DESCRIPTION

Title of Invention: COMBINATION THERAPY INVOLVING ANTIBODIES AGAINST CLAUDIN 18.2 AND IMMUNE CHECKPOINT INHIBITORS FOR TREATMENT OF CANCER

Cancer of the stomach and the esophagus (gastroesophageal; GE) is one of the malignancies with the highest unmet medical need. Gastric cancer is one of the leading causes of cancer death worldwide. The incidence of esophageal cancer has increased in recent decades, coinciding with a shift in histological type and primary tumor location. Adenocarcinoma of the esophagus is now more prevalent than squamous cell carcinoma in the United States and Western Europe, with most tumors located in the distal esophagus. The overall five-year survival rate for GE cancer is 20-25%, despite the aggressiveness of established standard treatment associated with substantial side effects.

The majority of patients presents with locally advanced or metastatic disease. For these patients, first line treatment is chemotherapy. Treatment regimens are based on a backbone of platinum and fluoropyrimidine derivatives mostly combined with a third compound (e.g. taxane or anthracyclines). Still, median progression free survival of 5 to 7 months and median overall survival of 9 to 11 months are the best that can be expected.

The lack of a major benefit from the various newer generation combination chemotherapy regimens for these cancers has stimulated research into the use of targeted agents. Recently, for Her2/neu-positive gastroesophageal cancers Trastuzumab has been approved. However, as only ~20% of patients are eligible for this treatment, the medical need is still high.

The tight junction molecule Claudin 18 splice variant 2 (Claudin 18.2 (CLDN18.2)) is a member of the claudin family of tight junction proteins. CLDN18.2 is a 27.8 kDa transmembrane protein comprising four membrane spanning domains with two small extracellular loops.

In normal tissues there is no detectable expression of CLDN18.2 by RT-PCR with exception of stomach. Immunohistochemistry with CLDN18.2 specific antibodies reveals stomach as the only positive tissue.

CLDN18.2 is a highly selective gastric lineage antigen expressed exclusively on short-lived differentiated gastric epithelial cells. CLDN18.2 is maintained in the course of malignant transformation and thus frequently displayed on the surface of human gastric cancer cells. Moreover, this pan-tumoral antigen is aberrantly expressed at significant levels in esophageal, pancreatic and lung adenocarcinomas. The CLDN18.2 protein is also localized in lymph node metastases of gastric cancer adenocarcinomas and in distant metastases especially into the ovary (so-called Krukenberg tumors).

The chimeric IgG1 antibody IMAB362 (Zolbetuximab [previously named Claudiximab]) which is directed against CLDN18.2 has been developed by Ganymed Pharmaceuticals AG. This antibody comprises a heavy chain having the sequence set forth in SEQ ID NO: 51 and a light chain having the sequence set forth in SEQ ID NO: 24. IMAB362 recognizes the first extracellular domain (ECD1) of CLDN18.2 with high affinity and specificity. IMAB362 does not bind to any other claudin family member including the closely related splice variant 1 of Claudin 18 (CLDN18.1). IMAB362 shows precise tumor cell specificity and bundles two independent highly potent mechanisms of action. Upon target binding IMAB362 mediates cell killing mainly by ADCC and CDC. Thus, IMAB362 lyses efficiently CLDN18.2-positive cells, including human gastric cancer cell lines *in vitro* and *in vivo*. Anti-tumor efficacy of IMAB362 was demonstrated in mice carrying xenografted tumors inoculated with CLDN18.2-positive cancer cell lines.

IgG1 antibodies typically engage the cellular immune system via interaction of the Fc domain with Fcγ receptors (FcγRs) expressed on various immune cells including natural killer cells which are the main agents of ADCC. However, IgG1 monoclonal antibodies (mAbs) triggering ADCC face several limitations including broad distribution of low affinity Fc receptor variants in the population (up to 80%) as well as *in vivo* IgG1 modifications reducing the mAb efficacy (Chames, P., et al., 2009, Br J Pharmacol, 157(2):220-233). Therapeutic antibodies also have to compete with the patients IgGs resulting in high doses of mAbs necessary *in vivo*. Furthermore, therapeutic antibodies may interact with FcγRIIb (an inhibitory FcγR expressed by B cells, macrophages, dendritic cells and neutrophils) resulting in negative signaling that decreases their efficacy.

In the last decades, immune checkpoint inhibitors have come into focus as potent cancer treatment therapeutics. These therapeutics block inhibitory immune checkpoint signaling that

restricts immune system functions. Thus, immune checkpoint inhibitors may lead to activation, proliferation and/or increase in signaling of T cells. However, immune checkpoint inhibitors have been found to have low activity in several cancers and to be beneficial only in a small fraction of patients (Darvin et al., 2018, Exp Mol Med 50(12):165). Approaches to overcome these limitations include co-administration of drugs such as drugs targeting co-inhibitory checkpoint receptors, anti-angiogenic therapeutics, small molecule inhibitors of tumor targets, and oncolytic viruses that faciliatate tumor cell lysis (Longo et al., 2019, Cancers 11(4):539). Due to high complexity of the tumor microenvironment (TME) and diverse mechanisms by which particular TME components induce immunosuppression in a synergistic manner in the light of substantial tumor heterogeneitety it is difficult to precisely assess the TME and to device anti-cancer strategies that are applicable to the general population (Duan et al., 2019, Cancer Med 7(9):4517-4529). Therefore, there is an urgent need to increase efficacy of immune checkpoint inhibitor therapy and the Society for Immunotherapy in Cancer (SITC) has convened the Combination Immunotherapy Task Force to address the promise and challenges of combining immune checkpoint blockade with other therapies.

Here we present data demonstrating that combined administration of an anti-CLDN18.2 antibody and an immune checkpoint inhibitor results in improved effects. In mouse tumor models, administration of an anti-CLDN18.2 antibody plus a checkpoint inhibitor displays superior efficacy compared to administration of an anti-CLDN18.2 antibody or an immune checkpoint inhibitor as single agent.

SUMMARY OF THE INVENTION

The present invention generally provides a combination therapy for effectively treating and/or preventing diseases associated with cells expressing CLDN18.2, including cancer diseases such as gastric cancer, esophageal cancer, pancreatic cancer, lung cancer such as non small cell lung cancer (NSCLC), ovarian cancer, colon cancer, hepatic cancer, head-neck cancer, and cancer of the gallbladder and metastases thereof, in particular gastric cancer metastasis such as Krukenberg tumors, peritoneal metastasis and lymph node metastasis. Particularly preferred cancer diseases are adenocarcinomas of the stomach, the esophagus, the pancreatic duct, the bile ducts, the lung and the ovary.

In one aspect, the present invention provides a method for treating a patient, comprising administering to the patient an anti-CLDN18.2 antibody and an immune checkpoint inhibitor.

In one aspect, the present invention provides a method for treating or preventing cancer in a patient, comprising administering to the patient an anti-CLDN18.2 antibody and an immune checkpoint inhibitor.

In a further aspect, the present invention provides a method for inhibiting growth of a tumor in a patient having cancer, comprising administering to the patient an anti-CLDN18.2 antibody and an immune checkpoint inhibitor.

In a further aspect, the present invention provides a method for inducing antibody-dependent cell-mediated cytotoxicity (ADCC) against cancer cells in a patient, e.g., a patient having cancer, comprising administering to the patient an anti-CLDN18.2 antibody and an immune checkpoint inhibitor.

In one embodiment of all aspects disclosed herein, the immune checkpoint inhibitor is selected from a PD-1 inhibitor, and a PD-L1 inhibitor. In one embodiment of all aspects disclosed herein, the immune checkpoint inhibitor is selected from an anti-PD-1 antibody, and an anti-PD-L1 antibody.

In one embodiment of all aspects disclosed herein, the immune checkpoint inhibitor is an anti-PD-1 antibody. In one embodiment of all aspects disclosed herein, the anti-PD-1 antibody is nivolumab (OPDIVO; BMS-936558), pembrolizumab (KEYTRUDA; MK-3475), pidilizumab (CT-011), cemiplimab (LIBTAYO, REGN2810), spartalizumab (PDR001), MEDI0680 (AMP-514), dostarlimab (TSR-042), cetrelimab (JNJ 63723283), toripalimab (JS001), AMP-224 (GSK-2661380), PF-06801591, tislelizumab (BGB-A317), ABBV-181, BI 754091, or SHR-1210.

In one embodiment of all aspects disclosed herein, the immune checkpoint inhibitor is an anti-PD-L1 antibody. In one embodiment of all aspects disclosed herein, the anti-PD-L1 antibody is atezolizumab (TECENTRIQ; RG7446; MPDL3280A; R05541267), durvalumab (MEDI4736), BMS-936559, avelumab (bavencio), lodapolimab (LY3300054), CX-072 (Proclaim-CX-072), FAZ053, KN035, or MDX-1105.

In one embodiment of all aspects disclosed herein, the immune checkpoint inhibitor is a CTLA-4 inhibitor. In one embodiment of all aspects disclosed herein, the immune checkpoint inhibitor is an anti-CTLA-4 antibody. In one embodiment of all aspects disclosed herein, the anti-CTLA-4 antibody is ipilimumab (Yervoy; Bristol Myers Squibb), tremelimumab (Pfizer/MedImmune), trevilizumab, AGEN-1884 (Agenus) or ATOR-1015.

In one embodiment of all aspects disclosed herein, the anti-CLDN18.2 antibody binds to native epitopes of CLDN18.2 present on the surface of living cells. In one embodiment of all aspects disclosed herein, the anti-CLDN18.2 antibody is a monoclonal, chimeric or humanized antibody, or a fragment of an antibody. In one embodiment of all aspects disclosed herein, the anti-CLDN18.2 antibody is coupled to a therapeutic agent such as a toxin, a radioisotope, a drug or a cytotoxic agent.

In one embodiment of all aspects disclosed herein, the anti-CLDN18.2 antibody binds to the first extracellular loop of CLDN18.2.

In one embodiment of all aspects disclosed herein, the anti-CLDN18.2 antibody mediates cell killing by one or more of complement-dependent cytotoxicity (CDC) mediated lysis, antibody-dependent cell-mediated cytotoxicity (ADCC) mediated lysis, induction of apoptosis and inhibition of proliferation.

In one embodiment of all aspects disclosed herein, the anti-CLDN18.2 antibody is an antibody selected from the group consisting of:

(i) an antibody produced by and/or obtainable from a clone deposited under the accession no. DSM ACC2737, DSM ACC2738, DSM ACC2739, DSM ACC2740, DSM ACC2741, DSM ACC2742, DSM ACC2743, DSM ACC2745, DSM ACC2746, DSM ACC2747, DSM ACC2748, DSM ACC2808, DSM ACC2809, or DSM ACC2810,

(ii) an antibody which is a chimerized or humanized form of the antibody under (i),

(iii) an antibody having the specificity of the antibody under (i), and

(iv) an antibody comprising the antigen binding portion or antigen binding site, in particular the variable region, of the antibody under (i) and preferably having the specificity of the antibody under (i).

In one embodiment of all aspects disclosed herein, the anti-CLDN18.2 antibody comprises a heavy chain variable region CDR1 comprising the sequence of positions 45-52 of the sequence set forth in SEQ ID NO: 17, a heavy chain variable region CDR2 comprising the sequence of positions 70-77 of the sequence set forth in SEQ ID NO: 17, a heavy chain variable region CDR3 comprising the sequence of positions 116-126 of the sequence set forth in SEQ ID NO: 17, a light chain variable region CDR1 comprising the sequence of positions 47-58 of the sequence set forth in SEQ ID NO: 24, a light chain variable region CDR2 comprising the sequence of positions 76-78 of the sequence set forth in SEQ ID NO: 24, and a light chain variable region CDR3 comprising the sequence of positions 115-123 of the sequence set forth in SEQ ID NO: 24.

In one embodiment of all aspects disclosed herein, the anti-CLDN18.2 antibody comprises a heavy chain variable region comprising the sequence set forth in SEQ ID NO: 32 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and/or a light chain variable region comprising the sequence set forth in SEQ ID NO: 39 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant.

In one embodiment of all aspects disclosed herein, the anti-CLDN18.2 antibody comprises a heavy chain constant region comprising the sequence set forth in SEQ ID NO: 13 or 52, or a functional variant thereof, or a fragment of the amino acid sequence or functional variant.

In one embodiment of all aspects disclosed herein, the anti-CLDN18.2 antibody comprises a heavy chain comprising the sequence set forth in SEQ ID NO: 17 or 51, or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and/or a light chain comprising the sequence set forth in SEQ ID NO: 24 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant.

In one embodiment of all aspects disclosed herein, the method comprises administering the anti-CLDN18.2 antibody at a dose of up to 1000 mg/m^2. In one embodiment of all aspects disclosed herein, the method comprises administering the anti-CLDN18.2 antibody repeatedly at a dose of 300 to 600 mg/m^2.

In one embodiment of all aspects disclosed herein, the cancer is CLDN18.2 positive. In one embodiment of all aspects disclosed herein, the cancer is selected from the group consisting of

gastric cancer, esophageal cancer, pancreatic cancer, lung cancer, ovarian cancer, colon cancer, hepatic cancer, head-neck cancer, cancer of the gallbladder and the metastasis thereof. In one embodiment of all aspects disclosed herein, the cancer is a Krukenberg tumor, peritoneal metastasis and/or lymph node metastasis. In one embodiment of all aspects disclosed herein, the cancer is an adenocarcinoma, in particular an advanced adenocarcinoma. In one embodiment of all aspects disclosed herein, the cancer is selected from the group consisting of cancer of the stomach, cancer of the esophagus, in particular the lower esophagus, cancer of the eso-gastric junction and gastroesophageal cancer.

In one embodiment of all aspects disclosed herein, CLDN18.2 has the amino acid sequence according to SEQ ID NO: 1.

In a further aspect, the present invention provides a medical preparation comprising an anti-CLDN18.2 antibody and an immune checkpoint inhibitor.

In one embodiment of all aspects disclosed herein, the medical preparation is a kit comprising a first container including the anti-CLDN18.2 antibody and a second container including the immune checkpoint inhibitor.

In one embodiment of all aspects disclosed herein, the medical preparation further includes printed instructions for use of the preparation for treatment of cancer.

In one embodiment of all aspects disclosed herein, the medical preparation is a composition comprising the anti-CLDN18.2 antibody and the immune checkpoint inhibitor.

In one embodiment of all aspects disclosed herein, the method of the invention further comprises administering a cytotoxic and/or cytostatic agent. In one embodiment of all aspects disclosed herein, the medical preparation of the invention further comprises a cytotoxic and/or cytostatic agent.

The cytotoxic and/or cytostatic agent may be an agent stabilizing or increasing expression of CLDN18.2. Expression of CLDN18.2 is preferably at the cell surface of a cancer cell. In one embodiment, the cytotoxic and/or cytostatic agent comprises an agent which induces a cell cycle arrest or an accumulation of cells in one or more phases of the cell cycle, preferably in one or

more phases of the cell cycle other than the G1-phase. The cytotoxic and/or cytostatic agent may comprise an agent selected from the group consisting of anthracyclines, platinum compounds, nucleoside analogs, taxanes, and camptothecin analogs, or prodrugs thereof, and combinations thereof. The cytotoxic and/or cytostatic agent may comprise an agent selected from the group consisting of epirubicin, oxaliplatin, cisplatin, 5-fluorouracil or prodrugs thereof such as capecitabine, docetaxel, irinotecan, and combinations thereof. The cytotoxic and/or cytostatic agent may comprise a combination of oxaliplatin and 5-fluorouracil or prodrugs thereof, a combination of cisplatin and 5-fluorouracil or prodrugs thereof, a combination of at least one anthracycline and oxaliplatin, a combination of at least one anthracycline and cisplatin, a combination of at least one anthracycline and 5-fluorouracil or prodrugs thereof, a combination of at least one taxane and oxaliplatin, a combination of at least one taxane and cisplatin, a combination of at least one taxane and 5-fluorouracil or prodrugs thereof, or a combination of at least one camptothecin analog and 5-fluorouracil or prodrugs thereof. The cytotoxic and/or cytostatic agent may be an agent inducing immunogenic cell death. The agent inducing immunogenic cell death may comprise an agent selected from the group consisting of anthracyclines, oxaliplatin and combinations thereof. The cytotoxic and/or cytostatic agent may comprise a combination of epirubicin and oxaliplatin. In one embodiment, the method of the invention comprises administering at least one anthracycline, at least one platinum compound and at least one of 5-fluorouracil and prodrugs thereof. In one embodiment, the medical preparation of the invention comprises at least one anthracycline, at least one platinum compound and at least one of 5-fluorouracil and prodrugs thereof. The anthracycline may be selected from the group consisting of epirubicin, doxorubicin, daunorubicin, idarubicin and valrubicin. Preferably, the anthracycline is epirubicin. The platinum compound may selected from the group consisting of oxaliplatin and cisplatin. The nucleoside analog may be selected from the group consisting of 5-fluorouracil and prodrugs thereof. The taxane may be selected from the group consisting of docetaxel and paclitaxel. The camptothecin analog may be selected from the group consisting of irinotecan and topotecan. In one embodiment, the method of the invention comprises administering (i) epirubicin, oxaliplatin and 5-fluorouracil, (ii) epirubicin, oxaliplatin and capecitabine, (iii) epirubicin, cisplatin and 5-fluorouracil, (iv) epirubicin, cisplatin and capecitabine, (v) folinic acid, oxaliplatin and 5-fluorouracil, (vi) folinic acid, oxaliplatin and capecitabine, or (vii) oxaliplatin and capecitabine. In one embodiment, the medical preparation of the invention comprises (i) epirubicin, oxaliplatin and 5-fluorouracil, (ii) epirubicin, oxaliplatin and capecitabine, (iii) epirubicin, cisplatin and 5-fluorouracil, (iv)

epirubicin, cisplatin and capecitabine, (v) folinic acid, oxaliplatin and 5-fluorouracil, (vi) folinic acid, oxaliplatin and capecitabine, or (vii) oxaliplatin and capecitabine.

The anti-CLDN18.2 antibody and the immune checkpoint inhibitor, and optionally the cytotoxic and/or cytostatic agent, may be present in the medical preparation in a mixture or separate from each other. The medical preparation may be a kit comprising a first container including the CLDN18.2 antibody and a container including the immune checkpoint inhibitor, and optionally a container including the cytotoxic and/or cytostatic agent. The medical preparation may further include printed instructions for use of the preparation for treatment of cancer, in particular for use of the preparation in a method of the invention. Different embodiments of the medical preparation, and, in particular, of the immune checkpoint inhibitor and the cytotoxic and/or cytostatic agent are as described above for the method of the invention.

The present invention also provides the agents described herein such as the anti-CLDN18.2 antibody and the immune checkpoint inhibitor for use in therapy. In one embodiment, such therapy comprises treating and/or preventing diseases associated with cells expressing CLDN18.2, including cancer diseases such as those described herein.

The present invention also provides the agents described herein such as the anti-CLDN18.2 antibody for use in the methods described herein, e.g. for administration in combination with an immune checkpoint inhibitor, and optionally a cytotoxic and/or cytostatic agent. The present invention also provides a use of the agents described herein such as the anti-CLDN18.2 antibody for the preparation of a pharmaceutical composition for use in the methods described herein, e.g. for administration in combination with an immune checkpoint inhibitor, and optionally a cytotoxic and/or cytostatic agent.

In one aspect, the present invention provides an anti-CLDN18.2 antibody for use in a method for treating or preventing cancer in a patient, comprising administering to the patient the anti-CLDN18.2 antibody and an immune checkpoint inhibitor. In a further aspect, the present invention provides an anti-CLDN18.2 antibody for use in a method for inhibiting growth of a tumor in a patient having cancer, comprising administering to the patient the anti-CLDN18.2 antibody and an immune checkpoint inhibitor. In a further aspect, the present invention provides an anti-CLDN18.2 antibody for use in a method for inducing antibody-dependent cell-mediated cytotoxicity (ADCC) against cancer cells in a patient, e.g., a patient having cancer, comprising

administering to the patient the anti-CLDN18.2 antibody and an immune checkpoint inhibitor. Preferred embodiments of these aspects are as described above for the methods of the invention.

In one aspect, the present invention provides an immune checkpoint inhibitor for use in a method for treating or preventing cancer in a patient, comprising administering to the patient an anti-CLDN18.2 antibody and the immune checkpoint inhibitor. In a further aspect, the present invention provides an immune checkpoint inhibitor for use in a method for inhibiting growth of a tumor in a patient having cancer, comprising administering to the patient an anti-CLDN18.2 antibody and the immune checkpoint inhibitor. In a further aspect, the present invention provides an immune checkpoint inhibitor for use in a method for inducing antibody-dependent cell-mediated cytotoxicity (ADCC) against cancer cells in a patient, e.g., a patient having cancer, comprising administering to the patient an anti-CLDN18.2 antibody and the immune checkpoint inhibitor. Preferred embodiments of these aspects are as described above for the methods of the invention.

In one aspect, the present invention provides an anti-CLDN18.2 antibody and an immune checkpoint inhibitor for use in a method for treating or preventing cancer in a patient. In a further aspect, the present invention provides an anti-CLDN18.2 antibody and an immune checkpoint inhibitor for use in a method for inhibiting growth of a tumor in a patient having cancer. In a further aspect, the present invention provides an anti-CLDN18.2 antibody and an immune checkpoint inhibitor for use in a method for inducing antibody-dependent cell-mediated cytotoxicity (ADCC) against cancer cells in a patient, e.g., a patient having cancer. Preferred embodiments of these aspects are as described above for the methods of the invention.

In one aspect, the present invention provides a use of an anti-CLDN18.2 antibody for the preparation of a pharmaceutical composition for treating or preventing cancer in a patient, wherein the anti-CLDN18.2 antibody is to be administered together with an immune checkpoint inhibitor. In a further aspect, the present invention provides a use of an anti-CLDN18.2 antibody for the preparation of a pharmaceutical composition for inhibiting growth of a tumor in a patient having cancer, wherein the anti-CLDN18.2 antibody is to be administered together with an immune checkpoint inhibitor. In a further aspect, the present invention provides a use of an anti-CLDN18.2 antibody for the preparation of a pharmaceutical composition for inducing antibody-dependent cell-mediated cytotoxicity (ADCC) against cancer cells in a patient, e.g., a patient having cancer, wherein the anti-CLDN18.2 antibody is to be administered together with an

immune checkpoint inhibitor. Preferred embodiments of these aspects are as described above for the methods of the invention.

In one aspect, the present invention provides a use of an immune checkpoint inhibitor for the preparation of a pharmaceutical composition for treating or preventing cancer in a patient, wherein the immune checkpoint inhibitor is to be administered together with an anti-CLDN18.2 antibody. In a further aspect, the present invention provides a use of an immune checkpoint inhibitor for the preparation of a pharmaceutical composition for inhibiting growth of a tumor in a patient having cancer, wherein the immune checkpoint inhibitor is to be administered together with an anti-CLDN18.2 antibody. In a further aspect, the present invention provides a use of an immune checkpoint inhibitor for the preparation of a pharmaceutical composition for inducing antibody-dependent cell-mediated cytotoxicity (ADCC) against cancer cells in a patient, e.g., a patient having cancer, wherein the immune checkpoint inhibitor is to be administered together with an anti-CLDN18.2 antibody. Preferred embodiments of these aspects are as described above for the methods of the invention.

In one aspect, the present invention provides a use of an anti-CLDN18.2 antibody and an immune checkpoint inhibitor for the preparation of a pharmaceutical composition for treating or preventing cancer in a patient. In a further aspect, the present invention provides a use of an anti-CLDN18.2 antibody and an immune checkpoint inhibitor for the preparation of a pharmaceutical composition for inhibiting growth of a tumor in a patient having cancer. In a further aspect, the present invention provides a use of an anti-CLDN18.2 antibody and an immune checkpoint inhibitor for the preparation of a pharmaceutical composition for inducing antibody-dependent cell-mediated cytotoxicity (ADCC) against cancer cells in a patient, e.g., a patient having cancer. Preferred embodiments of these aspects are as described above for the methods of the invention.

In one embodiment of the aspects described herein, a treatment described herein involves an immunotherapeutic treatment of a patient. In one embodiment of the aspects described herein, a treatment described herein involves inducing immune-mediated inhibition or destruction of cancer cells in a patient. In one embodiment of the aspects described herein, a treatment described herein involves inducing immune cell-mediated inhibition or destruction of cancer cells in a patient. In one embodiment of the aspects described herein, a treatment described herein involves inducing T cell-mediated inhibition or destruction of cancer cells in a patient. In one embodiment of the aspects described herein, a treatment described herein involves inducing

NK cell-mediated inhibition or destruction of cancer cells in a patient. In one embodiment of the aspects described herein, a treatment described herein involves inducing antibody-dependent cell-mediated cytotoxicity (ADCC) against cancer cells in a patient. In one embodiment, ADCC is mediated, at least in part, by NK cells. In one embodiment of the aspects described herein, a treatment described herein involves inducing complement dependent cytotoxicity (CDC) against cancer cells in a patient.

In one embodiment of the aspects described herein, administration of the immune checkpoint inhibitor increases anti-tumor efficacy of the anti-CLDN18.2 antibody. In one embodiment of the aspects described herein, administration of the immune checkpoint inhibitor increases the efficacy of the anti-CLDN18.2 antibody to induce an immune-mediated inhibition or destruction of cancer cells in a patient. In one embodiment of the aspects described herein, administration of the immune checkpoint inhibitor increases the efficacy of the anti-CLDN18.2 antibody to induce an immune cell-mediated inhibition or destruction of cancer cells in a patient. In one embodiment of the aspects described herein, administration of the immune checkpoint inhibitor increases the efficacy of the anti-CLDN18.2 antibody to induce a T cell-mediated inhibition or destruction of cancer cells in a patient. In one embodiment of the aspects described herein, administration of the immune checkpoint inhibitor increases the efficacy of the anti-CLDN18.2 antibody to induce a NK cell-mediated inhibition or destruction of cancer cells in a patient. In one embodiment of the aspects described herein, administration of the immune checkpoint inhibitor increases the efficacy of the anti-CLDN18.2 antibody to induce antibody-dependent cell-mediated cytotoxicity (ADCC) against cancer cells in a patient. In one embodiment of the aspects described herein, administration of the immune checkpoint inhibitor increases the efficacy of the anti-CLDN18.2 antibody to induce complement dependent cytotoxicity (CDC) against cancer cells in a patient. In one embodiment of the aspects described herein, administration of the immune checkpoint inhibitor increases efficacy of the anti-CLDN18.2 antibody in a synergistic manner.

Other features and advantages of the instant invention will be apparent from the following detailed description and claims.

BRIEF DESCRIPTION OF THE DRAWINGS

Figure 1. Effects of combination therapy with IMAB362 and Anti-mPD-1 Ab on CLS-103 LVT-murinCLDN18.2 syngeneic tumors

Figure 2. Effects of combination therapy with IMAB362 and Anti-mPD-1 Ab on CLS-103 LVT-murinCLDN18.2 syngeneic tumors – spider plot analysis for all treated mice

Figure 3. Long term effects of combination therapy with IMAB362 and Anti-mPD-1 Ab on CLS-103 LVT-murinCLDN18.2 syngeneic tumors – spider plot analysis for all treated mice

Figure 4. Long term effects of combination therapy with IMAB362 and Anti-mPD-1 Ab on CLS-103 LVT-murinCLDN18.2 syngeneic tumors

Figure 5. Effects of combination therapy with IMAB362 and Anti-mCTLA-4 Ab on CLS-103 LVT-murinCLDN18.2 syngeneic tumors

Figure 6. Effects of combination therapy with IMAB362 and Anti-mCTLA-4 Ab on CLS-103 LVT-murinCLDN18.2 syngeneic tumors – spider plot analysis for all treated mice

Figure 7. Effects of combination therapy with IMAB362 and Anti-mPD-L1 Ab on CLS-103 LVT-murinCLDN18.2 syngeneic tumors

Figure 8. Effects of combination therapy with IMAB362 and Anti-mPD-L1 Ab on CLS-103 LVT-murinCLDN18.2 syngeneic tumors – spider plot analysis for all treated mice

DETAILED DESCRIPTION OF THE INVENTION

Although the present invention is described in detail below, it is to be understood that this invention is not limited to the particular methodologies, protocols and reagents described herein as these may vary. It is also to be understood that the terminology used herein is for the purpose of describing particular embodiments only, and is not intended to limit the scope of the present invention which will be limited only by the appended claims. Unless defined otherwise, all

technical and scientific terms used herein have the same meanings as commonly understood by one of ordinary skill in the art.

In the following, the elements of the present invention will be described. These elements are listed with specific embodiments, however, it should be understood that they may be combined in any manner and in any number to create additional embodiments. The variously described examples and preferred embodiments should not be construed to limit the present invention to only the explicitly described embodiments. This description should be understood to support and encompass embodiments which combine the explicitly described embodiments with any number of the disclosed and/or preferred elements. Furthermore, any permutations and combinations of all described elements in this application should be considered disclosed by the description of the present application unless the context indicates otherwise.

Preferably, the terms used herein are defined as described in "A multilingual glossary of biotechnological terms: (IUPAC Recommendations)", H.G.W. Leuenberger, B. Nagel, and H. Kölbl, Eds., Helvetica Chimica Acta, CH-4010 Basel, Switzerland, (1995).

The practice of the present invention will employ, unless otherwise indicated, conventional methods of chemistry, biochemistry, cell biology, immunology, and recombinant DNA techniques which are explained in the literature in the field (cf., e.g., *Molecular Cloning: A Laboratory Manual*, 2nd Edition, J. Sambrook et al. eds., Cold Spring Harbor Laboratory Press, Cold Spring Harbor 1989).

Throughout this specification and the claims which follow, unless the context requires otherwise, the word "comprise", and variations such as "comprises" and "comprising", will be understood to imply the inclusion of a stated member, integer or step or group of members, integers or steps but not the exclusion of any other member, integer or step or group of members, integers or steps although in some embodiments such other member, integer or step or group of members, integers or steps may be excluded, i.e. the subject-matter consists in the inclusion of a stated member, integer or step or group of members, integers or steps. The terms "a" and "an" and "the" and similar reference used in the context of describing the invention (especially in the context of the claims) are to be construed to cover both the singular and the plural, unless otherwise indicated herein or clearly contradicted by context. Recitation of ranges of values herein is merely intended to serve as a shorthand method of referring individually to each separate value falling

within the range. Unless otherwise indicated herein, each individual value is incorporated into the specification as if it were individually recited herein. All methods described herein can be performed in any suitable order unless otherwise indicated herein or otherwise clearly contradicted by context. The use of any and all examples, or exemplary language (e.g., "such as"), provided herein is intended merely to better illustrate the invention and does not pose a limitation on the scope of the invention otherwise claimed. No language in the specification should be construed as indicating any non-claimed element essential to the practice of the invention.

Several documents are cited throughout the text of this specification. Each of the documents cited herein (including all patents, patent applications, scientific publications, manufacturer's specifications, instructions, etc.), whether *supra* or *infra*, are hereby incorporated by reference in their entirety. Nothing herein is to be construed as an admission that the invention is not entitled to antedate such disclosure by virtue of prior invention.

The term "CLDN18" relates to claudin 18 and includes any variants, including claudin 18 splice variant 1 (claudin 18.1 (CLDN18.1)) and claudin 18 splice variant 2 (claudin 18.2 (CLDN18.2)).

The term "CLDN18.2" preferably relates to human CLDN18.2, and, in particular, to a protein comprising, preferably consisting of the amino acid sequence according to SEQ ID NO: 1 of the sequence listing or a variant of said amino acid sequence.

The term "CLDN18.1" preferably relates to human CLDN18.1, and, in particular, to a protein comprising, preferably consisting of the amino acid sequence according to SEQ ID NO: 2 of the sequence listing or a variant of said amino acid sequence.

The term "variant" according to the invention refers, in particular, to mutants, splice variants, conformations, isoforms, allelic variants, species variants and species homologs, in particular those which are naturally present. An allelic variant relates to an alteration in the normal sequence of a gene, the significance of which is often unclear. Complete gene sequencing often identifies numerous allelic variants for a given gene. A species homolog is a nucleic acid or amino acid sequence with a different species of origin from that of a given nucleic acid or amino acid sequence. The term "variant" shall encompass any posttranslationally modified variants and conformation variants.

According to the invention, the term "CLDN18.2 positive cancer" means a cancer involving cancer cells expressing CLDN18.2, preferably on the surface of said cancer cells.

"Cell surface" is used in accordance with its normal meaning in the art, and thus includes the outside of the cell which is accessible to binding by proteins and other molecules.

CLDN18.2 is expressed on the surface of cells if it is located at the surface of said cells and is accessible to binding by CLDN18.2-specific antibodies added to the cells.

According to the invention, CLDN18.2 is not substantially expressed in a cell if the level of expression is lower compared to expression in stomach cells or stomach tissue. Preferably, the level of expression is less than 10%, preferably less than 5%, 3%, 2%, 1%, 0.5%, 0.1% or 0.05% of the expression in stomach cells or stomach tissue or even lower. Preferably, CLDN18.2 is not substantially expressed in a cell if the level of expression exceeds the level of expression in non-cancerous tissue other than stomach by no more than 2-fold, preferably 1,5-fold, and preferably does not exceed the level of expression in said non-cancerous tissue. Preferably, CLDN18.2 is not substantially expressed in a cell if the level of expression is below the detection limit and/or if the level of expression is too low to allow binding by CLDN18.2-specific antibodies added to the cells.

According to the invention, CLDN18.2 is expressed in a cell if the level of expression exceeds the level of expression in non-cancerous tissue other than stomach preferably by more than 2-fold, preferably 10-fold, 100-fold, 1000-fold, or 10000-fold. Preferably, CLDN18.2 is expressed in a cell if the level of expression is above the detection limit and/or if the level of expression is high enough to allow binding by CLDN18.2-specific antibodies added to the cells. Preferably, CLDN18.2 expressed in a cell is expressed or exposed on the surface of said cell.

According to the invention, the term "disease" refers to any pathological state, including cancer, in particular those forms of cancer described herein. Any reference herein to cancer or particular forms of cancer also includes cancer metastasis thereof. In a preferred embodiment, a disease to be treated according to the present application involves cells expressing CLDN18.2.

"Diseases associated with cells expressing CLDN18.2" or similar expressions means according to the invention that CLDN18.2 is expressed in cells of a diseased tissue or organ. In one embodiment, expression of CLDN18.2 in cells of a diseased tissue or organ is increased compared to the state in a healthy tissue or organ. An increase refers to an increase by at least 10%, in particular at least 20%, at least 50%, at least 100%, at least 200%, at least 500%, at least 1000%, at least 10000% or even more. In one embodiment, expression is only found in a diseased tissue, while expression in a healthy tissue is repressed. According to the invention, diseases associated with cells expressing CLDN18.2 include cancer diseases. Furthermore, according to the invention, cancer diseases preferably are those wherein the cancer cells express CLDN18.2.

As used herein, a "cancer disease" or "cancer" includes a disease characterized by aberrantly regulated cellular growth, proliferation, differentiation, adhesion, and/or migration. By "cancer cell" is meant an abnormal cell that grows by a rapid, uncontrolled cellular proliferation and continues to grow after the stimuli that initiated the new growth cease. Preferably, a "cancer disease" is characterized by cells expressing CLDN18.2 and a cancer cell expresses CLDN18.2. A cell expressing CLDN18.2 preferably is a cancer cell, preferably of the cancers described herein.

"Adenocarcinoma" is a cancer that originates in glandular tissue. This tissue is also part of a larger tissue category known as epithelial tissue. Epithelial tissue includes skin, glands and a variety of other tissue that lines the cavities and organs of the body. Epithelium is derived embryologically from ectoderm, endoderm and mesoderm. To be classified as adenocarcinoma, the cells do not necessarily need to be part of a gland, as long as they have secretory properties. This form of carcinoma can occur in some higher mammals, including humans. Well differentiated adenocarcinomas tend to resemble the glandular tissue that they are derived from, while poorly differentiated may not. By staining the cells from a biopsy, a pathologist will determine whether the tumor is an adenocarcinoma or some other type of cancer. Adenocarcinomas can arise in many tissues of the body due to the ubiquitous nature of glands within the body. While each gland may not be secreting the same substance, as long as there is an exocrine function to the cell, it is considered glandular and its malignant form is therefore named adenocarcinoma. Malignant adenocarcinomas invade other tissues and often metastasize given enough time to do so. Ovarian adenocarcinoma is the most common type of ovarian carcinoma. It includes the serous and mucinous adenocarcinomas, the clear cell adenocarcinoma

and the endometrioid adenocarcinoma.

By "metastasis" is meant the spread of cancer cells from its original site to another part of the body. The formation of metastasis is a very complex process and depends on detachment of malignant cells from the primary tumor, invasion of the extracellular matrix, penetration of the endothelial basement membranes to enter the body cavity and vessels, and then, after being transported by the blood, infiltration of target organs. Finally, the growth of a new tumor at the target site depends on angiogenesis. Tumor metastasis often occurs even after the removal of the primary tumor because tumor cells or components may remain and develop metastatic potential. In one embodiment, the term "metastasis" according to the invention relates to "distant metastasis" which relates to a metastasis which is remote from the primary tumor and the regional lymph node system. In one embodiment, the term "metastasis" according to the invention relates to lymph node metastasis. One particular form of metastasis which is treatable using the therapy of the invention is metastasis originating from gastric cancer as primary site. In preferred embodiments such gastric cancer metastasis is Krukenberg tumors, peritoneal metastasis and/or lymph node metastasis.

Krukenberg tumor is an uncommon metastatic tumor of the ovary accounting for 1% to 2% of all ovarian tumors. Prognosis of Krukenberg tumor is still very poor and there is no established treatment for Krukenberg tumors. Krukenberg tumor is a metastatic signet ring cell adenocarcinoma of the ovary. Stomach is the primary site in most Krukenberg tumor cases (70%). Carcinomas of colon, appendix, and breast (mainly invasive lobular carcinoma) are the next most common primary sites. Rare cases of Krukenberg tumor originating from carcinomas of the gallbladder, biliary tract, pancreas, small intestine, ampulla of Vater, cervix, and urinary bladder/urachus have been reported. The interval between the diagnosis of a primary carcinoma and the subsequent discovery of ovarian involvement is usually 6 months or less, but longer periods have been reported. In many cases, the primary tumor is very small and can escape detection. A history of a prior carcinoma of the stomach or another organ can be obtained in only 20% to 30% of the cases. Krukenberg tumor is an example of the selective spread of cancers, most commonly in the stomach-ovarian axis. This axis of tumor spread has historically drawn the attention of many pathologists, especially when it was found that gastric neoplasms selectively metastasize to the ovaries without involvement of other tissues. The route of metastasis of gastric carcinoma to the ovaries has been a mystery for a long time, but it is now evident that retrograde lymphatic spread is the most likely route of metastasis. Women with

Krukenberg tumors tend to be unusually young for patients with metastatic carcinoma as they are typically in the fifth decade of their lives, with an average age of 45 years. This young age of distribution can be related in part to the increased frequency of gastric signet ring cell carcinomas in young women. Common presenting symptoms are usually related to ovarian involvement, the most common of which are abdominal pain and distension (mainly because of the usually bilateral and often large ovarian masses). The remaining patients have nonspecific gastrointestinal symptoms or are asymptomatic. In addition, Krukenberg tumor is reportedly associated with virilization resulting from hormone production by ovarian stroma. Ascites is present in 50% of the cases and usually reveals malignant cells. Krukenberg tumors are bilateral in more than 80% of the reported cases. The ovaries are usually asymmetrically enlarged, with a bosselated contour. The sectioned surfaces are yellow or white; they are usually solid, although they are occasionally cystic. Importantly, the capsular surface of the ovaries with Krukenberg tumors is typically smooth and free of adhesions or peritoneal deposits. Of note, other metastatic tumors to the ovary tend to be associated with surface implants. This may explain why the gross morphology of Krukenberg tumor can deceptively appear as a primary ovarian tumor. However, bilateralism in Krukenberg tumor is consistent with its metastatic nature. Patients with Krukenberg tumors have an overall mortality rate that is significantly high. Most patients die within 2 years (median survival, 14 months). Several studies show that the prognosis is poor when the primary tumor is identified after the metastasis to the ovary is discovered, and the prognosis becomes worse if the primary tumor remains covert. No optimal treatment strategy for Krukenberg tumors has been clearly established in the literature. Whether a surgical resection should be performed has not been adequately addressed. Chemotherapy or radiotherapy has no significant effect on prognosis of patients with Krukenberg tumors.

In the present context, the term "treatment", "treating" or "therapeutic intervention" relates to the management and care of a subject for the purpose of combating a condition such as a disease or disorder. The term is intended to include the full spectrum of treatments for a given condition from which the subject is suffering, such as administration of the therapeutically effective compound to alleviate the symptoms or complications, to delay the progression of the disease, disorder or condition, to alleviate or relief the symptoms and complications, and/or to cure or eliminate the disease, disorder or condition as well as to prevent the condition, wherein prevention is to be understood as the management and care of an individual for the purpose of combating the disease, condition or disorder and includes the administration of the active compounds to prevent the onset of the symptoms or complications.

The term "therapeutic treatment" relates to any treatment which improves the health status and/or prolongs (increases) the lifespan of an individual. Said treatment may eliminate the disease in an individual, arrest or slow the development of a disease in an individual, inhibit or slow the development of a disease in an individual, decrease the frequency or severity of symptoms in an individual, and/or decrease the recurrence in an individual who currently has or who previously has had a disease.

The terms "prophylactic treatment" or "preventive treatment" relate to any treatment that is intended to prevent a disease from occurring in an individual. The terms "prophylactic treatment" or "preventive treatment" are used herein interchangeably.

The terms "individual" and "subject" are used herein interchangeably. They refer to a human or another mammal (e.g. mouse, rat, rabbit, dog, cat, cattle, swine, sheep, horse or primate) that can be afflicted with or is susceptible to a disease or disorder (e.g., cancer) but may or may not have the disease or disorder. In many embodiments, the individual is a human being. Unless otherwise stated, the terms "individual" and "subject" do not denote a particular age, and thus encompass adults, elderlies, children, and newborns. In embodiments of the present disclosure, the "individual" or "subject" is a "patient".

The term "patient" means an individual or subject for treatment, in particular a diseased individual or subject.

As used herein, "immune checkpoint" refers to regulators of the immune system, and, in particular, co-stimulatory and inhibitory signals that regulate the amplitude and quality of T cell receptor recognition of an antigen. In certain embodiments, the immune checkpoint is an inhibitory signal. In certain embodiments, the inhibitory signal is the interaction between PD-1 and PD-L1 and/or PD-L2. In certain embodiments, the inhibitory signal is the interaction between CTLA-4 and CD80 or CD86 to displace CD28 binding. In certain embodiments the inhibitory signal is the interaction between LAG-3 and MHC class II molecules. In certain embodiments, the inhibitory signal is the interaction between TIM-3 and one or more of its ligands, such as galectin 9, PtdSer, HMGB1 and CEACAM1. In certain embodiments, the inhibitory signal is the interaction between one or several KIRs and their ligands. In certain embodiments, the inhibitory signal is the interaction between TIGIT and one or more of its

ligands, PVR, PVRL2 and PVRL3. In certain embodiments, the inhibitory signal is the interaction between CD94/NKG2A and HLA-E. In certain embodiments, the inhibitory signal is the interaction between VISTA and its binding partner(s). In certain embodiments, the inhibitory signal is the interaction between one or more Siglecs and their ligands. In certain embodiments, the inhibitory signal is the interaction between GARP and one or more of it ligands. In certain embodiments, the inhibitory signal is the interaction between CD47 and SIRPα. In certain embodiments, the inhibitory signal is the interaction between PVRIG and PVRL2. In certain embodiments, the inhibitory signal is the interaction between CSF1R and CSF1. In certain embodiments, the inhibitory signal is the interaction between BTLA and HVEM. In certain embodiments, the inhibitory signal is part of the adenosinergic pathway, e.g., the interaction between A2AR and/or A2BR and adenosine, produced by CD39 and CD73. In certain embodiments, the inhibitory signal is the interaction between B7-H3 and its receptor and/or B7-H4 and its receptor. In certain embodiments, the inhibitory signal is mediated by IDO, CD20, NOX or TDO.

The "Programmed Death-1 (PD-1)" receptor refers to an immuno-inhibitory receptor belonging to the CD28 family. PD-1 is expressed predominantly on previously activated T cells *in vivo*, and binds to two ligands, PD-L1 (also known as B7-H1 or CD274) and PD-L2 (also known as B7-DC or CD273). The term "PD-1" as used herein includes human PD-1 (hPD-1), variants, isoforms, and species homologs of hPD-1, and analogs having at least one common epitope with hPD-1. "Programmed Death Ligand-1 (PD-L1)" is one of two cell surface glycoprotein ligands for PD-1 (the other being PD-L2) that downregulates T cell activation and cytokine secretion upon binding to PD-1. The term "PD-L1" as used herein includes human PD-L1 (hPD-L1), variants, isoforms, and species homologs of hPD-L1, and analogs having at least one common epitope with hPD-L1. The term "PD-L2" as used herein includes human PD-L2 (hPD-L2), variants, isoforms, and species homologs of hPD-L2, and analogs having at least one common epitope with hPD-L2. The ligands of PD-1 (PD-L1 and PD-L2) are expressed on the surface of antigen-presenting cells, such as dendritic cells or macrophages, and other immune cells. Binding of PD-1 to PD-L1 or PD-L2 results in downregulation of T cell activation. Cancer cells expressing PD-L1 and/or PD-L2 are able to switch off T cells expressing PD-1 what results in suppression of the anticancer immune response. The interaction between PD-1 and its ligands results in a decrease in tumor infiltrating lymphocytes, a decrease in T cell receptor mediated proliferation, and immune evasion by the cancerous cells. Immune suppression can be reversed

by inhibiting the local interaction of PD-1 with PD-L1, and the effect is additive when the interaction of PD-1 with PD-L2 is blocked as well.

"Cytotoxic T Lymphocyte Associated Antigen-4 (CTLA-4)" (also known as CD152) is a T cell surface molecule and is a member of the immunoglobulin superfamily. This protein downregulates the immune system by binding to CD80 (B7-1) and CD86 (B7-2). The term "CTLA-4" as used herein includes human CTLA-4 (hCTLA-4), variants, isoforms, and species homologs of hCTLA-4, and analogs having at least one common epitope with hCTLA-4. CTLA-4 is a homolog of the stimulatory checkpoint protein CD28 with much higher binding affinity for CD80 and CD86. CTLA4 is expressed on the surface of activated T cells and its ligands are expressed on the surface of professional antigen-presenting cells. Binding of CTLA-4 to its ligands prevents the co-stimulatory signal of CD28 and produces an inhibitory signal. Thus, CTLA-4 downregulates T cell activation.

"T cell Immunoreceptor with Ig and ITIM domains" (TIGIT, also known as WUCAM or Vstm3) is an immune receptor on T cells and Natural Killer (NK) cells and binds to PVR (CD155) on DCs, macrophages etc., and PVRL2 (CD112; nectin-2) and PVRL3 (CD113; nectin-3) and regulates T cell-mediated immunity. The term "TIGIT" as used herein includes human TIGIT (hTIGIT), variants, isoforms, and species homologs of hTIGIT, and analogs having at least one common epitope with hTIGIT. The term "PVR" as used herein includes human PVR (hPVR), variants, isoforms, and species homologs of hPVR, and analogs having at least one common epitope with hPVR. The term "PVRL2" as used herein includes human PVRL2 (hPVRL2), variants, isoforms, and species homologs of hPVRL2, and analogs having at least one common epitope with hPVRL2. The term "PVRL3" as used herein includes human PVRL3 (hPVRL3), variants, isoforms, and species homologs of hPVRL3, and analogs having at least one common epitope with hPVRL3.

The "B7 family" refers to inhibitory ligands with undefined receptors. The B7 family encompasses B7-H3 and B7-H4, both upregulated on tumor cells and tumor infiltrating cells. The terms "B7-H3" and "B7-H4" as used herein include human B7-H3 (hB7-H3) and human B7-H4 (hB7-H4), variants, isoforms, and species homologs thereof, and analogs having at least one common epitope with B7-H3 and B7-H4, respectively.

"B and T Lymphocyte Attenuator" (BTLA, also known as CD272) is a TNFR family member expressed in Th1 but not Th2 cells. BTLA expression is induced during activation of T cells and is in particular expressed on surfaces of CD8+ T cells. The term "BTLA" as used herein includes human BTLA (hBTLA), variants, isoforms, and species homologs of hBTLA, and analogs having at least one common epitope with hBTLA. BTLA expression is gradually downregulated during differentiation of human CD8+ T cells to effector cell phenotype. Tumor-specific human CD8+ T cells express high levels of BTLA. BTLA binds to "Herpesvirus entry mediator" (HVEM, also known as TNFRSF14 or CD270) and is involved in T cell inhibition. The term "HVEM" as used herein includes human HVEM (hHVEM), variants, isoforms, and species homologs of hHVEM, and analogs having at least one common epitope with hHVEM. BTLA-HVEM complexes negatively regulate T cell immune responses.

"Killer-cell Immunoglobulin-like Receptors" (KIRs) are receptors for MHC Class I molecules on NK T cells and NK cells that are involved in differentiation between healthy and diseased cells. KIRs bind to human leukocyte antigen (HLA) A, B and C, what suppresses normal immune cell activation. The term "KIRs" as used herein includes human KIRs (hKIRs), variants, isoforms, and species homologs of hKIRs, and analogs having at least one common epitope with a hKIR. The term "HLA" as used herein includes variants, isoforms, and species homologs of HLA, and analogs having at least one common epitope with a HLA. KIR as used herein in particular refers to KIR2DL1, KIR2DL2, and/or KIR2DL3.

"Lymphocyte Activation Gene-3 (LAG-3)" (also known as CD223) is an inhibitory receptor associated with inhibition of lymphocyte activity by binding to MHC class II molecules. This receptor enhances the function of Treg cells and inhibits CD8+ effector T cell function leading to immune response suppression. LAG-3 is expressed on activated T cells, NK cells, B cells and DCs. The term "LAG-3" as used herein includes human LAG-3 (hLAG-3), variants, isoforms, and species homologs of hLAG-3, and analogs having at least one common epitope.

"T Cell Membrane Protein-3 (TIM-3)" (also known as HAVcr-2) is an inhibitory receptor involved in the inhibition of lymphocyte activity by inhibition of Th1 cell responses. Its ligand is galectin 9 (GAL9), which is upregulated in various types of cancers. Other TIM-3 ligands include phosphatidyl serine (PtdSer), High Mobility Group Protein 1 (HMGB1) and Carcinoembryonic Antigen Related Cell Adhesion Molecule 1 (CEACAM1). The term "TIM-3" as used herein includes human TIM3 (hTIM-3), variants, isoforms, and species homologs of

hTIM-3, and analogs having at least one common epitope. The term "GAL9" as used herein includes human GAL9 (hGAL9), variants, isoforms, and species homologs of hGAL9, and analogs having at least one common epitope. The term "PdtSer" as used herein includes variants and analogs having at least one common epitope. The term "HMGB1" as used herein includes human HMGB1 (hHMGB1), variants, isoforms, and species homologs of hHMGB1, and analogs having at least one common epitope. The term "CEACAM1" as used herein includes human CEACAM1 (hCEACAM1), variants, isoforms, and species homologs of hCEACAM1, and analogs having at least one common epitope.

"CD94/NKG2A" is an inhibitory receptor predominantly expressed on the surface of natural killer cells and of CD8+ T cells. The term "CD94/NKG2A" as used herein includes human CD94/NKG2A (hCD94/NKG2A), variants, isoforms, and species homologs of hCD94/NKG2A, and analogs having at least one common epitope. The CD94/NKG2A receptor is a heterodimer comprising CD94 and NKG2A. It suppresses NK cell activation and CD8+ T cell function, probably by binding to ligands such as HLA-E. CD94/NKG2A restricts cytokine release and cytotoxic response of natural killer cells (NK cells), Natural Killer T cells (NK-T cells) and T cells (α/β and γ/δ). NKG2A is frequently expressed in tumor infiltrating cells and HLA-E is overexpressed in several cancers.

"Indoleamine 2,3-dioxygenase" (IDO) is a tryptophan catabolic enzyme with immune-inhibitory properties. The term "IDO" as used herein includes human IDO (hIDO), variants, isoforms, and species homologs of hIDO, and analogs having at least one common epitope. IDO is the rate limiting enzyme in tryptophan degradation catalyzing its conversion to kynurenine. Therefore, IDO is involved in depletion of essential amino acids. It is known to be involved in suppression of T and NK cells, generation and activation of Tregs and myeloid-derived suppressor cells, and promotion of tumor angiogenesis. IDO is overexpressed in many cancers and was shown to promote immune system escape of tumor cells and to facilitate chronic tumor progression when induced by local inflammation.

In the "adenosinergic pathway" or "adenosine signaling pathway" as used herein ATP is converted to adenosine by the ectonucleotidases CD39 and CD73 resulting in inhibitory signaling through adenosine binding by one or more of the inhibitory adenosine receptors "Adenosine A2A Receptor" (A2AR, also known as ADORA2A) and "Adenosine A2B Receptor" (A2BR, also known as ADORA2B). Adenosine is a nucleoside with immunosuppressive

properties and is present in high concentrations in the tumor microenvironment restricting immune cell infiltration, cytotoxicity and cytokine production. Thus, adenosine signaling is a strategy of cancer cells to avoid host immune system clearance. Adenosine signaling through A2AR and A2BR is an important checkpoint in cancer therapy that is activated by high adenosine concentrations typically present in the tumor microenvironment. CD39, CD73, A2AR and A2BR are expressed by most immune cells, including T cells, invariant natural killer cells, B cells, platelets, mast cells and eosinophils. Adenosine signaling through A2AR and A2BR counteracts T cell receptor mediated activation of immune cells and results in increased numbers of Tregs and decreased activation of DCs and effector T cells. The term "CD39" as used herein includes human CD39 (hCD39), variants, isoforms, and species homologs of hCD39, and analogs having at least one common epitope. The term "CD73" as used herein includes human CD73 (hCD73), variants, isoforms, and species homologs of hCD73, and analogs having at least one common epitope. The term "A2AR" as used herein includes human A2AR (hA2AR), variants, isoforms, and species homologs of hA2AR, and analogs having at least one common epitope. The term "A2BR" as used herein includes human A2BR (hA2BR), variants, isoforms, and species homologs of hA2BR, and analogs having at least one common epitope.

"V-domain Ig suppressor of T cell activation" (VISTA, also known as C10orf54) bears homology to PD-L1 but displays a unique expression pattern restricted to the hematopoietic compartment. The term "VISTA" as used herein includes human VISTA (hVISTA), variants, isoforms, and species homologs of hVISTA, and analogs having at least one common epitope. VISTA induces T cell suppression and is expressed by leukocytes within tumors.

The "Sialic acid binding immunoglobulin type lectin" (Siglec) family members recognize sialic acids and are involved in distinction between "self" and "non-self". The term "Siglecs" as used herein includes human Siglecs (hSiglecs), variants, isoforms, and species homologs of hSiglecs, and analogs having at least one common epitope with one or more hSiglecs. The human genome contains 14 Siglecs of which several are involved in immunosuppression, including, without limitation, Siglec-2, Siglec-3, Siglec-7 and Siglec-9. Siglec receptors bind glycans containing sialic acid, but differ in their recognition of the linkage regiochemistry and spatial distribution of sialic residues. The members of the family also have distinct expression patterns. A broad range of malignancies overexpress one or more Siglecs.

"CD20" is an antigen expressed on the surface of B and T cells. High expression of CD20 can be found in cancers, such as B cell lymphomas, hairy cell leukemia, B cell chronic lymphocytic leukemia, and melanoma cancer stem cells. The term "CD20" as used herein includes human CD20 (hCD20), variants, isoforms, and species homologs of hCD20, and analogs having at least one common epitope.

"Glycoprotein A repetitions predominant" (GARP) plays a role in immune tolerance and the ability of tumors to escape the patient's immune system. The term "GARP" as used herein includes human GARP (hGARP), variants, isoforms, and species homologs of hGARP, and analogs having at least one common epitope. GARP is expressed on lymphocytes including Treg cells in peripheral blood and tumor infiltrating T cells at tumor sites. It probably binds to latent "transforming growth factor β" (TGF-β). Disruption of GARP signaling in Tregs results in decreased tolerance and inhibits migration of Tregs to the gut and increased proliferation of cytotoxic T cells.

"CD47" is a transmembrane protein that binds to the ligand "signal-regulatory protein alpha" (SIRPα). The term "CD47" as used herein includes human CD47 (hCD47), variants, isoforms, and species homologs of hCD47, and analogs having at least one common epitope with hCD47. The term "SIRPα" as used herein includes human SIRPα (hSIRPα), variants, isoforms, and species homologs of hSIRPα, and analogs having at least one common epitope with hSIRPα. CD47 signaling is involved in a range of cellular processes including apoptosis, proliferation, adhesion and migration. CD47 is overexpressed in many cancers and functions as "don't eat me" signal to macrophages. Blocking CD47 signaling through inhibitory anti-CD47 or anti-SIRPα antibodies enables macrophage phagocytosis of cancer cells and fosters the activation of cancer-specific T lymphocytes.

"Poliovirus receptor related immunoglobulin domain containing" (PVRIG, also known as CD112R) binds to "Poliovirus receptor-related 2" (PVRL2). PVRIG and PVRL2 are overexpressed in a number of cancers. PVRIG expression also induces TIGIT and PD-1 expression and PVRL2 and PVR (a TIGIT ligand) are co-overexpressed in several cancers. Blockade of the PVRIG signaling pathway results in increased T cell function and CD8+ T cell responses and, therefore, reduced immune suppression and elevated interferon responses. The term "PVRIG" as used herein includes human PVRIG (hPVRIG), variants, isoforms, and species

homologs of hPVRIG, and analogs having at least one common epitope with hPVRIG. "PVRL2" as used herein includes hPVRL2, as defined above.

The "colony-stimulating factor 1" pathway is another checkpoint that can be targeted according to the disclosure. CSF1R is a myeloid growth factor receptor that binds CSF1. Blockade of the CSF1R signaling can functionally reprogram macrophage responses, thereby enhancing antigen presentation and anti-tumor T cell responses. The term "CSF1R" as used herein includes human CSF1R (hCSF1R), variants, isoforms, and species homologs of hCSF1R, and analogs having at least one common epitope with hCSF1R. The term "CSF1" as used herein includes human CSF1 (hCSF1), variants, isoforms, and species homologs of hCSF1, and analogs having at least one common epitope with hCSF1.

"Nicotinamide adenine dinucleotide phosphate NADPH oxidase" refers to an enzyme of the NOX family of enzymes of myeloid cells that generate immunosuppressive reactive oxygen species (ROS). Five NOX enzymes (NOX1 to NOX5) have been found to be involved in cancer development and immunosuppression. Elevated ROS levels have been detected in almost all cancers and promote many aspects of tumor development and progression. NOX produced ROS dampens NK and T cell functions and inhibition of NOX in myeloid cells improves anti-tumor functions of adjacent NK cells and T cells. The term "NOX" as used herein includes human NOX (hNOX), variants, isoforms, and species homologs of hNOX, and analogs having at least one common epitope with hNOX.

Another immune checkpoint that can be targeted according to the disclosure is the signal mediated by "tryptophan-2,3-dioxygenase" (TDO). TDO represents an alternative route to IDO in tryptophan degradation and is involved in immune suppression. Since tumor cells may catabolize tryptophan via TDO instead of IDO, TDO may represent an additional target for checkpoint blockade. Indeed, several cancer cell lines have been found to upregulate TDO and TDO may complement IDO inhibition. The term "TDO" as used herein includes human TDO (hTDO), variants, isoforms, and species homologs of hTDO, and analogs having at least one common epitope with hTDO.

Many of the immune checkpoints are regulated by interactions between specific receptor and ligand pairs, such as those described above. Thus, immune checkpoint proteins mediate immune checkpoint signaling. For example, checkpoint proteins directly or indirectly regulate T cell

activation, T cell proliferation and/or T cell function. Cancer cells often exploit these checkpoint pathways to protect themselves from being attacked by the immune system. Hence, the function of checkpoint proteins, which is modulated according to the present disclosure is typically the regulation of T cell activation, T cell proliferation and/or T cell function. Immune checkpoint proteins thus regulate and maintain self-tolerance and the duration and amplitude of physiological immune responses. Many of the immune checkpoint proteins belong to the B7:CD28 family or to the tumor necrosis factor receptor (TNFR) super family and, by binding to specific ligands, activate signaling molecules that are recruited to the cytoplasmic domain (Suzuki et al., 2016, Jap J Clin Onc, 46:191-203).

As used herein, the term "immune checkpoint modulator" or "checkpoint modulator" refers to a molecule or to a compound that modulates the function of one or more checkpoint proteins. Immune checkpoint modulators are typically able to modulate self-tolerance and/or the amplitude and/or the duration of the immune response. Preferably, the immune checkpoint modulator used according to the present disclosure modulates the function of one or more human checkpoint proteins and is, thus, a "human checkpoint modulator". In a preferred embodiment, the human checkpoint modulator as used herein is an immune checkpoint inhibitor.

As used herein, "immune checkpoint inhibitor" or "checkpoint inhibitor" refers to a molecule that totally or partially reduces, inhibits, interferes with or negatively modulates one or more checkpoint proteins or that totally or partially reduces, inhibits, interferes with or negatively modulates expression of one or more checkpoint proteins. In certain embodiments, the immune checkpoint inhibitor binds to one or more checkpoint proteins. In certain embodiments, the immune checkpoint inhibitor binds to one or more molecules regulating checkpoint proteins. In certain embodiments, the immune checkpoint inhibitor binds to precursors of one or more checkpoint proteins e.g., on DNA- or RNA-level. Any agent that functions as a checkpoint inhibitor according to the present disclosure can be used.

The term "partially" as used herein means at least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98% or 99% in the level, e.g., in the level of inhibition of a checkpoint protein.

In certain embodiments, the immune checkpoint inhibitor suitable for use in the methods disclosed herein, is an antagonist of inhibitory signals, e.g., an antibody which targets, for

example, PD-1, PD-L1, CTLA-4, LAG-3, B7-H3, B7-H4, or TIM-3. These ligands and receptors are reviewed in Pardoll, D., Nature. 12: 252-264, 2012. Further immune checkpoint proteins that can be targeted according the disclosure are described herein.

In certain embodiments, the immune checkpoint inhibitor prevents inhibitory signals associated with the immune checkpoint. In certain embodiments, the immune checkpoint inhibitor is an antibody, or fragment thereof that disrupts inhibitory signaling associated with the immune checkpoint. In certain embodiments, the immune checkpoint inhibitor is a small molecule inhibitor that disrupts inhibitory signaling. In certain embodiments, the immune checkpoint inhibitor is a peptide-based inhibitor that disrupts inhibitory signaling. In certain embodiments, the immune checkpoint inhibitor is an inhibitory nucleic acid molecule that disrupts inhibitory signaling.

In certain embodiments, the immune checkpoint inhibitor is an antibody, fragment thereof, or antibody mimic, that prevents the interaction between checkpoint blocker proteins, e.g., an antibody, or fragment thereof that prevents the interaction between PD-1 and PD-L1 or PD-L2. In certain embodiments, the immune checkpoint inhibitor is an antibody, fragment thereof, or antibody mimic, that prevents the interaction between CTLA-4 and CD80 or CD86. In certain embodiments, the immune checkpoint inhibitor is an antibody, fragment thereof, or antibody mimic, that prevents the interaction between LAG-3 and its ligands, or TIM-3 and its ligands. In certain embodiments, the immune checkpoint inhibitor prevents inhibitory signaling through CD39 and/or CD73 and/or the interaction of A2AR and/or A2BR with adenosine. In certain embodiments, the immune checkpoint inhibitor prevents interaction of B7-H3 with its receptor and/or of B7-H4 with its receptor. In certain embodiments, the immune checkpoint inhibitor prevents the interaction of BTLA with its ligand HVEM. In certain embodiments, the immune checkpoint inhibitor prevents the interaction of one or more KIRs with their respective ligands. In certain embodiments, the immune checkpoint inhibitor prevents the interaction of LAG-3 with one or more of its ligands. In certain embodiments, the immune checkpoint inhibitor prevents the interaction of TIM-3 with one or more of its ligands Galectin-9, PtdSer, HMGB1 and CEACAM1. In certain embodiments, the immune checkpoint inhibitor prevents the interaction of TIGIT with one or more of its ligands PVR, PVRL2 and PVRL3. In certain embodiments, the immune checkpoint inhibitor prevents the interaction of CD94/NKG2A with HLA-E. In certain embodiments, the immune checkpoint inhibitor prevents the interaction of VISTA with one or more of its binding partners. In certain embodiments, the immune checkpoint inhibitor prevents

the interaction of one or more Siglecs and their respective ligands. In certain embodiments, the immune checkpoint inhibitor prevents CD20 signaling. In certain embodiments, the immune checkpoint inhibitor prevents the interaction of GARP with one or more of its ligands. In certain embodiments, the immune checkpoint inhibitor prevents the interaction of CD47 with SIRPα. In certain embodiments, the immune checkpoint inhibitor prevents the interaction of PVRIG with PVRL2. In certain embodiments, the immune checkpoint inhibitor prevents the interaction of CSF1R with CSF1. In certain embodiments, the immune checkpoint inhibitor prevents NOX signaling. In certain embodiments, the immune checkpoint inhibitor prevents IDO and/or TDO signaling.

Inhibiting or blocking of inhibitory immune checkpoint signaling, as described herein, results in preventing or reversing immune-suppression and establishment or enhancement of T cell immunity against cancer cells. In one embodiment, inhibition of immune checkpoint signaling, as described herein, reduces or inhibits dysfunction of the immune system. In one embodiment, inhibition of immune checkpoint signaling, as described herein, renders dysfunctional immune cells less dysfunctional. In one embodiment, inhibition of immune checkpoint signaling, as described herein, renders a dysfunctional T cell less dysfunctional.

The term "dysfunction", as used herein, refers to a state of reduced immune responsiveness to antigenic stimulation. The term includes the common elements of both exhaustion and/or anergy in which antigen recognition may occur, but the ensuing immune response is ineffective to control infection or tumor growth. Dysfunction also includes a state in which antigen recognition is retarded due to dysfunctional immune cells.

The term "dysfunctional", as used herein, refers to an immune cell that is in a state of reduced immune responsiveness to antigen stimulation. Dysfunctional includes unresponsive to antigen recognition and impaired capacity to translate antigen recognition into downstream T cell effector functions, such as proliferation, cytokine production (e.g., IL-2) and/or target cell killing.

The term "anergy", as used herein, refers to the state of unresponsiveness to antigen stimulation resulting from incomplete or insufficient signals delivered through the T cell receptor (TCR). T cell anergy can also result upon stimulation with antigen in the absence of co-stimulation, resulting in the cell becoming refractory to subsequent activation by the antigen even in the

context of co-stimulation. The unresponsive state can often be overridden by the presence of IL-2. Anergic T cells do not undergo clonal expansion and/or acquire effector functions.

The term "exhaustion", as used herein, refers to immune cell exhaustion, such as T cell exhaustion as a state of T cell dysfunction that arises from sustained TCR signaling that occurs during many chronic infections and cancer. It is distinguished from anergy in that it arises not through incomplete or deficient signaling, but from sustained signaling. Exhaustion is defined by poor effector function, sustained expression of inhibitory receptors and a transcriptional state distinct from that of functional effector or memory T cells. Exhaustion prevents optimal control of diseases (e.g., infection and tumors). Exhaustion can result from both extrinsic negative regulatory pathways (e.g., immunoregulatory cytokines) as well as cell intrinsic negative regulatory pathways (inhibitory immune checkpoint pathways, such as described herein).

"Enhancing T cell function" means to induce, cause or stimulate a T cell to have a sustained or amplified biological function, or renew or reactivate exhausted or inactive T cells. Examples of enhancing T cell function include increased secretion of γ-interferon from CD8+ T cells, increased proliferation, increased antigen responsiveness (e.g., tumor clearance) relative to such levels before the intervention. In one embodiment, the level of enhancement is as least 5%, 10%, 15%, 20%, 25%, 30%, 35%, 40%, 45%, 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 100%, 110%, 120%, 130%, 140%, 150%, 200%, or more. Manners of measuring this enhancement are known to one of ordinary skill in the art.

The immune checkpoint inhibitor may be an inhibitory nucleic acid molecule. The term "inhibitory nucleic acid" or "inhibitory nucleic acid molecule" as used herein refers to a nucleic acid molecule, e.g., DNA or RNA, that totally or partially reduces, inhibits, interferes with or negatively modulates one or more checkpoint proteins. Inhibitory nucleic acid molecules include, without limitation, oligonucleotides, siRNA, shRNA, antisense DNA or RNA molecules, and aptamers (e.g., DNA or RNA aptamers).

The term "oligonucleotide" as used herein refers to a nucleic acid molecule that is able to decrease protein expression, in particular expression of a checkpoint protein, such as the checkpoint proteins described herein. Oligonucleotides are short DNA or RNA molecules, typically comprising from 2 to 50 nucleotides. Oligonucleotides maybe single-stranded or double-stranded. A checkpoint inhibitor oligonucleotide may be an antisense-oligonucleotide.

Antisense-oligonucleotides are single-stranded DNA or RNA molecules that are complementary to a given sequence, in particular to a sequence of the nucleic acid sequence (or a fragment thereof) of a checkpoint protein. Antisense RNA is typically used to prevent protein translation of mRNA, e.g., of mRNA encoding a checkpoint protein, by binding to said mRNA. Antisense DNA is typically used to target a specific, complementary (coding or non-coding) RNA. If binding takes place, such a DNA/RNA hybrid can be degraded by the enzyme RNase H. Moreover, morpholino antisense oligonucleotides can be used for gene knockdowns in vertebrates. For example, Kryczek et al., 2006 (J Exp Med, 203:871-81) designed B7-H4-specific morpholinos that specifically blocked B7-H4 expression in macrophages, resulting in increased T cell proliferation and reduced tumor volumes in mice with tumor associated antigen (TAA)-specific T cells.

The terms "siRNA" or "small interfering RNA" or "small inhibitory RNA" are used interchangeably herein and refer to a double-stranded RNA molecule with a typical length of 20-25 base pairs that interferes with expression of a specific gene, such as a gene coding for a checkpoint protein, with a complementary nucleotide sequence. In one embodiment, siRNA interferes with mRNA therefore blocking translation, e.g., translation of an immune checkpoint protein. Transfection of exogenous siRNA may be used for gene knockdown, however, the effect maybe only transient, especially in rapidly dividing cells. Stable transfection may be achieved, e.g., by RNA modification or by using an expression vector. Useful modifications and vectors for stable transfection of cells with siRNA are known in the art. siRNA sequences may also be modified to introduce a short loop between the two strands resulting in a "small hairpin RNA" or "shRNA". shRNA can be processed into a functional siRNA by Dicer. shRNA has a relatively low rate of degradation and turnover. Accordingly, the immune checkpoint inhibitor may be a shRNA.

The term "aptamer" as used herein refers to a single-stranded nucleic acid molecule, such as DNA or RNA, typically in a length of 25-70 nucleotides that is capable of binding to a target molecule, such as a polypeptide. In one embodiment, the aptamer binds to an immune checkpoint protein such as the immune checkpoint proteins described herein. For example, an aptamer according to the disclosure can specifically bind to an immune checkpoint protein or polypeptide, or to a molecule in a signaling pathway that modulates the expression of an immune checkpoint protein or polypeptide. The generation and therapeutic use of aptamers is well known in the art (see, e.g., US 5,475,096).

The terms "small molecule inhibitor" or "small molecule" are used interchangeably herein and refer to a low molecular weight organic compound, usually up to 1000 daltons, that totally or partially reduces, inhibits, interferes with, or negatively modulates one or more checkpoint proteins as described above. Such small molecular inhibitors are usually synthesized by organic chemistry, but may also be isolated from natural sources, such as plants, fungi, and microbes. The small molecular weight allows a small molecule inhibitor to rapidly diffuse across cell membranes. For example, various A2AR antagonists known in the art are organic compounds having a molecular weight below 500 daltons.

The immune checkpoint inhibitor may be an antibody, an antigen-binding fragment thereof, an antibody mimic or a fusion protein comprising an antibody portion with an antigen-binding fragment of the required specificity. Antibodies or antigen-binding fragments thereof are as described herein. Antibodies or antigen-binding fragments thereof that are immune checkpoint inhibitors include in particular antibodies or antigen-binding fragments thereof that bind to immune checkpoint proteins, such as immune checkpoint receptors or immune checkpoint receptor ligands. Antibodies or antigen-binding fragments may also be conjugated to further moieties, as described herein. In particular, antibodies or antigen-binding fragments thereof are chimerized, humanized or human antibodies. Preferably, immune checkpoint inhibitor antibodies or antigen-binding fragments thereof are antagonists of immune checkpoint receptors or of immune checkpoint receptor ligands.

In a preferred embodiment, an antibody that is an immune checkpoint inhibitor, is an isolated antibody.

The antibody that is an immune checkpoint inhibitor or the antigen-binding fragment thereof according to the present disclosure may also be an antibody that cross-competes for antigen binding with any known immune checkpoint inhibitor antibody. In certain embodiments, an immune checkpoint inhibitor antibody cross-competes with one or more of the immune checkpoint inhibitor antibodies described herein. The ability of antibodies to cross-compete for binding to an antigen indicates that these antibodies may bind to the same epitope region of the antigen or when binding to another epitope sterically hinder the binding of known immune checkpoint inhibitor antibodies to that particular epitope region. These cross-competing antibodies may have functional properties very similar to those they are cross-competing with as

they are expected to block binding of the immune checkpoint to its ligand either by binding to the same epitope or by sterically hindering the binding of the ligand. Cross-competing antibodies can be readily identified based on their ability to cross-compete with one or more of known antibodies in standard binding assays such as Surface Plasmon Resoncance analysis, ELISA assays or flow cytometry (see, e.g., WO 2013/173223).

In certain embodiments, antibodies or antigen binding fragments thereof that cross-compete for binding to a given antigen with, or bind to the same epitope region of a given antigen as, one or more known antibodies are monoclonal antibodies. For administration to human patients, these cross-competing antibodies can be chimeric antibodies, or humanized or human antibodies. Such chimeric, humanized or human monoclonal antibodies can be prepared and isolated by methods well known in the art.

The checkpoint inhibitor may also be in the form of the soluble form of the molecules (or variants thereof) themselves, e.g., a soluble PD-L1 or PD-L1 fusion.

In the context of the disclosure, more than one checkpoint inhibitor can be used, wherein the more than one checkpoint inhibitors are targeting distinct checkpoint pathways or the same checkpoint pathway. Preferably, the more than one checkpoint inhibitors are distinct checkpoint inhibitors. Preferably, if more than one distinct checkpoint inhibitor is used, in particular at least 2, 3, 4, 5, 6, 7, 8, 9 or 10 distinct checkpoint inhibitors are used, preferably 2, 3, 4 or 5 distinct checkpoint inhibitors are used, more preferably 2, 3 or 4 distinct checkpoint inhibitors are used, even more preferably 2 or 3 distinct checkpoint inhibitors are used and most preferably 2 distinct checkpoint inhibitors are used. Preferred examples of combinations of distinct checkpoint inhibitors include combination of an inhibitor of PD-1 signaling and an inhibitor of CTLA-4 signaling, an inhibitor of PD-1 signaling and an inhibitor of TIGIT signaling, an inhibitor of PD-1 signaling and an inhibitor of B7-H3 and/or B7-H4 signaling, an inhibitor of PD-1 signaling and an inhibitor of BTLA signaling, an inhibitor of PD-1 signaling and an inhibitor of KIR signaling, an inhibitor of PD-1 signaling and an inhibitor of LAG-3 signaling, an inhibitor of PD-1 signaling and an inhibitor of TIM-3 signaling, an inhibitor of PD-1 signaling and an inhibitor of CD94/NKG2A signaling, an inhibitor of PD-1 signaling and an inhibitor of IDO signaling, an inhibitor of PD-1 signaling and an inhibitor of adenosine signaling, an inhibitor of PD-1 signaling and an inhibitor of VISTA signaling, an inhibitor of PD-1 signaling and an inhibitor of Siglec signaling, an inhibitor of PD-1 signaling and an inhibitor of CD20 signaling, an inhibitor

of PD-1 signaling and an inhibitor of GARP signaling, an inhibitor of PD-1 signaling and an inhibitor of CD47 signaling, an inhibitor of PD-1 signaling and an inhibitor of PVRIG signaling, an inhibitor of PD-1 signaling and an inhibitor of CSF1R signaling, an inhibitor of PD-1 signaling and an inhibitor of NOX signaling, and an inhibitor of PD-1 signaling and an inhibitor of TDO signaling.

In certain embodiments, the inhibitory immunoregulator (immune checkpoint blocker) is a component of the PD-1/PD-L1or PD-1/PD-L2 signaling pathway. Accordingly, certain embodiments of the disclosure provide for administering to a subject a checkpoint inhibitor of the PD-1 signaling pathway. In certain embodiments, the checkpoint inhibitor of the PD-1 signaling pathway is a PD-1 inhibitor. In certain embodiments, the checkpoint inhibitor of the PD-1 signaling pathway is a PD-1 ligand inhibitor, such as a PD-L1 inhibitor or a PD-L2 inhibitor. In a preferred embodiment, the checkpoint inhibitor of the PD-1 signaling pathway is an antibody or an antigen-binding portion thereof that disrupts the interaction between the PD-1 receptor and one or more of its ligands, PD-L1 and/or PD-L2. Antibodies which bind to PD-1 and disrupt the interaction between PD-1 and one or more of its ligands are known in the art. In certain embodiments, the antibody or antigen-binding portion thereof binds specifically to PD-1. In certain embodiments, the antibody or antigen-binding portion thereof binds specifically to PD-L1 and inhibits its interaction with PD-1, thereby increasing immune activity. In certain embodiments, the antibody or antigen-binding portion thereof binds specifically to PD-L2 and inhibits its interaction with PD-1, thereby increasing immune activity.

In certain embodiments, the inhibitory immunoregulator is a component of the CTLA-4 signaling pathway. Accordingly, certain embodiments of the disclosure provide for administering to a subject a checkpoint inhibitor of the CTLA-4 signaling pathway. In certain embodiments, the checkpoint inhibitor of the CTLA-4 signaling pathway is a CTLA-4 inhibitor. In certain embodiments, the checkpoint inhibitor of the CTLA-4 signaling pathway is a CTLA-4 ligand inhibitor.

In certain embodiments, the inhibitory immunoregulator is a component of the TIGIT signaling pathway. Accordingly, certain embodiments of the disclosure provide for administering to a subject a checkpoint inhibitor of the TIGIT signaling pathway. In certain embodiments, the checkpoint inhibitor of the TIGIT signaling pathway is a TIGIT inhibitor. In certain

embodiments, the checkpoint inhibitor of the TIGIT signaling pathway is a TIGIT ligand inhibitor.

In certain embodiments, the inhibitory immunoregulator is a component of the B7 family signaling pathway. In certain embodiments, the B7 family members are B7-H3 and B7-H4. Certain embodiments of the disclosure provide for administering to a subject a checkpoint inhibitor of B7-H3 and/or B7-4. Accordingly, certain embodiments of the disclosure provide for administering to a subject an antibody or an antigen-binding portion thereof that targets B7-H3 or B7-H4. The B7 family does not have any defined receptors but these ligands are upregulated on tumor cells or tumor-infiltrating cells. Preclinical mouse models have shown that blockade of these ligands can enhance anti-tumor immunity.

In certain embodiments, the inhibitory immunoregulator is a component of the BTLA signaling pathway. Accordingly, certain embodiments of the disclosure provide for administering to a subject a checkpoint inhibitor of the BTLA signaling pathway. In certain embodiments, the checkpoint inhibitor of the BTLA signaling pathway is a BTLA inhibitor. In certain embodiments, the checkpoint inhibitor of the BTLA signaling pathway is a HVEM inhibitor.

In certain embodiments, the inhibitory immunoregulator is a component of one or more KIR signaling pathways. Accordingly, certain embodiments of the disclosure provide for administering to a subject a checkpoint inhibitor of one or more KIR signaling pathways. In certain embodiments, the checkpoint inhibitor of one or more KIR signaling pathways is a KIR inhibitor. In certain embodiments, the checkpoint inhibitor one or more KIR signaling pathways is a KIR ligand inhibitor. For example, the KIR inhibitor according to the present disclosure may be an anti-KIR antibody that binds to KIR2DL1, KIR2DL2, and/or KIR2DL3.

In certain embodiments, the inhibitory immunoregulator is a component of the LAG-3 signaling pathway. Accordingly, certain embodiments of the disclosure provide for administering to a subject a checkpoint inhibitor of LAG-3 signaling. In certain embodiments, the checkpoint inhibitor of the LAG-3 signaling pathway is a LAG-3 inhibitor. In certain embodiments, the checkpoint inhibitor of the LAG-3 signaling pathway is a LAG-3 ligand inhibitor.

In certain embodiments, the inhibitory immunoregulator is a component of the TIM-3 signaling pathway. Accordingly, certain embodiments of the disclosure provide for administering to a

subject a checkpoint inhibitor of the TIM-3 signaling pathway. In certain embodiments, the checkpoint inhibitor of the TIM-3 signaling pathway is a TIM-3 inhibitor. In certain embodiments, the checkpoint inhibitor of the TIM-3 signaling pathway is a TIM-3 ligand inhibitor.

In certain embodiments, the inhibitory immunoregulator is a component of the CD94/NKG2A signaling pathway. Accordingly, certain embodiments of the disclosure provide for administering to a subject a checkpoint inhibitor of the CD94/NKG2A signaling pathway. In certain embodiments, the checkpoint inhibitor of the CD94/NKG2A signaling pathway is a CD94/NKG2A inhibitor. In certain embodiments, the checkpoint inhibitor of the CD94/NKG2A signaling pathway is a CD94/NKG2A ligand inhibitor.

In certain embodiments, the inhibitory immunoregulator is a component of the IDO signaling pathway. Accordingly, certain embodiments of the disclosure provide for administering to a subject a checkpoint inhibitor of the IDO signaling pathway, e.g., an IDO inhibitor.

In certain embodiments, the inhibitory immunoregulator is a component of the adenosine signaling pathway. Accordingly, certain embodiments of the disclosure provide for administering to a subject a checkpoint inhibitor of the adenosine signaling pathway. In certain embodiments, the checkpoint inhibitor of the adenosine signaling pathway is a CD39 inhibitor. In certain embodiments, the checkpoint inhibitor of the adenosine signaling pathway is a CD73 inhibitor. In certain embodiments, the checkpoint inhibitor of the adenosine signaling pathway is an A2AR inhibitor. In certain embodiments, the checkpoint inhibitor of the adenosine signaling pathway is an A2BR inhibitor.

In certain embodiments, the inhibitory immunoregulator is a component of the VISTA signaling pathway. Accordingly, certain embodiments of the disclosure provide for administering to a subject a checkpoint inhibitor of the VISTA signaling pathway. In certain embodiments, the checkpoint inhibitor of the VISTA signaling pathway is a VISTA inhibitor.

In certain embodiments, the inhibitory immunoregulator is a component of one or more Siglec signaling pathways. Accordingly, certain embodiments of the disclosure provide for administering to a subject a checkpoint inhibitor of one or more Siglec signaling pathways. In certain embodiments, the checkpoint inhibitor of one or more Siglec signaling pathways is a

Siglec inhibitor. In certain embodiments, the checkpoint inhibitor of one or more Siglec signaling pathways is a Siglec ligand inhibitor.

In certain embodiments, the inhibitory immunoregulator is a component of the CD20 signaling pathway. Accordingly, certain embodiments of the disclosure provide for administering to a subject a checkpoint inhibitor of the CD20 signaling pathway. In certain embodiments, the checkpoint inhibitor of the CD20 signaling pathway is a CD20 inhibitor.

In certain embodiments, the inhibitory immunoregulator is a component of the GARP signaling pathway. Accordingly, certain embodiments of the disclosure provide for administering to a subject a checkpoint inhibitor of the GARP signaling pathway. In certain embodiments, the checkpoint inhibitor of the GARP signaling pathway is a GARP inhibitor.

In certain embodiments, the inhibitory immunoregulator is a component of the CD47 signaling pathway. Accordingly, certain embodiments of the disclosure provide for administering to a subject a checkpoint inhibitor of the CD47 signaling pathway. In certain embodiments, the checkpoint inhibitor of the CD47 signaling pathway is a CD47 inhibitor. In certain embodiments, the checkpoint inhibitor of the CD47 signaling pathway is a SIRPα inhibitor.

In certain embodiments, the inhibitory immunoregulator is a component of the PVRIG signaling pathway. Accordingly, certain embodiments of the disclosure provide for administering to a subject a checkpoint inhibitor of the PVRIG signaling pathway. In certain embodiments, the checkpoint inhibitor of the PVRIG signaling pathway is a PVRIG inhibitor. In certain embodiments, the checkpoint inhibitor of the PVRIG signaling pathway is a PVRIG ligand inhibitor.

In certain embodiments, the inhibitory immunoregulator is a component of the CSF1R signaling pathway. Accordingly, certain embodiments of the disclosure provide for administering to a subject a checkpoint inhibitor of the CSF1R signaling pathway. In certain embodiments, the checkpoint inhibitor of the CSF1R signaling pathway is a CSF1R inhibitor. In certain embodiments, the checkpoint inhibitor of the CSF1R signaling pathway is a CSF1 inhibitor.

In certain embodiments, the inhibitory immunoregulator is a component of the NOX signaling pathway. Accordingly, certain embodiments of the disclosure provide for administering to a subject a checkpoint inhibitor of the NOX signaling pathway, e.g., a NOX inhibitor.

In certain embodiments, the inhibitory immunoregulator is a component of the TDO signaling pathway. Accordingly, certain embodiments of the disclosure provide for administering to a subject a checkpoint inhibitor of the TDO signaling pathway, e.g., a TDO inhibitor.

Exemplary PD-1 inhibitors include, without limitation, anti-PD-1 antibodies such as BGB-A317 (BeiGene; see US 8,735,553, WO 2015/35606 and US 2015/0079109), cemiplimab (Regeneron; see WO 2015/112800) and lambrolizumab (e.g., disclosed as hPD109A and its humanized derivatives h409A1, h409A16 and h409A17 in WO2008/156712), AB137132 (Abcam), EH12.2H7 and RMP1-14 (#BE0146; Bioxcell Lifesciences Pvt. LTD.), MIH4 (Affymetrix eBioscience), nivolumab (OPDIVO, BMS-936558; Bristol Myers Squibb; see WO 2006/121168), pembrolizumab (KEYTRUDA; MK-3475; Merck; see WO 2008/156712), pidilizumab (CT-011; CureTech; see Hardy et al., 1994, Cancer Res., 54(22):5793-6 and WO 2009/101611), PDR001 (Novartis; see WO 2015/112900), MEDI0680 (AMP-514; AstraZeneca; see WO 2012/145493), TSR-042 (see WO 2014/179664), REGN-2810 (H4H7798N; cf. US 2015/0203579), JS001 (TAIZHOU JUNSHI PHARMA; see Si-Yang Liu et al., 2007, J. Hematol. Oncol. 70: 136), AMP-224 (GSK-2661380; cf. Li et al., 2016, Int J Mol Sci 17(7):1151 and WO 2010/027827 and WO 2011/066342), PF-06801591 (Pfizer), BGB-A317 (BeiGene; see WO 2015/35606 and US 2015/0079109), BI 754091, SHR-1210 (see WO2015/085847), and antibodies 17D8, 2D3, 4H1, 4A11, 7D3, and 5F4 as described in WO 2006/121168, INCSHR1210 (Jiangsu Hengrui Medicine; also known as SHR-1210; see WO 2015/085847), TSR-042 (Tesaro Biopharmaceutical; also known as ANB011; see W02014/179664), GLS-010 (Wuxi/Harbin Gloria Pharmaceuticals; also known as WBP3055; see Si-Yang et al., 2017, J. Hematol. Oncol. 70: 136), STI-1110 (Sorrento Therapeutics; see WO 2014/194302), AGEN2034 (Agenus; see WO 2017/040790), MGA012 (Macrogenics; see WO 2017/19846), IBI308 (Innovent; see WO 2017/024465, WO 2017/025016, WO 2017/132825, and WO 2017/133540), anti-PD-1 antibodies as described, e.g., in US 7,488,802, US 8,008,449, US 8,168,757, WO 03/042402, WO 2010/089411 (further disclosing anti-PD-L1 antibodies), WO 2010/036959, WO 2011/159877 (further disclosing antibodies against TIM-3), WO 2011/082400, WO 2011/161699, WO 2009/014708, WO 03/099196, WO 2009/114335, WO 2012/145493 (further disclosing antibodies against PD-L1), WO 2015/035606, WO

2014/055648 (further disclosing anti-KIR antibodies), US 2018/0185482 (further disclosing anti-PD-L1 and anti-TIGIT antibodies), US 8,008,449, US 8,779,105, US 6,808,710, US 8,168,757, US 2016/0272708, and US 8,354,509, small molecule antagonists to the PD-1 signaling pathway as disclosed, e.g., in Shaabani et al., 2018, Expert Op Ther Pat., 28(9):665-678 and Sasikumar and Ramachandra, 2018, BioDrugs, 32(5):481-497, siRNAs directed to PD-1 as disclosed, e.g., in WO 2019/000146 and WO 2018/103501, soluble PD-1 proteins as disclosed in WO 2018/222711 and oncolytic viruses comprising a soluble form of PD-1 as described, e.g., in WO 2018/022831.

In a certain embodiment, the PD-1 inhibitor is nivolumab (OPDIVO; BMS-936558), pembrolizumab (KEYTRUDA; MK-3475), pidilizumab (CT-011), PDR001, MEDI0680 (AMP-514), TSR-042, REGN2810, JS001, AMP-224 (GSK-2661380), PF-06801591, BGB-A317, BI 754091, or SHR-1210.

Exemplary PD-1 ligand inhibitors are PD-L1 inhibitors and PD-L2 inhibitors and include, without limitation, anti-PD-L1 antibodies such as MEDI4736 (durvalumab; AstraZeneca; see WO 2011/066389), MSB-0010718C (see US 2014/0341917), YW243.55.S70 (see SEQ ID NO: 20 of WO 2010/077634 and US 8,217,149), MIH1 (Affymetrix eBioscience; cf. EP 3 230 319), MDX-1105 (Roche/Genentech; see WO2013019906 and US 8,217,149) STI-1014 (Sorrento; see W02013/181634), CK-301 (Checkpoint Therapeutics), KN035 (3D Med/Alphamab; see Zhang et al., 2017, Cell Discov. 3:17004), atezolizumab (TECENTRIQ; RG7446; MPDL3280A; R05541267; see US 9,724,413), BMS-936559 (Bristol Myers Squibb; see US 7,943,743, WO 2013/173223), avelumab (bavencio; cf. US 2014/0341917), LY3300054 (Eli Lilly Co.), CX-072 (Proclaim-CX-072; also called CytomX; see WO2016/149201), FAZ053, KN035 (see WO2017020801 and WO2017020802), MDX-1105 (see US 2015/0320859), anti-PD-L1 antibodies disclosed in US 7,943,743, including 3G10, 12A4 (also referred to as BMS-936559), 10A5, 5F8, 10H10, 1B12, 7H1, 11E6, 12B7, and 13G4, anti-PD-L1 antibodies as described in WO 2010/077634, US 8,217,149, WO 2010/036959, WO 2010/077634, WO 2011/066342, US 8,217,149, US 7,943,743, WO 2010/089411, US 7,635,757, US 8,217,149, US 2009/0317368, WO 2011/066389, WO2017/034916, WO2017/020291, WO2017/020858, WO2017/020801, WO2016/111645, WO2016/197367, WO2016/061142, WO2016/149201, WO2016/000619, WO2016/160792, WO2016/022630, WO2016/007235, WO2015/ 179654, WO2015/173267, WO2015/181342, WO2015/109124, WO 2018/222711, WO2015/112805, WO2015/061668, WO2014/159562, WO2014/165082, WO2014/100079.

Exemplary CTLA-4 inhibitors include, without limitation, the monoclonal antibodies ipilimumab (Yervoy; Bristol Myers Squibb) and tremelimumab (Pfizer/Medlmmune), trevilizumab, AGEN-1884 (Agenus) and ATOR-1015, the anti-CTLA4 antibodies disclosed in WO 2001/014424, US 2005/0201994, EP 1212422, US 5,811,097, US 5,855,887, US 6,051,227, US 6,682,736, US 6,984,720, WO 01/14424, WO 00/37504, US 2002/0039581, US 2002/086014, WO 98/42752, US 6,207,156, US 5,977,318, US 7,109,003, and US 7,132,281, the dominant negative proteins abatacept (Orencia; see EP 2 855 533), which comprises the Fe region of IgG 1 fused to the CTLA-4 ECD, and belatacept (Nulojix; see WO 2014/207748), a second generation higher-affinity CTLA-4-Ig variant with two amino acid substitutions in the CTLA-4 ECD relative to abatacept, soluble CTLA-4 polypeptides, e.g., RG2077 and CTLA4-IgG4m (see US 6,750,334), anti-CTLA-4 aptamers and siRNAs directed to CTLA-4, e.g., as disclosed in US 2015/203848. Exemplary CTLA-4 ligand inhibitors are described in Pile et al., 2015 (Encyclopedia of Inflammatory Diseases, M. Parnham (ed.), doi: 10.1007/978-3-0348-0620-6_20).

Exemplary checkpoint inhibitors of the TIGIT signaling pathway include, without limitation, anti-TIGIT antibodies, such as BMS-986207, COM902 (CGEN-15137; Compugen), AB154 (Arcus Biosciences) or etigilimab (OMP-313M32; OncoMed Pharmaceuticals), or the antibodies disclosed in WO2017/059095, in particular "MAB10", US 2018/0185482, WO 2015/009856, and US 2019/0077864.

Exemplary checkpoint inhibitors of B7-H3 include, without limitation, the Fc-optimized monoclonal antibody enoblituzumab (MGA271; Macrogenics; see US 2012/0294796) and the anti-B7-H3 antibodies MGD009 (Macrogenics) and pidilizumab (see US 7,332,582).

Exemplary B7-H4 inhibitors include, without limitation, antibodies as described in Dangaj et al., 2013 (Cancer Research 73:4820-9) and in Smith et al., 2014 (Gynecol Oncol, 134:181-189), WO 2013/025779 (e.g., 2D1 encoded by SEQ ID NOs: 3 and 4, 2H9 encoded by SEQ ID NO: 37 and 39, and 2E11 encoded by SEQ ID NOs: 41 and 43) and in WO 2013/067492 (e.g., an antibody with an amino acid sequence selected from SEQ ID NOs: 1-8), morpholino antisense oligonucleotides, e.g., as described by Kryczek et al., 2006 (J Exp Med, 203:871-81), or soluble recombinant forms of B7-H4, such as disclosed in US 2012/0177645.

Exemplary BTLA inhibitors include, without limitation, the anti-BTLA antibodies described in Crawford and Wherry, 2009 (J Leukocyte Biol 86:5-8), WO 2011/014438 (e.g., 4C7 or an antibody comprising heavy and light chains according to SEQ ID NOs: 8 and 15 and/or SEQ ID NOs: 11 and 18), WO 2014/183885 (e.g., the antibody deposited under the number CNCM I-4752) and US 2018/155428.

Checkpoint inhibitors of KIR signaling include, without limitation, the monoclonal antibodies lirilumab (1-7F9; IPH2102; see see US 8,709,411), IPH4102 (Innate Pharma; see Marie-Cardine et al., 2014, Cancer 74(21): 6060-70), anti-KIR antibodies as disclosed, e.g., in US 2018/208652, US 2018/117147, US 2015/344576, WO 2005/003168, WO 2005/009465, WO 2006/072625, WO 2006/072626, WO 2007/042573, WO 2008/084106 (e.g., an antibody comprising heavy and light chains according to SEQ ID NOs: 2 and 3), WO 2010/065939, WO 2012/071411, WO 2012/160448 and WO 2014/055648.

LAG-3 inhibitors include, without limitation, the anti-LAG-3 antibodies BMS-986016 (Bristol-Myers Squibb; see WO 2014/008218 and WO 2015/116539), 25F7 (see US2011/0150892), IMP731 (see WO 2008/132601), H5L7BW (cf. WO2014140180), MK-4280 (28G-10; Merck; see WO 2016/028672), REGN3767 (Regneron/Sanofi), BAP050 (see WO 2017/019894), IMP-701 (LAG-525; Novartis) Sym022 (Symphogen), TSR-033 (Tesaro), MGD013 (a bispecific DART antibody targeting LAG-3 and PD-1 developed by MacroGenics), BI754111 (Boehringer Ingelheim), FS118 (a bispecific antibody targeting LAG-3 and PD-1 developed by F-star), GSK2831781 (GSK) and antibodies as disclosed in WO 2009/044273, WO 2008/132601, WO 2015/042246, EP 2 320 940, US 2019/169294, US 2019/169292, WO 2016/028672, WO 2016/126858, WO 2016/200782, WO 2015/200119, WO 2017/220569, WO 2017/087589, WO 2017/219995, WO 2017/019846, WO 2017/106129, WO 2017/062888, WO 2018/071500, WO 2017/087901, US 2017/0260271, WO 2017/198741, WO2017/220555, WO2017/015560, WO2017/025498, WO2017/149143, WO 2018/069500, WO2018/083087, WO2018/034227 WO2014/140180, the LAG-3 antagonistic protein AVA-017 (Avacta), the soluble LAG-3 fusion protein IMP321 (eftilagimod alpha; Immutep; see EP 2 205 257 and Brignone et al., 2007, J. Immunol., 179: 4202-4211), and soluble LAG-3 proteins disclosed in WO 2018/222711.

TIM-3 inhibitors include, without limitation, antibodies targeting TIM-3 such as F38-2E2 (BioLegend), cobolimab (TSR-022; Tesaro), LY3321367 (Eli Lilly), MBG453 (Novartis) and

antibodies as disclosed in, e.g., WO 2013/006490, WO 2018/085469 (e.g., antibodies comprising heavy and light chain sequences encoded by nucleic acid sequences according to SEQ ID NOs: 3 and 4), WO 2018/106588, WO 2018/106529 (e.g., an antibody comprising heavy and light chain sequences according to SEQ ID NOs: 8-11).

TIM-3 ligand inhibitors include, without limitation, CEACAM1 inhibitors such as the anti-CEACAM1 antibody CM10 (cCAM Biotherapeutics; see WO 2013/054331), antibodies disclosed in WO 2015/075725 (e.g., CM-24, 26H7, 5F4, TEC-11, 12-140-4, 4/3/17, COL-4, F36-54, 34B1, YG-C28F2, D14HD11, M8.7.7, D11-AD11, HEA81, B 1. 1, CLB-gran-10, F34-187, T84.1, B6.2, B 1.13, YG-C94G7, 12-140-5, scFv DIATHIS1, TET-2; cCAM Biotherapeutics), antibodies described by Watt et al., 2001 (Blood, 98: 1469-1479) and in WO 2010/12557 and PtdSer inhibitors such as bavituximab (Peregrine).

CD94/NKG2A inhibitors include, without limitation, monalizumab (IPH2201; Innate Pharma) and the antibodies and method for their production as disclosed in US 9,422,368 (e.g., humanized Z199; see EP 2 628 753), EP 3 193 929 and WO2016/032334 (e.g., humanized Z270; see EP 2 628 753).

IDO inhibitors include, without limitation, exiguamine A, epacadostat (INCB024360; InCyte; see US 9,624,185), indoximod (Newlink Genetics; CAS#: 110117-83-4), NLG919 (Newlink Genetics/Genentech; CAS#: 1402836-58-1), GDC-0919 (Newlink Genetics/Genentech; CAS#: 1402836-58-1), F001287 (Flexus Biosciences/BMS; CAS#: 2221034-29-1), KHK2455 (Cheong et al., 2018, Expert Opin Ther Pat. 28(4):317-330), PF-06840003 (see WO 2016/181348), navoximod (RG6078, GDC-0919, NLG919; CAS#: 1402837-78-8), linrodostat (BMS-986205; Bristol-Myers Suibb; CAS#: 1923833-60-6), small molecules such as 1-methyl-tryptophan, pyrrolidine-2,5-dione derivatives (see WO 2015/173764) and the IDO inhibitors disclosed by Sheridan, 2015, Nat Biotechnol 33:321-322.

CD39 inhibitors include, without limitation, A001485 (Arcus Biosciences), PSB 069 (CAS#: 78510-31-3) and the anti-CD39 monoclonal antibody IPH5201 (Innate Pharma; see Perrot et al., 2019, Cell Reports 8:2411-2425.E9).

CD73 inhibitors include, without limitation, anti-CD73 antibodies such as CPI-006 (Corvus Pharmaceuticals), MEDI9447 (MedImmune; see WO2016075099), IPH5301 (Innate Pharma;

see Perrot et al., 2019, Cell Reports 8:2411-2425.E9), the anti-CD73 antibodies described in WO2018/110555, the small molecule inhibitors PBS 12379 (Tocris Bioscience; CAS#: 1802226-78-3), A000830, A001190 and A001421 (Arcus Biosciences; see Becker et al., 2018, Cancer Research 78(13 Supplement):3691-3691, doi: 10.1158/1538-7445.AM2018-3691), CB-708 (Calithera Biosciences) and purine cytotoxic nucleoside analogue-based diphosphonates as described by Allard et al., 2018 (Immunol Rev., 276(1):121-144).

A2AR inhibitors include, without limitation, small molecule inhibitors such as istradefylline (KW-6002; CAS#: 155270-99-8), PBF-509 (Palobiopharma), ciforadenant (CPI-444: Corvus Pharma/Genentech; CAS#: 1202402-40-1), ST1535 ([2butyl-9-methyl-8-(2H-1,2,3-triazol 2-yl)-9H-purin-6-xylamine]; CAS#: 496955-42-1), ST4206 (see Stasi et al., 2015, Europ J Pharm 761:353-361; CAS#: 1246018-36-9), tozadenant (SYN115; CAS#: 870070-55-6), V81444 (see WO 2002/055082), preladenant (SCH420814; Merck; CAS#: 377727-87-2), vipadenant (BIIB014; CAS#: 442908-10-3), ST1535 (CAS#: 496955-42-1), SCH412348 (CAS#: 377727-26-9), SCH442416 (Axon 2283; Axon Medchem; CAS#: 316173-57-6), ZM241385 (4-(2-(7-amino-2-(2-furyl)-(1,2,4)triazolo(2,3-a)-(1,3,5)triazin-5-yl-amino)ethyl)phenol; Cas#: 139180-30-6), AZD4635 (AstraZeneca), AB928 (a dual A2AR/A2BR small molecule inhibitor; Arcus Biosciences) and SCH58261 (see Popoli et al., 2000, Neuropsychopharm 22:522-529; CAS#: 160098-96-4).

A2BR inhibitors include, without limitation, AB928 (a dual A2AR/A2BR small molecule inhibitor; Arcus Biosciences), MRS 1706 (CAS#: 264622-53-9), GS6201 (CAS#: 752222-83-6) and PBS 1115 (CAS#: 152529-79-8).

VISTA inhibitors include, without limitation, anti-VISTA antibodies such as JNJ-61610588 (onvatilimab; Janssen Biotech) and the small molecule inhibitor CA-170 (anti-PD-L1/L2 and anti-VISTA small molecule; CAS#: 1673534-76-3).

Siglec inhibitors include, without limitation, the anti-Sigle-7 antibodies disclosed in US 2019/023786 and WO 2018/027203 (e.g., an antibody comprising a variable heavy chain region according to SEQ ID NO: 1 and a variable light chain region according to SEQ ID NO: 15), the anti-Siglec-2 antibody inotuzumab ozogamicin (Besponsa; see US 8,153,768 and US 9,642,918), the anti-Siglec-3 antibody gemtuzumab ozogamicin (Mylotarg; see US 9,359,442) or the anti-Siglec-9 antibodies disclosed in US 2019/062427, US 2019/023786, WO 2019/011855, WO

2019/011852 (e.g., an antibody comprising the CDRs according to SEQ ID NOs: 171-176, or 3 and 4, or 5 and 6, or 7 and 8, or 9 and 10, or 11 and 12, or 13 and 14, or 15 and 16, or 17 and 18, or 19 and 20, or 21 and 22, or 23 and 24, or 25 and 26), US 2017/306014 and EP 3 146 979.

CD20 inhibitors include, without limitation, anti-CD20 antibodies such as rituximab (RITUXAN; IDEC-102; IDEC-C2B8; see US 5,843,439), ABP 798 (rituximab biosimilar), ofatumumab (2F2; see W02004/035607), obinutuzumab, ocrelizumab (2h7; see WO 2004/056312), ibritumomab tiuxetan (Zevalin), tositumomab, ublituximab (LFB-R603; LFB Biotechnologies) and the antibodies disclosed in US 2018/0036306 (e.g., an antibody comprising light and heavy chains according to SEQ ID NOs: 1-3 and 4-6, or 7 and 8, or 9 and 10).

GARP inhibitors include, without limitation, anti-GARP antibodies such as ARGX-115 (arGEN-X) and the antibodies and methods for their production as disclosed in US 2019/127483, US 2019/016811, US 2018/327511, US 2016/251438, EP 3 253 796.

CD47 inhibitors include, without limitation, anti-CD47 antibodies such as HuF9-G4 (Stanford University/Forty Seven), CC-90002/INBRX-103 (Celgene/Inhibrx), SRF231 (Surface Oncology), IBI188 (Innovent Biologics), AO-176 (Arch Oncology), bispecific antibodies targeting CD47 including TG-1801 (NI-1701; bispecific monoclonal antibody targeting CD47 and CD19; Novimmune/TG Therapeutics) and NI-1801 (bispecific monoclonal antibody targeting CD47 and mesothelin; Novimmune), and CD47 fusion proteins such as ALX148 (ALX Oncology; see Kauder et al., 2019, PLoS One, doi: 10.1371/journal.pone.0201832).

SIRPα inhibitors include, without limitation, anti-SIRPα antibodies such as OSE-172 (Boehringer Ingelheim/OSE), FSI-189 (Forty Seven), anti-SIRPα fusion proteins such as TTI-621 and TTI-662 (Trillium Therapeutics; see WO 2014/094122).

PVRIG inhibitors include, without limitation, anti-PVRIG antibodies such as COM701 (CGEN-15029) and antibodies and method for their manufacture as disclosed in, e.g., WO 2018/033798 (e.g., CHA.7.518.1H4(S241P), CHA.7.538.1.2.H4(S241P), CPA.9.086H4(S241P), CPA.9.083H4(S241P), CHA.9.547.7.H4(S241P), CHA.9.547.13.H4(S241P) and antibodies comprising a variable heavy domain according to SEQ ID NO: 5 and a variable light domain according to SEQ ID NO: 10 of WO 2018/033798 or antibodies comprising a heavy chain according to SEQ ID NO:9 and a light chain according to SEQ ID NO: 14; WO 2018/033798

further discloses anti-TIGIT antibodies and combination therapies with anti-TIGIT and anti-PVRIG antibodies), WO2016134333, WO2018017864 (e.g., an antibody comprising a heavy chain according to SEQ ID NOs: 5-7 having at least 90% sequence identity to SEQ ID NO: 11 and/or a light chain according to SEQ ID NOs: 8-10 having at least 90% sequence identity to SEQ ID NO: 12, or an antibody encoded by SEQ ID NOs: 13 and/or 14 or SEQ ID NOs: 24 and/or 29, or another antibody disclosed in WO 2018/017864) and anti-PVRIG antibodies and fusion peptides as disclosed in WO 2016/134335.

CSF1R inhibitors include, without limitation, anti-CSF1R antibodies cabiralizumab (FPA008; FivePrime; see WO 2011/140249, WO 2013/169264 and WO 2014/036357), IMC-CS4 (EiiLilly), emactuzumab (R05509554; Roche), RG7155 (WO 2011/70024, WO 2011/107553, WO 2011/131407, WO 2013/87699, WO 2013/119716, WO 2013/132044) and the small molecule inhibitors BLZ945 (CAS#: 953769-46-5) and pexidartinib (PLX3397; Selleckchem; CAS#: 1029044-16-3).

CSF1 inhibitors include, without limitation, anti-CSF1 antibodies disclosed in EP 1 223 980 and Weir et al., 1996 (J Bone Mineral Res 11: 1474-1481), WO 2014/132072, and antisense DNA and RNA as disclosed in WO 2001/030381.

Exemplary NOX inhibitors include, without limitation, NOX1 inhibitors such as the small molecule ML171 (Gianni et al., 2010, ACS Chem Biol 5(10):981-93, NOS31 (Yamamoto et al., 2018, Biol Pharm Bull. 41(3):419-426), NOX2 inhibitors such as the small molecules ceplene (histamine dihydrochloride; CAS#: 56-92-8), BJ-1301 (Gautam et al., 2017, Mol Cancer Ther 16(10):2144-2156; CAS#: 1287234-48-3) and inhibitors described by Lu et al., 2017, Biochem Pharmacol 143:25-38, NOX4 inhibitors such as the small molecule inhibitors VAS2870 (Altenhöfer et al., 2012, Cell Mol Life Sciences 69(14):2327-2343), diphenylene iodonium (CAS#: 244-54-2) and GKT137831 (CAS#: 1218942-37-0; see Tang et al., 2018, 19(10):578-585).

TDO inhibitors include, without limitation, 4-(indol-3-yl)-pyrazole derivatives (see US 9,126,984 and US 2016/0263087), 3-indol substituted derivatives (see WO 2015/140717, WO 2017/025868, WO 2016/147144), 3-(indol-3-yl)-pyridine derivatives (see US 2015/0225367 and WO 2015/121812), dual IDO/TDO antagonist, such as small molecule dual IDO/TDO inhibitors disclosed in WO 2015/150097, WO 2015/082499, WO 2016/026772, WO 2016/071283, WO

2016/071293, WO 2017/007700, and the small molecule inhibitor CB548 (Kim, C, et al., 2018, Annals Oncol 29 (suppl_8): viii400-viii441).

According to the disclosure, the immune checkpoint inhibitor is an inhibitor of an inhibitory checkpoint protein but preferably not an inhibitor of a stimulatory checkpoint protein. As described herein, a number of CTLA-4, PD-1, TIGIT, B7-H3, B7-H4, BTLA, KIR, LAG-3, TIM-3, CD94/NKG2A, IDO, A2AR, A2BR, VISTA, Siglec, CD20, CD39, CD73, GARP, CD47, PVRIG, CSF1R, NOX and TDO inhibitors and inhibitors of respective ligands are known and several of them are already in clinical trials or even approved. Based on these known immune checkpoint inhibitors, alternative immune checkpoint inhibitors may be developed. In particular, known inhibitors of the preferred immune checkpoint proteins may be used as such or analogues thereof may be used, in particular chimerized, humanized or human forms of antibodies and antibodies cross-competing with any of the antibodies described herein.

It will be understood by one of ordinary skill in the art that other immune checkpoint targets can also be targeted by antagonists or antibodies, provided that the targeting results in the stimulation of an immune response such as an anti-tumor immune response as reflected in an increase in T cell proliferation, enhanced T cell activation, and/or increased cytokine production (e.g., IFN-γ, IL2).

Checkpoint inhibitors may be administered in any manner and by any route known in the art. The mode and route of administration will depend on the type of checkpoint inhibitor to be used.

Checkpoint inhibitors may be administered in the form of any suitable pharmaceutical composition as described herein.

Checkpoint inhibitors may be administered in the form of nucleic acid, such DNA or RNA molecules, encoding an immune checkpoint inhibitor, e.g., an inhibitory nucleic acid molecule or an antibody or fragment thereof. For example, antibodies can be delivered encoded in expression vectors, as described herein. Nucleic acid molecules can be delivered as such, e.g., in the form of a plasmid or mRNA molecule, or complexed with a delivery vehicle, e.g., a liposome, lipoplex or nucleic-acid lipid particles. Checkpoint inhibitors may also be administered via an oncolytic virus comprising an expression cassette encoding the checkpoint inhibitor. Checkpoint inhibitors

may also be administered by administration of endogeneic or allogeneic cells able to express a checkpoint inhibitor, e.g., in the form of a cell based therapy.

The term "cell based therapy" refers to the transplantation of cells (e.g., T lymphocytes, dendritic cells, or stem cells) expressing an immune checkpoint inhibitor into a subject for the purpose of treating a disease or disorder (e.g., a cancer disease). In one embodiment, the cell based therapy comprises genetically engineered cells. In one embodiment, the genetically engineered cells express an immune checkpoint inhibitor, such as described herein. In one embodiment, the genetically engineered cells express an immune checkpoint inhibitor that is an inhibitory nucleic acid molecule, such as a siRNA, shRNA, an oligonucleotide, antisense DNA or RNA, an aptamer, an antibody or a fragment thereof or a soluble immune checkpoint protein or fusion. Genetically engineered cells may also express further agents that enhance T cell function. Such agents are known in the art. Cell based therapies for the use in inhibition of immune checkpoint signaling are disclosed, e.g., in WO 2018/222711, herein incorporated by reference in its entirety.

The term "oncolytic virus" as used herein, refers to a virus capable of selectively replicating in and slowing the growth or inducing the death of a cancerous or hyperproliferative cell, either *in vitro* or *in vivo*, while having no or minimal effect on normal cells. An oncolytic virus for the delivery of an immune checkpoint inhibitor comprises an expression cassette that may encode an immune checkpoint inhibitor that is an inhibitory nucleic acid molecule, such as a siRNA, shRNA, an oligonucleotide, antisense DNA or RNA, an aptamer, an antibody or a fragment thereof or a soluble immune checkpoint protein or fusion. The oncolytic virus preferably is replication competent and the expression cassette is under the control of a viral promoter, e.g., synthetic early/late poxvirus promoter. Exemplary oncolytic viruses include vesicular stomatitis virus (VSV), rhabdoviruses (e.g., picornaviruses such as Seneca Valley virus; SVV-001), coxsackievirus, parvovirus, Newcastle disease virus (NDV), herpes simplex virus (HSV; OncoVEX GMCSF), retroviruses (e.g., influenza viruses), measles virus, reovirus, Sinbis virus, vaccinia virus, as exemplarily described in WO 2017/209053 (including Copenhagen, Western Reserve, Wyeth strains), and adenovirus (e.g., Delta-24, Delta-24-RGD, ICOVIR-5, ICOVIR-7, Onyx-015, ColoAd1, H101, AD5/3-D24-GMCSF). Generation of recombinant oncolytic viruses comprising a soluble form of an immune checkpoint inhibitor and methods for their use are disclosed in WO 2018/022831, herein incorporated by reference in its entirety. Oncolytic viruses can be used as attenuated viruses.

As described herein, an anti-CLDN18.2 antibody is administered together, i.e., co-administered, with a checkpoint inhibitor to a subject, e.g., a patient. In certain embodiments, the checkpoint inhibitor and the anti-CLDN18.2 antibody are administered as a single composition to the subject. In certain embodiments, the checkpoint inhibitor and the anti-CLDN18.2 antibody are administered concurrently (as separate compositions at the same time) to the subject. In certain embodiments, the checkpoint inhibitor and the anti-CLDN18.2 antibody are administered separately to the subject. In certain embodiments, the checkpoint inhibitor is administered before the anti-CLDN18.2 antibody to the subject. In certain embodiments, the checkpoint inhibitor is administered after the anti-CLDN18.2 antibody to the subject. In certain embodiments, the checkpoint inhibitor and the anti-CLDN18.2 antibody are administered to the subject on the same day. In certain embodiments, the checkpoint inhibitor and the anti-CLDN18.2 antibody are administered to the subject on different days.

According to the invention, the term "cytotoxic and/or cytostatic agent" includes chemotherapeutic agents or combinations of chemotherapeutic agents such as cytostatic agents. Chemotherapeutic agents may affect cells in one of the following ways: (1) damage the DNA of the cells so they can no longer reproduce, (2) inhibit the synthesis of new DNA strands so that no cell replication is possible, (3) stop the mitotic processes of the cells so that the cells cannot divide into two cells. The cytotoxic and/or cytostatic agent may be an agent stabilizing or increasing expression of CLDN18.2.

The term "agent stabilizing or increasing expression of CLDN18.2" refers to an agent or a combination of agents the provision of which to cells results in increased RNA and/or protein levels of CLDN18.2, preferably in increased levels of CLDN18.2 protein on the cell surface, compared to the situation where the cells are not provided with the agent or the combination of agents. Preferably, the cell is a cancer cell, in particular a cancer cell expressing CLDN18.2, such as a cell of the cancer types desribed herein. The term "agent stabilizing or increasing expression of CLDN18.2" refers, in particular, to an agent or a combination of agents the provision of which to cells results in a higher density of CLDN18.2 on the surface of said cells compared to the situation where the cells are not provided with the agent or the combination of agents. "Stabilizing expression of CLDN18.2" includes, in particular, the situation where the agent or the combination of agents prevents a decrease or reduces a decrease in expression of CLDN18.2, e.g. expression of CLDN18.2 would decrease without provision of the agent or the combination of agents and provision of the agent or the combination of agents prevents said decrease or reduces

said decrease of CLDN18.2 expression. "Increasing expression of CLDN18.2" includes, in particular, the situation where the agent or the combination of agents increases expression of CLDN18.2, e.g. expression of CLDN18.2 would decrease, remain essentially constant or increase without provision of the agent or the combination of agents and provision of the agent or the combination of agents increases CLDN18.2 expression compared to the situation without provision of the agent or the combination of agents so that the resulting expression is higher compared to the situation where expression of CLDN18.2 would decrease, remain essentially constant or increase without provision of the agent or the combination of agents.

According to the invention, the term "agent stabilizing or increasing expression of CLDN18.2" preferably relates to an agent or a combination of agents such a cytostatic compound or a combination of cytostatic compounds the provision of which to cells, in particular cancer cells, results in the cells being arrested in or accumulating in one or more phases of the cell cycle, preferably in one or more phases of the cell cycle other than the G1- and G0-phases, preferably other than the G1-phase, preferably in one or more of the G2- or S-phase of the cell cycle such as the G1/G2-, S/G2-, G2- or S-phase of the cell cycle. The term "cells being arrested in or accumulating in one or more phases of the cell cycle" means that the precentage of cells which are in said one or more phases of the cell cycle increases. Each cell goes through a cycle comprising four phases in order to replicate itself. The first phase called G1 is when the cell prepares to replicate its chromosomes. The second stage is called S, and in this phase DNA synthesis occurs and the DNA is duplicated. The next phase is the G2 phase, when the RNA and protein duplicate. The final stage is the M stage, which is the stage of actual cell division. In this final stage, the duplicated DNA and RNA split and move to separate ends of the cell, and the cell actually divides into two identical, functional cells. Chemotherapeutic agents which are DNA damaging agents usually result in an accumulation of cells in the G1 and/or G2 phase. Chemotherapeutic agents which block cell growth by interfering with DNA synthesis such as antimetabolites usually result in an accumulation of cells in the S-phase. Examples of these drugs are 6-mercaptopurine and 5-fluorouracil.

According to the invention, the term "agent stabilizing or increasing expression of CLDN18.2" includes anthracyclines such as epirubicin, platinum compounds such as oxaliplatin and cisplatin, nucleoside analogs such as 5-fluorouracil or prodrugs thereof, taxanes such as docetaxel, and camptothecin analogs such as irinotecan and topotecan, and combinations of drugs such as combinations of drugs comprising one or more of anthracyclines such as epirubicin, oxaliplatin

and 5-fluorouracil such as a combination of drugs comprising oxaliplatin and 5-fluorouracil or other drug combinations described herein.

In one preferred embodiment, a "cytotoxic and/or cytostatic agent" is an "agent inducing immunogenic cell death".

In specific circumstances, cancer cells can enter a lethal stress pathway linked to the emission of a spatiotemporally defined combination of signals that is decoded by the immune system to activate tumor-specific immune responses (Zitvogel L. et al. (2010) Cell 140: 798–804). In such scenario cancer cells are triggered to emit signals that are sensed by innate immune effectors such as dendritic cells to trigger a cognate immune response that involves CD8+ T cells and IFN-γ signalling so that tumor cell death may elicit a productive anticancer immune response. These signals include the pre-apoptotic exposure of the endoplasmic reticulum (ER) chaperon calreticulin (CRT) at the cell surface, the pre-apoptotic secretion of ATP, and the post-apoptotic release of the nuclear protein HMGB1. Together, these processes constitute the molecular determinants of immunogenic cell death (ICD). Anthracyclines, oxaliplatin, and γ irradiation are able to induce all signals that define ICD, while cisplatin, for example, which is deficient in inducing CRT translocation from the ER to the surface of dying cells - a process requiring ER stress - requires complementation by thapsigargin, an ER stress inducer.

According to the invention, the term "agent inducing immunogenic cell death" refers to an agent or a combination of agents which when provided to cells, in particular cancer cells, is capable of inducing the cells to enter a lethal stress pathway which finally results in tumor-specific immune responses. In particular, an agent inducing immunogenic cell death when provided to cells induces the cells to emit a spatiotemporally defined combination of signals, including, in particular, the pre-apoptotic exposure of the endoplasmic reticulum (ER) chaperon calreticulin (CRT) at the cell surface, the pre-apoptotic secretion of ATP, and the post-apoptotic release of the nuclear protein HMGB1.

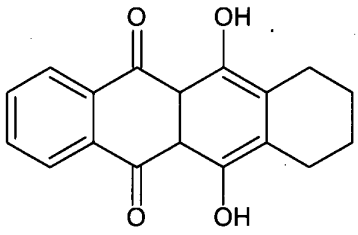
According to the invention, the term "agent inducing immunogenic cell death" includes anthracyclines and oxaliplatin.

Anthracyclines are a class of drugs commonly used in cancer chemotherapy that are also antibiotics. Structurally, all anthracyclines share a common four-ringed 7,8,9,10-tetrahydrotetracene-5,12-quinone structure and usually require glycosylation at specific sites.

Anthracyclines preferably bring about one or more of the following mechanisms of action: 1. Inhibiting DNA and RNA synthesis by intercalating between base pairs of the DNA/RNA strand, thus preventing the replication of rapidly-growing cancer cells. 2. Inhibiting topoisomerase II enzyme, preventing the relaxing of supercoiled DNA and thus blocking DNA transcription and replication. 3. Creating iron-mediated free oxygen radicals that damage the DNA and cell membranes.

According to the invention, the term "anthracycline" preferably relates to an agent, preferably an anticancer agent for inducing apoptosis, preferably by inhibiting the rebinding of DNA in topoisomerase II.

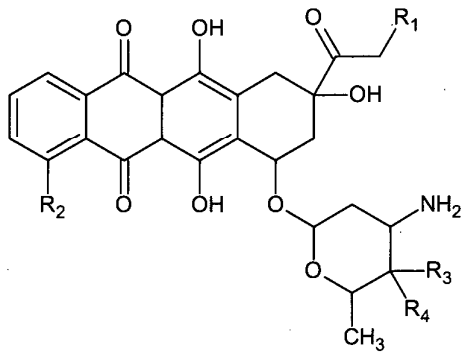
Preferably, according to the invention, the term "anthracycline" generally refers to a class of compounds having the following ring structure



including analogs and derivatives, pharmaceutical salts, hydrates, esters, conjugates and prodrugs thereof.

Examples of anthracyclines and anthracycline analogs include, but are not limited to, daunorubicin (daunomycin), doxorubicin (adriamycin), epirubicin, idarubicin, rhodomycin, pyrarubicin, valrubicin, N-trifluoro-acetyl doxorubicin-14-valerate, aclacinomycin, morpholinodoxorubicin (morpholino-DOX), cyanomorpholino-doxorubicin (cyanomorpholino-DOX), 2-pyrrolino-doxorubicin (2-PDOX), 5-iminodaunomycin, mitoxantrone and aclacinomycin A (aclarubicin). Mitoxantrone is a member of the anthracendione class of compounds, which are anthracycline analogs that lack the sugar moiety of the anthracyclines but retain the planar polycylic aromatic ring structure that permits intercalation into DNA.

Particularly preferred as anthracyline according to the invention is a compound of the following formula:

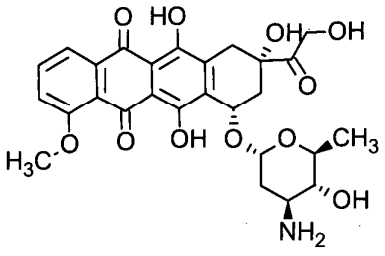


wherein

R_1 is selected from the group consisting of H and OH, R_2 is selected from the group consisting of H and OMe, R_3 is selected from the group consisting of H and OH, and R_4 is selected from the group consisting of H and OH.

In one embodiment, R_1 is H, R_2 is OMe, R_3 is H, and R_4 is OH. In another embodiment, R_1 is OH, R_2 is OMe, R_3 is H, and R_4 is OH. In another embodiment, R_1 is OH, R_2 is OMe, R_3 is OH, and R_4 is H. In another embodiment, R_1 is H, R_2 is H, R_3 is H, and R_4 is OH.

Specifically contemplated as anthracycline in the context of the present invention is epirubicin. Epirubicin is an anthracycline drug which has the following formula:

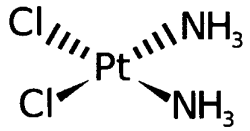


and is marketed under the trade name Ellence in the US and Pharmorubicin or Epirubicin Ebewe elsewhere. In particular, the term "epirubicin" refers to the compound (8R,10S)-10-[(2S,4S,5R,6S)-4-amino-5-hydroxy-6-methyl-oxan-2-yl]oxy-6,11-dihydroxy-8-(2-hydroxyacetyl)-1-methoxy-8-methyl-9,10-dihydro-7H-tetracen-5,12-dion. Epirubicin is favoured

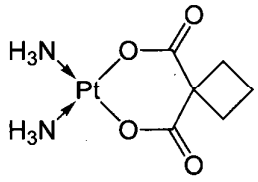
over doxorubicin, the most popular anthracycline, in some chemotherapy regimens as it appears to cause fewer side-effects.

According to the invention, the term "platinum compound" refers to compounds containing platinum in their structure such as platinum complexes and includes compounds such as cisplatin, carboplatin and oxaliplatin.

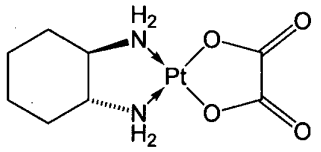
The term "cisplatin" or "cisplatinum" refers to the compound *cis*-diamminedichloroplatinum(II) (CDDP) of the following formula:



The term "carboplatin" refers to the compound cis-diammine(1,1-cyclobutanedicarboxylato)platinum(II) of the following formula:



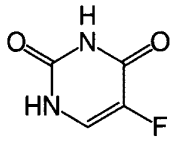
The term "oxaliplatin" refers to a compound which is a platinum compound that is complexed to a diaminocyclohexane carrier ligand of the following formula:



In particular, the term "oxaliplatin" refers to the compound [(1R,2R)-cyclohexane-1,2-diamine](ethanedioato-O,O')platinum(II). Oxaliplatin for injection is also marketed under the trade name Eloxatine.

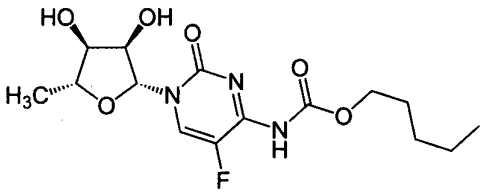
The term "nucleoside analog" refers to a structural analog of a nucleoside, a category that includes both purine analogs and pyrimidine analogs. In particular, the term "nucleoside analog" refers to fluoropyrimidine derivatives which includes fluorouracil and prodrugs thereof.

The term "fluorouracil" or "5-fluorouracil" (5-FU or f5U) (sold under the brand names Adrucil, Carac, Efudix, Efudex and Fluoroplex) is a compound which is a pyrimidine analog of the following formula:



In particular, the term refers to the compound 5-fluoro-1H-pyrimidine-2,4-dione.

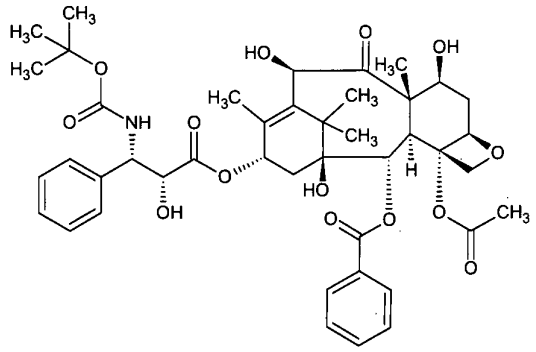
The term "capecitabine" (Xeloda, Roche) refers to a chemotherapeutic agent that is a prodrug that is converted into 5-FU in the tissues. Capecitabine which may be orally administered has the following formula:



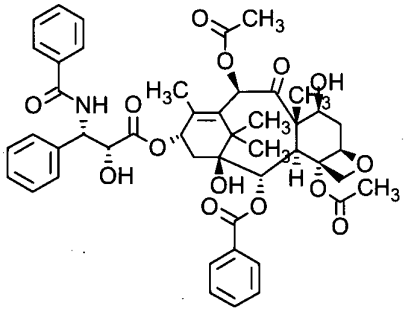
In particular, the term refers to the compound pentyl [1-(3,4-dihydroxy-5-methyltetrahydrofuran-2-yl)-5-fluoro-2-oxo-1H-pyrimidin-4-yl]carbamate.

Taxanes are a class of diterpene compounds that were first derived from natural sources such as plants of the genus Taxus, but some have been synthesized artificially. The principal mechanism of action of the taxane class of drugs is the disruption of microtubule function, thereby inhibiting the process of cell division. Taxanes include docetaxel (Taxotere) and paclitaxel (Taxol).

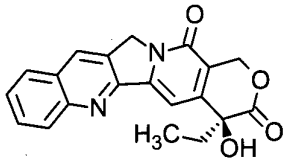
According to the invention, the term "docetaxel" refers to a compound having the following formula:



According to the invention, the term "paclitaxel" refers to a compound having the following formula:

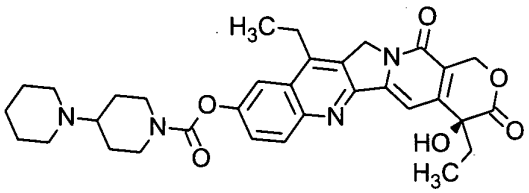


According to the invention, the term "camptothecin analog" refers to derivatives of the compound camptothecin (CPT; (S)-4-ethyl-4-hydroxy-1H-pyrano[3',4':6,7]indolizino[1,2-b] quinoline-3,14-(4H,12H)-dione). Preferably, the term "camptothecin analog" refers to compounds comprising the following structure:



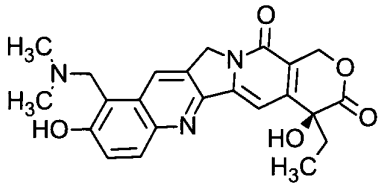
According to the invention, preferred camptothecin analogs are inhibitors of DNA enzyme topoisomerase I (topo I). Preferred camptothecin analogs according to the invention are irinotecan and topotecan.

Irinotecan is a drug preventing DNA from unwinding by inhibition of topoisomerase I. In chemical terms, it is a semisynthetic analogue of the natural alkaloid camptothecin having the following formula:



In particular, the term "irinotecan" refers to the compound (S)-4,11-diethyl-3,4,12,14-tetrahydro-4-hydroxy-3,14-dioxo1H-pyrano[3',4':6,7]-indolizino[1,2-b]quinolin-9-yl-[1,4'bipiperidine]-1'-carboxylate.

Topotecan is a topoisomerase inhibitor of the formula:



In particular, the term "topotecan" refers to the compound (S)-10-[(dimethylamino)methyl]-4-ethyl-4,9-dihydroxy-1H-pyrano[3',4':6,7]indolizino[1,2-b]quinoline-3,14(4H,12H)-dione monohydrochloride.

According to the invention, a cytotoxic and/or cytostatic agent may be a chemotherapeutic agent, in particular a chemotherapeutic agent established in cancer treatment and may be part of a combination of drugs such as a combination of drugs established for use in cancer treatment. Such combination of drugs may be a drug combination used in chemotherapy, and may be a drug combination as used in a chemotherapeutic regimen selected from the group consisting of **EOX** chemotherapy, **ECF** chemotherapy, **ECX** chemotherapy, **EOF** chemotherapy, **FLO** chemotherapy, **CAPOX** chemotherapy, **FOLFOX** chemotherapy, **FOLFIRI** chemotherapy, **DCF** chemotherapy and **FLOT** chemotherapy.

The drug combination used in **EOX** chemotherapy comprises of epirubicin, oxaliplatin and capecitabine. The drug combination used in **ECF** chemotherapy comprises of epirubicin, cisplatin and 5-fluorouracil. The drug combination used in **ECX** chemotherapy comprises of

epirubicin, cisplatin and capecitabine. The drug combination used in **EOF** chemotherapy comprises of epirubicin, oxaliplatin and 5-fluorouracil.

Epirubicin is normally given at a dose of 50 mg/m2, cisplatin 60 mg/m2, oxaliplatin 130 mg/m2, protracted venous infusion of 5-fluorouracil at 200 mg/m2/day and oral capecitabine 625 mg/m2 twice daily, for a total of eight 3-week cycles.

The drug combination used in **FLO** chemotherapy comprises of 5- fluorouracil, folinic acid and oxaliplatin (normally 5-fluorouracil 2,600 mg/m2 24-h infusion, folinic acid 200 mg/m2 and oxaliplatin 85 mg/m2, every 2 weeks).

FOLFOX is a chemotherapy regimen made up of folinic acid (leucovorin), 5-fluorouracil and oxaliplatin. The recommended dose schedule given every two weeks is as follows: Day 1: Oxaliplatin 85 mg/m² IV infusion and leucovorin 200 mg/m² IV infusion, followed by 5-FU 400 mg/m² IV bolus, followed by 5-FU 600 mg/m² IV infusion as a 22-hour continuous infusion; Day 2: Leucovorin 200 mg/m² IV infusion over 120 minutes, followed by 5-FU 400 mg/m² IV bolus given over 2-4 minutes, followed by 5-FU 600 mg/m² IV infusion as a 22-hour continuous infusion.

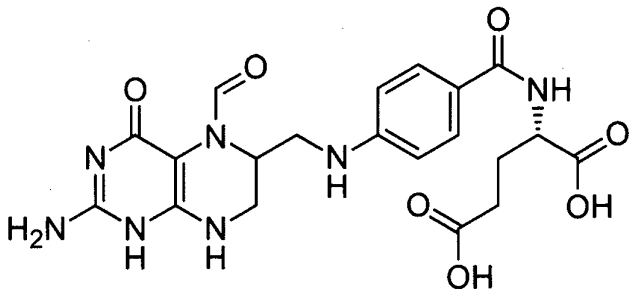
The drug combination used in **CAPOX** chemotherapy comprises of capecitabine and oxaliplatin.

The drug combination used in **FOLFIRI** chemotherapy comprises of 5-fluorouracil, leucovorin, and irinotecan.

The drug combination used in **DCF** chemotherapy comprises of docetaxel, cisplatin and 5-fluorouracil.

The drug combination used in **FLOT** chemotherapy comprises of docetaxel, oxaliplatin, 5-fluorouracil and folinic acid.

The term "folinic acid" or "leucovorin" refers to a compound useful in synergistic combination with the chemotherapy agent 5-fluorouracil. Folinic acid has the following formula:



In particular, the term refers to the compound (2S)-2-{[4-[(2-amino-5-formyl-4-oxo-5,6,7,8-tetrahydro-1H-pteridin-6-yl)methylamino]benzoyl]amino}pentanedioic acid.

The term "antigen" relates to an agent such as a protein or peptide comprising an epitope against which an immune response is directed and/or is to be directed. In a preferred embodiment, an antigen is a tumor-associated antigen, such as CLDN18.2, i.e., a constituent of cancer cells which may be derived from the cytoplasm, the cell surface and the cell nucleus, in particular those antigens which are produced, preferably in large quantity, intracellular or as surface antigens on cancer cells.

In the context of the present invention, the term "tumor-associated antigen" preferably relates to proteins that are under normal conditions specifically expressed in a limited number of tissues and/or organs or in specific developmental stages and are expressed or aberrantly expressed in one or more tumor or cancer tissues. In the context of the present invention, the tumor-associated antigen is preferably associated with the cell surface of a cancer cell and is preferably not or only rarely expressed in normal tissues.

The term "epitope" refers to an antigenic determinant in a molecule, i.e., to the part in a molecule that is recognized by the immune system, for example, that is recognized by an antibody. For example, epitopes are the discrete, three-dimensional sites on an antigen, which are recognized by the immune system. Epitopes usually consist of chemically active surface groupings of molecules such as amino acids or sugar side chains and usually have specific three dimensional structural characteristics, as well as specific charge characteristics. Conformational and non-conformational epitopes are distinguished in that the binding to the former but not the latter is lost in the presence of denaturing solvents. An epitope of a protein such as CLDN18.2 preferably comprises a continuous or discontinuous portion of said protein and is preferably between 5 and 100, preferably between 5 and 50, more preferably between 8 and 30, most preferably between

10 and 25 amino acids in length, for example, the epitope may be preferably 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, or 25 amino acids in length.

The term "antibody" refers to a glycoprotein comprising at least two heavy (H) chains and two light (L) chains inter-connected by disulfide bonds, and includes any molecule comprising an antigen binding portion thereof. The term "antibody" includes monoclonal antibodies and fragments or derivatives of antibodies, including, without limitation, human antibodies, humanized antibodies, chimeric antibodies, single chain antibodies, e.g., scFv's and antigen-binding antibody fragments such as Fab and Fab' fragments and also includes all recombinant forms of antibodies, e.g., antibodies expressed in prokaryotes, unglycosylated antibodies, and any antigen-binding antibody fragments and derivatives as described herein. Each heavy chain is comprised of a heavy chain variable region (abbreviated herein as VH) and a heavy chain constant region. Each light chain is comprised of a light chain variable region (abbreviated herein as VL) and a light chain constant region. The VH and VL regions can be further subdivided into regions of hypervariability, termed complementarity determining regions (CDR), interspersed with regions that are more conserved, termed framework regions (FR). Each VH and VL is composed of three CDRs and four FRs, arranged from amino-terminus to carboxy-terminus in the following order: FR1, CDR1, FR2, CDR2, FR3, CDR3, FR4. The variable regions of the heavy and light chains contain a binding domain that interacts with an antigen. The constant regions of the antibodies may mediate the binding of the immunoglobulin to host tissues or factors, including various cells of the immune system (e.g., effector cells) and the first component (Clq) of the classical complement system.

The antibodies described herein may be human antibodies. The term "human antibody", as used herein, is intended to include antibodies having variable and constant regions derived from human germline immunoglobulin sequences. The human antibodies described herein may include amino acid residues not encoded by human germline immunoglobulin sequences (e.g., mutations introduced by random or site-specific mutagenesis *in vitro* or by somatic mutation *in vivo*).

The term "humanized antibody" refers to a molecule having an antigen binding site that is substantially derived from an immunoglobulin from a non-human species, wherein the remaining immunoglobulin structure of the molecule is based upon the structure and/or sequence of a human immunoglobulin. The antigen binding site may either comprise complete variable

domains fused onto constant domains or only the complementarity determining regions (CDR) grafted onto appropriate framework regions in the variable domains. Antigen binding sites may be wild-type or modified by one or more amino acid substitutions, e.g. modified to resemble human immunoglobulins more closely. Some forms of humanized antibodies preserve all CDR sequences (for example a humanized mouse antibody which contains all six CDRs from the mouse antibody). Other forms have one or more CDRs which are altered with respect to the original antibody.

The term "chimeric antibody" refers to those antibodies wherein one portion of each of the amino acid sequences of heavy and light chains is homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular class, while the remaining segment of the chain is homologous to corresponding sequences in another. Typically the variable region of both light and heavy chains mimics the variable regions of antibodies derived from one species of mammals, while the constant portions are homologous to sequences of antibodies derived from another. One clear advantage to such chimeric forms is that the variable region can conveniently be derived from presently known sources using readily available B-cells or hybridomas from non-human host organisms in combination with constant regions derived from, for example, human cell preparations. While the variable region has the advantage of ease of preparation and the specificity is not affected by the source, the constant region being human, is less likely to elicit an immune response from a human subject when the antibodies are injected than would the constant region from a non human source. However the definition is not limited to this particular example.

The terms "antigen-binding portion" of an antibody (or simply "binding portion") or "antigen-binding fragment" of an antibody (or simply "binding fragment") or similar terms refer to one or more fragments of an antibody that retain the ability to specifically bind to an antigen. It has been shown that the antigen-binding function of an antibody can be performed by fragments of a full-length antibody. Examples of binding fragments encompassed within the term "antigen-binding portion" of an antibody include (i) Fab fragments, monovalent fragments consisting of the VL, VH, CL and CH domains; (ii) $F(ab')_2$ fragments, bivalent fragments comprising two Fab fragments linked by a disulfide bridge at the hinge region; (iii) Fd fragments consisting of the VH and CH domains; (iv) Fv fragments consisting of the VL and VH domains of a single arm of an antibody, (v) dAb fragments (Ward et al., (1989) Nature 341: 544-546), which consist of a VH domain; (vi) isolated complementarity determining regions (CDR), and (vii) combinations of

two or more isolated CDRs which may optionally be joined by a synthetic linker. Furthermore, although the two domains of the Fv fragment, VL and VH, are coded for by separate genes, they can be joined, using recombinant methods, by a synthetic linker that enables them to be made as a single protein chain in which the VL and VH regions pair to form monovalent molecules (known as single chain Fv (scFv); see e.g., Bird et al. (1988) Science 242: 423-426; and Huston et al. (1988) Proc. Natl. Acad. Sci. USA 85: 5879-5883). Such single chain antibodies are also intended to be encompassed within the term "antigen-binding fragment" of an antibody. A further example is binding-domain immunoglobulin fusion proteins comprising (i) a binding domain polypeptide that is fused to an immunoglobulin hinge region polypeptide, (ii) an immunoglobulin heavy chain CH2 constant region fused to the hinge region, and (iii) an immunoglobulin heavy chain CH3 constant region fused to the CH2 constant region. The binding domain polypeptide can be a heavy chain variable region or a light chain variable region. The binding-domain immunoglobulin fusion proteins are further disclosed in US 2003/0118592 and US 2003/0133939. These antibody fragments are obtained using conventional techniques known to those with skill in the art, and the fragments are screened for utility in the same manner as are intact antibodies.

The term "bispecific molecule" is intended to include any agent, e.g., a protein, peptide, or protein or peptide complex, which has two different binding specificities. For example, the molecule may bind to, or interact with (a) a cell surface antigen, and (b) an Fc receptor on the surface of an effector cell. The term "multispecific molecule" or "heterospecific molecule" is intended to include any agent, e.g., a protein, peptide, or protein or peptide complex, which has more than two different binding specificities. For example, the molecule may bind to, or interact with (a) a cell surface antigen, (b) an Fc receptor on the surface of an effector cell, and (c) at least one other component. Accordingly, the invention includes, but is not limited to, bispecific, trispecific, tetraspecific, and other multispecific molecules which are directed to CLDN18.2, and to other targets, such as Fc receptors on effector cells. The term "bispecific antibodies" also includes multivalent antibodies, such as trivalent antibodies with two different binding specificities, tetravalent antibodies with two or three different binding specificities, and so on. The term "bispecific antibodies" also includes diabodies. Diabodies are bivalent, bispecific antibodies in which the VH and VL domains are expressed on a single polypeptide chain, but using a linker that is too short to allow for pairing between the two domains on the same chain, thereby forcing the domains to pair with complementary domains of another chain and creating

two antigen binding sites (see e.g. , Holliger, P., et al. (1993) Proc. Natl. Acad. Sci. USA 90: 6444-6448; Poljak, R. J., et al. (1994) Structure 2: 1121-1123).

An antibody may be conjugated to a therapeutic moiety or agent, such as a cytotoxin, a drug (e.g., an immunosuppressant) or a radioisotope. A cytotoxin or cytotoxic agent includes any agent that is detrimental to and, in particular, kills cells. Examples include maytansins (e.g. mertansine, ravtansine or emtanside), auristatins (Monomethyl auristatin F (MMAF), Monomethyl auristatin E (MMAE)), dolastatins, calicheamicins (e.g. ozogamicin), pyrrolobenzidiazepine dimers (e.g. tesirine, tairine), duocarmycins (e.g. Duocarmycin SA, CC-1065, duocarmazine) and α-amanitin, irinotecan or its derivative SN-38, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, colchicin, doxorubicin, daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, and puromycin and analogs or homologs thereof, antimetabolites (e.g., methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, fludarabin, 5-fluorouracil decarbazine), alkylating agents (e.g., mechlorethamine, thioepa chlorambucil, melphalan, carmustine (BSNU) and lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (e.g., daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (e.g., dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC), and anti-mitotic agents (e.g., vincristine and vinblastine). In a preferred embodiment, the therapeutic agent is a cytotoxic agent or a radiotoxic agent. In another embodiment, the therapeutic agent is an immunosuppressant. In yet another embodiment, the therapeutic agent is GM-CSF. In a preferred embodiment, the therapeutic agent is doxorubicin, cisplatin, bleomycin, sulfate, carmustine, chlorambucil, cyclophosphamide or ricin A.

Antibodies also can be conjugated to a radioisotope, e.g., iodine-131, yttrium-90 or indium-111, to generate cytotoxic radiopharmaceuticals.

The antibody conjugates of the invention can be used to modify a given biological response, and the drug moiety is not to be construed as limited to classical chemical therapeutic agents. For example, the drug moiety may be a protein or polypeptide possessing a desired biological activity. Such proteins may include, for example, an enzymatically active toxin, or active fragment thereof, such as abrin, ricin A, pseudomonas exotoxin, or diphtheria toxin; a protein

such as tumor necrosis factor or interferon-γ; or, biological response modifiers such as, for example, lymphokines, interleukin-1 ("IL-1"), interleukin-2 ("IL-2"), interleukin-6 ("IL-6"), granulocyte macrophage colony stimulating factor ("GM-CSF"), granulocyte colony stimulating factor ("G-CSF"), or other growth factors.

Techniques for conjugating such therapeutic moiety to antibodies are well known, see, e.g., Arnon et al., "Monoclonal Antibodies For Immunotargeting Of Drugs In Cancer Therapy", in Monoclonal Antibodies And Cancer Therapy, Reisfeld et al. (eds.), pp. 243-56 (Alan R. Liss, Inc. 1985); Hellstrom et al., "Antibodies For Drug Delivery", in Controlled Drug Delivery (2nd Ed.), Robinson et al. (eds.), pp. 623-53 (Marcel Dekker, Inc. 1987); Thorpe, "Antibody Carriers Of Cytotoxic Agents In Cancer Therapy: A Review", in Monoclonal Antibodies '84: Biological And Clinical Applications, Pincheraet al. (eds.), pp. 475-506 (1985); "Analysis, Results, And Future Prospective Of The Therapeutic Use Of Radiolabeled Antibody In Cancer Therapy", in Monoclonal Antibodies For Cancer Detection And Therapy, Baldwin et al. (eds.), pp. 303-16 (Academic Press 1985), and Thorpe et al., "The Preparation And Cytotoxic Properties Of Antibody-Toxin Conjugates", Immunol. Rev., 62: 119-58 (1982).

As used herein, an antibody is "derived from" a particular germline sequence if the antibody is obtained from a system by immunizing an animal or by screening an immunoglobulin gene library, and wherein the selected antibody is at least 90%, more preferably at least 95%, even more preferably at least 96%, 97%, 98%, or 99% identical in amino acid sequence to the amino acid sequence encoded by the germline immunoglobulin gene. Typically, an antibody derived from a particular germline sequence will display no more than 10 amino acid differences, more preferably, no more than 5, or even more preferably, no more than 4, 3, 2, or 1 amino acid difference from the amino acid sequence encoded by the germline immunoglobulin gene.

As used herein, the term "heteroantibodies" refers to two or more antibodies, derivatives thereof, or antigen binding regions linked together, at least two of which have different specificities. These different specificities include a binding specificity for an Fc receptor on an effector cell, and a binding specificity for an antigen or epitope on a target cell, e.g., a tumor cell.

The antibodies described herein may be monoclonal antibodies. The term "monoclonal antibody" as used herein refers to a preparation of antibody molecules of single molecular composition. A monoclonal antibody displays a single binding specificity and affinity. In one embodiment, the

monoclonal antibodies are produced by a hybridoma which includes a B cell obtained from a non-human animal, e.g., mouse, fused to an immortalized cell.

The antibodies described herein may be recombinant antibodies. The term "recombinant antibody", as used herein, includes all antibodies that are prepared, expressed, created or isolated by recombinant means, such as (a) antibodies isolated from an animal (e.g., a mouse) that is transgenic or transchromosomal with respect to the immunoglobulin genes or a hybridoma prepared therefrom, (b) antibodies isolated from a host cell transformed to express the antibody, e.g., from a transfectoma, (c) antibodies isolated from a recombinant, combinatorial antibody library, and (d) antibodies prepared, expressed, created or isolated by any other means that involve splicing of immunoglobulin gene sequences to other DNA sequences.

Antibodies described herein may be derived from different species, including but not limited to mouse, rat, rabbit, guinea pig and human.

Antibodies described herein include polyclonal and monoclonal antibodies and include IgA such as IgA1 or IgA2, IgG1, IgG2, IgG3, IgG4, IgE, IgM, and IgD antibodies. In various embodiments, the antibody is an IgG1 antibody, more particularly an IgG1, kappa or IgG1, lambda isotype (i.e. IgG1, κ, λ), an IgG2a antibody (e.g. IgG2a, κ, λ), an IgG2b antibody (e.g. IgG2b, κ, λ), an IgG3 antibody (e.g. IgG3, κ, λ) or an IgG4 antibody (e.g. IgG4, κ, λ).

The term "transfectoma", as used herein, includes recombinant eukaryotic host cells expressing an antibody, such as CHO cells, NS/0 cells, HEK293 cells, HEK293T cells, plant cells, or fungi, including yeast cells.

As used herein, a "heterologous antibody" is defined in relation to a transgenic organism producing such an antibody. This term refers to an antibody having an amino acid sequence or an encoding nucleic acid sequence corresponding to that found in an organism not consisting of the transgenic organism, and being generally derived from a species other than the transgenic organism.

As used herein, a "heterohybrid antibody" refers to an antibody having light and heavy chains of different organismal origins. For example, an antibody having a human heavy chain associated with a murine light chain is a heterohybrid antibody.

The invention includes all antibodies and derivatives of antibodies as described herein which for the purposes of the invention are encompassed by the term "antibody". The term "antibody derivatives" refers to any modified form of an antibody, e.g., a conjugate of the antibody and another agent or antibody, or an antibody fragment.

The antibodies described herein are preferably isolated. "Isolated" means altered or removed from the natural state. For example, a nucleic acid or a peptide naturally present in a living animal is not "isolated", but the same nucleic acid or peptide partially or completely separated from the coexisting materials of its natural state is "isolated". An isolated nucleic acid or protein can exist in substantially purified form, or can exist in a non-native environment such as, for example, a host cell. An "isolated antibody" as used herein, is intended to include an antibody which is substantially free of other antibodies having different antigenic specificities (e.g., an isolated antibody that specifically binds to CLDN18.2 is substantially free of antibodies that specifically bind antigens other than CLDN18.2). An isolated antibody that specifically binds to an epitope, isoform or variant of human CLDN18.2 may, however, have cross-reactivity to other related antigens, e.g., from other species (e.g., CLDN18.2 species homologs). Moreover, an isolated antibody may be substantially free of other cellular material and/or chemicals. In one embodiment of the invention, a combination of "isolated" monoclonal antibodies relates to antibodies having different specificities and being combined in a well defined composition or mixture.

The term "binding" according to the invention preferably relates to a specific binding.

According to the present invention, an antibody is capable of binding to a predetermined target if it has a significant affinity for said predetermined target and binds to said predetermined target in standard assays. "Affinity" or "binding affinity" is often measured by equilibrium dissociation constant (K_D). Preferably, the term "significant affinity" refers to the binding to a predetermined target with a dissociation constant (K_D) of 10^{-5} M or lower, 10^{-6} M or lower, 10^{-7} M or lower, 10^{-8} M or lower, 10^{-9} M or lower, 10^{-10} M or lower, 10^{-11} M or lower, or 10^{-12} M or lower.

An antibody is not (substantially) capable of binding to a target if it has no significant affinity for said target and does not bind significantly, in particular does not bind detectably, to said target in standard assays. Preferably, the antibody does not detectably bind to said target if present in a

concentration of up to 2, preferably 10, more preferably 20, in particular 50 or 100 µg/ml or higher. Preferably, an antibody has no significant affinity for a target if it binds to said target with a K_D that is at least 10-fold, 100-fold, 10^3-fold, 10^4-fold, 10^5-fold, or 10^6-fold higher than the K_D for binding to the predetermined target to which the antibody is capable of binding. For example, if the K_D for binding of an antibody to the target to which the antibody is capable of binding is 10^{-7} M, the K_D for binding to a target for which the antibody has no significant affinity would be is at least 10^{-6} M, 10^{-5} M, 10^{-4} M, 10^{-3} M, 10^{-2} M, or 10^{-1} M.

An antibody is specific for a predetermined target if it is capable of binding to said predetermined target while it is not capable of binding to other targets, i.e. has no significant affinity for other targets and does not significantly bind to other targets in standard assays. According to the invention, an antibody is specific for CLDN18.2 if it is capable of binding to CLDN18.2 but is not (substantially) capable of binding to other targets. Preferably, an antibody is specific for CLDN18.2 if the affinity for and the binding to such other targets does not significantly exceed the affinity for or binding to CLDN18.2-unrelated proteins such as bovine serum albumin (BSA), casein, human serum albumin (HSA) or non-claudin transmembrane proteins such as MHC molecules or transferrin receptor or any other specified polypeptide. Preferably, an antibody is specific for a predetermined target if it binds to said target with a K_D that is at least 10-fold, 100-fold, 10^3-fold, 10^4-fold, 10^5-fold, or 10^6-fold lower than the K_D for binding to a target for which it is not specific. For example, if the K_D for binding of an antibody to the target for which it is specific is 10^{-7} M, the K_D for binding to a target for which it is not specific would be at least 10^{-6} M, 10^{-5} M, 10^{-4} M, 10^{-3} M, 10^{-2} M, or 10^{-1} M.

Binding of an antibody to a target can be determined experimentally using any suitable method; see, for example, Berzofsky et al., "Antibody-Antigen Interactions" In Fundamental Immunology, Paul, W. E., Ed., Raven Press New York, N Y (1984), Kuby, Janis Immunology, W. H. Freeman and Company New York, N Y (1992), and methods described herein. Affinities may be readily determined using conventional techniques, such as by equilibrium dialysis; by using the BIAcore 2000 instrument, using general procedures outlined by the manufacturer; by radioimmunoassay using radiolabeled target antigen; or by another method known to the skilled artisan. The affinity data may be analyzed, for example, by the method of Scatchard et al., Ann N.Y. Acad. ScL, 51:660 (1949). The measured affinity of a particular antibody-antigen interaction can vary if measured under different conditions, e.g., salt concentration, pH. Thus, measurements of affinity

and other antigen-binding parameters, e.g., K_D, IC_{50}, are preferably made with standardized solutions of antibody and antigen, and a standardized buffer.

As used herein, "isotype" refers to the antibody class (e.g., IgM or IgG1) that is encoded by heavy chain constant region genes.

As used herein, "isotype switching" refers to the phenomenon by which the class, or isotype, of an antibody changes from one Ig class to one of the other Ig classes.

The term "naturally occurring" as used herein as applied to an object refers to the fact that an object can be found in nature. For example, a polypeptide or polynucleotide sequence that is present in an organism (including viruses) that can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory is naturally occurring.

The term "rearranged" as used herein refers to a configuration of a heavy chain or light chain immunoglobulin locus wherein a V segment is positioned immediately adjacent to a D-J or J segment in a conformation encoding essentially a complete VH or VL domain, respectively. A rearranged immunoglobulin (antibody) gene locus can be identified by comparison to germline DNA; a rearranged locus will have at least one recombined heptamer/nonamer homology element.

The term "unrearranged" or "germline configuration" as used herein in reference to a V segment refers to the configuration wherein the V segment is not recombined so as to be immediately adjacent to a D or J segment.

According to the invention an anti-CLDN18.2 antibody is an antibody capable of binding to an epitope present in CLDN18.2, preferably an epitope located within the extracellular domains of CLDN18.2, in particular the first extracellular domain, preferably amino acid positions 29 to 78 of CLDN18.2. In particular embodiments, an anti-CLDN18.2 antibody is an antibody capable of binding to (i) an epitope on CLDN18.2 which is not present on CLDN18.1, preferably SEQ ID NO: 3, 4, and 5, (ii) an epitope localized on the CLDN18.2-loop1, preferably SEQ ID NO: 8, (iii) an epitope localized on the CLDN18.2-loop2, preferably SEQ ID NO: 10, (iv) an epitope localized on the CLDN18.2-loopD3, preferably SEQ ID NO: 11, (v) an epitope, which

encompass CLDN18.2-loop1 and CLDN18.2-loopD3, or (vi) a non-glycosylated epitope localized on the CLDN18.2-loopD3, preferably SEQ ID NO: 9.

According to the invention an anti-CLDN18.2 antibody preferably is an antibody binding to CLDN18.2 but not to CLDN18.1. Preferably, an anti-CLDN18.2 antibody is specific for CLDN18.2. Preferably, an anti-CLDN18.2 antibody is an antibody binding to CLDN18.2 expressed on the cell surface. In particular preferred embodiments, an anti-CLDN18.2 antibody binds to native epitopes of CLDN18.2 present on the surface of living cells. Preferably, an anti-CLDN18.2 antibody binds to one or more peptides selected from the group consisting of SEQ ID NOs: 1, 3-11, 44, 46, and 48-50. Preferably, an anti-CLDN18.2 antibody is specific for the afore mentioned proteins, peptides or immunogenic fragments or derivatives thereof. An anti-CLDN18.2 antibody may be obtained by a method comprising the step of immunizing an animal with a protein or peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 1, 3-11, 44, 46, and 48-50, or a nucleic acid or host cell expressing said protein or peptide. Preferably, the antibody binds to cancer cells, in particular cells of the cancer types mentioned above and, preferably, does not bind substantially to non-cancerous cells.

Preferably, binding of an anti-CLDN18.2 antibody to cells expressing CLDN18.2 induces or mediates killing of cells expressing CLDN18.2. The cells expressing CLDN18.2 are preferably cancer cells and are, in particular, selected from the group consisting of tumorigenic gastric, esophageal, pancreatic, lung, ovarian, colon, hepatic, head-neck, and gallbladder cancer cells. Preferably, the antibody induces or mediates killing of cells by inducing one or more of complement dependent cytotoxicity (CDC) mediated lysis, antibody dependent cellular cytotoxicity (ADCC) mediated lysis, apoptosis, and inhibition of proliferation of cells expressing CLDN18.2. Preferably, ADCC mediated lysis of cells takes place in the presence of effector cells, which in particular embodiments are selected from the group consisting of monocytes, mononuclear cells, NK cells and PMNs. Inhibiting proliferation of cells can be measured *in vitro* by determining proliferation of cells in an assay using bromodeoxyuridine (5-bromo-2-deoxyuridine, BrdU). BrdU is a synthetic nucleoside which is an analogue of thymidine and can be incorporated into the newly synthesized DNA of replicating cells (during the S phase of the cell cycle), substituting for thymidine during DNA replication. Detecting the incorporated chemical using, for example, antibodies specific for BrdU indicates cells that were actively replicating their DNA.

In preferred embodiments, antibodies described herein can be characterized by one or more of the following properties:

a) specificity for CLDN18.2;

b) a binding affinity to CLDN18.2 of about 100 nM or less, preferably, about 5-10 nM or less and, more preferably, about 1-3 nM or less,

c) the ability to induce or mediate CDC on CLDN18.2 positive cells;

d) the ability to induce or mediate ADCC on CLDN18.2 positive cells;

e) the ability to inhibit the growth of CLDN18.2 positive cells;

f) the ability to induce apoptosis of CLDN18.2 positive cells.

In a particularly preferred embodiment, an anti-CLDN18.2 antibody is produced by a hybridoma deposited at the DSMZ (Mascheroder Weg 1b, 38124 Braunschweig, Germany; new address: Inhoffenstr. 7B, 38124 Braunschweig, Germany) and having the following designation and accession number:

a. 182-D1106-055, accesssion no. DSM ACC2737, deposited on October 19, 2005

b. 182-D1106-056, accesssion no. DSM ACC2738, deposited on October 19, 2005

c. 182-D1106-057, accesssion no. DSM ACC2739, deposited on October 19, 2005

d. 182-D1106-058, accesssion no. DSM ACC2740, deposited on October 19, 2005

e. 182-D1106-059, accesssion no. DSM ACC2741, deposited on October 19, 2005

f. 182-D1106-062, accesssion no. DSM ACC2742, deposited on October 19, 2005,

g. 182-D1106-067, accesssion no. DSM ACC2743, deposited on October 19, 2005

h. 182-D758-035, accesssion no. DSM ACC2745, deposited on Nov. 17, 2005

i. 182-D758-036, accesssion no. DSM ACC2746, deposited on Nov. 17, 2005

j. 182-D758-040, accesssion no. DSM ACC2747, deposited on Nov. 17, 2005

k. 182-D1106-061, accesssion no. DSM ACC2748, deposited on Nov. 17, 2005

l. 182-D1106-279, accesssion no. DSM ACC2808, deposited on Oct. 26, 2006

m. 182-D1106-294, accesssion no. DSM ACC2809, deposited on Oct. 26, 2006,

n. 182-D1106-362, accesssion no. DSM ACC2810, deposited on Oct. 26, 2006.

Preferred antibodies according to the invention are those produced by and obtainable from the above-described hybridomas; i.e. 37G11 in the case of 182-D1106-055, 37H8 in the case of 182-D1106-056, 38G5 in the case of 182-D1106-057, 38H3 in the case of 182-D1106-058, 39F11 in the case of 182-D1106-059, 43A11 in the case of 182-D1106-062, 61C2 in the case of 182-D1106-067, 26B5 in the case of 182-D758-035, 26D12 in the case of 182-D758-036, 28D10 in

the case of 182-D758-040, 42E12 in the case of 182-D1106-061, 125E1 in the case of 182-D1106-279, 163E12 in the case of 182-D1106-294, and 175D10 in the case of 182-D1106-362; and the chimerized and humanized forms thereof.

Preferred chimerized antibodies and their sequences are shown in the following table.

	clone	mAb	Isotype	variable region	chimerized antibody
heavy chain	43A11	182-D1106-062	IgG2a	SEQ ID NO:29	SEQ ID NO:14
	163E12	182-D1106-294	IgG3	SEQ ID NO:30	SEQ ID NO:15
	125E1	182-D1106-279	IgG2a	SEQ ID NO:31	SEQ ID NO:16
	166E2	182-D1106-308	IgG3	SEQ ID NO:33	SEQ ID NO:18
	175D10	182-D1106-362	IgG1	SEQ ID NO:32	SEQ ID NO:17
	45C1	182-D758-187	IgG2a	SEQ ID NO:34	SEQ ID NO:19
light chain	43A11	182-D1106-062	IgK	SEQ ID NO:36	SEQ ID NO:21
	163E12	182-D1106-294	IgK	SEQ ID NO:35	SEQ ID NO:20
	125E1	182-D1106-279	IgK	SEQ ID NO:37	SEQ ID NO:22
	166E2	182-D1106-308	IgK	SEQ ID NO:40	SEQ ID NO:25
	175D10	182-D1106-362	IgK	SEQ ID NO:39	SEQ ID NO:24
	45C1	182-D758-187	IgK	SEQ ID NO:38	SEQ ID NO:23
	45C1	182-D758-187	IgK	SEQ ID NO:41	SEQ ID NO:26
	45C1	182-D758-187	IgK	SEQ ID NO:42	SEQ ID NO:27
	45C1	182-D758-187	IgK	SEQ ID NO:43	SEQ ID NO:28

In preferred embodiments, antibodies, in particular chimerised forms of antibodies according to the invention include antibodies comprising a heavy chain constant region (CH) comprising an amino acid sequence derived from a human heavy chain constant region such as the amino acid sequence represented by SEQ ID NO: 13 or 52, or a functional variant thereof, or a fragment of the amino acid sequence or functional variant. In further preferred embodiments, antibodies, in particular chimerised forms of antibodies according to the invention include antibodies comprising a light chain constant region (CL) comprising an amino acid sequence derived from a human light chain constant region such as the amino acid sequence represented by SEQ ID NO: 12 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant.

In a particular preferred embodiment, antibodies, in particular chimerised forms of antibodies according to the invention include antibodies which comprise a CH comprising an amino acid sequence derived from a human CH such as the amino acid sequence represented by SEQ ID NO: 13 or 52, or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and which comprise a CL comprising an amino acid sequence derived from a human CL such as the amino acid sequence represented by SEQ ID NO: 12 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant.

In one embodiment, an anti-CLDN18.2 antibody is a chimeric mouse/human IgG1 monoclonal antibody comprising kappa, murine variable light chain, human kappa light chain constant region allotype Km(3), murine heavy chain variable region, human IgG1 constant region, allotype G1m(3).

In certain preferred embodiments, chimerised forms of antibodies include antibodies comprising a heavy chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 15, 16, 17, 18, 19, 51, and a functional variant thereof, or a fragment of the amino acid sequence or functional variant and/or comprising a light chain comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 20, 21, 22, 23, 24, 25, 26, 27, 28, and a functional variant thereof, or a fragment of the amino acid sequence or functional variant.

In certain preferred embodiments, chimerised forms of antibodies include antibodies comprising a combination of heavy chains and light chains selected from the following possibilities (i) to (ix):

(i) the heavy chain comprises an amino acid sequence represented by SEQ ID NO: 14 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and the light chain comprises an amino acid sequence represented by SEQ ID NO: 21 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant,

(ii) the heavy chain comprises an amino acid sequence represented by SEQ ID NO: 15 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and the light chain comprises an amino acid sequence represented by SEQ ID NO: 20 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant,

(iii) the heavy chain comprises an amino acid sequence represented by SEQ ID NO: 16 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and the

light chain comprises an amino acid sequence represented by SEQ ID NO: 22 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant,

(iv) the heavy chain comprises an amino acid sequence represented by SEQ ID NO: 18 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and the light chain comprises an amino acid sequence represented by SEQ ID NO: 25 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant,

(v) the heavy chain comprises an amino acid sequence represented by SEQ ID NO: 17 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and the light chain comprises an amino acid sequence represented by SEQ ID NO: 24 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant,

(vi) the heavy chain comprises an amino acid sequence represented by SEQ ID NO: 19 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and the light chain comprises an amino acid sequence represented by SEQ ID NO: 23 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant,

(vii) the heavy chain comprises an amino acid sequence represented by SEQ ID NO: 19 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and the light chain comprises an amino acid sequence represented by SEQ ID NO: 26 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant,

(viii) the heavy chain comprises an amino acid sequence represented by SEQ ID NO: 19 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and the light chain comprises an amino acid sequence represented by SEQ ID NO: 27 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant,

(ix) the heavy chain comprises an amino acid sequence represented by SEQ ID NO: 19 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and the light chain comprises an amino acid sequence represented by SEQ ID NO: 28 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant, and

(x) the heavy chain comprises an amino acid sequence represented by SEQ ID NO: 51 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and the light chain comprises an amino acid sequence represented by SEQ ID NO: 24 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant.

In one particularly preferred embodiment, an anti-CLDN18.2 antibody comprises a heavy chain comprising an amino acid sequence represented by SEQ ID NO: 17 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and a light chain

comprising an amino acid sequence represented by SEQ ID NO: 24 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant.

In one particularly preferred embodiment, an anti-CLDN18.2 antibody comprises a heavy chain comprising an amino acid sequence represented by SEQ ID NO: 51 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and a light chain comprising an amino acid sequence represented by SEQ ID NO: 24 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant.

A fragment of an amino acid sequence selected from the group consisting of SEQ ID NOs: 14, 15, 16, 17, 18, 19, 51, 20, 21, 22, 23, 24, 25, 26, 27, and 28 preferably relates to said sequence wherein 17, 18, 19, 20, 21, 22 or 23 amino acids at the N-terminus are removed.

In a preferred embodiment, an anti-CLDN18.2 antibody comprises a heavy chain variable region (VH) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 29, 30, 31, 32, 33, 34, and a functional variant thereof, or a fragment of the amino acid sequence or functional variant.

In a preferred embodiment, an anti-CLDN18.2 antibody comprises a light chain variable region (VL) comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 35, 36, 37, 38, 39, 40, 41, 42, 43, and a functional variant thereof, or a fragment of the amino acid sequence or functional variant.

In certain preferred embodiments, an anti-CLDN18.2 antibody comprises a combination of heavy chain variable region (VH) and light chain variable region (VL) selected from the following possibilities (i) to (ix):

(i) the VH comprises an amino acid sequence represented by SEQ ID NO: 29 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and the VL comprises an amino acid sequence represented by SEQ ID NO: 36 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant,

(ii) the VH comprises an amino acid sequence represented by SEQ ID NO: 30 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and the VL comprises an amino acid sequence represented by SEQ ID NO: 35 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant,

(iii) the VH comprises an amino acid sequence represented by SEQ ID NO: 31 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and the VL comprises an amino acid sequence represented by SEQ ID NO: 37 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant,

(iv) the VH comprises an amino acid sequence represented by SEQ ID NO: 33 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and the VL comprises an amino acid sequence represented by SEQ ID NO: 40 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant,

(v) the VH comprises an amino acid sequence represented by SEQ ID NO: 32 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and the VL comprises an amino acid sequence represented by SEQ ID NO: 39 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant,

(vi) the VH comprises an amino acid sequence represented by SEQ ID NO: 34 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and the VL comprises an amino acid sequence represented by SEQ ID NO: 38 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant,

(vii) the VH comprises an amino acid sequence represented by SEQ ID NO: 34 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and the VL comprises an amino acid sequence represented by SEQ ID NO: 41 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant,

(viii) the VH comprises an amino acid sequence represented by SEQ ID NO: 34 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and the VL comprises an amino acid sequence represented by SEQ ID NO: 42 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant,

(ix) the VH comprises an amino acid sequence represented by SEQ ID NO: 34 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and the VL comprises an amino acid sequence represented by SEQ ID NO: 43 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant.

In one particularly preferred embodiment, an anti-CLDN18.2 antibody comprises a VH comprising an amino acid sequence represented by SEQ ID NO: 32 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant and a VL comprising an amino acid sequence represented by SEQ ID NO: 39 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant. In a more preferred embodiment, the

anti-CLDN18.2 antibody comprises a VH comprising an amino acid sequence represented by SEQ ID NO: 32 and a VL comprises an amino acid sequence represented by SEQ ID NO: 39, such as IMAB362 (Zolbetuximab).

The term "fragment" refers, in particular, to one or more of the complementarity-determining regions (CDRs), preferably at least the CDR3 sequence, optionally in combination with the CDR1 sequence and/or the CDR2 sequence, of the heavy chain variable region (VH) and/or of the light chain variable region (VL). In one embodiment said one or more of the complementarity-determining regions (CDRs) are selected from a set of complementarity-determining regions CDR1, CDR2 and CDR3. In a particularly preferred embodiment, the term "fragment" refers to the complementarity-determining regions CDR1, CDR2 and CDR3 of the heavy chain variable region (VH) and/or of the light chain variable region (VL).

In a preferred embodiment, an anti-CLDN18.2 antibody comprises a VH comprising a set of complementarity-determining regions CDR1, CDR2 and CDR3 selected from the following embodiments (i) to (vi):

(i) CDR1: positions 45-52 of SEQ ID NO: 14, CDR2: positions 70-77 of SEQ ID NO: 14, CDR3: positions 116-125 of SEQ ID NO: 14,

(ii) CDR1: positions 45-52 of SEQ ID NO: 15, CDR2: positions 70-77 of SEQ ID NO: 15, CDR3: positions 116-126 of SEQ ID NO: 15,

(iii) CDR1: positions 45-52 of SEQ ID NO: 16, CDR2: positions 70-77 of SEQ ID NO: 16, CDR3: positions 116-124 of SEQ ID NO: 16,

(iv) CDR1: positions 45-52 of SEQ ID NO: 17, CDR2: positions 70-77 of SEQ ID NO: 17, CDR3: positions 116-126 of SEQ ID NO: 17,

(v) CDR1: positions 44-51 of SEQ ID NO: 18, CDR2: positions 69-76 of SEQ ID NO: 18, CDR3: positions 115-125 of SEQ ID NO: 18, and

(vi) CDR1: positions 45-53 of SEQ ID NO: 19, CDR2: positions 71-78 of SEQ ID NO: 19, CDR3: positions 117-128 of SEQ ID NO: 19.

In a preferred embodiment, an anti-CLDN18.2 antibody comprises a VH comprising at least one, preferably two, more preferably all three of the CDR sequences of a set of complementarity-determining regions CDR1, CDR2 and CDR3 selected from the above embodiments (i) to (vi).

In a preferred embodiment, an anti-CLDN18.2 antibody comprises a VL comprising a set of complementarity-determining regions CDR1, CDR2 and CDR3 selected from the following embodiments (i) to (ix):

(i) CDR1: positions 47-58 of SEQ ID NO: 20, CDR2: positions 76-78 of SEQ ID NO: 20, CDR3: positions 115-123 of SEQ ID NO: 20,

(ii) CDR1: positions 49-53 of SEQ ID NO: 21, CDR2: positions 71-73 of SEQ ID NO: 21, CDR3: positions 110-118 of SEQ ID NO: 21,

(iii) CDR1: positions 47-52 of SEQ ID NO: 22, CDR2: positions 70-72 of SEQ ID NO: 22, CDR3: positions 109-117 of SEQ ID NO: 22,

(iv) CDR1: positions 47-58 of SEQ ID NO: 23, CDR2: positions 76-78 of SEQ ID NO: 23, CDR3: positions 115-123 of SEQ ID NO: 23,

(v) CDR1: positions 47-58 of SEQ ID NO: 24, CDR2: positions 76-78 of SEQ ID NO: 24, CDR3: positions 115-123 of SEQ ID NO: 24,

(vi) CDR1: positions 47-58 of SEQ ID NO: 25, CDR2: positions 76-78 of SEQ ID NO: 25, CDR3: positions 115-122 of SEQ ID NO: 25,

(vii) CDR1: positions 47-58 of SEQ ID NO: 26, CDR2: positions 76-78 of SEQ ID NO: 26, CDR3: positions 115-123 of SEQ ID NO: 26,

(viii) CDR1: positions 47-58 of SEQ ID NO: 27, CDR2: positions 76-78 of SEQ ID NO: 27, CDR3: positions 115-123 of SEQ ID NO: 27, and

(ix) CDR1: positions 47-52 of SEQ ID NO: 28, CDR2: positions 70-72 of SEQ ID NO: 28, CDR3: positions 109-117 of SEQ ID NO: 28.

In a preferred embodiment, an anti-CLDN18.2 antibody comprises a VL comprising at least one, preferably two, more preferably all three of the CDR sequences of a set of complementarity-determining regions CDR1, CDR2 and CDR3 selected from the above embodiments (i) to (ix).

In a preferred embodiment, an anti-CLDN18.2 antibody comprises a combination of VH and VL each comprising a set of complementarity-determining regions CDR1, CDR2 and CDR3 selected from the following embodiments (i) to (ix):

(i) VH: CDR1: positions 45-52 of SEQ ID NO: 14, CDR2: positions 70-77 of SEQ ID NO: 14, CDR3: positions 116-125 of SEQ ID NO: 14, VL: CDR1: positions 49-53 of SEQ ID NO: 21, CDR2: positions 71-73 of SEQ ID NO: 21, CDR3: positions 110-118 of SEQ ID NO: 21,

(ii) VH: CDR1: positions 45-52 of SEQ ID NO: 15, CDR2: positions 70-77 of SEQ ID NO: 15, CDR3: positions 116-126 of SEQ ID NO: 15, VL: CDR1: positions 47-58 of SEQ ID NO: 20, CDR2: positions 76-78 of SEQ ID NO: 20, CDR3: positions 115-123 of SEQ ID NO: 20,
(iii) VH: CDR1: positions 45-52 of SEQ ID NO: 16, CDR2: positions 70-77 of SEQ ID NO: 16, CDR3: positions 116-124 of SEQ ID NO: 16, VL: CDR1: positions 47-52 of SEQ ID NO: 22, CDR2: positions 70-72 of SEQ ID NO: 22, CDR3: positions 109-117 of SEQ ID NO: 22,
(iv) VH: CDR1: positions 44-51 of SEQ ID NO: 18, CDR2: positions 69-76 of SEQ ID NO: 18, CDR3: positions 115-125 of SEQ ID NO: 18, VL: CDR1: positions 47-58 of SEQ ID NO: 25, CDR2: positions 76-78 of SEQ ID NO: 25, CDR3: positions 115-122 of SEQ ID NO: 25,
(v) VH: CDR1: positions 45-52 of SEQ ID NO: 17, CDR2: positions 70-77 of SEQ ID NO: 17, CDR3: positions 116-126 of SEQ ID NO: 17, VL: CDR1: positions 47-58 of SEQ ID NO: 24, CDR2: positions 76-78 of SEQ ID NO: 24, CDR3: positions 115-123 of SEQ ID NO: 24,
(vi) VH: CDR1: positions 45-53 of SEQ ID NO: 19, CDR2: positions 71-78 of SEQ ID NO: 19, CDR3: positions 117-128 of SEQ ID NO: 19, VL: CDR1: positions 47-58 of SEQ ID NO: 23, CDR2: positions 76-78 of SEQ ID NO: 23, CDR3: positions 115-123 of SEQ ID NO: 23,
(vii) VH: CDR1: positions 45-53 of SEQ ID NO: 19, CDR2: positions 71-78 of SEQ ID NO: 19, CDR3: positions 117-128 of SEQ ID NO: 19, VL: CDR1: positions 47-58 of SEQ ID NO: 26, CDR2: positions 76-78 of SEQ ID NO: 26, CDR3: positions 115-123 of SEQ ID NO: 26,
(viii) VH: CDR1: positions 45-53 of SEQ ID NO: 19, CDR2: positions 71-78 of SEQ ID NO: 19, CDR3: positions 117-128 of SEQ ID NO: 19, VL: CDR1: positions 47-58 of SEQ ID NO: 27, CDR2: positions 76-78 of SEQ ID NO: 27, CDR3: positions 115-123 of SEQ ID NO: 27, and
(ix) VH: CDR1: positions 45-53 of SEQ ID NO: 19, CDR2: positions 71-78 of SEQ ID NO: 19, CDR3: positions 117-128 of SEQ ID NO: 19, VL: CDR1: positions 47-52 of SEQ ID NO: 28, CDR2: positions 70-72 of SEQ ID NO: 28, CDR3: positions 109-117 of SEQ ID NO: 28.

In a preferred embodiment, an anti-CLDN18.2 antibody comprises a VH comprising at least one, preferably two, more preferably all three of the VH CDR sequences of a set of complementarity-determining regions CDR1, CDR2 and CDR3 selected from the above embodiments (i) to (ix) and a VL comprising at least one, preferably two, more preferably all three of the VL CDR sequences of a set of complementarity-determining regions CDR1, CDR2 and CDR3 from the same embodiment (i) to (ix).

The term "at least one, preferably two, more preferably all three of the CDR sequences" preferably relates to at least the CDR3 sequence, optionally in combination with the CDR1 sequence and/or the CDR2 sequence.

In one particularly preferred embodiment, an anti-CLDN18.2 antibody comprises a combination of VH and VL each comprising a set of complementarity-determining regions CDR1, CDR2 and CDR3 as follows:
VH: CDR1: positions 45-52 of SEQ ID NO: 17, CDR2: positions 70-77 of SEQ ID NO: 17, CDR3: positions 116-126 of SEQ ID NO: 17, VL: CDR1: positions 47-58 of SEQ ID NO: 24, CDR2: positions 76-78 of SEQ ID NO: 24, CDR3: positions 115-123 of SEQ ID NO: 24.

In further preferred embodiments, an anti-CLDN18.2 antibody preferably comprises one or more of the complementarity-determining regions (CDRs), preferably at least the CDR3 variable region, of the heavy chain variable region (VH) and/or of the light chain variable region (VL) of a monoclonal antibody against CLDN18.2, preferably of a monoclonal antibody against CLDN18.2 described herein, and preferably comprises one or more of the complementarity-determining regions (CDRs), preferably at least the CDR3 variable region, of the heavy chain variable regions (VH) and/or light chain variable regions (VL) described herein. In one embodiment said one or more of the complementarity-determining regions (CDRs) are selected from a set of complementarity-determining regions CDR1, CDR2 and CDR3 described herein. In a particularly preferred embodiment, an anti-CLDN18.2 antibody preferably comprises the complementarity-determining regions CDR1, CDR2 and CDR3 of the heavy chain variable region (VH) and/or of the light chain variable region (VL) of a monoclonal antibody against CLDN18.2, preferably of a monoclonal antibody against CLDN18.2 described herein, and preferably comprises the complementarity-determining regions CDR1, CDR2 and CDR3 of the heavy chain variable regions (VH) and/or light chain variable regions (VL) described herein.

In one embodiment an antibody comprising one or more CDRs, a set of CDRs or a combination of sets of CDRs as described herein comprises said CDRs together with their intervening framework regions. Preferably, the portion will also include at least about 50% of either or both of the first and fourth framework regions, the 50% being the C-terminal 50% of the first framework region and the N-terminal 50% of the fourth framework region. Construction of antibodies made by recombinant DNA techniques may result in the introduction of residues N- or C-terminal to the variable regions encoded by linkers introduced to facilitate cloning or other

manipulation steps, including the introduction of linkers to join variable regions or join variable regions to further protein sequences including sequences as described herein.

In one embodiment an antibody comprising one or more CDRs, a set of CDRs or a combination of sets of CDRs as described herein comprises said CDRs in a human antibody framework.

Reference herein to an antibody comprising with respect to the heavy chain thereof a particular chain, or a particular region or sequence preferably relates to the situation wherein all heavy chains of said antibody comprise said particular chain, region or sequence. This applies correspondingly to the light chain of an antibody.

In one embodiment, an anti-CLDN18.2 antibody competes for CLDN18.2 binding with an anti-CLDN18.2 antibody described herein and/or has the specificity for CLDN18.2 of an anti-CLDN18.2 antibody described herein. In these and other embodiment, an anti-CLDN18.2 antibody may be highly homologous to an anti-CLDN18.2 antibody described herein. It is contemplated that a preferred anti-CLDN18.2 antibody has CDR regions either identical or highly homologous to the CDR regions of an anti-CLDN18.2 antibody described herein. By "highly homologous" it is contemplated that from 1 to 5, preferably from 1 to 4, such as 1 to 3 or 1 or 2 substitutions may be made in each CDR region.

The term "compete" refers to the competition between two binding molecules, e.g., antibodies, for binding to a target antigen. If two binding molecules do not block each other for binding to a target antigen, such binding molecules are non-competing and this is an indication that said binding molecules do not bind to the same part, i.e. epitope, of the target antigen. It is well known to a person skilled in the art how to test for competition of binding molecules such as antibodies for binding to a target antigen. An example of such a method is a so-called cross-competition assay, which may e.g. be performed as an ELISA or by flow-cytometry. For example an ELISA-based assay may be performed by coating ELISA plate wells with one of the antibodies; adding the competing antibody and His-tagged antigen/target and detecting whether the added antibody inhibited binding of the His-tagged antigen to the coated antibody, e.g., by adding biotinylated anti-His antibody, followed by Streptavidin-poly-HRP, and further developing the reaction with ABTS and measuring the absorbance at 405 nm. For example a flow-cytometry assay may be performed by incubating cells expressing the antigen/target with an excess of unlabeled antibody, incubating the cells with a sub-optimal concentration of biotin-

labelled antibody, followed by incubation with fluorescently labeled streptavidin and analyzing by flow cytometry.

Two binding molecules have the "same specificity" if they bind to the same antigen and to the same epitope. Whether a molecule to be tested recognizes the same epitope as a certain binding molecule, i.e., the binding molecules bind to the same epitope, can be tested by different methods known to the skilled person, e.g., based on the competition of the binding molecules such as antibodies for the same epitope. The competition between the binding molecules can be detected by a cross-blocking assay. For example, a competitive ELISA assay may be used as a cross-blocking assay. For example, target antigen may be coated on the wells of a microtiter plate and antigen binding antibody and candidate competing test antibody may be added. The amount of the antigen binding antibody bound to the antigen in the well indirectly correlates with the binding ability of the candidate competing test antibody that competes therewith for binding to the same epitope. Specifically, the larger the affinity of the candidate competing test antibody is for the same epitope, the smaller the amount of the antigen binding antibody bound to the antigen-coated well. The amount of the antigen binding antibody bound to the well can be measured by labeling the antibody with detectable or measurable labeling substances.

"Homologous" refers to the sequence similarity or sequence identity between two polypeptides or between two nucleic acid molecules. When a position in both of the two compared sequences is occupied by the same base or amino acid monomer subunit, then the molecules are homologous at that position. The percent of homology between two sequences is a function of the number of matching or homologous positions shared by the two sequences divided by the number of positions compared X 100. For example, if 6 of 10 of the positions in two sequences are matched or homologous then the two sequences are 60% homologous. Generally, a comparison is made when two sequences are aligned to give maximum homology. Homologous sequences exhibit according to the disclosure at least 40%, in particular at least 50%, at least 60%, at least 70%, at least 80%, at least 90% and preferably at least 95%, at least 98 or at least 99% identity of the amino acid or nucleotide residues.

"Fragment", with reference to an amino acid sequence (peptide or protein), relates to a part of an amino acid sequence, i.e. a sequence which represents the amino acid sequence shortened at the N-terminus and/or C-terminus. A fragment shortened at the C-terminus (N-terminal fragment) is obtainable e.g. by translation of a truncated open reading frame that lacks the 3'-end of the open

reading frame. A fragment shortened at the N-terminus (C-terminal fragment) is obtainable e.g. by translation of a truncated open reading frame that lacks the 5'-end of the open reading frame, as long as the truncated open reading frame comprises a start codon that serves to initiate translation. A fragment of an amino acid sequence comprises e.g. at least 50 %, at least 60 %, at least 70 %, at least 80%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, or at least 99% of the amino acid residues from an amino acid sequence. A fragment of an amino acid sequence preferably comprises at least 6, in particular at least 8, at least 12, at least 15, at least 20, at least 30, at least 50, or at least 100 consecutive amino acids from an amino acid sequence.

A "fragment" of an antibody sequence, when it replaces said antibody sequence in an antibody, preferably retains binding of said antibody to CLDN18.2 and preferably functions of said antibody as described herein, e.g. CDC mediated lysis or ADCC mediated lysis.

By "variant" or "variant protein" or "variant polypeptide" herein is meant a protein that differs from a parent protein by virtue of at least one amino acid modification. The parent polypeptide may be a naturally occurring or wild type (WT) polypeptide, or may be a modified version of a wild type polypeptide. Preferably, the variant polypeptide has at least one amino acid modification compared to the parent polypeptide, e.g. from 1 to about 20 amino acid modifications, and preferably from 1 to about 10 or from 1 to about 5 amino acid modifications compared to the parent.

By "parent polypeptide", "parent protein", "precursor polypeptide", or "precursor protein" as used herein is meant an unmodified polypeptide that is subsequently modified to generate a variant. A parent polypeptide may be a wild type polypeptide, or a variant or engineered version of a wild type polypeptide.

By "wild type" or "WT" or "native" herein is meant an amino acid sequence that is found in nature, including allelic variations. A wild type protein or polypeptide has an amino acid sequence that has not been intentionally modified.

For the purposes of the present disclosure, "variants" of an amino acid sequence (peptide, protein or polypeptide) comprise amino acid insertion variants, amino acid addition variants, amino acid deletion variants and/or amino acid substitution variants. The term "variant" includes all mutants,

splice variants, posttranslationally modified variants, conformations, isoforms, allelic variants, species variants, and species homologs, in particular those which are naturally occurring.

Amino acid insertion variants comprise insertions of single or two or more amino acids in a particular amino acid sequence. In the case of amino acid sequence variants having an insertion, one or more amino acid residues are inserted into a particular site in an amino acid sequence, although random insertion with appropriate screening of the resulting product is also possible. Amino acid addition variants comprise amino- and/or carboxy-terminal fusions of one or more amino acids, such as 1, 2, 3, 5, 10, 20, 30, 50, or more amino acids. Amino acid deletion variants are characterized by the removal of one or more amino acids from the sequence, such as by removal of 1, 2, 3, 5, 10, 20, 30, 50, or more amino acids. The deletions may be in any position of the protein. Amino acid deletion variants that comprise the deletion at the N-terminal and/or C-terminal end of the protein are also called N-terminal and/or C-terminal truncation variants. Amino acid substitution variants are characterized by at least one residue in the sequence being removed and another residue being inserted in its place. Preference is given to the modifications being in positions in the amino acid sequence which are not conserved between homologous proteins or peptides and/or to replacing amino acids with other ones having similar properties. Preferably, amino acid changes in peptide and protein variants are conservative amino acid changes, i.e., substitutions of similarly charged or uncharged amino acids. A conservative amino acid change involves substitution of one of a family of amino acids which are related in their side chains. Naturally occurring amino acids are generally divided into four families: acidic (aspartate, glutamate), basic (lysine, arginine, histidine), non-polar (alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), and uncharged polar (glycine, asparagine, glutamine, cysteine, serine, threonine, tyrosine) amino acids. Phenylalanine, tryptophan, and tyrosine are sometimes classified jointly as aromatic amino acids. In one embodiment, conservative amino acid substitutions include substitutions within the following groups:

glycine, alanine;

valine, isoleucine, leucine;

aspartic acid, glutamic acid;

asparagine, glutamine;

serine, threonine;

lysine, arginine; and

phenylalanine, tyrosine.

Preferably the degree of similarity, preferably identity between a given amino acid sequence and an amino acid sequence which is a variant of said given amino acid sequence will be at least about 60%, 65%, 70%, 80%, 81%, 82%, 83%, 84%, 85%, 86%, 87%, 88%, 89%, 90%, 91%, 92%, 93%, 94%, 95%, 96%, 97%, 98%, or 99%. The degree of similarity or identity is given preferably for an amino acid region which is at least about 10%, at least about 20%, at least about 30%, at least about 40%, at least about 50%, at least about 60%, at least about 70%, at least about 80%, at least about 90% or about 100% of the entire length of the reference amino acid sequence. For example, if the reference amino acid sequence consists of 200 amino acids, the degree of similarity or identity is given preferably for at least about 20, at least about 40, at least about 60, at least about 80, at least about 100, at least about 120, at least about 140, at least about 160, at least about 180, or about 200 amino acids, preferably continuous amino acids. In preferred embodiments, the degree of similarity or identity is given for the entire length of the reference amino acid sequence. The alignment for determining sequence similarity, preferably sequence identity can be done with art known tools, preferably using the best sequence alignment, for example, using Align, using standard settings, preferably EMBOSS::needle, Matrix: Blosum62, Gap Open 10.0, Gap Extend 0.5.

"Sequence similarity" indicates the percentage of amino acids that either are identical or that represent conservative amino acid substitutions. "Sequence identity" between two amino acid sequences indicates the percentage of amino acids that are identical between the sequences.

The term "percentage identity" is intended to denote a percentage of amino acid residues which are identical between the two sequences to be compared, obtained after the best alignment, this percentage being purely statistical and the differences between the two sequences being distributed randomly and over their entire length. Sequence comparisons between two amino acid sequences are conventionally carried out by comparing these sequences after having aligned them optimally, said comparison being carried out by segment or by "window of comparison" in order to identify and compare local regions of sequence similarity. The optimal alignment of the sequences for comparison may be produced, besides manually, by means of the local homology algorithm of Smith and Waterman, 1981, Ads App. Math. 2, 482, by means of the local homology algorithm of Neddleman and Wunsch, 1970, J. Mol. Biol. 48, 443, by means of the similarity search method of Pearson and Lipman, 1988, Proc. Natl Acad. Sci. USA 85, 2444, or by means of computer programs which use these algorithms (GAP, BESTFIT, FASTA, BLAST

P, BLAST N and TFASTA in Wisconsin Genetics Software Package, Genetics Computer Group, 575 Science Drive, Madison, Wis.).

The percentage identity is calculated by determining the number of identical positions between the two sequences being compared, dividing this number by the number of positions compared and multiplying the result obtained by 100 so as to obtain the percentage identity between these two sequences.

The teaching given herein with respect to specific amino acid sequences, e.g. those shown in the sequence listing, is to be construed so as to also relate to variants of said specific sequences resulting in sequences which are functionally equivalent to said specific sequences, e.g. amino acid sequences exhibiting properties identical or similar to those of the specific amino acid sequences. One important property is to retain binding of an antibody to its target or to sustain effector functions of an antibody. Preferably, a sequence which is a variant with respect to a specific sequence, when it replaces the specific sequence in an antibody retains binding of said antibody to CLDN18.2 and preferably functions of said antibody as described herein, e.g. CDC mediated lysis or ADCC mediated lysis.

It will be appreciated by those skilled in the art that in particular the sequences of the CDR, hypervariable and variable regions can be modified without losing the ability to bind CLDN18.2. For example, CDR regions will be either identical or highly homologous to the regions of antibodies specified herein. By "highly homologous" it is contemplated that from 1 to 5, preferably from 1 to 4, such as 1 to 3 or 1 or 2 substitutions may be made in the CDRs. In addition, the hypervariable and variable regions may be modified so that they show substantial homology with the regions of antibodies specifically disclosed herein.

The term "functional variant", as used herein, refers to a variant molecule or sequence that comprises an amino acid sequence that is altered by one or more amino acids compared to the amino acid sequence of the parent molecule or sequence and that is still capable of fulfilling one or more of the functions of the parent molecule or sequence, e.g., binding to a target molecule or contributing to binding to a target molecule. If the parent molecule or sequence is an antibody molecule or sequence, the alteration is preferably not in the variable regions of the antibody, more preferably not in the CDR regions of the antibody. In one embodiment, a functional variant either alone or in combination with other elements competes for binding to a target molecule

with the parent molecule or sequence. In other words, the modifications in the amino acid sequence of the parent molecule or sequence do not significantly affect or alter the binding characteristics of the molecule or sequence. In different embodiments, binding of the functional variant may be reduced but still significantly present, e.g., binding of the functional variant may be at least 50%, at least 60%, at least 70%, at least 80%, or at least 90% of the parent molecule or sequence. However, in other embodiments, binding of the functional variant may be enhanced compared to the parent molecule or sequence.

An amino acid sequence (peptide, protein or polypeptide) "derived from" a designated amino acid sequence (peptide, protein or polypeptide) refers to the origin of the first amino acid sequence. Preferably, the amino acid sequence which is derived from a particular amino acid sequence has an amino acid sequence that is identical, essentially identical or homologous to that particular sequence or a fragment thereof. Amino acid sequences derived from a particular amino acid sequence may be variants of that particular sequence or a fragment thereof.

The term "nucleic acid", as used herein, is intended to include DNA and RNA. A nucleic acid may be single-stranded or double-stranded, but preferably is double-stranded DNA.

According to the invention, the term "expression" is used in its most general meaning and comprises the production of RNA or of RNA and protein/peptide. It also comprises partial expression of nucleic acids. Furthermore, expression may be carried out transiently or stably.

The term "transgenic animal" refers to an animal having a genome comprising one or more transgenes, preferably heavy and/or light chain transgenes, or transchromosomes (either integrated or non-integrated into the animal's natural genomic DNA) and which is preferably capable of expressing the transgenes. For example, a transgenic mouse can have a human light chain transgene and either a human heavy chain transgene or human heavy chain transchromosome, such that the mouse produces human anti-CLDN18.2 antibodies when immunized with CLDN18.2 antigen and/or cells expressing CLDN18.2. The human heavy chain transgene can be integrated into the chromosomal DNA of the mouse, as is the case for transgenic mice, e.g., HuMAb mice, such as HCo7 or HCol2 mice, or the human heavy chain transgene can be maintained extrachromosomally, as is the case for transchromosomal (e.g., KM) mice as described in WO 02/43478. Such transgenic and transchromosomal mice may be

capable of producing multiple isotypes of human monoclonal antibodies to CLDN18.2 (e.g., IgG, IgA and/or IgE) by undergoing V-D-J recombination and isotype switching.

"Reduce", "decrease" or "inhibit" as used herein means an overall decrease or the ability to cause an overall decrease, preferably of 5% or greater, 10% or greater, 20% or greater, more preferably of 50% or greater, and most preferably of 75% or greater, in the level, e.g. in the level of expression or in the level of proliferation of cells.

Terms such as "increase" or "enhance" preferably relate to an increase or enhancement by about at least 10%, preferably at least 20%, preferably at least 30%, more preferably at least 40%, more preferably at least 50%, even more preferably at least 80%, and most preferably at least 100%, at least 200%, at least 500%, at least 1000%, at least 10000% or even more.

"Inducing" when used in relation to a certain activity or function such as antibody-dependent cell-mediated cytotoxicity (ADCC) may mean that there was no such activity or function present before induction, but it may also mean that there was a certain level of such activity or function present before induction and after induction said activity or function is enhanced. Thus, the term "inducing" also includes "enhancing".

Mechanisms of mAb action

Although the following provides considerations regarding the mechanism underlying the therapeutic efficacy of antibodies of the invention it is not to be considered as limiting to the invention in any way.

The antibodies described herein preferably interact with components of the immune system, preferably through ADCC or CDC. Antibodies described herein can also be used to target payloads (e.g., radioisotopes, drugs or toxins) to directly kill tumor cells or can be used with traditional chemotherapeutic agents, attacking tumors through complementary mechanisms of action that may include anti-tumor immune responses that may have been compromised owing to a chemotherapeutic's cytotoxic side effects on T lymphocytes. However, antibodies described herein may also exert an effect simply by binding to CLDN18.2 on the cell surface, thus, e.g. blocking proliferation of the cells.

Antibody-dependent cell-mediated cytotoxicity

ADCC describes the cell-killing ability of effector cells as described herein, in particular lymphocytes, which preferably requires the target cell being marked by an antibody.

ADCC preferably occurs when antibodies bind to antigens on tumor cells and the antibody Fc domains engage Fc receptors (FcR) on the surface of immune effector cells. Several families of Fc receptors have been identified, and specific cell populations characteristically express defined Fc receptors. ADCC can be viewed as a mechanism to directly induce a variable degree of immediate tumor destruction that leads to antigen presentation and the induction of tumor-directed T-cell responses. Preferably, *in vivo* induction of ADCC will lead to tumor-directed T-cell responses and host-derived antibody responses.

Complement-dependent cytotoxicity

CDC is another cell-killing method that can be directed by antibodies. IgM is the most effective isotype for complement activation. IgG1 and IgG3 are also both very effective at directing CDC via the classical complement-activation pathway. Preferably, in this cascade, the formation of antigen-antibody complexes results in the uncloaking of multiple C1q binding sites in close proximity on the C_H2 domains of participating antibody molecules such as IgG molecules (C1q is one of three subcomponents of complement C1). Preferably these uncloaked C1q binding sites convert the previously low-affinity C1q–IgG interaction to one of high avidity, which triggers a cascade of events involving a series of other complement proteins and leads to the proteolytic release of the effector-cell chemotactic/activating agents C3a and C5a. Preferably, the complement cascade ends in the formation of a membrane attack complex, which creates pores in the cell membrane that facilitate free passage of water and solutes into and out of the cell.

Antibodies described herein can be produced by a variety of techniques, including conventional monoclonal antibody methodology, e.g., the standard somatic cell hybridization technique of Kohler and Milstein, Nature 256: 495 (1975). Although somatic cell hybridization procedures are preferred, in principle, other techniques for producing monoclonal antibodies can be employed, e.g., viral or oncogenic transformation of B-lymphocytes or phage display techniques using libraries of antibody genes.

The preferred animal system for preparing hybridomas that secrete monoclonal antibodies is the murine system. Hybridoma production in the mouse is a very well established procedure. Immunization protocols and techniques for isolation of immunized splenocytes for fusion are

known in the art. Fusion partners (e.g., murine myeloma cells) and fusion procedures are also known.

Other preferred animal systems for preparing hybridomas that secrete monoclonal antibodies are the rat and the rabbit system (e.g. described in Spieker-Polet et al., Proc. Natl. Acad. Sci. U.S.A. 92:9348 (1995), see also Rossi et al., Am. J. Clin. Pathol. 124: 295 (2005)).

In yet another preferred embodiment, human monoclonal antibodies can be generated using transgenic or transchromosomal mice carrying parts of the human immune system rather than the mouse system. These transgenic and transchromosomic mice include mice known as HuMAb mice and KM mice, respectively, and are collectively referred to herein as "transgenic mice." The production of human antibodies in such transgenic mice can be performed as described in detail for CD20 in WO2004 035607

Yet another strategy for generating monoclonal antibodies is to directly isolate genes encoding antibodies from lymphocytes producing antibodies of defined specificity e.g. see Babcock et al., 1996; A novel strategy for generating monoclonal antibodies from single, isolated lymphocytes producing antibodies of defined specificities. For details of recombinant antibody engineering see also Welschof and Kraus, Recombinant antibodes for cancer therapy ISBN-0-89603-918-8 and Benny K.C. Lo Antibody Engineering ISBN 1-58829-092-1.

To generate antibodies, mice can be immunized with carrier-conjugated peptides derived from the antigen sequence, i.e. the sequence against which the antibodies are to be directed, an enriched preparation of recombinantly expressed antigen or fragments thereof and/or cells expressing the antigen, as described. Alternatively, mice can be immunized with DNA encoding the antigen or fragments thereof. In the event that immunizations using a purified or enriched preparation of the antigen do not result in antibodies, mice can also be immunized with cells expressing the antigen, e.g., a cell line, to promote immune responses.

The immune response can be monitored over the course of the immunization protocol with plasma and serum samples being obtained by tail vein or retroorbital bleeds. Mice with sufficient titers of immunoglobulin can be used for fusions. Mice can be boosted intraperitonealy or intravenously with antigen expressing cells 3 days before sacrifice and removal of the spleen to increase the rate of specific antibody secreting hybridomas.

To generate hybridomas producing monoclonal antibodies, splenocytes and lymph node cells from immunized mice can be isolated and fused to an appropriate immortalized cell line, such as a mouse myeloma cell line. The resulting hybridomas can then be screened for the production of antigen-specific antibodies. Individual wells can then be screened by ELISA for antibody secreting hybridomas. By Immunofluorescence and FACS analysis using antigen expressing cells, antibodies with specificity for the antigen can be identified. The antibody secreting hybridomas can be replated, screened again, and if still positive for monoclonal antibodies can be subcloned by limiting dilution. The stable subclones can then be cultured *in vitro* to generate antibody in tissue culture medium for characterization.

Antibodies also can be produced in a host cell transfectoma using, for example, a combination of recombinant DNA techniques and gene transfection methods as are well known in the art (Morrison, S. (1985) Science 229: 1202).

For example, in one embodiment, the gene(s) of interest, e.g., antibody genes, can be ligated into an expression vector such as a eukaryotic expression plasmid such as used by the GS gene expression system disclosed in WO 87/04462, WO 89/01036 and EP 338 841 or other expression systems well known in the art. The purified plasmid with the cloned antibody genes can be introduced in eukaryotic host cells such as CHO cells, NS/0 cells, HEK293T cells or HEK293 cells or alternatively other eukaryotic cells like plant derived cells, fungal or yeast cells. The method used to introduce these genes can be methods described in the art such as electroporation, lipofectine, lipofectamine or others. After introduction of these antibody genes in the host cells, cells expressing the antibody can be identified and selected. These cells represent the transfectomas which can then be amplified for their expression level and upscaled to produce antibodies. Recombinant antibodies can be isolated and purified from these culture supernatants and/or cells.

Alternatively, the cloned antibody genes can be expressed in other expression systems, including prokaryotic cells, such as microorganisms, e.g. E. coli. Furthermore, the antibodies can be produced in transgenic non-human animals, such as in milk from sheep and rabbits or in eggs from hens, or in transgenic plants; see e.g. Verma, R., et al. (1998) J. Immunol. Meth. 216: 165-181; Pollock, et al. (1999) J. Immunol. Meth. 231: 147-157; and Fischer, R., et al. (1999) Biol. Chem. 380: 825-839.

Chimerization

Murine monoclonal antibodies can be used as therapeutic antibodies in humans when labeled with toxins or radioactive isotopes. Nonlabeled murine antibodies are highly immunogenic in man when repetitively applied leading to reduction of the therapeutic effect. The main immunogenicity is mediated by the heavy chain constant regions. The immunogenicity of murine antibodies in man can be reduced or completely avoided if respective antibodies are chimerized or humanized. Chimeric antibodies are antibodies, the different portions of which are derived from different animal species, such as those having a variable region derived from a murine antibody and a human immunoglobulin constant region. Chimerisation of antibodies is achieved by joining of the variable regions of the murine antibody heavy and light chain with the constant region of human heavy and light chain (e.g. as described by Kraus et al., in Methods in Molecular Biology series, Recombinant antibodies for cancer therapy ISBN-0-89603-918-8). In a preferred embodiment chimeric antibodies are generated by joining human kappa-light chain constant region to murine light chain variable region. In an also preferred embodiment chimeric antibodies can be generated by joining human lambda-light chain constant region to murine light chain variable region. The preferred heavy chain constant regions for generation of chimeric antibodies are IgG1, IgG3 and IgG4. Other preferred heavy chain constant regions for generation of chimeric antibodies are IgG2, IgA, IgD and IgM.

Humanization

Antibodies interact with target antigens predominantly through amino acid residues that are located in the six heavy and light chain complementarity determining regions (CDRs). For this reason, the amino acid sequences within CDRs are more diverse between individual antibodies than sequences outside of CDRs. Because CDR sequences are responsible for most antibody-antigen interactions, it is possible to express recombinant antibodies that mimic the properties of specific naturally occurring antibodies by constructing expression vectors that include CDR sequences from the specific naturally occurring antibody grafted onto framework sequences from a different antibody with different properties (see, e.g., Riechmann, L. et al. (1998) Nature 332: 323-327; Jones, P. et al. (1986) Nature 321: 522-525; and Queen, C. et al. (1989) Proc. Natl. Acad. Sci. U. S. A. 86: 10029-10033). Such framework sequences can be obtained from public DNA databases that include germline antibody gene sequences. These germline sequences will differ from mature antibody gene sequences because they will not include completely assembled variable genes, which are formed by V (D) J joining during B cell maturation. Germline gene

sequences will also differ from the sequences of a high affinity secondary repertoire antibody at individual evenly across the variable region.

The ability of antibodies to bind an antigen can be determined using standard binding assays (e.g., ELISA, Western Blot, Immunofluorescence and flow cytometric analysis).

To purify antibodies, selected hybridomas can be grown in two-liter spinner-flasks for monoclonal antibody purification. Alternatively, antibodies can be produced in dialysis based bioreactors. Supernatants can be filtered and, if necessary, concentrated before affinity chromatography with protein G-sepharose or protein A-sepharose. Eluted IgG can be checked by gel electrophoresis and high performance liquid chromatography to ensure purity. The buffer solution can be exchanged into PBS, and the concentration can be determined by OD280 using 1.43 extinction coefficient. The monoclonal antibodies can be aliquoted and stored at -80°C.

To determine if the selected monoclonal antibodies bind to unique epitopes, site-directed or multi-site directed mutagenesis can be used.

To determine the isotype of antibodies, isotype ELISAs with various commercial kits (e.g. Zymed, Roche Diagnostics) can be performed. Wells of microtiter plates can be coated with anti-mouse Ig. After blocking, the plates are reacted with monoclonal antibodies or purified isotype controls, at ambient temperature for two hours. The wells can then be reacted with either mouse IgG1, IgG2a, IgG2b or IgG3, IgA or mouse IgM-specific peroxidase-conjugated probes. After washing, the plates can be developed with ABTS substrate (1 mg/ml) and analyzed at OD of 405-650. Alternatively, the IsoStrip Mouse Monoclonal Antibody Isotyping Kit (Roche, Cat. No. 1493027) may be used as described by the manufacturer.

In order to demonstrate presence of antibodies in sera of immunized mice or binding of monoclonal antibodies to living cells expressing antigen, flow cytometry can be used. Cell lines expressing naturally or after transfection antigen and negative controls lacking antigen expression (grown under standard growth conditions) can be mixed with various concentrations of monoclonal antibodies in hybridoma supernatants or in PBS containing 1% FBS, and can be incubated at 4°C for 30 min. After washing, the APC- or Alexa647-labeled anti IgG antibody can bind to antigen-bound monoclonal antibody under the same conditions as the primary antibody staining. The samples can be analyzed by flow cytometry with a FACS instrument using light

and side scatter properties to gate on single, living cells. In order to distinguish antigen-specific monoclonal antibodies from non-specific binders in a single measurement, the method of co-transfection can be employed. Cells transiently transfected with plasmids encoding antigen and a fluorescent marker can be stained as described above. Transfected cells can be detected in a different fluorescence channel than antibody-stained cells. As the majority of transfected cells express both transgenes, antigen-specific monoclonal antibodies bind preferentially to fluorescence marker expressing cells, whereas non-specific antibodies bind in a comparable ratio to non-transfected cells. An alternative assay using fluorescence microscopy may be used in addition to or instead of the flow cytometry assay. Cells can be stained exactly as described above and examined by fluorescence microscopy.

In order to demonstrate presence of antibodies in sera of immunized mice or binding of monoclonal antibodies to living cells expressing antigen, immunofluorescence microscopy analysis can be used. For example, cell lines expressing either spontaneously or after transfection antigen and negative controls lacking antigen expression are grown in chamber slides under standard growth conditions in DMEM/F12 medium, supplemented with 10 % fetal calf serum (FCS), 2 mM L-glutamine, 100 IU/ml penicillin and 100 µg/ml streptomycin. Cells can then be fixed with methanol or paraformaldehyde or left untreated. Cells can then be reacted with monoclonal antibodies against the antigen for 30 min. at 25°C. After washing, cells can be reacted with an Alexa555-labelled anti-mouse IgG secondary antibody (Molecular Probes) under the same conditions. Cells can then be examined by fluorescence microscopy.

Cell extracts from cells expressing antigen and appropriate negative controls can be prepared and subjected to sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis. After electrophoresis, the separated antigens will be transferred to nitrocellulose membranes, blocked, and probed with the monoclonal antibodies to be tested. IgG binding can be detected using anti-mouse IgG peroxidase and developed with ECL substrate.

Antibodies can be further tested for reactivity with antigen by Immunohistochemistry in a manner well known to the skilled person, e.g. using paraformaldehyde or acetone fixed cryosections or paraffin embedded tissue sections fixed with paraformaldehyde from non-cancer tissue or cancer tissue samples obtained from patients during routine surgical procedures or from mice carrying xenografted tumors inoculated with cell lines expressing spontaneously or after transfection antigen. For immunostaining, antibodies reactive to antigen can be incubated

followed by horseradish-peroxidase conjugated goat anti-mouse or goat anti-rabbit antibodies (DAKO) according to the vendors instructions.

Antibodies can be tested for their ability to mediate phagocytosis and killing of cells expressing CLDN18.2. The testing of monoclonal antibody activity *in vitro* will provide an initial screening prior to testing *in vivo* models.

Antibody dependent cell-mediated cytotoxicity (ADCC):

Briefly, polymorphonuclear cells (PMNs), NK cells, monocytes, mononuclear cells or other effector cells, from healthy donors can be purified by Ficoll Hypaque density centrifugation, followed by lysis of contaminating erythrocytes. Washed effector cells can be suspended in RPMI supplemented with 10% heat-inactivated fetal calf serum or, alternatively with 5% heat-inactivated human serum and mixed with ^{51}Cr labeled target cells expressing CLDN18.2, at various ratios of effector cells to target cells. Alternatively, the target cells may be labeled with a fluorescence enhancing ligand (BATDA). A highly fluorescent chelate of Europium with the enhancing ligand which is released from dead cells can be measured by a fluorometer. Another alternative technique may utilize the transfection of target cells with luciferase. Added lucifer yellow may then be oxidated by viable cells only. Purified anti-CLDN18.2 IgGs can then be added at various concentrations. Irrelevant human IgG can be used as negative control. Assays can be carried out for 4 to 20 hours at 37°C depending on the effector cell type used. Samples can be assayed for cytolysis by measuring ^{51}Cr release or the presence of the EuTDA chelate in the culture supernatant. Alternatively, luminescence resulting from the oxidation of lucifer yellow can be a measure of viable cells.

Anti-CLDN18.2 monoclonal antibodies can also be tested in various combinations to determine whether cytolysis is enhanced with multiple monoclonal antibodies.

Complement dependent cytotoxicity (CDC):

Monoclonal anti-CLDN18.2 antibodies can be tested for their ability to mediate CDC using a variety of known techniques. For example, serum for complement can be obtained from blood in a manner known to the skilled person. To determine the CDC activity of mAbs, different methods can be used. ^{51}Cr release can for example be measured or elevated membrane permeability can be assessed using a propidium iodide (PI) exclusion assay. Briefly, target cells can be washed and 5 x 10^5/ml can be incubated with various concentrations of mAb for 10-30 min. at room temperature or at 37°C. Serum or plasma can then be added to a final concentration

of 20% (v/v) and the cells incubated at 37°C for 20-30 min. All cells from each sample can be added to the PI solution in a FACS tube. The mixture can then be analyzed immediately by flow cytometry analysis using FACSArray.

In an alternative assay, induction of CDC can be determined on adherent cells. In one embodiment of this assay, cells are seeded 24 h before the assay with a density of 3 x 10^4/well in tissue-culture flat-bottom microtiter plates. The next day growth medium is removed and the cells are incubated in triplicates with antibodies. Control cells are incubated with growth medium or growth medium containing 0.2% saponin for the determination of background lysis and maximal lysis, respectively. After incubation for 20 min. at room temperature supernatant is removed and 20% (v/v) human plasma or serum in DMEM (prewarmed to 37°C) is added to the cells and incubated for another 20 min. at 37°C. All cells from each sample are added to propidium iodide solution (10 µg/ml). Then, supernatants are replaced by PBS containing 2.5 µg/ml ethidium bromide and fluorescence emission upon excitation at 520 nm is measured at 600 nm using a Tecan Safire. The percentage specific lysis is calculated as follows: % specific lysis = (fluorescence sample-fluorescence background)/ (fluorescence maximal lysis-fluorescence background) x 100.

Induction of apoptosis and inhibition of cell proliferation by monoclonal antibodies:

To test for the ability to initiate apoptosis, monoclonal anti-CLDN18.2 antibodies can, for example, be incubated with CLDN18.2 positive tumor cells, e.g., SNU-16, DAN-G, KATO-III or CLDN18.2 transfected tumor cells at 37°C for about 20 hours. The cells can be harvested, washed in Annexin-V binding buffer (BD biosciences), and incubated with Annexin V conjugated with FITC or APC (BD biosciences) for 15 min. in the dark. All cells from each sample can be added to PI solution (10 µg/ml in PBS) in a FACS tube and assessed immediately by flow cytometry (as above). Alternatively, a general inhibition of cell-proliferation by monoclonal antibodies can be detected with commercially available kits. The DELFIA Cell Proliferation Kit (Perkin-Elmer, Cat. No. AD0200) is a non-isotopic immunoassay based on the measurement of 5-bromo-2'-deoxyuridine (BrdU) incorporation during DNA synthesis of proliferating cells in microplates. Incorporated BrdU is detected using europium labelled monoclonal antibody. To allow antibody detection, cells are fixed and DNA denatured using Fix solution. Unbound antibody is washed away and DELFIA inducer is added to dissociate europium ions from the labelled antibody into solution, where they form highly fluorescent chelates with components of the DELFIA Inducer. The fluorescence measured - utilizing time-

resolved fluorometry in the detection - is proportional to the DNA synthesis in the cell of each well.

Preclinical studies

Monoclonal antibodies which bind to CLDN18.2 also can be tested in an *in vivo* model (e.g. in immune deficient mice carrying xenografted tumors inoculated with cell lines expressing CLDN18.2, e.g. DAN-G, SNU-16, or KATO-III, or after transfection, e.g. HEK293) to determine their efficacy in controlling growth of CLDN18.2-expressing tumor cells.

In vivo studies after xenografting CLDN18.2 expressing tumor cells into immunocompromised mice or other animals can be performed using antibodies described herein. Antibodies can be administered to tumor free mice followed by injection of tumor cells to measure the effects of the antibodies to prevent formation of tumors or tumor-related symptoms. Antibodies can be administered to tumor-bearing mice to determine the therapeutic efficacy of respective antibodies to reduce tumor growth, metastasis or tumor related symptoms. Antibody application can be combined with application of other substances as immune checkpoint inhibitors, cystostatic drugs, growth factor inhibitors, cell cycle blockers, angiogenesis inhibitors or other antibodies to determine efficacy and potential toxicity of combinations. To analyze toxic side effects mediated by antibodies animals can be inoculated with antibodies or control reagents and thoroughly investigated for symptoms possibly related to CLDN18.2-antibody therapy. Possible side effects of *in vivo* application of CLDN18.2 antibodies particularly include toxicity at CLDN18.2 expressing tissues including stomach. Antibodies recognizing CLDN18.2 in human and in other species, e.g. mice, are particularly useful to predict potential side effects mediated by application of monoclonal CLDN18.2-antibodies in humans.

Mapping of epitopes recognized by antibodies can be performed as described in detail in "Epitope Mapping Protocols (Methods in Molecular Biology) by Glenn E. Morris ISBN-089603-375-9 and in "Epitope Mapping: A Practical Approach" Practical Approach Series, 248 by Olwyn M. R. Westwood, Frank C. Hay.

The compounds and agents described herein may be administered in the form of any suitable pharmaceutical composition.

The term "pharmaceutical composition" relates to a formulation comprising a therapeutically effective agent, preferably together with pharmaceutically acceptable carriers, diluents and/or excipients. Said pharmaceutical composition is useful for treating, preventing, or reducing the severity of a disease or disorder by administration of said pharmaceutical composition to a subject. A pharmaceutical composition is also known in the art as a pharmaceutical formulation.

Pharmaceutical compositions are usually provided in a uniform dosage form and may be prepared in a manner known per se. A pharmaceutical composition may e.g. be in the form of a solution or suspension.

The pharmaceutical compositions according to the present disclosure are generally applied in a "pharmaceutically effective amount" and in "a pharmaceutically acceptable preparation".

The term "pharmaceutically acceptable" refers to the non-toxicity of a material which does not interact with the action of the active component of the pharmaceutical composition.

The term "pharmaceutically effective amount" or "therapeutically effective amount" refers to the amount which achieves a desired reaction or a desired effect alone or together with further doses. In the case of the treatment of a particular disease, the desired reaction preferably relates to inhibition of the course of the disease. This comprises slowing down the progress of the disease and, in particular, interrupting or reversing the progress of the disease. The desired reaction in a treatment of a disease may also be delay of the onset or a prevention of the onset of said disease or said condition. An effective amount of the compositions described herein will depend on the condition to be treated, the severeness of the disease, the individual parameters of the patient, including age, physiological condition, size and weight, the duration of treatment, the type of an accompanying therapy (if present), the specific route of administration and similar factors. Accordingly, the doses administered of the compositions described herein may depend on various of such parameters. In the case that a reaction in a patient is insufficient with an initial dose, higher doses (or effectively higher doses achieved by a different, more localized route of administration) may be used.

The pharmaceutical compositions of the present disclosure may contain salts, buffers, preservatives, and optionally other therapeutic agents. In one embodiment, the pharmaceutical

compositions of the present disclosure comprise one or more pharmaceutically acceptable carriers, diluents and/or excipients.

Suitable preservatives for use in the pharmaceutical compositions of the present disclosure include, without limitation, benzalkonium chloride, chlorobutanol, paraben and thimerosal.

The term "excipient" as used herein refers to a substance which may be present in a pharmaceutical composition of the present disclosure but is not an active ingredient. Examples of excipients, include without limitation, carriers, binders, diluents, lubricants, thickeners, surface active agents, preservatives, stabilizers, emulsifiers, buffers, flavoring agents, or colorants.

The term "diluent" relates a diluting and/or thinning agent. Moreover, the term "diluent" includes any one or more of fluid, liquid or solid suspension and/or mixing media. Examples of suitable diluents include ethanol, glycerol and water.

The term "carrier" refers to a component which may be natural, synthetic, organic, inorganic in which the active component is combined in order to facilitate, enhance or enable administration of the pharmaceutical composition. A carrier as used herein may be one or more compatible solid or liquid fillers, diluents or encapsulating substances, which are suitable for administration to subject. Suitable carrier include, without limitation, sterile water, Ringer, Ringer lactate, sterile sodium chloride solution, isotonic saline, polyalkylene glycols, hydrogenated naphthalenes and, in particular, biocompatible lactide polymers, lactide/glycolide copolymers or polyoxyethylene/polyoxy-propylene copolymers. In one embodiment, the pharmaceutical composition of the present disclosure includes isotonic saline.

Pharmaceutically acceptable carriers, excipients or diluents for therapeutic use are well known in the pharmaceutical art, and are described, for example, in Remington's Pharmaceutical Sciences, Mack Publishing Co. (A. R Gennaro edit. 1985).

Pharmaceutical carriers, excipients or diluents can be selected with regard to the intended route of administration and standard pharmaceutical practice.

In one embodiment, pharmaceutical compositions described herein may be administered intravenously, intraarterially, subcutaneously, intradermally or intramuscularly. In certain

embodiments, the pharmaceutical composition is formulated for local administration or systemic administration. Systemic administration may include enteral administration, which involves absorption through the gastrointestinal tract, or parenteral administration. As used herein, "parenteral administration" refers to the administration in any manner other than through the gastrointestinal tract, such as by intravenous injection. In a preferred embodiment, the pharmaceutical compositions is formulated for systemic administration. In another preferred embodiment, the systemic administration is by intravenous administration. The compositions may be injected directly into a tumor or lymph node.

The term "co-administering" as used herein means a process whereby different compounds or compositions are administered to the same patient. For example, the anti-CLDN18.2 antibody and the immune checkpoint inhibitor described herein may be administered simultaneously, at essentially the same time, or sequentially. If administration takes place sequentially, the anti-CLDN18.2 antibody may be administered before or after administration of the immune checkpoint inhibitor. If administration takes place simultaneously the anti-CLDN18.2 antibody and the immune checkpoint inhibitor need not be administered within the same composition. The anti-CLDN18.2 antibody and the immune checkpoint inhibitor may be administered one or more times and the number of administrations of each component may be the same or different. In addition, the anti-CLDN18.2 antibody and the immune checkpoint inhibitor need not be administered at the same site.

The agents and compositions described herein can be administered to patients, e.g., *in vivo*, to treat or prevent a variety of disorders such as those described herein. Preferred patients include human patients having disorders that can be corrected or ameliorated by administering the agents and compositions described herein. This includes disorders involving cells characterized by expression of CLDN18.2.

For example, in one embodiment, agents described herein can be used to treat a patient with a cancer disease, e.g., a cancer disease such as described herein characterized by the presence of cancer cells expressing CLDN18.2.

The pharmaceutical compositions and methods of treatment described according to the invention may also be used for immunization or vaccination to prevent a disease described herein.

As used herein, an "instructional material" or "instructions" includes a publication, a recording, a diagram, or any other medium of expression which can be used to communicate the usefulness of the compositions and methods of the invention. The instructional material of the kit of the invention may, for example, be affixed to a container which contains the compositions of the invention or be shipped together with a container which contains the compositions. Alternatively, the instructional material may be shipped separately from the container with the intention that the instructional material and the compositions be used cooperatively by the recipient.

The present invention is further illustrated by the following examples which are not be construed as limiting the scope of the invention.

EXAMPLES

Example 1: Efficacy studies of the combination of anti-CLDN18.2 antibodies and immune checkpoint inhibitors *in vivo*

In order to determine whether a combination of an anti-CLDN18.2 antibody and an immune checkpoint inhibitor provides improved anti-tumor activity over the single agents alone *in vivo*, anti-tumor activity of IMAB362 in combination with an anti-mPD-1 antibody was examined in a subcutaneously transplanted syngeneic gastric carcinoma model in immunocompetent outbred Crl:NMRI(Han) mice using CLS-103 cells with lentiviral transduction of murine CLDN18.2 (CLS-103 LVT-murinCLDN18.2). Rituximab was used as an isotype control of IMAB362.

Test antibodies

- Anti-CLDN18.2 antibody: IMAB362 (Astellas Pharma Inc.)
- control antibody: Rituximab BS Intravenous Infusion [KHK] 500 mg (Kyowa Hakko Kirin Co., Ltd., Cat# 22900AMX00971000)
- Anti-mPD-1 antibody: InVivoMAb anti-mouse PD-1, clone RMP1-14 (BioXCell, Cat#BE0146)
- isotype control antibody: InVivoMAb rat IgG2a isotype control, anti-trinitrophenol, clone 2A3 (BioXCell, Cat#BE0089)

CLS-103 LVT-murinCLDN18.2 gastric cancer mouse model

Lentivirally transduced mouse CLDN18.2 expressing tumor cells (CLS-103 LVT-

murinCLDN18.2) were engrafted subcutaneously into the right flank of female Crl:NMRI(Han) mice (10-week-old) at 2×10^6 cells/mouse. Mice were randomized based on tumor volume measured 2 days after engraftment into 4 groups (n = 12 per group). The day of randomization was defined as day 0. IMAB362 or control antibody Rituximab was administered at 800 μg/mouse. Anti-mPD-1 antibody or isotype control antibody was administered at 100 μg/mouse. All antibodies were administered by intraperitoneal injections twice per week starting on day 0. Tumors were measured twice per week. The study endpoint was defined as day 14. Tumor volume was determined by length × width × width × 0.5. Tumor growth inhibition (TGI[%]) of each group was calculated using the equation described below.

TGI[%]=100 × (1 - increase of mean tumor volume of each group※ ÷ increase of mean tumor volume of control group※)

※: increase of tumor volume [mm^3] = mean tumor volume of the last measurement of each group - mean tumor volume at randomization

#, ##: $p<0.05$, $p<0.01$, compared with each single agent group (Student's t-test).

As can be seen in Figure 1, in the mouse CLS-103 LVT-murinCLDN18.2 tumor study, the combination treatment of IMAB362 and anti-mPD-1 antibody inhibited tumor growth to a much greater degree than their single agent controls. On day 14, treatment with 800 μg of IMAB362 or 100 μg of anti-mPD-1 antibody produced 50% or 60% TGI, respectively, whereas the combination treatment comprising 800 μg of IMAB362 + 100 μg of anti-mPD-1 antibody produced 91% TGI. On day 14, the mean tumor volume of the combination group was significantly smaller compared with each single agent groups (Student's t-test). The individual CLS-103 LVT-murinCLDN18.2 tumor volumes for each of the 12 mice per group are shown for all treatment groups (Figure 2). This spider plot analysis of individual tumor growth for all treated mice showed a marked delay and/or inhibition of tumor growth in mice treated with the combination of IMAB362 and anti-mPD-1 antibody.

Example 2: Long-term efficacy studies of the combination of anti-CLDN18.2 antibodies and immune checkpoint inhibitors *in vivo*

In order to determine whether a combination of an anti-CLDN18.2 antibody and an immune checkpoint inhibitor improves anti-tumor activity over the single agents alone *in vivo* in long-

term period, anti-tumor activity of IMAB362 in combination with an anti-mPD-1 antibody was examined up to day 28 in a subcutaneously transplanted syngeneic gastric carcinoma model in immunocompetent outbred Crl:NMRI(Han) mice using CLS-103 cells with lentiviral transduction of murine CLDN18.2 (CLS-103 LVT-murinCLDN18.2). Rituximab was used as an isotype control of IMAB362.

Test antibodies

- Anti-CLDN18.2 antibody: IMAB362 (Astellas Pharma Inc.)
- control antibody: Rituximab BS Intravenous Infusion [KHK] 500 mg (Kyowa Kirin Co., Ltd., Cat# 22900AMX00971000)
- Anti-mPD-1 antibody: InVivoMAb anti-mouse PD-1, clone RMP1-14 (BioXCell, Cat#BE0146)
- isotype control antibody: InVivoMAb rat IgG2a isotype control, anti-trinitrophenol, clone 2A3 (BioXCell, Cat#BE0089)

CLS-103 LVT-murinCLDN18.2 gastric cancer mouse model

Lentivirally transduced mouse CLDN18.2 expressing tumor cells (CLS-103 LVT-murinCLDN18.2) were engrafted subcutaneously into the right flank of female Crl:NMRI(Han) mice (11-week-old) at 2×10^6 cells/mouse. Mice were randomized based on tumor volume measured 2 days after engraftment into 4 groups (n = 12 per group). The day of randomization was defined as day 0. IMAB362 or control antibody Rituximab was administered at 800 μg/mouse. Anti-mPD-1 antibody or isotype control antibody was administered at 100 μg/mouse. All antibodies were administered by intraperitoneal injections twice per week starting on day 0. Tumors were measured twice per week. The study endpoint was defined as day 28. Tumor volume was determined by length × width × width × 0.5. Mean tumor volume was calculated up to day 21 since 1 of 12 mice in the Rituximab/isotype treated group, as well as in the anti-mPD-1 antibody single agent group, was sacrificed due to tumor size being over 2000 mm^3. Tumor growth inhibition (TGI [%]) of each group was calculated based on the measurement obtained up to day 21 using the equation described below. Complete regression (CR) was determined up to day 28 as the tumor volume of individual regressed to zero.

TGI [%] = 100 × (1 - increase of mean tumor volume of each group# ÷ increase of mean tumor volume of control group#)

#: increase of mean tumor volume [mm^3] = mean tumor volume of day 21 measurement of each

group - mean tumor volume at randomization (day 0)

* $p<0.05$, ** $p<0.01$, compared with each single agent group (Student's t-test).

Results

In this mouse CLS-103 LVT-murinCLDN18.2 tumor study, IMAB362 in combination with anti-mPD-1 antibody improved the long-term anti-tumor effect which was determined by the number of CR up to day 28 in a synergistic manner. As shown in Figure 3, the individual tumor growth for each of the 12 mice per group and the number of CR mice are shown for all treatment groups. On day 28, treatment with 800 µg of IMAB362 or 100 µg of anti-mPD-1 antibody resulted in 2 or 1 CR in the group of 12 mice, respectively, whereas the combination treatment comprising 800 µg of IMAB362 + 100 µg of anti-mPD-1 antibody resulted in 6 CR in the group of 12 mice. Our results showed an increased number of mice with CR in the treatment group of the combination of IMAB362 and anti-mPD-1 antibody compared to that of single agent group in a synergistic manner. As shown in Figure 4, on day 21, treatment with 800 µg of IMAB362 or 100 µg of anti-mPD-1 antibody produced 40% TGI or no tumor growth inhibition, respectively, whereas the combination treatment comprising 800 µg of IMAB362 + 100 µg of anti-mPD-1 antibody produced 87% TGI. The mean tumor volume of the combination group was significantly smaller compared with each single agent groups and demonstrates that IMAB362 and an anti-mPD-1 antibody act in a synergistic manner.

Example 3: Efficacy studies of the combination of anti-CLDN18.2 antibodies and immune checkpoint inhibitors *in vivo*

In order to determine whether a combination of an anti-CLDN18.2 antibody and an immune checkpoint inhibitor improves anti-tumor activity over the single agents alone *in vivo,* anti-tumor activity of IMAB362 in combination with an anti-mCTLA-4 antibody was examined in a subcutaneously transplanted syngeneic gastric carcinoma model in immunocompetent outbred Crl:NMRI(Han) mice using CLS-103 cells with lentiviral transduction of murine CLDN18.2 (CLS-103 LVT-murinCLDN18.2). Rituximab was used as an isotype control of IMAB362.

Test antibodies

- Anti-CLDN18.2 antibody: IMAB362 (Astellas Pharma Inc.)

- control antibody: Rituximab BS Intravenous Infusion [KHK] 500 mg (Kyowa Kirin Co., Ltd., Cat# 22900AMX00971000)
- Anti-mCTLA-4 antibody: InVivoMAb anti-mouse CTLA-4, clone 9D9 (BioXCell, Cat#BE0164)
- isotype control antibody: InVivoMAb mouse IgG2b isotype control, unknown specificity, clone MPC-11 (BioXCell, Cat#BE0086)

CLS-103 LVT-murinCLDN18.2 gastric cancer mouse model

Lentivirally transduced mouse CLDN18.2 expressing tumor cells (CLS-103 LVT-murinCLDN18.2) were engrafted subcutaneously into the right flank of female Crl:NMRI(Han) mice (11-week-old) at 2×10^6 cells/mouse. Mice were randomized based on tumor volume measured 2 days after engraftment into 4 groups (n = 12 per group). The day of randomization was defined as day 0. IMAB362 or control antibody Rituximab was administered at 800 µg/mouse. Anti-mCTLA-4 antibody or isotype control antibody was administered at 300 µg/mouse. All antibodies were administered by intraperitoneal injections twice per week starting on day 0. Tumors were measured twice per week. The study endpoint was defined as day 14. Tumor volume was determined by length × width × width × 0.5. Tumor growth inhibition (TGI [%]) or tumor regression rate (TRR [%]) of each group was calculated using the equations described below.

TGI [%] = 100 × (1 - increase of mean tumor volume of each group# ÷ increase of mean tumor volume of control group#)

#: increase of mean tumor volume [mm^3] = mean tumor volume of day 14 measurement of each group - mean tumor volume at randomization (day 0)

TRR [%] = 100 × (1 - mean tumor volume of day14 measurement of each group ÷ mean tumor volume at randomization of each group)

*: $p<0.05$, compared with each single agent group (Student's t-test).

Results

As can be seen in Figure 5, in the mouse CLS-103 LVT-murinCLDN18.2 tumor study, the combination treatment of IMAB362 and anti-mCTLA-4 antibody inhibited tumor growth to a greater degree than their single agent groups. On day 14, treatment with 800 µg of IMAB362 or

300 μg of anti-mCTLA-4 antibody produced 72% or 87% TGI, respectively, whereas the combination treatment comprising 800 μg of IMAB362 + 300 μg of anti-mCTLA-4 antibody not only inhibited, but also regressed tumor to 3% TRR. The individual CLS-103 LVT-murinCLDN18.2 tumor volumes for each of the 12 mice per group are shown for all treatment groups (Figure 6). In mice treated with the combination of IMAB362 and anti-mCTLA-4 antibody, the individual tumor growth for all treated mice showed an inhibition or even regression of tumor growth.

Example 4: Efficacy studies of the combination of anti-CLDN18.2 antibodies and immune checkpoint inhibitors *in vivo*

In order to determine whether a combination of an anti-CLDN18.2 antibody and an immune checkpoint inhibitor improves anti-tumor activity over the single agents alone *in vivo*, anti-tumor activity of IMAB362 in combination with an anti-mPD-L1 antibody was examined in a subcutaneously transplanted syngeneic gastric carcinoma model in immunocompetent outbred Crl:NMRI(Han) mice using CLS-103 cells with lentiviral transduction of murine CLDN18.2 (CLS-103 LVT-murinCLDN18.2). Rituximab was used as an isotype control of IMAB362.

Test antibodies

- Anti-CLDN18.2 antibody: IMAB362 (Astellas Pharma Inc.)
- control antibody: Rituximab BS Intravenous Infusion [KHK] 500 mg (Kyowa Kirin Co., Ltd., Cat# 22900AMX00971000)
- Anti-mPD-L1 antibody: InVivoMAb anti-mouse PD-L1, clone 10F.9G2 (BioXCell, Cat#BE0101)
- isotype control antibody: InVivoMAb rat IgG2b isotype control, anti-keyhole limpet hemocyanin, clone LTF-2 (BioXCell, Cat#BE0090)

CLS-103 LVT-murinCLDN18.2 gastric cancer mouse model

Lentivirally transduced mouse CLDN18.2 expressing tumor cells (CLS-103 LVT-murinCLDN18.2) were engrafted subcutaneously into the right flank of female Crl:NMRI(Han) mice (11-week-old) at 2×10^6 cells/mouse. Mice were randomized based on tumor volume measured 2 days after engraftment into 4 groups (n = 12 per group). The Rituximab/Isotype control group consisted of the data from 11 out of 12 mice due to 1 death occurring on day 11. The day of randomization was defined as day 0. IMAB362 or control antibody Rituximab was

administered at 800 μg/mouse. Anti-mPD-L1 antibody or isotype control antibody was administered at 300 μg/mouse. All antibodies were administered by intraperitoneal injections twice per week starting on day 0. Tumors were measured twice per week. The study endpoint was defined as day 14. Tumor volume was determined by length × width × width × 0.5. Tumor growth inhibition (TGI [%]) or tumor regression rate (TRR [%]) of each group was calculated using the equations described below.

TGI [%] = 100 × (1 - increase of mean tumor volume of each group# ÷ increase of mean tumor volume of control group#)

#: increase of mean tumor volume [mm^3] = mean tumor volume of day 14 measurement of each group - mean tumor volume at randomization (day 0)

TRR [%] = 100 × (1 - mean tumor volume of day 14 measurement of each group ÷ mean tumor volume at randomization of each group)

*, **: $p<0.05$, $p<0.01$, compared with each single agent group (Student's t-test).

Results

In Figure 7, in the mouse CLS-103 LVT-murinCLDN18.2 tumor study, the combination treatment of IMAB362 and anti-mPD-L1 antibody inhibited tumor growth to a much greater degree than their single agent groups. On day 14, treatment with 800 μg of IMAB362 or 300 μg of anti-mPD-L1 antibody produced 60% or 62% TGI, respectively, whereas the combination treatment comprising 800 μg of IMAB362 + 300 μg of anti-mPD-L1 antibody not only inhibited, but also regressed the tumor to 13% TRR. On day 14, mean tumor volume of the combination group was significantly smaller compared with each single agent groups and demonstrates that IMAB362 and an anti-mPD-L1 antibody act in a synergistic manner. The individual CLS-103 LVT-murinCLDN18.2 tumor volumes of each mouse per group are shown for all treatment groups (Figure 8). In mice treated with the combination of IMAB362 and anti-mPD-L1 antibody, the individual tumor growth for all treated mice showed an inhibition or even regression of tumor growth in a synergistic manner.

SEQUENCE LISTING

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<120> COMBINATION THERAPY INVOLVING ANTIBODIES AGAINST CLAUDIN 18.2 AND IMMUNE CHECKPOINT INHIBITORS FOR TREATMENT OF CANCER

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Met Ala Val Thr Ala Cys Gln Gly Leu Gly Phe Val Val Ser Leu Ile
1 5 10 15

Gly Ile Ala Gly Ile Ile Ala Ala Thr Cys Met Asp Gln Trp Ser Thr
20 25 30

Gln Asp Leu Tyr Asn Asn Pro Val Thr Ala Val Phe Asn Tyr Gln Gly
35 40 45

Leu Trp Arg Ser Cys Val Arg Glu Ser Ser Gly Phe Thr Glu Cys Arg
50 55 60

Gly Tyr Phe Thr Leu Leu Gly Leu Pro Ala Met Leu Gln Ala Val Arg
65 70 75 80

Ala Leu Met Ile Val Gly Ile Val Leu Gly Ala Ile Gly Leu Leu Val
85 90 95

Ser Ile Phe Ala Leu Lys Cys Ile Arg Ile Gly Ser Met Glu Asp Ser
100 105 110

Ala Lys Ala Asn Met Thr Leu Thr Ser Gly Ile Met Phe Ile Val Ser
115 120 125

Gly Leu Cys Ala Ile Ala Gly Val Ser Val Phe Ala Asn Met Leu Val

130 135 140

Thr Asn Phe Trp Met Ser Thr Ala Asn Met Tyr Thr Gly Met Gly Gly
145 150 155 160

Met Val Gln Thr Val Gln Thr Arg Tyr Thr Phe Gly Ala Ala Leu Phe
165 170 175

Val Gly Trp Val Ala Gly Gly Leu Thr Leu Ile Gly Gly Val Met Met
180 185 190

Cys Ile Ala Cys Arg Gly Leu Ala Pro Glu Glu Thr Asn Tyr Lys Ala
195 200 205

Val Ser Tyr His Ala Ser Gly His Ser Val Ala Tyr Lys Pro Gly Gly
210 215 220

Phe Lys Ala Ser Thr Gly Phe Gly Ser Asn Thr Lys Asn Lys Lys Ile
225 230 235 240

Tyr Asp Gly Gly Ala Arg Thr Glu Asp Glu Val Gln Ser Tyr Pro Ser
245 250 255

Lys His Asp Tyr Val
260

<210> 2
<211> 261
<212> PRT
<213> Homo sapiens

<400> 2

Met Ser Thr Thr Thr Cys Gln Val Val Ala Phe Leu Leu Ser Ile Leu
1 5 10 15

Gly Leu Ala Gly Cys Ile Ala Ala Thr Gly Met Asp Met Trp Ser Thr
20 25 30

Gln Asp Leu Tyr Asp Asn Pro Val Thr Ser Val Phe Gln Tyr Glu Gly
35 40 45

Leu Trp Arg Ser Cys Val Arg Gln Ser Ser Gly Phe Thr Glu Cys Arg
50 55 60

Pro Tyr Phe Thr Ile Leu Gly Leu Pro Ala Met Leu Gln Ala Val Arg
65 70 75 80

Ala Leu Met Ile Val Gly Ile Val Leu Gly Ala Ile Gly Leu Leu Val
85 90 95

Ser Ile Phe Ala Leu Lys Cys Ile Arg Ile Gly Ser Met Glu Asp Ser
100 105 110

Ala Lys Ala Asn Met Thr Leu Thr Ser Gly Ile Met Phe Ile Val Ser
115 120 125

Gly Leu Cys Ala Ile Ala Gly Val Ser Val Phe Ala Asn Met Leu Val
130 135 140

Thr Asn Phe Trp Met Ser Thr Ala Asn Met Tyr Thr Gly Met Gly Gly
145 150 155 160

Met Val Gln Thr Val Gln Thr Arg Tyr Thr Phe Gly Ala Ala Leu Phe
165 170 175

Val Gly Trp Val Ala Gly Gly Leu Thr Leu Ile Gly Gly Val Met Met
180 185 190

Cys Ile Ala Cys Arg Gly Leu Ala Pro Glu Glu Thr Asn Tyr Lys Ala
195 200 205

Val Ser Tyr His Ala Ser Gly His Ser Val Ala Tyr Lys Pro Gly Gly
210 215 220

Phe Lys Ala Ser Thr Gly Phe Gly Ser Asn Thr Lys Asn Lys Lys Ile
225 230 235 240

Tyr Asp Gly Gly Ala Arg Thr Glu Asp Glu Val Gln Ser Tyr Pro Ser
245 250 255

Lys His Asp Tyr Val
260

〈210〉 3
〈211〉 10
〈212〉 PRT
〈213〉 Homo sapiens

<400> 3

Asp Gln Trp Ser Thr Gln Asp Leu Tyr Asn
1 5 10

<210> 4
<211> 11
<212> PRT
<213> Homo sapiens

<400> 4

Asn Asn Pro Val Thr Ala Val Phe Asn Tyr Gln
1 5 10

<210> 5
<211> 14
<212> PRT
<213> Homo sapiens

<400> 5

Ser Thr Gln Asp Leu Tyr Asn Asn Pro Val Thr Ala Val Phe
1 5 10

<210> 6
<211> 13
<212> PRT
<213> Homo sapiens

<400> 6

Thr Asn Phe Trp Met Ser Thr Ala Asn Met Tyr Thr Gly
1 5 10

<210> 7
<211> 13
<212> PRT
<213> Homo sapiens

<400> 7

Asp Ser Ala Lys Ala Asn Met Thr Leu Thr Ser Gly Ile
1 5 10

<210> 8
<211> 55
<212> PRT
<213> Homo sapiens

<400> 8

Met Asp Gln Trp Ser Thr Gln Asp Leu Tyr Asn Asn Pro Val Thr Ala
1 5 10 15

Val Phe Asn Tyr Gln Gly Leu Trp Arg Ser Cys Val Arg Glu Ser Ser
20 25 30

Gly Phe Thr Glu Cys Arg Gly Tyr Phe Thr Leu Leu Gly Leu Pro Ala
35 40 45

Met Leu Gln Ala Val Arg Ala
50 55

<210> 9
<211> 24
<212> PRT
<213> Homo sapiens

<400> 9

Phe Ala Leu Lys Cys Ile Arg Ile Gly Ser Met Glu Asp Ser Ala Lys
1 5 10 15

Ala Asn Met Thr Leu Thr Ser Gly
20

<210> 10
<211> 40
<212> PRT
<213> Homo sapiens

<400> 10

Ala Asn Met Leu Val Thr Asn Phe Trp Met Ser Thr Ala Asn Met Tyr
1 5 10 15

Thr Gly Met Gly Gly Met Val Gln Thr Val Gln Thr Arg Tyr Thr Phe
20 25 30

Gly Ala Ala Leu Phe Val Gly Trp
35 40

<210> 11
<211> 153
<212> PRT
<213> Homo sapiens

<400> 11

Met Asp Gln Trp Ser Thr Gln Asp Leu Tyr Asn Asn Pro Val Thr Ala
1 5 10 15

Val Phe Asn Tyr Gln Gly Leu Trp Arg Ser Cys Val Arg Glu Ser Ser
20 25 30

Gly Phe Thr Glu Cys Arg Gly Tyr Phe Thr Leu Leu Gly Leu Pro Ala
35 40 45

Met Leu Gln Ala Val Arg Ala Leu Met Ile Val Gly Ile Val Leu Gly
50 55 60

Ala Ile Gly Leu Leu Val Ser Ile Phe Ala Leu Lys Cys Ile Arg Ile
65 70 75 80

Gly Ser Met Glu Asp Ser Ala Lys Ala Asn Met Thr Leu Thr Ser Gly
85 90 95

Ile Met Phe Ile Val Ser Gly Leu Cys Ala Ile Ala Gly Val Ser Val
100 105 110

Phe Ala Asn Met Leu Val Thr Asn Phe Trp Met Ser Thr Ala Asn Met
115 120 125

Tyr Thr Gly Met Gly Gly Met Val Gln Thr Val Gln Thr Arg Tyr Thr
130 135 140

Phe Gly Ala Ala Leu Phe Val Gly Trp
145 150

<210> 12
<211> 107
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: Translation of PCR product

<400> 12

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
1 5 10 15

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
20 25 30

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
35 40 45

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
50 55 60

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
65 70 75 80

Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
85 90 95

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
100 105

<210> 13
<211> 326
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: Translation of PCR product

<400> 13

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
1 5 10 15

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
20 25 30

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
35 40 45

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
50 55 60

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
65 70 75 80

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro
85 90 95

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu

```
                100                 105                 110

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
        115                 120                 125

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
    130                 135                 140

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
145                 150                 155                 160

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
                165                 170                 175

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
            180                 185                 190

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
        195                 200                 205

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
    210                 215                 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn
225                 230                 235                 240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
                245                 250                 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
            260                 265                 270

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
        275                 280                 285

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys
    290                 295                 300

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
305                 310                 315                 320

Ser Leu Ser Pro Gly Lys
                325
```

<210> 14
<211> 466
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: chimeric monoclonal antibody

<400> 14

Met Glu Trp Thr Trp Val Phe Leu Phe Leu Leu Ser Val Thr Ala Gly
1 5 10 15

Val His Ser Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Met Lys
20 25 30

Pro Gly Ala Ser Val Lys Ile Ser Cys Lys Ala Thr Gly Tyr Thr Phe
35 40 45

Ser Ser Tyr Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly Leu
50 55 60

Glu Trp Ile Gly Glu Ile Leu Pro Gly Ser Gly Ser Thr Asn Tyr Asn
65 70 75 80

Glu Lys Phe Lys Gly Lys Ala Thr Phe Thr Ala Asp Thr Ser Ser Asn
85 90 95

Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
100 105 110

Tyr Tyr Cys Ala Arg Tyr Asp Tyr Pro Trp Phe Ala Tyr Trp Gly Gln
115 120 125

Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys Gly Pro Ser Val
130 135 140

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
145 150 155 160

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
165 170 175

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val

180 185 190

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
195 200 205

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
210 215 220

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
225 230 235 240

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
245 250 255

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
260 265 270

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
275 280 285

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
290 295 300

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
305 310 315 320

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
325 330 335

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
340 345 350

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
355 360 365

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
370 375 380

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
385 390 395 400

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
405 410 415

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
420 425 430

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
435 440 445

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
450 455 460

Gly Lys
465

<210> 15
<211> 467
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: chimeric monoclonal antibody

<400> 15

Met Asp Trp Leu Trp Asn Leu Leu Phe Leu Met Ala Ala Ala Gln Ser
1 5 10 15

Ile Gln Ala Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys
20 25 30

Pro Gly Glu Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Asn Tyr Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu
50 55 60

Lys Trp Met Gly Trp Ile Asn Thr Asn Thr Gly Glu Pro Thr Tyr Ala
65 70 75 80

Glu Glu Phe Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser
85 90 95

Thr Ala Tyr Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr
100 105 110

Tyr Phe Cys Ala Arg Leu Gly Phe Gly Asn Ala Met Asp Tyr Trp Gly

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        115                 120                 125

Gln Gly Thr Ser Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
    130                 135                 140

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
145                 150                 155                 160

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
                165                 170                 175

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
            180                 185                 190

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
        195                 200                 205

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
    210                 215                 220

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
225                 230                 235                 240

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
                245                 250                 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
            260                 265                 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
        275                 280                 285

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
    290                 295                 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
305                 310                 315                 320

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
                325                 330                 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
            340                 345                 350
```

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
355 360 365

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
370 375 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385 390 395 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
405 410 415

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
420 425 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
435 440 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
450 455 460

Pro Gly Lys
465

<210> 16
<211> 465
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: chimeric monoclonal antibody

<400> 16

Met Glu Trp Ile Trp Ile Phe Leu Phe Ile Leu Ser Gly Thr Ala Gly
1 5 10 15

Val His Ser Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg
20 25 30

Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Asp Tyr Tyr Ile Asn Trp Val Lys Gln Arg Thr Gly Gln Gly Leu

```
    50                  55                  60

Glu Trp Ile Gly Glu Ile Tyr Pro Gly Ser Gly Asn Thr Tyr Tyr Asn
65                  70                  75                  80

Glu Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser
                85                  90                  95

Thr Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val
            100                 105                 110

Tyr Phe Cys Ala Arg Ser Tyr Gly Ala Phe Asp Tyr Trp Gly Gln Gly
        115                 120                 125

Thr Thr Leu Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val Phe
    130                 135                 140

Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala Leu
145                 150                 155                 160

Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser Trp
                165                 170                 175

Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val Leu
            180                 185                 190

Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro Ser
        195                 200                 205

Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys Pro
    210                 215                 220

Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp Lys
225                 230                 235                 240

Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly Pro
                245                 250                 255

Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile Ser
            260                 265                 270

Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu Asp
        275                 280                 285
```

Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His Asn
290 295 300

Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg Val
305 310 315 320

Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys Glu
325 330 335

Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu Lys
340 345 350

Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr Thr
355 360 365

Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu Thr
370 375 380

Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp Glu
385 390 395 400

Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val Leu
405 410 415

Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp Lys
420 425 430

Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His Glu
435 440 445

Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro Gly
450 455 460

Lys
465

<210> 17
<211> 467
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: chimeric monoclonal antibody

<400> 17

```
Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1               5                  10                  15

Val His Ser Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Arg
            20                  25                  30

Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe
        35                  40                  45

Thr Ser Tyr Trp Ile Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
    50                  55                  60

Glu Trp Ile Gly Asn Ile Tyr Pro Ser Asp Ser Tyr Thr Asn Tyr Asn
65                  70                  75                  80

Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser
                85                  90                  95

Thr Ala Tyr Met Gln Leu Ser Ser Pro Thr Ser Glu Asp Ser Ala Val
            100                 105                 110

Tyr Tyr Cys Thr Arg Ser Trp Arg Gly Asn Ser Phe Asp Tyr Trp Gly
        115                 120                 125

Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
    130                 135                 140

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
145                 150                 155                 160

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
                165                 170                 175

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
            180                 185                 190

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
        195                 200                 205

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
    210                 215                 220
```

Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys
225 230 235 240

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
245 250 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
260 265 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
275 280 285

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
290 295 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
305 310 315 320

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
325 330 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
340 345 350

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val
355 360 365

Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser
370 375 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385 390 395 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
405 410 415

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
420 425 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
435 440 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
450 455 460

Pro Gly Lys
465

<210> 18
<211> 466
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: chimeric monoclonal antibody

<400> 18

Met Glu Trp Arg Ile Phe Leu Phe Ile Leu Ser Gly Thr Ala Gly Val
1 5 10 15

His Ser Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro
20 25 30

Gly Ala Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr
35 40 45

Asp Tyr Val Ile Ser Trp Val Lys Gln Arg Thr Gly Gln Gly Leu Glu
50 55 60

Trp Ile Gly Glu Ile Tyr Pro Gly Ser Gly Ser Thr Tyr Tyr Asn Glu
65 70 75 80

Lys Phe Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Asn Thr
85 90 95

Ala Tyr Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr
100 105 110

Phe Cys Ala Arg Gly Val Leu Leu Arg Ala Met Asp Tyr Trp Gly Gln
115 120 125

Gly Thr Ser Val Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser Val
130 135 140

Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala Ala
145 150 155 160

Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val Ser
165 170 175

Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala Val
180 185 190

Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val Pro
195 200 205

Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His Lys
210 215 220

Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys Ser Cys Asp
225 230 235 240

Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly Gly
245 250 255

Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met Ile
260 265 270

Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His Glu
275 280 285

Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val His
290 295 300

Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr Arg
305 310 315 320

Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly Lys
325 330 335

Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile Glu
340 345 350

Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val Tyr
355 360 365

Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln Val Ser Leu
370 375 380

Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu Trp
385 390 395 400

Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro Val
405 410 415

Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val Asp
420 425 430

Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met His
435 440 445

Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser Pro
450 455 460

Gly Lys
465

<210> 19
<211> 469
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: chimeric monoclonal antibody

<400> 19

Met Asp Trp Ile Trp Ile Met Leu His Leu Leu Ala Ala Ala Thr Gly
1 5 10 15

Ile Gln Ser Gln Val His Leu Gln Gln Ser Gly Ser Glu Leu Arg Ser
20 25 30

Pro Gly Ser Ser Val Lys Leu Ser Cys Lys Asp Phe Asp Ser Glu Val
35 40 45

Phe Pro Phe Ala Tyr Met Ser Trp Ile Arg Gln Lys Pro Gly His Gly
50 55 60

Phe Glu Trp Ile Gly Asp Ile Leu Pro Ser Ile Gly Arg Thr Ile Tyr
65 70 75 80

Gly Glu Lys Phe Glu Asp Lys Ala Thr Leu Asp Ala Asp Thr Val Ser
85 90 95

Asn Thr Ala Tyr Leu Glu Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala
100 105 110

Ile Tyr Tyr Cys Ala Arg Gly Glu Gly Tyr Gly Ala Trp Phe Ala Tyr
115 120 125

Trp Gly Gln Gly Thr Leu Val Thr Val Ser Ala Ala Ser Thr Lys Gly
130 135 140

Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly
145 150 155 160

Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val
165 170 175

Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe
180 185 190

Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val
195 200 205

Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val
210 215 220

Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Lys Val Glu Pro Lys
225 230 235 240

Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu
245 250 255

Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr
260 265 270

Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val
275 280 285

Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val
290 295 300

Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser
305 310 315 320

Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu
325 330 335

Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala
340 345 350

Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro
355 360 365

Gln Val Tyr Thr Leu Pro Pro Ser Arg Asp Glu Leu Thr Lys Asn Gln
370 375 380

Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala
385 390 395 400

Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr
405 410 415

Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu
420 425 430

Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser
435 440 445

Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser
450 455 460

Leu Ser Pro Gly Lys
465

<210> 20
<211> 240
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: chimeric monoclonal antibody

<400> 20

Met Glu Ser Gln Thr Gln Val Leu Met Ser Leu Leu Phe Trp Val Ser
1 5 10 15

Gly Thr Cys Gly Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Thr
20 25 30

Val Thr Ala Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser
35 40 45

Leu Leu Asn Ser Gly Asn Gln Lys Asn Tyr Leu Thr Trp Tyr Gln Gln
50 55 60

Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg
65 70 75 80

Glu Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp
85 90 95

Phe Thr Leu Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr
100 105 110

Tyr Cys Gln Asn Asp Tyr Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr
115 120 125

Lys Leu Glu Leu Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe
130 135 140

Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys
145 150 155 160

Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
165 170 175

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln
180 185 190

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser
195 200 205

Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His
210 215 220

Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235 240

<210> 21
<211> 235
<212> PRT

<213> Artificial

<220>
<223> Description of artificial sequence: chimeric monoclonal antibody

<400> 21

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Met His Phe Gln Val Gln Ile Phe Ser Phe Leu Leu Ile Ser Ala Ser
1               5                  10                  15

Val Ile Met Ser Arg Gly Gln Ile Val Leu Thr Gln Ser Pro Ala Ile
            20                  25                  30

Met Ser Ala Ser Pro Gly Glu Lys Val Thr Ile Thr Cys Ser Ala Ser
        35                  40                  45

Ser Ser Val Ser Tyr Met His Trp Phe Gln Gln Lys Pro Gly Thr Ser
    50                  55                  60

Pro Lys Leu Trp Ile Tyr Ser Thr Ser Asn Leu Ala Ser Gly Val Pro
65                  70                  75                  80

Ala Arg Phe Ser Gly Ser Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile
                85                  90                  95

Ser Arg Met Glu Ala Glu Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Arg
            100                 105                 110

Ser Ser Tyr Pro Pro Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
        115                 120                 125

Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu
    130                 135                 140

Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe
145                 150                 155                 160

Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln
                165                 170                 175

Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser
            180                 185                 190

Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu
        195                 200                 205
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Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser
210 215 220

Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 22
<211> 234
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: chimeric monoclonal antibody

<400> 22

Met Glu Phe Gln Thr Gln Val Phe Val Phe Val Leu Leu Trp Leu Ser
1 5 10 15

Gly Val Asp Gly Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser
20 25 30

Thr Ser Val Gly Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asn
35 40 45

Val Arg Thr Ala Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro
50 55 60

Lys Ala Leu Ile Tyr Leu Ala Ser Asn Arg His Thr Gly Val Pro Asp
65 70 75 80

Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Asn Val Gln Ser Glu Asp Leu Ala Asp Tyr Phe Cys Leu Gln His Trp
100 105 110

Asn Tyr Pro Leu Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys Arg
115 120 125

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
130 135 140

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr

145 150 155 160

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
165 170 175

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
180 185 190

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
195 200 205

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
210 215 220

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230

<210> 23
<211> 240
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: chimeric monoclonal antibody

<400> 23

Met Asp Ser Gln Ala Gln Val Leu Met Leu Leu Leu Trp Val Ser
1 5 10 15

Gly Thr Cys Gly Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala
20 25 30

Val Ser Val Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser
35 40 45

Leu Leu Tyr Ser Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln
50 55 60

Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg
65 70 75 80

Glu Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp
85 90 95

Phe Thr Leu Thr Ile Ser Ser Val Lys Ala Glu Asp Leu Ala Val Tyr
100 105 110

Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr
115 120 125

Lys Leu Glu Leu Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe
130 135 140

Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys
145 150 155 160

Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
165 170 175

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln
180 185 190

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser
195 200 205

Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His
210 215 220

Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235 240

<210> 24
<211> 240
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: chimeric monoclonal antibody

<400> 24

Met Glu Ser Gln Thr Gln Val Leu Met Ser Leu Leu Phe Trp Val Ser
1 5 10 15

Gly Thr Cys Gly Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Thr
20 25 30

Val Thr Ala Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser
35 40 45

Leu Leu Asn Ser Gly Asn Gln Lys Asn Tyr Leu Thr Trp Tyr Gln Gln
50 55 60

Lys Pro Gly Gln Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg
65 70 75 80

Glu Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp
85 90 95

Phe Thr Leu Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr
100 105 110

Tyr Cys Gln Asn Asp Tyr Ser Tyr Pro Phe Thr Phe Gly Ser Gly Thr
115 120 125

Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe
130 135 140

Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys
145 150 155 160

Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
165 170 175

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln
180 185 190

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser
195 200 205

Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His
210 215 220

Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235 240

〈210〉 25
〈211〉 239
〈212〉 PRT
〈213〉 Artificial

〈220〉
〈223〉 Description of artificial sequence: chimeric monoclonal antibody

<400> 25

Met Asp Ser Gln Ala Gln Val Leu Ile Leu Leu Leu Leu Trp Val Ser
1 5 10 15

Gly Thr Cys Gly Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala
20 25 30

Val Ser Ala Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser
35 40 45

Leu Leu Asn Ser Arg Thr Arg Lys Asn Tyr Leu Ala Trp Tyr Gln Gln
50 55 60

Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg
65 70 75 80

Glu Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp
85 90 95

Phe Thr Leu Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr
100 105 110

Tyr Cys Lys Gln Ser Tyr Asn Leu Tyr Thr Phe Gly Gly Gly Thr Lys
115 120 125

Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro
130 135 140

Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu
145 150 155 160

Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp
165 170 175

Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp
180 185 190

Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys
195 200 205

Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His Gln
210 215 220

Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235

<210> 26
<211> 240
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: chimeric monoclonal antibody

<400> 26

Met Asp Ser Gln Ala Gln Val Leu Met Leu Leu Leu Trp Val Ser
1 5 10 15

Gly Thr Cys Gly Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala
20 25 30

Val Ser Val Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser
35 40 45

Leu Leu Tyr Ser Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln
50 55 60

Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg
65 70 75 80

Glu Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Ala Thr Asp
85 90 95

Phe Thr Leu Thr Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Asp Tyr
100 105 110

His Cys Gly Gln Gly Tyr Ser Tyr Pro Tyr Thr Phe Gly Gly Gly Thr
115 120 125

Lys Leu Glu Ile Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe
130 135 140

Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys
145 150 155 160

Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
165 170 175

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln
180 185 190

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser
195 200 205

Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His
210 215 220

Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235 240

<210> 27
<211> 240
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: chimeric monoclonal antibody

<400> 27

Met Asp Ser Gln Ala Gln Val Leu Met Leu Leu Leu Leu Trp Val Ser
1 5 10 15

Gly Thr Cys Gly Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala
20 25 30

Val Ser Val Gly Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser
35 40 45

Leu Leu Tyr Ser Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln
50 55 60

Lys Pro Gly Gln Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg
65 70 75 80

Glu Ser Gly Val Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp
85 90 95

Phe Thr Leu Thr Ile Ser Ser Val Lys Ala Glu Asp Leu Ala Val Tyr
100 105 110

Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr

115 120 125

Lys Leu Glu Leu Lys Arg Thr Val Ala Ala Pro Ser Val Phe Ile Phe
130 135 140

Pro Pro Ser Asp Glu Gln Leu Lys Ser Gly Thr Ala Ser Val Val Cys
145 150 155 160

Leu Leu Asn Asn Phe Tyr Pro Arg Glu Ala Lys Val Gln Trp Lys Val
165 170 175

Asp Asn Ala Leu Gln Ser Gly Asn Ser Gln Glu Ser Val Thr Glu Gln
180 185 190

Asp Ser Lys Asp Ser Thr Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser
195 200 205

Lys Ala Asp Tyr Glu Lys His Lys Val Tyr Ala Cys Glu Val Thr His
210 215 220

Gln Gly Leu Ser Ser Pro Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230 235 240

<210> 28
<211> 234
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: chimeric monoclonal antibody

<400> 28

Met Glu Ser Gln Thr Leu Val Phe Ile Ser Ile Leu Leu Trp Leu Tyr
1 5 10 15

Gly Ala Asp Gly Asn Ile Val Met Thr Gln Ser Pro Lys Ser Met Ser
20 25 30

Met Ser Val Gly Glu Arg Val Thr Leu Thr Cys Lys Ala Ser Glu Asn
35 40 45

Val Val Thr Tyr Val Ser Trp Tyr Gln Gln Lys Pro Glu Gln Ser Pro
50 55 60

Lys Leu Leu Ile Tyr Gly Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp
65 70 75 80

Arg Phe Thr Gly Ser Gly Ser Ala Thr Asp Phe Thr Leu Thr Ile Ser
85 90 95

Ser Val Lys Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln Tyr Tyr
100 105 110

Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys Arg
115 120 125

Thr Val Ala Ala Pro Ser Val Phe Ile Phe Pro Pro Ser Asp Glu Gln
130 135 140

Leu Lys Ser Gly Thr Ala Ser Val Val Cys Leu Leu Asn Asn Phe Tyr
145 150 155 160

Pro Arg Glu Ala Lys Val Gln Trp Lys Val Asp Asn Ala Leu Gln Ser
165 170 175

Gly Asn Ser Gln Glu Ser Val Thr Glu Gln Asp Ser Lys Asp Ser Thr
180 185 190

Tyr Ser Leu Ser Ser Thr Leu Thr Leu Ser Lys Ala Asp Tyr Glu Lys
195 200 205

His Lys Val Tyr Ala Cys Glu Val Thr His Gln Gly Leu Ser Ser Pro
210 215 220

Val Thr Lys Ser Phe Asn Arg Gly Glu Cys
225 230

<210> 29
<211> 117
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: Translation of PCR product

<400> 29

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Met Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Ile Ser Cys Lys Ala Thr Gly Tyr Thr Phe Ser Ser Tyr
20 25 30

Trp Ile Glu Trp Val Lys Gln Arg Pro Gly His Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Leu Pro Gly Ser Gly Ser Thr Asn Tyr Asn Glu Lys Phe
50 55 60

Lys Gly Lys Ala Thr Phe Thr Ala Asp Thr Ser Ser Asn Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Ala Arg Tyr Asp Tyr Pro Trp Phe Ala Tyr Trp Gly Gln Gly Thr Leu
100 105 110

Val Thr Val Ser Ala
115

<210> 30
<211> 118
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: Translation of PCR product

<400> 30

Gln Ile Gln Leu Val Gln Ser Gly Pro Glu Leu Lys Lys Pro Gly Glu
1 5 10 15

Thr Val Lys Ile Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asn Tyr
20 25 30

Gly Met Asn Trp Val Lys Gln Ala Pro Gly Lys Gly Leu Lys Trp Met
35 40 45

Gly Trp Ile Asn Thr Asn Thr Gly Glu Pro Thr Tyr Ala Glu Glu Phe
50 55 60

Lys Gly Arg Phe Ala Phe Ser Leu Glu Thr Ser Ala Ser Thr Ala Tyr
65 70 75 80

Leu Gln Ile Asn Asn Leu Lys Asn Glu Asp Thr Ala Thr Tyr Phe Cys
85 90 95

Ala Arg Leu Gly Phe Gly Asn Ala Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Ser Val Thr Val Ser Ser
115

<210> 31
<211> 116
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: Translation of PCR product

<400> 31

Gln Val Gln Leu Gln Gln Ser Gly Ala Glu Leu Ala Arg Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Tyr Ile Asn Trp Val Lys Gln Arg Thr Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Tyr Pro Gly Ser Gly Asn Thr Tyr Tyr Asn Glu Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Ser Tyr Gly Ala Phe Asp Tyr Trp Gly Gln Gly Thr Thr Leu
100 105 110

Thr Val Ser Ser
115

<210> 32

<211> 118
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: Translation of PCR product

<400> 32

Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Arg Pro Gly Ala
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Ser Tyr
20 25 30

Trp Ile Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Asn Ile Tyr Pro Ser Asp Ser Tyr Thr Asn Tyr Asn Gln Lys Phe
50 55 60

Lys Asp Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Pro Thr Ser Glu Asp Ser Ala Val Tyr Tyr Cys
85 90 95

Thr Arg Ser Trp Arg Gly Asn Ser Phe Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Thr Leu Thr Val Ser Ser
115

<210> 33
<211> 118
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: Translation of PCR product

<400> 33

Gln Val Gln Leu Gln Gln Ser Gly Pro Glu Leu Val Lys Pro Gly Ala
1 5 10 15

Ser Val Lys Met Ser Cys Lys Ala Ser Gly Tyr Thr Phe Thr Asp Tyr
20 25 30

Val Ile Ser Trp Val Lys Gln Arg Thr Gly Gln Gly Leu Glu Trp Ile
35 40 45

Gly Glu Ile Tyr Pro Gly Ser Gly Ser Thr Tyr Tyr Asn Glu Lys Phe
50 55 60

Lys Gly Lys Ala Thr Leu Thr Ala Asp Lys Ser Ser Asn Thr Ala Tyr
65 70 75 80

Met Gln Leu Ser Ser Leu Thr Ser Glu Asp Ser Ala Val Tyr Phe Cys
85 90 95

Ala Arg Gly Val Leu Leu Arg Ala Met Asp Tyr Trp Gly Gln Gly Thr
100 105 110

Ser Val Thr Val Ser Ser
115

<210> 34
<211> 120
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: Translation of PCR product

<400> 34

Gln Val His Leu Gln Gln Ser Gly Ser Glu Leu Arg Ser Pro Gly Ser
1 5 10 15

Ser Val Lys Leu Ser Cys Lys Asp Phe Asp Ser Glu Val Phe Pro Phe
20 25 30

Ala Tyr Met Ser Trp Ile Arg Gln Lys Pro Gly His Gly Phe Glu Trp
35 40 45

Ile Gly Asp Ile Leu Pro Ser Ile Gly Arg Thr Ile Tyr Gly Glu Lys
50 55 60

Phe Glu Asp Lys Ala Thr Leu Asp Ala Asp Thr Val Ser Asn Thr Ala
65 70 75 80

Tyr Leu Glu Leu Asn Ser Leu Thr Ser Glu Asp Ser Ala Ile Tyr Tyr

85 90 95

Cys Ala Arg Gly Glu Gly Tyr Gly Ala Trp Phe Ala Tyr Trp Gly Gln
100 105 110

Gly Thr Leu Val Thr Val Ser Ala
115 120

<210> 35
<211> 113
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: Translation of PCR product

<400> 35

Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Thr Val Thr Ala Gly
1 5 10 15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
20 25 30

Gly Asn Gln Lys Asn Tyr Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn
85 90 95

Asp Tyr Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
100 105 110

Lys

<210> 36
<211> 106
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: Translation of PCR product

<400> 36

Gln Ile Val Leu Thr Gln Ser Pro Ala Ile Met Ser Ala Ser Pro Gly
1 5 10 15

Glu Lys Val Thr Ile Thr Cys Ser Ala Ser Ser Ser Val Ser Tyr Met
20 25 30

His Trp Phe Gln Gln Lys Pro Gly Thr Ser Pro Lys Leu Trp Ile Tyr
35 40 45

Ser Thr Ser Asn Leu Ala Ser Gly Val Pro Ala Arg Phe Ser Gly Ser
50 55 60

Gly Ser Gly Thr Ser Tyr Ser Leu Thr Ile Ser Arg Met Glu Ala Glu
65 70 75 80

Asp Ala Ala Thr Tyr Tyr Cys Gln Gln Arg Ser Ser Tyr Pro Pro Thr
85 90 95

Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 37
<211> 107
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: Translation of PCR product

<400> 37

Asp Ile Val Met Thr Gln Ser Gln Lys Phe Met Ser Thr Ser Val Gly
1 5 10 15

Asp Arg Val Ser Ile Thr Cys Lys Ala Ser Gln Asn Val Arg Thr Ala
20 25 30

Val Ala Trp Tyr Gln Gln Lys Pro Gly Gln Ser Pro Lys Ala Leu Ile
35 40 45

Tyr Leu Ala Ser Asn Arg His Thr Gly Val Pro Asp Arg Phe Thr Gly

50 55 60

Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr Ile Ser Asn Val Gln Ser
65 70 75 80

Glu Asp Leu Ala Asp Tyr Phe Cys Leu Gln His Trp Asn Tyr Pro Leu
85 90 95

Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105

<210> 38
<211> 113
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: Translation of PCR product

<400> 38

Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala Val Ser Val Gly
1 5 10 15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30

Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Val Lys Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
85 90 95

Tyr Tyr Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
100 105 110

Lys

<210> 39
<211> 113
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: Translation of PCR product

<400> 39

Asp Ile Val Met Thr Gln Ser Pro Ser Ser Leu Thr Val Thr Ala Gly
1 5 10 15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser
20 25 30

Gly Asn Gln Lys Asn Tyr Leu Thr Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Pro Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Asn
85 90 95

Asp Tyr Ser Tyr Pro Phe Thr Phe Gly Ser Gly Thr Lys Leu Glu Ile
100 105 110

Lys

<210> 40
<211> 112
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: Translation of PCR product

<400> 40

Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala Val Ser Ala Gly
1 5 10 15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Asn Ser

20 25 30

Arg Thr Arg Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Val Tyr Tyr Cys Lys Gln
85 90 95

Ser Tyr Asn Leu Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile Lys
100 105 110

<210> 41
<211> 113
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: Translation of PCR product

<400> 41

Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala Val Ser Val Gly
1 5 10 15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30

Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Ala Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Val Gln Ala Glu Asp Leu Ala Asp Tyr His Cys Gly Gln
85 90 95

Gly Tyr Ser Tyr Pro Tyr Thr Phe Gly Gly Gly Thr Lys Leu Glu Ile
100 105 110

Lys

<210> 42
<211> 113
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: Translation of PCR product

<400> 42

Asp Ile Val Met Ser Gln Ser Pro Ser Ser Leu Ala Val Ser Val Gly
1 5 10 15

Glu Lys Val Thr Met Ser Cys Lys Ser Ser Gln Ser Leu Leu Tyr Ser
20 25 30

Ser Asn Gln Lys Asn Tyr Leu Ala Trp Tyr Gln Gln Lys Pro Gly Gln
35 40 45

Ser Pro Lys Leu Leu Ile Tyr Trp Ala Ser Thr Arg Glu Ser Gly Val
50 55 60

Pro Asp Arg Phe Thr Gly Ser Gly Ser Gly Thr Asp Phe Thr Leu Thr
65 70 75 80

Ile Ser Ser Val Lys Ala Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln
85 90 95

Tyr Tyr Ser Tyr Pro Leu Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu
100 105 110

Lys

<210> 43
<211> 107
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: Translation of PCR product

<400> 43

Asn Ile Val Met Thr Gln Ser Pro Lys Ser Met Ser Met Ser Val Gly
1 5 10 15

Glu Arg Val Thr Leu Thr Cys Lys Ala Ser Glu Asn Val Val Thr Tyr
20 25 30

Val Ser Trp Tyr Gln Gln Lys Pro Glu Gln Ser Pro Lys Leu Leu Ile
35 40 45

Tyr Gly Ala Ser Asn Arg Tyr Thr Gly Val Pro Asp Arg Phe Thr Gly
50 55 60

Ser Gly Ser Ala Thr Asp Phe Thr Leu Thr Ile Ser Ser Val Lys Ala
65 70 75 80

Glu Asp Leu Ala Val Tyr Tyr Cys Gln Gln Tyr Tyr Ser Tyr Pro Leu
85 90 95

Thr Phe Gly Ala Gly Thr Lys Leu Glu Leu Lys
100 105

<210> 44
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Epitope

<400> 44

Met Asp Gln Trp Ser Thr Gln Asp Leu Tyr Asn Asn Pro Val Thr
1 5 10 15

<210> 45
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Epitope

<400> 45

Ser Thr Gln Asp Leu Tyr Asn Asn Pro Val Thr Ala Val Phe Asn
1 5 10 15

<210> 46
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Epitope

<400> 46

Leu Tyr Asn Asn Pro Val Thr Ala Val Phe Asn Tyr Gln Gly Leu
1 5 10 15

<210> 47
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Epitope

<400> 47

Pro Val Thr Ala Val Phe Asn Tyr Gln Gly Leu Trp Arg Ser Cys
1 5 10 15

<210> 48
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Epitope

<400> 48

Val Phe Asn Tyr Gln Gly Leu Trp Arg Ser Cys Val Arg Glu Ser
1 5 10 15

<210> 49
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Epitope

<400> 49

Gln Gly Leu Trp Arg Ser Cys Val Arg Glu Ser Ser Gly Phe Thr
1 5 10 15

<210> 50
<211> 15
<212> PRT
<213> Artificial

<220>
<223> Epitope

<400> 50

Arg Ser Cys Val Arg Glu Ser Ser Gly Phe Thr Glu Cys Arg Gly
1 5 10 15

<210> 51
<211> 467
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: chimeric monoclonal antibody

<400> 51

Met Gly Trp Ser Cys Ile Ile Leu Phe Leu Val Ala Thr Ala Thr Gly
1 5 10 15

Val His Ser Gln Val Gln Leu Gln Gln Pro Gly Ala Glu Leu Val Arg
20 25 30

Pro Gly Ala Ser Val Lys Leu Ser Cys Lys Ala Ser Gly Tyr Thr Phe
35 40 45

Thr Ser Tyr Trp Ile Asn Trp Val Lys Gln Arg Pro Gly Gln Gly Leu
50 55 60

Glu Trp Ile Gly Asn Ile Tyr Pro Ser Asp Ser Tyr Thr Asn Tyr Asn
65 70 75 80

Gln Lys Phe Lys Asp Lys Ala Thr Leu Thr Val Asp Lys Ser Ser Ser
85 90 95

Thr Ala Tyr Met Gln Leu Ser Ser Pro Thr Ser Glu Asp Ser Ala Val
100 105 110

Tyr Tyr Cys Thr Arg Ser Trp Arg Gly Asn Ser Phe Asp Tyr Trp Gly
115 120 125

Gln Gly Thr Thr Leu Thr Val Ser Ser Ala Ser Thr Lys Gly Pro Ser
130 135 140

Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly Gly Thr Ala
145 150 155 160

Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro Val Thr Val
165 170 175

Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr Phe Pro Ala
180 185 190

Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val Val Thr Val
195 200 205

Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn Val Asn His
210 215 220

Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro Lys Ser Cys
225 230 235 240

Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu Leu Leu Gly
245 250 255

Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp Thr Leu Met
260 265 270

Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp Val Ser His
275 280 285

Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly Val Glu Val
290 295 300

His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn Ser Thr Tyr
305 310 315 320

Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp Leu Asn Gly
325 330 335

Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro Ala Pro Ile
340 345 350

Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu Pro Gln Val

355 360 365

Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn Gln Val Ser
370 375 380

Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile Ala Val Glu
385 390 395 400

Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr Thr Pro Pro
405 410 415

Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys Leu Thr Val
420 425 430

Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys Ser Val Met
435 440 445

His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu Ser Leu Ser
450 455 460

Pro Gly Lys
465

<210> 52
<211> 326
<212> PRT
<213> Artificial

<220>
<223> Description of artificial sequence: Translation of PCR product

<400> 52

Gly Pro Ser Val Phe Pro Leu Ala Pro Ser Ser Lys Ser Thr Ser Gly
1 5 10 15

Gly Thr Ala Ala Leu Gly Cys Leu Val Lys Asp Tyr Phe Pro Glu Pro
20 25 30

Val Thr Val Ser Trp Asn Ser Gly Ala Leu Thr Ser Gly Val His Thr
35 40 45

Phe Pro Ala Val Leu Gln Ser Ser Gly Leu Tyr Ser Leu Ser Ser Val
50 55 60

Val Thr Val Pro Ser Ser Ser Leu Gly Thr Gln Thr Tyr Ile Cys Asn
65 70 75 80

Val Asn His Lys Pro Ser Asn Thr Lys Val Asp Lys Arg Val Glu Pro
85 90 95

Lys Ser Cys Asp Lys Thr His Thr Cys Pro Pro Cys Pro Ala Pro Glu
100 105 110

Leu Leu Gly Gly Pro Ser Val Phe Leu Phe Pro Pro Lys Pro Lys Asp
115 120 125

Thr Leu Met Ile Ser Arg Thr Pro Glu Val Thr Cys Val Val Val Asp
130 135 140

Val Ser His Glu Asp Pro Glu Val Lys Phe Asn Trp Tyr Val Asp Gly
145 150 155 160

Val Glu Val His Asn Ala Lys Thr Lys Pro Arg Glu Glu Gln Tyr Asn
165 170 175

Ser Thr Tyr Arg Val Val Ser Val Leu Thr Val Leu His Gln Asp Trp
180 185 190

Leu Asn Gly Lys Glu Tyr Lys Cys Lys Val Ser Asn Lys Ala Leu Pro
195 200 205

Ala Pro Ile Glu Lys Thr Ile Ser Lys Ala Lys Gly Gln Pro Arg Glu
210 215 220

Pro Gln Val Tyr Thr Leu Pro Pro Ser Arg Glu Glu Met Thr Lys Asn
225 230 235 240

Gln Val Ser Leu Thr Cys Leu Val Lys Gly Phe Tyr Pro Ser Asp Ile
245 250 255

Ala Val Glu Trp Glu Ser Asn Gly Gln Pro Glu Asn Asn Tyr Lys Thr
260 265 270

Thr Pro Pro Val Leu Asp Ser Asp Gly Ser Phe Phe Leu Tyr Ser Lys
275 280 285

Leu Thr Val Asp Lys Ser Arg Trp Gln Gln Gly Asn Val Phe Ser Cys

290 295 300

Ser Val Met His Glu Ala Leu His Asn His Tyr Thr Gln Lys Ser Leu
305 310 315 320

Ser Leu Ser Pro Gly Lys
325

Claims:

1. A method for treating or preventing cancer in a patient, comprising administering to the patient an anti-CLDN18.2 antibody and an immune checkpoint inhibitor.

2. A method for inhibiting growth of a tumor in a patient having cancer, comprising administering to the patient an anti-CLDN18.2 antibody and an immune checkpoint inhibitor.

3. A method for inducing antibody-dependent cell-mediated cytotoxicity (ADCC) against cancer cells in a patient having cancer, comprising administering to the patient an anti-CLDN18.2 antibody and an immune checkpoint inhibitor.

4. The method of any one of claims 1 to 3, wherein the immune checkpoint inhibitor is selected from a PD-1 inhibitor, and a PD-L1 inhibitor.

5. The method of any one of claims 1 to 4, wherein the immune checkpoint inhibitor is selected from an anti-PD-1 antibody, and an anti-PD-L1 antibody.

6. The method of any one of claims 1 to 5, wherein the immune checkpoint inhibitor is an anti-PD-1 antibody.

7. The method of claim 6, wherein the anti-PD-1 antibody is nivolumab (OPDIVO; BMS-936558), pembrolizumab (KEYTRUDA; MK-3475), pidilizumab (CT-011), cemiplimab (LIBTAYO, REGN2810), spartalizumab (PDR001),, MEDI0680 (AMP-514), dostarlimab (TSR-042), cetrelimab (JNJ 63723283), toripalimab (JS001), AMP-224 (GSK-2661380), PF-06801591, tislelizumab (BGB-A317), ABBV-181, BI 754091, or SHR-1210.

8. The method of any one of claims 1 to 5, wherein the immune checkpoint inhibitor is an anti-PD-L1 antibody.

9. The method of claim 8, wherein the anti-PD-L1 antibody is atezolizumab (TECENTRIQ; RG7446; MPDL3280A; R05541267), durvalumab (MEDI4736), BMS-936559, avelumab (bavencio), lodapolimab (LY3300054),, CX-072 (Proclaim-CX-072), FAZ053, KN035, or MDX-1105.

10. The method of any one of claims 1 to 3, wherein the immune checkpoint inhibitor is a CTLA-4 inhibitor.

11. The method of any one of claims 1 to 3, and 10, wherein the immune checkpoint inhibitor is an anti-CTLA-4 antibody.

12. The method of claim 11, wherein the anti-CTLA-4 antibody is ipilimumab (Yervoy; Bristol Myers Squibb), tremelimumab (Pfizer/Medlmmune), trevilizumab, AGEN-1884 (Agenus) or ATOR-1015.

13. The method of any one of claims 1 to 12, wherein the anti-CLDN18.2 antibody binds to the first extracellular loop of CLDN18.2.

14. The method of any one of claims 1 to 13, wherein the anti-CLDN18.2 antibody mediates cell killing by one or more of complement-dependent cytotoxicity (CDC) mediated lysis, antibody-dependent cell-mediated cytotoxicity (ADCC) mediated lysis, induction of apoptosis and inhibition of proliferation.

15. The method of any one of claims 1 to 14, wherein the anti-CLDN18.2 antibody is an antibody selected from the group consisting of:
(i) an antibody produced by and/or obtainable from a clone deposited under the accession no. DSM ACC2737, DSM ACC2738, DSM ACC2739, DSM ACC2740, DSM ACC2741, DSM ACC2742, DSM ACC2743, DSM ACC2745, DSM ACC2746, DSM ACC2747, DSM ACC2748, DSM ACC2808, DSM ACC2809, or DSM ACC2810,
(ii) an antibody which is a chimerized or humanized form of the antibody under (i),
(iii) an antibody having the specificity of the antibody under (i), and
(iv) an antibody comprising the antigen binding portion or antigen binding site, in particular the variable region, of the antibody under (i) and preferably having the specificity of the antibody under (i).

16. The method of any one of claims 1 to 15, wherein the anti-CLDN18.2 antibody comprises a heavy chain variable region CDR1 comprising the sequence of positions 45-52 of the sequence set forth in SEQ ID NO: 17, a heavy chain variable region CDR2 comprising the

sequence of positions 70-77 of the sequence set forth in SEQ ID NO: 17, a heavy chain variable region CDR3 comprising the sequence of positions 116-126 of the sequence set forth in SEQ ID NO: 17, a light chain variable region CDR1 comprising the sequence of positions 47-58 of the sequence set forth in SEQ ID NO: 24, a light chain variable region CDR2 comprising the sequence of positions 76-78 of the sequence set forth in SEQ ID NO: 24, and a light chain variable region CDR3 comprising the sequence of positions 115-123 of the sequence set forth in SEQ ID NO: 24.

17. The method of any one of claims 1 to 16, wherein the anti-CLDN18.2 antibody comprises a heavy chain variable region comprising the sequence set forth in SEQ ID NO: 32 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant.

18. The method of any one of claims 1 to 17, wherein the anti-CLDN18.2 antibody comprises a light chain variable region comprising the sequence set forth in SEQ ID NO: 39 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant.

19. The method of any one of claims 1 to 18, wherein the anti-CLDN18.2 antibody comprises a heavy chain constant region comprising the sequence set forth in SEQ ID NO: 13 or 52, or a functional variant thereof, or a fragment of the amino acid sequence or functional variant.

20. The method of any one of claims 1 to 19, wherein the anti-CLDN18.2 antibody comprises a heavy chain comprising the sequence set forth in SEQ ID NO: 17 or 51, or a functional variant thereof, or a fragment of the amino acid sequence or functional variant.

21. The method of any one of claims 1 to 20, wherein the anti-CLDN18.2 antibody comprises a light chain comprising the sequence set forth in SEQ ID NO: 24 or a functional variant thereof, or a fragment of the amino acid sequence or functional variant.

22. The method of any one of claims 1 to 21, wherein the method comprises administering the anti-CLDN18.2 antibody at a dose of up to 1000 mg/m^2.

23. The method of any one of claims 1 to 22, wherein the method comprises administering the anti-CLDN18.2 antibody repeatedly at a dose of 300 to 600 mg/m^2.

24. The method of any one of claims 1 to 23, wherein the cancer is CLDN18.2 positive.

25. The method of any one of claims 1 to 24, wherein the cancer is an adenocarcinoma, in particular an advanced adenocarcinoma.

26. The method of any one of claims 1 to 25, wherein the cancer is selected from the group consisting of cancer of the stomach, cancer of the esophagus, in particular the lower esophagus, cancer of the eso-gastric junction and gastroesophageal cancer.

27. The method of any one of claims 1 to 26, wherein CLDN18.2 has the amino acid sequence according to SEQ ID NO: 1.

28. A medical preparation comprising an anti-CLDN18.2 antibody and an immune checkpoint inhibitor.

29. The medical preparation of claim 28, which is a kit comprising a first container including the anti-CLDN18.2 antibody and a second container including the immune checkpoint inhibitor.

30. The medical preparation of claim 28 or 29, further including printed instructions for use of the preparation for treatment of cancer.

31. The medical preparation of claim 28, which is a composition comprising the anti-CLDN18.2 antibody and the immune checkpoint inhibitor.