The present invention demonstrates the existence of anti-CLDN18.2 monoclonal antibodies that can be highly efficiently internalized upon CLDN18.2 binding on CLND18.2-expressing cells and therefore are suitable for ADC development. Furthermore, the successful conjugation of such antibodies to the drugs DM4 and MMAE using cleavable SPDB or Val-Cit (vc) linkers, respectively, is disclosed. *In vitro*, the antibody conjugates reduce viability of gastric and pancreatic cancer cells expressing CLDN18.2. IMAB362-vcMMAE and IMAB362-DM4 do not bind to or influence viability of CLDN18.2 negative cells. Both, the DM4 and vcMMAE conjugates exert bystander killing effects on CLDN18.2 negative cancer cells co-cultured with CLDN18.2 positive cancer cells *in vitro*. Furthermore, *in vivo*, intravenous administration of the antibody conjugates in nude mice with CLDN18.2-positive gastric or pancreatic xenograft tumors results in dose-dependent tumor growth inhibition, survival benefit and even complete regression of early and advanced tumors. Significant therapeutic effects are observed at single dose intravenous application of ~4-8 mg/kg; optimal therapeutic effects are achieved at 15-16 mg/kg. The maximum tolerated single dose of both conjugates could not be determined as the highest possible tested doses of 15.2 and 16 mg/kg did not result in liver toxicities or other toxic effects.

From the data presented herein it can be concluded that anti-CLDN18.2 antibody-drug conjugates such as those described herein are highly potent drugs for the treatment of CLDN18.2-positive human carcinomas such as gastric and pancreatic carcinomas.

SUMMARY

The present invention generally provides a therapy for effectively treating and/or preventing cancer associated with cells expressing CLDN18.2 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 refers to the use of an antibody-drug conjugate for the preparation of a medicament for treating a CLDN18.2-expressing cancer wherein the antibody-drug conjugate is to be administered to a patient, said antibody-drug conjugate comprising an antibody having the ability of binding to CLDN18.2 covalently attached to at least one toxin drug moiety, wherein the antibody-drug conjugate, upon binding to cells expressing CLDN18.2 is internalized into the cells, wherein (a) the antibody having the ability of binding to CLDN18.2 recognizes the same or essentially the same epitope as a CLDN18.2-binding antibody comprising an antibody heavy chain comprising an amino acid sequence represented by SEQ ID NO: 32 and an antibody light chain comprising an amino acid sequence represented by SEQ ID NO: 39, and/or competes with said CLDN18.2-binding antibody for binding to CLDN18.2; or (b) the antibody having the ability of binding to CLDN18.2 recognizes the same or essentially the same epitope as a CLDN18.2-binding antibody comprising an antibody heavy chain comprising an amino acid sequence represented by SEQ ID NO: 30 and an antibody light chain comprising an amino acid sequence represented by SEQ ID NO: 35, and/or competes with said CLDN18.2-binding antibody for binding to CLDN18.2.

Also disclosed herein is a a method of treating or preventing a CLDN18.2-expressing cancer comprising administering an antibody-drug conjugate comprising an antibody having the ability of binding to CLDN18.2 covalently attached to at least one toxin drug moiety to a cancer patient.

In one example, the antibody-drug conjugate is internalized into cells following binding to CLDN18.2 expressed by the cells.

In one example, the antibody having the ability of binding to CLDN18.2 specifically binds to CLDN18.2. In one example, the antibody-drug conjugate specifically binds to CLDN18.2.

In one example, the antibody having the ability of binding to CLDN18.2 is a monoclonal, chimeric or humanized antibody, or a fragment of an antibody. In one example, the antibody having the ability of binding to CLDN18.2 is a monoclonal antibody.

In one example, the antibody having the ability of binding to CLDN18.2 binds to native epitopes of CLDN18.2 present on the surface of living cells. In one example, the antibody having the ability of binding to CLDN18.2 binds to an extracellular domain of CLDN18.2. In one example, the antibody having the ability of binding to CLDN18.2 binds to the first extracellular loop of CLDN18.2.

In one example, the antibody having the ability of binding to CLDN18.2 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 example, the antibody having the ability of binding to CLDN18.2 comprises a heavy chain comprising an amino acid sequence represented by SEQ ID NO: 32 or a fragment thereof or a variant of said amino acid sequence or fragment and a light chain comprising an

amino acid sequence represented by SEQ ID NO: 39 or a fragment thereof or a variant of said amino acid sequence or fragment. In one example, the antibody having the ability of binding to CLDN18.2 comprises a heavy chain comprising an amino acid sequence represented by SEQ ID NO: 17 or 51 or a fragment thereof or a variant of said amino acid sequence or fragment and a light chain comprising an amino acid sequence represented by SEQ ID NO: 24 or a fragment thereof or a variant of said amino acid sequence or fragment. In one example, the antibody having the ability of binding to CLDN18.2 comprises a heavy chain comprising an amino acid sequence represented by SEQ ID NO: 30 or a fragment thereof or a variant of said amino acid sequence or fragment and a light chain comprising an amino acid sequence represented by SEQ ID NO: 35 or a fragment thereof or a variant of said amino acid sequence or fragment. In one example, the antibody having the ability of binding to CLDN18.2 comprises a heavy chain comprising an amino acid sequence represented by SEQ ID NO: 15 or a fragment thereof or a variant of said amino acid sequence or fragment and a light chain comprising an amino acid sequence represented by SEQ ID NO: 20 or a fragment thereof or a variant of said amino acid sequence or fragment. In one example, the antibody having the ability of binding to CLDN18.2 recognizes the same or essentially the same epitope as a CLDN18.2-binding antibody comprising a heavy chain comprising an amino acid sequence represented by SEQ ID NO: 32 or a fragment thereof or a variant of said amino acid sequence or fragment and a light chain comprising an amino acid sequence represented by SEQ ID NO: 39 or a fragment thereof or a variant of said amino acid sequence or fragment, and/or competes with said CLDN18.2-binding antibody for binding to CLDN18.2. In one example, the antibody having the ability of binding to CLDN18.2 recognizes the same or essentially the same epitope as a CLDN18.2-binding antibody comprising a heavy chain comprising an amino acid sequence represented by SEQ ID NO: 17 or 51 or a fragment thereof or a variant of said amino acid sequence or fragment and a light chain comprising an amino acid sequence represented by SEQ ID NO: 24 or a fragment thereof or a variant of said amino acid sequence or fragment, and/or competes with said CLDN18.2-binding antibody for binding to CLDN18.2. In one example, the antibody having the ability of binding to CLDN18.2 recognizes the same or essentially the same epitope as a CLDN18.2-binding antibody comprising a heavy chain comprising an amino acid sequence represented by SEQ ID NO: 30 or a fragment thereof or a variant of said amino acid sequence or fragment and a light chain comprising an amino acid sequence represented by SEQ ID NO: 35 or a fragment thereof or a variant of said amino acid sequence or fragment, and/or competes with said CLDN18.2-binding antibody for binding to CLDN18.2. In one

example, the antibody having the ability of binding to CLDN18.2 recognizes the same or essentially the same epitope as a CLDN18.2-binding antibody comprising a heavy chain comprising an amino acid sequence represented by SEQ ID NO: 15 or a fragment thereof or a variant of said amino acid sequence or fragment and a light chain comprising an amino acid sequence represented by SEQ ID NO: 20 or a fragment thereof or a variant of said amino acid sequence or fragment, and/or competes with said CLDN18.2-binding antibody for binding to CLDN18.2. An antibody which competes with a second antibody for binding to a target preferably is antagonistic to said second antibody.

In one example, the toxin drug moiety is cell membrane-permeable. In one example, the toxin drug moiety is a cytotoxic or cytostatic agent. In one example, the toxin drug moiety is a maytansinoid or an auristatin. In one example, the maytansinoid is selected from the group consisting of DM1 and DM4. In one example, the auristatin is selected from the group consisting of monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF).

In one example, the antibody having the ability of binding to CLDN18.2 is covalently attached to the toxin drug moiety by a linker. In one example, the linker is a cleavable linker. In one example, the linker is cleavable under intracellular conditions. In one example, the linker is hydrolyzable at a pH of less than 5.5. In one example, the linker is cleavable by an intracellular protease. In one example, the linker is a cathepsin-cleavable linker. In one example, the linker comprises a dipeptide. In one example, the dipeptide is val-cit or phe-lys. In one example, the antibody is attached to the linker through a cysteine thiol of the antibody. In one example, the antibody is attached to the linker through amine groups, in particular amine groups of lysine residues of the antibody.

In one example, the antibody-drug conjugate is administered in an amount effective for the treatment or prevention of the CLDN18.2-expressing cancer. In one example, the antibody-drug conjugate is administered at a dose of between 3 to 30 mg/kg body weight, such as between 4 to 25, 5 to 20, 10 to 18, or 15 to 16 mg/kg body weight. In one example, the antibody-drug conjugate is administered at a dose of between 8 to 150, 9 to 100 or 9 to 90 mg/m^2 body surface of a human patient, such as between 12 to 75, 15 to 60, 30 to 54, or 45 to 48 mg/m^2 body surface of a human patient. In one example, a single dose of the antibody-drug conjugate or two or more doses of the antibody-drug conjugate are

administered. In one example, the antibody-drug conjugate is administered by intravenous injection.

In one example, the method described herein further comprises administering surgery, chemotherapy and/or radiation therapy.

In one example, expression of CLDN18.2 is at the cell surface of cancer cells. In one example, the cancer is an adenocarcinoma, in particular an advanced adenocarcinoma. In one example, the cancer is selected from the group consisting of gastric cancer, esophageal cancer, pancreatic cancer, lung cancer such as non small cell lung cancer (NSCLC), breast cancer, ovarian cancer, colon cancer, hepatic cancer, head-neck cancer, cancer of the gallbladder and the metastasis thereof, a Krukenberg tumor, peritoneal metastasis and/or lymph node metastasis. In one example, 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 example, the patient is a HER2/neu negative patient or a patient with HER2/neu positive status but not eligible to trastuzumab therapy.

In one example, CLDN18.2 has the amino acid sequence according to SEQ ID NO: 1.

Also disclosed herein is an antibody-drug conjugate comprising an antibody having the ability of binding to CLDN18.2 covalently attached to at least one toxin drug moiety.

In one example, the antibody-drug conjugate is internalized into cells following binding to CLDN18.2 expressed by the cells.

In one example, the antibody having the ability of binding to CLDN18.2 specifically binds to CLDN18.2. In one example, the antibody-drug conjugate specifically binds to CLDN18.2.

In one example, the antibody having the ability of binding to CLDN18.2 is a monoclonal, chimeric or humanized antibody, or a fragment of an antibody. In one example, the antibody having the ability of binding to CLDN18.2 is a monoclonal antibody.

In one example, the antibody having the ability of binding to CLDN18.2 binds to native epitopes of CLDN18.2 present on the surface of living cells. In one example, the antibody having the ability of binding to CLDN18.2 binds to an extracellular domain of CLDN18.2. In one example, the antibody having the ability of binding to CLDN18.2 binds to the first extracellular loop of CLDN18.2.

In one example, the antibody having the ability of binding to CLDN18.2 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 example, the antibody having the ability of binding to CLDN18.2 comprises a heavy chain comprising an amino acid sequence represented by SEQ ID NO: 32 or a fragment thereof or a variant of said amino acid sequence or fragment and a light chain comprising an amino acid sequence represented by SEQ ID NO: 39 or a fragment thereof or a variant of said amino acid sequence or fragment. In one example, the antibody having the ability of binding to CLDN18.2 comprises a heavy chain comprising an amino acid sequence represented by SEQ ID NO: 17 or 51 or a fragment thereof or a variant of said amino acid sequence or fragment and a light chain comprising an amino acid sequence represented by SEQ ID NO: 24 or a fragment thereof or a variant of said amino acid sequence or fragment. In one example, the antibody having the ability of binding to CLDN18.2 comprises a heavy chain comprising an amino acid sequence represented by SEQ ID NO: 30 or a fragment thereof or a variant of said amino acid sequence or fragment and a light chain comprising an amino acid sequence represented by SEQ ID NO: 35 or a fragment thereof or a variant of said amino acid sequence or fragment. In one example, the antibody having the ability of binding to CLDN18.2 comprises a heavy chain comprising an amino acid sequence represented by SEQ ID NO: 15 or a fragment thereof or a variant of said amino acid sequence or fragment and a light chain comprising an amino acid sequence represented by SEQ ID NO: 20 or a fragment thereof or a variant of said amino acid sequence or fragment. In one

example, the antibody having the ability of binding to CLDN18.2 recognizes the same or essentially the same epitope as a CLDN18.2-binding antibody comprising a heavy chain comprising an amino acid sequence represented by SEQ ID NO: 32 or a fragment thereof or a variant of said amino acid sequence or fragment and a light chain comprising an amino acid sequence represented by SEQ ID NO: 39 or a fragment thereof or a variant of said amino acid sequence or fragment, and/or competes with said CLDN18.2-binding antibody for binding to CLDN18.2. In one example, the antibody having the ability of binding to CLDN18.2 recognizes the same or essentially the same epitope as a CLDN18.2-binding antibody comprising a heavy chain comprising an amino acid sequence represented by SEQ ID NO: 17 or 51 or a fragment thereof or a variant of said amino acid sequence or fragment and a light chain comprising an amino acid sequence represented by SEQ ID NO: 24 or a fragment thereof or a variant of said amino acid sequence or fragment, and/or competes with said CLDN18.2-binding antibody for binding to CLDN18.2. In one example, the antibody having the ability of binding to CLDN18.2 recognizes the same or essentially the same epitope as a CLDN18.2-binding antibody comprising a heavy chain comprising an amino acid sequence represented by SEQ ID NO: 30 or a fragment thereof or a variant of said amino acid sequence or fragment and a light chain comprising an amino acid sequence represented by SEQ ID NO: 35 or a fragment thereof or a variant of said amino acid sequence or fragment, and/or competes with said CLDN18.2-binding antibody for binding to CLDN18.2. In one example, the antibody having the ability of binding to CLDN18.2 recognizes the same or essentially the same epitope as a CLDN18.2-binding antibody comprising a heavy chain comprising an amino acid sequence represented by SEQ ID NO: 15 or a fragment thereof or a variant of said amino acid sequence or fragment and a light chain comprising an amino acid sequence represented by SEQ ID NO: 20 or a fragment thereof or a variant of said amino acid sequence or fragment, and/or competes with said CLDN18.2-binding antibody for binding to CLDN18.2. An antibody which competes with a second antibody for binding to a target preferably is antagonistic to said second antibody.

In one example, the toxin drug moiety is cell membrane-permeable. In one example, the toxin drug moiety is a cytotoxic or cytostatic agent. In one example, the toxin drug moiety is a maytansinoid or an auristatin. In one example, the maytansinoid is selected from the group consisting of DM1 and DM4. In one example, the auristatin is selected from the group consisting of monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF).

In one example, the antibody having the ability of binding to CLDN18.2 is covalently attached to the toxin drug moiety by a linker. In one example, the linker is a cleavable linker. In one example, the linker is cleavable under intracellular conditions. In one example, the linker is hydrolyzable at a pH of less than 5.5. In one example, the linker is cleavable by an intracellular protease. In one example, the linker is a cathepsin-cleavable linker. In one example, the linker comprises a dipeptide. In one example, the dipeptide is val-cit or phe-lys. In one example, the antibody is attached to the linker through a cysteine thiol of the antibody. In one example, the antibody is attached to the linker through amine groups, in particular amine groups of lysine residues of the antibody.

In one example, CLDN18.2 has the amino acid sequence according to SEQ ID NO: 1.

Also disclosed herein is a pharmaceutical formulation comprising the antibody-drug conjugate disclosed herein, and a pharmaceutically acceptable diluent, carrier or excipient.

Also disclosed herein is a medical preparation comprising the antibody-drug conjugate as disclosed herein. In one example, the medical preparation is present in the form of a kit comprising a container including the antibody-drug conjugate. In one example, the medical preparation further includes printed instructions for use of the preparation in a method of treating or preventing cancer, in particular a CLDN18.2-expressing cancer.

Also disclosed herein is the antibody-drug conjugate as described herein, the pharmaceutical composition as described herein or the medical preparation as described herein for use in therapy, in particular for use in a method of treating or preventing cancer, in particular a CLDN18.2-expressing cancer. In one example, the method of treating or preventing cancer is a method of treating or preventing a CLDN18.2-expressing cancer.

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

CLAIMS

1. Use of an antibody-drug conjugate for the preparation of a medicament for treating a CLDN18.2-expressing cancer wherein the antibody-drug conjugate is to be administered to a patient, said antibody-drug conjugate comprising an antibody having the ability of binding to CLDN18.2 covalently attached to at least one toxin drug moiety, wherein the antibody-drug conjugate, upon binding to cells expressing CLDN18.2 is internalized into the cells, wherein
(a) the antibody having the ability of binding to CLDN18.2 recognizes the same or essentially the same epitope as a CLDN18.2-binding antibody comprising an antibody heavy chain comprising an amino acid sequence represented by SEQ ID NO: 32 and an antibody light chain comprising an amino acid sequence represented by SEQ ID NO: 39, and/or competes with said CLDN18.2-binding antibody for binding to CLDN18.2; or
(b) the antibody having the ability of binding to CLDN18.2 recognizes the same or essentially the same epitope as a CLDN18.2-binding antibody comprising an antibody heavy chain comprising an amino acid sequence represented by SEQ ID NO: 30 and an antibody light chain comprising an amino acid sequence represented by SEQ ID NO: 35, and/or competes with said CLDN18.2-binding antibody for binding to CLDN18.2.

2. The use of claim 1, wherein the antibody comprises an antibody heavy chain comprising an amino acid sequence represented by SEQ ID NO: 32 and an antibody light chain comprising an amino acid sequence represented by SEQ ID NO: 39.

3. The use of claim 1 or 2, wherein the antibody comprises an antibody heavy chain comprising an amino acid sequence represented by SEQ ID NO: 17 or 51 and an antibody light chain comprising an amino acid sequence represented by SEQ ID NO: 24.

4. The use of claim 1, wherein the antibody comprises an antibody heavy chain comprising an amino acid sequence represented by SEQ ID NO: 30 and an antibody light chain comprising an amino acid sequence represented by SEQ ID NO: 35.

5. The use of any one of claims 1 to 4, wherein the toxin drug moiety is a cytotoxic or cytostatic agent.

6. The use of any one of claims 1 to 5, wherein the toxin drug moiety is a maytansinoid or an auristatin, wherein the maytansinoid is preferably selected from the group consisting of DM1 and DM4 and , wherein the auristatin is preferably selected from the group consisting of monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF).

7. The use of any one of claims 1 to 6, wherein the antibody having the ability of binding to CLDN18.2 is covalently attached to the toxin drug moiety by a linker, wherein the linker is preferably a cleavable linker.

8. The use of claim 7, wherein the linker is cleavable by an intracellular protease.

9. The use of claims 7 or 8, wherein the linker is a cathepsin-cleavable linker.

10. The use of any one of claims 7 to 9, wherein the linker comprises a dipeptide.

11. The use of claim 10, wherein the dipeptide is val-cit or phe-lys.

12. The use of any one of claims 1 to 11 which further comprises administration of surgery, chemotherapy and/or radiation therapy.

13. The use of any one of claims 1 to 12, wherein the cancer is an adenocarcinoma, in particular an advanced adenocarcinoma.

14. The use of any one of claims 1 to 13, wherein the cancer is selected from the group consisting of gastric cancer, esophageal cancer, pancreatic cancer, lung cancer such as non small cell lung cancer (NSCLC), breast cancer, ovarian cancer, colon cancer, hepatic cancer, head-neck cancer, cancer of the gallbladder and the metastasis thereof, a Krukenberg tumor, peritoneal metastasis and/or lymph node metastasis.

15. The use of any one of claims 1 to 14, 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.