
Tumor-specific Claudin 18.2 antibody-drug conjugates

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 has the NCBI reference sequence: NP_001002026.1 The sequence can also be derived from SEQ ID NO: 135.

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 STQDLYNNPVTAVF (SEQ ID NO: 59), N-terminal extracellular domain of CLDN18.2, unglycosylated. It also disclosed polyclonal rabbit antibodies screened with a pan-CLDN18

5 peptide TNFWMSTANMYTG (SEQ ID NO: 60) in the C-terminal extracellular domain 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

10 by immunization with the peptide METDTLLLWVLLWVPGSTGDAAQPARRARRTKLGTELGSTPVWWSADGRMDQ WSTQDLYNNPVTAVFNYQGLWRSCVRESSGFTECRGYFTLLGLPAMLQAVRAAIQH SGGRRRARTKTHLRRGSE (SEQ ID NO: 63), including the first extracellular domain of CLDN18.2 with N- and C-terminal extensions. Antibodies obtained by this immunization

15 mediate cell killing by complement dependent cytotoxicity (CDC) and antibody-dependent 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

20 to overlapping peptides within the first extracellular domain (MDQWSTQDLYNNPVT (SEQ ID NO: 64), LYNNPVTAVFNYQGL (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,

25 WO2013/167259 discloses antibodies binding to C-terminal epitopes of CLDN18.2. The 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

30 cytotoxin, a drug (e.g. an immunosuppressant) or a radioisotope. WO2014/075788 discloses a 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
5 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
10 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-
15 CLDN18.2 monoclonal antibodies binding to a different epitope than IMAB362. WO2019/219089 discloses monoclonal antibodies binding to a mutant of CLDN18.2. Other antibodies binding to CLDN18.2 have been disclosed in WO2019/242505, WO2020/038404, WO2020/043044, WO2020/063988, WO2020/082209, WO2020/018852, WO2020/023679, WO2020/135674, WO2020/135201, WO2020/139956, WO2020/025792, WO2020160560,
20 CN111808194 and WO2020200196.

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
25 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
30 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
- 5 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.
- 10 As already mentioned above, IMAB362 has also been developed as an antibody-drug conjugate (ADC) (disclosed in WO2016/165762), where the antibody has been conjugated to the MMAE or DM4 drugs. The DM4 drug was coupled to IMAB362 via SPBD (N-succinimidyl-3-(2-pyridyldithio)butyrate), an amino and sulfhydryl reactive heterobifunctional protein crosslinker which reacts via an N-hydroxysuccinimide (NHS) ester with primary amines (as found in lysine
- 15 side chains or the N-terminus of proteins) of the antibody. The valine-citrulline-MMAE drug was coupled to thiolated IMAB362. In that case, IMAB362 was initially thiolated with the heterobifunctional linker 2-IT (2-iminothilane) which reacts with the free amines of lysine residues. The valine-citrulline is a linker cleavable by cathepsins. All the caveats listed above related to IMAB362 also apply to an ADC based on the same antibody.
- 20 Therefore, there is a need for improved antibodies and ADCs 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
- 25 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
- 30 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 receptor (FcRn).

- 5 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, which is a monovalent fragment consisting of the variable light (VL), variable heavy (VH),
10 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) a variable fragment (Fv) fragment, which consists of the VL and VH domains of a single arm
15 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
20 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
25 or in any combination.

- 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
30 receptor components with all or parts of the immunoglobulin CH domains, (ii) binding proteins, 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.

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

The term "Antibody-Drug conjugate" or "ADC" refers to an antibody or antibody fragment to which toxins (or drugs) have been linked. In an ADC, toxins are conjugated to the antibody or antibody fragment by cleavable or non-cleavable linkers. Cleavable linker may be designed to be cleaved extracellularly in the tumor environment or intracellularly within the cytosol. Cleavable linkers exploit differential conditions of reducing power or enzymatic degradation that can be present either outside or inside the target cell. Non-cleavable linkers require the ADC to be internalized, the antibody-linker component needs to be degraded by lysosomal proteases for the toxins to be released. Conjugation of the linker to the antibody may also vary. Conjugation may rely on the presence of lysine and cysteine residues within the polypeptide structure of the antibody as the point of conjugation. Reactive groups on the linker can e.g. be conjugated to the side chain of lysine residues through amide or amidine bond formation. Conjugation via cysteine residues requires a partial reduction of the antibody. Alternatively, site-specific enzymatic conjugation can be used. This requires enzymes that react with the antibody and can induce site- or amino acid sequence-specific modifications. Peptide sequences recognized by these enzymes may have to be inserted into the genetically engineered antibodies or fragments to be conjugated. Enzymes which have been used for such purpose are sortase, transglutaminase, galactosyltransferase, sialyltransferase and tubulin-tyrosine ligase. An overview of ADC linker conjugation and toxins can be found in Ponziani et al, 2020 (Ponziani et al. 2020). An overview of conjugation of toxins to antibody fragments can be found in Aguiar et al, 2018 (Aguiar et al. 2018). The type of linker and the method of conjugation used to

conjugate the toxin to the antibody or antibody fragment may determine the drug-to-antibody ratio (DAR).

The term “toxin” refers to a cytotoxic and/or cytostatic agent that can be based on a synthetic, plant, fungal, or bacterial molecule. Cytotoxic or cytostatic means that they inhibit the growth of and/or inhibit the replication of and/or kill cells, particularly malignant cells typically due to their increased turnover. In a preferred embodiment, the toxin is selected from the group consisting of anthracyclines and derivatives thereof. Anthracyclines are antibiotic compounds that exhibit cytotoxic activity, and may kill cells by different mechanisms, including intercalation of the drug molecules into the DNA of the cell or DNA severing activity thereby inhibiting DNA-dependent nucleic acid synthesis, generation of free radicals by the drug which react with cellular macromolecules to cause damage to the cells, DNA alkylation and/or interactions of the drug molecules with the cell membrane. Anthracyclines include doxorubicin, epirubicin, idarubicin, daunomycin, nemorubicin, and derivatives thereof. A well-known and preferred anthracycline derivative is PNU-159682, or in short PNU, CAS No. 202350-68-3. It is a highly potent metabolite of nemorubicin having outstanding cytotoxicity. Anthracycline derivatives are understood as including also the toxin as a result of conjugation to specific ligands, where due to the conjugation chemistry used, some atoms of the original toxin may be missing (Broggini 2008; Quintieri et al. 2005). In some instances, the term anthracycline derivatives may be understood as a result of lysosomal degradation, where fragment of the linker may remain attached to the anthracycline molecule. The term “anthracyclines” as used herein refers to anthracyclines and anthracycline derivatives.

The term “selectively binds to CLDN18.2” or “selective binding to CLDN18.2” as referred to herein refers to an antibody exhibiting binding to CLDN18.2, while exhibiting no (specific) binding to CLDN18.1. Hence, the antibodies selectively binding to CLDN18.2 do not exhibit cross-reactivity to CLDN18.1.

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 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.

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

5 The inventors have surprisingly identified novel antibody-drug conjugates (ADCs) involving anti-CLDN18.2 antibodies and a toxin as further described herein, 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.

10 The invention provides an ADC based on an antibody 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.

Increased binding to tumor tissue by the antibody or fragment thereof provided herein may be
15 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-
20 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.

Increased binding to CLDN18.2 expressed in tumor tissue may be due to posttranslational
25 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
30 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 antibody or fragment thereof binds 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 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 the antibody 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 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 may be between 1.1 times higher and 2.5 times higher, between 1.2 times 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 antibody or fragment thereof binds to CLDN18.2 with an EC₅₀ value of above 0.6 µg/ml, above 1 µg/ml, preferably above 1.5 µg/ml, 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, 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 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 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, 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 antibody or fragment thereof binds to CLDN18.2 with a maxMFI values within +/- 40% of the maxMFI value of IMAB362 when measured by flow cytometry on HEK293T cells overexpressing CLDN18.2. The antibody or fragment thereof may also bind 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.

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

The invention also provides an ADC comprising 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 SEQ ID NO: 26, respectively, and a toxin. In one embodiment, the toxin is an anthracycline.

In another embodiment, the inventors have engineered novel ADCs based on the novel anti CLDN18.2 antibodies from above, which surprisingly exhibit better cytotoxic activity on tumor
5 cells compared to a similar ADC based on IMAB362.

The ADC of the invention has the general formula $A - (L-T)_n$, wherein

- a. A is an antibody or fragment thereof binding to CLDN18.2 comprising the heavy chain complementarity-determining regions (CDRs) HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 21, SEQ ID NO: 22, and SEQ ID NO: 23, respectively and
10 the light chain CDRs LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26, respectively,
- b. L is a linker, and
- c. T is a toxin,

wherein the toxin is an anthracycline.

- 15 In one embodiment, n is an integer ≥ 1 and ≤ 10 . The invention also relates to a pharmaceutical acceptable salt or ester of the ADC.

The invention also provides an ADC comprising an antibody 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.

- 20 The respective consensus sequences can be found in Table 1. It is understood that any ADC comprising an antibody 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

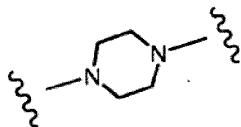
CDRs	Sequence	SEQ ID
HCDR1	DYAMX X in 5 th position is H or Y	SEQ ID NO: 21
HCDR2	WINXYTGKPTYXXXFXG X in 4 th position is T or A; X in 12 th position is A or S;	SEQ ID NO: 22

	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	
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

In one embodiment, the linker L of the ADC of the invention comprises at least one non-cleavable linker element. A non-cleavable linker element may be defined as a linker element that is only subjected to lysosomal degradation, that is not the substrate of specific enzymes and
5 that is stable in plasma and cytosol.

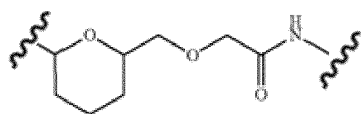
The non-cleavable linker element may be selected from the group consisting of:

- a. ethylenediamine (EDA),
- b. N-formyl-N,N'-dimethylethylenediamine,
- c. diethylamine (DEA),
- 10 d. a piperazine-derived compound of the following formula:



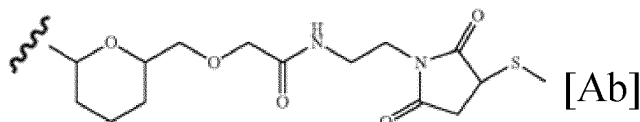
wherein the wavy lines indicate attachments to the toxin and another linker element,

- e. the compound of the following formula:



wherein the wavy lines indicate attachments to the toxin and another linker element,

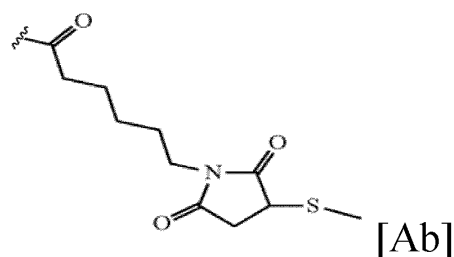
f. the compound of the following formula:



5

wherein the wavy line indicates attachment to the toxin and [Ab] indicates the antibody or fragment thereof,

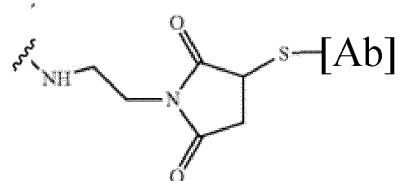
g. a maleimidocaproyl compound of the following formula:



10

wherein the wavy line indicates attachment to another linker element and [Ab] indicates the antibody or fragment thereof,

h. the compound of the following formula:



15

wherein the wavy line indicates attachment to a toxin and [Ab] indicates the antibody or fragment thereof,

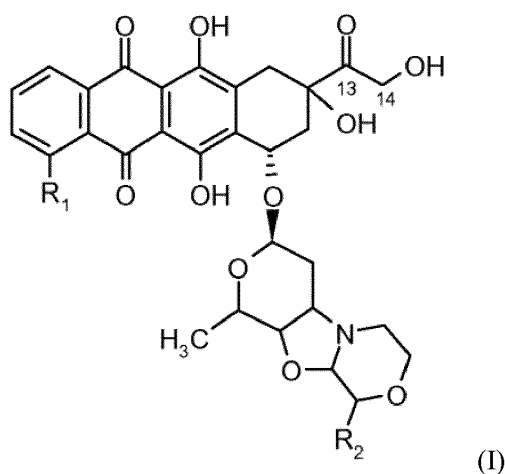
and wherein the non-cleavable linker element is conjugated to the toxin by means of an amide bond or an ether bond.

The non-cleavable linker element may be directly covalently attached to the antibody (and thereby form the linker) or it may be attached via other linker elements such as oligopeptide

linker elements. Alternatively, or additionally, cleavable linker elements may be present in the linker.

The non-cleavable linker element may be linked to the antibody via amino-acids of the antibody sequence that have side-chains with available nucleophilic groups such as ϵ -NH₂ of lysine and the sulfhydryl SH group of cysteine. Maleimide chemistry allows linkage to the cysteine side-chain while acylation chemistry is usually used for linkage to the lysine side-chain. Ample information on such linkages can be found in Jain *et al*, 2015 (Jain et al. 2015). Linkage of a non-cleavable linker element to an oligopeptide linker element may be carried out by carbodiimide crosslinking chemistry. Guidance for such crosslinking chemistry may be found in the Thermo Scientific Crosslinking Technical Handbook (2012) ("Crosslinking Technical Handbook" 2012).

The non-cleavable linker element may also be directly attached to the anthracycline. In one embodiment, the non-cleavable linker element is attached to the anthracycline of formula I by means of an amide bond to C₁₃ or an ether bond to C₁₄, wherein R₁ is hydrogen atom, hydroxy or methoxy group and R₂ is a C₁-C₅ alkoxy group.



It is understood that a combination of one or more linker elements may be used to form the linker in order to link the antibody to the toxin, including enzyme-cleavable linker elements.

In another element, the linker further comprises an oligopeptide linker element and/or enzyme-cleavable linker element and/or a spacer element.

The oligopeptide linker element is understood as being an oligopeptide that is present in addition to the peptidic chain forming the antibodies or fragment thereof. The oligopeptide

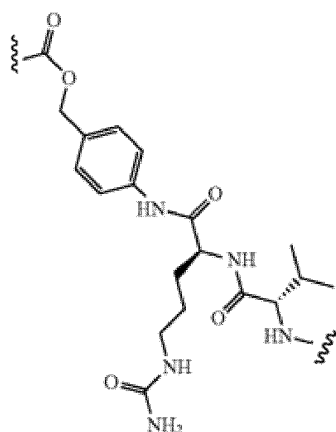
linker element may be directly attached to the C-termini of the heavy and/or light chains forming the antibody, or the fragments thereof. In one embodiment, the DNA coding sequence of the oligopeptide linker element may be part of the DNA coding for the respective heavy and/or light chain forming the antibody or fragment thereof.

- 5 In another embodiment, the oligopeptide linker element may be the result of peptide ligation used to link two or more oligopeptide linker elements. Ligation may be catalyzed by peptide ligases such as sortases (i.e. Sortase A), asparaginyl endoproteases (i.e. Butelase 1), trypsin related enzymes (i.e. Trypsiligase) or subtilisin-derived variants (i.e. Peptiligase) (Nuijens et al. 2019). The oligopeptide linker elements may thus include peptide ligase recognition motifs.
- 10 The term spacer element, in the context of the invention, is to be understood as spacers added to the linker to avoid steric hindrance and to allow proper conjugation of the toxin to the antibody or fragment thereof.

- In one embodiment, the oligopeptide linker element comprises a sortase recognition motif oligopeptide selected from: -LPXTG_m-, -LPXAG_m-, -LPXSG_m-, -LAXTG_m-, -LPXTG_m-, -
 15 LPXTA_m-, -NPQTG_m- or -NPQTN_m-, with G_m being an oligoglycine with m being an integer between ≥ 1 and ≤ 21 , A_m being an oligoalanine with m being an integer between ≥ 1 and ≤ 21 , N_m being an oligoasparagine with m being an integer between ≥ 1 and ≤ 21 and X being any conceivable amino acid. Preferably, m is 2 or 3, especially 2. In a preferred embodiment, the sortase recognition motif oligopeptide is -LPQTGG- or -LPETGG-.
 20 motif oligopeptide may be present at the C-termini of the heavy and/or light chains, of the antibody or of fragments thereof, preferably at the C-termini of the light chains.

- In a further preferred embodiment, the oligopeptide linker element of the ADC comprises the sequence SEQ ID NO: 131. In one embodiment, the sequence SEQ ID NO: 131 is at the C-terminus of the antibody heavy chain and in another preferred embodiment at the C-terminus
 25 of the antibody light chain.

In another embodiment, an enzyme-cleavable linker element is present in the linker. The enzyme-cleavable linker element may comprise a val-cit-PAB linker according to the compound of the following formula:



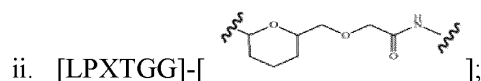
wherein the wavy lines indicate attachments to other linker elements or the antibody or the toxin. The enzyme-cleavable linker element may be attached to another linker element or the antibody or the toxin by known crosslinker chemistry as described above for the non-cleavable linker elements.

In yet another embodiment, the linker further comprises a spacer element. In one embodiment, the spacer element comprises a peptidic flexible oligopeptide. Flexible linker elements can be applied when the linked components require a certain degree of movement or interaction. Flexible oligopeptides are generally composed of small, non-polar (e.g. G) or polar (e.g. S or T) amino acids. The small size of these amino acids provides flexibility and allows for mobility of the connected functional components. The incorporation of S or T can maintain stability of the linker in aqueous solutions by forming hydrogen bonds with water molecules, and therefore reduces the unfavorable interaction between the linker and protein moieties. Further guidance on peptidic flexible oligopeptides may be found in Chen et al, 2013 (Chen, Zaro, and Shen 2013).

Preferably the spacer element comprises a peptidic flexible oligopeptide consisting of G and S, more preferably the peptidic flexible oligopeptide is (GGGGS)_o with o being 1, 2, 3, 4 or 5.

The invention also provides ADCs of the following structures:

- a. $A - ([\text{oligopeptide linker element} - \text{non-cleavable linker element}] - T)_n$ and preferably wherein the linker is selected from:
 - i. [LPXTGG]-[ethylenediamine], and



- b. A – ([oligopeptide linker element – enzyme cleavable linker element – non-cleavable linker element] – T)_n and preferably wherein the linker is selected from:

- 5 i. [LPXTGG]-[vc-PAB]-[N-formyl-N,N'-dimethylethylenediamine], and
 ii. [LPXTGG]-[vc-PAB]-[piperazine];

- c. A – ([spacer element – oligopeptide linker element – non-cleavable linker element] – T)_n and preferably wherein the linker is selected from:

- i. [GGGGS]-[LPXTGG]-[ethylenediamine], and



- d. A – ([spacer element – oligopeptide linker element – enzyme cleavable linker element – non-cleavable linker element] – T)_n and preferably wherein the linker is selected from:

- 15 i. [GGGGS]-[LPXTGG]-[vc-PAB]-[N-formyl-N,N'-
 dimethylethylenediamine], and
 ii. [GGGGS]-[LPXTGG]-[vc-PAB]-[piperazine].

where A is 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, and T is a anthracycline.

In one embodiment, n is an integer ≥ 1 and ≤ 10 . The invention also relates to a pharmaceutical acceptable salt or ester of the ADC.

It is understood that the toxin may be conjugated via the linker to the C-termini of the antibody heavy and/or light chains, or at the C-termini of the antibody fragments.

25 In a preferred embodiment, the non-cleavable linker element is ethylenediamine and the oligopeptide linker element is LPXTGG wherein X is Q or E, preferably wherein X is Q.

In one embodiment,

- a. (L-T) is covalently linked to both light chains of the antibody,
- b. (L-T) is covalently linked to both heavy chains of the antibody, or
- c. (L-T) is covalently linked to both light chains and both heavy chains of the antibody.

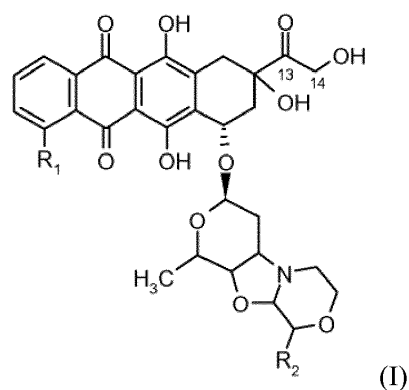
5 In one embodiment, (L-T)

- a. is linked to the C-terminus of the antibody light chain or antibody heavy chain, or
- b. is linked to an amino acid side chain of the antibody light chain or antibody heavy chain.

10 In case the toxin is linked to the C-terminus of the antibody light chain or antibody heavy chain, an oligopeptide linker element and an optional spacer element may be part of the antibody amino-acid sequence when the antibody is recombinantly expressed with such C-terminal tag. In case the toxin is linked to an amino-acid side chain of the antibody amino acid sequence, the linker element may be linked by maleimide chemistry or acylation chemistry, depending on the amino acid side chain of choice.

15 Surprisingly, the ADCs of the invention, with the toxin either conjugated via an oligopeptide peptide linker element – non-cleavable enzyme linker element at the HC only, via a spacer element - oligopeptide peptide linker element – non-cleavable enzyme linker element at the LC only, or such linker-toxin combinations at the HC and LC have a higher cytotoxic activity on cells expressing CLDN18.2 than a similar ADC based on IMAB362 (see Figure 11 to 19 and
20 Example 8) showing the superiority of the newly identified antibodies over the prior art antibody also in the ADC context. The ADCs of the invention have also a higher cytotoxic activity than an ADC based on IMAB362 and conjugated to MMAE via an MC-vc-PAB linker as previously disclosed in WO2016/165762 (see Figure 11).

In one embodiment, the anthracycline has the following formula (I):



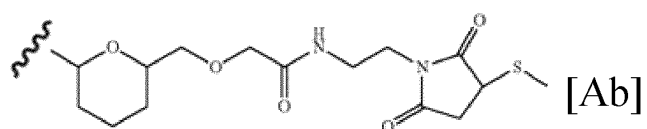
wherein R_1 is a hydrogen atom, a hydroxy or methoxy group, and wherein R_2 is a C_1 - C_5 alkoxy group. In one embodiment, the anthracycline is attached to the linker via C_{13} resulting in the loss of C_{14} and the hydroxyl group or via C_{14} resulting in loss of the hydroxyl group.

- 5 It is understood that linking the toxin (via C_{13} or C_{14}) to an antibody will not affect the cytotoxic activity of the toxin.

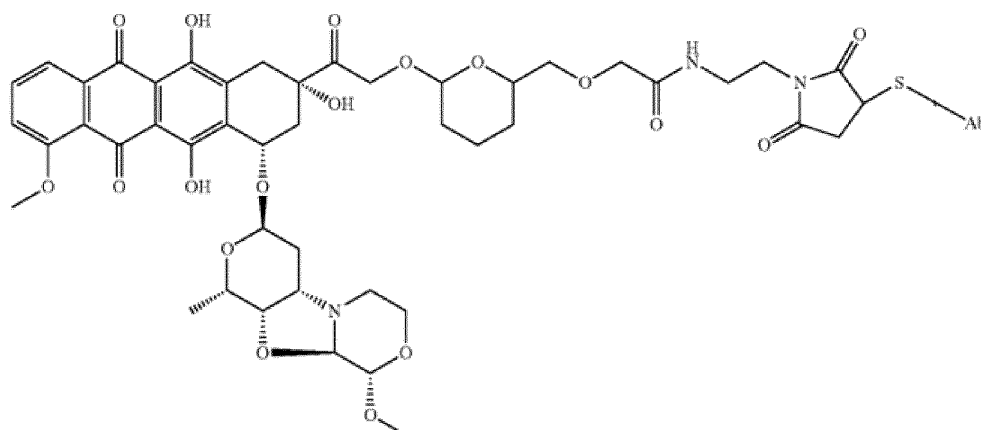
Further information on the synthesis of PNU-159682 and its use as toxin in ADCs may be found in Holte D *et al* 2020 (Holte et al. 2020).

- 10 PNU-159682 may be linked to the antibody by non-cleavable or enzyme-cleavable linkers as shown below.

The linker may be a maleimide acetal linker:

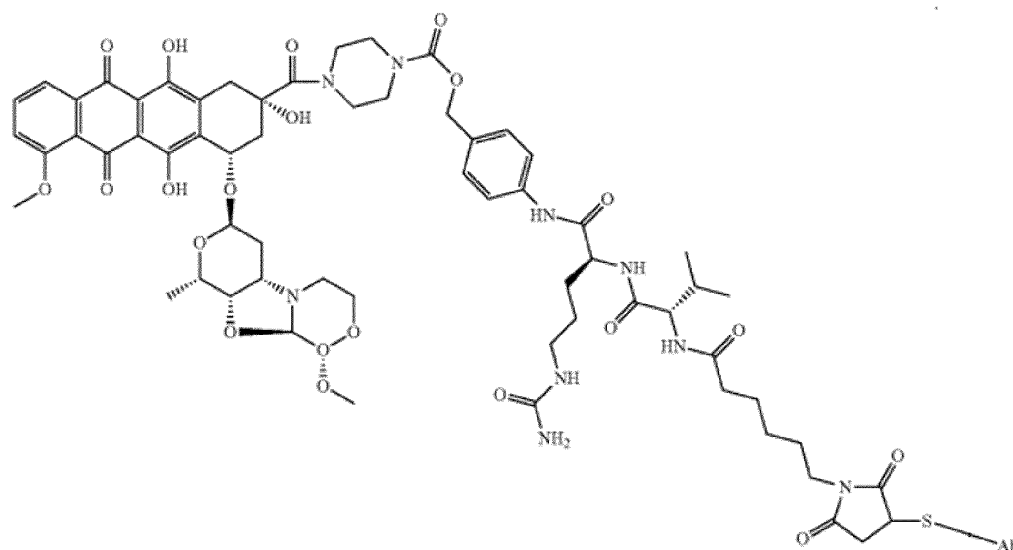


Such a linker was used in an PNU-159682-maleimide acetal-Ab ADC shown below:



Such a PNU-159682 maleimide acetal-Ab ADC has been disclosed in US 10,435,471, column 90. The PNU-159682 maleimide acetal compound has been disclosed as compound 51 in WO2010/009124 and may be prepared as disclosed in Example 3d (paragraphs [0576] to [0578]), based on the compound prepared in Example 2 (paragraphs [0542] to [0550]) of the same application.

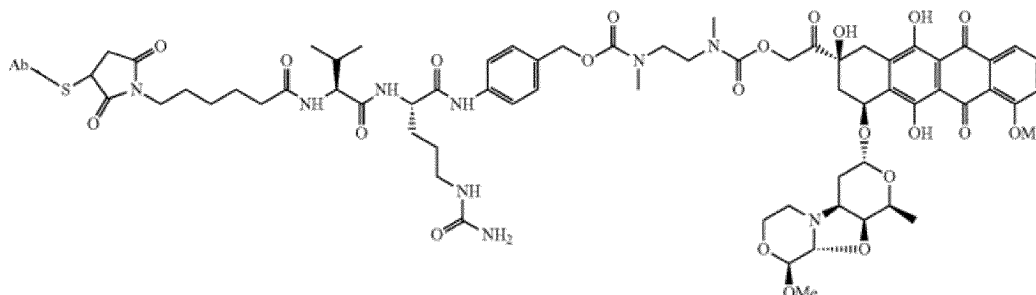
PNU-159682 may also be linked to the antibody by a val-cit-PAB enzyme-cleavable linkers to form a PNU-159682-val-cit-PAB-Ab ADC as shown below:



Such an ADC has been disclosed in US 10,435,471, column 91-92. The PNU-159682-val-cit-

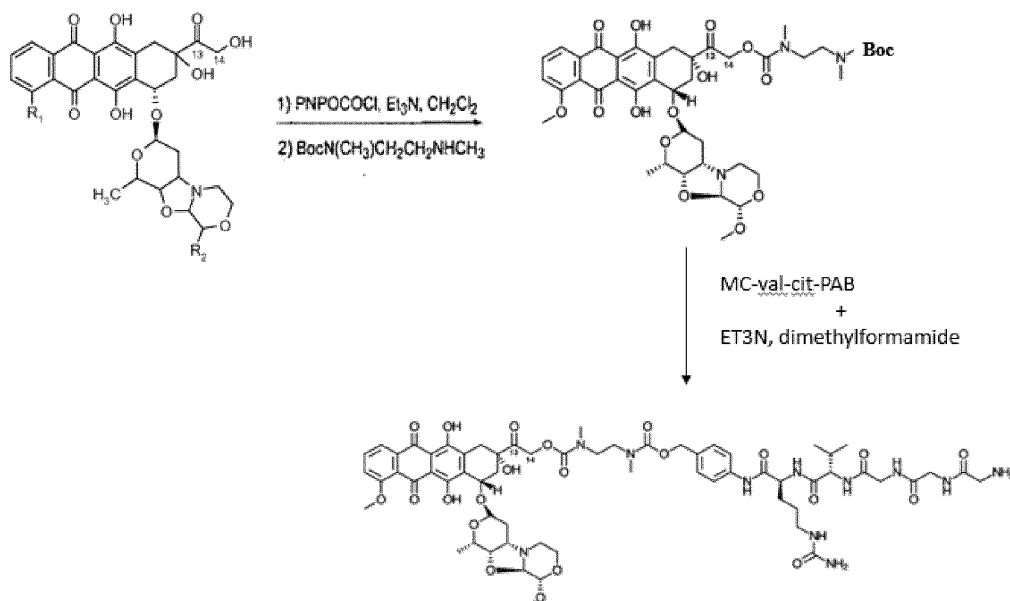
PAB compound is disclosed as compound 55 in WO2010/009124 and may be prepared as shown in Example 3b (paragraph [0567]-[0573] and Figure 7d) of the same application.

PNU-159682 may also be linked to the antibody via an enzyme cleavable linker val-cit-PAB and an additional non-cleavable linker element as shown below:



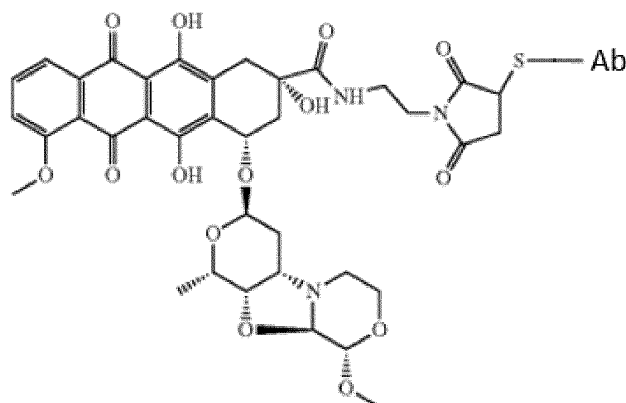
5

Such an ADC has been disclosed in US 10,435,471, column 91-92 and Yu SF et Clin Cancer Research 2015 (Yu et al. 2015). The PNU-159682-val-cit-PAB + non-cleavable linker compound may be prepared as follows:



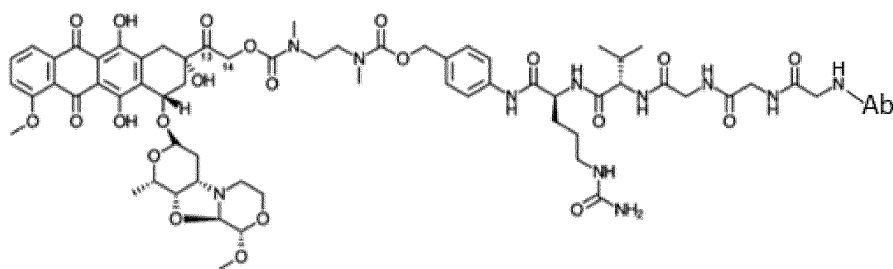
10 wherein MC-val-cit-PAB is commercially available (MedChemExpress Cat. No.: HY-78738) and Boc is a tert-butyloxycarbonyl protecting group.

PNU-159682 may also be linked to the antibody via a non-cleavable maleimide linker as shown below:



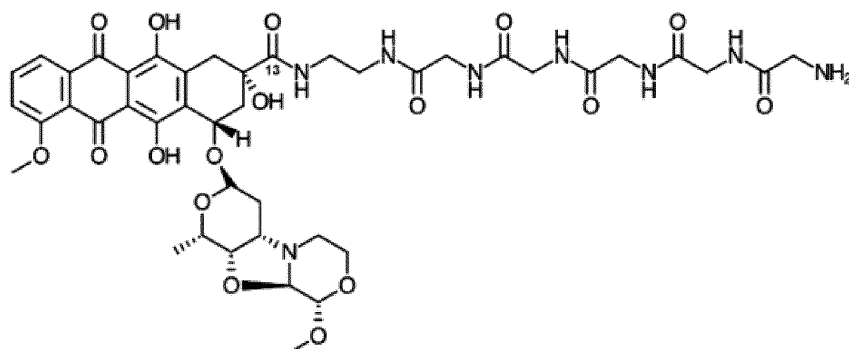
Such ADC has been disclosed in US 10,435,471, column 93. The PNU-159682-maleimid compound is disclosed as compound 55 in WO2010/009124 and its preparation in Example 3a (paragraphs [0564] to [0566] of the same application).

- 5 A combination of non-cleavable, enzyme-cleavable and oligopeptide linker elements has also been used to link PNU-159682 to an antibody. Such ADC is shown below:



- Such a compound is disclosed in Stefan et al. (Stefan et al. 2017). Such an ADC may be synthesized as disclosed above for the PNU-159682-val-cit-PAB + non-cleavable linker ADC, substituting MC-Val-Cit-PAB by Fmoc-Gly3-Val-Cit-PAB (commercially available from MedChemExpress Cat No.: HY-136106), and the resulting linker-toxin compound may be conjugated to an antibody as disclosed in WO2016/102679, page 34, 2nd paragraph.
- 10

PNU-159682 may also be linked to an antibody via a non-cleavable EDA linker element combined with an oligopeptide linker element (-GGGGG-) as shown below:



Such a compound is disclosed in WO2016/102679, Figure 3A. It may be prepared as disclosed in the scheme on Figure 3B and page 33, last paragraph to page 34, 1st paragraph of WO2016/102679 and the resulting linker-toxin compound may be conjugated to an antibody as disclosed in WO2016/102679, page 34, 2nd paragraph. The oligo peptide linker element used
 5 disclosed in WO2016/102679, page 34, 2nd paragraph. The oligo peptide linker element used above may also be (-GGG-) or preferably (-GG-).

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
 10 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.
 15 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
 20 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
 25 cytometry on cells expressing CLDN18.2 of antibodies with CDRs comprised in the consensus sequences of Table 1.

The cytotoxic activity of ADCs can be characterized by EC50 values retrieved from an ADC cytotoxic assay. Example 8 and Table 9 below relates to the calculation of EC50 values of the ADCs of the invention using cytotoxic assays with cells expressing CLDN18.2.

Although the antibody binding EC50 ($\mu\text{g/ml}$) values of all the hCl antibodies measured on the HEK293T cells lines overexpressing CLDN18.2 and on the PA-TU-8988S-High cell lines is higher than the antibody binding EC50 value of the reference antibody IMAB362 on the same cell lines (see Table 4 and Example 2), i.e. the hCl antibodies provided herein bind CLDN18.2 with lower affinity compared to IMAB362, the inventors have now surprisingly shown that the ADC cytotoxicity EC50 (ng/ml) value of the ADCs of the invention measured on the HEK293T and A549 cells lines overexpressing CLDN18.2 and on the PA-TU-8988S-High cell lines were lower than the cytotoxicity EC50 value of an ADC based on IMAB362 on the same cell lines (see Table 9 and Example 8). This shows that the ADCs of the invention have a higher cytotoxic activity than an ADC based on IMAB362, despite the antibodies having a lower binding affinity to the target than IMAB362.

Likewise, the ADCs of the invention showed higher *in-vivo* efficacy in patient-derived tumor xenograft models than an ADC based on IMAB362 (see Example 9).

In one embodiment, the antibody or fragment thereof binds to CLDN18.2 and 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 LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 24, SEQ ID NO: 25, and SEQ ID NO: 26, respectively.

In one embodiment, the antibody or fragment thereof binds to CLDN18.2 and comprises:

- 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;
- 5 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;
- 10 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
- 15 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.

- 20 In another preferred embodiment, the ADCs based on the antibodies have a higher cytotoxic activity on CLDN18.2-expressing cells than the corresponding ADC based on IMAB362 as for example shown by the EC50 values for cytotoxic activity.

In yet another embodiment, the antibody or fragment thereof binds to CLDN18.2 and comprises:

- 25 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.

In yet another embodiment, the antibody or fragment thereof binds to CLDN18.2 and comprises:

- 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.

In another embodiment, the antibody or fragment thereof binds to CLDN18.2 and comprises:

- 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;
- 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;
- h. a VL sequence of SEQ ID NO: 40; or
- i. a VL sequence of SEQ ID NO: 41.

In a further embodiment, the antibody or fragment thereof binds to CLDN18.2 and comprises:

- 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;
- 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.

In another embodiment, the antibody binds to CLDN18.2 and comprises:

- 10 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;
- 15 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;
- 20 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;
- 25 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.

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.

The ADCs of the present invention, with an anthracycline conjugated to the light chain only, have a higher cytotoxic activity on cells expressing CLDN18.2 than IMAB362 with an anthracycline derivative conjugated to the light chain only (see Figures 11 to 19). The ADCs of the invention with an anthracycline conjugated either to the heavy and light chain or only to the heavy chain or only to the light chain have also a higher cytotoxic activity than IMAB362-MC-vc-PAB-MMAE previously disclosed in WO2016/165762 (see Figure 11).

The inventors have also shown that the ADCs of the present have a higher *in-vivo* cytotoxic activity on patient-derived gastric tumor xenograft models, colon tumor xenograft models, pancreatic tumor xenograft models and lung tumor xenograft models than an identical ADC based on IMAB362 (see Figure 21 to 24, respectively and Example 9). In a preferred embodiment, the antibody binds to CLDN18.2 and comprises the heavy chain sequence of SEQ ID NO: 46 and light chain sequence of SEQ ID NO: 51.

In a further preferred embodiment, the antibody binds to CLDN18.2 and consists of the heavy chain sequence of SEQ ID NO: 46 and light chain sequence of SEQ ID NO: 51.

The antibody may have 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 antibody binds to CLDN18.2 and has 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: 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.

In a further embodiment, the antibody binds to CLDN18.2 and has 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 antibody binds to CLDN18.2 and has 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 antibody or fragment thereof that binds to CLDN18.2 is 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
- 25 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 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 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 FcγR binding.

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 FcγRIIIa binding	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • (Stavenhagen et al. 2007) • (Lazar et al. 2006) • (Shields et al. 2001) • (Mimoto et al. 2013)
Increased FcγRIIIa binding, decreased FcγRIIb binding	S239D/I332E/A330L	(Lazar et al. 2006)
<i>Enhance ADCP</i>		
Increased FcγRIIa binding, Increased FcγRIIIa binding	G236A/S239D/I332E	(Richards et al. 2008)
<i>Enhance CDC</i>		
Increased C1q binding	<ul style="list-style-type: none"> • K326W/E333S • S267E/H268F/S324T • IgG1/IgG3 cross subclass 	<ul style="list-style-type: none"> • (Idusogie et al. 2001) • (Moore et al. 2010)

		<ul style="list-style-type: none"> • (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	<ul style="list-style-type: none"> • 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 	<ul style="list-style-type: none"> • (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	<ul style="list-style-type: none"> • M252Y/S254T/T256E • M428L/N434S 	<ul style="list-style-type: none"> • (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)

In vivo 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
5 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
10 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
15 listed in Table 3, while the other scFv domain is from an antibody that binds e.g. to CD3, CD16, 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 antibody or fragment thereof binds to CLDN18.2, the antibody
20 having the constant light chain region (CL) of SEQ ID NO: 127 and 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 antibody has 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
25 even further reduced FcγR binding.

The inventors have now surprisingly shown that ADCs of the present invention based on antibodies having the L234A/L235A mutations in the constant heavy chain region CH2 have a higher *in-vivo* cytotoxic activity on patient-derived tumor xenograft models than an identical ADC based on IMAB362 (see Figure 21C and 23B and Example 9).

30 In a another preferred embodiment, the antibody or fragment thereof binds to CLDN18.2 and comprises 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 antibody or fragment thereof binds to CLDN18.2 and consists of the VH sequence of SEQ ID NO: 33, the VL sequence of SEQ ID NO: 38, the
5 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 antibody or fragment thereof binds to CLDN18.2, wherein the antibody or fragment thereof is humanized. Humanization of monoclonal antibodies is well-established. The Handbook of Therapeutic Antibodies, Second Edition, gives ample
10 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 fragment binding to CLDN18.2.

15 In a further embodiment, the antibody or fragment thereof binds 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
20 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
25 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.

In another embodiment, the ADC is bound to another moiety. The binding of the antibody or
30 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.

In yet another embodiment, the -specific antibody or fragment thereof binds to CLDN18.2, wherein the antibody is less susceptible to posttranslational deamidation than IMAB362. In a further embodiment, the tumor-specific antibody or fragment thereof binds to CLDN18.2, wherein the antibody does not undergo posttranslational deamidation. Posttranslational modifications (PTM) are an important concern 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 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*).

10 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.

15 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.

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.

20 In another embodiment, the invention relates to a method of producing an ADC of the invention.

In one embodiment, the method comprises the following steps:

- a. providing A, an antibody or fragment thereof with one or more linker elements,
- b. providing one or more toxins T with one or more linker elements, and
- c. conjugating the antibody and the toxin resulting in the antibody-drug conjugate.

30 In one embodiment, the method comprises the following steps:

- d. providing A, an antibody or fragment thereof with an oligopeptide linker element preferably at its C-terminus, optionally preceded by a spacer element at the antibody light and/or heavy chains,
- e. providing one or more toxins T with a non-cleavable linker element optionally followed by an oligopeptide linker element, and
- f. conjugating the antibody and the toxin resulting in the antibody-drug conjugate.

It is understood that any antibody A herein disclosed may be provided with any oligopeptide linker element and optional spacer element herein disclosed. Likewise, any anthracycline toxin T may be linked with any non-cleavable linker element herein disclosed. The type of conjugation may depend on the linker element and/or on the method for preparing the ADC. A representation of an ADC produced by this method can be found in Figure 25.

In a preferred embodiment, the ADC of the invention consists of:

- the antibody consisting of two heavy chains of the amino acid sequence according to SEQ ID NO: 46, and two light chains of the amino acid sequence according to SEQ ID NO: 51, wherein the antibody binds to CLDN18.2,
- the linker [GGGGS]-[LPQTGG]-[ethylenediamine] at the C-terminus of the light chains, and
- the anthracycline-based small molecule toxin 3'-deamino-3'',4'-anhydro-[2''(S)-methoxy-3''(R)-oxy-4''-morpholinyl]doxorubicin (PNU-159682), linked covalently to the ethylenediamine of the linker at C₁₃, resulting in the loss of C₁₄ and of the hydroxyl group.

In another preferred embodiment, the ADC of the invention consists of:

- the antibody consisting of two heavy chains of the amino acid sequence according to SEQ ID NO: 133, and two light chains of the amino acid sequence according to SEQ ID NO: 51, wherein the antibody binds to CLDN18.2,
- the linker [GGGGS]-[LPQTGG]-[ethylenediamine] at the C-terminus of the light chains, and
- the anthracycline-based small molecule toxin 3'-deamino-3'',4'-anhydro-[2''(S)-methoxy-3''(R)-oxy-4''-morpholinyl]doxorubicin (PNU-159682), linked covalently to the ethylenediamine of the linker at C₁₃, resulting in the loss of C₁₄ and of the hydroxyl group.

In yet another preferred embodiment, the ADC of the invention consists of:

- the antibody consisting of two heavy chains of the amino acid sequence according to SEQ ID NO: 134 and two light chains of the amino acid sequence according to SEQ ID NO: 51, wherein the antibody binds to CLDN18.2,
- 5 • the linker [GGGGS]-[LPQTGG]-[ethylenediamine] at the C-terminus of the light chains, and
- the anthracycline-based small molecule toxin 3'-deamino-3'',4'-anhydro-[2''(S)-methoxy-3''(R)-oxy-4''-morpholinyl]doxorubicin (PNU-159682),
10 linked covalently to the ethylenediamine of the linker at C₁₃, resulting in the loss of C₁₄ and of the hydroxyl group.

The invention also relates to a pharmaceutical composition comprising the disclosed ADCs and an excipient.

Also provided are nucleic acid sequences encoding the isolated tumor-specific antibodies or functional fragments thereof that bind CLDN18.2 for their use in the manufacture of an ADC.

- 15 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, 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
20 may be an IgA1, IgA2, IgD, IgE, IgG1, IgG2, IgG3, IgG4, synthetic IgG, IgM or mutated and Fc-engineered versions thereof. The nucleic acids may additionally comprise coding sequences for oligopeptide linker elements that are directly fused to the C-termini of the antibody heavy chains and or the antibody light chains.

- Also provided are expression vectors comprising a nucleic acid of the invention or a degenerate
25 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). Preferred are expression
30 vectors for mammalian cells, especially CHO cells.

Also provided are host cells 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. Preferred are mammalian cells, especially CHO cells.

In another embodiment, the invention relates to an ADC of the invention binding to CLDN18.2
5 for use in treatment.

In another embodiment of the invention relates to an ADC of the invention for use in the treatment of a subject that is suffering from a cancer/neoplastic disease.

In another embodiment, the invention relates to an ADC 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
10 being diagnosed for a neoplastic disease.

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

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
15 disease to be treated expresses CLDN18.2.

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

Another embodiment of the invention provides a method for treating a neoplastic disease, including pancreatic, gastric, esophageal, ovarian or lung cancer, with an ADC as provided
20 herein, wherein the method comprises administering a pharmaceutically effective amount of the ADC 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.

The amino acid sequence of human CLDN18.2 protein has the NCBI reference sequence: NP_001002026.1. The sequence can also be derived from SEQ ID NO: 135.

DESCRIPTION OF DRAWINGS

25 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.

Figure 11: *In-vitro* cytotoxic assay on HEK-293T-CLDN18.2 cells of the ADCs were PNU is conjugated either to the HC or LC or HC and LC of the chimeric antibodies cC11-1 (A), cC11-2 (B) or cC11-3 (C). The cytotoxic activity of the ADCs is compared to the cytotoxic activity of and ADC based on IMAB362 or the isotype control Ac10 conjugated to PNU is the same manner, or, when shown, to an ADC based on IMAB362 were the toxin MMAE is conjugated to the antibody via a MC-vc-PAB enzyme-cleavable linker. Figure legend: the ADCs are labeled HC-LC-PNU when PNU is conjugated to the heavy and light chains of the antibody, labeled HC-PNU when PNU is conjugated to the heavy chains only and labeled LC-PNU when PNU is conjugated to the light chains only. All ADCs conjugated with PNU have a -LPQTGG-oligopeptide linker and an ethylenediamine linker. A flexible oligopeptide -GGGGS- is also present when PNU is conjugated to the light chains. When present in the label, G2 stands for the two glycine in the oligopeptide linker.

Figure 12: *In-vitro* cytotoxic assay on HEK-293T-CLDN18.1 cells of the ADCs were PNU is conjugated either to the HC or LC or HC and LC of the chimeric antibodies cC11-1 (A), cC11-2 (B) or cC11-3 (C). The cytotoxic activity of the ADCs is compared to the cytotoxic activity of IMAB362 or the isotype control Ac10 conjugated to PNU is the same manner. Figure legend: the ADCs are labeled HC-LC-PNU when PNU is conjugated to the heavy and light chains of the antibody, labeled HC-PNU when PNU is conjugated to the heavy chains only and labeled LC-PNU when PNU is conjugated to the light chains only. All ADCs conjugated with PNU have a -LPQTGG- oligopeptide linker and an ethylenediamine linker. A flexible oligopeptide -GGGGS- is also present when PNU is conjugated to the light chains. When present in the label, G2 stands for the two glycine in the oligopeptide linker.

Figure 13: *In-vitro* cytotoxic assay on BxPC-3-CLDN18.2 cells of the ADCs were PNU is conjugated either to the HC or LC or HC and LC of the chimeric antibodies cC11-1 (A), cC11-2 (B) or cC11-3 (C). The cytotoxic activity of the ADCs is compared to the cytotoxic activity of IMAB362 or the isotype control Ac10 conjugated to PNU is the same manner. Figure legend: the ADCs are labeled HC-LC-PNU when PNU is conjugated to the heavy and light chains of the antibody, labeled HC-PNU when PNU is conjugated to the heavy chains only and labeled LC-PNU when PNU is conjugated to the light chains only. All ADCs conjugated with PNU have a -LPQTGG- oligopeptide linker and an ethylenediamine linker. A flexible oligopeptide -

GGGGS- is also present when PNU is conjugated to the light chains. When present in the label, G2 stands for the two glycine in the oligopeptide linker.

Figure 14: *In-vitro* cytotoxic assay on A549-CLDN18.2 cells of the ADCs were PNU is conjugated either to the HC or LC or HC and LC of the chimeric antibodies cC11-1 (A), cC11-2 (B) or cC11-3 (C). The cytotoxic activity of the ADCs is compared to the cytotoxic activity of IMAB362 or the isotype control Ac10 conjugated to PNU in the same manner, or to an ADC based on IMAB362 where the toxin MMAE is conjugated to the antibody via a MC-vc-PAB enzyme-cleavable linker. Figure legend: the ADCs are labeled HC-LC-PNU when PNU is conjugated to the heavy and light chains of the antibody, labeled HC-PNU when PNU is conjugated to the heavy chains only and labeled LC-PNU when PNU is conjugated to the light chains only. All ADCs conjugated with PNU have a -LPQTGG- oligopeptide linker and an ethylenediamine linker. A flexible oligopeptide -GGGGS- is also present when conjugated PNU is to the light chains. When present in the label, G2 stands for the two glycine in the oligopeptide linker.

Figure 15: *In-vitro* cytotoxic assay on A549-CLDN18.1 cells of the ADCs were PNU is conjugated either to the HC or LC or HC and LC of the chimeric antibodies cC11-1 (A), cC11-2 (B) or cC11-3 (C). The cytotoxic activity of the ADCs is compared to the cytotoxic activity of IMAB362 or the isotype control Ac10 conjugated to PNU in the same manner, or to an ADC based on IMAB362 where the toxin MMAE is conjugated to the antibody via a MC-vc-PAB enzyme-cleavable linker. Figure legend: the ADCs are labeled HC-LC-PNU when PNU is conjugated to the heavy and light chains of the antibody, labeled HC-PNU when PNU is conjugated to the heavy chains only and labeled LC-PNU when PNU is conjugated to the light chains only. All ADCs conjugated with PNU have a -LPQTGG- oligopeptide linker and an ethylenediamine linker. A flexible oligopeptide -GGGGS- is also present when PNU is conjugated to the light chains. When present in the label, G2 stands for the two glycine in the oligopeptide linker.

Figure 16: *In-vitro* cytotoxic assay on PATU-8988-S-High cells of the ADCs were PNU is conjugated either to the HC or LC or HC and LC of the chimeric antibodies cC11-1 (A), cC11-2 (B) or cC11-3 (C). The cytotoxic activity of the ADCs is compared to the cytotoxic activity of IMAB362 or the isotype control Ac10 conjugated to PNU in the same manner. Figure legend: the ADCs are labeled HC-LC-PNU when PNU is conjugated to the heavy and light chains of the antibody, labeled HC-PNU when PNU is conjugated to the heavy chains only and labeled

LC-PNU when PNU is conjugated to the light chains only. All ADCs conjugated with PNU have a -LPQTGG- oligopeptide linker and an ethylenediamine linker. A flexible oligopeptide -GGGGS- is also present when PNU is conjugated to the light chains. When present in the label, G2 stands for the two glycine in the oligopeptide linker.

5 Figure 17: *In-vitro* cytotoxic assay on A549-CLDN18.2 cells of the ADCs were PNU is conjugated to the LC of the humanized antibodies hC11a to hC11c (A), hC11d to hC11f (B), hC11g to hC11i (C) and hC11j (D). The cytotoxic activity of the ADCs is compared to the cytotoxic activity of ADCs were PNU is conjugated to the LC of the chimeric cC11-1 antibody or IMAB362. Figure legend: the ADCs, labeled LC-PNU, have PNU conjugated to the light
10 chains only via a [GGGGS]-[LPQTGG]-[ethylenediamine] linker. When present in the label, G2 stands for the two glycine in the oligopeptide linker.

Figure 18: *In-vitro* cytotoxic assay on HEK-293T-CLDN18.2 cells of the ADCs were PNU is conjugated to the LC of the humanized antibodies hC11a to hC11c (A), hC11d to hC11f (B), hC11g to hC11i (C) and hC11j (D). The cytotoxic activity of the ADCs is compared to the
15 cytotoxic activity of ADCs were PNU is conjugated to the LC of the chimeric cC11-1 antibody or IMAB362. Figure legend: the ADCs, labeled LC-PNU, have PNU conjugated to the light chains only via a [GGGGS]-[LPQTGG]-[ethylenediamine] linker. When present in the label, G2 stands for the two glycine in the oligopeptide linker.

Figure 19: *In-vitro* cytotoxic assay on HEK-293T-CLDN18.1 cells of the ADCs were PNU is conjugated to the LC of the humanized antibodies hC11a to hC11c (A), hC11d to hC11f (B), hC11g to hC11i (C) and hC11j (D). The cytotoxic activity of the ADCs is compared to the
20 cytotoxic activity of ADCs were PNU is conjugated to the LC of the chimeric cC11-1 antibody or IMAB362. Figure legend: the ADCs, labeled LC-PNU, have PNU conjugated to the light chains only via a [GGGGS]-[LPQTGG]-[ethylenediamine] linker. When present in the label,
25 G2 stands for the two glycine in the oligopeptide linker.

Figure 20: *In-vitro* cytotoxic assay on PATU-8988-S-High cells of the ADCs were PNU is conjugated to the LC of the humanized antibodies hC11a to hC11c (A), hC11d to hC11f (B), hC11g to hC11i (C) and hC11j (D). The cytotoxic activity of the ADCs is compared to the
30 cytotoxic activity of ADCs were PNU is conjugated to the LC of the chimeric cC11-1 antibody or IMAB362. Figure legend: the ADCs, labeled LC-PNU, have PNU conjugated to the light chains only via a [GGGGS]-[LPQTGG]-[ethylenediamine] linker. When present in the label, G2 stands for the two glycine in the oligopeptide linker.

Figure 21: *In-vivo* efficacy of ADC hC11a-LC-G2-PNU (A), hC11f-LC-G2-PNU (B) and hC11a(LALA)-LC-G2-PNU (C) in the gastric patient-derived tumor xenograft model GXA 3037, compared to the ADC IMAB362-LC-G2-PNU. Each ADC is tested either at 0.2 mg/kg/day, 0.6 mg/kg/day or 2 mg/kg/day. Figure legend: all ADCs have PNU conjugated to the light chains only via a [GGGGS]-[LPQTGG]-[ethylenediamine] linker.

Figure 22: *In-vivo* efficacy of ADC hC11a-LC-G2-PNU in the colon cancer patient-derived tumor xenograft model CXF 742, compared to the isotype control ADC Ac10-LC-G2-PNU. Each ADC is tested at 2 mg/kg/day. Figure legend: all ADCs have PNU conjugated to the light chains only via a [GGGGS]-[LPQTGG]-[ethylenediamine] linker.

Figure 23: *In-vivo* efficacy of ADC hC11a-LC-G2-PNU (A) and hC11a(LALA)-LC-G2-PNU (B) in the pancreatic cancer patient-derived tumor xenograft model PAXF 2175, compared to the ADC IMAB362-LC-G2-PNU. Each ADC is tested either at 0.2 mg/kg/day or 0.6 mg/kg/day. Figure legend: all ADCs have PNU conjugated to the light chains only via a [GGGGS]-[LPQTGG]-[ethylenediamine] linker.

Figure 24: *In-vivo* efficacy of ADC hC11a-LC-G2-PNU in the lung cancer patient-derived tumor xenograft model LIXFC 2050, compared to the isotype control ADC Ac10-LC-G2-PNU. Each ADC is tested at 2 mg/kg/day. Figure legend: all ADCs have PNU conjugated to the light chains only via a [GGGGS]-[LPQTGG]-[ethylenediamine] linker.

Figure 25: Graphical representation of and ADC were PNU has been conjugated to the antibody LC via a spacer element -GGGGS-, an oligopeptide linker element -LPQTGG- and a non-cleavable linker element EDA, linked to the C₁₃ of PNU. Figure legend: all ADCs have PNU conjugated to the light chains only via a [GGGGS]-[LPQTGG]-[ethylenediamine] linker.

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 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 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 in 10 humanized clones named hC11a, hC11b, hC11c, hC11d, hC11e, hC11f, hC11g, hC11h, hC11i and hC11j antibodies (see Table 3). These antibodies were also used to generate ADCs.
- 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 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
cC11-1		
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	gccgtcttctacggatatacgatggacgcg	SEQ ID NO: 73
VH	cagatccagctcgtccagagcgggcccggagctgaagaagccgg gggagagcgtgaagatctcgtgcaaggcgagcggatatacgtt cacggactacgcgatgcactgggtcaagcaagcgccggggaaa gggctgaagtggatgggtggatcaacacgtacacggggaagc cgacatacgcggacgacttcaagggcgattcgtgttctcgtc ggaggcgagcgcgagcacggcgaacctgcaaattctcgaacctg aagaacgaggacacggcgacgtacttctgcgcgcgggccgtct tctacggatatacgatggacgcgtgggggcagggtaccagcgt gacggtctcgagc	SEQ ID NO: 74
LCDR1	RASEDIYSNLA	SEQ ID NO: 4
LCDR2	SVKRLQD	SEQ ID NO: 5
LCDR3	LQGSNFPLT	SEQ ID NO: 6

NAME	SEQUENCE	SEQ ID NO
VL	DIQMTQSPASLSASLGETISIACRASEDIYSNLAWEYQQKSGKS PQLLI FSVKRLQDGVPSRFSGSGSGTQYSLKISGMQPEDEGDY FCLQGSNFPLTFGSGTKLEIK	SEQ ID NO: 28
LCDR1	cgggcgagcgaggacatctactcgaacctggcg	SEQ ID NO: 75
LCDR2	tccgtcaagcggctgcaagac	SEQ ID NO: 76
LCDR3	ctgcaaggagcaacttcccgtgacg	SEQ ID NO: 77
VL	gacatccagatgacgcagagcccggcgctcgctgagcgcgagcc tgggggagacgatctcgatcgctgcccggcgagcgaggacat ctactcgaacctggcggttatcaacagaagagcgggaagagc ccgcagctgctgatcttctccgtcaagcggctgcaagacggcg tcccagccgattctcggggagcgggagcgggacgcagtactc gctgaagatctcggggatgcagccggaggacgagggggactac ttctgcctgcaaggagcaacttcccgtgacgttcgggtcgg 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	QIQLVQSGPELKKPGESVKISCKTSGYTFDYAMHWVKQGP GK GMKWMGINAYTGKPTYADDFKGRFVLSLEASASTANLQISNL KNEDTATYFCARAVYYGYTMDAWGQGTSVIVSS	SEQ ID NO: 29
HCDR1	gactacgcgatgcac	SEQ ID NO: 71
HCDR2	tggatcaacgcgtacacggggaagccgacctacgcggacgact tcaagggg	SEQ ID NO: 79
HCDR3	gccgtctactacggatatacgatggac	SEQ ID NO: 80
VH	cagatccagctcgtccagagcgggcccggagctgaagaagccgg gggagagcgtgaagatctcgtgcaagacgagcggatatacggt cacggactacgcgatgcactgggtcaagcaggggcccagggaaa gggatgaagtggatgggtggatcaacgcgtacacggggaagc cgacctacgcggacgacttcaaggggcgattcgtgctgagcct ggaggcgagcgcctcgacggcgaacctgcaaatctcgaacctg aagaacgaggacacggcgacgtacttctgcgcgcggggcgtct actacggatatacgatggacgcgtgggggaggggtaccagcgt gatcgtctcgagc	SEQ ID NO: 81
LCDR1	RTSEDIYSNFA	SEQ ID NO: 9
LCDR2	SVNRLQD	SEQ ID NO: 10
LCDR3	LQGSKFPLT	SEQ ID NO: 11
VL	DIQMTQSPASLSASLGETISIECRTSEDIYSNFAWFQQKSGKS PQLLIYSVNRLQDGVPSRFSGSGSGTQYSLKISGMQPEDEGDY FCLQGSKFPLTFGSGTKLEIK	SEQ ID NO: 30
LCDR1	cggacgagcgaggacatctactcgaacttcgcg	SEQ ID NO: 82
LCDR2	tcagtcaaccggctgcaagac	SEQ ID NO: 83
LCDR3	ctgcaaggagcaagttcccgtgacg	SEQ ID NO: 84
VL	gacatccagatgacgcagagcccggcgagcctgagcgcgagcc tgggggagacgatctcgatcgagtgcgggacgagcgaggacat ctactcgaacttcgctgggttcacagagaagagcgggaagagc ccgcagctgctgatctactcagtcaaccggctgcaagacggcg tcccagccgattctcggggagcgggagcgggacgcagtactc gctgaagatctcggggatgcagccggaggacgagggggactac ttctgcctgcaaggagcaagttcccgtgacgttcgggagcg gtaccaaactcgagatcaaa	SEQ ID NO: 85

NAME	SEQUENCE	SEQ ID NO
cCII-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	gccgtcttctacggatatacgatggacgcg	SEQ ID NO: 73
VH	cagatccagctcgtccagagcgggcccggagctgaagaagccgg gggagagcgtgaagatctcgtgcaaggcgagcggatatacgtt cacggactacgcgatgtactgggtcaagcaagtgcgggggaaa gggctgcgatggatgggtggatcaacacgtacacggggaagc cgacctacgcggacgacttcaaggggagattcgtgttctcgtc ggaggcgcgcgcgagcacggcgcaacctgcaaattctcgaacctg aagaacgaggacacgcgcgacttctcgcgcggggccgtct tctacggatatacgatggacgcgtgggggcagggtaccagcgt gacgggtctcgagc	SEQ ID NO: 88
LCDR1	RTSEDIYSNLA	SEQ ID NO: 13
LCDR2	AIKRLQD	SEQ ID NO: 14
LCDR3	LQGSKFPLT	SEQ ID NO: 11
VL	DIQMTQSPASLSASLGETISIACTSEDIYSNLAWYQQKSGKS PQLLI FAIKRLQDGVPSRFSGSGSGTQYSLKISGMQPEDEGDY FCLQGSKFPLTFSGGTKLEIK	SEQ ID NO: 32
LCDR1	cggacgagcgcgaggacatctactcgaacctggcg	SEQ ID NO: 89
LCDR2	gcgatcaagcggctgcaagac	SEQ ID NO: 90
LCDR3	ctgcaagggagcaagttcccgcgtgacg	SEQ ID NO: 84
VL	gacatccagatgacgcagagcccggcgagcctgagcgcgagcc tgggggagacgatctcgatcgctgcccgcgagcgcgaggacat ctactcgaacctggcgtggtatcaacagaagagcgggaagagc ccgcagctgctgatcttcgcgatcaagcggctgcaagacggcg tcccgcgagccgattctcggggagcgggagcgggacgcagtactc gctgaagatctcggggatgcagccggaggacgagggggactac ttctgcctgcaaggagcaagttcccgcgtgacgttcgggtcgg 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	QVQLVQSGAEVKKPGASVKVSCASGYTFTDYAMHWVRQAPGQ RLEWMGWINTYTGKPTYAQKFQGRVTITRDTSASTAYMELSSL RSED TAVYYCARAVFYGYTMDAWGQGT LVT VSS	SEQ ID NO: 33
Heavy chain	QVQLVQSGAEVKKPGASVKVSCASGYTFTDYAMHWVRQAPGQ RLEWMGWINTYTGKPTYAQKFQGRVTITRDTSASTAYMELSSL RSED TAVYYCARAVFYGYTMDAWGQGT LVT VSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKENWYVDGVEVHNAKTKPREEQYNST	SEQ ID NO: 46

NAME	SEQUENCE	SEQ ID NO
	YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSQSV HEALHNHYTQKSLSLSPGX X is R or K	
HCDR1	gactacgcgatgcac	SEQ ID NO: 71
HCDR2	tggatcaatacatcacaggggaagccgacttatgcgcaaaaat tccaagga	SEQ ID NO: 92
HCDR3	gcggtcttctacggatatacgatggatgcc	SEQ ID NO: 93
VH	caggtccaactagtccaaagcggggcgggaagtcaagaagccc gagcatccgtcaaagtcagctgcaaggcgagcgatatacatt tacggactacgcgatgcactgggtcaggcaagcccctgggcaa aggctcgaatggatggatggatcaatacatcacaggggaagc cgacttatgcgcaaaaattccaaggaagagtcacaattacgcg ggatacatccgcatctaccgcctacatggagctaagctcgctg cggagcgaggatacggcggtctactattgcgcccgagcgggtct tctacggatatacgatggatgcctgggggcagggtaccctggt cacggtctcgagc	SEQ ID NO: 94
LCDR1	RASEDIYSNLA	SEQ ID NO: 4
LCDR2	SVKRLQD	SEQ ID NO: 5
LCDR3	LQGSNFPLT	SEQ ID NO: 6
VL	DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAQYQQKPGKA PKLLIFSVKRLQDGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCLQGSNFPLTFGQGTKVEIK	SEQ ID NO: 38
Light chain	DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAQYQQKPGKA PKLLIFSVKRLQDGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCLQGSNFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 51
LCDR1	agggcctccgaagacatctactccaacctggca	SEQ ID NO: 95
LCDR2	agcgtcaaaagactacaagat	SEQ ID NO: 96
LCDR3	ttgcaaggaagcaatttccccttgact	SEQ ID NO: 97
VL	gacattcaaatgacgcaaagcccatcatcgctgagcgcacatcg tcggggatagagtaccataacatgcagggcctccgaagacat ctactccaacctggcatggatcaacaaaaaccggggaaggct ccgaagctgctgatatcttagcgtcaaaagactacaagatggag taccgagccgattttcggaagcgggagcgggacggatttcac 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	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAPGQ RLEWMGWINTYTGKPTYSQKFQGRVTITRDTASASTAYMELSSL RSEDTAVYYCARAVFYGYTMDAWGQGLTVTVSS	SEQ ID NO: 34
Heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAPGQ RLEWMGWINTYTGKPTYSQKFQGRVTITRDTASASTAYMELSSL RSEDTAVYYCARAVFYGYTMDAWGQGLTVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHCTCPPELLEGGPSVFLFPPKPKDTLMISRT	SEQ ID NO: 47

NAME	SEQUENCE	SEQ ID NO
	PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVTLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSGSEFFLYSKLTVDKSRWQQGNVFSVSV HEALHNHYTQKSLSLSPGX X is R or K	
HCDR1	gattatgcaatgcac	SEQ ID NO: 99
HCDR2	tggattaacacctacacgggcaagccacataactccccaaaat tccaagga	SEQ ID NO: 100
HCDR3	gctgtattctatggatatacaatggatgcc	SEQ ID NO: 101
VH	caggtccaattagtccaaagcggggcggaagtcaagaagccgg ggcgagcggtcaaagtcctcatgcaaagcgagcgatacacatt taccgattatgcaatgcactgggtcaggcaagcaccggacaa aggctggaatggatggatggattaacacctacacgggcaagc ccacataactccccaaaattccaaggaagggtcacgataacgag agacacgagcgcgagcaccggaatggatggggtggattaacac ctacacgggcaagccacataactccccaaaattccaaggaagg gtcacgataacgagagacacgagcgcgagcaccgtaccctggt caccgtctcgagc	SEQ ID NO: 102
LCDR1	RASEDIYSNLA	SEQ ID NO: 4
LCDR2	SVKRLQD	SEQ ID NO: 5
LCDR3	LQGSNFPLT	SEQ ID NO: 6
VL	DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAWYQQKPGKA PKLLIFSVKRLQDGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCLQGSNFPLTFGQGTKVEIK	SEQ ID NO: 38
Light chain	DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAWYQQKPGKA PKLLIFSVKRLQDGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCLQGSNFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST 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 tcggggatagagtaccataacatgcagggcctccgaagacat ctactccaacctggcatggatcaacaaaaaccggggaaggct ccgaagctgctgatatttagcgtcaaaagactacaagatggag taccgagccgattttcgggaagcgggagcgggacggatttcac gctgaccatatcaagtttgcaaccggaggattttgacacatac tattgcttgcaaggaagcaatttccccttgactttcgggcaag gtaccaaggtcgagatcaaa	SEQ ID NO: 98
hCI1c		
HCDR1	DYAMH	SEQ ID NO: 1
HCDR2	WINTYTGKPTYSQKFQG	SEQ ID NO: 16
HCDR3	AVFYGYTMDA	SEQ ID NO: 3
VH	QVQLVQSGAEVKKPGASVKVSKASGYTFTDYAMHWVRQAPGQ RLEWMGWINTYTGKPTYSQKFQGRVTITRDTASTAYMELSSL RSEDVAVYYCARAVFYGYTMDAWGQGLVTVSS	SEQ ID NO: 34
Heavy chain	QVQLVQSGAEVKKPGASVKVSKASGYTFTDYAMHWVRQAPGQ RLEWMGWINTYTGKPTYSQKFQGRVTITRDTASTAYMELSSL RSEDVAVYYCARAVFYGYTMDAWGQGLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK	SEQ ID NO: 47

NAME	SEQUENCE	SEQ ID NO
	KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVTLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVM HEALHNHYTQKSLSLSPGX X is R or K	
HCDR1	gattatgcaatgcac	SEQ ID NO: 99
HCDR2	tggattaacacctacacgggcaagccacatactcccaaaaat tccaagga	SEQ ID NO: 100
HCDR3	gctgtattctatggatatacaatggatgcc	SEQ ID NO: 101
VH	caggtccaattagttccaaagcggggcggaagtcaagaagccgg ggcgagcgtcaaagtctcatgcaaagcgagcggatacacatt tacggattatgcaatgcactgggtcaggcaagcaccgacaa aggctggaatggatggatggattaacacctacacgggcaagc ccacatactcccaaaaattccaaggaagggtcacgataacgag agacacgagcgcgagcaccggaatggatggatggatggattaacac ctacacgggcaagccacatactcccaaaaattccaaggaagg gtcacgataacgagagacacgagcgcgagcaccgtaccctggt caccgtctcgagc	SEQ ID NO: 102
LCDR1	RTSEDIYSNLA	SEQ ID NO: 17
LCDR2	AIKRLQD	SEQ ID NO: 14
LCDR3	LQGSKFPLT	SEQ ID NO: 11
VL	DIQMTQSPSSLSASVGDRVTITCRTSEDIYSNLA WYQQKPGKA PKLLIFAIKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSKFPLTFGQGTKVEIK	SEQ ID NO: 39
Light chain	DIQMTQSPSSLSASVGDRVTITCRTSEDIYSNLA WYQQKPGKA PKLLIFAIKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSKFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 52
LCDR1	cgaacgagcaggacatactcaaaccttgca	SEQ ID NO: 103
LCDR2	gcgataaagaggctgcaagac	SEQ ID NO: 104
LCDR3	ttgcaaggctccaaatttcccctgaca	SEQ ID NO: 105
VL	gacatccaaatgactcaaagcccatcatcgctatcggcacg tcggggatagagtcacgataacatgccgaacgagcaggacat atactcaaaccttgcatggtatcaacaaagccggggaaggcc ccgaagctactgatattcgcgataaagaggctgcaagacggag ttccatcacgattttcgggatctggctcggggaccgattttac gctgactatatcatcgctgcaaccggaagattttgcaacatac tactgcttgcaaggctccaaatttcccctgacattcggaacaag gtaccaaggtcgagatcaaa	SEQ ID NO: 106
hC11d		
HCDR1	DYAMH	SEQ ID NO: 1
HCDR2	WINTYTGKPTYSQKFQG	SEQ ID NO: 16
HCDR3	AVFYGYTMDA	SEQ ID NO: 3
VH	QVQLVQSGAEVKKPGASVKVSKASGYTFTDYAMHWVRQAPGQ RLEWMGWINTYTGKPTYSQKFQGRVTITRDTASTAYMELSSL RSED TAVYYCARAVFYGYTMDAWGQGLTVTVSS	SEQ ID NO: 34
Heavy chain	QVQLVQSGAEVKKPGASVKVSKASGYTFTDYAMHWVRQAPGQ RLEWMGWINTYTGKPTYSQKFQGRVTITRDTASTAYMELSSL RSED TAVYYCARAVFYGYTMDAWGQGLTVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF	SEQ ID NO: 47

NAME	SEQUENCE	SEQ ID NO
	PAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVM HEALHNHYTQKSLSLSPGX X is R or K	
HCDR1	gattatgcaatgcac	SEQ ID NO: 99
HCDR2	tggattaacacctacacgggcaagccacatactcccaaaaat tccaagga	SEQ ID NO: 100
HCDR3	gctgtattctatggatatacaatggatgcc	SEQ ID NO: 101
VH	caggtccaattagtcacaaagcggggcggaagtcaagaagccgg ggcgagcgtcaaagtctcatgcaaagcgagcggatacacatt tacggattatgcaatgcactgggtcaggcaagcaccgggacaa aggctggaatggatggatggattaacacctacacgggcaagc ccacatactcccaaaaattccaaggaagggtcacgataacgag agacacgagcgcgagcaccggaatggatggggtggaataacac ctacacgggcaagccacatactcccaaaaattccaaggaagg gtcacgataacgagagacacgagcgcgagcaccgtaccctggt caccgtctcgagc	SEQ ID NO: 102
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 ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 53
LCDR1	cggacgagcaggatatttattcgaactttgca	SEQ ID NO: 107
LCDR2	cagtcaatcggctacaagat	SEQ ID NO: 108
LCDR3	ctacaagggagcaaattcccgtgaca	SEQ ID NO: 84
VL	gacatccaaatgacgcaatcaccgagctcgctgagcgcacatctg tcggggaccgtgtcacaatcacatgccggacgagcaggatat ttattcgaactttgcatggtatcaacaaaaaccgggcaaggct ccgaaacttttgatttattcagtcgaatcggctacaagatggcg tcccgagccgatttagcgggagcggatcggggaaccgactttac gctgacgatatcatcgctacaaccggaggacttcgcgacttat tactgcctacaaggagcaaaattcccgtgacattcggacaag 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	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMYWVRQAPGQ RLEWMGWINTYTGKPTYAQKFQGRVTITRDTSASTAYMELSSL RSED TAVYYCARAVFYGYTMDAWGQGLVTVSS	SEQ ID NO: 35
Heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMYWVRQAPGQ RLEWMGWINTYTGKPTYAQKFQGRVTITRDTSASTAYMELSSL RSED TAVYYCARAVFYGYTMDAWGQGLVTVSSASTKGPSVFP	SEQ ID NO: 48

NAME	SEQUENCE	SEQ ID NO
	LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHCTCPPELPGGSPVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFCFSVMHEALHNHYTQKSLSLSPGX X is R or K	
HCDR1	gattacgcaatgtac	SEQ ID NO: 110
HCDR2	tggataaatacctatacgggaaagccaacatacgcacaaaaat tccaaggc	SEQ ID NO: 111
HCDR3	gccgtcttttatggatatacgcagtggaagca	SEQ ID NO: 112
VH	cagggtccaactgggtccaatcgggggctgaagtcacaaaagccgg gggagcgagcgtcaaagtcagctgcaaagcatcgggatacacatt tacggattacgcaatgtactgggtcaggcaagcaccggccaa cgactggaatggatgggtggataaatacctatacgggaaagc caacatacgcacaaaaattccaaggccgcgtcacaataacgcg ggacacgagcgcatcgacggcttatatggaactatcatcgctg cgatcggaagacacggcggtctattattgcgcacgcgcgctct tttatggatatacgcagtcacgcatgggggcagggtaccctggt cacggtctcgagc	SEQ ID NO: 113
LCDR1	RASEDIYSNLA	SEQ ID NO: 4
LCDR2	SVKRLQD	SEQ ID NO: 5
LCDR3	LQGSNFPLT	SEQ ID NO: 6
VL	DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAHWYQQKPGKA PKLLIFSVKRLQDGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCLQGSNFPLTFGQGTKVEIK	SEQ ID NO: 38
Light chain	DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAHWYQQKPGKA PKLLIFSVKRLQDGVPSRFSGSGSGTDFTLTISSLQPEDFATY YCLQGSNFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCCLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 51
LCDR1	agggcctccgaagacatctactccaacctggca	SEQ ID NO: 95
LCDR2	agcgtcaaaagactacaagat	SEQ ID NO: 96
LCDR3	ttgcaaggaagcaatttccccttgact	SEQ ID NO: 97
VL	gacattcaaatgacgcaaaagcccatcatcgctgagcgcacatcg tcggggatagagtcaccataacatgcagggcctccgaagacat ctactccaacctggcatggtatcaacaaaaaccggggaaggct ccgaagctgctgatatttagcgtcaaaagactacaagatggag taccgagccgatttccgggaagcgggagcgggacggatttcac gctgaccatatcaagtttgcaaccggaggattttgcgacatac tattgcttgcaaggaagcaatttccccttgactttcgggcaag gtaccaaggctcgagatcaaa	SEQ ID NO: 98
hCI1f		
HCDR1	DYAMH	SEQ ID NO: 1
HCDR2	WINAYTGKPTYAQKFQG	SEQ ID NO: 20
HCDR3	AVFYGYTMDA	SEQ ID NO: 3
VH	QVQLVQSGAEVKKPGASVKVSCASGYTFTDYAMHWVRQAPGQ RLEWMGWINAYTGKPTYAQKFQGRVTITRDTASTAYMELSSL RSEDYAVYYCARAVFYGYTMDAWGQGTITVTVSS	SEQ ID NO: 36
Heavy chain	QVQLVQSGAEVKKPGASVKVSCASGYTFTDYAMHWVRQAPGQ RLEWMGWINAYTGKPTYAQKFQGRVTITRDTASTAYMELSSL	SEQ ID NO: 49

NAME	SEQUENCE	SEQ ID NO
	RSEDTAVYYCARAVFYGYTMDAWGQGTLLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHCTCPPELGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVHLQDNLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSVSM HEALHNHYTQKSLSLSPGX X is R or K	
HCDR1	gactacgcaatgcac	SEQ ID NO: 114
HCDR2	tggattaatgcctacacggggaagccgacctacgcacaaaaat tccaagga	SEQ ID NO: 115
HCDR3	gccgtcttctatggatatacatggatgct	SEQ ID NO: 116
VH	caggtccaattggtccaaagcggggcgagggtcaagaagccgg ggcgagcgtcaaagtctcatgcaaggcaagcggatatacatt tacggactacgcaatgcactgggtccggcaagcccctgggcaa cggctggaatggatggatggattaatgcctacacggggaagc cgacctacgcacaaaaattccaaggacgagtcacgattacgcg ggatactagcgcgagcaccgcatatatggagctaagctcgctg cgatctgaggataccgctgtatactactgcgcgagagccgtct tctatggatatacatggatgcttgggggcagggtaccctggg cacggctcgcgac	SEQ ID NO: 117
LCDR1	RASEDIYSNLA	SEQ ID NO: 4
LCDR2	SVKRLQD	SEQ ID NO: 5
LCDR3	LQGSNFPLT	SEQ ID NO: 6
VL	DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAWYQQKPGKA PKLLIYSVKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSNFPLTFGQGTKVEIK	SEQ ID NO: 41
Light chain	DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAWYQQKPGKA PKLLIYSVKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSNFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 54
LCDR1	cgagcttcggaggacatctatagcaacttggt	SEQ ID NO: 118
LCDR2	agcgtcaaaaggctccaagac	SEQ ID NO: 119
LCDR3	ctacaaggctctaacttcccattgaca	SEQ ID NO: 120
VL	gatatccaaatgacgcaatcaccatctagcctatcggcctctg tgggggaccgagtcaccatcacatgccgagcttcggaggacat ctatagcaacttggttggtatcaacaaaagccggggaaagca ccaaagctgctgatatatagcgtcaaaaggctccaagacggag tcccaagccgattctcgggctccggctccgggacggattttac 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	QVQLVQSGAEVKKPGASVKVSCKASGYTFDYAMHWVRQAPGQ RLEWMGWINAYTGKPTYAQKFQGRVTITRDTASASTAYMELSSL RSEDTAVYYCARAVFYGYTMDAWGQGTLLVTVSS	SEQ ID NO: 36

NAME	SEQUENCE	SEQ ID NO
Heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAPGQ RLEWMGWINAYTGKPTYAQKFQGRVTITRDTASASTAYMELSSL RSED TAVYYCARAVFYGYTMDAWGQGT LVT VSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVT VSWNSGALTSGVHTF PAVLQSSGLYSLSSVTV PSSSLGTQTYICNVNHKPSNTKV KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLT VLVHQLDNLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNV FSCSV HEALHNHYTQKSLSLSPGX X is R or K	SEQ ID NO: 49
HCDR1	gactacgcaatgcac	SEQ ID NO: 114
HCDR2	tggattaatgcctacacggggaagccgacctacgcacaaaaat tccaagga	SEQ ID NO: 115
HCDR3	gccgtcttctatggatatacgaatggatgct	SEQ ID NO: 116
VH	caggtccaattggtccaaagcggggcgagggtcaagaagccgg ggcgagcgtcaaagtctcatgcaaggcaagcggatatacatt tacggactacgcaatgcactgggtccggcaagcccctgggcaa cggctggaatggatggatggatgaatgcctacacggggaagc cgacctacgcacaaaaattccaaggacgagtcacgattacgcg ggatactagcgcgagcaccgcataatggagctaagctcgctg cgatctgaggataccgctgtatactactgcgcgagagccgtct tctatggatatacgaatggatgcttgggggcagggtaccctggt cacggtctcgagc	SEQ ID NO: 117
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 ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 53
LCDR1	cggacgagcggaggaatatttattcgaactttgca	SEQ ID NO: 107
LCDR2	cagtcaatcggctacaagat	SEQ ID NO: 108
LCDR3	ctacaaggagcaaatccccgctgaca	SEQ ID NO: 84
VL	gacatccaaatgacgcaatcaccgagctcgctgagcgcacatctg tcggggaccgtgtcacaatcacatgccggacgagcggaggaat ttattcgaactttgcatggatcaacaaaaaccgggcaaggct ccgaaacttttgatttattcagtcaatcggctacaagatggcg tcccgagccgatttagcgggagcggatcgggaaccgactttac gtgacgatatcatcgctacaaccggaggacttcgcgacttat tactgcctacaaggagcaaatccccgctgacattcggacaag gtaccaaggtcgagatcaaa	SEQ ID NO: 109
hC11h		
HCDR1	DYAMY	SEQ ID NO: 12
HCDR2	WINAYTGKPTYAQKFQG	SEQ ID NO: 20
HCDR3	AVYYGYTMDA	SEQ ID NO: 8

NAME	SEQUENCE	SEQ ID NO
VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMYWVRQAPGQ RLEWMGWINAYTGKPTYAQKFQGRVTITRDTSASTAYMELSSL RSEDYAVYYCARAVYYGYTMDAWGQGTLVTVSS	SEQ ID NO: 37
Heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMYWVRQAPGQ RLEWMGWINAYTGKPTYAQKFQGRVTITRDTSASTAYMELSSL RSEDYAVYYCARAVYYGYTMDAWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHCTPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSGSEFLYSKLTVDKSRWQQGNVFSFSVM HEALHNHYTQKSLSLSPGX X is R or K	SEQ ID NO: 50
HCDR1	gactacgctatgtat	SEQ ID NO: 122
HCDR2	tggattaatgcctacaccgggaagccgacttatgcgcaaaaat ttcaagga	SEQ ID NO: 123
HCDR3	gcgggtctactatggatatacgatggacgca	SEQ ID NO: 124
VH	caggtccaactggttcaatctggagcggaagtcaagaagcccg gagcatccgtcaaagtctcgtgcaaggcatctggatacacatt caccgactacgctatgtattgggtccggcaagcccccgacaa cggctggaatggatggatggattaatgcctacaccgggaagc cgacttatgcgcaaaaatttcaaggaagggtcacgattacgag ggacacgagcgctcaaccgcatacatggagctatcgagcctg cgaagcgaggacaccgcgggtctactactgcgcggggcggtct actatggatatacgatggacgcattgggggcagggtaccctggt cacggtctcgagc	SEQ ID NO: 125
LCDR1	RASEDIYSNLA	SEQ ID NO: 4
LCDR2	SVKRLQD	SEQ ID NO: 5
LCDR3	LQGSNFPLT	SEQ ID NO: 6
VL	DIQMTQSPSSLSASVGDRTITCRASEDIYSNLAAYQQKPGKA PKLLIYSVKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSNFPLTFGQGTKVEIK	SEQ ID NO: 41
Light chain	DIQMTQSPSSLSASVGDRTITCRASEDIYSNLAAYQQKPGKA PKLLIYSVKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSNFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDST YLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 54
LCDR1	cgagcttcggaggacatctatagcaacttggct	SEQ ID NO: 118
LCDR2	agcgtcaaaaggctccaagac	SEQ ID NO: 119
LCDR3	ctacaaggctctaacttcccattgaca	SEQ ID NO: 120
VL	gatatccaaatgacgcaatcaccatctagcctatcggcctctg tgggggaccgagtcaccatcacatgccgagcttcggaggacat ctatagcaacttggcttggatcaacaaaagccggggaaagca ccaaagctgctgatataatagcgtcaaaaggctccaagacggag tcccaagccgattctcgggctccggctccgggacggattttac gctgacaatttcgagcctgcaaccggaggactttgcaacctac tattgcctacaaggctctaacttcccattgacatttgggcaag gtaccaaggtcgagatcaaa	SEQ ID NO: 121
hCIIi		
HCDR1	DYAMY	SEQ ID NO: 12
HCDR2	WINAYTGKPTYAQKFQG	SEQ ID NO: 20

NAME	SEQUENCE	SEQ ID NO
HCDR3	AVYYGYTMDA	SEQ ID NO: 8
VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMYWVRQAPGQ RLEWMGWINAYTGKPTYAQKFQGRVTITRDTASASTAYMELSSL RSEDTAVYYCARAVYYGYTMDAWGQGTLVTVSS	SEQ ID NO: 37
Heavy chain	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMYWVRQAPGQ RLEWMGWINAYTGKPTYAQKFQGRVTITRDTASASTAYMELSSL RSEDTAVYYCARAVYYGYTMDAWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTPVSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSFSVM HEALHNHYTQKSLSLSPGX X is R or K	SEQ ID NO: 50
HCDR1	gactacgctatgtat	SEQ ID NO: 122
HCDR2	tggattaatgcctacaccgggaagccgacttatgcgcaaaaat ttcaagga	SEQ ID NO: 123
HCDR3	gcgggtctactatggatatacgatggacgca	SEQ ID NO: 124
VH	caggtccaactggttcaatctggagcggaagtcaagaagcccg gagcatccgtcaaagtctcgtgcaaggcatctggatacacatt caccgactacgctatgtattgggtccggcaagcccccgacaa cggctggaatggatggatggattaatgcctacaccgggaagc cgacttatgcgcaaaaatttcaaggaagggtcacgattacgcg ggacacgagcgctcaaccgcatacatggagctatcgagcctg cgaagcgaggacaccgcgggtctactactgcgcgcgggcggtct actatggatatacgatggacgcatgggggcagggtaccctggt cacggtctcgagc	SEQ ID NO: 125
LCDR1	RASEDIYSNLA	SEQ ID NO: 4
LCDR2	SVKRLQD	SEQ ID NO: 5
LCDR3	LQGSNFPLT	SEQ ID NO: 6
VL	DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAWYQQKPGKA PKLLIFSVKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSNFPLTFGQGTKVEIK	SEQ ID NO: 38
Light chain	DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAWYQQKPGKA PKLLIFSVKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSNFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS TYSLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 51
LCDR1	agggcctccgaagacatctactccaacctggca	SEQ ID NO: 95
LCDR2	agcgtcaaaagactacaagat	SEQ ID NO: 96
LCDR3	ttgcaaggaagcaatttccccttgact	SEQ ID NO: 97
VL	gacattcaaatgacgcaaaagcccatcatcgctgagcgcacg tcggggatagagtaccataacatgcagggcctccgaagacat ctactccaacctggcatggtatcaacaaaaaccggggaaggct ccgaagctgctgatatttagcgtcaaaagactacaagatggag taccgagccgattttcgggaagcgggagcgggacggatttcac gctgaccatatcaagtttgcaaccggaggattttgacacatac tattgcttgcaaggaagcaatttccccttgactttcgggcaag gtaccaaggtcgagatcaaa	SEQ ID NO: 98
hClj		
HCDR1	DYAMY	SEQ ID NO: 12

NAME	SEQUENCE	SEQ ID NO
HCDR2	WINAYTGKPTYAQKFQG	SEQ ID NO: 20
HCDR3	AVYYGYTMDA	SEQ ID NO: 8
VH	QVQLVQSGAEVKKPGASVKVSKASGYTFTDYAMYWVRQAPGQ RLEWMGWINAYTGKPTYAQKFQGRVTITRDTSASTAYMELSSL RSED TAVYYCARAVYYGYTMDAWGQGTLVTVSS	SEQ ID NO: 37
Heavy chain	QVQLVQSGAEVKKPGASVKVSKASGYTFTDYAMYWVRQAPGQ RLEWMGWINAYTGKPTYAQKFQGRVTITRDTSASTAYMELSSL RSED TAVYYCARAVYYGYTMDAWGQGTLVTVSSASTKGPSVFP LAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTF PAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKHTHTCPPCPAPPELLGGPSVFLFPPKPKDTLMISRT PEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVHLQDNLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNG QPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSFSVM HEALHNHYTQKSLSLSPGX X is R or K	SEQ ID NO: 50
HCDR1	gactacgctatgtat	SEQ ID NO: 122
HCDR2	tggattaatgcctacaccgggaagccgacttatgcgcaaaaat ttcaagga	SEQ ID NO: 123
HCDR3	gcgggtctactatggatatacgatggacgca	SEQ ID NO: 124
VH	caggtccaactggttcaatctggagcgggaagtcaagaagcccg gagcatccgtcaaagtctcgtgcaaggcatctggatacacatt caccgactacgctatgtattgggtccggcaagcccccgacaa cggctggaatggatggatggatgaatgcctacaccgggaagc cgacttatgcgcaaaaatttcaaggaagggtcacgattacgcg ggacacgagcgctcaaccgcatacatggagctatcgagcctg cgaagcgaggacaccgcgggtctactactgcgcgcgggcgggtct actatggatatacgatggacgcgatgggggcaggggtaccctggt cacgggtctcgagc	SEQ ID NO: 125
LCDR1	RTSEDIYSNLA	SEQ ID NO: 17
LCDR2	AIKRLQD	SEQ ID NO: 14
LCDR3	LQGSKFPLT	SEQ ID NO: 11
VL	DIQMTQSPSSLSASVGDRVTITCRTSEDIYSNLA WYQQKPGKA PKLLIFAIKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSKFPLTFGQGTKVEIK	SEQ ID NO: 39
Light chain	DIQMTQSPSSLSASVGDRVTITCRTSEDIYSNLA WYQQKPGKA PKLLIFAIKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATY YCLQGSKFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGT ASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDS T YLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	SEQ ID NO: 52
LCDR1	cgaacgagcaggacatatactcaaacccttgca	SEQ ID NO: 103
LCDR2	gcgataaagaggctgcaagac	SEQ ID NO: 104
LCDR3	ttgcaagggtccaaatttcccctgaca	SEQ ID NO: 105
VL	gacatccaaatgactcaaagcccatcatcgctatcggcacg tcggggatagagtcacgataacatgccgaacgagcaggacat atactcaaacccttgcattggtatcaacaaaagccggggaaggcc ccgaagctactgatattcgcgataaagaggctgcaagacggag ttccatcacgattttcgggatctggctcggggaccgattttac gctgactatacatcgctgcaaccggaagattttgcaacatac tactgcttgcaaggctccaaatttcccctgacattcggacaag gtaccaaggtcgagatcaaa	SEQ ID NO: 106

The antibodies described in further Examples 2 to 5 were modified to contain a LPQTGG 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
5 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-
10 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
15 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
20 affinity as could be expected and even increased its affinity for 6 out of 10 antibodies, compared to the parental chimeric cCl1-1 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
25 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
30 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 FACS Aria™ 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 × 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 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	EC ₅₀ (μg/ml)	Max MFI	EC ₅₀ (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

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

5 The pre-B cell L11 cell line (Waldmeier et al. 2016), BxPC-3 (ATCC CRL-1687TM) cell line and HEK293T (ATCC CRL-3216TM) and A549 (ATCC CCL-185TM) cell line do not endogenously express CLDN18.1 or CLDN18.2. Therefore, in order to test antibody binding, CLDN18.1 or CLDN18.2 were recombinantly overexpressed in the HEK293T and A549 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 and A549 cells. 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 CLDN18.1 and CLDN18.2 in the transfected cells was analyzed by FC. (see Figure 3). In brief, trypsinized HEK293T and A549 cells, and L11 cells grown in suspension 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 (eBioscience) as secondary antibody for 30 min on ice. Upon further wash, resuspended stained cells in ice-cold FC buffer were analyzed using a FACSCaliburTM instrument (see Figure 4 and Figure 5). Un-transfected parental cells, not expressing CLDN18.2, were used as negative control. The 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 CLDN18 Antibody from ProSci (catalog number 63-847).

5 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, 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
10 (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

15 The A549 (ATCC CCL-185TM) 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
20 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
25 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.
30 Upon further wash, resuspended stained cells in ice-cold FC buffer were analyzed using a FACSCaliburTM 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 1×10^6 A549 cells expressing CLDN18.2
in 100 μ l of 50% Matrigel and tumors growth was monitored over a few weeks until the tumor
5 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
10 resuspended and strained through a 70 μ m 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
15 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
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 μ l/well of staining mix
consisting of the antibody of choice (cCl1-1, hCl1a, hCl1b, hCl1c and hCl1f at 4 μ g/ml;
20 IMAB364 at 2 μ g/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
were washed twice in PBS and centrifuged (400 g for 2 min at 4°C). Pellets were resuspended
in 50 μ l/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
25 twice in PBS. Pellets were resuspended in 100 μ l of PBS containing DAPI. Plates were kept on
ice until FC analysis. For FC analysis, live cells were separated from dead cells by forward
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.

30 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,
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 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

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 (-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% H₂O₂ 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 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 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.

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, 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 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

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-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 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	
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	

- 5 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
- 10 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,
- 5 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.
- 10 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 aM values of the non-stressed samples (except for IMAB362) were already noticeably high.
- 15 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.

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	

mAb	stressed yes (+) / no (-)	Proportion of aM (%)	Increase proportion of aM after stress test (%)
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	

Example 7: Conjugation of mAbs with glycine-modified toxin to form ADCs using sortase-mediated conjugation.

Sortase A enzyme: Recombinant and affinity purified Sortase A enzyme from *Staphylococcus aureus* was produced in *E. coli* as disclosed in W02014140317A1.

Generation of glycine-modified toxins: the biglycine-modified EDA-anthracycline derivative GG-EDA-PNU-159682 (see also Figure 25) was manufactured by Levena Biopharma, San Diego, USA. Here the toxin PNU-159682 was synthesized to already include the non-cleavable linker EDA and an oligopeptide linker GG. The identity and the purity of the glycine-modified toxin was confirmed by mass-spectrometry and HPLC. The glycine-modified toxins exhibited > 95% purity, as determined by HPLC chromatography.

Sortase-mediated antibody conjugation: the above-mentioned toxins were conjugated to the heavy chain and light chain or only light chain LPQTG-tagged anti-CLDN18.2 antibodies as per Table 3 and comparative antibodies (IMAB362, the CD30-specific antibody AC10). Alternatively, toxins were conjugated only to the light chain of the antibodies. The antibodies were conjugated to the toxins by incubating heavy and light chains or light chain-only LPQTG-tagged mAbs at 20 μ M with glycine-modified toxin at 100 μ M and Sortase A at 4 μ M in the conjugation buffer (50 mM HEPES pH 7.5, 150 mM NaCl, 1 mM CaCl₂, 10% glycerol) for 3.5h at 25°C or overnight at 4°C. The reaction was stopped by passage through a rProtein A GraviTrap column (GE Healthcare). The column was washed with 36 column volumes of wash buffer (25 mM HEPES pH 7.5, 150 mM NaCl, 10 % (v/v) Glycerol). Bound conjugate was eluted with 5 column volumes of elution buffer (0.1 M glycine pH 2.7, 50 mM NaCl, 10% (v/v) Glycerol), with 0.5 column volume fractions collected into tubes containing 1M HEPES pH 8 to neutralize the acid. Protein containing fractions were pooled and formulated in Histidine

buffer (15 mM Histidine, pH 6.5, 175 mM Sucrose, 0.02% Tween 20) using a Zeba Spin (Thermo Fisher) desalting column. Endotoxins were removed using Pierce High Capacity Endotoxin Removal Resin (Thermo Fisher) and sterile filtered through a 0.22 µm filter. The final concentration of the ADCs was measured by UV-visible spectroscopy.

- 5 The ADC IMAB362-MC-vc-PAB-MMAE was generated as disclosed in WO2016/166122 (Example 1, section 3, page 75-76).

ADC analytics: DAR was assessed by Reverse Phase Chromatography performed on a PLRP-S, 300 Å, 2.1 x 150 mm, 3 µm column (Agilent) run at 0.7 ml/min at 60°C with a 9-minute linear gradient (25-40 %) followed by a 4-minute linear gradient (40-75 %) between 0.1%
10 TFA/3% CH₃CN/H₂O and 0.1% TFA/CH₃CN. Samples were first reduced by incubation with 10% v/v 0.5 M DTT, pH 8.0 at 37°C for 15 minutes. All generated ADCs had a DAR LC = 2 or a DAR HC-LC = 4.

Example 8: *in-vitro* cytotoxic assays of anti-CLDN18.2 antibody-based ADCs on CLDN18.2-expressing cells [data from NBET'2483]

- 15 In Example 8 and following Example 9, an ADC of the formula [antibody]-HC-LC-PNU is an ADC where the antibody is conjugated at the heavy and light chain with the toxin PNU-159682 and has a DAR = 4; an ADC of the formula [antibody]-HC-PNU or [antibody]-LC-PNU is conjugated at the heavy or light chain, respectively, with the toxin PNU-159682 and has a DAR = 2. All these ADCs also have an -LPQTGG- oligopeptide linker and ethylenediamine non-
20 cleavable linker. The structure of an ADC of the formula [antibody]-LC-PNU can be seen in Figure 25.

Cytotoxicity of anti-CLDN18.2 ADCs was investigated using A549 cells or HEK293T cells or BxPC-3 engineered to overexpress hCLDN18.2 (see Example 3 and 4) or PA-TU-8988S-high cells (see Example 2) endogenously expressing hCLDN18.2 and compared to IMAB362-HC-G2-PNU, IMAB362-LC-G2-PNU, IMAB362-HC-LC-G2-PNU or IMAB362-MC-vc-PAB-MMAE. HEK293T and A549 cells engineered to overexpress hCLDN18.1 (see Example 3)
25 were used to show specificity to CLDN18.2 and not to CLDN18.1.

In brief, 1000 cells/well of A549 cells or HEK293T cells, 5000 cells/well of BxPC-3 cells or 10000 cells/well of PA-TU-8988S-high cells were plated in white clear bottom 96-well plates
30 (Greiner) (excluding edge wells, which contained water) in 75 µl DMEM high glucose, 10% FCS, 100 IU/ml Pen/Step/Fungizone, 2mM L-Glutamine and were grown at 37°C in a

humidified incubator at 7.5% CO₂ atmosphere. After one day of incubation, each ADC was added to respective wells in an amount of 25 µl of 4-fold serial dilution in complete growth medium resulting in concentration of ADCs from 5000 to 0.076 ng/ml for A549 cells, from 1000 to 0.015 ng/ml for HEK293-T cells, from 20000 to 0.25 ng/ml for BxPC-3 cells and from 20000 to 0.31 ng/ml for PA-TU-8988S cells. After 4 additional days, plates were removed from the incubator and equilibrated to room temperature. After approximately 30 min, 50 µl of CellTiter-Glo® 2.0 Luminescent Solution (Promega) was added to each well. After shaking the plates at 450 rpm for 5 min followed by 10 min incubation without shaking, luminescence was measured on a Tecan Spark 10M plate reader with an integration time of 250 ms per well. Curves of luminescence versus ADC concentration (ng/ml) were fitted with the Graphpad Prism Software (see Figures 11 to 19).

The *in-vitro* cytotoxicity assays show that cC11-1, cC11-2 and cC11-3, either conjugated at the HC only, at the LC only or at both HC and LC showed a better cytotoxic activity than IMAB362 comparably conjugated and IMAB362-MC-vc-PAB-MMAE on HEK293T cells overexpressing CLDN18.2 (see Figure 11), on BxPC-3 cells overexpressing CLDN18.2 (see Figure 13), on A549 cells overexpressing CLDN18.2 (see Figure 14) or on PA-TU-8988S-High cells (see Figure 16), while the cytotoxic activity was only observed on very high concentrations of ADCs in HEK293T cells overexpressing CLDN18.1 (see Figure 12) or A549 cells overexpressing CLDN18.1 (see Figure 15). Any cytotoxic activity on cells overexpressing CLDN18.1 was attributed to the at least 1000 x higher concentration of toxins and was only observed for a DAR4 conjugation (toxins conjugated at the antibody heavy and light chains). Likewise, the control ADC based on the Ac10 antibody not targeting CLDN18.2 had only cytotoxic activity at very high concentration of ADC (see Figure 14, 15).

The *in-vitro* cytotoxicity assays also show that ADC based on the antibodies hC11a to hC11j, with the toxin conjugated to the LC only (resulting in a DAR 2), had a superior cytotoxic activity on A549 cells overexpressing CLDN18.2 (see Figure 17), HEK293T cells overexpressing CLDN18.2 (see Figure 18) or PA-TU-8988S cells (see Figure 20) than the ADC based on IMAB362 likewise with the toxin conjugated at the LC. The cytotoxic activity of the ADC based on the antibodies hC11a to hC11j was selective for cell overexpressing CLDN18.2, they had no cytotoxic activity on HEK293T cells overexpressing CLDN18.1 (see Figure 19).

EC₅₀ values for humanized antibodies conjugated at their LC, determined using built-in “log(inhibitor) vs. response – variable slope (four parameters)” EC₅₀ determination function of the Prism Software, are reported in Table 9.

Table 9: EC₅₀ (ng/ml) values for tested ADCs based on *in-vitro* cytotoxicity assays

Cell line ADC	A549-CLDN18.2		HEK293T- CLDN18.2	PATU8988S-high	
hCL1a-LC-G2-PNU	4.8	4.4	2.4	56.5	83.0
hCL1b-LC-G2-PNU	6.4	4.4	2.1	64.3	96.8
hCL1c-LC-G2-PNU	30.0	30.6	6.1	727	1165
hCL1d-LC-G2-PNU	11.6	11.3	2.7	234	293
hCL1e-LC-G2-PNU	4.3	4.0	2.7	42.7	59.1
hCL1f-LC-G2-PNU	14.0	11.1	3.8	234.2	331.1
hCL1g-LC-G2-PNU	70.7	57.3	6.9	1753	2213
hCL1h-LC-G2-PNU	3.3	2.9	1.8	37.9	37.2
hCL1i-LC-G2-PNU	3.8	2.4	1.8	35.1	34.8
hCL1j-LC-G2-PNU	5.3	4.0	1.6	64.2	91.8
IMAB362-LC-G2-PNU	70.25	61.41	15.23	3071	4264

5

Overall, all the of the invention have a high *in vitro* cytotoxic potential, with a higher cytotoxic activity than IMAB362-LC-G2-PNU.

Example 9: Analysis of *in-vivo* efficacy of ADC hCL1a-LC-G2-PNU and hCL1f-LC-G2-PNU in patient-derived tumor xenograft models

10 The following studies were performed at Charles River GmbH (Freiburg, Germany).

Table 10: Patient-derived tumor xenograft models used for evaluation of anti-CLDN18.2 ADC hCL1a-LC-G2-PNU and hCL1f-LC-G2-PNU

Model	Mouse Strain and Sex	Tumor Establishment
GXA 3037 (Gastric adenocarcinoma)	Female NMR1 nude mice, implantation at 5-7 weeks of age	Tumor implantation unilateral subcutaneous.

Model	Mouse Strain and Sex	Tumor Establishment
CXF 742 (Colon adenocarcinoma)		Tumor volume at randomization 50 to 250 mm ³ (preferably 80 – 200 mm ³)
PAXF 2175 (Pancreatic ductal carcinoma)		
LIXFC 2050 (Lung adenocarcinoma)		

The anti-CLDN18.2 ADCs hC11a-LC-G2-PNU, hC11a(LALA)-LC-G2 and hC11f-LC-G2-PNU were investigated in the patient-derived tumor xenograph (PDX) models according to the following study protocol:

- 5 Table 11: Protocols used for evaluation of anti-CLDN18.2 hC11a-LC-G2-PNU, hC11a(LALA)-LC-G2 and hC11f-LC-G2-PNU ADCs in PDX models.

PDX model	Group	No. of Mice	Total Daily Dose	Dosing days	Route
GXA 3037	Control vehicle	3	5 ml/kg of saline	1, 8, 15	intravenous
	hC11a-LC-G2-PNU	3	0.6 mg/kg	1, 8, 15	intravenous
	hC11a-LC-G2-PNU	3	2 mg/kg	1, 8, 15	intravenous
	hC11a(LALA)-LC-G2-PNU	3	0.2 mg/kg	1, 8, 15	intravenous
	hC11f-LC-G2-PNU	3	0.6 mg/kg	1, 8, 15	intravenous
	hC11f-LC-G2-PNU	3	2 mg/kg	1, 8, 15	intravenous
	IMAB362-LC-G2-PNU	3	0.2 mg/kg	1, 8, 15	intravenous
	IMAB362-LC-G2-PNU	3	0.6 mg/kg	1, 8, 15	intravenous
	IMAB362-LC-G2-PNU	3	2 mg/kg	1, 8, 15	intravenous
CXF 742	Control vehicle	3	5 ml/kg of saline	1, 8, 15	intravenous
	hC11a-LC-G2-PNU	3	2 mg/kg	1, 8, 15	intravenous
	AC10-LC-G2-PNU (isotype control)	3	2 mg/kg	1, 8, 15	intravenous

PDX model	Group	No. of Mice	Total Daily Dose	Dosing days	Route
PAXF 2175	Control vehicle	3	5 ml/kg of saline	1, 8, 15	intravenous
	hCl1a-LC-G2-PNU	3	0.2 mg/kg	1, 8, 15	intravenous
	hCl1a-LC-G2-PNU	3	0.6 mg/kg	1, 8, 15	intravenous
	hCl1a(LALA)-LC-G2-PNU	3	0.2 mg/kg	1, 8, 15	intravenous
	IMAB362-LC-G2-PNU	3	0.2 mg/kg	1, 8, 15	intravenous
LIXFC 2050	Control vehicle	3	5 ml/kg of saline	1, 8, 15	intravenous
	hCl1a-LC-G2-PNU	3	2 mg/kg	1, 8, 15	intravenous
	AC10-LC-G2-PNU (isotype control)	3	2 mg/kg	1, 8, 15	intravenous

Mice were subcutaneously implanted unilaterally with PDX material. Mice allocated into groups when tumors reached randomization criteria and were treated with ADCs as indicated in Table 11 or vehicle for a total of 3 times. Tumor volumes were determined by caliper measurements and body weight was recorded twice weekly. Mice were euthanized on reaching a tumor burden of 2000 mm³, or on significant body weight loss (overall more than 30%, or more than 20% in two days).

Figures 20 to 23 show the relative tumor volume evolution over the studies in the different PDX models. Tumor xenografts established with patient-derived tumor material having CLDN18.2 expression responded significantly to treatment with the ADCs of the invention. The response (delayed tumor growth or tumor shrinkage) with the ADCs of the invention when administered at lower doses (0.2 mg/kg or 0.6 mg/kg) was better than the similar ADC based on the anti-CLDN18.2 antibody IMAB362 administered at the same doses and comparably good when administered at the higher dose of 2 mg/kg.

EMBODIMENTS

1. An antibody-drug conjugate having the general formula $A - (L-T)_n$, wherein

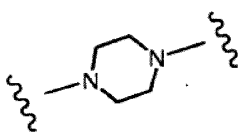
- a. A is an antibody or fragment thereof binding to CLDN18.2 comprising the HCDR1, HCDR2 and HCDR3 sequences of SEQ ID NO: 21, SEQ ID NO: 22 and SEQ ID NO: 23 respectively, and the LCDR1, LCDR2 and LCDR3 sequences of SEQ ID NO: 24, SEQ ID NO: 25 and SEQ ID NO: 26 respectively,
- b. L is a linker, and
- c. T is a toxin,

wherein the toxin is an anthracycline,

wherein n is an integer between ≥ 1 and ≤ 10 ;

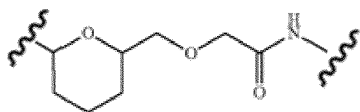
or a pharmaceutically acceptable salt or ester thereof.

2. The antibody-drug conjugate of embodiment 1, wherein the linker L comprises at least one a non-cleavable linker element.
3. The antibody-drug conjugate of embodiment 2, wherein the non-cleavable linker element is selected from the group consisting of
 - i. ethylenediamine (EDA),
 - j. N-formyl-N,N'-dimethylethylenediamine,
 - k. diethylamine (DEA),
 - l. a piperazine-derived compound of the following formula:



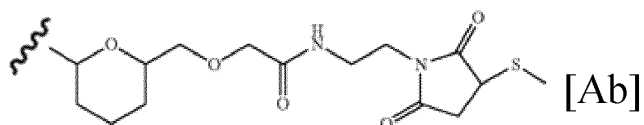
wherein the wavy lines indicate attachments to the toxin and another linker element,

- m. the compound of the following formula:



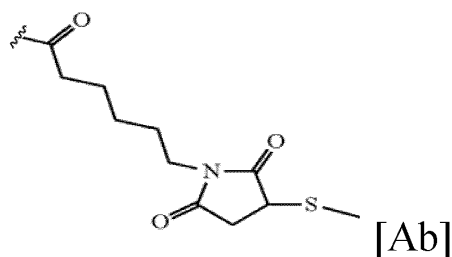
wherein the wavy lines indicate attachments to the toxin and another linker element,

n. the compound of the following formula:



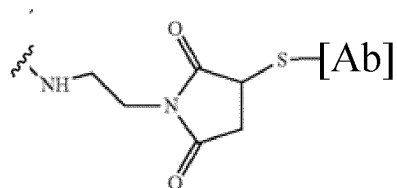
5 wherein the wavy lines indicate attachments to the toxin and [Ab] indicates the antibody or fragment thereof,

o. a maleimidocaproyl compound of the following formula:



10 wherein the wavy lines indicate attachments to another linker element and [Ab] indicates the antibody or fragment thereof,

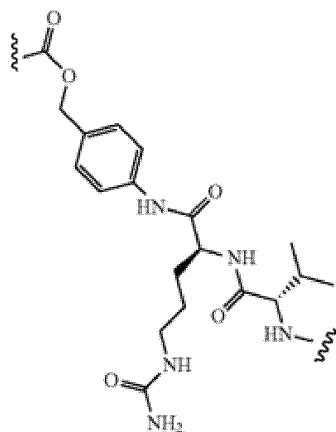
p. the compound of the following formula:



wherein the wavy lines indicate attachments to a toxin and [Ab] indicates the antibody or fragment thereof,

15 and wherein the non-cleavable linker element is conjugated to the toxin by means of an amide bond or an ether bond.

4. The antibody-drug conjugate of embodiment 2 or embodiment 3, wherein the linker further comprises an oligopeptide linker element and/or enzyme-cleavable linker element and/or a spacer element.
5. The antibody-drug conjugate of embodiment 4, wherein one oligopeptide linker element comprises a sortase recognition motif oligopeptide selected from: -LPXTG_m-, -LPXAG_m-, -LPXSG_m-, -LAXTG_m-, -LPXTG_m-, -LPXTA_m-, -NPQTG_m- or -NPQTN_m-, with G_m being an oligoglycine with m being an integer between ≥ 1 and ≤ 21 , A_m being an oligoalanine with m being an integer between ≥ 1 and ≤ 21 , N_m being an oligoasparagine with m being an integer between ≥ 1 and ≤ 21 and X being any conceivable amino acid, preferably the sortase recognition motif oligopeptide being -LPQTGG- or -LPETGG-.
6. The antibody-drug conjugate of embodiment 5, wherein the oligopeptide linker element comprises:
 - a. the sequence SEQ ID NO: 131, or
 - b. the sequence SEQ ID NO: 132.
7. The antibody-drug conjugate of any of embodiments 4 to 6, wherein the enzyme-cleavable linker element comprises a val-cit-PAB linker according to the compound of the following formula:



wherein the wavy lines indicate attachments to other linker elements.

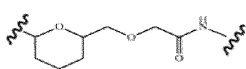
8. The antibody-drug conjugate of any of embodiments 4 to 7, wherein the spacer element comprises a peptidic flexible oligopeptide, preferably wherein the peptidic flexible

oligopeptide consists of G and S, more preferably wherein the peptidic flexible oligopeptide is (GGGGS)_o with o being 1, 2, 3, 4 or 5.

9. The antibody-drug conjugate of any of embodiments 1 to 8, wherein the antibody drug conjugate has the following structure:

5 a. A – ([oligopeptide linker element – non-cleavable linker element] – T)_n and preferably wherein the linker is selected from:

i. [LPXTGG]-[ethylenediamine], and

ii. [LPXTGG]-[];

10 b. A – ([oligopeptide linker element – enzyme cleavable linker element – non-cleavable linker element] – T)_n and preferably wherein the linker is selected from:

i. [LPXTGG]-[vc-PAB]-[N-formyl-N,N'-dimethylethylenediamine], and

ii. [LPXTGG]-[vc-PAB]-[piperazine];

15 c. A – ([spacer element – oligopeptide linker element – non-cleavable linker element] – T)_n and preferably wherein the linker is selected from:

i. [GGGGS]-[LPXTGG]-[ethylenediamine], and

ii. [GGGGS]-[LPXTGG]-[]; or

20 d. A – ([spacer element – oligopeptide linker element – enzyme cleavable linker element – non-cleavable element] – T)_n and preferably wherein the linker is selected from:

i. [GGGGS]-[LPXTGG]-[vc-PAB]-[N-formyl-N,N'-dimethylethylenediamine], and

ii. [GGGGS]-[LPXTGG]-[vc-PAB]-[piperazine].

25 10. The antibody-drug conjugate of embodiment 9, wherein the non-cleavable linker element is ethylenediamine and wherein the oligopeptide linker element is LPXTGG wherein X is Q or E, preferably wherein X is Q.

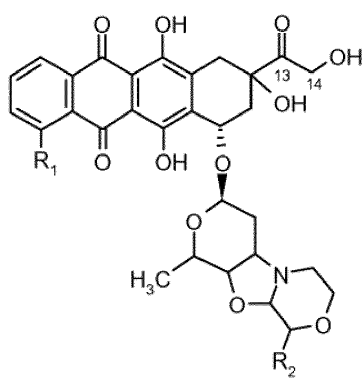
11. The antibody-drug conjugate of any of embodiments 1 to 10, wherein

- a. (L-T) is covalently linked to both light chains of the antibody,
- b. (L-T) is covalently linked to both heavy chains of the antibody, or
- c. (L-T) is covalently linked to both light chains and both heavy chains of the antibody.

12. The antibody-drug conjugate of any of embodiments 1 to 11, wherein (L-T)

- a. is linked to the C-terminus of the antibody light chain or antibody heavy chain, or
- b. is linked to an amino acid side chain of the antibody light chain or antibody heavy chain.

13. The antibody-drug conjugate of any of embodiments 1 to 12, wherein the anthracycline derivative has the following formula (I), and is covalently linked to the non-cleavable linker element by the C₁₃ resulting in the loss of the C₁₄ and the hydroxyl group, or is covalently linked to the non-cleavable linker element by the hydroxyl group on C₁₄:



and wherein R₁ is a hydrogen atom, a hydroxy or methoxy group,

and wherein R₂ is a C₁-C₅ alkoxy group.

14. The antibody-drug conjugate of any of embodiments 1 to 13, wherein the anthracycline derivative is a derivative of 3'-deamino-3'',4'-anhydro-[2''(S)-methoxy-3''(R)-oxy-4''-morpholinyl]doxorubicin (PNU-159682).

15. The antibody-drug conjugate of any of embodiments 1 to 14, wherein A, the antibody or fragment thereof, comprises:
- 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.

16. The antibody-drug conjugate of any of embodiments 1 to 14, wherein A, the antibody or fragment thereof comprises:
- 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.
17. The antibody-drug conjugate of any of embodiments 1 to 15, wherein A, the antibody or fragment thereof comprises:
- 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;
 - 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
 - j. a VH sequence of SEQ ID NO: 37 and a VL sequence of SEQ ID NO: 39.
18. The antibody-drug conjugate of any of embodiments 1 to 14 or 16, wherein A, the antibody or fragment thereof comprises:
- 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.

19. The antibody-drug conjugate of any of embodiments 1 to 15 or 17, wherein A, the antibody or fragment thereof, comprises:

- 5 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;
- 10 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;
- 15 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;
- 20 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

or versions thereof with an engineered Fc domain.

25 20. A method of producing an antibody-drug conjugate according to any of embodiments 1 to 19, wherein the method comprises the following steps:

- g. providing A, an antibody or fragment thereof with an oligopeptide linker element preferably at its C-terminus, optionally preceded by a spacer element at the antibody light and/or heavy chains,
- h. providing one or more toxins T with a non-cleavable linker element, and
- 5 i. conjugating the antibody and the toxin resulting in the antibody-drug conjugate.

21. An antibody-drug conjugate consisting of:

- the antibody consisting of two heavy chains of the amino acid sequence according to SEQ ID NO: 46, and two light chains of the amino acid sequence according to SEQ ID NO: 51, wherein the antibody binds to CLDN18.2,
- 10 • the linker [GGGGS]-[LPQTGG]-[ethylenediamine] at the C-terminus of the light chains, and
- the anthracycline-based small molecule toxin 3'-deamino-3'',4'-anhydro-[2''(S)-methoxy-3''(R)-oxy-4''-morpholinyl]doxorubicin (PNU-159682), linked covalently to the ethylenediamine of the linker at C₁₃, resulting in the loss
15 of C₁₄ and of the hydroxyl group.

22. An antibody-drug conjugate consisting of:

- the antibody consisting of two heavy chains of the amino acid sequence according to SEQ ID NO: 133, and two light chains of the amino acid sequence according to SEQ ID NO: 51, wherein the antibody binds to CLDN18.2,
- 20 • the linker [GGGGS]-[LPQTGG]-[ethylenediamine] at the C-terminus of the light chains, and
- the anthracycline-based small molecule toxin 3'-deamino-3'',4'-anhydro-[2''(S)-methoxy-3''(R)-oxy-4''-morpholinyl]doxorubicin (PNU-159682), linked covalently to the ethylenediamine of the linker at C₁₃, resulting in the loss
25 of C₁₄ and of the hydroxyl group.

23. An antibody-drug conjugate consisting of:

- the antibody consisting of two heavy chains of the amino acid sequence according to SEQ ID NO: 134 and two light chains of the amino acid sequence according to SEQ ID NO: 51, wherein the antibody binds to CLDN18.2,
 - the linker [GGGGS]-[LPQXTGG]-[ethylenediamine] at the C-terminus of the light chains, and
 - the anthracycline-based small molecule toxin 3'-deamino-3'',4'-anhydro-[2''(S)-methoxy-3''(R)-oxy-4''-morpholinyl]doxorubicin (PNU-159682), linked covalently to the ethylenediamine of the linker at C₁₃, resulting in the loss of C₁₄ and of the hydroxyl group.
24. A pharmaceutical composition comprising the antibody-drug conjugate of any of embodiments 1 to 23 and an excipient.
25. The antibody-drug conjugate of any of embodiments 1 to 23 for use in treatment.
26. The antibody-drug conjugate of any of embodiments 1 to 23 for use in the treatment of cancer.
27. The antibody-drug conjugate of embodiment 24, wherein the cancer is selected from pancreatic, gastric, esophageal, ovarian, and lung cancer.

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

	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
5	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 DIQMTQSPASLSASLGETISIACRASEDIYSNLAWEYQKSGKSPQ LLISVKRLQDGVPSRFSGSGSGTQYSLKISGMQPEDEGDYFCLQ GSNFPLTFGSGTKLEIK
20	SEQ ID NO: 29	cC11-2 HC variable region QIQLVQSGPELKKPGESVKISCKTSGYTFTDYAMHWVKQGPVK GMKWMGWINAYTGKPTYADDFKGRFVFSLEASASTANLQISN LKNEATATYFCARAVFYGYTMDAWGQGTSVIVSS
25	SEQ ID NO: 30	cC11-2 LC variable region DIQMTQSPASLSASLGETISIECRTSEDIYSNFAWFQKSGKSPQL LIYSVNRLQDGVPSRFSGSGSGTQYSLKISGMQPEDEGDYFCLQ GSKFPLTFGSGTKLEIK
30	SEQ ID NO: 31	cC11-3 HC variable region QIQLVQSGPELKKPGESVKISCKASGYTFTDYAMYWVKQVPGK GLRWMGWINTYTGKPTYADDFKGRFVFSLEASASTANLQISNL KNEDTATYFCARAVFYGYTMDAWGQGTSVTVSS
35	SEQ ID NO: 32	cC11-3 LC variable region DIQMTQSPASLSASLGETISIACRTSEDIYSNLAWEYQKSGKSPQ LLIFAIKRLQDGVPSRFSGSGSGTQYSLKISGMQPEDEGDYFCLQ GSKFPLTFGSGTKLEIK
40	SEQ ID NO: 33	hCL1a HC variable region QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAP GQRLEWMGWINTYTGKPTYAQKFQGRVTITRDTSASTAYMELS SLRSEDATVYYCARAVFYGYTMDAWGQGTLLVTVSS
	SEQ ID NO: 34	hCL1b, c and d HC variable region QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAP GQRLEWMGWINTYTGKPTYSQKFQGRVTITRDTSASTAYMELS SLRSEDATVYYCARAVFYGYTMDAWGQGTLLVTVSS
	SEQ ID NO: 35	hCL1e HC variable region QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMYWVRQAP GQRLEWMGWINTYTGKPTYAQKFQGRVTITRDTSASTAYMELS SLRSEDATVYYCARAVFYGYTMDAWGQGTLLVTVSS

	SEQ ID NO: 36	hCL1f and g HC variable region QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAP GQRLEWMGWINAYTGKPTYAQKFQGRVTITRDTSASTAYMEL SSLRSEDVAVYYCARAVFYGYTMDAWGQGTLVTVSS
5	SEQ ID NO: 37	hCL1h, i and j HC variable region QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMYWVRQAP GQRLEWMGWINAYTGKPTYAQKFQGRVTITRDTSASTAYMEL SSLRSEDVAVYYCARAVYYGYTMDAWGQGTLVTVSS
10	SEQ ID NO: 38	hCL1a, b, e and i LC variable region DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAWEYQQKPGKAP KLLIFSVKRLQDGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQ GSNFPPLTFGQGTKVEIK
15	SEQ ID NO: 39	hCL1c and j LC variable region DIQMTQSPSSLSASVGDRVTITCRTSEDIYSNLAWEYQQKPGKAP KLLIFAIKRLQDGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCLQ GSKFPLTFGQGTKVEIK
20	SEQ ID NO: 40	hCL1d and g LC variable region DIQMTQSPSSLSASVGDRVTITCRTSEDIYSNFAWEYQQKPGKAP KLLIYSVNRLQDGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCL QGSKFPLTFGQGTKVEIK
	SEQ ID NO: 41	hCL1f and h LC variable region DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAWEYQQKPGKAP KLLIYSVKRLQDGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCL QGSNFPPLTFGQGTKVEIK
25	SEQ ID NO: 42	hCL3a, b and c HC variable region QVQLQESGPGLVKPSSETLSLTCAVSGYSVSSNYRWHWIRQPPG KGLEWIGYINIAGSTNYPNPSLKSRTISVDTSKNQFSLKLSSVTA ADTAVYYCARNPSITRAMDAWGQGTLVTVSS
30	SEQ ID NO: 43	hCL3a LC variable region DIQMTQSPSSLSASVGDRVTITCKSSQNIFKNLEWYQQKPGKAP KLLIYYTNNLQGTGVPSSRFSGSGSGTDFTLTISSLQPEDFATYYCY QYNSGPFTFGQGTKVEIK
35	SEQ ID NO: 44	hCL3b LC variable region DIQMTQSPSSLSASVGDRVTITCRSSQNIFKNLEWYQQKPGKAP KLLIYYTNNLQGTGVPSSRFSGSGSGTDFTLTISSLQPEDFATYYCY QYNSGPFTFGQGTKVEIK
40	SEQ ID NO: 45	hCL3c LC variable region DIQMTQSPSSLSASVGDRVTITCRSSQNIFKNLEWYQQKPGKAP KLLIYYTNNLQSGVPSRFSGSGSGTDFTLTISSLQPEDFATYYCY QYNSGPFTFGQGTKVEIK
	SEQ ID NO: 46	hCL1a HC full QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAP GQRLEWMGWINTYTGKPTYAQKFQGRVTITRDTSASTAYMELS SLRSEDVAVYYCARAVFYGYTMDAWGQGTLVTVSSASTKGPS

5		VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE ALHNHYTQKSLSLSPGX X is K or R
10	SEQ ID NO: 47	hCL1b, c and d HC full QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAP GQRLEWMGWINTYTGKPTYSQKFQGRVTITRDTASTAYMELS SLRSEDТАVYYCARAVFYGYTMDAWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK
15		KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
20		ALHNHYTQKSLSLSPGX X is K or R
25	SEQ ID NO: 48	hCL1e HC full QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMYWVRQAP GQRLEWMGWINTYTGKPTYAQKFQGRVTITRDTASTAYMELS SLRSEDТАVYYCARAVFYGYTMDAWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK
30		KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
35		ALHNHYTQKSLSLSPGX X is K or R
40	SEQ ID NO: 49	hCL1f and g HC full QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAP GQRLEWMGWINAYTGKPTYAQKFQGRVTITRDTASTAYMEL SSLRSEDТАVYYCARAVFYGYTMDAWGQGTLVTVSSASTKGPS VFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVH TFPAVLQSSGLYSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDK
45		KVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPE VTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTY RVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQP REPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQP ENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHE
		ALHNHYTQKSLSLSPGX X is K or R
	SEQ ID NO: 50	hCL1h, i and j HC full QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMYWVRQAP GQRLEWMGWINAYTGKPTYAQKFQGRVTITRDTASTAYMEL

5		SSLRSEDVAVYYCARAVYYGYTMDAWGQGTLLTVSSASTKGP SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVD KKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRT EVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQ PREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQ PENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMH EALHNHYTQKSLSLSPGX X is K or R
10	SEQ ID NO: 51	hCL1a, b, e and i LC full DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAWYQQKPGKAP KLLIFSVKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATYYCLQ GSNFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCL LNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSST LTLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
15	SEQ ID NO: 52	hCL1c and j LC full DIQMTQSPSSLSASVGDRVTITCRTSEDIYSNLAWYQQKPGKAP KLLIFAIKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATYYCLQ GSKFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVCL LNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLSST LTLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
20	SEQ ID NO: 53	hCL1d and g LC full DIQMTQSPSSLSASVGDRVTITCRTSEDIYSNFAWYQQKPGKAP KLLIYSVNRQLQDGVPSRFSGSGSGTDFTLTISLQPEDFATYYCL QGSKFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLS TLTLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
25	SEQ ID NO: 54	hCL1f and h LC full DIQMTQSPSSLSASVGDRVTITCRASEDIYSNLAWYQQKPGKAP KLLIYSVKRLQDGVPSRFSGSGSGTDFTLTISLQPEDFATYYCL QGSNFPLTFGQGTKVEIKRTVAAPSVFIFPPSDEQLKSGTASVVC LLNNFYPPREAKVQWKVDNALQSGNSQESVTEQDSKDSSTYSLS TLTLISKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC
30	SEQ ID NO: 55	IMAB362 HC full QVQLQQPGAELVRPGASVKLSCKASGYTFTSYWINWVKQRP QGLEWIGNIYPSDSYTNYNQKFKDKATLTVDKSSSTAYMQLSSP TSEDSAVYYCTRSWRGNSFDYWGQGTTLTVSSASTKGPSVFPL APSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPA VLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVE PKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTC VVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREP QVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENN YKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFSCSVMHEALH NHYTQKSLSLSPGX X is K or R
45		

5	SEQ ID NO: 56	IMAB362 LC full DIVMTQSPSSLTVTAGEKVTMSCKSSQSLLNSGNQKNYLTWYQ QKPGQPPKLLIWASTRESGVPDRFTGSGSGTDFTLTISSVQAED LAVYYCQNDYSYPFTFGSGTKLEIKRTVAAPSVFIFPPSDEQLKS GTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSK DSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGE C
	SEQ ID NO: 57	DQWSTQDLYN
10	SEQ ID NO: 58	NNPVTAVFNYQ
	SEQ ID NO: 59	STQDLYNNPVTAVF
	SEQ ID NO: 60	TNFWMSTANMYTG
	SEQ ID NO: 61	ALMIVGIVLGAIGLLV
	SEQ ID NO: 62	RIGSMEDSAKANMTLTSGIMFIVS
	SEQ ID NO: 63	METDTLLLWVLLLWVPGSTGDAAQPARRARRTKLGTELGSTPV WWNSADGRMDQWSTQDLYNNPVTAVFNYQGLWRSCVRESSG FTECRGYFTLLGLPAMLQAVRAAIQHSGGRSRRARTKTHLRG SE
20	SEQ ID NO: 64	MDQWSTQDLYNNPVT
	SEQ ID NO: 65	LYNNPVTAVFNYQGL
	SEQ ID NO: 66	VFNYQGLWRSCVRES
	SEQ ID NO: 67	QGLWRSCVRESSGFT
	SEQ ID NO: 68	RSCVRESSGFTECRG
	SEQ ID NO: 69	TEDEVQSYPSKHDYV
25	SEQ ID NO: 70	EVQSYPSKHDYV
	SEQ ID NO: 71	gactacgcatgcac
	SEQ ID NO: 72	tggatcaacacgtacacggggaagccgacatacgcggacgacttcaagggg
	SEQ ID NO: 73	gccgtcttctacggatatacgtggacgcg
30	SEQ ID NO: 74	cagatccagctcgtccagagcgggcccggagctgaagaagccgggggagagcgtgaagatctcgt gcaaggcgagcggatatacgttcacggactacgcgactgactgggtcaagcaagcgcgggggaaa gggctgaagtggatggggtggatcaacacgtacacggggaagccgacatacgcggacgacttcaa ggggcgattcgtgttctcgtggaggcgagcgcgagcacggcgaacctgcaaattctgaacctgaa gaacgaggacacggcgacgtacttctgcgcgcccggcgtcttctacggatatacgtggacgcgtg ggggcaggggtaccagcgtgacgggtctcgagc
	SEQ ID NO: 75	cgggcgagcgaggacatctactcgaacctggcg
35	SEQ ID NO: 76	tccgtcaagcggctgcaagac

	SEQ ID NO: 77	ctgcaaggagcaacttcccgtgacg
	SEQ ID NO: 78	
5		gacatccagatgacgcagagcccggtcgctgagcgcgagcctgggggagacgatctcgatcg gtgccgggagcgcgaggacatctactgaacctggcgtggtatcaacagaagagcgggaagagcc cgagctgctgatcttctccgtcaagcggctgcaagacggcgtcccgagccgattctcggggagcg ggagcgggacgcagctactcgctgaagatctcgggatgcagccggaggacgaggggactacttc tgcttgaaggagcaacttcccgtgacgttcgggtcgggtaccaaactcgagatcaaa
	SEQ ID NO: 79	tggatcaacgcgtacacggggaagccgacctacgcggacgacttcaagggg
	SEQ ID NO: 80	gccgtctactacggatatacatgagac
10	SEQ ID NO: 81	
		cagatccagctcgtccagagcgggcccggagctgaagaagccgggggagagcgtgaagatctcgt gcaagacgagcggatatacgttcacggactacgcgatgcactgggtcaagcaggggccaaggaaa gggatgaagtggatgggtggatcaacgcgtacacggggaagccgacctacgcggacgacttcaa ggggcgattcgtgctgagcctggagcgcgagcgcctcgacggcgaacctgcaaactctgaacctgaa 15 gaacgaggacacggcgacgtacttctgcgcgggcccgtctactacggatatacatgagcgcgtg ggggcaggggtaccagcgtgatctctcgagc
	SEQ ID NO: 82	cggacgagcgcgaggacatctactgaacttcgcg
	SEQ ID NO: 83	tcagtcaaccggctgcaagac
	SEQ ID NO: 84	ctgcaaggagcaagttcccgtgacg
20	SEQ ID NO: 85	
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	SEQ ID NO: 86	gactacgcgatgtac
	SEQ ID NO: 87	tggatcaacacgtacacggggaagccgacctacgcggacgacttcaagggg
	SEQ ID NO: 88	
30		cagatccagctcgtccagagcgggcccggagctgaagaagccgggggagagcgtgaagatctcgt gcaaggcgcgagcggatatacgttcacggactacgcgatgtactgggtcaagcaagtccggggaaa gggctgcgatggatgggtggatcaacacgtacacggggaagccgacctacgcggacgacttcaa ggggcgattcgtgttctcgtggagcgcgagcgcgagcacggcgaacctgcaaactctgaacctgaa gaacgaggacacggcgacgtacttctgcgcgggcccgtcttctacggatatacatgagcgcgtg ggggcaggggtaccagcgtgacgggtctcgagc
35	SEQ ID NO: 89	cggacgagcgcgaggacatctactgaacctggcg
	SEQ ID NO: 90	gcgatcaagcggctgcaagac
	SEQ ID NO: 91	
40		gacatccagatgacgcagagcccggtgagcctgagcgcgagcctgggggagacgatctcgatcg cgtgccggagcgcgaggacatctactgaacctggcgtggtatcaacagaagagcgggaagagc ccgcagctgctgatcttgcgatcaagcggctgcaagacggcgtcccgagccgattctcggggagc gggagcgggacgcagctactcgctgaagatctcgggatgcagccggaggacgagggggactactt ctgcctgcaaggagcaagttcccgtgacgttcgggtcgggtaccaaactcgagatcaaa

	SEQ ID NO: 92	tggatcaatacacacggggaagccgacttatgcgcaaaaattccaagga
	SEQ ID NO: 93	gcggtcttctacggatatacgaatggatgcc
	SEQ ID NO: 94	
5		cagggtccaactagtcctaaagcggggcggaagtcaagaagcccgagcatccgtcaagtcagctg caaggcgagcggatatacattacggactacgcgatgcactgggtcaggcaagccctgggcaaag gctcgaatggatgggatggatcaatacacacggggaagccgacttatgcgcaaaaattccaagga agagtcacaattacgcgggatacatccgcactaccgcctacatggagctaagctcgtcgggagcg aggatacggcggtctactattgcgcccagcgggtcttctacggatatacgaatggatgcctgggggca gggtaccctgggtcacggtctcgagc
10	SEQ ID NO: 95	agggcctccgaagacatctactccaacctggca
	SEQ ID NO: 96	agcgtcaaaagactacaagat
	SEQ ID NO: 97	ttgcaaggaagcaatttccccttgact
	SEQ ID NO: 98	
15		gacattcaaatgacgcaaagcccatcatcgctgagcgcacggtcggggatagagtcaccataacat gcaggggcctccgaagacatctactccaacctggcatggatcaacaaaaccggggaaggctccga agctgctgatattagcgtcaaaagactacaagatggagtagcgcgagccgatttgcgggaagcgggag cgggacgggatttcacgctgacatatcaagtttgcaaccggaggatttgcgacatactattgcttgcaa ggaagcaatttccccttgacttccgggcaaggtaccaaggtcgagatcaaa
	SEQ ID NO: 99	gattatgcaatgcac
20	SEQ ID NO: 100	tggattaacacctacacgggcaagccacatactcccaaaaattccaagga
	SEQ ID NO: 101	gctgtattctatggatatacaatggatgcc
	SEQ ID NO: 102	
25		cagggtccaattagtcctaaagcggggcggaagtcaagaagccggggcgagcgtcaaagtctcatg caaagcgagcgggatacacattacggattatgcaatgcactgggtcaggcaagcaccgggacaaaag gctggaatggatgggatggattaacacctacacgggcaagccacatactcccaaaaattccaagga agggtcacgataacgagagacagcgcgagcaccggaatggatgggatggattaacacctaca cgggcaagccacatactcccaaaaattccaaggaagggtcacgataacgagagacagcgcgcg agcaccgtaccctgggtcacggtctcgagc
	SEQ ID NO: 103	cgaacgagcgcaggacatactcaaaccttgca
30	SEQ ID NO: 104	gcgataaagaggctgcaagac
	SEQ ID NO: 105	ttgcaaggtccaaatttcccctgaca
	SEQ ID NO: 106	
35		gacatccaaatgactcaaagcccatcatcgctatcggcacggtcggggatagagtcacgataacatg ccgaacgagcgcaggacatactcaaaccttgcatggatcaacaaaaccgggggaaggcccgaa gctactgatattcgcgataaagaggctgcaagacggagttccatcacgatttgcgggatctggctcgg ggaccgattttacgctgactatatcatcgctgcaaccggaagatttgcacatactactgcttgcaagg ctccaaatttcccctgacattcggacaaggtaccaaggtcgagatcaaa
	SEQ ID NO: 107	cggacgagcgcaggatatttattcgaactttgca
	SEQ ID NO: 108	cagtcaatcggtacaagat

SEQ ID NO: 109	
5	gacatccaaatgacgcaatcaccgagctcgtgagcgcatctgtcggggaccgtgtcacaatcacat gccggacgagcgaggatatttattcgaactttgcatggatatcaacaaaaccgggcaaggctccgaa acttttgattattcagtcgaatcggctacaagatggcgtcccagcgatttagcgggagcggatcggg aaccgactttacgtgacgatatcatcgtacaaccggaggacttcgcgacttattactgcctacaagg gagcaaattcccgtgacattcggacaaggtaggacgaagtcgagatcaaa
SEQ ID NO: 110	gattacgcaatgtac
SEQ ID NO: 111	tggataaatcctatacgggaaagccaacatacgcccaaaaattccaaggg
SEQ ID NO: 112	gccgtcttttatggatatacgtggacgca
10	SEQ ID NO: 113
15	cagggtccaactgggtccaatcgggggctgaagtcaaaaagccgggggagcgtcaaagtgcgctg caaagcatcgggatacacatttacggattacgcaatgtactgggtcaggcaagcacccggccaacga ctggaatggatgggctggataaacctatacgggaaagccaacatacgcccaaaaattccaagggc gcgtcacataacgcgggacacgagcgcacgcacggcttatatggaactatcatcgtgcgacgcga agacacggcggtctattattgcgcacgcgccgtcttttatggatatacgtggacgcacgtggggcagg gtaccctgggtcacgggtcgcgagc
SEQ ID NO: 114	gactacgcaatgcac
SEQ ID NO: 115	tggattaatgcctacacggggaagccgacctacgcacaaaaattccaagga
SEQ ID NO: 116	gccgtcttctatggatatacgtggatgct
20	SEQ ID NO: 117
25	cagggtccaattgggtccaaagcggggcgagggtcaagaagccgggggagcgtcaaagtctcatg caaggcaagcggatatacatttacggactacgcaatgcactgggtccggcaagcccctgggcaacg gctggaatggatgggatggattaatgcctacacggggaagccgacctacgcacaaaaattccaagg acgagtcacgattacgcgggatactagcgcgagcaccgcatatatggagctaagctcgtgcgacgt gaggataccgctgtatactactgcgcgagagccgtcttctatggatatacgtggatgcttgggggca gggtaccctgggtcacgggtcgcgagc
SEQ ID NO: 118	cgagcttcggaggacatctatagcaacttggct
SEQ ID NO: 119	agcgtcaaaaggctccaagac
SEQ ID NO: 120	ctacaaggctctaacttccattgaca
30	SEQ ID NO: 121
35	gatatccaaatgacgcaatcaccatctagcctatcggcctctgtgggggaccgagtcaccatcacatg ccgagcttcggaggacatctatagcaacttggcttggtatcaacaaaagccggggaagcaccaaaag ctgctgatatatagcgtcaaaagggtccaagacggagtcgaagccgatttctgggctccggctccg ggacggattttacgtgacaatttcgagcctgcaaccggaggacttgcacactactattgcctacaag gctctaacttccattgacatttgggcaaggtaggacgaagtcgagatcaaa
SEQ ID NO: 122	gactacgctatgtat
SEQ ID NO: 123	tggattaatgcctacacggggaagccgacttatgcgcacaaaatttcaagga
SEQ ID NO: 124	gcgggtctactatggatatacgtggacgca
40	SEQ ID NO: 125
	cagggtccaactgggtccaatctggagcgggaagtcaagaagcccgagcatccgtcaaagtctcgtgca

5		aggcacatctggatacacattaccgactacgctatgtattgggtccggcaagccccggacaacggct ggaatggatgggatggaatgcctacaccgggaagccgacttatgcgcaaaattcaaggaagg gtcacgattacgcgggacacgagcgctcaaccgcatacatggagctatcgagcctgcgaagcgag gacaccgcgggtctactactgcgcgcggcggtctactatggatatacatggacgcacatgggggcag ggtaccctggtcacgggtctcgagc
	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]
10	SEQ ID NO: 127	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVD NALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACE VTHQGLSSPVTKSFNRGEC [constant light chain - CL domain]
15	SEQ ID NO: 128	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPK SNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTL MISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREE QYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTIS KAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEW ESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFS CSVMHEALHNHYTQKSLSLSPGX X is K or R [constant heavy chain - CH1 + Fc domain]
25	SEQ ID NO: 129	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPK SNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDT LMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPRE EQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTI SKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVE WESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVF SCSVMHEALHNHYTQKSLSLSPGX X is K or R [L234A/L235A mutation in constant heavy chain - CH1 + Fc domain]
35	SEQ ID NO: 130	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSG ALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPK SNTKVDKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDT LMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNAKTKPR EEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEK TISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAV EWESNGQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNV FSCVMHEALHNHYTQKSLSLSPGX X is K or R [L236A/L236A/P329G mutation in constant heavy chain - CH1 + Fc domain]
45	SEQ ID NO: 131	LPQTGG [sortase tag]
	SEQ ID NO: 132	GGGGS-LPQTGG [sortase tag]

SEQ ID NO: 133	hCL1a HC full LALA
5	<p>QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAP</p> <p>GQRLEWMGWINTYTGKPTYAQKFQGRVTITRDTSASTAYMEL</p> <p>SSLRSEDVAVYYCARAVFYGYTMDAWGQGTLVTVSSASTKGP</p> <p>SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV</p> <p>HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKV</p> <p>DKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISR</p> <p>TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN</p> <p>STYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAK</p> <p>10 GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN</p> <p>GQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFS</p> <p>CSVMHEALHNHYTQKSLSLSPGX X is K or R</p>
SEQ ID NO: 134	hCL1a HC full LALAPG
15	<p>QVQLVQSGAEVKKPGASVKVSCKASGYTFTDYAMHWVRQAP</p> <p>GQRLEWMGWINTYTGKPTYAQKFQGRVTITRDTSASTAYMEL</p> <p>SSLRSEDVAVYYCARAVFYGYTMDAWGQGTLVTVSSASTKGP</p> <p>SVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGV</p> <p>HTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKV</p> <p>DKKVEPKSCDKTHTCPPCPAPEAAGGPSVFLFPPKPKDTLMISR</p> <p>20 TPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYN</p> <p>STYRVVSVLTVLHQDWLNGKEYKCKVSNKALGAPIEKTISKAK</p> <p>GQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESN</p> <p>GQPENNYKTTTPVLDSGDSFFLYSKLTVDKSRWQQGNVFS</p> <p>CSVMHEALHNHYTQKSLSLSPGX X is K or R</p>
25	SEQ ID NO: 135 CLDN18.2
30	<p>MAVTACQGLGFVVSLLIGIAGIIAATCMDQWSTQDLYNNPVTAV</p> <p>FNYQGLWRSCVRESSGFTECRGYFTLLGLPAMLQAVRALMIVG</p> <p>IVLGAIGLLVSIFALKCIRIGSMEDSAKANMTLTSGIMFIVSGLCA</p> <p>IAGVSVFANMLVTNFWMSTANMYTGMGGMVQTVQTRYTFGA</p> <p>ALFVGWVAGGLTLIGGVMMCIACRGLAPEETNYKAVSYHASG</p> <p>HSVAYKPGGFKASTGFGSNTKNKKIYDGGARTEDEVQSYPSKH</p> <p>DYV</p>
SEQ ID NO: 136	LPXTG _m [sortase tag]
	X in 3rd position is any of the 20 natural amino acids, m = 1-21.
35	SEQ ID NO: 137 LPXAG _m [sortase tag]
	X in 3rd position is any of the 20 natural amino acids, m = 1-21.
SEQ ID NO: 138	LPXSG _m [sortase tag]
	X in 3rd position is any of the 20 natural amino acids, m = 1-21.

	SEQ ID NO: 139	LAXTG _m [sortase tag]
		X in 3rd position is any of the 20 natural amino acids, m = 1-21.
	SEQ ID NO: 140	LPXTA _m [sortase tag]
		X in 3rd position is any of the 20 natural amino acids, m = 1-21.
5	SEQ ID NO: 141	NPQTG _m [sortase tag]
	SEQ ID NO: 142	NPQTN _m [sortase tag]
	SEQ ID NO: 143	LPETGG [sortase tag]
	SEQ ID NO: 144	(GGGGS) _o [oligopeptide], o = 1-5
	SEQ ID NO: 145	LPXTGG [sortase tag]
	10	X in 3rd position is any of the 20 natural amino acids.
	SEQ ID NO: 146	GGGGLPXTGG [sortase tag]
		X in 8th position is any of the 20 natural amino acids.
	SEQ ID NO: 147	GGGGG [oligopeptide]
	SEQ ID NO: 148	LPQTG [sortase tag]

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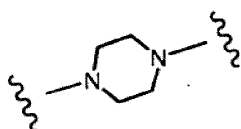
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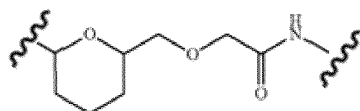
CLAIMS

1. An antibody-drug conjugate having the general formula $A - (L-T)_n$, wherein
- A is 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,
 - L is a linker, and
 - T is a toxin,
- wherein the toxin is an anthracycline,
- wherein n is an integer between ≥ 1 and ≤ 10 ;
- or a pharmaceutically acceptable salt or ester thereof.
2. The antibody-drug conjugate of claim 1, wherein the linker L comprises at least one a non-cleavable linker element, preferably wherein the non-cleavable linker element is
- ethylenediamine (EDA),
 - N-formyl-N,N'-dimethylethylenediamine,
 - diethylamine (DEA),
 - a piperazine-derived compound of the following formula:



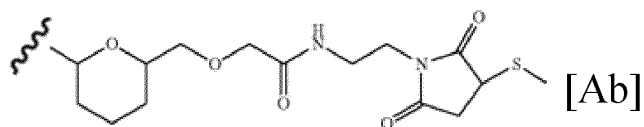
wherein the wavy lines indicate attachments to the toxin and another linker element,

- the compound of the following formula:



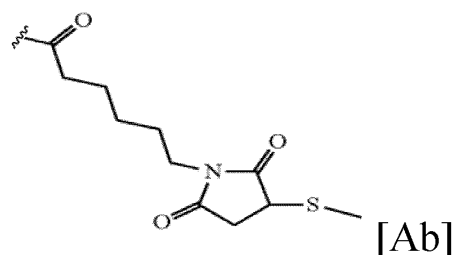
wherein the wavy lines indicate attachments to the toxin and another linker element,

- the compound of the following formula:



wherein the wavy lines indicate attachments to the toxin and [Ab] indicates the antibody or fragment thereof,

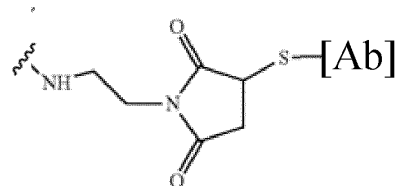
g. a maleimidocaproyl compound of the following formula:



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wherein the wavy lines indicate attachments to another linker element and [Ab] indicates the antibody or fragment thereof,

h. the compound of the following formula:



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wherein the wavy lines indicate attachments to a toxin and [Ab] indicates the antibody or fragment thereof,

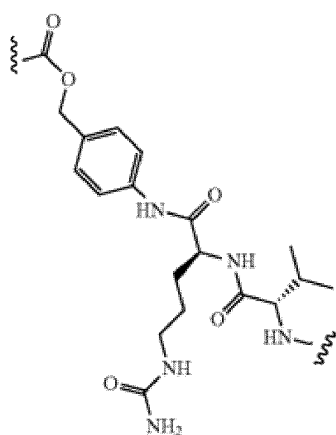
and wherein the non-cleavable linker element is conjugated to the toxin by means of an amide bond or an ether bond.

3. The antibody-drug conjugate of claim 2, wherein the linker further comprises an oligopeptide linker element and/or enzyme-cleavable linker element and/or a spacer element.
4. The antibody-drug conjugate of claim 3, wherein one oligopeptide linker element comprises a sortase recognition motif oligopeptide selected from: -LPXTG_m-, -LPXAG_m-, -LPXSG_m-, -LAXTG_m-, -LPXTG_m-, -LPXTA_m-, -NPQTG_m- or -NPQTN_m-, with G_m being an oligoglycine with m being an integer between ≥ 1 and ≤ 21 , A_m being an oligoalanine with m being an integer between ≥ 1 and ≤ 21 , N_m being an oligoasparagine with m being an integer between ≥ 1 and ≤ 21 and X being any

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conceivable amino acid, preferably the sortase recognition motif oligopeptide being -LPQTGG- or -LPETGG-, preferably wherein the oligopeptide linker element comprises:

- a. the sequence SEQ ID NO: 131, or
 - b. the sequence SEQ ID NO: 132.
- 5
5. The antibody-drug conjugate of any of claim 3 or claim 4, wherein the enzyme-cleavable linker element comprises a val-cit-PAB linker according to the compound of the following formula:



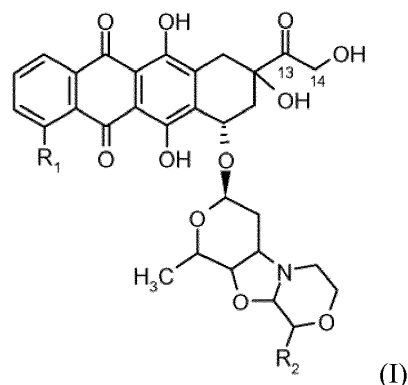
10 wherein the wavy lines indicate attachments to other linker elements.

6. The antibody-drug conjugate of any of claims 3 to 5, wherein the spacer element comprises a peptidic flexible oligopeptide, preferably wherein the peptidic flexible oligopeptide consists of G and S, more preferably wherein the peptidic flexible oligopeptide is (GGGGS)_o with o being 1, 2, 3, 4 or 5.
- 15 7. The antibody-drug conjugate of any of claims 1 to 6, wherein the antibody drug conjugate has the following structure:
- a. A – ([oligopeptide linker element – non-cleavable linker element] – T)_n and preferably wherein the linker is selected from:

i. [LPXTGG]-[ethylenediamine], and

20 ii. [LPXTGG]-[102

- b. A – ([oligopeptide linker element – enzyme cleavable linker element – non-cleavable linker element] – T)_n and preferably wherein the linker is selected from:
- i. [LPXTGG]-[vc-PAB]-[N-formyl-N,N'-dimethylethylenediamine], and
 - ii. [LPXTGG]-[vc-PAB]-[piperazine];
- c. A – ([spacer element – oligopeptide linker element – non-cleavable linker element] – T)_n and preferably wherein the linker is selected from:
- i. [GGGGS]-[LPXTGG]-[ethylenediamine], and
 - ii. [GGGGS]-[LPXTGG]-[]; or
- d. A – ([spacer element – oligopeptide linker element – enzyme cleavable linker element – non-cleavable element] – T)_n and preferably wherein the linker is selected from:
- i. [GGGGS]-[LPXTGG]-[vc-PAB]-[N-formyl-N,N'-dimethylethylenediamine], and
 - ii. [GGGGS]-[LPXTGG]-[vc-PAB]-[piperazine].
8. The antibody-drug conjugate of claim 7, wherein the non-cleavable linker element is ethylenediamine and wherein the oligopeptide linker element is LPXTGG wherein X is Q or E, preferably wherein X is Q.
9. The antibody-drug conjugate of any of claims 1 to 8, wherein
- a. (L-T) is covalently linked to both light chains of the antibody,
 - b. (L-T) is covalently linked to both heavy chains of the antibody, or
 - c. (L-T) is covalently linked to both light chains and both heavy chains of the antibody.
10. The antibody-drug conjugate of any of claims 1 to 9, wherein the anthracycline derivative has the following formula (I), and is covalently linked to the non-cleavable linker element by the C₁₃ resulting in the loss of the C₁₄ and the hydroxyl group, or is covalently linked to the non-cleavable linker element by the hydroxyl group on C₁₄:



and wherein R₁ is a hydrogen atom, a hydroxy or methoxy group,

and wherein R₂ is a C₁-C₅ alkoxy group, preferably wherein the anthracycline

derivative is a derivative of 3'-deamino-3'',4'-anhydro-[2''(S)-methoxy-3''(R)-oxy-4''-morpholinyl]doxorubicin (PNU-159682).

11. The antibody-drug conjugate of any of claims 1 to 10, wherein A, the antibody or fragment thereof, comprises:

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;

- 5 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
- 10 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.
12. The antibody-drug conjugate of any of claims 1 to 10, wherein A, the antibody or fragment thereof comprises:
- 15 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
- 20 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,
- preferably wherein A, the antibody or fragment thereof comprises:
- 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; or
- a VH sequence of SEQ ID NO: 31 and a VL sequence of SEQ ID NO: 32.

13. The antibody-drug conjugate of any of claims 1 to 11, wherein A, the antibody or fragment thereof comprises:

- 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;
 - 5 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;
 - 10 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,
- preferably wherein A, the antibody or fragment thereof, comprises:
- 15 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;
 - 20 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
- or versions thereof with an engineered Fc domain.
- 10 14. A method of producing an antibody-drug conjugate according to any of claims 1 to 19, wherein the method comprises the following steps:
- a. providing A, an antibody or fragment thereof with an oligopeptide linker element preferably at its C-terminus, optionally preceded by a spacer element at the antibody light and/or heavy chains,
- 15 b. providing one or more toxins T with a non-cleavable linker element, and
- c. conjugating the antibody and the toxin resulting in the antibody-drug conjugate.
15. An antibody-drug conjugate consisting of:
- the antibody consisting of two heavy chains of the amino acid sequence according to SEQ ID NO: 46, and two light chains of the amino acid sequence according to SEQ ID NO: 51, wherein the antibody binds to CLDN18.2,

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 - the linker [GGGGS]-[LPQTGG]-[ethylenediamine] at the C-terminus of the light chains, and
 - the anthracycline-based small molecule toxin 3'-deamino-3'',4'-anhydro-[2''(S)-methoxy-3''(R)-oxy-4''-morpholinyl]doxorubicin (PNU-159682),

25 linked covalently to the ethylenediamine of the linker at C₁₃, resulting in the loss of C₁₄ and of the hydroxyl group; or

 - the antibody consisting of two heavy chains of the amino acid sequence according to SEQ ID NO: 133, and two light chains of the amino acid sequence according to SEQ ID NO: 51, wherein the antibody binds to CLDN18.2,

- the linker [GGGGS]-[LPQTGG]-[ethylenediamine] at the C-terminus of the light chains, and
 - the anthracycline-based small molecule toxin 3'-deamino-3'',4'-anhydro-[2''(S)-methoxy-3''(R)-oxy-4''-morpholinyl]doxorubicin (PNU-159682),
5 linked covalently to the ethylenediamine of the linker at C₁₃, resulting in the loss of C₁₄ and of the hydroxyl group; or
 - the antibody consisting of two heavy chains of the amino acid sequence according to SEQ ID NO: 134 and two light chains of the amino acid sequence according to SEQ ID NO: 51, wherein the antibody binds to CLDN18.2,
 - 10 • the linker [GGGGS]-[LPQXTGG]-[ethylenediamine] at the C-terminus of the light chains, and
 - the anthracycline-based small molecule toxin 3'-deamino-3'',4'-anhydro-[2''(S)-methoxy-3''(R)-oxy-4''-morpholinyl]doxorubicin (PNU-159682),
15 linked covalently to the ethylenediamine of the linker at C₁₃, resulting in the loss of C₁₄ and of the hydroxyl group.
16. A pharmaceutical composition comprising the antibody-drug conjugate of any of claims 1 to 15 and an excipient.
17. The antibody-drug conjugate of any of claims 1 to 15 for use in treatment.
18. The antibody-drug conjugate of any of claims 1 to 15 for use in the treatment of
20 cancer, preferably wherein the cancer is selected from pancreatic, gastric, esophageal, ovarian, and lung cancer.