromide or chloride), vinyl sulfone and pyridylthio.

[0130] The drug can be linked to the antibody or fragment by a linker. Suitable linkers include, for example, cleavable and non-cleavable linkers. A cleavable linker is typically susceptible to cleavage under intracellular conditions. Suitable cleavable linkers include, for example, a peptide linker cleavable by an intracellular protease, such as lysosomal protease or an endosomal protease. In exemplary embodiments, the linker can be a dipeptide linker, such as a valine-citrulline (val-cit), a phenylalanine-lysine (phe-lys) linker, or maleimidocapronic-valine-citrulline-p-aminobenzyloxycarbonyl (mc-Val-Cit-PABA) linker. Another linker is Sulfosuccinimidyl-4-[N-maleimidomethyl]cyclohexane-1-carboxylate (smcc). Sulfo-smcc conjugation occurs via a maleimide group which reacts with sulfhydryls (thiols, —SH), while its Sulfo-NHS ester is reactive toward primary amines (as found in Lysine and the protein or peptide N-terminus). Yet another linker is maleimidocaproyl (mc). Other suitable linkers include linkers hydrolyzable at a specific pH or a pH range, such as a hydrazone linker. Additional suitable cleavable linkers include disulfide linkers. The linker may be covalently bound to the antibody to such an extent that the antibody must be degraded intracellularly in order for the drug to be released e.g. the mc linker and the like.

[0131] A linker can include a group for linkage to the antibody. For example, linker can include an amino, hydroxyl, carboxyl or sulfhydryl reactive groups (e.g., maleimide, haloacetamides (e.g., iodo, bromo or chloro), haloesters (e.g., iodo, bromo or chloro), halomethyl ketones (e.g., iodo, bromo or chloro), benzylic halides (e.g., iodide, bromide or chloride), vinyl sulfone and pyridylthio).

[0132] In some embodiments, the drug moiety is a cytotoxic or cytostatic agent, an immunosuppressive agent, a radioisotope, a toxin, or the like. The conjugate can be used for inhibiting the multiplication of a tumor cell or cancer cell, causing apoptosis in a tumor or cancer cell, or for treating cancer in a patient. The conjugate can be used accordingly in a variety of settings for the treatment of animal cancers. The conjugate can be used to deliver a drug to a tumor cell or cancer cell. Without being bound by theory, in some embodiments, the conjugate binds to or associates with a cancer cell expressing claudin 18.2, and the conjugate and/or drug can be taken up inside a tumor cell or cancer cell through receptor-mediated endocytosis.

[0133] Once inside the cell, one or more specific peptide sequences within the conjugate (e.g., in a linker) are hydrolytically cleaved by one or more tumor-cell or cancer-cell-associated proteases, resulting in release of the drug. The released drug is then free to migrate within the cell and induce cytotoxic or cytostatic or other activities. In some embodiments, the drug is cleaved from the antibody outside the tumor cell or cancer cell, and the drug subsequently penetrates the cell, or acts at the cell surface.

[0134] Examples of drug moieties or payloads are selected from the group consisting of DM1 (maytansine, N2'-deacetyl-N2'-(3-mercapto-1-oxopropyl)- or N2'-deacetyl-N2'-(3-mercapto-1-oxopropyl)-maytansine), mc-MMAD (6-maleimidocaproyl-monomethylauristatin-D or N-methyl-L-valyl-N-[(1S,2R)-2-methoxy-4-[(2S)-2-[(1R,2R)-1-methoxy-2-methyl-3-oxo-3-[[[(1S)-2-phenyl-1-(2-thiazolyl)ethyl]amino]propyl]-1-pyrrolidinyl]-1-[(1S)-1-methylpropyl]-4-oxobutyl]-N-methyl-(9Cl)-L-valinamide), mc-MMAF (maleimidocaproyl-monomethylauristatin F or N-[6-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-1-oxohexyl]-N-methyl-L-valyl-L-valyl-(3R,4S,5S)-3-methoxy-5-methyl-4-(methylamino)heptanoyl-(α R, β R,2S)- β -methoxy- α -methyl-2-pyrrolidinepropanoyl-L-phenylalanine) and mc-Val-Cit-PABA-MMAE (6-maleimidocaproyl-ValCit-(p-aminobenzyloxycarbonyl)-monomethylauristatin E or N-[[[4-[[N-[6-(2,5-dihydro-2,5-dioxo-1H-pyrrol-1-yl)-1-oxohexyl]-L-valyl-N5-(aminocarbonyl)-L-ornithyl]amino]phenyl]methoxy]carbonyl]-N-methyl-L-valyl-N-[(1S,2R)-4-[(2S)-2-[(1R,2R)-3-[(1R,2S)-2-hydroxy-1-methyl-2-phenylethyl]amino]-1-methoxy-2-methyl-3-oxopropyl]-1-pyrrolidinyl]-2-methoxy-1-[(1S)-1-methylpropyl]-4-oxobutyl]-N-methyl-L-valinamide). DM1 is a derivative of the tubulin inhibitor maytansine while MMAD, MMAE, and MMAF are auristatin derivatives. In some embodiments, the drug moiety is selected from the group consisting of mc-MMAF and mc-Val-Cit-PABA-MMAE. In some embodiments, the drug moiety is a maytansinoid or an auristatin.

[0135] The antibodies or fragments may be conjugated or fused to a therapeutic agent, which may include detectable labels such as radioactive labels, an immunomodulator, a hormone, an enzyme, an oligonucleotide, a photoactive therapeutic or diagnostic agent, a cytotoxic agent, which may be a drug or a toxin, an ultrasound enhancing agent, a non-radioactive label, a combination thereof and other such agents known in the art.

[0136] The antibodies can be detectably labeled by coupling it to a chemiluminescent compound. The presence of the chemiluminescent-tagged antigen-binding polypeptide is

then determined by detecting the presence of luminescence that arises during the course of a chemical reaction. Examples of particularly useful chemiluminescent labeling compounds are luminol, isoluminol, therrromatic acridinium ester, imidazole, acridinium salt and oxalate ester.

[0137] The antibodies can also be detectably labeled using fluorescence emitting metals such as ^{152}Eu , or others of the lanthanide series. These metals can be attached to the antibody using such metal chelating groups as diethylenetriaminepentacetic acid (DTPA) or ethylenediaminetetraacetic acid (EDTA). Techniques for conjugating various moieties to an antibody are well known, *see, e.g.*, Arnon *et al.*, "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in *Monoclonal Antibodies And Cancer Therapy*, Reisfeld *et al.* (eds.), pp. 243-56 (Alan R. Liss, Inc. (1985); Hellstrom *et al.*, "Antibodies For Drug Delivery", in *Controlled Drug Delivery* (2nd Ed.), Robinson *et al.*, (eds.), Marcel Dekker, Inc., pp. 623- 53 (1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in *Monoclonal Antibodies '84: Biological And Clinical Applications*, Pinchera *et al.* (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in *Monoclonal Antibodies For Cancer Detection And Therapy*, Baldwin *et al.* (eds.), Academic Press pp. 303-16 (1985), and Thorpe *et al.*, "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", *Immunol. Rev.* (52:119-58 (1982)).

Polynucleotides Encoding the Antibodies and Methods of Preparing the Antibodies

[0138] The present disclosure also provides isolated polynucleotides or nucleic acid molecules encoding the antibodies, variants or derivatives thereof of the disclosure. The polynucleotides of the present disclosure may encode the entire heavy and light chain variable regions of the antigen-binding polypeptides, variants or derivatives thereof on the same polynucleotide molecule or on separate polynucleotide molecules. Additionally, the polynucleotides of the present disclosure may encode portions of the heavy and light chain variable regions of the antigen-binding polypeptides, variants or derivatives thereof on the same polynucleotide molecule or on separate polynucleotide molecules.

[0139] Methods of making antibodies are well known in the art and described herein. In certain embodiments, both the variable and constant regions of the antigen-binding polypeptides of the present disclosure are fully human. Fully human antibodies can be made

using techniques described in the art and as described herein. For example, fully human antibodies against a specific antigen can be prepared by administering the antigen to a transgenic animal which has been modified to produce such antibodies in response to antigenic challenge, but whose endogenous loci have been disabled. Exemplary techniques that can be used to make such antibodies are described in U.S. patents: 6,150,584; 6,458,592; 6,420,140 which are incorporated by reference in their entireties.

Treatment Methods

[0140] As described herein, the antibodies, variants, derivatives or antibody-drug conjugates of the present disclosure may be used in certain treatment and diagnostic methods.

[0141] The present disclosure is further directed to antibody-based therapies which involve administering the antibodies, fragments, or antibody-drug conjugates of the disclosure to a patient such as an animal, a mammal, and a human for treating one or more of the disorders or conditions described herein. Therapeutic compounds of the disclosure include, but are not limited to, antibodies of the disclosure (including variants and derivatives thereof as described herein) and nucleic acids or polynucleotides encoding antibodies of the disclosure (including variants and derivatives thereof as described herein).

[0142] The antibodies of the disclosure can also be used to treat or inhibit cancer. As provided above, claudin 18.2 can be overexpressed in tumor cells, in particular gastric, pancreatic, esophageal, ovarian, and lung tumors. Inhibition of claudin 18.2 has been shown to be useful for treating the tumors.

[0143] Accordingly, in some embodiments, provided are methods for treating a cancer in a patient in need thereof. The method, in one embodiment, entails administering to the patient an effective amount of an antibody, fragment, or antibody-drug conjugate of the present disclosure. In some embodiments, at least one of the cancer cells (e.g., stromal cells) in the patient over-express claudin 18.2.

[0144] Cellular therapies, such as chimeric antigen receptor (CAR) T-cell therapies, are also provided in the present disclosure. A suitable cell can be used, that is put in contact with an anti-claudin 18.2 antibody of the present disclosure (or alternatively engineered to express an anti-claudin 18.2 antibody of the present disclosure). Upon such contact or engineering, the cell can then be introduced to a cancer patient in need of a treatment. The cancer patient may

have a cancer of any of the types as disclosed herein. The cell (e.g., T cell) can be, for instance, a tumor-infiltrating T lymphocyte, a CD4⁺ T cell, a CD8⁺ T cell, or the combination thereof, without limitation.

[0145] In some embodiments, the cell was isolated from the cancer patient him- or her-self. In some embodiments, the cell was provided by a donor or from a cell bank. When the cell is isolated from the cancer patient, undesired immune reactions can be minimized.

[0146] Non-limiting examples of cancers include bladder cancer, breast cancer, colorectal cancer, endometrial cancer, esophageal cancer, head and neck cancer, kidney cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, pancreatic cancer, prostate cancer, and thyroid cancer. In some embodiments, the cancer is one or more of gastric, pancreatic, esophageal, ovarian, and lung cancers.

[0147] Additional diseases or conditions associated with increased cell survival, that may be treated, prevented, diagnosed and/or prognosed with the antibodies or variants, or derivatives thereof of the disclosure include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (*e.g.*, acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (*e.g.*, chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (*e.g.*, Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyo sarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma,

hemangioblastoma, acoustic neuroma, oligodendroglioma, meningioma, melanoma, neuroblastoma and retinoblastoma.

[0148] A specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the particular antibodies, variant or derivative thereof used, the patient's age, body weight, general health, sex, and diet, and the time of administration, rate of excretion, drug combination, and the severity of the particular disease being treated. Judgment of such factors by medical caregivers is within the ordinary skill in the art. The amount will also depend on the individual patient to be treated, the route of administration, the type of formulation, the characteristics of the compound used, the severity of the disease, and the desired effect. The amount used can be determined by pharmacological and pharmacokinetic principles well known in the art.

[0149] Methods of administration of the antibodies, fragments, or antibody-drug conjugates or include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The antigen-binding polypeptides or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Thus, pharmaceutical compositions containing the antigen-binding polypeptides of the disclosure may be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), buccally, or as an oral or nasal spray.

[0150] The term “parenteral” as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intra-articular injection and infusion.

[0151] Administration can be systemic or local. In addition, it may be desirable to introduce the antibodies of the disclosure into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0152] It may be desirable to administer the antigen-binding polypeptides or compositions of the disclosure locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, *e.g.*, in conjunction, with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the disclosure, care must be taken to use materials to which the protein does not absorb.

[0153] The amount of the antibodies, fragments, or antibody-drug conjugates of the disclosure which will be effective in the treatment, inhibition and prevention of an inflammatory, immune or malignant disease, disorder or condition can be determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease, disorder or condition, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0154] As a general proposition, the dosage administered to a patient of the antibodies, fragments, or antibody-drug conjugates of the present disclosure is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight, between 0.1 mg/kg and 20 mg/kg of the patient's body weight, or 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of antibodies of the disclosure may be reduced by enhancing uptake and tissue penetration (*e.g.*, into the brain) of the antibodies by modifications such as, for example, lipidation.

[0155] In an additional embodiment, the compositions of the disclosure are administered in combination with cytokines. Cytokines that may be administered with the compositions of the disclosure include, but are not limited to, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, anti-CD40, CD40L, and TNF- α .

[0156] In additional embodiments, the compositions of the disclosure are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

Compositions

[0157] The present disclosure also provides pharmaceutical compositions. Such compositions comprise an effective amount of an antibody, fragment, or antibody-drug conjugate, and an acceptable carrier. In some embodiments, the composition further includes a second anticancer agent (e.g., an immune checkpoint inhibitor).

[0158] In a specific embodiment, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans. Further, a “pharmaceutically acceptable carrier” will generally be a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

[0159] The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents such as acetates, citrates or phosphates. Antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; and agents for the adjustment of tonicity such as sodium chloride or dextrose are also envisioned. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch,

magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences by E. W. Martin, incorporated herein by reference. Such compositions will contain a therapeutically effective amount of the antigen-binding polypeptide, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0160] In an embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0161] The compounds of the disclosure can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

EXAMPLES

Example 1: Generation of murine monoclonal antibodies against human claudin 18 isoform 2 (CLD 18A2)

a. Immunizations:

[0162] Balb/c and C57/BL6 mice were immunized with eukaryotic expression vectors, encoding human claudin 18.2 (CLD 18A2) fragments. 50 µg of plasmid DNA was injected into the quadriceps (intramuscular, i.m.) on day 1 and 10. The presence of antibodies directed against human CLD 18A2 in the serum of the mice was monitored on day 20 by flow cytometry, using HEK293 cells transiently transfected with a nucleic acid encoding human CLD 18A2. Mice with detectable immune responses (**FIG. 1**) were boosted three and two days prior to fusion by intraperitoneal injection of 5×10^7 HEK293 cells transiently transfected with a nucleic acid encoding human CLD 18A2.

b. Generation of hybridomas producing human monoclonal antibodies to CLD18A2:

[0163] Mouse splenocytes were isolated and fused with PEG to a mouse myeloma cell line based on standard protocols. The resulting hybridomas were then screened for production of immunoglobulins with CLD 18A2 specificity using HEK293 cells transfected with a nucleic acid encoding human CLD18 by cell ELISA.

[0164] Single cell suspensions of splenic lymphocytes from immunized mice were fused with P3X63Ag8U.1 non-secreting mouse myeloma cells (ATCC, CRL 1597) in a 2:1 ratio using 50% PEG (Roche Diagnostics, CRL 738641). Cells were plated at approximately 3×10^4 /well in flat bottom microtiter plates, followed by about two weeks incubation in selective medium containing 10% fetal bovine serum, 2% hybridoma fusion and cloning supplement (HFCS, Roche Diagnostics, CRL 1 363 735) plus 10 mM HEPES, 0.055 mM 2-mercaptoethanol, 50 µg/ml gentamycin and 1x HAT (Sigma, CRL H0262). After 10 to 14 days individual wells were screened by Cell ELISA for anti-CLD 18A2 monoclonal antibodies (**FIG. 2**). The antibody secreting hybridomas were re-plated, screened again with HEK293 expressing CLD 18A2 or CLD 18A1 by FACS and, if still positive for CLD18A2 and negative for CLD18A1, were subcloned by limiting dilution. The stable subclones were then cultured in vitro to generate small amounts of antibody in tissue culture medium for characterization. At least one clone from each hybridoma, which retained the reactivity of parent cells (by FACS), was chosen. Three vial cell banks were generated for each clone and stored in liquid nitrogen.

c. Selection of monoclonal antibodies binding to CLD 18A2 not to CLD18A1:

[0165] To determine the isotype of antibodies, an isotype ELISA was performed. The mouse monoAB ID Kit (Zymed, CRL 90-6550) was used to determine Ig subclasses of the identified

CLD18A2 reactive monoclonal antibodies. Thirty-two hybridoma cell lines were generated: 64G11B4, 65G8B8, 56E8F10F4, 54A2C4, 44F6B11, 15C2B7, 20F1E10, 72C1B6A3, 58G2C2, 101C4F12, 103A10B2, 40C10E3, 78E8G9G6, 4F11E2, 10G7G11, 12F1F4, 78C10B6G4, 119G11D9, 113G12E5E6, 116A8B7, 105F7G12, 84E9E12, 103F4D4, 110C12B6, 85H12E8, 103H2B4, 103F6D3, 113E12F7, 120B7B2, 111B12D11, 111E7E2, and 100F4G12, with further details as shown below:

64G11B4, mouse monoclonal IgG1, κ antibody
65G8B8, mouse monoclonal IgG1, κ antibody
56E8F10F4, mouse monoclonal IgG1, κ antibody
54A2C4, mouse monoclonal IgG1, κ antibody
44F6B11, mouse monoclonal IgG1, κ antibody
15C2B7, mouse monoclonal IgG1, κ antibody
20F1E10, mouse monoclonal IgG1, κ antibody
72C1B6A3, mouse monoclonal IgG1, κ antibody
58G2C2, mouse monoclonal IgG2a, κ antibody
101C4F12, mouse monoclonal IgG2b, κ antibody
103A10B2, mouse monoclonal IgG2b, κ antibody
40C10E3, mouse monoclonal IgG1, λ antibody
78E8G9G6, mouse monoclonal IgG1, κ antibody
4F11E2, mouse monoclonal IgG1, κ antibody
10G7G11, mouse monoclonal IgG1, κ antibody
12F1F4, mouse monoclonal IgG1, κ antibody
78C10B6G4, mouse monoclonal IgG1, κ antibody
119G11D9, mouse monoclonal IgG1, κ antibody
113G12E5E6, mouse monoclonal IgG1, κ antibody
116A8B7, mouse monoclonal IgG1, κ antibody
105F7G12, mouse monoclonal IgG1, κ antibody
84E9E12, mouse monoclonal IgG1, κ antibody
103F4D4, mouse monoclonal IgG1, κ antibody
110C12B6, mouse monoclonal IgG1, κ antibody
85H12E8, mouse monoclonal IgG1, κ antibody
103H2B4, mouse monoclonal IgG1, κ antibody
103F6D3, mouse monoclonal IgG1, κ antibody

113E12F7, mouse monoclonal IgG2a, κ antibody
 120B7B2, mouse monoclonal IgG2a, κ antibody
 111B12D11, mouse monoclonal IgG2a, κ antibody
 111E7E2, mouse monoclonal IgG2a, κ antibody
 100F4G12, mouse monoclonal IgG3, κ antibody.

Example 2. Hybridoma Sequencing

[0166] Hybridoma cells (1×10^7) were harvested and total RNA was extracted using Tri Reagent as described above for spleen tissue. cDNA was prepared using SuperScript III kit according to the manufacturer's instruction, described above. The resulting cDNA product was used as template for PCR with primers VhRevU and VhForU, the resulting 300 bp PCR product was cleaned up using a PCR clean-up kit and sequenced with the same primer. PCR reaction was also performed with light chain V-region specific primer VkRev7 and VkFor (for variable region only) or KappaFor primers (for entire kappa light chain). Sequencing reactions were performed on cleaned PCR product to obtain DNA sequences for the antibodies, 64G11B4, 65G8B8, 56E8F10F4, 54A2C4, 44F6B11, 15C2B7, 20F1E10, 72C1B6A3, 58G2C2, 101C4F12, 103A10B2, 40C10E3, 78E8G9G6, 4F11E2, 10G7G11, 12F1F4, 78C10B6G4, 119G11D9, 113G12E5E6, 116A8B7, 105F7G12, 84E9E12, 103F4D4, 110C12B6, 85H12E8, 103H2B4, 103F6D3, 113E12F7, 120B7B2, 111B12D11, 111E7E2, and 100F4G12. Their variable (VH and VL) sequences are shown in Table 1 below.

Table 1. Sequences of the variable regions of the antibodies

Antibody chain	Sequence	SEQ ID NO:
Light chains		
64G11B4	DIVMTQSPSSLTVTAGEKVTMCKSSQSLNLSGNQKRYLTWYQQKPGQPP KLLIYWASTRESGVPDRFTGSGSGTDFTLTISVQADDLAVYYCQNDYFY PFTFGAGTNLELK	117
65G8B8	DIMMTQSPSSLTVTGTEKVTLTCKSSQSLNLSGNLKNYLTWYQQKPGHPP KLLIYWASTRESGVPVRFRTGSGSGTDFTLTISVQAEDLTVYYCQNVYIY PFTFGSGTKLEMR	118
56E8F10F4	DIVMTQSPSSLTVTAGEKVTMSCKSSQSLNLSGNQKRYLTWYQQKPGQPP KLLIYWASTRESGVPDRFTGSGSGTYFTLTISVQAADLAVYYCQNDYFY PFTFGSGTKLEIK	119
54A2C4	DTVMTQFPSSLSVSAGEKVTMSCKSSQSLNLSGNQKRYLTWYQQKPGQPP KLLIYGASTRESGVPDRFTGSGSGTDFTLTISVQAEDLAVYYCQNDLYY PWTFGGGKLEFK	120
44F6B11	DIVMTQSPSSLTVTAGEKVTMSCKSNQSLNLSGNQKRYLTWYQQKPGQSP KLLIYWASTRESGVPDRFTGSESGTDFTLTISVRAEDLAVYYCQNGYSY	121

	PFTFGSGTKLEMK	
15C2B7	DIVMTQSPSSSLTVTAGEKVTMSCKSSQSLNLSGNQKNYLTWYQQKPGQPP KLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVQTEDLAVYYCQNNYYF PLTFGAGTKLELK	122
20F1E10	DIVMTQSPSSSLTVTAGEKVTMSCKSSQSLNLSGNQKNYLTWYQQKPGQPP KLLIYWASTRESGVPDRFTGSGSGTDFILTITKVQAEDLAVYYCQNVYSY PLTFGAGTKLELK	123
72C1B6A3	DIVMTQSPSSSLTVTAGEKVTMSCKSSQSLNLSGNQKNYLTWYQQRPGQPP KLLIYRASSRESGVPVRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDYIY PYTFGGGTKLEMN	124
58G2C2	DIVMTQSPSSSLTVTAGEKVTMSCKSSQSLNLSGNQKNYLTWYQQKPGQPP TLIIFWAFTRESGVPDRFTGSGSGTDFTLTINSVQAEDLAVYYCQNSYSY PFTFGSGTKLEIK	125
101C4F12	DIVMTQSPSSSLTVTAGEKVTMSCKSSQSLNLSGNQKNYLTWYQQKPGQPP RLIIYWSSTRDSGVPDRFTGSGSRTDFTLTISSVQAEDLAVYYCQNNFIY PLTFGAGTKLELK	126
103B2	DIVMTQSPSSSLTVTPGEKVTMSCRSSMSLFNSGNQKSYLSWYHQKPGQPP KLLIYWASTRDSGVPVRFTGSGSGTDFTLTISSVQAEDLAVYYCHNDYIY PLTFGAGTKLELK	127
78E8G9G6	DIVMTQSPSSSLTVTAGEKVTMNCRSIQSLNLSGNQKNYLSWYQQKPGQPP KLLIYWASTRESGVPDRFTGSGSGTDFTLTIRSVLDEDLAVYYCQNSYSY PFTFGSGTKLEMK	128
4F11E2	DIVMTQSPSSSLTVTAGEKVTLCRSSQSLNLSGNRKNYLTWYQQIPGQPP KLLIYWASTRESGVPDRFTGSGSGTYFTLTISVQAEDLAVYYCQNAYSY PFTFGSGTKLEKK	129
10G7G11	DIVMTQSPSSSLTVTAGEKVTMTCKSSQSLNLSGNQKNYLTWYQKPGQPP KLLIYWASTRESGVPDRFTGSGSGTYFTLTVSSVQAEDLAVYYCQNAYYF PFTFGSGTKLEKK	130
12F1F4	DIVMTQSPSSSLTVTARERVSMTCSSQSLNLSGNQKNYLTWYQQKPGQPP KLLIYWSSTRRESGVPDRFTGSGSGTDFTLTISSVQAEDLAIYFCQNNYYY PFTFGSGTKLEIK	131
78C10B6G4	DIVMTQSPSSSLTVTAGEKVTMSCKSSQSLNLSGNQKNYLTWYQQRPGQPP KLLIYRASSRESGVPVRFTGSGSGTDFTLTISSVQAEDLAVYYCQNDYIY PYTFGGGTKLEMN	124
119G11D9	DIVMTQSPSSSLTVTAGERVMTMRCRSTQSLNLSGNQKNYLTWYQQKPGQPP KLLIYWASTRESGVPDRFTGGGSGTDFTLTISSVQAEDLAVYYCQNAYYY PLTFGAGTKLERK	132
113G12E5E6	DIVMTQSPSSSLTVTAGERVMTMCKPSQSLNLSGNQKNYLAWYQQKPGQPP KLLLYWASTRESGVPDRFKGSGSGTDFTLTISSVQAEDLAVYYCQNAIFY PCTFGGGTKLEMK	133
116A8B7	DIVMTQSPSSSLTVTAGEKVTMRCRSTQSLNLSGNQKNYLTWYQQKPGQPP KLLIYWASTRESGVPDRFTGSGSGTDFTLTISSVQAEDLAVYYCQNAYYY PLTFGVGTLERK	134
105F7G12	DIVMTQSPSSSLTVTAGERVMTMCKSSQSLNLSGNQKNYLAWYQQKPGQPP KLLLYWASTRESGVPDRFKGSGSGTDFTLTISSVQAEDLAVYYCQNAIFY PCTFGGGTKLEMK	135
84E9E12	DIVMTQSPSSSLTVTTGEKVTMSCKSSQSVFNSGNQKNYLTWYQQKPGQPP KLLVYWASTRESGVPARFTGSGSGTVFTLTISVQAEDLAVYYCQNDYIF PLTFGAGTRLELK	136
103F4D4	DIVMTQSPSSQTVTAGEKVTLSRCSQSLNLSGNQKNYLTWYQQKPGQPP KLLIYWASTRESGVPDRFTGSGSGTYFTFTISVQAEDLAVYYCQNAIFY PFTFGAGTKLELK	137
110C12B6	DIVMTQSPSSSLTVTAGEKVTMRCRSTQSLNLSGNQKNYLTWYQQKPGQPP	134

	KLLIYWASTRESGVPDRFTGSGSGTDFTLTISVQAEDLAVYYCQNAYYY PLTFGVGKLELK	
85H12E8	DIVMTQSPSSSLTVTAGEKVTMCKSSQSLNLSGNQKNYLSWYQQEPGQPP KLLIYWASTRESGVPDRFTGSGSGTDFTLTISNIQAEDLALYFCQNAIFY PFTFGSGTKLELK	138
103H2B4	DILMTQSPSSSLTVTAGEKVTMCKSSQSLNLSGNQKNYLTWYQQKPGQSP KLLIYWASTRESGVPDRFTGSGSGTDFTLTISVQAEDLAVYYCQNNIFY PLTFGVGKLELK	139
103F6D3	DIVMTQSPSSSLTVTAGEKVTLSRCSQSLNLSGNQKNYLTWYQQKPGQPP KLLIYWASTRESGVPDRFTGSGSGTYFTFTISVQAEDLAVYYCQNAIFY PFTFGAGTKLELK	137
113E12F7	DIVMTQSPSSSLTVTAGEKVTMCKSSQSLFNSGNQKNYLTWYQQKPGQPP KLLIYWASTRESGVPDRFTGSGSGTYFTLTISVQAEDLAVYYCQNNIY PLAFGTGKLELK	140
120B7B2	DIVMTQSPSSSLTVTAGEKVTMCKSSQSLNLSGNQKNYLTWYQQRPQPP KLLMYWASTRESGVPDRFTGSGSGTDFTLTISGQAEDLAIYFCQNGYYF PFTFGSGTKLETK	141
111B12D11	DIVMTQSPSSSLTVTAGEKVTMCRCSQSLFNSGNQKNYLTWYQQKPGQPP KLLIYWASTRESGVPDRFTGSGSGTDFTLTISVQAEDLAVYYCQNNIY PLAFGAGTKLELK	142
111E7E2	DIVMTQSPSSSLTVTAGEKVTMCKSSQSLFNSGNQKNYLTWYQQKPGQPP KLLIYWASTRESGVPDRFTGSGSGTDFTLTISVQAEDLAVYYCQNNIY PLAFGAGTKLELK	143
100F4G12	DIVMTQSPSSSLTVTAGEKVTMRCKSTQSLNLSGNQKNYLTWYQQKPGQSP KLLIYWASTRESGVPDRFTGSGSGTDFTLTISVQAEDLAVYYCQNAYYY PLTFGPGTKLELK	144
Heavy Chain		
64G11B4	QVQLHQSGTELVPRGTSVKVSKASGYAFTNYLLEWVKQRPQGGLWEWIGE INPGNGGSNYNEKFKGKATLTADKSSSTAYMQLSSLTSVDSAVYFCARIY YGNSFAYWGQGLVTVSA	145
65G8B8	QVQLKESGPGVLVAPSQSLITCTVSGFSLTSYGVSWVRQPPGKGLEWLG IWGDGNTIYHSALKSRLSISRDNKRQVFLKVNLSQIDDTATYYCAKQGL YGHAMDYWGQTSVIVSS	146
56E8F10F4	DVQLVESGGGLVQPGGSRKLSCTASGFTFNSFGMNWVRQAPKGLEWVAF ISGGSNTIHYLDTVKGRFTISRDNPKNTLFLQMTSLRSEDATAMYYCTRLA LGNAMDYWGQTSVIVSS	147
54A2C4	EVQHVETGGGLVQPKGSLKLSAASGFTFNTNAMNWVRQAPKGLEWVAR IRSKSNYATYYADSVKDRFTISRDDSQSMYLVQMNNLKTEDTAMYYCVS GAYYGNSKAFDYWGQGLVTVSA	148
54A2C4'	QVQLHQSGTELVPRGTSVKVSKASGYAFTNYLLEWVKQRPQGGLWEWIGE INPGNGGSNYNEKFKGKATLTADKSSSTAYMQLSSLTSVDSAVYFCARIY YGNSFAYWGQGLVTVSA	145
54A2C4''	QVQLQQSGAELARPGASVKMSCKASGYFTFTYSIHWLKQGGPGGLEWIGY INPSTIYTNYNQKFKYKATLTADKSSSTAYIQLSSLTSDDS AVYYCAREG YGRGNAMDYWGQTSVTVSS	149
44F6B11	EVQLVESGGDLVQPGGSLKLSAASGFTFNSYGMNWVRQTPDKRLEWVAT FSYGD SHNYYSDSVKGRFTISRDIADALYLQMSLSRSEDATAIYYCARFG RGNTMDYWGQTSVTVSL	150
15C2B7	QIQLVQSGPELRKPGETVKISCKASGYFTFTNYGMNWVKQAPGKGLKWMW INANTGEPTYAEEFKGRFAFSLETSARSAYLQINSKLNEDTATYFCARLT RGNSFDYWGQTTTLTVSS	151
20F1E10	QIQLVQSGPELKKPGETVKISCKASGYFTFTKYGMNWVRQAPGKGLKWMGW	152

	ISTNTGEPTYAEEFKGRFAFSLETSASTAFLQINNKNEDTATYFCARLV RGNSFDFWQGQGITLTVSS	
72C1B6A3	QVQLQQSGGELVKPGASVKMSCKAFGYTFTTYPIEWMKQNHGKSLEWIGN FHPYNDDTKYNEKFKGKAKLTVEKSSSTVYLEVSRLTSDDSAVYYCARRA YGYPYAMDYWGQGTSTVTVSS	153
58G2C2	QVHLQQSGAEVVRPGTSVKVSCASGYAFTNYLIEWIKKRPQGQGLEWVGV INPGRSGTNYNEKFKGKATLTADKSSSTAYMQLSSLTSDDSAVYFCARTR YGGNAMDYWGQGTSTVTVSS	154
101C4F12	QVQLKESGPGQVAPSQSLSIACTVSGFSLSSYGVHWVRQPPGKGLEWLG IWAGGSTNYDSALMSRLTISKDNSRTRVFLKMNSLQTDDETAIYYCARSLY GNSLDSWGPQTTLTVSS	155
103B2	QVQLKESGPGVLVAPSQSLSITCTVSGSLTSTFGVHWIRQPPGKGLEWLG IWAGGSTNYSALMSRLSISKDNSKSKVYFLKMNSLQTDDETAIYYCARSLY GNSFDYWGQGTALTTVSS	156
40C10E3	QVQLKESGPGVLVAPSQSLSITCTVSGFSLSSYGVNWRQPPGKGLEWLAA IRSDGIITYNSVLKSRRLRISKDNSKSKVFLKMNSLQTDDETAIYYCARWFR GNVLDYWGQGTSTVTVSS	157
78E8G9G6	QVQLKESGPGVLVAPSQSLSITCTVSGFSLISYGVHWVRQPPGKGLEWLG IWAGGSTNYSALMSRLSISKDNSKSKVFLKMNSLQTYDTAMYYCARDY GGNSLDYWGQGTSTVTVSS	158
4F11E2	DVQLVESGGGLVQPGGSRKLSAASGFTFTFGMHWRQAPKQGLEWVAY ITSGNSPIYFTDTVKGRFTISRDNPKNTLFLQMTSLGSEDTAVYYCARSS YYGNSMDYWGQGTSTVTVSS	159
10G7G11	QVQLKESGPGVLVAPSQSLSITCTISGFSLNTYGVHWVRQPPGKGLEWL MLSDGNTVYNSSLKSRRLSLTKDNSKSKQLLLKMNSLQTDDETAIYYCARHKA YGNAMDYWGQGTSTVTVSS	160
12F1F4	QVQLKESGPGVLVAPSQSLSITCTVSGFSLINYGVSWRQPPGKGLEWLG IWGDGNTNYQSALRSRLSIRKDTSKSKVFLKLNSVHTDGTATYYCAKVGR GNAMDHWGQGISVIVSS	161
78C10B6G4	QVQLKESGPGVLVAPSQSLSITCTVSGFSLINYGVSWRQPPGKGLEWLG IRGDGNTNYQSALRSRLSIRKDTSKSKVFLKLNSVHTDGTATYYCAKVGR GNAMDHWGQGISVIVSS	162
119G11D9	EVQLQQSGPELVKPGASVKMSCKASGYTFTGFLMHVVKQKPGQGLEWIGY INPYNDGTYSEKFKGKATLTSDKSSSTAFMELSSLTSDDSAVYYCARLD YGNAMDYWGQGTSTVTVSS	163
113G12E5E6	QVQLKQSGPGLVQPSQSLSITCTVSDFSLTKEYGVHWFRQSPGKGLEWLG IWTGGNTDYNPALIPRLSFRKDNSKSKVFFKMNSLQSSDTAVYYCARNGY YGNAMDYWGQGTSTVTVSS	164
116A8B7	EVQLQQSGPELVKPGASVKMSCKSSGYTFTGFLMHVVKQKPGQGLEWIGY INPYNDGTYSEKFKGKATLTSDKSSSTAYMELSSLTSDDSAVYYCGRLD YGNAMDYWGQGTSTVTVSS	165
105F7G12	QVQLKQSGPGLVQPSQSLSITCTVSDFSLTKEYGVHWFRQSPGKGLEWLG IWTGGNTDYNPALIPRLSFRKDNSKSKVFFKMNSLQSSDTAVYYCARNGY YGNAMDYWGQGTSTVTVSS	164
84E9E12	DVQLQESGPGVLKPSQSLSLSCSVTGYSTSGYFWTWFRQPPGNKLEWMMG YISYDGSNNYNPSLKNRISITRDTSKNQFFLKLNSVTEDTATYYCASFR FFAYWGQGTTLTVSA	166
103F4D4	QVQLQQSGAELARPGASVKMSCKASGYTFTYTIHWLQKPGQGLEWIGY INPSTIYTNYNQKFKYKATLTADKSSSTAYIQLSLSTSDDSAVYYCAREG YGRGNAMDYWGQGTSTVTVSS	149
110C12B6	EVQLQQSGPELVKPGASVKMSCKSSGYTFTGFLMHVVKQKPGQGLEWIGY INPYNDGTYSEKFKGKATLTSDKSSSTAYMELSSLTSDDSAVYYCGRLD YGNAMDYWGQGTSTVTVSS	165

85H12E8	QVQLKESGPGLVAPSQSLITCTVSGFSLSNYGVSWVRQPPGKGLEWLGVIWAGGNTNYSALMSRLRISKDNSKQVFLKMNSLQTDITARYYCARHGYGKGNAMDNWGQGTSTTVSS	167
103H2B4	QVQLQQPGAEPVKPGASVKLSCKASGYSTNFTLHWVRQRPQGQGLEWIGGINPTNGRTYYNEKFKRKATLTVDKSSSTVYMQLSNLTPEDSAVFYCARIYYGNSMDYWGQGTSLTVSA	168
103F6D3	QVQLQQSGAELARPGASVKMSCKASGYTFPTYSIHVLKQGPQGQGLEWIGYINPTIYTNYNQKFKYKTLTADKSSSTAYIQLSSLTSDDSAVYYCAREGYGRGNAMDYWGQGTSTTVSS	169
113E12F7	QVQLKESGPGLVAPSQSLITCTVTGFSLSYGVHVRQPPGKGLEWLGVIWAGGSTNYDSALMSRLSISKDRSKQVFLKMTSLQTDITAMYYCARSLYGNSFDHWGQGTSLTVSS	170
120B7B2	EVQLQQSGPELVKPGASVKMSCKASGYTFTGYIIQWMKQKPGQGLEWIGFINPYNDGTYNEQFKGKATLTSDKSSNAAYMELSSLTSEDSAVYYCARAYFGNSFAYWGQGTSLTVSA	171
111B12D11	QVQLKESGPGLVAPSQSLITCTVSGFSLTSYGVHVRQPPGKGLEWLGVIWAGGSTNYDSTLMSRLSISKDRSKQVFLKMTSLQTDITAMYYCARSLYGNSFDHWGQGTSLTVSS	172
111E7E2	QVQLKESGPGLVAPSQSLITCTVSGFSLTSYGAHVRQPPGKGLEWLGVIWAGGSTNYDSALMSRLSISKDRSKQVFLKMTSLQTDITAMYYCARSLYGNSFDHWGQGTSLTVSS	173
100F4G12	EVQLQQSGPELVKPGASVKMSCKASGYTFTGFLMHVVKQKPGQGLEWIGYINPYNDGTYSERFKGKATLTSDKSSSTAYMELSSLTSDDSAVYYCARLDYGNAMDYWGQGTSTTVSS	174

Example 3. Production and purification of monoclonal antibodies reactive to CLD18A2

[0167] To produce mg amounts of antibody for functional characterization, hybridoma cells were seeded in dialysis based bioreactors (CELLLine CL1000, Integra, Chur, CH) at 2×10^6 cells / ml. Antibody-containing supernatant was harvested once weekly. Each mouse monoclonal antibody was purified using Melon Gel (Pierce, Rockford, USA) and concentrated by ammonium sulphate precipitation. Antibody concentration and purity was estimated by sodium dodecylsulphate gel electrophoresis and coomassie staining.

Example 4. Binding of murine monoclonal antibodies reactive to CLD18A2

[0168] MKN45 cells that over-expressed CLD18A2 were harvested from flasks. 100 μ l of 1×10^6 cells/ml of cells were incubated with primary antibodies indicated as **FIG. 3** in 3-fold serial dilutions starting from 100 nM to 0.003 nM for 30 minutes on ice. After being washed with 200 μ l of FACS buffer twice, cells were incubated with secondary antibody for 30 minutes on ice. Cells were washed with 200 μ l of FACS buffer twice and transferred to BD Falcon 5 ml tube and analyzed by FACS. The results of the study showed that the purified murine antibody could bind to MKN45 cells transfected with human CLD18A2 by flow cytometry with high EC₅₀, compared with positive reference antibody.

Example 5. Binding of murine monoclonal antibodies reactive to CLD18A2 mutant

[0169] SU620 cells that endogenously expressed CLD18A2 bearing the M149L mutation were harvested from flasks. 100 μ l of 1×10^6 cells/ml of cells were incubated with primary antibodies indicated as **FIG. 4** in 3-fold serial dilutions starting from 100 nM to 0.003 nM for 30 minutes on ice. After being washed with 200 μ l of FACS buffer twice, cells were incubated with secondary antibody for 30 minutes on ice. Cells were washed with 200 μ l of FACS buffer twice and transferred to BD Falcon 5 ml tube and analyzed by FACS. The results of the study showed that the purified murine antibody could bind to SU620 cells endogenously expressing human CLD18A2 bearing M149L mutation by flow cytometry with high EC50, while the reference antibody did not (**FIG. 4**).

Example 6. Binding of murine monoclonal antibodies reactive to mouse and cyno CLD18A2

[0170] To evaluate these antibodies' cross-reactivities with mouse and cyno CLD18A2, HEK293 cells that over-expressed mouse, cyno or human CLD18A2 were harvested from flasks. 100 μ l of 1×10^6 cells/ml of cells were incubated with primary antibodies indicated as **FIG. 3** in 3-fold serial dilutions starting from 100 nM to 0.003 nM for 30 minutes on ice. After being washed with 200 μ l of FACS buffer twice, cells were incubated with secondary antibody for 30 minutes on ice. Cells were washed with 200 μ l of FACS buffer twice and transferred to BD Falcon 5 ml tube and analyzed by FACS. The results of the study showed that the purified murine antibodies can bind to mouse and cyno CLD18A2 by flow cytometry with high EC50, at least similar to the reference antibodies (**FIG. 5 , 6 and 7**).

Example 7. Binding of chimeric antibodies reactive to CLD18A2

[0171] The murine VH and VK genes were produced synthetically and then respectively cloned into vectors containing the human gamma 1 and human kappa constant domains. The purified chimeric antibodies were produced from transfected CHO cells.

[0172] MKN45 cells that stably expressed human CLD18A2 or CLD18A1 were harvested from flasks. 100 μ l of 1×10^6 cells/ml of cells were incubated with primary chimeric antibodies indicated as **FIG. 4** in 3-fold serial dilutions starting from 100 nM to 0.003 nM for 30 minutes on ice. After being washed with 200 μ l of FACS buffer twice, cells were

incubated with secondary antibody for 30 minutes on ice. Cells were washed with 200 μ l of FACS buffer twice and transferred to BD Falcon 5 ml tube and analyzed by FACS. The results of the study showed that the chimeric antibodies can bind to human CLD18A2 with high EC50, but not CLD18A1 (**FIG. 8 and 9**).

Example 8. Antibody-Dependent Cellular Cytotoxicity (ADCC) of Chimeric antibodies

[0173] The ADCC Reporter Bioassay uses an alternative readout at an earlier point in ADCC MOA pathway activation: the activation of gene transcription through the NFAT (nuclear factor of activated T-cells) pathway in the effector cell. In addition, the ADCC Reporter Bioassay uses engineered Jurkat cells stably expressing the Fc γ RIIIa receptor, V158 (high affinity) variant, and an NFAT response element driving expression of firefly luciferase as effector cells. Antibody biological activity in ADCC MOA is quantified through the luciferase produced as a result of NFAT pathway activation; luciferase activity in the effector cell was quantified with luminescence readout (**FIG. 1**). Signal was high, and assay background was low.

[0174] Serial dilutions of claudin 18.2 chimeric monoclonal antibody or Ref. Ab were incubated for 6 hours of induction at 37°C with engineered Jurkat effector cells (ADCC Bioassay Effector Cells), with or without ADCC Bioassay Target Cells (expressing claudin 18.2). Luciferase activity was quantified using Bio-Glo™ Reagent (**Table 2**). The results show that these chimeric antibodies have very strong ADCC activities.

Table 2. EC50 of the tested antibodies

Antibody	EC50 (pM)
4F11E2	22.18
12F1F4	36.77
64G11B4	125.7
72C186A3	46.32
78E8G9G6	15.86
103F6D3	79.53
120B7B2	5.806
Ref. Ab	458.5

Example 9. Humanization of the 4F11E2, 72C1B6A3 and 120B7B2 mouse mAbs

[0175] The mAb 4F11E2, 72C1B6A3 and 120B7B2 variable region genes were employed to create humanized MAbs. In the first step of this process, the amino acid sequences of the VH and VL of MAb were compared against the available database of human Ig gene sequences to find the overall best-matching human germline Ig gene sequences.

[0176] The amino acid sequences of the humanized antibodies are listed in **Table 3** below.

Table 3. Humanized sequences

Name	Sequence	SEQ ID NO:
4F11VH_1 (grafted VH)	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTFGMHVVRQAPGKGLEWVS YITSGNSPIYFTDTVKGRFTISRDNANKSLYLQMNSLRAEDTAVYYCARSS YYGNSMDYWGQGTLLTVSS	175
4F11VH_2	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTFGMHVVRQAPGKGLEWVAY ITSGNSPIYFTDTVKGRFTISRDNANKSLYLQMNSLRAEDTAVYYCARSS YYGNSMDYWGQGTLLTVSS	176
4F11VH_3	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTFGMHVVRQAPGKGLEWVAY ITSGNSPIYFTDTVKGRFTISRDNANKSLYLQMNSLRAEDTAVYYCARSS YYGNSMDYWGQGTLLTVSS	177
4F11VL_1 (grafted VL)	DIVMTQSPDSLAVSLGERATINCRSSQSLNLSGNRKNYLTWYQQKPGQPP KLLIYWASTRESGVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQNAYSY PFTFGGGTKLEIK	178
4F11VL_2	DTVMTQSPDSLAVSLGERATINCRSSQSLNLSGNRKNYLTWYQQKPGQPP KLLIYWASTRESGVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQNAYSY PFTFGGGTKLEIK	179
4F11VL_3	DTVMTQSPDSLAVSLGERVTINCRSSQSLNLSGNRKNYLTWYQQKPGQPP KLLIYWASTRESGVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQNAYSY PFTFGGGTKLEIK	180
72C1VH_1 (grafted VH)	QVQLVQSGAEVVKPGASVKVSKASGYTFTTYPIEWMRQAPGQRLEWMGN FHPYNDDTKYNEKFKGRVTITRDTSASTAYMELSSLRSEDVAVYYCARRA YGYPYAMDYWGQGTLLTVSS	181
72C1VH_2	QVQLVQSGAEVVKPGASVKVSKASGYTFTTYPIEWMRQAPGQRLEWMGN FHPYNDDTKYNEKFKGRVTITVDTASTAYMELSSLRSEDVAVYYCARRA YGYPYAMDYWGQGTLLTVSS	182
72C1VH_3	QVQLVQSGAEVVKPGASVKVSKASGYTFTTYPIEWMKQAPGQRLEWMGN FHPYNDDTKYNEKFKGRVTITVDTASTAYMEVSSLRSEDVAVYYCARRA YGYPYAMDYWGQGTLLTVSS	183
72C1VH_4	QVQLVQSGAEVVKPGASVKMSKASGYTFTTYPIEWMKQAPGQRLEWMGN FHPYNDDTKYNEKFKGRVTITVDTASTVYLEVSSLRSEDVAVYYCARRA YGYPYAMDYWGQGTLLTVSS	184
72C1VL_1 (grafted VL)	DIVMTQSPDSLAVSLGERATINCKSSQSLNLSGNQKNYLTWYQQKPGQPP KLLIYRASSRESGVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQNDYIY PYTFGGGKLEIK	185
72C1VL_2	DIVMTQSPDSLAVSLGERATISCKSSQSLNLSGNQKNYLTWYQQKPGQPP KLLIYRASSRESGVPDRFSGSGSGTDFTLTISLQAEDVAVYYCQNDYIY PYTFGGGKLEIK	186

72C1VL_3	DIVMTQSPDSLAVSLGERVTMSCKSSQSLNSGNQKNYLTWYQQKPGQPP KLLIYRASSRESGVPDRFSGSGSGTDFTLTISSVQAEDVAVYYCQNDYIY PYTFGGGTGLEIK	187
120B7VH_1 (grafted VH)	QVQLVQSGAEVVKPGASVKVSCASGYTFTGYIIQWVRQAPGQRLEWMGF INPYNDGTYNEQFKGRVTITRDTASTAYMELSSLRSEDTAVYYCARAY FGNSFAYWGQGTLLTVSS	188
120B7VH_2	QVQLVQSGAEVVKPGASVKVSCASGYTFTGYIIQWVRQAPGQRLEWMGF INPYNDGTYNEQFKGRVTITSDTSASAAAYMELSSLRSEDTAVYYCARAY FGNSFAYWGQGTLLTVSS	189
120B7VH_3	QVQLVQSGAEVVKPGASVKVSCASGYTFTGYIIQWMKQAPGQRLEWIGF INPYNDGTYNEQFKGRATITSDTSASAAAYMELSSLRSEDTAVYYCARAY FGNSFAYWGQGTLLTVSS	190
120B7VH_4	EVQLVQSGAEVVKPGASVKMSCKASGYTFTGYIIQWMKQAPGQRLEWIGF INPYNDGTYNEQFKGRATITSDTSASAAAYMELSSLRSEDTAVYYCARAY FGNSFAYWGQGTLLTVSS	191
120B7VL_1 (grafted VL)	DIVMTQSPDSLAVSLGERATINCKSSQSLNSGNQKNYLTWYQQKPGQPP KLLIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNGYYF PFTFGGGTKLEIK	192
120B7VL_2	DIVMTQSPDSLAVSLGERATISCKSSQSLNSGNQKNYLTWYQQKPGQPP KLLMYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNGYYF PFTFGGGTKLEIK	193
120B7VL_3	DIVMTQSPDSLAVSLGERATISCKSSQSLNSGNQKNYLTWYQQKPGQPP KLLMYWASTRESGVPDRFSGSGSGTDFTLTISSGQAEDVAVYFCQNGYYF PFTFGGGTKLEIK	194
120B7VL_4	DIVMTQSPDSLAVSLGERVTMSCKSSQSLNSGNQKNYLTWYQQKPGQPP KLLMYWASTRESGVPDRFSGSGSGTDFTLTISSGQAEDVAVYFCQNGYYF PFTFGGGTKLEIK	195

[0177] The humanized VH and VL genes were produced synthetically and then respectively cloned into vectors containing the human gamma 1 and human kappa constant domains. The pairing of the human VH and the human VL created the humanized antibodies (see **Table 4**).

Table 4. Humanized antibodies with their VH and VL regions

4F11E2

VH	4F11VH_1	4F11VH_2	4F11VH_3
VL			
4F11VL_1	hu4F11.1	hu4F11.4	hu4F11.7
4F11VL_2	hu4F11.2	hu4F11.5	hu4F11.8
4F11VL_3	hu4F11.3	hu4F11.6	hu4F11.9

72C1B6A3

VH	72C1VH_1	72C1VH_2	72C1VH_3	72C1VH_4
VL				
72C1VL_1	hu72C1.10	hu72C1.13	hu72C1.16	hu72C1.19
72C1VL_2	hu72C1.11	hu72C1.14	hu72C1.17	hu72C1.20
72C1VL_3	hu72C1.12	hu72C1.15	hu72C1.18	hu72C1.21

120B7B2

VH	120B7VH_1	120B7VH_2	120B7VH_3	120B7VH_4
VL				
120B7VL_1	hu120B7.22	hu120B7.26	hu120B7.30	hu120B7.34
120B7VL_2	hu120B7.23	hu120B7.27	hu120B7.31	hu120B7.35
120B7VL_3	hu120B7.24	hu120B7.28	hu120B7.32	hu120B7.36
120B7VL_4	hu120B7.25	hu120B7.29	hu120B7.33	hu120B7.37

Example 10. Binding of humanized antibodies reactive to CLD18A2

[0178] MKN45 cells that stably expressed human CLD18A2 or CLD18A1 were harvested from flasks. 100 μ l of 1×10^6 cells/ml of cells were incubated with primary humanized antibodies indicated as FIG. 4 in 3-fold serial dilutions starting from 100 nM to 0.003 nM for 30 minutes on ice. After being washed with 200 μ l of FACS buffer twice, cells were incubated with secondary antibody for 30 minutes on ice. Cells were washed with 200 μ l of FACS buffer twice and transferred to BD Falcon 5 ml tube and analyzed by FACS. The results of the study showed that the indicated humanized antibodies can bind to human CLD18A2 with high EC₅₀, but not CLD18A1 (FIG. 10 and 11).

Example 11. Binding of PTM (post-translational modification) de-risk humanized antibodies reactive to CLD18A2

[0179] Post-translational modifications (PTMs) can cause problems during the development of a therapeutic protein such as increased heterogeneity, reduced bioactivity, reduced stability, immunogenicity, fragmentation and aggregation. The potential impact of PTMs depends on their location and in some cases on solvent exposure. The CDRs of sequence were analyzed for the following potential PTMs: asparagine deamidation, aspartate isomerization, free cysteine thiol groups, N-glycosylation, oxidation, fragmentation by potential hydrolysis site etc.

[0180] To reduce the risk of developing PTM in 4F11E2, 72C1B6A3 and 120B7B2, some concerned amino acids in the VH and VL were mutated. And then nine antibodies were generated:

Clones	HC:LC*	No.
4F11E2	HC N55Q-LC N31E	1
	HC N55Q-LC S32A	2
	HC N55E-LC S32A	3
	HC N55E&N104Q-LC S32A	8
	HC N55E&N104E-LC S32A	9

	HC N55E&S105A-LC S32A	10
72C1B6A3	HC WT-LC N31E	4
	HC WT-LC S32A	5
120B7B2	HC G57D&S104A-LC-N96E&N31E	6
	HC G57D&S104A-LC-S32A&G97A	7

*The amino acid location (e.g., N55) is according to the amino acid residue number in the corresponding VH or VL amino acid sequence, not Kabat or Chothia.

Antibody	Mutant	Sequence (mutation highlighted)	SEQ ID NO:
4F11E2	HC N55Q	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTFGMHWRQAPGKGLEWVS YITSG Q SPIYFTDTVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARSS YYGN Q SMDYWGQGTLLTVSS	196
	HC N55E	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTFGMHWRQAPGKGLEWVS YITSG E SPIYFTDTVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARSS YYGN E SMDYWGQGTLLTVSS	197
	HC N55E & N104Q	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTFGMHWRQAPGKGLEWVS YITSG E SPIYFTDTVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARSS YYG Q SMDYWGQGTLLTVSS	198
	HC N55E & N104E	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTFGMHWRQAPGKGLEWVS YITSG E SPIYFTDTVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARSS YYG E SMDYWGQGTLLTVSS	199
	HC N55E & S105A	EVQLVESGGGLVQPGGSLRLSCAASGFTFSTFGMHWRQAPGKGLEWVS YITSG E SPIYFTDTVKGRFTISRDNAKNSLYLQMNSLRAEDTAVYYCARSS YYGN A SMDYWGQGTLLTVSS	200
	LC N31E	DIVMTQSPDLSAVSLGERATINCRSSQSL E SGNRKNYLTWYQQKPGQPP KLLIYWASTRESGVPDRFSGSGSGTDFTLTITSS LQ AEDVAVYYCQNAYSY PFTFGGGTKLEIK	201
	LC S32A	DIVMTQSPDLSAVSLGERATINCRSSQSL N AGNRKNYLTWYQQKPGQPP KLLIYWASTRESGVPDRFSGSGSGTDFTLTITSS LQ AEDVAVYYCQNAYSY PFTFGGGTKLEIK	202
72C1B6A3	HC WT	QVQLVQSGAEVKKPGASVKVSKASGYTFTTYPIEWVRQAPGQRLEWMGN FHPYND D TKYNEKFGRVTITRDTASTAYMELSSLRSEDVAVYYCARRA YGYPYAMDYWGQGTLLTVSS	181
	LC S32A	DIVMTQSPDLSAVSLGERATINCKSSQSL N AGNQKNYLTWYQQKPGQPP KLLIYRASSRESGVPDRFSGSGSGTDFTLTITSS LQ AEDVAVYYCQNDYIY PYTFGGGTKLEIK	203
	LC N31E	DIVMTQSPDLSAVSLGERATINCKSSQSL E SGNQKNYLTWYQQKPGQPP KLLIYRASSRESGVPDRFSGSGSGTDFTLTITSS LQ AEDVAVYYCQNDYIY PYTFGGGTKLEIK	204
120B7B2	HC G57D & S104A	QVQLVQSGAEVKKPGASVKVSKASGYTFTGYIIQWVRQAPGQRLEWMGF INPYND D TKYNEQFKGRVTITRDTASTAYMELSSLRSEDVAVYYCARAY FGN A FAYWGQGTLLTVSS	205
	LC-S32A & G97A	DIVMTQSPDLSAVSLGERATINCKSSQSL N AGNQKNYLTWYQQKPGQPP KLLIYWASTRESGVPDRFSGSGSGTDFTLTITSS LQ AEDVAVYYCQ A YYF PFTFGGGTKLEIK	206
	LC-N96E & N31E	DIVMTQSPDLSAVSLGERATINCKSSQSL E SGNQKNYLTWYQQKPGQPP KLLIYWASTRESGVPDRFSGSGSGTDFTLTITSS LQ AEDVAVYYCQ E GYF PFTFGGGTKLEIK	207

[0181] MKN45 cells that stably expressed human CLD18A2 or CLD18A1 were harvested from flasks. 100 μ l of 1×10^6 cells/ml of cells were incubated with primary mutated humanized antibodies indicated as fig. 4 in 3-fold serial dilutions starting from 100 nM to 0.003 nM for 30 minutes on ice. After being washed with 200 μ l of FACS buffer twice, cells were incubated with secondary antibody for 30 minutes on ice. Cells were washed with 200

ul of FACS buffer twice and transferred to BD Falcon 5 ml tube and analyzed by FACS. The results of the study showed that the indicated antibodies can bind to human CLD18A2 with high EC₅₀, but not CLD18A1 (**FIG. 12 and 13**).

[0182] To evaluate the de-risked variants of 4F11E2d (HC N55E/LC S32A) and 4F11E2d (H N55E N104Q/LC S32A) antigen binding potency, the variants were tested in a cell-based binding assay. Serially diluted anti-CLDN18.2 antibodies, starting from 100nM, were incubated with 10⁵ cells for 30 minutes on ice. After being washed with FACS buffer, cells were then incubated with APC labeled secondary antibody for another 30 minutes on ice. Cells bound with antibodies were analyzed by FACS. The variants showed potent binding to cell surface claudin 18.2 (**FIG. 14**).

Example 12. Antibody-Dependent Cellular Cytotoxicity (ADCC) of PTM de-risked humanized antibodies

[0183] Serial dilutions of claudin 18.2 PTM de-risked humanized antibodies or Ref. Ab were incubated for 6 hours of induction at 37°C with engineered Jurkat effector cells (ADCC Bioassay Effector Cells), with or without ADCC Bioassay Target Cells (expressing claudin 18.2). Luciferase activity was quantified using Bio-Glo™ Reagent (Table 5). The results show that these humanized antibodies have very strong ADCC activities.

Table 5. ADCC

No.	Tested antibody	EC ₅₀ (pM)
1	4F11E2 -HC N55Q-LC N31E	238.1
2	4F11E2 -HC N55Q-LC S32A	413.9
3	4F11E2 -HC N55E-LCS32A	148.1
4	72C1B6A3-HC WT-LC N31E	1651
5	72C1B6A3-HC WT- LCS32A	190.5
6	120B7B2-HC G57D&104A- LC-N96E&N31E	492.6
7	120B7B2-HC G57D&10 4A- LC-S32A&G97A	113.9
Ref. Ab	Ref. Ab	158.3

Example 13. Epitope mapping

[0184] All amino acids of extracellular domain of claudin 18.2 were individually mutated to A. Each mutated or wildtype claudin 18.2 was transfected into Hek293 cells. The expression

of claudin 18.2 was evaluated by indicated antibodies. The results are shown in FIG. 15 (only amino acid residues at which the mutation reduced bindings are shown).

[0185] As shown in **FIG. 15**, amino acids W30, N45, Y46, G48, L49, W50, C53, V54, R55, E56, S58, F60, E62, C63, R80, Y169, and G172 are involved in the binding of the three tested antibodies, 4F11E2 (H4F), 72C186A3 (H72C1) and 120B7B2 (120), or the reference antibody 175D10 (IMAB362). W30 appeared to form a cluster of residues at the first half of the first extracellular domain of the claudin 18.2 protein. N45, Y46, G48, L49, W50, C53, V54, R55, E56, S58, F60, E62 and C63 appeared to be a second cluster of residues within the same extracellular domain. Y169 and G172, on the other hand, are located at or close to the second extracellular domain.

[0186] The crystal structures of various claudin proteins have been resolved. As shown in **FIG. 20** (adapted from Suzuki *et al.*, *Ann. N.Y. Acad. Sci.*, 1397: 25-34), claudin proteins include four transmembrane segments, a short intracellular N-terminus, a large first extracellular loop (loop 1, or ECS1) that contains a consensus W-LW-C-C motif, a shorter second extracellular loop (loop 2, or ECS2), and an intracellular C-terminal tail. Loop 1 includes four β strands β 1, β 2, β 3, and β 4, and loops includes one β strand, β 5.

[0187] The mutations of W30, L49 and W50 to alanine likely destabilized the conformation of loop 1. The mutation of C53 or C63 likely disrupted the disulfide bond between β 3 and β 4. R80 is likely important for maintaining the interaction between parallel claudin 18.2 molecules on the cell surface, or for stabilizing the conformation of loop 1. The remaining residues, including N45, Y46, G48, V54, R55, E56, S58, F60, and E62 (in the β 3 to β 4 loop), and Y169 and G172 (in β 5), likely present the interface for binding to the antibodies tested here.

Example 14. Comparison of humanized 4F11E2, 72C1B6A3 and 120B7B2 antibodies with benchmark 175D10 claudin 18.2 antibody

Cell based binding

[0188] To compare the humanized anti-claudin18.2 antibodies: 4F11E2 (HC N55E/LC S32A), 72C1B6A3(HC WT/ LC S32A) and 120B7B2 (HC G57D S104A/LC S32A G97A) to benchmark antibody 175D10 (IMAB362), this example determined the cell-based binding in human claudin 18.2 expressed cells. CHO-K1 cells that stably expressed human CLD18A2

were sorted for high expressor and low expressor based on the level of human CLDN18.2 expression. Serially diluted anti-CLDN18.2 antibodies, starting from 100nM, were incubated with 10^5 cells for 30 minutes on ice. After being washed with FACS buffer, cells were then incubated with APC labeled secondary antibody for another 30 minutes on ice. Cells bound with antibodies were analyzed by FACS.

[0189] As shown in the **FIG 16**, 4F11E2, 72C1B6A3 and 120B7B2 showed superior binding to 175D10 in both claudin 18.2 high and low CHO-K1 cells.

ADCC assay

[0190] To further compare the ADCC effect of humanized anti-claudin18.2 antibodies: 4F11E2 (HC N55E/LC S32A), 72C1B6A3(HC WT/ LC S32A) and 120B7B2 (HC G57D S104A/LC S32A G97A) to benchmark antibody 175D10 (IMAB362), this example performed a cell based ADCC assay. Briefly, NK92 cells were cocultured with claudin18.2 overexpressed 293 cells in the presence of different dose anti-claudin 18.2 antibodies. As shown in **FIG. 17**, 4F11E2, 72C1B6A3 and 120B7B2 showed superior ADCC potency to the 175D10 antibody.

[0191] For certain therapeutic antibodies, enhanced ADCC may increase the therapeutic window to antibody-based target therapy. Enhanced ADCC may be achieved through engineering the Fc region such as with the S239D/I332E mutations. In a NK92 cell based ADCC assay, the 4H11E2, 72C1B6A3 and 120B7B2 antibodies with S239D/I332E mutations in the Fc region mediated stronger NK92-mediated cell killing of claudin 18.2 overexpressed 293 cells as compared to the control antibody 175D10 with the same S239D/I332E mutations (**FIG. 18**).

Antibody-dependent cellular phagocytosis (ADCP)

[0192] The effect of anti-CLDN 18.2 mAbs on the tumor cell phagocytosis by macrophages was evaluated in an *in vitro* assay in which CLDN18.2 positive NUG-C4 cells were co-cultured with human differentiated macrophages in the presence of different concentration of anti-CLDN18.2 mAbs. In short, CD14⁺ monocytes were purified from human peripheral blood mononuclear cells (PBMCs) and *in vitro* differentiated into mature macrophages for 6 days. The monocyte derived macrophages (MDMs) were collected and re-plated in 24-well dishes overnight as effector cells. NUG-C4 expressing CLDN 18.2-eGFP as target cells were

added to MDMs at a ratio of 5 tumor cells per phagocyte in the presence of different concentrations of anti-CLDN18.2 mAbs. After 3 hours' incubation, non-phagocytosed target cells were washed away with PBS and the remaining phagocytes were collected and stained with macrophage marker CD14 followed by flow cytometry analysis. Phagocytosis index was calculated by quantitating the percent of GFP⁺ cells in CD14⁺ cells, normalized to that of IgG control.

[0193] As shown in **FIG. 19**, all C18.2 mAbs significantly enhanced the phagocytosis of NUG-C4 cells in a concentration-dependent manner. In both wildtype IgG1 and S239D/I332E mutated IgG1 formats, 4H11E2, 72C1B6A3 and 120B7B2 antibodies showed stronger ADCP effect than the reference antibody 175D10.

[0194] Taken together, this example demonstrates that the newly developed 4F11E2, 72C1B6A3 and 120B7B2 antibodies had stronger cell-based binding and ADCC/ADCP potency than the reference antibody 175D10. It is contemplated that the improved properties of these new antibodies can be attributed to the higher binding specificity of these antibodies as compared to that of the reference antibody 175D10. For instance, 175D10's interaction with claudin 18.2 is strong across the spectrum in **FIG. 15**, which includes strong binding to D28, Q33, N38 and V43, and then G59 and V79. The new antibodies, 4F11E2, 72C1B6A3 and 120B7B2, by contrast, have higher specificity to W30 within the first half of the first extracellular domain, and higher specificity to G48 through E56 within the second half of the first extracellular domain. The new antibodies also have slightly stronger binding to Y46 which is also in the second half. Their binding to D28, Q33, N38, V43, G59 and V79 is considerably weaker, which likely contributed to the improved ADCC and ADCP of the new antibodies.

Example 15: pHAb conjugation to claudin 18.2 antibodies

[0195] Internalization of CLDN18.2 bound anti-Claudin 18.2 antibodies was determined using pHAbReactive Dyes based internalization assay. pHAb Dyes are pH sensor dyes that have very low fluorescence at pH > 7 and a dramatic increase in fluorescence as the pH of the solution becomes acidic. pHAb Dyes have excitation maxima (Ex) at 532nm and emission maxima (Em) at 560nm. pHAb Dye-conjugated antibodies can be used for monitoring receptor-mediated antibody internalization. When an antibody-pHAb Dye conjugate binds to its receptor on the cell membrane, it exhibits minimal fluorescence. However, upon receptor-

mediated internalization, antibody-pHAb Dye conjugates traffic to the endosome and lysosomal vesicles where pH is acidic, causing the pHAb Dye to fluoresce. This fluorescence can be detected using various techniques, including cell imaging, flow cytometry and fluorescent plate-based readers with appropriate filters.

[0196] Experimental protocol:

[0197] A. Antibodies Production

[0198] 27 chimeric antibodies, 3 humanized antibodies and a control IgG1 were produced by transiently transfecting ExpiCHO cells and purified by Protein A affinity chromatography.

[0199] B. On-Bead Antibody Conjugation Using pHAb Thiol Reactive Dye

[0200] 1. Gently shake or use an end-over-end mixer to uniformly resuspend the AmMag® Protein A Beads (LC00695). Keep the suspension uniform when making aliquots of beads.

[0201] 2. Add 50µl of bead slurry to a 1.5ml microcentrifuge tube. Place the tube on the magnetic stand for 10 seconds.

[0202] 3. Remove and discard the storage buffer.

[0203] 4. Add 250µl of PBS (pH7.4). Mix and place tube on the magnetic stand for 10 seconds. Remove and discard the buffer.

[0204] 5. Add 1.0ml of sample containing 100µg of antibody to the beads.

[0205] 6. Mix the sample for 60 minutes at room temperature. Keep the beads in suspension by mixing continuously.

[0206] 7. Place the tube in the magnetic stand for 10 seconds. Remove the supernatant.

[0207] 8. Add 250µl of thiol conjugation buffer (10mM phosphate buffer containing 1mM EDTA, pH 7.0) and mix. Place the tube in the magnetic stand for 10 seconds. Remove and discard the buffer. Repeat this step for a total of two washes.

[0208] 9. Add 100µl of thiol conjugation buffer.

[0209] 10. Add DTT to a final concentration of 2.5mM.

- [0210] 11. Mix sample for 60 minutes at room temperature. Keep the beads in suspension by mixing continuously.
- [0211] 12. Place the tube in the magnetic stand for 10 seconds and discard the buffer.
- [0212] 13. Add 250µl of thiol conjugation buffer and mix. Place the tube in the magnetic stand for 10 seconds. Remove and discard the buffer. Repeat this step for a total of two washes.
- [0213] 14. Add 100µl of thiol conjugation buffer.
- [0214] 15. Quickly centrifuge the pHAb Thiol Reactive Dye(G9835) (i.e., $14,000 \times g$ in a tabletop centrifuge for 5–10 seconds) and dissolve at 10mg/ml by adding 25µl of 1:1 DMSO-water mix to 0.25mg of dye. Mix by vortexing. It may take 1–3 minutes for the dye to dissolve completely. Make this solution just before use.
- [0215] 16. Add 1.2µl of pHAb Thiol Reactive Dye for 100µg of antibody to make a 20 molar excess of dye.
- [0216] 17. Mix for 60 minutes. Keep the beads in suspension by mixing continuously.
- [0217] 18. Place the tube in the magnetic stand for 10 seconds. Remove and discard the supernatant. 19. Add 250µl of thiol conjugation buffer and mix. Place in the magnetic stand for 10 seconds. Remove and discard the bind/wash buffer (PBS.pH7.4).
- [0218] 20. Repeat Step 19 for a total of two washes.
- [0219] 21. Add 100µl of elution buffer (0.1M Glycine, pH3.0) to the beads.
- [0220] 22. Mix for 5 minutes at room temperature.
- [0221] 23. Place the tube in the magnetic stand for 10 seconds. Remove eluted sample and transfer to a new microcentrifuge tube containing 5µl of neutralization buffer (1M Tris-HCl, pH9.0).
- [0222] The antibody concentration and dye-to-antibody ratio (DAR) for the tested antibodies are shown in **Table 6**.

Table 6. Dye-to-antibody ratio (DAR)

Clone	DAR
64G11B4	2.5
65G8B8	4.2
56E8F10F4	3.2
44F6B11	3.0
15C2B7	3.0
20F1E10	2.7
58G2C2	3.1
101C4F12	2.4
103A10B2	3.5
78E8G9G6	2.3
10G7G11	3.1
12F1F4	2.4
78C10B6G4	2.9
119G11D9	3.2
113G12E5E6	2.9
116A8B7	2.6
105F7G12	2.8
84E9E12	2.8
103F4D4	2.1
110C12B6	2.8
85H12E8	3.8
103H2B4	2.7
103F6D3	3.1
113E12F7	3.9
111B12D11	2.7
111E7E2	3.5
100F4G12	3.0
4F11E2 HC N55E-LC S32A (BG2001-C)	3.0
72C1B6A3 HC WT-LC S32A(BG2001-D)	2.6
120B7B2 HC G57D&S104A-LC S32A&G97A (BG2001-E)	3.0
IMAB362 (Ref. Ab)	3.3
IgG (Control)	2.9

Example 16: Screen for claudin 18.2 antibodies' internalization

[0223] Stably transfected human CLDN18.2 MKN45 cells were harvested with 0.05% Trypsin/EDTA (Gibco, 25300-054) and plated in a 96-well black plate (Thermo Scientific #165305) at the density of 20 K per 90 μ l per well. Plates were incubated for 20–24 h before treatment with pHAb labeled antibodies.

[0224] For internalization, pHAb conjugated claudin 18.2 antibodies were added to the cells at two concentrations (20nM and 100nM) and mixed gently for 1–2 min on a plate mixer and

then incubated overnight to allow internalization (internalization can be detected in a few hours). Plates were read on a fluorescent plate reader at Ex/Em: 532 nm/560 nm on a Tecan Infinity M1000 Pro. To achieve higher sensitivity, media was replaced by PBS before reading the plate.

[0225] The results normalized by DAR are shown in **Table 7**. The internalization efficiencies of the tested antibodies were greater than the reference antibody IMAB362.

Table 7. Internalization Results

Clone	Fluorescence	
	20nM	100nM
64G11B4	24030	33742
65G8B8	15101	20466
56E8F10F4	19865	28672
44F6B11	18438	26089
15C2B7	19400	33174
20F1E10	21146	45093
58G2C2	18645	33059
101C4F12	15179	44394
103A10B2	11998	31328
78E8G9G6	26815	41148
10G7G11	9603	35549
12F1F4	29457	43854
78C10B6G4	16190	29146
119G11D9	21438	34049
113G12E5E6	11003	28896
116A8B7	21356	41720
105F7G12	14848	43122
84E9E12	18710	35080
103F4D4	31176	49098
110C12B6	19335	40139
85H12E8	19710	29070
103H2B4	23459	35834
103F6D3	23023	41266
113E12F7	13181	29861
111B12D11	15926	37384
111E7E2	12335	28040
100F4G12	17093	33537
4F11E2 HC N55E-LC S32A (BG2001-C)	19008	28208
72C1B6A3 HC WT-LC S32A (BG2001-D)	26203	36535
120B7B2 HC G57D&S104A-LC S32A&G97A (BG2001-E)	19204	31321
IMAB362 (Ref. Ab)	7951	18907
IgG (Control)	854	2705

Example 17: EC50 of internalization of chimeric claudin 18.2 antibodies on CHO-claudin 18.2 cells

[0226] Stably transfected human CLDN18.2 CHO cells were harvested with 0.05% Trypsin/EDTA (Gibco, 25300-054) and plated in a 96-well black plate (Thermo Scientific #165305) at the density of 10 K per 90 μ l per well. Plates were incubated for 20–24 h before treatment with pHAb labeled antibodies.

[0227] For internalization, pHAb conjugated chimeric claudin 18.2 antibodies were added to the cells at different concentrations (100nM, 30nM, 10nM, 3nM, 1nM, 0.3nM, 0.1nM, 0.03nM and 0.01nM) and mixed gently for 1–2 min on a plate mixer and then incubated overnight to allow internalization (internalization can be detected in a few hours). Plates were read on a fluorescent plate reader at Ex/Em: 532 nm/560 nm on a Tecan Infinity M1000 Pro. To achieve higher sensitivity, media was replaced by PBS before reading the plate.

[0228] The results normalized with DAR are shown in the **FIG. 21**. Again, the internalization efficiencies of the tested antibodies were greater than the reference antibody IMAB362.

Example 18: EC50 of internalization of humanized claudin 18.2 antibodies on CHO-claudin 18.2 cells

[0229] Stably transfected human CLDN18.2 CHO cells were harvested with 0.05% Trypsin/EDTA (Gibco, 25300-054) and plated in a 96-well black plate (Thermo Scientific #165305) at the density of 10 K per 90 μ l per well. Plates were incubated for 20–24 h before treatment with pHAb labeled antibodies.

[0230] For internalization, pHAb conjugated humanized claudin 18.2 antibodies were added to the cells at different concentrations (100nM, 30nM, 10nM, 3nM, 1nM, 0.3nM, 0.1nM, 0.03nM and 0.01nM) and mixed gently for 1–2 min on a plate mixer and then incubated overnight to allow internalization (internalization can be detected in a few hours). Plates were read on a fluorescent plate reader at Ex/Em: 532 nm/560 nm on a Tecan Infinity M1000 Pro. To achieve higher sensitivity, media was replaced by PBS before reading the plate.

[0231] The results normalized with DAR are shown in the **FIG. 22**, which shows that the internalization efficiencies of the tested antibodies were greater than the reference antibody IMAB362.

Example 19: EC50 of internalization of humanized claudin 18.2 antibodies on MKN45-caludin 18.2 cells

[0232] Stably transfected human CLDN18.2 MKN45 cells were harvested with 0.05% Trypsin/EDTA (Gibco, 25300-054) and plated in a 96-well black plate (Thermo Scientific #165305) at the density of 10 K per 90 μ l per well. Plates were incubated for 20–24 h before treatment with pHAb labeled antibodies.

[0233] For internalization, pHAb conjugated humanized claudin 18.2 antibodies were added to the cells at different concentrations (100nM, 30nM, 10nM, 3nM, 1nM, 0.3nM, 0.1nM, 0.03nM and 0.01nM) and mixed gently for 1–2 min on a plate mixer and then incubated overnight to allow internalization (internalization can be detected in a few hours). Plates were read on a fluorescent plate reader at Ex/Em: 532 nm/560 nm on a Tecan Infinity M1000 Pro. To achieve higher sensitivity, media was replaced by PBS before reading the plate.

[0234] The results normalized with DAR are shown in the **FIG. 23**, which shows that the internalization efficiencies of the tested antibodies were greater than the reference antibody IMAB362.

Example 20: Antibody Drug Conjugates

[0235] Each antibody was mixed with approximately three-fold TCEP and was stirred for 2 h at 37 °C. The reaction system was quickly dropped over eight-fold for VC - MMAE and was incubated for 1 h on ice, and 20-fold excess of cysteine was added over the drug linker to extinguish the reaction. Finally, the ADC product was purified by elution through Sephadex G-25 equilibrated in PBS and concentrated by centrifugal ultrafiltration. The conjugate was filtered through a 0.2 μ m filter under sterile conditions and stored at -80 °C for analysis and testing. Drug antibody ratio was analyzed by UV spectrometry, the monomer content by SEC-HPLC and free drug content by RP-HPLC. The (DAR) of vcMMAE conjugated antibodies are shown in Table 8.

Table 8. DAR of vcMMAE conjugated antibodies

Antibodies	DAR
4F11E2 HC N55E-LC S32A	3.76
72C1B6A3 HC WT-LC S32A	3.93

IMAB362 (Ref. Ab)	4.00
IgG1 (Control)	3.81

Example 21: Relative binding affinities and specificities of anti-CLDN18.2 naked antibodies and antibody drug conjugates

[0236] This example determined the relative binding affinities and specificities of anti-CLDN18.2 naked antibodies and antibody drug conjugates by flow cytometry using CLDN18.2 positive and negative cell lines.

[0237] Cells from an exponentially growing culture were harvested with 0.05% Trypsin/EDTA (Gibco, 25300-054), and counted using a Neubauer counting chamber. Cells were centrifuged 5 min at 1,500 rpm (468×g), the supernatant was discarded and cells were resuspended in FACS buffer (PBS containing 2% FCS (Gibco, 10270-106) for analysis with toxin-conjugated antibodies, PBS containing 2% FCS and 2 mM EDTA for screening of CLDN18.2 reactive naked antibodies) at 2×10^6 cells/ml. 100 µl of the cell suspension per well (correspond to 2×10^5 cells/well) were transferred to a round bottom 96-well microtiter plate. After centrifugation for 1 min at 1500 rpm, supernatant was discarded and cells were resuspended in FACS buffer containing toxin-conjugated or naked antibodies at appropriate concentrations (up to 20 µg/ml for relative affinity measurement or 50 µg/ml for expression control) and incubated for 30-45 min at 4° C. (Table 8). The cells were centrifuged for 1 min at 1500 rpm and the supernatant was discarded. After the cells were washed three times with FACS buffer, they were resuspended in FACS buffer containing APC-conjugated anti-human IgG (Jackson Immuno Research, 109-136-170) or APC-conjugated goat-anti-mouse IgG (Jackson Immuno Research, 115-136-146) or Protein L-FITC (1 µg/ml, analysis of chim mAB294) and incubated for 30 min at 4° C. (Table 3). After incubation, 100 µl FACS buffer were added to each sample, the cells were centrifuged for 1 min at 1500 rpm and the supernatant was discarded. The washing step with FACS buffer was repeated twice. Finally, the cells were resuspended in 100 µl FACS buffer and binding was determined using a BD FACS Array Bioanalyzer.

[0238] It should be noted that toxin-conjugated and naked antibodies were applied at equal concentrations. The results are shown in **FIG. 24**.

Example 22: Cell toxicity of Claudin 18.2 humanized antibodies with MMAE is more potent than IMAB362 with MMAE in DAN-G, NUGC or SCG-7901 transfectants

[0239] Cells overexpressing human claudin 18.2 (DAN-G, NUGC or SCG-7901 transfectants) were harvested with 0.05% Trypsin/EDTA (Gibco, 25300-054), resuspended in cell culture medium and 50 µl of the cell suspension with the corresponding amount of cells were seeded per well in 96-well cell culture plates. After 24 h, toxin conjugated IMAB362 or control antibodies diluted in 50 µl medium at appropriate concentrations were added and cells were cultured for another 72 h. The effect of Claudin 18.2 humanized antibodies with MMAE on cell viability was determined using a CellTiter-Glo® Luminescent Cell Viability Assay (G7572).

[0240] CellTiter-Glo® Luminescent Cell Viability Assay (G7572) protocol used was as follows:

[0241] 1. Prepare opaque-walled multiwell plates with mammalian cells in culture medium, 100µl per well for 96-well plates or 25µl per well for 384-well plates. Multiwell plates must be compatible with the luminometer used.

[0242] 2. Prepare control wells containing medium without cells to obtain a value for background luminescence.

[0243] 3. Add the test compound to experimental wells, and incubate according to culture protocol. 4. Equilibrate the plate and its contents at room temperature for approximately 30 minutes

[0244] 5. Add a volume of CellTiter-Glo® Reagent equal to the volume of cell culture medium present in each well (e.g., add 100µl of reagent to 100µl of medium containing cells for a 96-well plate, or add 25µl of reagent to 25µl of medium containing cells for a 384-well plate).

[0245] 6. Mix contents for 2 minutes on an orbital shaker to induce cell lysis.

[0246] 7. Allow the plate to incubate at room temperature for 10 minutes to stabilize luminescent signal. Note: Uneven luminescent signal within standard plates can be caused by temperature gradients, uneven seeding of cells or edge effects in multiwell plates.

[0247] 8. Record luminescence.

[0248] The testing results are shown in **FIG. 25A-C**. BG2001-C and BG2001-D both, when conjugated with MMAE, had greatly increased cytotoxicity as compared to the reference IMAB362-NMAE conjugate, in all tested cells. These results therefore demonstrate the improved ability of the presently disclosed antibodies in internalizing conjugated drugs.

Example 23: Cell toxicity of Claudin 18.2 humanized antibodies with MMAE is more potent than IMAB362 with MMAE in SNU620 endogenously expressing human claudin 18.2

[0249] SNU620 cells were resuspended in cell culture medium and 50 μ l of the cell suspension with the corresponding amount of cells were seeded per well in 96-well cell culture plates. After 24 h, toxin conjugated antibodies, including reference antibody IMAB362, diluted in 50 μ l medium at appropriate concentrations were added and cells were cultured for another 72 h. The effect of Claudin 18.2 humanized antibodies with MMAE on cell viability was determined using a CellTiter-Glo® Luminescent Cell Viability Assay (G7572). As shown in **FIG. 26**, BG2001-C and BG2001-D both were greatly more effective in delivering the conjugated MMAE into the SNU620 cells as compared to the reference antibody IMAB362, a lead anti-claudin 18.2 antibody under clinical development.

Example 24: *In vivo* Efficacy of the Antibody Drug Conjugates

[0250] This example tested the efficacy of one of the antibody-drug conjugates (ADC), as compared to the antibody alone (mAb), in reducing tumor growth in nude mice transplanted with human tumor cells.

[0251] 0.1 mL (5×10^5 cells) of human patient derived cells (mixed with Matrigel 1:1) were subcutaneously inoculated on the right back of each mouse. When the average tumor volume reached 60~80 mm³, 30 mice were selected for treatment experiments.

[0252] Eighteen days following inoculation, 5 mice having a tumor size in the range of 330-520 mm³ were selected for each treatment (1 mg/kg, 3 mg/mk, 10 mg/kg or 20 mg/kg ADC, QW) for three weeks. For comparison, antibody (mAb) only treatment was at 10 mg/kg (BIW).

[0253] The results are presented in FIG. 27. ADC at both 10 mg/kg and 20 mg/kg completely inhibited tumor growth, without reducing the animals' body weights. FIG. 28 shows the

average and individual tumor reduction effect in each animal. The tumor reduction effect of the ADC, therefore, is considerably greater than antibody alone.

* * *

[0254] The present disclosure is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the disclosure, and any compositions or methods which are functionally equivalent are within the scope of this disclosure. It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present disclosure without departing from the spirit or scope of the disclosure. Thus, it is intended that the present disclosure cover the modifications and variations of this disclosure provided they come within the scope of the appended claims and their equivalents.

[0255] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.