
Tumor-specific Claudin 18.2 antibodies

BACKGROUND

5 Tight junctions are multiprotein complexes connecting adjacent epithelial or endothelial cells to form a barrier, preventing molecules from passing in between the cells, and helping to maintain the cell and tissue polarity. Tight junctions consist of three main groups of transmembrane proteins: claudins and occludin, cytoplasmic plaque proteins, and cingulin. They also contain cytoskeletal and signaling proteins, e.g. actin, myosin II, and PKC ζ . These
10 proteins interact to maintain the tight junction structure (Yu and Turner 2008).

Claudins form a family of 23 proteins (Hewitt, Agarwal, and Morin 2006). Claudin 18 is a human protein encoded by the CLDN18 gene which forms tight junction strands in epithelial cells. The human CLDN18 can be alternatively spliced with two alternative first exons, resulting in two protein isoforms, CLDN18.1 (or Claudin 18.1) and CLDN18.2 (or Claudin
15 18.2). CLDN18.2 was first disclosed as Zsig28 protein in WO2000/015659. The two isoforms differ in the N-terminal 69 amino acids encompassing the first extracellular loop. The first extracellular domain spans from amino acid 28 to amino acid 80. Within this stretch there are 8 amino acid differences between CLDN18.1 and CLDN18.2. The two different isoforms are expressed in different tissues, with CLDN18.1 being predominantly expressed in lung tissue
20 whereas CLDN18.2 displays stomach specificity (Niimi et al. 2001). CLDN18.2 expression in normal stomach is restricted to the differentiated short-lived cells of stomach epithelium. CLDN18.2 expression has further been identified in various tumor tissues. For example, CLDN18.2 has been found to be expressed in pancreatic, esophageal, ovarian, and lung tumors, correlating with distinct histologic subtypes (Sahin et al. 2008). The amino acid sequence of
25 human CLDN18.2 protein can be derived from NCBI reference sequence: NP_001002026.1. The sequence is also disclosed as SEQ ID NO: 133.

In view of its restricted expression pattern in normal tissues, and of its ectopic expression in human cancers, CLDN18.2 is an attractive cancer target for antibody therapy of epithelial tumors. A number of studies have been made towards such an antibody therapy.
30 WO2004/047863 identified the splice variants of CLDN18 and screened antibodies against different peptides derived from CLDN18.2: peptide DQWSTQDLYN (SEQ ID NO: 57), N-terminal extracellular of CLDN18.2, independent of glycosylation; peptide NNPVTAVFNYQ

(SEQ ID NO: 58), N-terminal extracellular of CLDN18.2, mainly unglycosylated; and peptide STQDLYNNPVTA VF (SEQ ID NO: 59), N-terminal extracellular domain of CLDN18.2, unglycosylated. It also disclosed polyclonal rabbit antibodies screened with a pan-CLDN18 peptide TNFWMSTANMYTG (SEQ ID NO: 60) in the C-terminal extracellular domain

5 common to both CLDN18.1 and CLDN18.2 isoforms. WO2005/113587 discloses antibodies against specific epitopes of CLDN18.2 defined by the peptide sequences: ALMIVGIVLGAIGLLV (SEQ ID NO: 61) and RIGSMEDSAKANMTLTSGIMFIVS (SEQ ID NO: 62). WO2007/059997 discloses CLDN18.2 specific monoclonal antibodies obtained by immunization with the peptide

10 METDTLLLWVLLLWVPGSTGDAAQPARRARRTKLGTELGSTPVWWSADGRMDQ WSTQDLYNNPVTA VFNYQGLWRSCVRESSGFTECRGYFTLLGLPAMLQAVRAAIQH SGGRSRRARTKTHLRRGSE (SEQ ID NO: 63), including the first extracellular domain of CLDN18.2 with N- and C-terminal extensions. Antibodies obtained by this immunization mediate cell killing by complement dependent cytotoxicity (CDC) and antibody-dependent

15 cell-mediated cytotoxicity (ADCC). Antibody IMAB362, also known as Claudiximab or Zolbetuximab, is disclosed in WO2007/059997 and WO2016/165762. IMAB362 is an IgG1 antibody derived from a murine monoclonal antibody and has been chimerized to display the human IgG1 constant region for clinical use. WO2008/145338 also discloses antibodies binding to overlapping peptides within the first extracellular domain (MDQWSTQDLYNNPVT (SEQ

20 ID NO: 64), LYNNPVTA VFNYQGL (SEQ ID NO: 65), VFNYQGLWRSCVRES (SEQ ID NO: 66), QGLWRSCVRESSGFT (SEQ ID NO: 67), and RSCVRESSGFTECRG (SEQ ID NO: 68)). In an effort to produce antibodies targeting the C-terminal portion of CLDN18.2 for diagnostic purposes to detect CLDN18.2 expression in cells of cancer tissue sections, WO2013/167259 discloses antibodies binding to C-terminal epitopes of CLDN18.2. The

25 sequences of the two epitopes are TEDEVQSYPSKHDYV (SEQ ID NO: 69) and EVQSYPSKHDYV (SEQ ID NO: 70). WO2013/174509 presents combinations of anti-CLDN18.2 antibodies with agents stabilizing $\gamma\delta$ T cells or with agents stabilizing or increasing the expression of CLDN18.2. Antibodies may be conjugated to a therapeutic moiety such as a cytotoxin, a drug (e.g. an immunosuppressant) or a radioisotope. WO2014/075788 discloses a

30 method of treatment a cancer disease using a bispecific antibody binding CLDN18.2 and CD3. WO2014/127906 discloses combination agents stabilizing or increasing the expression of CLDN18.2. WO2016/166122 discloses anti-CLDN18.2 monoclonal antibodies that can be highly efficiently internalized upon CLDN18.2 binding and therefore are suitable for antibody-

drug conjugate (ADC) development. Furthermore, the conjugation of such antibodies to the drugs DM4 and MMAE using cleavable SPDB or Valine-Citrulline linkers, respectively, is disclosed. However, despite all the antibodies disclosed in the patent applications, only the chimeric IMAB362, disclosed in WO2007/059997 and WO2016/165762, is currently tested in clinical trial. In addition to these antibodies and ADCs, WO2018/006882 discloses chimeric antigen receptors (CAR) based on anti-CLDN18.2 monoclonal antibodies. Antibodies of WO2018/006882 have been humanized and their sequence is disclosed in the Supplementary Materials section associated with Jiang et al 2018 (Jiang et al. 2018). CAR T-cells based on the humanized antibody are currently tested in a phase I clinical trial (ClinicalTrials.gov Identifier: NCT03159819) in patients with advanced gastric adenocarcinoma and pancreatic adenocarcinoma. CN109762067 discloses other anti-CLDN18.2 monoclonal antibodies mediating cell killing by CDC and ADCC. WO2019/173420 discloses anti-CLDN18.2 humanized monoclonal antibodies with ADCC activity. WO2019/175617 discloses anti-CLDN18.2 monoclonal antibodies binding to a different epitope than IMAB362. WO2019/219089 discloses monoclonal antibodies binding to a mutant of CLDN18.2.

CLDN18.2 has been described to exist in different conformations and contains a potential extracellular N-glycosylation site (see WO2007/059997 page 3, first para.), which may lead to potentially different topologies/differential glycosylation between normal and tumor cells (see WO2007/059997 page 4, second para.). However, none of the reported antibodies is preferentially targeting CLDN18.2 expressed on tumor cells. Since CLDN18.2 is expressed not only in tumors, but also in healthy tissue, namely in stomach tissue (Sahin et al. 2008), it clearly would be beneficial to have antibodies targeting only CLDN18.2 expressed in tumor in order to avoid safety issues and side effect very often associated with the on-target effect of therapeutic antibodies to healthy organs/tissues (Hansel et al. 2010), in particular as reported for IMAB362 (Sahin et al. 2018; Tureci et al. 2019).

In addition to binding to targets with high affinity, therapeutic antibodies should maintain their desired properties during development, production, storage and clinical application (*in vivo*). Antibody stability may be compromised by post-translational modifications (PTM) (Lu et al. 2019; Gervais 2016). Since uncontrolled PTM may lead to antibodies with less than desired efficacy, activity, potency or stability, it is therefore very important while developing therapeutic antibodies to design them with the minimal possible PTMs. PTMs can also have a profound effect on regulatory acceptance, technology transfer or processes and development of

biosimilars. The predominant modifications are oxidation, deamidation and isomerization. Further, IMAB362 is a chimeric antibody still having extended mouse sequence, which could lead to antidrug antibodies in some patients, which, e.g. upon repeated application, may lead to decreased efficacy of the treatment.

- 5 Therefore, there is a need for improved antibodies specific to CLDN18.2 for use in the treatment of tumor patients.

DEFINITIONS

"Antibodies" or "antibody", also called "immunoglobulins" (Ig), generally comprise four polypeptide chains, two heavy (H) chains and two light (L) chains, and are therefore multimeric
 10 proteins, or comprise an equivalent Ig homologue thereof (e.g., a camelid antibody comprising only a heavy chain, single-domain antibodies (sdAb) or nanobody which can be either be derived from a heavy or light chain). The term "antibodies" includes antibody-based binding protein, modified antibody format retaining target binding capacity. The term "antibodies" also includes full length functional mutants, variants, or derivatives thereof (including, but not
 15 limited to, murine, chimeric, humanized and fully human antibodies) which retain the essential epitope binding features of an Ig molecule, and includes dual specific, bispecific, multispecific, and dual variable domain Igs. Ig molecules can be of any class (e.g., IgG, IgE, IgM, IgD, IgA, and IgY), or subclass (e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2) and allotype. Ig molecules may also be mutated e.g. to enhance or reduce affinity for Fcγ receptors or the neonatal Fc
 20 receptor (FcRn).

An "antibody fragment", as used herein, relates to a molecule comprising at least one polypeptide chain derived from an antibody that is not full length and exhibits target binding. Antibody fragments are capable of binding to the same epitope or target as their corresponding full-length antibody. Antibody fragments include, but are not limited to (i) a Fab fragment,
 25 which is a monovalent fragment consisting of the variable light (VL), variable heavy (VH), constant light (CL) and constant heavy 1 (CH1) domains; (ii) a F(ab')₂ fragment, which is a bivalent fragment comprising two Fab fragments linked by a disulfide bridge at the hinge region (reduction of a F(ab')₂ fragment result in two Fab' fragment with a free sulfhydryl group); (iii) a heavy chain portion of a Fab (Fa) fragment, which consists of the VH and CH1 domains; (iv)
 30 a variable fragment (Fv) fragment, which consists of the VL and VH domains of a single arm of an antibody; (v) a domain antibody (dAb) fragment, which comprises a single variable domain; (vi) an isolated complementarity determining region (CDR); (vii) a single chain Fv

- fragment (scFv); (viii) a diabody, which is a bivalent, bispecific antibody in which VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with the complementarity domains of another chain and creating two antigen binding sites; (ix) a linear antibody, which comprises a pair of tandem Fv segments (VH-CH1-VH-CH1) which, together with complementarity light chain polypeptides, form a pair of antigen binding regions; (x) Dual-Variable Domain Immunoglobulin; (xi) other non-full length portions of immunoglobulin heavy and/or light chains, or mutants, variants, or derivatives thereof, alone or in any combination.
- 10 An "antibody-based binding protein", as used herein, may represent any protein that contains at least one antibody-derived VH, VL, or CH immunoglobulin domain in the context of other non-immunoglobulin, or non-antibody derived components. Such antibody-based proteins include, but are not limited to (i) Fc-fusion proteins of binding proteins, including receptors or receptor components with all or parts of the immunoglobulin CH domains, (ii) binding proteins, 15 in which VH and or VL domains are coupled to alternative molecular scaffolds, or (iii) molecules, in which immunoglobulin VH, and/or VL, and/or CH domains are combined and/or assembled in a fashion not normally found in naturally occurring antibodies or antibody fragments.

- The term "modified antibody format", as used herein, encompasses antibody-drug-conjugates (ADCs), polyalkylene oxide-modified scFv, monobodies, diabodies, camelid antibodies, domain antibodies, bi- or trispecific antibodies, IgA, or two IgG structures joined by a J chain and a secretory component, shark antibodies, new world primate framework and non-new world primate CDR, IgG4 antibodies with hinge region removed, IgG with two additional binding sites engineered into the CH3 domains, antibodies with altered Fc region to enhance or reduce affinity for Fc gamma receptors, dimerized constructs comprising CH3, VL, and VH, and the like.
- 20 25

The Kabat numbering scheme (Martin and Allemn 2014) has been applied to the disclosed antibodies.

- Where the term "comprising" is used in the present description and claims, it does not exclude other elements. For the purposes of the present invention, the term "consisting of" is considered to be a preferred embodiment of the term "comprising of". If hereinafter a group is defined to
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comprise at least a certain number of embodiments, this is also to be understood to disclose a group, which preferably consists only of these embodiments.

Where an indefinite or definite article is used when referring to a singular noun, e.g. “a”, “an” or “the”, this includes a plural of that noun unless something else is specifically stated.

- 5 Technical terms are used by their common sense. If a specific meaning is conveyed to certain terms, definitions of terms will be given in the following in the context of which the terms are used.

DESCRIPTION OF THE INVENTION

10 The inventors have surprisingly identified novel anti-CLDN18.2 antibodies as further described in the following embodiments, which exhibit increased binding to tumor cells expressing CLDN18.2 compared to healthy stomach cells expressing CLDN18.2 and/or have improved stability and/or are humanized while retaining their improved properties.

15 Therefore, in one embodiment of the invention, the invention provides an antibody or fragment thereof binding to CLDN18.2, wherein the antibody or fragment thereof exhibits increased binding to tumor tissue expressing CLDN18.2 over healthy tissue expressing CLDN18.2. In one embodiment, the healthy cells or tissue used for the comparison are healthy stomach cells or healthy stomach tissue.

20 Increased binding to tumor tissue by the antibody or fragment thereof provided herein may be shown by bioanalytical methods such as flow cytometry (FC) or immunohistochemistry (IHC), as shown in Examples 4 and 5, respectively. A tumor expressing CLDN18.2 may be generated by subcutaneously injecting CLDN18.2-expressing A549 cells into a Balb/c mouse. The CLDN18.2-expressing A549 cells may be generated as shown in Example 4 and are available under the accession number DSM ACC3360 deposited on 6 December 2019 at the DSMZ-
25 Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH Inhoffenstr. 7B 38124 Braunschweig DE. The healthy tissue (e.g. healthy stomach tissue) may also originate from the mouse bearing the tumor. Increased binding to tumor tissue over healthy tissue may thus be shown on the tumor tissue and healthy tissue obtained from the same animal.

30 Increased binding to CLDN18.2 expressed in tumor tissue may be due to posttranslational modification such as differential glycosylation of CLDN18.2, or misfolding of CLDN18.2, when compared to CLDN18.2 expressed in healthy tissue.

Flow cytometry (FC) may be used as a bioanalytical method to test antibody binding. The percentage of CLDN18.2-positive cells can for example be measured by FC for a specific anti-CLDN18.2 antibody. Another possible binding read-out may for example be the ratio of the percentage of CLDN18.2-positive cells in a tumor cell sample versus the percentage of CLDN18.2-positive cells in a cell sample obtained from healthy tissue, such as healthy stomach tissue. Increased binding of an antibody to tumor cells expressing CLDN18.2 generated from CLDN18.2-expressing A549 cells compared to healthy cells, such as healthy stomach cells, may be shown by a ratio of > 2 , > 5 , ≥ 10 , preferably ≥ 15 , and more preferably ≥ 20 .

Increased binding of an antibody to tumor cells expressing CLDN18.2 generated from CLDN18.2-expressing A549 cells compared to healthy cells, such as healthy stomach cells, may also be described by showing that the antibody binds at least 2 times more, at least 5 times more, at least 10 times more, preferably at least 15 times more, preferably at least 20 times more tumor cells than healthy cells, such as healthy stomach cells.

Immunohistochemistry (IHC) may be used as a bioanalytical method to test antibody binding. The tissue sample used for IHC should preferably be snap frozen after resection and, once thawed, fixed in acetone as, e.g., shown in Example 5. Since CLDN18.2 is a tight-junction protein in healthy tissue, positive CLDN18.2 staining should result in visualization of a predominantly membranous staining at the cell-cell interface in healthy tissue and/or tumor tissue. Negative CLDN18.2 staining or weak staining should therefore result in absence of membranous staining.

In another embodiment, the invention provides an antibody or fragment thereof binding to CLDN18.2 with a half maximal effective concentration (EC₅₀) value of above 0.4 µg/ml, above 0.5 µg/ml, preferably above 0.6 µg/ml, but not above 1 µg/ml when measured by flow cytometry (FC) titration on HEK293T cells overexpressing CLDN18.2. HEK293T cells overexpressing CLDN18.2 may be generated as described in Example 3. The EC₅₀ value of the antibody of the invention may be, when measured by flow cytometry (FC) titration on HEK293T cells overexpressing CLDN18.2, between 0.4 and 1 µg/ml, between 0.5 and 1 µg/ml or preferably between 0.6 and 1 µg/ml.

Alternatively, the EC₅₀ value of an antibody of the invention may be compared to the EC₅₀ value of IMAB362 when measured by flow cytometry on HEK293T cells overexpressing CLDN18.2, wherein the EC₅₀ value of the antibody of the invention is at least 1.1 times higher,

at least 1.2 times higher, preferably at least 1.5 times higher, more preferably at least 2 times higher, even more preferably at least 2.5 times higher than the EC50 value of IMAB362 but not more than 5 times higher than the EC50 value of IMAB362. The EC50 value of the antibody of the invention may be between 1.1 times higher and 2.5 times higher, between 1.2 times
5 higher and 2.5 times higher, preferably between 1.5 times higher and 2.5 times higher, or more preferably between 2 times higher and 2.5 times higher than the EC50 value of IMAB362 when measured by flow cytometry on HEK293T cells overexpressing CLDN18.2.

In another embodiment, the invention provides an antibody or fragment thereof binding to CLDN18.2 with an EC50 value of above 0.6 µg/ml, above 1 µg/ml, preferably above 1.5 µg/ml,
10 more preferably above 2 µg/ml, but not above 3 µg/ml when measured by flow cytometry titration on PA-TU-8988S-High cells. PA-TU-8988S-High cells may be generated as described in Example 2. The EC50 value of the antibody of the invention, when measured by flow cytometry titration on PA-TU-8988S-High cells, may be between 0.6 and 3 µg/ml, between 1 and 3 µg/ml, preferably between 1.5 and 3 µg/ml, or more preferably between 2 and 3 µg/ml.

Alternatively, the EC50 value of the antibody of the invention may be compared to the EC50 value of IMAB362 when measured by flow cytometry on PA-TU-8988S-High cells, wherein the EC50 value of the antibody of the invention is at least 1.5 times higher, at least 2 times higher, preferably at least 3 times higher, more preferably at least 4 times higher, but not more than 5 times higher than the EC50 value of IMAB362. The EC50 value of the antibody of the
20 invention, when measured by flow cytometry on PA-TU-8988S-High cells, may be between 1.5 times higher and 5 times higher, between 2 times higher and 5 times higher, between 3 times higher and 5 times higher or between 4 times higher and 5 times higher than the EC50 value of IMAB362.

In another embodiment, the invention provides an antibody or fragment thereof binding to CLDN18.2 with a maxMFI values within +/- 40% of the maxMFI value of IMAB362 when
25 measured by flow cytometry on HEK293T cells overexpressing CLDN18.2. The invention also provides an antibody or fragment thereof binding to CLDN18.2 with maxMFI values equal or up to 2 times higher than the maxMFI value of IMAB362 when measured by flow cytometry on PA-TU-8988S-High cells.

30 An antibody or functional fragment thereof with increased binding to tumor tissue expressing CLDN18.2 compared to healthy tissue expressing CLDN18.2 may have therapeutic advantages

over antibodies unable to discriminate healthy tissue expressing CLDN18.2 from tumor tissue expressing CLDN18.2. Tumor-specific antibodies may not lead to safety issues and side effects, which are very often associated with the on-target effect of therapeutic antibodies in healthy organs/tissues (Hansel et al. 2010). Such undesirable effects have been reported for, e.g.,

5 IMAB362 (Sahin et al. 2018; Tureci et al. 2019).

The invention also provides an antibody or fragment thereof binding to CLDN18.2 comprising the heavy chain complementarity determining region (HCDR) HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 21, SEQ ID NO: 22, and SEQ ID NO: 23, respectively and the light chain CDR LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 24, SEQ ID NO: 25, and

10 SEQ ID NO: 26, respectively.

The invention also provides an antibody or fragment thereof binding to CLDN18.2 comprising the heavy chain HCDR3 sequence of SEQ ID NO: 23 and the light chain LCDR3 sequence of SEQ ID NO: 26.

The respective consensus sequences can be found in Table 1. It is understood that any antibody

15 or fragment thereof based on any combination of CDRs derived from the consensus sequences and binding to CLDN18.2 is part of the invention.

Table 1: isolated antibody CDR consensus sequences

CDRs	Sequence	SEQ ID
HCDR1	DYAMX X in 5 th position is H or Y	SEQ ID NO: 21
HCDR2	WINXYTGKPTYXXXFXG X in 4 th position is T or A; X in 12 th position is A or S; X in 13 th position is D or Q; X in 14 th position is D or K; X in 16 th position is K or Q	SEQ ID NO: 22
HCDR3	AVXYGYTMDA X in 3 rd position is F or Y	SEQ ID NO: 23
LCDR1	RXSEDIYSNXA X in 2 nd position is A or T; X in 10 th position is L or F	SEQ ID NO: 24

LCDR2	XXXRLQD X in 1 st position is S or A; X in 2 nd position is V or I; X in 3 rd position is K or N	SEQ ID NO: 25
LCDR3	LQGSXFPLT X in 5 th position is K or N	SEQ ID NO: 26

Antibody binding or binding affinity is generally expressed in terms of equilibrium association or dissociation constants (K_a or K_d , respectively), which are in turn reciprocal ratios of dissociation and association rate constants (k_{off} and k_{on} , respectively). Thus, equivalent affinities

5 may correspond to different rate constants, so long as the ratio of the rate constants remains the same. Binding affinities and/or rate constants can be determined using techniques well known in the art or described herein, such as ELISA, flow cytometry titration, isothermal titration calorimetry (ITC), Biacore (SPR), biolayer interferometry or fluorescent polarization. In some cases, due to the nature of the antigen, the K_a or K_d of antibodies may be difficult to measure.

10 This is especially true for integral membrane proteins such as Claudins (Hashimoto et al. 2018). In such cases, the integral membrane protein may be expressed as proteoliposomes or lipoparticles. Such lipoparticles may be immobilized on plastic and used in ELISA assay to determine the binding affinity of antibodies to the immobilized antigen. Instead of K_a or K_d values, half maximal effective concentration (EC50) values may thus be calculated for each

15 tested antibody or functional fragment thereof, reflecting its binding affinity (or strength of binding) to the antigen. Example 2 and Figure 1 below exemplify ELISA assay binding affinity curves of antibodies with CDRs comprised in the consensus sequences of Table 1. The EC50 value and the maximal binding value can be used for quantification of the binding of the antibodies to CLDN18.2. Example 3 below relates to the calculation of EC50 values by flow

20 cytometry on cells expressing CLDN18.2 of antibodies with CDRs comprised in the consensus sequences of Table 1.

In another embodiment, the invention provides an antibody or fragment thereof binding to CLDN18.2 which comprises the heavy chain CDRs HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 21, SEQ ID NO: 126, and SEQ ID NO: 23, respectively and the light chain CDRs

25 LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26, respectively.

In one embodiment, the invention relates to an antibody or fragment thereof binding to CLDN18.2, comprising:

- 5 a. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 1, SEQ ID NO: 15 and SEQ ID NO: 3, respectively, and the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, respectively;
- b. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 1, SEQ ID NO: 16 and SEQ ID NO: 3, respectively, and the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, respectively;
- 10 c. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 1, SEQ ID NO: 16 and SEQ ID NO: 3, respectively, and the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 17, SEQ ID NO: 14 and SEQ ID NO: 11, respectively;
- d. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 1, SEQ ID NO: 16 and SEQ ID NO: 3, respectively, and the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 11, respectively;
- 15 e. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 12, SEQ ID NO: 15 and SEQ ID NO: 3, respectively, and the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, respectively;
- f. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 1, SEQ ID NO: 20, and SEQ ID NO: 3, respectively, and the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, respectively;
- 20 g. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 1, SEQ ID NO: 20 and SEQ ID NO: 3, respectively, and the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 11, respectively;
- h. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 12, SEQ ID NO: 20 and SEQ ID NO: 8, respectively, and the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, respectively; or
- 25 i. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 12, SEQ ID NO: 20 and SEQ ID NO: 8, respectively, and the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 17, SEQ ID NO: 14 and SEQ ID NO: 11, respectively.

In yet another embodiment, the invention provides an antibody or fragment thereof binding to CLDN18.2, comprising:

- 5 a. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 1, SEQ ID NO: 2 and SEQ ID NO: 3, respectively, and the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, respectively;
- b. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 1, SEQ ID NO: 7 and SEQ ID NO: 8, respectively, and the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11, respectively; or
- 10 c. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 12, SEQ ID NO: 2 and SEQ ID NO: 3, respectively, and the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 13, SEQ ID NO: 14 and SEQ ID NO: 11, respectively.

In yet another embodiment, the invention relates to an antibody or fragment thereof binding to CLDN18.2, comprising:

- a. a VH sequence of SEQ ID NO: 27 and a VL sequence of SEQ ID NO: 28;
- 15 b. a VH sequence of SEQ ID NO: 29 and a VL sequence of SEQ ID NO: 30;
- c. a VH sequence of SEQ ID NO: 31 and a VL sequence of SEQ ID NO: 32.

In another embodiment, the invention relates to an antibody or fragment thereof binding to CLDN18.2, comprising:

- a. a VH sequence of: SEQ ID NO: 33;
- 20 b. a VH sequence of SEQ ID NO: 34;
- c. a VH sequence of SEQ ID NO: 35;
- d. a VH sequence of SEQ ID NO: 36; or
- e. a VH sequence of SEQ ID NO: 37;

and

- 25 f. a VL sequence of SEQ ID NO: 38;
- g. a VL sequence of SEQ ID NO: 39;
- h. a VL sequence of SEQ ID NO: 40; or

- i. a VL sequence of SEQ ID NO: 41.

In a further embodiment, the invention relates to an antibody or fragment thereof binding to CLDN18.2, comprising:

- a. a VH sequence of SEQ ID NO: 33 and a VL sequence of SEQ ID NO: 38;
- 5 b. a VH sequence of SEQ ID NO: 34 and a VL sequence of SEQ ID NO: 38;
- c. a VH sequence of SEQ ID NO: 34 and a VL sequence of SEQ ID NO: 39;
- d. a VH sequence of SEQ ID NO: 34 and a VL sequence of SEQ ID NO: 40;
- e. a VH sequence of SEQ ID NO: 35 and a VL sequence of SEQ ID NO: 38;
- f. a VH sequence of SEQ ID NO: 36 and a VL sequence of SEQ ID NO: 41;
- 10 g. a VH sequence of SEQ ID NO: 36 and a VL sequence of SEQ ID NO: 40;
- h. a VH sequence of SEQ ID NO: 37 and a VL sequence of SEQ ID NO: 41;
- i. a VH sequence of SEQ ID NO: 37 and a VL sequence of SEQ ID NO: 38; or
- j. a VH sequence of SEQ ID NO: 37 and a VL sequence of SEQ ID NO: 39.

15 In another embodiment, the invention relates to an antibody binding to CLDN18.2, comprising:

- a. the heavy chain sequence of SEQ ID NO: 46 and light chain sequence of SEQ ID NO: 51;
- b. the heavy chain sequence of SEQ ID NO: 47 and light chain sequence of SEQ ID NO: 51;
- 20 c. the heavy chain sequence of SEQ ID NO: 47 and light chain sequence of SEQ ID NO: 52;
- d. the heavy chain sequence of SEQ ID NO: 47 and light chain sequence of SEQ ID NO: 53;
- e. the heavy chain sequence of SEQ ID NO: 48 and light chain sequence of SEQ ID NO: 51;
- 25 f. the heavy chain sequence of SEQ ID NO: 47 and light chain sequence of SEQ ID NO: 54;

- g. the heavy chain sequence of SEQ ID NO: 49 and light chain sequence of SEQ ID NO: 53;
- h. the heavy chain sequence of SEQ ID NO: 50 and light chain sequence of SEQ ID NO: 54;
- 5 i. the heavy chain sequence of SEQ ID NO: 50 and light chain sequence of SEQ ID NO: 51; or
- j. the heavy chain sequence of SEQ ID NO: 50 and light chain sequence of SEQ ID NO: 52.

10 The constant light chain region CL and the constant heavy chain region CH1 and Fc region of the disclosed antibodies may have the amino acid sequence of SEQ ID NO: 127 and SEQ ID NO: 128, respectively.

In a preferred embodiment, the invention relates to an antibody binding to CLDN18.2, comprising the heavy chain sequence of SEQ ID NO: 46 and light chain sequence of SEQ ID NO: 51.

15 In a further preferred embodiment, the invention relates to an antibody binding to CLDN18.2, consisting of the heavy chain sequence of SEQ ID NO: 46 and light chain sequence of SEQ ID NO: 51.

20 The invention also relates to an antibody having an amino acid sequence with at least 80% identity, at least 85%, at least 90%, at least 95% or at least 98% identity to the amino acid sequence of the antibody of the invention, exhibiting increased binding to tumor cells expressing CLDN18.2 compared to healthy stomach cells expressing CLDN18.2.

In one embodiment, the invention relates to an antibody binding to CLDN18.2 and having an amino acid sequence with at least 80% identity, at least 85%, at least 90%, at least 95% or at least 98% identity to an antibody comprising:

- 25 a. a VH sequence of SEQ ID NO: 27 and a VL sequence of SEQ ID NO: 28;
- b. a VH sequence of SEQ ID NO: 29 and a VL sequence of SEQ ID NO: 30;
- c. a VH sequence of SEQ ID NO: 31 and a VL sequence of SEQ ID NO: 32.

30 In a further embodiment, the invention relates to an antibody binding to CLDN18.2 and having an amino acid sequence with at least 80% identity, at least 85%, at least 90%, at least 95% or at least 98% identity to an antibody comprising:

- a. a VH sequence of SEQ ID NO: 33 and a VL sequence of SEQ ID NO: 38;
- b. a VH sequence of SEQ ID NO: 34 and a VL sequence of SEQ ID NO: 38;
- c. a VH sequence of SEQ ID NO: 34 and a VL sequence of SEQ ID NO: 39;
- d. a VH sequence of SEQ ID NO: 34 and a VL sequence of SEQ ID NO: 40;
- 5 e. a VH sequence of SEQ ID NO: 35 and a VL sequence of SEQ ID NO: 38;
- f. a VH sequence of SEQ ID NO: 36 and a VL sequence of SEQ ID NO: 41;
- g. a VH sequence of SEQ ID NO: 36 and a VL sequence of SEQ ID NO: 40;
- h. a VH sequence of SEQ ID NO: 37 and a VL sequence of SEQ ID NO: 41;
- i. a VH sequence of SEQ ID NO: 37 and a VL sequence of SEQ ID NO: 38; or
- 10 j. a VH sequence of SEQ ID NO: 37 and a VL sequence of SEQ ID NO: 39.

In yet a further embodiment, the invention relates to an antibody binding to CLDN18.2 and having an amino acid sequence with at least 80% identity, at least 85%, at least 90%, at least 95% or at least 98% identity to an antibody consisting of the heavy chain sequence of SEQ ID NO: 46 and light chain sequence of SEQ ID NO: 51.

- 15 In another embodiment, the Fc domain of the antibody (or antibody fragment when present) may comprise modifications or mutations, such as the modifications or mutations listed in Table 2 below. Such a modification or mutation may be introduced to modulate the effector activity of the Fc domain of the antibody. Modification of antibodies may also include peptide tags added to the C-terminal end of the antibody HC and/or LC chain. Such tags may be used e.g.
- 20 for protein purification or protein conjugation.

- In another embodiment, the invention provides an antibody or fragment thereof binding to CLDN18.2, the antibody being an IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, synthetic IgG, IgM, F(ab)₂, Fv, scFv, IgGACH2, F(ab')₂, scFvCH3, Fab, VL, VH, scFv4, scFv3, scFv2, dsFv, Fv, scFv-Fc, (scFv)₂, a non-depleting IgG, a diabody, a bivalent antibody or Fc-engineered versions thereof. In a preferred embodiment, the antibody is an IgG1 type of antibody. The Fc region of immunoglobulins interacts with multiple Fcγ receptors (FcγR) and complement proteins (e.g. C1q), and mediates immune effector functions, such as elimination of targeted cells via antibody-dependent cellular cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP) or complement-dependent cytotoxicity (CDC). For therapeutic
- 25

- approaches, it may be beneficial to enhance or silence Fc related effector functions. The type of immunoglobulin (IgA, IgD, IgE, IgG, IgM) may be selected according to the desired effector function of the antibody related to the Fc domain. One may also employ a synthetic immunoglobulin, such as an immunoglobulin with the IgG2 amino acids 118 to 260 and the
- 5 IgG4 amino acids 261 to 447 or an IgG2 variant with point mutations from IgG4 (e.g. H268Q/V309L/A30S/P331S). Such synthetic immunoglobulins reduce effector functions of the antibody. Fc-engineered immunoglobulins may also be employed to modulate antibody effector function. Table 2 shows example of such Fc engineering. Expression in production cell lines with altered fucosylation may also impact Fc γ R binding.
- 10 Table 2: Examples of modifications to modulate antibody effector function. Unless otherwise noted, the mutations are on the IgG1 subclass (Wang, Mathieu, and Brezski 2018).

Engineering and intended function	Mutation	Reference
<i>Enhance ADCC</i>		
Increased Fc γ RIIIa binding	• F243L/R292P/Y300L/V305I/P396L • S239D/I332E • S298A/E333A/K334A • in one heavy chain: L234Y/L235Q/G236W/S239M/H268D/D270E/S298A, in the opposing heavy chain: D270E/K326D/A330M/K334E	• (Stavenhagen et al. 2007) • (Lazar et al. 2006) • (Shields et al. 2001) • (Mimoto et al. 2013)
Increased Fc γ RIIIa binding, decreased Fc γ RIIb binding	S239D/I332E/A330L	(Lazar et al. 2006)
<i>Enhance ADCP</i>		
Increased Fc γ RIIa binding, Increased Fc γ RIIIa binding	G236A/S239D/I332E	(Richards et al. 2008)
<i>Enhance CDC</i>		
Increased C1q binding	• K326W/E333S • S267E/H268F/S324T • IgG1/IgG3 cross subclass	• (Idusogie et al. 2001) • (Moore et al. 2010) • (Natsume et al. 2008)
Hexamerization	E345R/E430G/S440Y	(Diebolder et al. 2014)
<i>Reduce effector function</i>		
Aglycosylated	N297A or N297Q or N297G	(Bolt et al. 1993; Leabman et al. 2013; Tao

		and Morrison 1989; Walker et al. 1989)
Reduced FcγR and C1q binding	• L235E • IgG1: L234A/L235A or L234A/L235A/P329G • IgG4:F234A/L235A • IgG2/IgG4 cross isotype • IgG2: H268Q/V309L/A330S/P331S • IgG2: V234A/G237A/P238S/H268A/V309L/A330S/P331S	• (Alegre et al. 1992) • (Xu et al. 2000; Lo et al. 2017) • (Xu et al. 2000) • (Rother et al. 2007) • (An et al. 2009) • (Vafa et al. 2014)
<i>Increase half-life</i>		
Increased FcRn Binding at pH 6.0	• M252Y/S254T/T256E • M428L/N434S	• (Dall'Acqua et al. 2002) • (Zalevsky et al. 2010)
<i>Increased coengagement</i>		
Increased FcγRIIb binding	S267E/L328F	(Chu et al. 2008)
Increased FcγRIIa binding, decreased FcγRIIIa binding	N325S/L328F	(Shang et al. 2014)

Half-life of antibodies may also be modulated. The Fc domain plays a central role in the stability and serum half-life of antibodies. For therapeutic approaches, antibody half-life may be reduced by using an antibody fragment missing the Fc domain or with a truncated Fc domain, such as F(ab)₂, Fv, scFv, IgGACH2, F(ab')₂, scFvCH3, Fab, VL, VH, scFv4, scFv3, scFv2, dsFv, Fv, scFv-Fc or (scFv)₂. The antibodies may also be in the form of diabodies or bivalent antibodies. Diabodies or bivalent antibodies may be used to increase the affinity to the target allowing lower dosage. Functional fragments missing the Fc domain or with truncated Fc domains may also be used in the development of other therapeutic approaches such as chimeric antigen receptor T cell (CAR T cells) or bispecific T cell engagers (BiTEs). In CAR constructs, one VH and one VL domain are typically connected by a short peptide linker to form a single-chain variable fragment (scFv), and the scFv fragment is further linked to a transmembrane domain and an intracytoplasmic T cell immunoreceptor tyrosine-based activation motif (from e.g. CD3ζ) and further domains of co-stimulatory molecules (from e.g. CD28, 4-1BB (CD127), or

OX40) (Chang and Chen 2017). The VH and VL domains used in the scFv fragment may be the ones of the antibodies listed in Table 3. BiTEs typically consist of the fusion of two scFv of two different antibodies. One scFv domain may be of the isolated antibodies binding CLDN18.2 listed in Table 3, while the other scFv domain is from an antibody that binds e.g. to CD3, CD16,
5 NKG2D, NKp46, CD2, CD28 or CD25. Ample guidance on BiTEs antibody formats and other bispecific antibody formats used for T-cell redirecting may be found in the review by Diego Ellerman (2019).

In another embodiment, the invention provides an antibody or fragment thereof binding to CLDN18.2, the antibody having the constant light chain region (CL) of SEQ ID NO: 127 and
10 preferably the constant heavy chain region CH1 and Fc region of SEQ ID NO: 129 with reduced FcγR binding having the L234A/L235A mutations in the constant heavy chain region CH2. More preferably, the invention provides for an antibody with the constant heavy chain region CH1 and Fc region of SEQ ID NO: 130 having a L234A/L235A/P329G mutation in the constant heavy chain region CH1 and Fc region with even further reduced FcγR binding.

15 In a another preferred embodiment, the invention relates to an antibody or fragment thereof binding to CLDN18.2, comprising the VH sequence of SEQ ID NO: 33, the VL sequence of SEQ ID NO: 38, the constant light chain region (CL) of SEQ ID NO: 127 and the constant heavy chain region CH1 and Fc region of SEQ ID NO: 129 with L234A/L235A.

In a another preferred embodiment, the invention relates to an antibody or fragment thereof
20 binding to CLDN18.2, consisting of the VH sequence of SEQ ID NO: 33, the VL sequence of SEQ ID NO: 38, the constant light chain region (CL) of SEQ ID NO: 127 and the constant heavy chain region CH1 and Fc region of SEQ ID NO: 129 with L234A/L235A.

In another embodiment, the invention provides an antibody or fragment thereof binding to CLDN18.2, wherein the antibody or fragment thereof is humanized. Humanization of
25 monoclonal antibodies is well-established. The Handbook of Therapeutic Antibodies, Second Edition, gives ample information on humanization of monoclonal antibodies (Saldanha 2014), bioinformatics tools for analysis of such antibodies (Martin and Allemn 2014) and development and manufacture of therapeutic antibodies (Jacobi et al. 2014).

In another embodiment, the antibody or fragment thereof is an isolated antibody or isolated
30 fragment binding to CLDN18.2.

- In a further embodiment, the invention provides an antibody or fragment thereof binding to CLDN18.2, wherein the antibody or fragment thereof does not bind to CLDN18.1. Hence, the antibody does not exhibit cross-reactivity or cross-binding to CLDN18.1. Binding of an antibody to a target protein can be tested by flow cytometry on cells expressing the target protein. Specific binding of a tested antibody to its target protein can be visualized on a histogram plot. Such plot results in a peak with high fluorescent signal when the antibody specifically binds to the expressed target protein, and in a peak with low fluorescent signal when the antibody does not, or only very weakly bind to the expressed target protein. The degree of binding can also be expressed in a bar graph showing the maximal mean fluorescent intensity (maxMFI) measured by flow cytometry, with high maxMFI reflecting strong binding and low/no maxMFI reflecting no binding or very weak binding. Comparing maxMFI values for different antibodies in a same experimental set up may also be indicative of the affinity of the antibodies to the target, with a higher maxMFI indicating a lower off rate and higher affinity. Examples of such binding assays can be found in Example 3 and Figures 4 and 5.
- 15 In another embodiment, the invention provides an antibody or fragment thereof binding to CLDN18.2, the antibody being bound to another moiety. The binding of the antibody or fragment thereof to another moiety may be covalent or no-covalent. The moiety may include radioisotopes, fluorescent tags, histological markers, cytotoxins or cytokines. Covalent binding of the moiety to the antibody may be facilitated by linkers known in the art.
- 20 In yet another embodiment, the invention relates to a tumor-specific antibody or fragment thereof that binds to CLDN18.2, wherein the antibody is less susceptible to posttranslational deamidation than IMAB362. In a further embodiment, the invention relates to a tumor-specific antibody or fragment thereof that binds to CLDN18.2, wherein the antibody does not undergo posttranslational deamidation. Posttranslational modifications (PTM) are an important concern
- 25 in both antibody development and antibody production and storage. Uncontrolled PTM may lead to antibodies with less efficacy, activity, potency or stability. PTMs may be N-glycosylation, lysine glycation and cysteines capped with other cysteines, glutathione, or other sulfhydryl-containing compounds from cell culture media during bioprocessing, or formation of dimers and higher oligomers due to cysteines linked by covalent disulfide bridges. Among
- 30 PTMs, deamidation of asparagine (Asn, N) residues, isomerization of aspartate (aspartic acid, Asp, D) residues, and formation of succinimide intermediates are the most frequent modification reactions for therapeutic antibodies during production, storage or *in vivo* after

administration. Deamidation of Asn and isomerization of Asp depend on sequence liabilities, the structural environment and on the storage conditions, particularly the solution pH and storage temperature. These modifications may lead to decreased or even loss of function or biological activity, especially if the affected residues are involved in target binding. Asn and Asp residues are at risk for modifications particularly when they are located in structurally flexible regions such as CDR loops, and when certain other structural prerequisites are met, whereas framework regions have been observed to be comparatively resistant to modifications. In addition to the structural location of Asn and Asp residues, canonic motifs of Asn deamidation and of Asp isomerization have also been identified. These canonical motifs are NG, NS, NN, NT, NH, and DG, DS, DD, DT and DH, respectively (Lu et al. 2019). Upon in-silico analysis, the disclosed antibodies present a DG Asp-isomerization motif in the last amino acid of CDR2 of the VL domain and in the CH2 and CH3 regions of the HC (VL-CDR2 (at position 62), CH2 (at position 282), CH3 (at position 403)).

Isomerization of Asp can be tested by subjecting the antibodies to low pH (i.e. pH 5.5) and heat (i.e. 40°C) for two weeks, while Asn deamidation of antibodies can be tested by subjecting the antibodies to high pH (i.e. pH 8.0) and heat (i.e. 40°C) for one week, mimicking production and storage conditions.

The inventors have now shown that the disclosed antibodies, under these harsh conditions, albeit containing Asn and Asp in their CDRs, and bearing an Asp-Gly (DG) Asp-isomerization motif, surprisingly were free of Asn deamidation (see Table 6) and Asp isomerization (see Table 7) and that their binding affinity to CLDN18.2 was not affected. IMAB362 on the other hand showed Asn deamidation under such conditions, inducing a loss of binding affinity (as seen in Table 6 and Figure 10). The invention thus provides isolated antibodies or fragments thereof that bind to CLDN18.2 and which are less prone than IMAB362 to PTMs during production, storage and clinical application (*in vivo*) and that warrants for maintained binding affinity to CLDN18.2 during production, storage and clinical application (*in vivo*).

The invention also provides an antibody binding to the same epitope as an antibody described herein. In one embodiment, the antibody binds to the same epitope as an antibody comprising a heavy chain sequence of SEQ ID NO: 46 and a light chain sequence of SEQ ID NO: 51.

The invention further provides an antibody competing for binding with an antibody described herein. In one embodiment, the antibody competes for binding with an antibody comprising a heavy chain sequence of SEQ ID NO: 46 and a light chain sequence of SEQ ID NO: 51.

The invention further provides an antibody that competitively inhibits binding of an antibody described herein to Claudin 18.2. In one embodiment, the antibody competitively inhibits binding of an antibody comprising a heavy chain sequence of SEQ ID NO: 46 and a light chain sequence of SEQ ID NO: 51 to Claudin 18.2.

- 5 Suitable methods to detect binding of antibodies to the same antigen include approaches to map the antigen-antibody interactions. Such approaches have been described in Abbott 2014 (Abbott, Damschroder, and Lowe 2014). Suitable methods to detect competition include competitive assays by epitope binning, as described in Abdiche 2009 (Abdiche et al. 2009). Suitable method for detecting competitive inhibition include ELISA assays.
- 10 According to one embodiment, the invention provides nucleic acid sequences encoding the isolated tumor-specific antibodies or functional fragments thereof that bind CLDN18.2. The nucleic acid sequences may encode for the CDRs alone, for the VH and VL regions, or for the entire heavy and light chains of the antibodies. These nucleic acid sequences may be found in Table 3. The nucleic acid sequence may also encode for F(ab)₂, Fv, scFv, IgGACH2, F(ab')₂,
15 scFvCH3, Fab, VL, VH, scFv4, scFv3, scFv2, dsFv, Fv, scFv-Fc, (scFv)₂, a non-depleting IgG, a diabody, a bivalent antibody or Fc-engineered versions thereof. The encoded immunoglobulin may be an IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, synthetic IgG, IgM or mutated and Fc-engineered versions thereof.

- In yet another embodiment, the nucleic acid sequence may also encode a CAR construct that
20 binds to CLDN18.2. Ample guidance on construction of CAR T cells may be found in Chang and Chen (2017) or June and Sadelain (2018). In one embodiment, the invention provides a T cell that has been genetically engineered to produce an artificial T-cell receptor, e.g. a chimeric antigen receptor (CAR), wherein the artificial T-cell receptor comprises the antibody or functional fragment thereof of the present invention that binds to CLDN18.2.

- 25 In yet another embodiment, the invention provides a tumor-specific antibody-based binding protein that specifically binds to CLDN18.2. Such binding protein may contain at least a CLDN18.2 binding domain of the disclosed antibodies and another protein domain not related to antibodies. The invention also provides a modified antibody format that binds to CLDN18.2.

- The invention also provides an expression vector comprising a nucleic acid of the invention or
30 a degenerate nucleic acid as a result of codon degeneracy. The expression vector may be an expression vector for protein expression in mammalian cells, bacteria, fungal or insect cells,

and chosen for the type of host cell bearing the expression vector comprising the nucleic acid encoding the antibodies or functional fragments thereof. Ample guidance for the construction of such vectors may be found in Green and Sambrook (Green and Sambrook 2012).

5 In another embodiment, the invention provides a host cell comprising a nucleic acid or an expression vector of the present invention. The host cell may be a mammalian cell or cell line, a bacterial cell, a fungal cell or an insect cell.

10 In another embodiment, the invention relates to an antibody or fragment thereof binding to CLDN18.2, the nucleic acid encoding the antibody or fragment thereof, the vector comprising the nucleic acid or the host cells comprising the nucleic acid or the vector, for use in the treatment of a subject that is suffering from a neoplastic disease.

15 In another embodiment, the invention relates to an antibody or fragment thereof binding to CLDN18.2, the nucleic acid encoding the antibody or fragment thereof, the vector comprising the nucleic acid or the host cells comprising the nucleic acid or the vector, for use in the treatment of a subject that is at risk of developing a neoplastic disease, and/or for use in the treatment of a subject being diagnosed for a neoplastic disease.

The disclosed antibodies or fragments thereof may be used as monotherapy. In a preferred embodiment, the disclosed antibodies or fragments thereof are used in combination with the established standard of care of the neoplastic disease.

20 The neoplastic disease may be at least one disease selected from the group consisting of pancreatic, gastric, esophageal, ovarian and lung cancer. It is understood that the neoplastic disease to be treated expresses CLDN18.2.

In one embodiment, the subject is a mammal. In a preferred embodiment, the subject is a human.

25 Another embodiment of the invention provides a method for treating a neoplastic disease, including pancreatic, gastric, esophageal, ovarian or lung cancer, with an antibody or functional fragment thereof that binds to CLDN18.2, wherein the method comprises administering a pharmaceutically effective amount of the antibody or functional fragment thereof to a subject in need thereof. The method of treatment may be a monotherapy or preferably a combination therapy with the established standard of care of the neoplastic disease.

30 The amino acid sequence of human CLDN18.2 protein can be derived from NCBI reference sequence: NP_001002026.1. The sequence is also disclosed as SEQ ID NO: 133.

DESCRIPTION OF DRAWINGS

Figure 1: Evaluation by ELISA of the binding to lipoparticles containing CLDN18.2 or null-lipoparticles of selected chimeric and humanized anti-CLDN18.2 antibodies as indicated. A. Chimeric antibodies cC11-1, cC11-2, cC11-3, IMAB362 and only secondary antibody; B. Humanized antibodies hC11a to hC11j, chimeric cC11-1, IMAB362 and only secondary antibody. All newly generated antibodies bind to liposomal CLDN18.2.

Figure 2: Sorting of PA-TU-8988S cells for expression levels of CLDN18.2. A. FC profile of PA-TU-8988S stained with IMAB362. B. FC profile of PA-TU-8988S cells sorted by FACS for high expression of CLDN18.2.

Figure 3: Generation of HEK293T cells overexpressing huCLDN18.2. HEK293T cells, not expressing endogenously CLDN18.2, were transfected with a plasmid coding for huCLDN18.2 to stably express CLDN18.2 or coding for huCLDN18.1 to stably express CLDN18.1. The expression was analyzed by FC after staining with IMAB362, and a panCLDN18.1 antibody or an anti-human IgG secondary antibody only. A. FC profile of un-transfected HEK293T cells. B. FC profile of transfected HEK293T cells stably expressing CLDN18.1. C. FC profile of transfected HEK293T cells stably expressing CLDN18.2.

Figure 4: Flow cytometry binding assay of chimeric cC11-1, cC11-2 and cC11-3 antibodies to pre-B cell L11 cells overexpressing CLDN18.1 or CLDN18.2. The chimeric antibodies bind to CLDN18.2 and not to CLDN18.1. IMAB362 was used as positive binding control.

Figure 5: Flow cytometry binding assay of humanized hC11a to hC11j antibodies to HEK293T cells overexpressing CLDN18.1 or CLDN18.2. The humanized antibodies bind to CLDN18.2 and not to CLDN18.1. IMAB362 and cC11-1 were used as positive binding control.

Figure 6: FACS expression profiles of A549 cells overexpressing CLDN18.2. A549 cells, not expressing endogenously CLDN18.2, were stably transfected with a plasmid coding for CLDN18.2 and the expression of CLDN18.2 was analyzed by FACS using IMAB362.

Figure 7: Flow cytometry live-cell staining. Graph representing the percentage of isolated single cells bound by CLDN18.2 antibodies (cC11-1, hC11a, hC11b, hC11c, hC11f and IMAB362). Single cells were isolated either from a mouse tumor expressing CLDN18.2 arising from injected A549 cells overexpressing CLDN18.2 (solid bars) or from a mouse healthy stomach expressing CLDN18.2 (open bars).

Figure 8: Staining of frozen stomach tissue. Frozen tissue slides of mouse healthy stomach tissue expressing CLDN18.2 have been stained with hC11a (A), hC11b (B), hC11c (C), hC11f (D) or IMAB362 (E) antibodies. Pictures are representative IHC images.

Figure 9: Staining of frozen tumor tissue arising from injected A549 cells overexpressing CLDN18.2. Frozen tissue slides of mouse tumor expressing CLDN18.2 have been stained with hC11a (A), hC11f (B), IMAB362 (C) or the Abcam 34H14L15 pan-CLDN18 antibodies. Pictures are representative IHC images.

Figure 10: Effect of deamidation on the binding activity of IMAB362. The affinity of IMAB362 to CLDN18.2 decreases after deamidation.

10 EXAMPLES

Example 1: Generation of chimeric and humanized antibodies

Techniques to generate monoclonal antibodies have been well-established. The Handbook of Therapeutic Antibodies, Second Edition (2014), gives ample information on these techniques, such as the production of monoclonal antibodies by immunization of mice or rats (Moldenhauer 15 2014), humanization of monoclonal antibodies (Saldanha 2014), bioinformatics tools for analysis of antibodies (Martin and Allemn 2014) or development and manufacture of therapeutic antibodies (Jacobi et al. 2014). In brief, monoclonal antibodies against CLDN18.2 were generated by DNA immunization of rats with a plasmid coding for the human CLDN18.2 cDNA (huCLDN18.2) (NCBI Reference Sequence: NM_001002026.3). The specific reactivity 20 of rat immune sera against huCLDN18.2 was analyzed by flow cytometry (FC analysis) and ELISA. Hybridoma clones were subsequently generated from lymphocytes isolated from the immunized rats to obtain chimeric antibodies. Three clones were identified as being CLDN18.2-specific, resulting in the chimeric antibodies named cC11-1, cC11-2 and cC11-3 with similar CDRs (see Table 3). Subsequently, cC11-1 cC11-2 and cC11-3 were humanized, resulting 25 in 10 humanized clones named hC11a, hC11b, hC11c, hC11d, hC11e, hC11f, hC11g, hC11h, hC11i and hC11j antibodies (see Table 3).

As a control, the IMAB362 antibody was synthesized using the sequences of the heavy (SEQ ID NO: 55) and light chain (SEQ ID NO: 56) as published in WO2013/174509 and designated as monoclonal antibody 182-D1106-362, accession no. DSM ACC2810, deposited on 26 30 October, 2006 at the DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH Inhoffenstr. 7 B 38124 Braunschweig DE.

Table 3: antibody nucleic acid and amino acid sequences

NAME	SEQUENCE	SEQ ID NO
cCII-1		
HCDR1	DYAMH	SEQ ID NO: 1
HCDR2	WINTYTGKPTYADDFKG	SEQ ID NO: 2
HCDR3	AVFYGYTMDA	SEQ ID NO: 3
VH	QIQLVQSGPELKKPGESVKISCKASGYTFTDYAMHWVKQAPGK GLKWMGWINTYTGKPTYADDFKGRFVFSLEASASTANLQISNL KNEDTATYFCARAVFYGYTMDAWGQGTSTVTVSS	SEQ ID NO: 27
HCDR1	gactacgcgatgcac	SEQ ID NO: 71
HCDR2	tggatcaacacgtacacggggaagccgacatacgcggacgact tcaagggg	SEQ ID NO: 72
HCDR3	gccgtcttctacggatatacgcgatggacgcg	SEQ ID NO: 73
VH	cagatccagctcgtccagagcgggcccggagctgaagaagccgg gggagagcgtgaagatctcgtgcaaggcgagcggatatacgtt cacggactacgcgatgcactgggtcaagcaagcgcgggggaaa gggctgaagtggatgggtggatcaacacgtacacggggaagc cgacatacgcggacgacttcaaggggcgattcgtgttctcgt ggaggcgagcgcgagcacggcgaaacctgcaaactctgaacctg aagaacgaggacacggcgacgtacttctgcgcgcgggcccgtct tctacggatatacgcgatggacgcgtggggggcaggggtaccagcgt gacgggtctcgagc	SEQ ID NO: 74
LCDR1	RASEDIYSNLA	SEQ ID NO: 4
LCDR2	SVKRLQD	SEQ ID NO: 5
LCDR3	LQGSNFPLT	SEQ ID NO: 6
VL	DIQMTQSPASLSASLGETISIACRASEDIYSNLAWEYQQKSGKS PQLLIFSVKRLQDGVPSRFSGSGSGTQYSLKISGMQPEDEGDY FCLQGSNFPLTFGSGTKLEIK	SEQ ID NO: 28
LCDR1	cgggcgagcgcgaggacatctactcgaacctggcg	SEQ ID NO: 75
LCDR2	tccgtcaagcggctgcaagac	SEQ ID NO: 76
LCDR3	ctgcaagggagcaacttcccgcgtgacg	SEQ ID NO: 77
VL	gacatccagatgacgcagagcccggcgctcgtgagcgcgagcc tgggggagacgatctcgatcgcggtgcccggcgagcgcgaggacat ctactcgaacctggcgtggatcaacagaagagcgggaagagc ccgcagctgctgatcttctccgtcaagcggctgcaagacggcg tcccagcgcgatctcggggagcgggagcgggacgcagctactc gctgaagatctcggggatgcagccggaggacgagggggactac ttctgcctgcaagggagcaacttcccgcgtgacgttcgggtcgg gtaccaaactcgagatcaaa	SEQ ID NO: 78
cCII-2		
HCDR1	DYAMH	SEQ ID NO: 1
HCDR2	WINAYTGKPTYADDFKG	SEQ ID NO: 7
HCDR3	AVYYGYTMDA	SEQ ID NO: 8
VH	QIQLVQSGPELKKPGESVKISCKTSGYTFTDYAMHWVKQGPGK GMKWMGWINAYTGKPTYADDFKGRFVLSLEASASTANLQISNL KNEDTATYFCARAVYYGYTMDAWGQGTSTVIVSS	SEQ ID NO: 29
HCDR1	gactacgcgatgcac	SEQ ID NO: 71
HCDR2	tggatcaacgcgtacacggggaagccgacctacgcggacgact tcaagggg	SEQ ID NO: 79
HCDR3	gccgtctactacggatatacgcgatggac	SEQ ID NO: 80

NAME	SEQUENCE	SEQ ID NO
VH	cagatccagctcgtccagagcgggcccggagctgaagaagccgg gggagagcgtgaagatctcgtgcaagacgagcggatatacgtt cacggactacgcgatgcactgggtcaagcaggggcccagggaaa gggatgaagtggatgggtggatcaacgcgtacacggggaagc cgacctacgcggacgacttcaaggggcgattcgtgctgagcct ggaggcagcgcctcgacggcgaacctgcaaattctcgaacctg aagaacgaggacacggcgacgtacttctgcgcgcgggcccgtct actacggatatacgtggacgcgtgggggacaggtaccagcgt gatcgtctcgagc	SEQ ID NO: 81
LCDR1	RTSEDIYSNFA	SEQ ID NO: 9
LCDR2	SVNRLQD	SEQ ID NO: 10
LCDR3	LQGSKFPLT	SEQ ID NO: 11
VL	DIQMTQSPASLSASLGETISIECRTSEDIYSNFAWFQKSGKS PQLLIYSVNRLQDGVPSRFSGSGSGTQYSLKISGMQPEDEGDY FCLQGSKFPLTFGSGTKLEIK	SEQ ID NO: 30
LCDR1	cggacgagcaggacatctactcgaacttcgcg	SEQ ID NO: 82
LCDR2	tcagtcaaccggctgcaagac	SEQ ID NO: 83
LCDR3	ctgcaagggagcaagttcccgcgtgacg	SEQ ID NO: 84
VL	gacatccagatgacgcagagcccggcgagcctgagcgcgagcc tgggggagacgatctcgatcgagtgcgggacgagcaggacat ctactcgaacttcgcgtggttccagcagaagagcgggaagagc ccgcagctgctgatctactcagtcaaccggctgcaagacggcg tcccagagccgattctcggggagcgggagcgggacgcagtactc gctgaagatctcggggatgcagccggaggacgagggggactac ttctgcctgcaaggagcaagttcccgcgtgacgttcgggagcg gtaccaaactcgagatcaaa	SEQ ID NO: 85
cCI1-3		
HCDR1	DYAMY	SEQ ID NO: 12
HCDR2	WINTYTGKPTYADDFKG	SEQ ID NO: 2
HCDR3	AVFYGYTMDA	SEQ ID NO: 3
VH	QIQLVQSGPELKKPGESVKISCKASGYTFTDYAMYWVKQVPGK GLRWMGWINTYTGKPTYADDFKGRFVFSLEASASTANLQISNL KNEDTATYFCARAVFYGYTMDAWGQGTSTVTVSS	SEQ ID NO: 31
HCDR1	gactacgcgatgtac	SEQ ID NO: 86
HCDR2	tggatcaacacgtacacggggaagccgacctacgcggacgact tcaagggg	SEQ ID NO: 87
HCDR3	gccgtcttctacggatatacgtggacgcg	SEQ ID NO: 73
VH	cagatccagctcgtccagagcgggcccggagctgaagaagccgg gggagagcgtgaagatctcgtgcaaggcgagcggatatacgtt cacggactacgcgatgtactgggtcaagcaagtgcgggggaaa gggctgcgatggatgggtggatcaacacgtacacggggaagc cgacctacgcggacgacttcaaggggcgattcgtgttctcgtc ggaggcagcgcgagcacggcgaacctgcaaattctcgaacctg aagaacgaggacacggcgacgtacttctgcgcgcgggcccgtct tctacggatatacgtggacgcgtgggggacaggtaccagcgt gacggtctcgagc	SEQ ID NO: 88
LCDR1	RTSEDIYSNLA	SEQ ID NO: 13
LCDR2	AIKRLQD	SEQ ID NO: 14
LCDR3	LQGSKFPLT	SEQ ID NO: 11

NAME	SEQUENCE	SEQ ID NO
VL	DIQMTQSPASLSASLGETISIA CRTSEDIYSNLAWYQQKSGKS PQLLI FAIKRLQDGVPSRFSGSGSGTQYSLKISGMQPEDEGDY FCLQGSKFPLTFGSGTKLEIK	SEQ ID NO: 32
LCDR1	cggacgagcggagacatctactcgaacctggcg	SEQ ID NO: 89
LCDR2	gcgatcaagcggctgcaagac	SEQ ID NO: 90
LCDR3	ctgcaagggagcaagttcccgcgtgacg	SEQ ID NO: 84
VL	gacatccagatgacgcagagcccggcgagcctgagcgcgagcc tgggggagacgatctcgatcgctgcccggacgagcggagacat ctactcgaacctggcgtggtatcaacagaagagcgggaagagc ccgcagctgctgatcttcgcgatcaagcggctgcaagacggcg tcccgagccgattctcggggagcgggagcgggacgcagtactc gctgaagatctcggggatgcagccggaggacgagggggactac ttctgcctgcaagggagcaagttcccgcgtgacgttcgggtcgg gtaccaaactcgagatcaaa	SEQ ID NO: 91
hCIIa		
HCDR1	DYAMH	SEQ ID NO: 1
HCDR2	WINTYTGKPTYAQKFQG	SEQ ID NO: 15
HCDR3	AVFYGYTMDA	SEQ ID NO: 3
VH	QVQLVQSGAEVKKPGASVKVSKASGYTFTDYAMHWVRQAPGQ RLEWMGWINTYTGKPTYAQKFQGRVTITRDTASTAYMELSSL RSEDTAVYYCARAVFYGYTMDAWGQGLTVTVSS	SEQ ID NO: 33
Heavy chain	QVQLVQSGAEVKKPGASVKVSKASGYTFTDYAMHWVRQAPGQ RLEWMGWINTYTGKPTYAQKFQGRVTITRDTASTAYMELSSL RSEDTAVYYCARAVFYGYTMDAWGQGLTVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYITLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVSM HEALHNHYTQKSLSLSPGK	SEQ ID NO: 46
HCDR1	gactacgcgatgcac	SEQ ID NO: 71
HCDR2	tggatcaatacatcacgggggaagccgacttatgcgcaaaaat tccaagga	SEQ ID NO: 92
HCDR3	gcggtcttctacggatatacgatggatgcc	SEQ ID NO: 93
VH	caggtccaactagtcctaaagcggggcggaagtcaagaagcccg gagcatccgtcaaagtcagctgcaaggcgagcggatatacatt tacggactacgcgatgcactgggtcaggcaagcccctgggcaa aggctcgaatggatggatggatcaatacatcacgggggaagc cgacttatgcgcaaaaattccaaggaagagtcacaattacgcg ggatacatccgcacatctaccgcctacatggagctaagctcgctg cggagcggagatacggcgggtctactattgcgcccgcgagcgggtct tctacggatatacgatggatgcctgggggcagggtaccctgggt cacgggtctcgagc	SEQ ID NO: 94
LCDR1	RASEDIYSNLA	SEQ ID NO: 4
LCDR2	SVKRLQD	SEQ ID NO: 5
LCDR3	LQGSNFPLT	SEQ ID NO: 6
VL	DIQMTQSPSSLSASVGDRTITCRASEDIYSNLAWYQQKPGKA PKLLIFSVKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSNFPLTFGQGTKVEIK	SEQ ID NO: 38

NAME	SEQUENCE	SEQ ID NO
Light chain	DIQMTQSPSSLSASVGDRTITCRASEDIYSNLAWYQQKPGKA PKLLIFSVKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSNFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKST YLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 51
LCDR1	agggcctccgaagacatctactccaacctggca	SEQ ID NO: 95
LCDR2	agcgtcaaaagactacaagat	SEQ ID NO: 96
LCDR3	ttgcaaggaagcaatttccccttgact	SEQ ID NO: 97
VL	gacattcaaatgacgcaaagcccatcatcgctgagcgcatcgg tcggggatagagtcaccataacatgcaggcctccgaagacat ctactccaacctggcatggtatcaacaaaaaccggggaaggct ccgaagctgctgatatttagcgtcaaaagactacaagatggag taccgagccgattttcgggaagcgggagcgggacggatttcac gctgaccatatcaagtttgcaaccggaggattttgacacatac tattgcttgcaaggaagcaatttccccttgactttcgggcaag gtaccaaggtcgagatcaaa	SEQ ID NO: 98
hC11b		
HCDR1	DYAMH	SEQ ID NO: 1
HCDR2	WINTYTGKPTYSQKFQG	SEQ ID NO: 16
HCDR3	AVFYGYTMDA	SEQ ID NO: 3
VH	QVQLVQSGAEVKKPGASVKVSCASGYTFTDYAMHWVRQAPGQ RLEWMGWINTYTGKPTYSQKFQGRVTITRDTASTAYMELSSL RSEDTAVYYCARAVFYGYTMDAWGQGTTLVTSS	SEQ ID NO: 34
Heavy chain	QVQLVQSGAEVKKPGASVKVSCASGYTFTDYAMHWVRQAPGQ RLEWMGWINTYTGKPTYSQKFQGRVTITRDTASTAYMELSSL RSEDTAVYYCARAVFYGYTMDAWGQGTTLVTSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKHTHTCPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVSVLTFLHQLDNLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSFSVM HEALHNHYTQKSLSLSPGK	SEQ ID NO: 47
HCDR1	gattatgcaatgcac	SEQ ID NO: 99
HCDR2	tggattaacacctacacgggcaagccacatactcccaaaaat tccaagga	SEQ ID NO: 100
HCDR3	gctgtattctatggatatacaatggatgcc	SEQ ID NO: 101
VH	caggtccaattagtccaaagcggggcggaagtcaagaagccgg ggcgagcgtcaaagtctcatgcaaagcgagcggatacacatt tacggattatgcaatgcactgggtcaggcaagcaccggacaa aggctggaatggatggatggattaacacctacacgggcaagc ccacatactcccaaaaattccaaggaaggtcacgataacgag agacacgagcgcgagcaccggaatggatggatggatggattaacac ctacacgggcaagccacatactcccaaaaattccaaggaag gtcacgataacgagagacacgagcgcgagcaccgtaccctggt caccgtctcgagc	SEQ ID NO: 102
LCDR1	RASEDIYSNLA	SEQ ID NO: 4
LCDR2	SVKRLQD	SEQ ID NO: 5
LCDR3	LQGSNFPLT	SEQ ID NO: 6

NAME	SEQUENCE	SEQ ID NO
VL	DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAWEYQQKPGKA PKLLIFSVKRLQDGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCLQGSNFPPLTFGQGTKVEIK	SEQ ID NO: 38
Light chain	DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAWEYQQKPGKA PKLLIFSVKRLQDGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCLQGSNFPPLTFGQGTKVEIKRTVAAPSVFI FPPSDEQLKSGT ASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS YLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 51
LCDR1	agggcctccgaagacatctactccaacctggca	SEQ ID NO: 95
LCDR2	agcgtcaaaagactacaagat	SEQ ID NO: 96
LCDR3	ttgcaaggaagcaatttccccttgact	SEQ ID NO: 97
VL	gacattcaaatgacgcaaagcccatcatcgctgagcgcacatcg tcggggatagagtaccataacatgcaggcctccgaagacat ctactccaacctggcatggatcaacaaaaaccggggaaggct ccgaagctgctgatatttagcgtcaaaagactacaagatggag taccgagccgattttcgggaagcgggagcgggacggatttcac gctgaccatatcaagtttgcaaccggaggattttgacacatac tattgcttgcaaggaagcaatttccccttgactttcgggcaag gtaccaaggtcgagatcaaa	SEQ ID NO: 98
hC11c		
HCDR1	DYAMH	SEQ ID NO: 1
HCDR2	WINTYTGKPTYSQKFQG	SEQ ID NO: 16
HCDR3	AVFYGYTMDA	SEQ ID NO: 3
VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAPGQ RLEWMGWINTYTGKPTYSQKFQGRVTITRDTASTAYMELSSL RSEDTAVYYCARAVFYGYTMDAWGQGLVTVSS	SEQ ID NO: 34
Heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAPGQ RLEWMGWINTYTGKPTYSQKFQGRVTITRDTASTAYMELSSL RSEDTAVYYCARAVFYGYTMDAWGQGLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKHTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVQLHQLDNLNGKEYCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSFSVM HEALHNHYTQKSLSLSPGK	SEQ ID NO: 47
HCDR1	gattatgcaatgcac	SEQ ID NO: 99
HCDR2	tggattaacacctacacgggcaagcccacatactcccaaaat tccaagga	SEQ ID NO: 100
HCDR3	gctgtattctatggatatacaatggatgcc	SEQ ID NO: 101
VH	cagggtccaattagtcctaaagcggggcggaagtcaagaagccgg ggcgagcgtcaaagtctcatgcaaagcgagcggatacacatt tacggattatgcaatgcactgggtcaggcaagcaccgggacaa aggctggaatggatggatggattaacacctacacgggcaagc ccatactcccaaaattccaaggaagggtcacgataacgag agacacgagcgcgagcaccggaatggatggggtggaattaacac ctacacgggcaagcccacatactcccaaaattccaaggaagg gtcacgataacgagagacacgagcgcgagcaccgtaccctggt caccgtctcgagc	SEQ ID NO: 102
LCDR1	RTSEDIYSNLA	SEQ ID NO: 17
LCDR2	AIKRLQD	SEQ ID NO: 14

NAME	SEQUENCE	SEQ ID NO
LCDR3	LQGSKFPLT	SEQ ID NO: 11
VL	DIQMTQSPSSLSASVGDRVTITCRTSEDIYSNLAWYQQKPGKA PKLLIFAIKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSKFPLTFGQGTKVEIK	SEQ ID NO: 39
Light chain	DIQMTQSPSSLSASVGDRVTITCRTSEDIYSNLAWYQQKPGKA PKLLIFAIKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSKFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKST YSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 52
LCDR1	cgaacgagcgaggacatatatactcaaaccttgca	SEQ ID NO: 103
LCDR2	gcgataaagaggtcgcaagac	SEQ ID NO: 104
LCDR3	ttgcaaggctccaaatttcccctgaca	SEQ ID NO: 105
VL	gacatccaaatgactcaaagcccatcatcgctatcggtcatcggt tcggggatagagtcacgataaactgcccgaacgagcgaggacat atactcaaaccttgcatggtatcaacaaaagccggggaaggcc ccgaagctactgatattcgcgataaagaggtcgcaagacggag ttccatcacgatttttcgggatctggtcggggaccgattttac gctgactatatcatcgctgcaaccggaagattttgcaacatac tactgcttgcaaggctccaaatttcccctgacattcggaacg gtaccaaggtcgagatcaaa	SEQ ID NO: 106
hCIId		
HCDR1	DYAMH	SEQ ID NO: 1
HCDR2	WINTYTGKPTYSQKFQG	SEQ ID NO: 16
HCDR3	AVFYGYTMDA	SEQ ID NO: 3
VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAPGQ RLEWMGWINTYTGKPTYSQKFQGRVTITRDTASASTAYMELSSL RSEDTAVYYCARAVFYGYTMDAWGQGTLVTVSS	SEQ ID NO: 34
Heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAPGQ RLEWMGWINTYTGKPTYSQKFQGRVTITRDTASASTAYMELSSL RSEDTAVYYCARAVFYGYTMDAWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSV HEALHNHYTQKSLSLSPGK	SEQ ID NO: 47
HCDR1	gattatgcaatgcac	SEQ ID NO: 99
HCDR2	tggattaacacctacacgggcaagccacatactccccaaaat tccaagga	SEQ ID NO: 100
HCDR3	gctgtattctatggatatacaatggatgcc	SEQ ID NO: 101
VH	caggtccaattagtccaaagcggggcggaagtcaagaagccgg ggcgagcgtcaaagtctcatgcaaagcgagcggatacacatt tacggattatgcaatgcactgggtcaggcaagcaccgggacaa aggctggaatggatggatggattaacacctacacgggcaagc ccacatactccccaaaattccaaggaagggtcacgataacgag agacacgagcgcgagcaccggaatggatgggatggattaacac ctacacgggcaagccacatactccccaaaattccaaggaagg gtcacgataacgagagacacgagcgcgagcaccgtaccctggt caccgtctcgagc	SEQ ID NO: 102
LCDR1	RTSEDIYSNFA	SEQ ID NO: 18

NAME	SEQUENCE	SEQ ID NO
LCDR2	SVNRLQD	SEQ ID NO: 19
LCDR3	LQGSKFPLT	SEQ ID NO: 11
VL	DIQMTQSPSSLSASVGDRVTITCRTSEDIYSNFAWYQQKPGKA PKLLIYSVNRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSKFPLTFGQGTKVEIK	SEQ ID NO: 40
Light chain	DIQMTQSPSSLSASVGDRVTITCRTSEDIYSNFAWYQQKPGKA PKLLIYSVNRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSKFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST YLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 53
LCDR1	cggacgagcgaggatatttattcgaactttgca	SEQ ID NO: 107
LCDR2	cagtcaatcggctacaagat	SEQ ID NO: 108
LCDR3	ctacaaggagcaaattcccgtgaca	SEQ ID NO: 84
VL	gacatccaaatgacgcaatcaccgagctcgctgagcgcacatctg tcggggaccgtgtcacaatcacatgccggacgagcgaggatat ttattcgaactttgcatggtatcaacaaaaaccgggcaaggct ccgaaacttttgatttattcagtcaatcggctacaagatggcg tcccgagccgatttagcgggagcggatcggaaccgactttac gctgacgatatcatcgctacaaccgaggacttcgcgacttat tactgcctacaaggagcaaattcccgtgacattcggacaag gtaccaaggtcgagatcaaa	SEQ ID NO: 109
hCIIe		
HCDR1	DYAMY	SEQ ID NO: 12
HCDR2	WINTYTGKPTYAQKFQG	SEQ ID NO: 15
HCDR3	AVFYGYTMDA	SEQ ID NO: 3
VH	QVQLVQSGAEVKKPGASVKVSCASGYTFTDYAMYWVRQAPGQ RLEWMGWINTYTGKPTYAQKFQGRVTITRDTASTAYMELSSL RSEDTAVYYCARAVFYGYTMDAWGQGLVTVSS	SEQ ID NO: 35
Heavy chain	QVQLVQSGAEVKKPGASVKVSCASGYTFTDYAMYWVRQAPGQ RLEWMGWINTYTGKPTYAQKFQGRVTITRDTASTAYMELSSL RSEDTAVYYCARAVFYGYTMDAWGQGLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKHTCTPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSV HEALHNHYTQKSLSLSPGK	SEQ ID NO: 48
HCDR1	gattacgcaatgtac	SEQ ID NO: 110
HCDR2	tggataaatacctatacgggaaagccaacatacgcggcaaaat tccaaggc	SEQ ID NO: 111
HCDR3	gccgtcttttatggatatacgcgagcga	SEQ ID NO: 112
VH	caggtccaactggtccaatcgggggctgaagtcaaaaagccgg ggcgagcgtcaaagtcagctgcaaagcatcgggatacacatt tacggattacgcaatgtactgggtcaggcaagcaccggccaa cgactggaatggatggctggataaatacctatacgggaaagc caacatacgcggcaaaattccaaggccgcgtcacaataacgcg ggacacgagcgcacgcagcgttatatggaactatcatcgctg cgatcggaagacacggcggtctattattgcgcacgcgcgctct tttatggatatacgcgagcgcgtgggggcagggtaccctggt cacggtctcgagc	SEQ ID NO: 113

NAME	SEQUENCE	SEQ ID NO
LCDR1	RASEDIYSNLA	SEQ ID NO: 4
LCDR2	SVKRLQD	SEQ ID NO: 5
LCDR3	LQGSNFPLT	SEQ ID NO: 6
VL	DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLA WYQQKPGKA PKLLIFSVKRLQDGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCLQGSNFPLTFGQGTKVEIK	SEQ ID NO: 38
Light chain	DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLA WYQQKPGKA PKLLIFSVKRLQDGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCLQGSNFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS YLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 51
LCDR1	agggcctccgaagacatctactccaacctggca	SEQ ID NO: 95
LCDR2	agcgtcaaaagactacaagat	SEQ ID NO: 96
LCDR3	ttgcaaggaagcaatttccccttgact	SEQ ID NO: 97
VL	gacattcaaatgacgcaaagcccatcatcgctgagcgcacggt tcggggatagagtcaccataacatgcagggcctccgaagacat ctactccaacctggcatggtatcaacaaaaacggggaaggct ccgaagctgctgatatttagcgtcaaaagactacaagatggag taccgagccgattttcgggaagcgggagcgggacggatttcac gctgaccatatcaagtttgcaaccggaggattttgacacatac tattgcttgcaaggaagcaatttccccttgactttcgggcaag gtaccaaggtcgagatcaaa	SEQ ID NO: 98
hCII1f		
HCDR1	DYAMH	SEQ ID NO: 1
HCDR2	WINAYTGKPTYAQKFQG	SEQ ID NO: 20
HCDR3	AVFYGYTMDA	SEQ ID NO: 3
VH	QVQLVQSGAEVKKPGASVKVSCASGYTFTDYAMHWVRQAPGQ RLEWMGINAYTGKPTYAQKFQGRVTITRDTASTAYMELSSL RSEDTAVYYCARAVFYGYTMDAWGQGTLLVTVSS	SEQ ID NO: 36
Heavy chain	QVQLVQSGAEVKKPGASVKVSCASGYTFTDYAMHWVRQAPGQ RLEWMGINAYTGKPTYAQKFQGRVTITRDTASTAYMELSSL RSEDTAVYYCARAVFYGYTMDAWGQGTLLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVFSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDITLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVM HEALHNHYTQKSLSLSPGK	SEQ ID NO: 49
HCDR1	gactacgcaatgcac	SEQ ID NO: 114
HCDR2	tggattaatgcctacacggggaagccgacctacgcacaaaaat tccaagga	SEQ ID NO: 115
HCDR3	gccgtcttctatggatatacgatggatgct	SEQ ID NO: 116
VH	caggtccaattggtccaaagcggggcgagggtcaagaagccgg ggcgagcgtcaaagtctcatgcaaggcaagcgatatacatt tacggactacgcaatgcactgggtccggcaagcccctgggcaa cggctggaatggatggatggattaatgcctacacggggaagc cgacctacgcacaaaaattccaaggacgagtcacgattacgcg ggatactagcgcgagcaccgcataatggagctaagctcgctg cgatctgaggataccgctgtatactactgcgcgagagccgtct	SEQ ID NO: 117

NAME	SEQUENCE	SEQ ID NO
	tctatggatatacgaatggatgcttgggggcagggtaccctggt cacggtctcgagc	
LCDR1	RASEDIYSNLA	SEQ ID NO: 4
LCDR2	SVKRLQD	SEQ ID NO: 5
LCDR3	LQGSNFPLT	SEQ ID NO: 6
VL	DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLA WYQQKPGKA PKLLIYSVKRLQDGVPSRFSGSGSGTDFTLT TISSLQPEDFATY YCLQGSNFPLTFGQGTKVEIK	SEQ ID NO: 41
Light chain	DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLA WYQQKPGKA PKLLIYSVKRLQDGVPSRFSGSGSGTDFTLT TISSLQPEDFATY YCLQGSNFPLTFGQGTKVEIK RTVAAPSVFIFPPSDEQLKSGT ASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 54
LCDR1	cgagcttcgaggacatctatagcaacttggt	SEQ ID NO: 118
LCDR2	agcgtcaaaaggctccaagac	SEQ ID NO: 119
LCDR3	ctacaaggctctaacttcccattgaca	SEQ ID NO: 120
VL	gatatccaaatgacgcaatcaccatctagcctatcggcctctg tgggggaccgagtcacatcacatgcccagcttcgaggacat ctatagcaacttggttggtatcaacaaagccggggaaagca ccaaagctgctgatatatagcgtcaaaaggctccaagacggag tccaagccgattctcgggctccggctccgggacggattttac gctgacaatttcgagcctgcaaccggaggactttgcaacctac tattgcctacaaggctctaacttcccattgacatttgggcaag gtaccaaggctcgagatcaaa	SEQ ID NO: 121
hCIIg		
HCDR1	DYAMH	SEQ ID NO: 1
HCDR2	WINAYTGKPTYAQKFQG	SEQ ID NO: 20
HCDR3	AVFYGYTMDA	SEQ ID NO: 3
VH	QVQLVQSGAEVKKPGASVKVSKASGYTFTDYAMHWVRQAPGQ RLEWMGINAYTGKPTYAQKFQGRVTITRDTASTAYMELSSL RSEDTAVYYCARAVFYGYTMDAWGQGLVTVSS	SEQ ID NO: 36
Heavy chain	QVQLVQSGAEVKKPGASVKVSKASGYTFTDYAMHWVRQAPGQ RLEWMGINAYTGKPTYAQKFQGRVTITRDTASTAYMELSSL RSEDTAVYYCARAVFYGYTMDAWGQGLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKHTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVM HEALHNHYTQKSLSLSPGK	SEQ ID NO: 49
HCDR1	gactacgcaatgcac	SEQ ID NO: 114
HCDR2	tggattaatgcctacacggggaagccgacctacgcacaaaaat tccaagga	SEQ ID NO: 115
HCDR3	gccgtcttctatggatatacgaatggatgct	SEQ ID NO: 116
VH	caggtccaattggtccaaagcggggcggaggtcaagaagccgg ggcgagcgtcaaagtctcatgcaaggcaagcggatatacatt tacggactacgcaatgcaactgggtccggcaagcccctgggcaa cggctggaatggatggatggaatgaatgcctacacggggaagc cgacctacgcacaaaaattccaaggacgagtcacgattacgag ggatactagcgcgagcaccgcataatggagctaagctcgctg	SEQ ID NO: 117

NAME	SEQUENCE	SEQ ID NO
	cgatctgaggataccgctgtatactactgcgcgagagccgtct tctatggatatacgaatggatgcttgggggcagggtaccctggt cacggtctcgagc	
LCDR1	RTSEDIYSNFA	SEQ ID NO: 18
LCDR2	SVNRLQD	SEQ ID NO: 19
LCDR3	LQGSKFPLT	SEQ ID NO: 11
VL	DIQMTQSPSSLSASVGDRVTITCRTSEDIYSNFAWYQQKPGKA PKLLIYSVNRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSKFPLTFGQGTKVEIK	SEQ ID NO: 40
Light chain	DIQMTQSPSSLSASVGDRVTITCRTSEDIYSNFAWYQQKPGKA PKLLIYSVNRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSKFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKST YLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 53
LCDR1	cggacgagcgaggatatttattcgaactttgca	SEQ ID NO: 107
LCDR2	cagtcaatcggctacaagat	SEQ ID NO: 108
LCDR3	ctacaaggagcaaattcccgcgtgaca	SEQ ID NO: 84
VL	gacatccaaatgacgcaatcaccgagctcgctgagcgcatctg tcggggaccgtgtcacaatcacatgccggacgagcgaggatat ttattcgaactttgcatggtatcaaaaaaacgggcaaggct ccgaaacttttgatttattcagtcaatcggctacaagatggcg tcccgagccgatttagcgggagcggatcggaaccgactttac gctgacgatatcatcgctacaaccggaggacttcgcgacttat tactgcctacaaggagcaaattcccgcgtgacattcggacaag gtaccaaggtcgagatcaaa	SEQ ID NO: 109
hCIIh		
HCDR1	DYAMY	SEQ ID NO: 12
HCDR2	WINAYTGKPTYAQKFQG	SEQ ID NO: 20
HCDR3	AVYYGYTMDA	SEQ ID NO: 8
VH	QVQLVQSGAEVKKPGASVKVSKASGYTFTDYAMYWVRQAPGQ RLEWMGWINAYTGKPTYAQKFQGRVTITRDTASASTAYMELSSL RSED TAVYYCARAVYYGYTMDAWGQGT LVT VSS	SEQ ID NO: 37
Heavy chain	QVQLVQSGAEVKKPGASVKVSKASGYTFTDYAMYWVRQAPGQ RLEWMGWINAYTGKPTYAQKFQGRVTITRDTASASTAYMELSSL RSED TAVYYCARAVYYGYTMDAWGQGT LVT VSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLT VLVHQLDNLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV M HEALHNHYTQKSLSLSPGK	SEQ ID NO: 50
HCDR1	gactacgctatgtat	SEQ ID NO: 122
HCDR2	tggattaatgcctacaccgggaagccgacttatgcgcaaaaat ttcaagga	SEQ ID NO: 123
HCDR3	gcggtctactatggatatacgaatggacgca	SEQ ID NO: 124
VH	caggtccaactggttcaatctggagcggaagtcaagaagcccg gagcatccgtcaaagtctcgtgcaaggcatctggatacacatt caccgactacgctatgtattgggtccggcaagcccccgacaa cggctggaatggatggatggattaatgcctacaccgggaagc cgacttatgcgcaaaaatttcaaggaagggtcacgattacgcg	SEQ ID NO: 125

NAME	SEQUENCE	SEQ ID NO
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LCDR1	RASEDIYSNLA	SEQ ID NO: 4
LCDR2	SVKRLQD	SEQ ID NO: 5
LCDR3	LQGSNFPLT	SEQ ID NO: 6
VL	DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLA PKLLIYSVKRLQDGVPSRFSGSGSGTDFTLT YCLQGSNFPLTFGQGTKVEIK	SEQ ID NO: 41
Light chain	DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLA PKLLIYSVKRLQDGVPSRFSGSGSGTDFTLT YCLQGSNFPLTFGQGTKVEIKRTVAAPSVFI ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK YSLSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 54
LCDR1	cgagcttcggaggacatctatagcaacttggt	SEQ ID NO: 118
LCDR2	agcgtcaaaaggctccaagac	SEQ ID NO: 119
LCDR3	ctacaaggctctaacttcccattgaca	SEQ ID NO: 120
VL	gatatccaaatgacgcaatcaccatctagcctatcggcctctg tgaggagcagtcaccatcacatgccgagcttcggaggacat ctatagcaacttggttggtatcaacaaagccggggaaagca ccaaagctgctgatataatagcgtcaaaaggctccaagacggag tccaagccgattctcgggctccggctccgggacggattttac gctgacaatttcgagcctgcaaccggaggactttgcaacctac tattgcctacaaggctctaacttcccattgacatttgggcaag gtaccaaggtcgagatcaaa	SEQ ID NO: 121
hCIIi		
HCDR1	DYAMY	SEQ ID NO: 12
HCDR2	WINAYTGKPTYAQKFQG	SEQ ID NO: 20
HCDR3	AVYYGYTMDA	SEQ ID NO: 8
VH	QVQLVQSGAEVKKPGASVKVSKASGYTFTDYAMYWVRQAPGQ RLEWMGWINAYTGKPTYAQKFQGRVTITRDTASTAYMELSSL RSEDTAVYYCARAVYYGYTMDAWGQGLVTVSS	SEQ ID NO: 37
Heavy chain	QVQLVQSGAEVKKPGASVKVSKASGYTFTDYAMYWVRQAPGQ RLEWMGWINAYTGKPTYAQKFQGRVTITRDTASTAYMELSSL RSEDTAVYYCARAVYYGYTMDAWGQGLVTVSSASTKGPSVFP LAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKHTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVHLQDNLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSV HEALHNHYTQKSLSLSPGK	SEQ ID NO: 50
HCDR1	gactacgctatgtat	SEQ ID NO: 122
HCDR2	tggattaatgcctacaccgggaagccgacttatgcgcaaaaat ttcaagga	SEQ ID NO: 123
HCDR3	gcggtctactatggatatacgatggacgca	SEQ ID NO: 124
VH	caggtccaactggttcaatctggagcggaagtcaagaagcccg gagcatccgtcaaagtctcgtgcaaggcatctggatacacatt caccgactacgctatgtattgggtccggcaagccccgggaca cggctggaatggatggatggatgattaatgcctacaccgggaagc	SEQ ID NO: 125

NAME	SEQUENCE	SEQ ID NO
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LCDR1	RASEDIYSNLA	SEQ ID NO: 4
LCDR2	SVKRLQD	SEQ ID NO: 5
LCDR3	LQGSNFPLT	SEQ ID NO: 6
VL	DIQMTQSPSSLSASVGDRTTITCRASEDIYSNLA WYQQKPGKA PKLLIFSVKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSNFPLTFGQGTKVEIK	SEQ ID NO: 38
Light chain	DIQMTQSPSSLSASVGDRTTITCRASEDIYSNLA WYQQKPGKA PKLLIFSVKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSNFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 51
LCDR1	agggcctccgaagacatctactccaacctggca	SEQ ID NO: 95
LCDR2	agcgtcaaaagactacaagat	SEQ ID NO: 96
LCDR3	ttgcaaggaagcaattttccccttgact	SEQ ID NO: 97
VL	gacattcaaagtacgcaaaagcccatcatcgctgagcgcatcgg tcggggatagagtcaccataacatgcagggcctccgaagacat ctactccaacctggcatggtatcaacaaaaaccggggaaggct ccgaagctgctgatatttagcgtcaaaagactacaagatggag taccgagccgattttcgggaagcgggagcgggacggatttcac gctgaccatatcaagtttgcaaccggaggatttttgcgacatac tattgcttgcaaggaagcaattttccccttgactttcgggcaag gtaccaaggtcgagatcaaa	SEQ ID NO: 98
hC11j		
HCDR1	DYAMY	SEQ ID NO: 12
HCDR2	WINAYTGKPTYAQKFQG	SEQ ID NO: 20
HCDR3	AVYYGYTMDA	SEQ ID NO: 8
VH	QVQLVQSGAEVKKPGASVKVSKASGYTFTDYAMYWVRQAPGQ RLEWMGINAYTGKPTYAQKFQGRVTITRDTASTAYMELSSL RSEDTAVYYCARAVYYGYTMDAWGQGLVTVSS	SEQ ID NO: 37
Heavy chain	QVQLVQSGAEVKKPGASVKVSKASGYTFTDYAMYWVRQAPGQ RLEWMGINAYTGKPTYAQKFQGRVTITRDTASTAYMELSSL RSEDTAVYYCARAVYYGYTMDAWGQGLVTVSSASTKGPSVFP LAPSSKSTSGGTAAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTFLHQLDNLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSFSVM HEALHNHYTQKSLSLSPGK	SEQ ID NO: 50
HCDR1	gactacgctatgtat	SEQ ID NO: 122
HCDR2	tggattaatgcctacaccgggaagccgacttatgcgcaaaaat ttcaagga	SEQ ID NO: 123
HCDR3	gcgggtctactatggatatacatgatggacgca	SEQ ID NO: 124
VH	caggtccaactgggtcaatctggagcgggaagtcaagaagcccg gagcatccgtcaaagtctcgtgcaaggcatctggatacacatt caccgactacgctatgtattgggtccggcaagccccggacaa	SEQ ID NO: 125

NAME	SEQUENCE	SEQ ID NO
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LCDR1	RTSEDIYSNLA	SEQ ID NO: 17
LCDR2	AIKRLQD	SEQ ID NO: 14
LCDR3	LQGSKFPLT	SEQ ID NO: 11
VL	DIQMTQSPSSLSASVGDRVTITCRTSEDIYSNLAWYQQKPGKA PKLLIFAIKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSKFPLTFGQGTKVEIK	SEQ ID NO: 39
Light chain	DIQMTQSPSSLSASVGDRVTITCRTSEDIYSNLAWYQQKPGKA PKLLIFAIKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSKFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 52
LCDR1	cgaacgagcgaggacataactcaaacccttgca	SEQ ID NO: 103
LCDR2	gcgataaagaggctgcaagac	SEQ ID NO: 104
LCDR3	ttgcaaggctccaaatttcccctgaca	SEQ ID NO: 105
VL	gacatccaaatgactcaaagcccatcatcgctatcggcatcgg tcggggatagagtcacgataacatgccgaacgagcgaggacat atactcaaacccttgcattggtatcaacaaaagccggggaaggcc ccgaagctactgatattcgcgataaagaggctgcaagacggag ttccatcacgattttcgggatctggctcggggaccgattttac gctgactatatcatcgctgcaaccggaagattttgcaacatac tactgcttgcaaggctccaaatttcccctgacattcggacaag gtaccaaggctcgagatcaaa	SEQ ID NO: 106

The antibodies described in further Examples 2 to 5 were modified to contain a RLPQTGG tag (SEQ ID NO: 131) at the C-terminal end of the HC and/or a GGGGSLPQTGG tag (SEQ ID NO: 132) at the C-terminal end of the LC. The C-terminal lysine (K) on the HC was in this case replaced by the Arg (R) of the tag. The addition of the tags did not change the affinity to and specificity for CLDN18.2 of the antibodies.

Example 2: ELISA assay and FC titration to confirm the binding to CLDN18.2 of chimeric and humanized antibody variants

The binding affinity to CLDN18.2 of the chimeric and humanized antibodies (hCl) was tested in an ELISA assay with lipoparticles bearing CLDN18.2 as source of antigen. CLDN18.2-lipoparticles and Null-lipoparticles (without bound antigens as a negative control) were used to coat 96-well plates at a final concentration of 10 U/ml. Upon washing with PBS/0.05% Tween-20 (PBS-T) and blocking with PBS-T/3% BSA for at least 1h at 37°C, 1:3 serial dilutions of the tested antibodies with a starting concentration of 2 µg/ml were added to the coated wells

and incubated for at least 1h at 37°C. The presence of bound antibodies was revealed through binding of an HRP-goat anti-human secondary antibody, development with SIGMAFAST™ OPD as peroxidase substrate and the reaction was stopped by adding 2M H₂SO₄, followed by reading the OD at 490 nm on an ELISA plate reader. Representative binding curves are shown in Figure 1. All tested antibodies of the invention bind specifically to CLDN18.2 containing lipoparticles. Interestingly, humanization of the chimeric antibody did not result in decreased affinity as could be expected and even increased its affinity for 6 out of 10 antibodies, compared to the parental chimeric cC11-1 antibody.

The binding of the chimeric and humanized antibodies to CLDN18.2 was also tested by FC titration with PA-TU-8988S cells (Creative Bioarray, catalog number CSC-C0326) and HEK293T (ATCC, CRL-3216™) cells overexpressing CLDN18.2. FC titration allow to measure the half maximal effective concentration (EC₅₀) of tested antibodies. PA-TU-8988S cells expressing high levels of CLDN18.2 were selected by FACS. Herein, these cells are designated as PA-TU-8988S-High cells. Based on FACS staining with IMAB362, the PA-TU-8988S cell population expresses different levels of CLDN18.2, with a high and a medium level of expression (see Figure 2A). In order to have a more homogenous cell population, the cells were sorted by FACS to select only cells with a the higher CLDN18.2 expression. In brief, PA-TU-8988S cells suspended in FACS buffer (PBS, 2% FCS) were incubated on ice for 30 min with IMAB362 at 2µg/ml. After wash in FACS buffer, the cells were incubated with the PE-labeled Fcγ specific IgG goat anti-human secondary antibody (eBioscience) on ice for 30 min. After wash, the stained cells were resuspended in FACS buffer, analyzed and sorted by a FACSaria™ instrument, separating medium expressing cells from high expressing cells (Figure 2B). After sorting the collected PA-TU-8988S-High cells were resuspended in growth media, expanded and frozen aliquots were preserved in liquid N₂. HEK293T cells overexpressing CLDN18.2 or CLDN18.1 were generated as described in Example 3 and the expression of CLDN18.2 was analyzed by flow cytometry (Figure 3).

In order to quantify the binding of the antibodies to CLDN18.2, 250 x 10³ cells/well of HEK293T cells overexpressing CLDN18.2 or PA-TU-8988-High cells were seeded in FC buffer (PBS/2% FBS) into 96-well plates and allowed to settle by centrifugation. IMAB362 and the hCl antibodies to be tested were diluted at 20µg/ml, followed by 1:4 serial dilutions and incubated with the plated cells for 30 min at 4°C. A PE-coupled secondary anti-human IgG antibody was added to the cells for additional 30 min at 4°C after washes with the FC buffer,

followed by further washes with FC buffer. The cells were then resuspended in 100 µl FC buffer and measured with a FACSCalibur™ cell analyzer (BD Biosciences, USA). The FC analysis (see Figure 5 and Table 4) shows that the hCl antibodies have a higher EC50 value than IMAB362, although having a maxMFI value in the same range as IMAB362. The similar
5 maxMFI values may be indicative of a similar on/off rate for IMAB362 and the hCl antibodies.

Table 4: Maximum MFI and EC50 (µg/ml) measured on all the hCl and IMAB362 antibodies on the HEK293T cells lines overexpressing CLDN18.2 and on the PA-TU-8988S-High cell lines.

Antibody	HEK293T-CLDN18.2		PA-TU-8988S-High	
	Max MFI	EC50 (µg/ml)	Max MFI	EC50 (µg/ml)
IMAB362	1968	0.3878	1046	0.5082
hCl1a	1879	0.5976	1649	2.431
hCl1b	1859	0.5715	1724	1.984
hCl1c	1233	0.7531	1048	1.472
hCl1d	1642	0.5411	1530	1.933
hCl1e	1935	0.5583	1862	2.241
hCl1f	1721	0.7948	1602	2.144
hCl1g	1438	0.6779	1254	1.77
hCl1h	2076	0.4325	1949	1.75
hCl1i	2175	0.4437	2087	1.231
hCl1j	1848	0.4081	1705	1.157

10 Example 3: Generation of pre-B cell L11 cells and HEK293 T cells stably expressing hCLDN18.1 and hCLDN18.2; test of binding specificity of the chimeric and humanized antibodies.

The pre-B cell L11 cell line (Waldmeier et al. 2016) and the HEK293T (ATCC CRL-3216™) cell line do not endogenously express CLDN18.1 or CLDN18.2. Therefore, in order to test
15 antibody binding, CLDN18.1 and CLDN18.2 were recombinantly overexpressed in these cell lines. Cells were co-transfected by electroporation with a transposase expression construct (pcDNA3.1-hy-mPB), a construct bearing transposable full-length huCLDN18.1 (pPB-Puro-huCLDN18.1) or huCLDN18.2 (pPB-Puro-huCLDN18.2) along with a puromycin resistance

cassette and a construct carrying EGFP as transfection control (pEGFP-N3) (Waldmeier et al. 2016). Upon electroporation, cells were allowed to recover for two days in growth media at 37°C in a humidified incubator in a 7.5% CO₂ atmosphere for L11 cells and 5% CO₂ atmosphere for HEK293T cells. Transfection was verified by FC analysis of the EGFP expression. Cells
5 expressing CLDN18.1 or CLDN18.2 were then selected by the addition of puromycin into culture at 1 µg/ml, and further expanded to allow the generation of frozen stocks in FCS with 10% DMSO. The expression of CLDN18.1 and CLDN18.2 in the transfected cells was analyzed by FC (see Figure 3). In brief, trypsinized HEK293T cells and L11 cells grown in suspension were collected by centrifugation, resuspended in PBS/2% FCS and stained for CLDN18.2 using
10 IMAB362 as primary antibody at 2 µg/ml on ice for 30 min and, upon washing in PBS/2% FCS, stained with anti-human IgG (Fc gamma-specific) PE goat antibody (eBioscience) as secondary antibody for 30 min on ice. Upon further wash, resuspended stained cells in ice-cold FC buffer were analyzed using a FACSCalibur™ instrument (see Figure 4 and Figure 5). Untransfected parental cells, not expressing CLDN18.2, were used as negative control. The
15 expression of CLDN18.1 was analyzed in a similar fashion, using a proprietary pan-CLDN18 antibody recognizing CLDN18.1 and CLDN18.2 (see Figure 3). Any pan-CLDN18 antibody usable for flow cytometry measurement would also be adequate such as antibody anti-Claudin-18/CLDN18 (C-term) provided by OriGene Technologies (catalog number AP50944PU-N), CLDN18 (C-Term) Rabbit pAb from MyBioSource (catalog number MBS8555451) or the
20 CLDN18 Antibody from ProSci (catalog number 63-847).

The L11 and HEK293T cells stably expressing huCLDN18.1 and huCLDN18.2 were consequently used to test the binding specificity of the chimeric antibodies cC11-1, cC11-2, cC11-3 and the humanized antibodies to CLDN18.2 and not to CLDN18.1. The cells were stained on ice for 30 min using the antibodies at 2 µg/ml and, upon washing in PBS/2% FCS,
25 stained with anti-human IgG (Fc gamma-specific) PE goat antibody (eBioscience) as secondary antibody for 30 min on ice. All three chimeric antibodies (Figure 4) and humanized antibodies (Figure 5) bind to huCLDN18.2 expressed by L11 or HEK293T cells, and not to huCLDN18.1. Furthermore, the humanized antibodies bind to huCLDN18.2 with a similar affinity as IMAB362 and with an at least as good affinity as cC11-1 (Figure 5).

Example 4: Testing of humanized CLDN18.2 antibodies binding activity by flow cytometry on live tumor tissue and live stomach tissue

The A549 (ATCC CCL-185™) cell line does not endogenously express CLDN18.1 or CLDN18.2. In order to test antibody binding to CLDN18.2, CLDN18.2 was expressed in A549 cells. A549 cells were co-transfected by electroporation with a transposase expression construct (pcDNA3.1-hy-mPB) (Klose et al. 2017), a construct bearing transposable full-length huCldn18.2 (pPB-Puro-huCldn18.1) along with puromycin expression cassette and a construct carrying EGFP as transfection control (pEGFP-N3) (Waldmeier et al. 2016). Upon electroporation, cells were allowed to recover for two days in growth media at 37°C in a humidified incubator in a 5% CO₂ atmosphere. Transfection was verified by FC analysis of the EGFP expression. Cells expressing CLDN18.1 or CLDN18.2 were then selected by the addition of puromycin into culture at 1 µg/ml, and further expanded to allow the generation of frozen stocks in FCS with 10% DMSO. The expression of CLDN18.2 in the transfected cells was analyzed by FC. In brief, trypsinized A549 cells were collected by centrifugation, resuspended in PBS/2% FCS and stained for CLDN18.2 using IMAB362 as primary antibody at 2 µg/ml on ice for 30 min and, upon washing in PBS/2% FCS, stained with anti-human IgG (Fc gamma-specific) PE goat antibody at 2.5 µg/ml (eBioscience) as secondary antibody for 30 min on ice. Upon further wash, resuspended stained cells in ice-cold FC buffer were analyzed using a FACSCalibur™ instrument (see Figure 6). Un-transfected parental cells, not expressing CLDN18.2, were used as negative control. The cells were deposited on 6 December 2019 at the DSMZ-Deutsche Sammlung von Mikroorganismen und Zellkulturen GmbH Inhoffenstr. 7B 38124 Braunschweig DE and are available under the accession number DSM ACC3360.

Two Balb/c mice were implanted subcutaneously with 1x10⁶ A549 cells expressing CLDN18.2 in 100 µl of 50% Matrigel and tumors growth was monitored over a few weeks until the tumor reached the desired size between 150-450 mm³. Healthy stomach tissue and tumor tissue was collected for FC analysis. The collected tissues were cut into small pieces and digested with the Miltenyi tumor dissociation kit (MACS Miltenyi Biotec, Germany). Tissue pieces were incubated with dissociation buffer (prepared according to the manufacturer instruction) in 6 well plates for 30 min in 37°C under permanent gentle rocking motion. Samples were resuspended and strained through a 70 µm cell strainer (Corning, USA) followed by a wash with 20 ml FC buffer (PBS + 2% FBS). Cell suspensions were centrifuged (5 min at 400 g for 4°C) and the supernatants were discarded. If needed, cell suspensions were passed through a

strainer and centrifuged repeatedly and pellets resuspended in 5 ml of red blood cell lysis buffer (Biolegend, USA), incubated on ice for 4 min. After incubation, 25 ml of PBS was added, and the suspensions were centrifuged again (5 min at 400 g for 4°C). Pellets were resuspended in FC buffer (0.5 - 3 ml based on pellets). Equal number of cells were transferred into 96 well
 5 plates and further processed for FC analysis. The cells in the plates were washed with PBS and centrifuged (400 g for 2 min at 4°C). Pellets were resuspended in 50 µl/well of staining mix consisting of the antibody of choice (cCl1-1, hCl1a, hCl1b, hCl1c and hCl1f at 4 µg/ml; IMAB364 at 2 µg/ml) and the AF488-labelled AE1/AE3 pan-cytokeratin antibody (Thermo Fisher Scientific, USA) diluted in PBS and incubated for 25 min on ice. After incubation, cells
 10 were washed twice in PBS and centrifuged (400 g for 2 min at 4°C). Pellets were resuspended in 50 µl/well of secondary staining mix (PBS + PE-labelled anti-human antibody) (Thermo Fisher Scientific, USA), and incubated 25 min on ice. After incubation cells were washed again twice in PBS. Pellets were resuspended in 100 µl of PBS containing DAPI. Plates were kept on ice until FC analysis. For FC analysis, live cells were separated from dead cells by forward
 15 scatter and DAPI stain. Live cells were then gated for the presence of cytokeratin (AF888 positive) and bound CLDN18.2 antibodies (PE positive cells). Results of the FC analysis can be seen in Figure 7 and Table 5. The results are the average of data obtained from two mice.

All the tested antibodies (cCl1-1, hCl1a, hCl1b, hCl1c, hCl1f and IMAB364) bound to a similar percentage of tumor cells bearing CLDN18.2, approximately between 20% and 30%. However,
 20 surprisingly, only IMAB362 bound to healthy stomach cells bearing CLDN18.2 while binding of cCl1-1, hCl1a, hCl1b, hCl1c and hCl1f was barely detectable, binding less than 1% of healthy stomach cells. The difference in the binding capacity between CLDN18.2 expressed in tumor cells originating for injected A549 cells expressing CLDN18.2 and healthy stomach cells was also expressed as a ratio of the % of positive tumor cells divided by the % of positive
 25 stomach cells (see last column in Table 5). This ratio was below 5 and on average close to 1 for IMAB362, and above 15, on average above 30, for the tested humanized clones of cCl1-1 (hCl1a, hCl1b, hCl1c and hCl1f).

Table 5: FC binding data and binding ratio of selected antibodies to healthy stomach cells and tumor cells.

	% of positive tumor cells			% of positive healthy stomach cells			Ratio tumor/stomach		
	Mouse 1	Mouse 2	Average	Mouse 1	Mouse 2	Average	Ratio Mouse 1	Ratio Mouse 2	Ratio Average
cCl1-1	37	15	26	0.4	0.3	0.35	92.5	50	74.3
hCl1a	34	18	26	1.2	0.3	0.75	28.3	60	34.7
hCl1b	43	17	30	1	0.13	0.565	43	130.7	53.1
hCl1c	29	8	18.5	0.1	0.4	0.25	290	20	74
hCl1f	32	14	23	0.04	0.1	0.07	800	140	328.6
IMAB362	33	11	22	13	37	25	2.53	0.29	0.88

Therefore, cCl1-1 and the tested humanized clones of cCl1-1 (hCl1a, hCl1b, hCl1c and hCl1f) show increased binding to tumor cells vs. healthy stomach cells and are therefore tumor-specific CLDN18.2 antibodies. In contrast IMAB362 does not allow to discriminate tumor cells bearing CLDN18.2 from healthy stomach cells bearing CLDN18.2.

Example 5: Testing of humanized CLDN18.2 antibodies by immunohistochemistry (IHC) on frozen tissue samples

- 10 Fresh stomach and tumor tissue samples expressing CLDN18.2 obtained from Balb/c mice subcutaneously implanted with 1×10^6 A549 cells expressing CLDN18.2 were snap-frozen in OCT in a suitable tissue mold. 5-15 μ m thick tissue sections were cut with a cryostat at -20°C , transferred to microscope slide at room temperature (RT) and subsequently kept frozen until IHC staining. Before staining, slides were brought back to RT and fixed in pre-cooled acetone
- 15 (- 20°C) for 10 min. After evaporation of the acetone at RT, the slides were rinsed in TBS and processed to block non-specific staining sites: slides were incubated in 0.3% H_2O_2 for 15 min at RT, followed by TBS washes and incubation in a peroxidase-blocking solution (Agilent, USA) for 60 min at RT. After blocking, the slides were processed for antibody staining: the slides were incubated with the primary antibodies (hCL1a, hCl1b, hCl1c, hCl1f, IMAB362 and
- 20 the 34H14L15 pan-CLDN18 antibody (Abcam, USA)) for 120 min at RT, washed in TBS,

- followed by incubation with an HRP-conjugated anti-human antibody (or anti-rabbit antibody for the pan-CLDN18 antibody) for 30 min at RT. Antibody binding to CLDN18.2 or pan-CLDN18 on the tissue sections was revealed by treating the slides with the DAB+ substrate Chromogen system (Agilent, USA) according the manufacturer's instructions. After subsequent
- 5 TBS washes, the slides were counterstained in hematoxylin, rinsed in dH₂O for 15 min, dehydrated in sequential 95% and 100% ethanol washes, further followed by cleaning of the slides in xylene. Finally, the slides were mounted with a coverslip in a glycerol mounting medium (Agilent, USA). Representative microscopy images of the staining of healthy mouse stomach tissue and mouse tumor tissue can be found in Figure 8 and Figure 9, respectively.
- 10 Figure 8 shows representative staining of healthy stomach tissue. Only hematoxylin stain of the nuclei is visible in tissue co-stained with hCL1a, hCL1b, hCL1c and hCL1f (respectively panels A, B, C and D), while tissue stained co-stained with IMAB362 (panel E) shows membranous CLDN18.2 DAB stain. Therefore, the tested humanized clones of cCL1-1 (hCL1a, hCL1b, hCL1c and hCL1f) do not bind healthy stomach tissue expressing CLDN18.2 in contrast to IMAB362,
- 15 which binds healthy stomach tissue expressing CLDN18.2. Furthermore, Figure 9 shows representative staining of tumor tissue, panel A, B, C and D are representative image of tumor tissue stained with hCL1a, hCL1f, IMAB362 and the Abcam 34H14L15 pan-CLDN18 antibody, respectively. All the tumor stained with the tested antibodies show strong membranous CLDN18.2 DAB stain. The tested humanized clones of cCL1-1 (hCL1a and hCL1f) bound to
- 20 mouse tumor tissue expressing CLDN18.2 in similarly to IMAB362 or the pan-CLDN18 antibody. Therefore, the humanized clones of cCL1-1 exhibit increased binding to tumor tissue expressing CLDN18.2 compared to healthy stomach tissue expressing CLDN18.2.

Example 6: Asn-deamidation and Asp-isomerization liability analysis of humanized antibody (hCL) variants and IMAB362

- 25 Deamidation of Asn (N) residues and isomerization of Asp (D) residues may occur during biopharmaceutical manufacturing, storage or clinical application (*in vivo*). Deamidation and isomerization may lead to potential changes in protein structure, function, activity, stability and immunogenicity. Therefore, it must be minimized and controlled, particularly in a regulatory context. The presence of Asn deamidation and Asp isomerization motifs can be analyzed *in-*
- 30 *silico*. The most common Asn deamidation motif is the NG motif and the most common Asp-isomerization motif in the DG motif.

Such *in-silico* analysis revealed that all hCl antibodies had a potential DG Asp-isomerization motif in the 2nd CDR of the VL, and that none of the hCl antibodies or IMAB362 had potential NG deamidation motifs in their CDRs. To verify the *in-silico* predictions, hCl antibodies and IMAB362 were stressed under high pH or low pH and heat to accelerate the modification that may to occur during manufacturing processes and long-term storage. In brief, antibody samples were buffer exchanged with Amicon centrifugal filters to 20 mM sodium phosphate buffer, pH 8.0 for the Asn-deamidation stress test or 20 mM citrate buffer, pH 5.5 for the Asp-isomerization stress test, and the samples were diluted to a final concentration of 3.0 mg/ml. 30 µl of sample was incubated for 1 week (Asn-deamidation) or 2 weeks (Asp-isomerization) at 40°C in a thermoblock with a heated anti-condensation lid. The stressed and non-stressed sample was stored at -80°C. Asn-deamidation and Asp-isomerization of the samples was analyzed by strong cation exchange (SCX) chromatography. Deamidation of Asn leads in a SCX chromatogram to an increase of the peak area before the main peak (bM), while Asp-isomerization leads in a SCX chromatograph to an increase of the peak area after the main peak (aM) (Du et al. 2012). SCX chromatography was performed on a MAbPac SCX-10 Column (ThermoFisher Scientific, Basel, CH), with buffer A at pH 4.0 and buffer B at pH 11.0. The flow rate was of 0.5 ml/min with a pH gradient of 30-80 % buffer B. 10 µg of the sample in 20 µl of buffer A was injected into the column. Sample detection was performed by protein absorbance at 280 nm. The hCl antibodies showed only an increase of bM of about 27.9-32.2 % (see Table 6), which was not rated as critical. However, IMAB362 showed a pronounced increase in bM of 40.9% (see Table 6), even though this antibody does not have a NG motif in the variable domains. In contrast to the anti-CLDN18.2 monoclonal antibodies of the invention, IMAB362 has two NS motifs at positions HC CDR3 (aa 103-104) (SEQ ID NO: 55) and LC CDR 1 (aa 31-32) (SEQ ID NO: 56). NS motifs are the second most liable motifs for deamidation.

Table 6: Deamidation stress test of mAB, strong cation exchange (SCX) chromatography

mAb	stressed yes (+) / no (-)	Proportion of bM (%)	Increase proportion of bM after stress test (%)
hCl1a	-	20.9	27.9
	+	48.8	
hCl1b	-	19.7	29.1
	+	48.8	
hCl1c	-	19.4	31.2
	+	50.6	

mAb	stressed yes (+) / no (-)	Proportion of bM (%)	Increase proportion of bM after stress test (%)
hCl1d	–	18.2	32.2
	+	50.4	
hCl1e	–	21.4	28.1
	+	49.5	
hCl1f	–	18.7	28.9
	+	47.6	
hCl1g	–	18.8	28.6
	+	47.4	
hCl1h	–	17.5	31.6
	+	49.1	
hCl1i	–	20.5	30.0
	+	50.5	
hCl1j	–	20.2	30.0
	+	50.2	
IMAB362	–	26.0	40.9
	+	66.9	

The impact of the Asn-deamidation stress test on binding affinity to CLDN18.2 of hCl1a, hCl1i and IMAB362 was tested in an ELISA assay with lipoparticles bearing CLDN18.2 as source of antigen. CLDN18.2-lipoparticles and Null-lipoparticles (without antigens) were used to coat 96-well plates at a final concentration of 10 U/ml in 100 mM sodium carbonate, pH 9.6. Upon washing with PBS/0.05% Tween-20 (PBS-T) and blocking with PBS-T/3% BSA for at least 1h at 37°C, 1:3 serial dilutions of hCl antibodies with a starting concentration of 2 µg/ml were added and incubated for at least 1h at 37°C. The presence of bound antibodies was revealed through binding an HRP-goat anti-human secondary antibody, developed with Sigma-Fast OPD as peroxidase substrate, the reaction was stopped by adding 2 M H₂SO₄ and reading was performed at OD-490 on an ELISA plate reader. The IMAB362 EC₅₀ value was 1.8 times higher after the deamidation stress test (non-stressed reference: EC₅₀ of 51.5 ng/ml, stressed: EC₅₀ of 95.09 ng/ml) (see Figure 10). This might be related to the increase of bM of 40.9 % in SCX after deamidation stress test (see Table 6). Confirming the SCX Asn-deamidation results, no significant difference in antigen binding was observed after deamidation stress test for hCl1a and hCl1i (see Table 6). The deamidation stress test thus shows that the hCl antibodies are less prone to deamidation and potential decreased target binding than IMAB362 and predictably are more stable during manufacturing, storage and clinical application (*in vivo*) resulting in a more uniform and active antibody/product.

- Although all hCl antibodies had a potential DG Asp-isomerization motif in the 2nd CDR of the VL and in the CH2 and CH3 domain of the HC (VL-CDR2 (at position 62), CH2 (at position 282), CH3 (at position 403)), the Asp-isomerization stress test did not reveal Asp-isomerization (see Table 7) contrary to what could have been predicted from Du et al (Du et al. 2012). The
- 5 aM values of the non-stressed samples (except for IMAB362) were already noticeably high. This may be due to lysine clipping variants of the heavy chain. IMAB362 was the only antibody without a high aM in the non-stressed sample. IMAB362 is the only tested anti-CLDN18.2 antibody without C-terminal Lys, implying that for the hCl antibodies the C-terminal Lys clipping is the most probable reason for increased aM in non-stressed and stressed samples.
- 10 Table 7: Asp-isomerization stress test of mAbs, strong cation exchange (SCX) chromatography

mAb	stressed yes (+) / no (-)	Proportion of aM (%)	Increase proportion of aM after stress test (%)
hCl1a	–	45.1	-6.5
	+	38.6	
hCl1b	–	45.2	-5.7
	+	39.5	
hCl1c	–	40.3	-2.3
	+	38.1	
hCl1d	–	41.3	-4.6
	+	36.7	
hCl1e	–	44.4	-4.2
	+	40.2	
hCl1f	–	43.5	-1.8
	+	41.7	
hCl1g	–	44.5	-6.4
	+	38.0	
hCl1h	–	43.2	-4.7
	+	38.5	
hCl1i	–	44.1	-4.6
	+	39.5	
hCl1j	–	43.7	-7.7
	+	36.0	
IMAB362	–	1.5	4.1
	+	5.6	

The invention is also described by the following embodiments:

1. An antibody or fragment thereof binding to CLDN18.2, wherein the antibody or fragment thereof exhibits increased binding to tumor tissue expressing CLDN18.2 over healthy tissue expressing CLDN18.2.
2. An antibody or fragment thereof binding to CLDN18.2 comprising the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 21, SEQ ID NO: 22, and SEQ ID NO: 23, respectively, and the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26, respectively.
3. The antibody or fragment thereof of embodiment 1 or 2, comprising:
 - a. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 1, SEQ ID NO: 15 and SEQ ID NO: 3, respectively, and the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, respectively;
 - b. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 1, SEQ ID NO: 16 and SEQ ID NO: 3, respectively, and the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, respectively;
 - c. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 1, SEQ ID NO: 16 and SEQ ID NO: 3, respectively, and the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 17, SEQ ID NO: 14 and SEQ ID NO: 11, respectively;
 - d. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 1, SEQ ID NO: 16 and SEQ ID NO: 3, respectively, and the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 11, respectively;
 - e. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 12, SEQ ID NO: 15 and SEQ ID NO: 3, respectively, and the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, respectively;
 - f. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 1, SEQ ID NO: 20 and SEQ ID NO: 3, respectively, and the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, respectively;
 - g. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 1, SEQ ID NO: 20 and SEQ ID NO: 3, respectively, and the LCDR1, LCDR2 and LCDR3

sequences of SEQ ID NO: 18, SEQ ID NO: 19 and SEQ ID NO: 11,
respectively;

h. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 12, SEQ ID NO:
20 and SEQ ID NO: 8, respectively, and the LCDR1, LCDR2 and LCDR3
sequences of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, respectively;
or

i. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 12, SEQ ID NO:
20 and SEQ ID NO: 8, respectively, and the LCDR1, LCDR2 and LCDR3
sequences of SEQ ID NO: 17, SEQ ID NO: 14 and SEQ ID NO: 11,
respectively.

4. The antibody or fragment thereof of embodiment 1 or 2, comprising:

a. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 1, SEQ ID NO: 2
and SEQ ID NO: 3, respectively, and the LCDR1, LCDR2 and LCDR3
sequences of SEQ ID NO: 4, SEQ ID NO: 5 and SEQ ID NO: 6, respectively;

b. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 1, SEQ ID NO: 7
and SEQ ID NO: 8, respectively, and the LCDR1, LCDR2 and LCDR3
sequences of SEQ ID NO: 9, SEQ ID NO: 10 and SEQ ID NO: 11,
respectively; or

c. the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 12, SEQ ID NO:
2 and SEQ ID NO: 3, respectively, and the LCDR1, LCDR2 and LCDR3
sequences of SEQ ID NO: 13, SEQ ID NO: 14 and SEQ ID NO: 11,
respectively.

5. The antibody of fragment thereof of embodiment 1 or 2, comprising:

a. a VH sequence of SEQ ID NO: 27 and a VL sequence of SEQ ID NO: 28;

b. a VH sequence of SEQ ID NO: 29 and a VL sequence of SEQ ID NO: 30; or

c. a VH sequence of SEQ ID NO: 31 and a VL sequence of SEQ ID NO: 32.

6. The antibody or fragment thereof of any one of embodiments 1-3, comprising:

a. a VH sequence having at least 80%, at least 85%, at least 90%, at least 95% or at
least 98% sequence identity with the amino acid sequence of SEQ ID NO: 33;

b. a VH sequence having at least 80%, at least 85%, at least 90%, at least 95% or at
least 98% sequence identity with the amino acid sequence of SEQ ID NO: 34;

- c. a VH sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 35;
- d. a VH sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 36; or
- 5 e. a VH sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 37;
- and
- f. a VL sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 38;
- 10 g. a VL sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 39;
- h. a VL sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the amino acid sequence of SEQ ID NO: 40; or
- i. a VL sequence having at least 80%, at least 85%, at least 90%, at least 95% or at
- 15 least 98% sequence identity with the amino acid sequence of SEQ ID NO: 41.
7. The antibody or fragment thereof of embodiment 1 or 2, comprising:
- a. a VH sequence of: SEQ ID NO: 33;
- b. a VH sequence of SEQ ID NO: 34;
- c. a VH sequence of SEQ ID NO: 35;
- 20 d. a VH sequence of SEQ ID NO: 36; or
- e. a VH sequence of SEQ ID NO: 37;
- and
- f. a VL sequence of SEQ ID NO: 38;
- g. a VL sequence of SEQ ID NO: 39;
- 25 h. a VL sequence of SEQ ID NO: 40; or
- i. a VL sequence of SEQ ID NO: 41.
8. The antibody or fragment thereof of embodiment 1 or 2, comprising:
- a. a VH sequence of SEQ ID NO: 33 and a VL sequence of SEQ ID NO: 38;
- b. a VH sequence of SEQ ID NO: 34 and a VL sequence of SEQ ID NO: 38;
- 30 c. a VH sequence of SEQ ID NO: 34 and a VL sequence of SEQ ID NO: 39;

- d. a VH sequence of SEQ ID NO: 34 and a VL sequence of SEQ ID NO: 40;
 - e. a VH sequence of SEQ ID NO: 35 and a VL sequence of SEQ ID NO: 38;
 - f. a VH sequence of SEQ ID NO: 36 and a VL sequence of SEQ ID NO: 41;
 - g. a VH sequence of SEQ ID NO: 36 and a VL sequence of SEQ ID NO: 40;
 - 5 h. a VH sequence of SEQ ID NO: 37 and a VL sequence of SEQ ID NO: 41;
 - i. a VH sequence of SEQ ID NO: 37 and a VL sequence of SEQ ID NO: 38; or
 - j. a VH sequence of SEQ ID NO: 37 and a VL sequence of SEQ ID NO: 39.
9. The antibody of any one of embodiments 1-3, comprising:
- a. a heavy chain sequence having at least 80%, at least 85%, at least 90%, at least
10 95% or at least 98% sequence identity with the amino acid sequence of the heavy
chain sequence of SEQ ID NO: 46 and a light chain sequence having at least 80%,
at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the
amino acid sequence of the light chain sequence of SEQ ID NO: 51;
 - b. a heavy chain sequence having at least 80%, at least 85%, at least 90%, at least
15 95% or at least 98% sequence identity with the amino acid sequence of the heavy
chain sequence of SEQ ID NO: 47 and a light chain sequence having at least 80%,
at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the
amino acid sequence of the light chain sequence of SEQ ID NO: 51;
 - c. a heavy chain sequence having at least 80%, at least 85%, at least 90%, at least
20 95% or at least 98% sequence identity with the amino acid sequence of the heavy
chain sequence of SEQ ID NO: 47 and a light chain sequence having at least 80%,
at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the
amino acid sequence of the light chain sequence of SEQ ID NO: 52;
 - d. a heavy chain sequence having at least 80%, at least 85%, at least 90%, at least
25 95% or at least 98% sequence identity with the amino acid sequence of the heavy
chain sequence of SEQ ID NO: 47 and a light chain sequence having at least 80%,
at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the
amino acid sequence of the light chain sequence of SEQ ID NO: 53;
 - e. a heavy chain sequence having at least 80%, at least 85%, at least 90%, at least
30 95% or at least 98% sequence identity with the amino acid sequence of the heavy
chain sequence of SEQ ID NO: 48 and a light chain sequence having at least 80%,

- at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the amino acid sequence of the light chain sequence of SEQ ID NO: 51;
- 5 f. a heavy chain sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the amino acid sequence of the heavy chain sequence of SEQ ID NO: 47 and a light chain sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the amino acid sequence of the light chain sequence of SEQ ID NO: 54;
- 10 g. a heavy chain sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the amino acid sequence of the heavy chain sequence of SEQ ID NO: 49 and a light chain sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the amino acid sequence of the light chain sequence of SEQ ID NO: 53;
- 15 h. a heavy chain sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the amino acid sequence of the heavy chain sequence of SEQ ID NO: 50 and a light chain sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the amino acid sequence of the light chain sequence of SEQ ID NO: 54;
- 20 i. a heavy chain sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the amino acid sequence of the heavy chain sequence of SEQ ID NO: 50 and a light chain sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the amino acid sequence of the light chain sequence of SEQ ID NO: 51;
- 25 j. a heavy chain sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the amino acid sequence of the heavy chain sequence of SEQ ID NO: 50 and a light chain sequence having at least 80%, at least 85%, at least 90%, at least 95% or at least 98% sequence identity with the amino acid sequence of the light chain sequence of SEQ ID NO: 52, or versions thereof with an engineered Fc domain.
10. The antibody of embodiment 1 or 2, comprising:
- 30 a. the heavy chain sequence of SEQ ID NO: 46 and light chain sequence of SEQ ID NO: 51;

- b. the heavy chain sequence of SEQ ID NO: 47 and light chain sequence of SEQ ID NO: 51;
 - c. the heavy chain sequence of SEQ ID NO: 47 and light chain sequence of SEQ ID NO: 52;
 - 5 d. the heavy chain sequence of SEQ ID NO: 47 and light chain sequence of SEQ ID NO: 53;
 - e. the heavy chain sequence of SEQ ID NO: 48 and light chain sequence of SEQ ID NO: 51;
 - f. the heavy chain sequence of SEQ ID NO: 47 and light chain sequence of SEQ ID NO: 54;
 - 10 g. the heavy chain sequence of SEQ ID NO: 49 and light chain sequence of SEQ ID NO: 53;
 - h. the heavy chain sequence of SEQ ID NO: 50 and light chain sequence of SEQ ID NO: 54;
 - 15 i. the heavy chain sequence of SEQ ID NO: 50 and light chain sequence of SEQ ID NO: 51;
 - j. the heavy chain sequence of SEQ ID NO: 50 and light chain sequence of SEQ ID NO: 52,
or versions thereof with an engineered Fc domain.
- 20 11. The antibody or fragment thereof of any one of embodiments 1 to 10, wherein the antibody or fragment thereof is IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, synthetic IgG, IgM, F(ab)₂, Fv, scFv, IgGACH2, F(ab')₂, scFvCH3, Fab, VL, VH, scFv4, scFv3, scFv2, dsFv, Fv, scFv-Fc, (scFv)₂, a non-depleting IgG, a diabody, a bivalent antibody or Fc-engineered versions thereof.
- 25 12. The antibody or fragment thereof of any one of embodiments 1 to 11, wherein the antibody or fragment thereof is humanized.
13. The antibody or fragment thereof of any one of embodiments 1 to 12, wherein the antibody or fragment thereof does not bind to CLDN18.1.

14. The antibody or fragment thereof of any one of embodiments 1 to 13, wherein the antibody or fragment thereof is less susceptible to posttranslational deamidation than IMAB362.
- 5 15. The antibody or fragment thereof of any one of embodiments 1 to 14, wherein the antibody or fragment thereof labels at least 2 times more, at least 5 times more, at least 10 times more, or at least 20 times more tumor cells expressing CLDN18.2 over healthy tissue cells expressing CLDN18.2 during flow cytometry measurement.
- 10 16. The antibody or fragment thereof of any one of embodiments 1 to 14, wherein the increased binding to tumor tissue expressing CLDN18.2 over healthy tissue expressing CLDN18.2 is measured by flow cytometry or by immunohistochemistry.
- 15 17. The antibody or fragment thereof of any one of embodiments 1 to 16, wherein the antibody or fragment thereof binds to CLDN18.2 expressed in HEK293T cells or PA-TU-8988-High cells with an EC50 value that is at least 1.1 times higher, at least 1.2 times higher, at least 1.5 times higher, at least 2 times higher or at least 2.5 times higher but not more than 3 times higher than the EC50 value of IMAB362 binding to CLDN18.2 expressed in HEK293T cells or PA-TU-8988-High cells.
18. The antibody or fragment thereof of embodiment 17, wherein binding is measured by flow cytometry (FC) titration.
- 20 19. The antibody or fragment thereof of any one of embodiments 1 to 18, wherein the antibody or fragment thereof is isolated.
20. A nucleic acid encoding the antibody or fragment thereof of any of embodiments 1 to 19.
21. A vector comprising the nucleic acid of embodiment 20.
22. A host cell comprising the nucleic acid of embodiment 20 or the vector of embodiment 21.

23. The antibody or fragment thereof of any one of embodiments 1 to 19, the nucleic acid of embodiment 20, the vector of embodiment 21 or the host cell of embodiment 22 for use in the treatment of a subject
- 5 a. suffering from,
 b. at risk of developing, and/or
 c. being diagnosed for
 a neoplastic disease.
24. The antibody or fragment thereof for the use of embodiment 23, wherein the neoplastic disease is selected from the group consisting of pancreatic, gastric, esophageal, ovarian
10 and lung cancer.
25. An antibody or fragment thereof binding to CLDN18.2, wherein the antibody or fragment thereof
- (i) binds to the same epitope as an antibody comprising a heavy chain sequence of SEQ ID NO: 46 and a light chain sequence of SEQ ID NO: 51;
- 15 (ii) competes for binding with an antibody comprising a heavy chain sequence of SEQ ID NO: 46 and a light chain sequence of SEQ ID NO: 51; and/or
- (iii) competitively inhibits binding of an antibody comprising a heavy chain sequence of SEQ ID NO: 46 and a light chain sequence of SEQ ID NO: 51 to CLDN18.2.

Sequences

	SEQ ID NO: 1	DYAMH
	SEQ ID NO: 2	WINTYTGKPTYADDFKG
	SEQ ID NO: 3	AVFYGYTMDA
5	SEQ ID NO: 4	RASEDIYSNLA
	SEQ ID NO: 5	SVKRLQD
	SEQ ID NO: 6	LQGSNFPLT
	SEQ ID NO: 7	WINAYTGKPTYADDFKG
	SEQ ID NO: 8	AVYYGYTMDA
10	SEQ ID NO: 9	RTSEDIYSNFA
	SEQ ID NO: 10	SVNRLQD
	SEQ ID NO: 11	LQGSKFPLT
	SEQ ID NO: 12	DYAMY
	SEQ ID NO: 13	RTSEDIYSNLA
15	SEQ ID NO: 14	AIKRLQD
	SEQ ID NO: 15	WINTYTGKPTYAQKFQG
	SEQ ID NO: 16	WINTYTGKPTYSQKFQG
	SEQ ID NO: 17	RTSEDIYSNLA
	SEQ ID NO: 18	RTSEDIYSNFA
20	SEQ ID NO: 19	SVNRLQD
	SEQ ID NO: 20	WINAYTGKPTYAQKFQG
	SEQ ID NO: 21	DYAMX X in 5 th position is H or Y
25	SEQ ID NO: 22	WINXYTGKPTYXXXFXG X in 4 th position is T or A; X in 12 th position is A or S; X in 13 th position is D or Q; X in 14 th position is D or K; X in 16 th position is K or Q
30	SEQ ID NO: 23	AVXYGYTMDA X in 3 rd position is F or Y

	SEQ ID NO: 24	RXSEDIYSNXA X in 2 nd position is A or T; X in 10 th position is L or F
5	SEQ ID NO: 25	XXXRLQD X in 1 st position is S or A; X in 2 nd position is V or I; X in 3 rd position is K or N
	SEQ ID NO: 26	LQGSXFPLT X in 5 th position is K or N
10	SEQ ID NO: 27	cC11-1 HC variable region QIQLVQSGPELKKPGESVKISCKASGYTFTDYAMHWVKQAPGK GLKWMGWINTYTGKPTYADDFKGRFVFSLEASASTANLQISNL KNEDTATYFCARAVFYGYTMDAWGQGTSVTVSS
15	SEQ ID NO: 28	cC11-1 LC variable region DIQMTQSPASLSASLGETISIACRASEDIYSNLAWEYQQKSGKSPQ LLIFSVKRLQDGVPSRFSGSGSGTQYSLKISGMQPEDEGDYFCLQ GSNFPLTFGSGTKLEIK
20	SEQ ID NO: 29	cC11-2 HC variable region QIQLVQSGPELKKPGESVKISCKTSGYTFTDYAMHWVKQGPVK GMKWMGWINAYTGKPTYADDFKGRFVLSLEASASTANLQISN LKNEDTATYFCARAVYYGYTMDAWGQGTSVIVSS
25	SEQ ID NO: 30	cC11-2 LC variable region DIQMTQSPASLSASLGETISIECRTSEDIYSNFAWFQQKSGKSPQL LIYSVNRLQDGVPSRFSGSGSGTQYSLKISGMQPEDEGDYFCLQ GSKFPLTFGSGTKLEIK
	SEQ ID NO: 31	cC11-3 HC variable region QIQLVQSGPELKKPGESVKISCKASGYTFTDYAMYWVKQVPGK GLRWMGWINTYTGKPTYADDFKGRFVFSLEASASTANLQISNL KNEDTATYFCARAVFYGYTMDAWGQGTSVTVSS
30	SEQ ID NO: 32	cC11-3 LC variable region DIQMTQSPASLSASLGETISIACRTSEDIYSNLAWEYQQKSGKSPQ LLIFAIKRLQDGVPSRFSGSGSGTQYSLKISGMQPEDEGDYFCLQ GSKFPLTFGSGTKLEIK
35	SEQ ID NO: 33	hCL1a HC variable region QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAP GQRLEWMGWINTYTGKPTYAQKFQGRVTITRDTSASTAYMELS SLRSEDNAVYYCARAVFYGYTMDAWGQGTLVTVSS
40	SEQ ID NO: 34	hCL1b, c and d HC variable region QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAP GQRLEWMGWINTYTGKPTYSQKFQGRVTITRDTSASTAYMELS SLRSEDNAVYYCARAVFYGYTMDAWGQGTLVTVSS

	SEQ ID NO: 35	hCL1e HC variable region QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMYWVRQAP GQRLEWMGWINTYTGKPTYAQKFQGRVTITRDTSASTAYMELS SLRSEDNAVYYCARAVFYGYTMDAWGQGTLVTVSS
5	SEQ ID NO: 36	hCL1f and g HC variable region QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAP GQRLEWMGWINAYTGKPTYAQKFQGRVTITRDTSASTAYMEL SSLRSEDNAVYYCARAVFYGYTMDAWGQGTLVTVSS
10	SEQ ID NO: 37	hCL1h, i and j HC variable region QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMYWVRQAP GQRLEWMGWINAYTGKPTYAQKFQGRVTITRDTSASTAYMEL SSLRSEDNAVYYCARAVYYGYTMDAWGQGTLVTVSS
15	SEQ ID NO: 38	hCL1a, b, e and i LC variable region DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAWEYQQKPGKAP KLLIFSVKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATYYCLQ GSNFPPLTFGQGTKVEIK
20	SEQ ID NO: 39	hCL1c and j LC variable region DIQMTQSPSSLSASVGDRVTITCRTSEDIYSNLAWEYQQKPGKAP KLLIFAIKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATYYCLQ GSKFPLTFGQGTKVEIK
	SEQ ID NO: 40	hCL1d and g LC variable region DIQMTQSPSSLSASVGDRVTITCRTSEDIYSNFAWEYQQKPGKAP KLLIYSVNRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATYYCL QGSKFPLTFGQGTKVEIK
25	SEQ ID NO: 41	hCL1f and h LC variable region DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAWEYQQKPGKAP KLLIYSVKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATYYCL QGSNFPPLTFGQGTKVEIK
30	SEQ ID NO: 42	hCL3a, b and c HC variable region QVQLQESGPGLVKPSETLSLTCAVSGYSVSSNYRWHWIRQPPG KGLEWIGYINIAGSTNYPNPSLKSRVTISVDTSKNQFSLKLSSVTA ADNAVYYCARNPSITRAMDAWGQGTLVTVSS
35	SEQ ID NO: 43	hCL3a LC variable region DIQMTQSPSSLSASVGDRVTITCKSSQNIFKNLEWEYQQKPGKAP KLLIYYTNNLQTGVPSRFSGSGSGTDFTLTISLQPEDFATYYCY QYNSGPFTFGQGTKVEIK
40	SEQ ID NO: 44	hCL3b LC variable region DIQMTQSPSSLSASVGDRVTITCRSSQNIFKNLEWEYQQKPGKAP KLLIYYTNNLQTGVPSRFSGSGSGTDFTLTISLQPEDFATYYCY QYNSGPFTFGQGTKVEIK

	SEQ ID NO: 45	hCL3c LC variable region DIQMTQSPSSLSASVGDRVTITCRSSQNIFKNLEWYQQKPGKAP KLLIYYTNNLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCY QYNSGPFTFGQGTKVEIK
5	SEQ ID NO: 46	hCL1a HC full QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAP GQRLEWMGWINTYTGKPTYAQKFQGRVTITRDTSASTAYMELS SLRSEDNAVYYCARAVFYGYTMDAWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK
10		
15		
	SEQ ID NO: 47	hCL1b, c and d HC full QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAP GQRLEWMGWINTYTGKPTYSQKFQGRVTITRDTSASTAYMELS SLRSEDNAVYYCARAVFYGYTMDAWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK
20		
25		
	SEQ ID NO: 48	hCL1e HC full QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMYWVRQAP GQRLEWMGWINTYTGKPTYAQKFQGRVTITRDTSASTAYMELS SLRSEDNAVYYCARAVFYGYTMDAWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGK
30		
35		
40		
	SEQ ID NO: 49	hCL1f and g HC full QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAP GQRLEWMGWINAYTGKPTYAQKFQGRVTITRDTSASTAYMEL SSLRSEDNAVYYCARAVFYGYTMDAWGQGTLVTVSSASTKGPS

5		VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
10	SEQ ID NO: 50	hCL1h, i and j HC full QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMYWVRQAPGQRLEWMGWINAYTGKPTYAQKFQGRVTITRDTASTAYMELSSLRSEDTAVYYCARAVYYGYTMDAWGQGLTVTVSSASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVD
15		KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGK
20	SEQ ID NO: 51	hCL1a, b, e and i LC full DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAWEYQQKPGKAPKLLIFSVKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATYYCLQGSKFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
25	SEQ ID NO: 52	hCL1c and j LC full DIQMTQSPSSLSASVGDRVTITCRSEDYISNLAWEYQQKPGKAPKLLIFAIKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATYYCLQGSKFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
30	SEQ ID NO: 53	hCL1d and g LC full DIQMTQSPSSLSASVGDRVTITCRSEDYISNFAWEYQQKPGKAPKLLIYSVNRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATYYCLQGSKFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
35	SEQ ID NO: 54	hCL1f and h LC full DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAWEYQQKPGKAPKLLIYSVKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATYYCLQGSKFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
40		

		LLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDYSLSS TLTLKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
	SEQ ID NO: 55	IMAB362 HC full
5		QVQLQQPGAELVRPGASVKLSCKASGYTFTSYWINWVKQRP QGLEWIGNIYPSDSYTNYNQKFKDKATLTVDKSSSTAYMQLSSP TSEDSAVYYCTRSWRGNSFDYWGGQTTLTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC 10 VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALH NHYTQKSLSLSPGK
15	SEQ ID NO: 56	IMAB362 LC full
20		DIVMTQSPSSLTVTAGEKVTMSCKSSQSLNLSGNQKNYLTWYQ QKPGQPPKLLIYWASTRESGVPDRFTGSGSGTDFLTISVQAED LAVYYCQNDYSYPFTFGSGTKLEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYSLSSLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C
	SEQ ID NO: 57	DQWSTQDLYN
	SEQ ID NO: 58	NNPVTAVFNYQ
	SEQ ID NO: 59	STQDLYNNPVTAVF
25	SEQ ID NO: 60	TNFWMSTANMYTG
	SEQ ID NO: 61	ALMIVGIVLGAIGLLV
	SEQ ID NO: 62	RIGSMEDSAKANMTLTSGIMFIVS
	SEQ ID NO: 63	
30		METDTLLLWVLLLWVPGSTGDAAQPARRARRTKLGTGLSTPV WWNSADGRMDQWSTQDLYNNPVTAVFNYQGLWRSCVRESSG FTECRGYFTLLGLPAMLQAVRAAIQHSGGRSRRARTKTHLRRG SE
	SEQ ID NO: 64	MDQWSTQDLYNNPVT
	SEQ ID NO: 65	LYNNPVTAVFNYQGL
35	SEQ ID NO: 66	VFNYQGLWRSCVRES
	SEQ ID NO: 67	QGLWRSCVRESSGFT
	SEQ ID NO: 68	RSCVRESSGFTECRG
	SEQ ID NO: 69	TEDEVQSYPSKHDYV

	SEQ ID NO: 70	EVQSYPSKHDYV
	SEQ ID NO: 71	gactacgcgatgcac
	SEQ ID NO: 72	tggatcaacacgtacacggggaagccgacatacgcggacgacttcaagggg
	SEQ ID NO: 73	gccgtcttctacggatatac gatggacgcg
5	SEQ ID NO: 74	cagatccagctcgtccagagcgggcccggagctgaagaagccgggggagagcgtgaagatctcgt gcaaggcgagcggatatacgttcacggactacgcgatgcactgggtcaagcaagcggccgggaaa gggctgaagtggatgggtggatcaacacgtacacggggaagccgacatacgcggacgacttcaa ggggcgattcgtgttctcgtggagcgagcgcgagcacggcgaacctgcaaatctcgaacctgaa 10 gaacgaggacacggcgacgtacttctgcgcggggccgttctacggatatac gatggacgcgtg ggggcaggggtaccagcgtgacggctcgcgac
	SEQ ID NO: 75	cggcgagcgcgaggacatctactcgaacctggcg
	SEQ ID NO: 76	tccgtcaagcggctgcaagac
	SEQ ID NO: 77	ctgcaaggagcaacttcccgtgcg
15	SEQ ID NO: 78	gacatccagatgacgcagagccccggcgtcgtgagcgcgagcctgggggagacgatctcgatcgc gtgccggcgagcgcgaggacatctactcgaacctggcgtggtatcaacagaagagcgggaagagcc cgcagctgctgatcttctccgtcaagcggctgcaagacggcgtcccagccgattctcggggagcg ggagcgggacgcagtactcgtgaagatctcggggatgcagccggaggacgagggggactactt 20 tgcttgaaggagcaacttcccgtgacgttcgggtcgggtaccaaactcgagatcaaa
	SEQ ID NO: 79	tggatcaacgcgtacacggggaagccgacctacgcggacgacttcaagggg
	SEQ ID NO: 80	gccgtctactacggatatac gatggac
	SEQ ID NO: 81	cagatccagctcgtccagagcgggcccggagctgaagaagccgggggagagcgtgaagatctcgt 25 gcaagacgagcggatatacgttcacggactacgcgatgcactgggtcaagcaggggccagggaaa gggatgaagtggatgggtggatcaacgcgtacacggggaagccgacctacgcggacgacttcaa ggggcgattcgtgctgagcctggagcgagcgcctcgacggcgaacctgcaaatctcgaacctgaa gaacgaggacacggcgacgtacttctgcgcggggccgttactacggatatac gatggacgcgtg ggggcaggggtaccagcgtgatcgtctcgagc
30	SEQ ID NO: 82	cggacgagcgcgaggacatctactcgaacttcgcg
	SEQ ID NO: 83	tcagtcaaccggctgcaagac
	SEQ ID NO: 84	ctgcaaggagcaagtcccgtgcg
	SEQ ID NO: 85	gacatccagatgacgcagagccccggcgcgagcctgagcgcgagcctgggggagacgatctcgatcg 35 agtgccggacgagcgcgaggacatctactcgaacttcgcgtggttccagcagaagagcgggaagagc ccgcagctgctgatctactcagtcgaacggctgcaagacggcgtcccagccgattctcggggagc gggagcgggacgcagtactcgtgaagatctcggggatgcagccggaggacgagggggactactt ctgctgcaaggagcaagtcccgtgacgttcgggagcgggtaccaaactcgagatcaaa

	SEQ ID NO: 86	gactacgcgatgtac
	SEQ ID NO: 87	tggatcaacacgtacacggggaagccgacctacgcggacgacttcaagggg
	SEQ ID NO: 88	cagatccagctcgtccagagcggggcggagctgaagaagccgggggagagcgtgaagatctcgt
5		gcaaggcgagcggatatacgttcacggactacgcgatgtactgggtcaagcaagtgccggggaaa
		gggctgcgatggatgggtggatcaacacgtacacggggaagccgacctacgcggacgacttcaa
		ggggcgattcgtgttctcgtggagggcagcgcgagcacggcgaacctgcaaattctgaacctgaa
		gaacgaggacacggcgacttctgcgcgcggggcgtcttctacggatatacgtggacgcgtg
		ggggcagggtaccagcgtgacgggtctcgagc
10	SEQ ID NO: 89	cggacgagcggagacatctactcgaacctggcg
	SEQ ID NO: 90	gcgatcaagcggctgcaagac
	SEQ ID NO: 91	gacatccagatgacgcagagcccgaggcctgagcgcgagcctgggggagacgatctcgatcg
15		cgtgccggagcggagacatctactcgaacctggcgtgggtatcaacagaagagcgggaagagc
		ccgcagctgctgatcttcgcgatcaacggctgcaagacggcgtcccgagccgattctcggggagc
		gggagcgggacgcagtactcgtgaagatctcggggatgcagccggaggacgaggggactactt
		ctgcctgcaagggagcaagttcccgtgacgttcgggtcgggtaccaaaactcgagatcaaa
	SEQ ID NO: 92	tggatcaatacacacggggaagccgacttatgcgcaaaaattccaagga
	SEQ ID NO: 93	gcgggtcttctacggatatacgtggatgcc
20	SEQ ID NO: 94	cagggtccaactagtccaaagcggggcggaaagtaagaagcccgagcatccgtcaaagtcagctg
		caaggcgagcggatatacatttacggactacgcgatgcactgggtcaggcaagcccctgggcaaaag
		gctcgaatggatgggatggatcaatacacacggggaagccgacttatgcgcaaaaattccaagga
		agagtcacaattacgcgggatacatccgcattacccgctacatggagctaagctcgtcgggagcg
25		aggatacggcgtctactattgcgcccagcgggtcttctacggatatacgtggatgcctgggggca
		gggtaccctggtcacgggtctcgagc
	SEQ ID NO: 95	agggcctccgaagacatctactccaacctggca
	SEQ ID NO: 96	agcgtcaaaagactacaagat
	SEQ ID NO: 97	ttgcaaggaagcaattccccttgact
30	SEQ ID NO: 98	gacattcaaatgacgcaaagcccacatcgtcgtgagcgcacgcgtcggggatagagtcaccataacat
		gcagggcctccgaagacatctactccaacctggcatgggtatcaaaaaaccggggaaggctccga
		agctgctgatatttagcgtcaaaagactacaagatggagtaccgagccgattttcggaagcgggag
		cgggacggatttcacgctgaccatatcaagtttgcaaccggaggattttgcgacatactattgcttgcaa
35		ggaagcaatttccccttgactttcgggcaaggtaccaaggtcgagatcaaa
	SEQ ID NO: 99	gattatgcaatgcac
	SEQ ID NO: 100	tggattaacacctacacgggcaagcccacatactcccaaaaattccaagga
	SEQ ID NO: 101	gctgtattctatggatatacaatggatgcc

	SEQ ID NO: 102	cagggtccaattagtccaaagcggggcggaagtcaagaagccggggcgagcgtcaaagtctcatg caaagcgagcggatacacatttacggattatgcaatgcactgggtcaggcaagcaccgggacaag gctggaatggatgggatggattaacacctacacgggcaagccacatactccaaaaattccaagga 5 agggtcacgataacgagagacacgagcgcgagcaccggaatggatgggatggattaacacctaca cgggcaagccacatactccaaaaattccaaggaagggtcacgataacgagagacacgagcgcg agcaccgtaccctggtcaccgtctcgagc
	SEQ ID NO: 103	cgaacgagcggagcatatactcaaaccttgca
	SEQ ID NO: 104	gcgataaagaggctgcaagac
10	SEQ ID NO: 105	ttgcaaggctccaaatttccctgaca
	SEQ ID NO: 106	gacatccaaatgactcaaagcccatcatcgctatcggtcatcggtcggggatagagtcacgataacatg ccgaacgagcggagcatatactcaaaccttgcatgggatcaaaaaagccggggaaggcccgaa gctactgatattcgcgataaagaggctgcaagacggagttccatcacgattttcgggatctggctcgg 15 ggaccgattttacgctgactatatcatcgctgcaaccggaagattttgcaacatactactgcttgaagg ctccaaatttccctgacattcggacaagggtaccaagggtcgagatcaaa
	SEQ ID NO: 107	cggacgagcggagatatttattcgaactttgca
	SEQ ID NO: 108	cagtcaatcggtacacaagat
20	SEQ ID NO: 109	gacatccaaatgacgcaatcacgagctcgctgagcgcacatctgtcggggaccgtgtcacaatcacat gccggacgagcggagatatttattcgaactttgcatgggatcaaaaaaccgggcaaggctccgaa acttttgatttattcagtcaatcggtacaaagatggcgtcccagccgatttagcgggagcggatcggg aaccgactttacgctgacgatatcatcgctacaaccggaggacttcgcgacttattactgcctacaagg gagcaaattcccgtgacattcggacaagggtaccaagggtcgagatcaaa
25	SEQ ID NO: 110	gattacgcaatgtac
	SEQ ID NO: 111	tggataaacctatacgggaaagccaacatacgcccaaaaattccaaggc
	SEQ ID NO: 112	gccgtctttatggatatacgtggacgca
	SEQ ID NO: 113	cagggtccaactgggtccaatcgggggctgaagtcaaaaagccggggcgagcgtcaaagtcagctg caaagcatcgggatacacatttacggattacgcaatgtactgggtcaggcaagcaccggccaacga ctggaatggatgggctggataaacctatacgggaaagccaacatacgcccaaaaattccaaggcc 30 gcgtcacaataacggggacacgagcgcacatcgacggcttatatggaactatcatcgctgcgacgga agacacggcgggtctattattgcgcacgcgccgtctttatggatatacgtggacgcatgggggcagg gtaccctggtcacgggtctcgagc
35	SEQ ID NO: 114	gactacgcaatgcac
	SEQ ID NO: 115	tggattaatgcctacacggggaagccgacctacgcacaaaaattccaagga
	SEQ ID NO: 116	gccgtcttctatggatatacgtggatgct

5	SEQ ID NO: 117	cagggtccaattggtccaaagcggggcggagggtcaagaagccggggcgagcgtcaaagtctcatg caaggcaagcggatatacattacggactacgcaatgcactgggtccggcaagccctgggcaacg gctggaatggatgggatggattaatgcctacacggggaagccgacctacgcacaaaaattccaagg acgagtcacgattacgcggtactagcgcgagcaccgcatatatggagctaagctcgtgcgatct gaggataccgctgtatactactgcgcgagagccgtcttctatggatatacgatggatgctgggggca gggtaccctggtcacggctcgcgac
	SEQ ID NO: 118	cgagcttcggaggacatctatagcaactggct
	SEQ ID NO: 119	agcgtcaaaaggctccaagac
10	SEQ ID NO: 120	ctacaaggctctaactccattgaca
	SEQ ID NO: 121	gatatccaaatgacgcaatcaccatctagcctatcggcctctgtgggggaccgagtcaccatcacatg ccgagcttcggaggacatctatagcaactggcttggtatcaacaaaagccggggaagcaccaaaag ctgctgatatatagcgtcaaaaggctccaagacggagtgccaagccgattctcgggtccgggtccg ggacggattttacgctgacaatttcgagcctgcaaccggaggactttgcaacctactattgcctacaag gctctaactccattgacatttgggcaaggtagcaaggtcgagatcaaa
15		
	SEQ ID NO: 122	gactacgctatgtat
	SEQ ID NO: 123	tggattaatgcctacacgggaagccgacttatgcgcaaaaatttcaagga
	SEQ ID NO: 124	gcgggtctactatggatatacgatggacgca
20	SEQ ID NO: 125	cagggtccaactgggtcaatctggagcgggaagtcaagaagcccgagcatccgtcaaagtctcgtgca aggcatctggatacacattcaccgactacgctatgtattgggtccggcaagccccggacaacggct ggaatggatggatggattaatgcctacacgggaagccgacttatgcgcaaaaatttcaaggaagg gtcacgattacgcgggacacgagcgcctcaaccgcatacatggagctatcgagcctgcgaagcgag gacaccgcggtctactactgcgcgcgggcggtctactatggatatacgatggacgcatggggcgag ggtaccctggtcacgggtcgcgac
25		
	SEQ ID NO: 126	WINXYTGKPTYXQKFQG X in 4 th position is T or A; X in 12 th position is A or S [HC CDR2 for hC11x only, not chimeric clones cC11-1,2,3]
30		
	SEQ ID NO: 127	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKSTYSLSSLTSLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC [constant light chain - CL domain]
35	SEQ ID NO: 128	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS
40		

		KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGK [constant heavy chain - CH1 + Fc domain]
5	SEQ ID NO: 129	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDT LMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE 10 EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGK [L234A/L235A mutation in constant heavy chain - CH1 + Fc domain]
15	SEQ ID NO: 130	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKP SNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDT LMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR 20 EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTPPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSVMHEALHNHYTQKSLSLSPGK [L236A/L236A/P329G mutation in constant heavy chain - CH1 + Fc domain]
25	SEQ ID NO: 131	RLPQTGG [sortase tag]
	SEQ ID NO: 132	GGGGS-LPQTGG [sortase tag]
	SEQ ID NO: 133	CLDN18.2
30		MAVTACQGLGFVVSLIGIAGIIAATCMDQWSTQDLYNNPVTAV FNYQGLWRSCVRESSGFTECRGYFTLLGLPAMLQAVRALMIVG IVLGAIGLLVSIFALKCIRIGSMEDSAKANMTLTSGIMFIVSGLCA IAGVSVFANMLVTNFWMSTANMYTGMGGMVQTVQTRYTFGA ALFVGWVAGGLTLIGGVMMCIACRGLAPEETNYKAVSYHASG HSVAYKPGGFKASTGFGSNTKNKKIYDGGARTEDEVQSYPSKH DYV
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