

ANTI-GPC1 ANTIBODIES AND USES THEREOF

TECHNICAL FIELD

This disclosure relates to anti-GPC1 (Glypican-1) antibodies, antigen-binding fragments thereof, and the uses thereof.

BACKGROUND

Glypicans (GPCs) are a family of heparan sulfate proteoglycans (HSPGs) that interact with the plasma membrane through a glycosylphosphatidyl inositol anchor. In humans, six GPC family members have been identified, including GPC1, GPC2, GPC3, GPC4, GPC5 and GPC6. Glypicans are predominantly expressed during embryonic development and have been reported to play an important role in organ morphological development by influencing signaling pathways including Wnt, hedgehog, transforming growth factor- β , and fibroblast growth factor. Glypicans participate in many important processes, including cellular proliferation, migration, differentiation, extracellular matrix, and tumor microenvironment remodeling. Studies have suggested that aberrant expression of GPC1 is detected in multiple cancers and that disturbance of GPC1 influences cancer progression. GPC1 has been proven to be a useful biomarker for multiple cancerous tissues, such as prostate, hepatocellular, and pancreatic carcinomas. Considering the important role of GPC1 in various tumors, there is a need to develop a therapeutic agent targeting GPC1.

SUMMARY

This disclosure relates to anti-GPC1 antibodies, antigen-binding fragment thereof, and the uses thereof.

In one aspect, the disclosure is related to an antibody or antigen-binding fragment thereof that binds to GPC1 (Glypican-1) comprising:

a heavy chain variable region (VH) comprising complementarity determining regions (CDRs) 1, 2 and 3, wherein the VH CDR1 region comprises an amino acid sequence that is at least 85% identical to a selected VH CDR1 amino acid sequence, the VH CDR2 region comprises an amino acid sequence that is at least 85% identical to a selected VH CDR2 amino

acid sequence, and the VH CDR3 region comprises an amino acid sequence that is at least 85% identical to a selected VH CDR3 amino acid sequence; and

a light chain variable region (VL) comprising CDRs 1, 2 and 3, wherein the VL CDR1 region comprises an amino acid sequence that is at least 85% identical to a selected VL CDR1 amino acid sequence, the VL CDR2 region comprises an amino acid sequence that is at least 85% identical to a selected VL CDR2 amino acid sequence, and the VL CDR3 region comprises an amino acid sequence that is at least 85% identical to a selected VL CDR3 amino acid sequence,

wherein the selected VH CDRs 1, 2, and 3 amino acid sequences and the selected VL CDRs 1, 2 and 3 amino acid sequences are one of the following:

(1) the selected VH CDRs 1, 2, 3 amino acid sequences are set forth in SEQ ID NOs: 1-3, respectively, and the selected VL CDRs 1, 2, 3 amino acid sequences are set forth in SEQ ID NOs: 4-6, respectively; and

(2) the selected VH CDRs 1, 2, 3 amino acid sequences are set forth in SEQ ID NOs: 7-9, respectively, and the selected VL CDRs 1, 2, 3 amino acid sequences are set forth in SEQ ID NOs: 4-6, respectively.

In some embodiments, the VH comprises CDRs 1, 2, 3 with the amino acid sequences set forth in SEQ ID NOs: 1-3, respectively, and the VL comprises CDRs 1, 2, 3 with the amino acid sequences set forth in SEQ ID NOs: 4-6, respectively, according to Kabat definition.

In some embodiments, the VH comprises CDRs 1, 2, 3 with the amino acid sequences set forth in SEQ ID NOs: 7-9, respectively, and the VL comprises CDRs 1, 2, 3 with the amino acid sequences set forth in SEQ ID NOs: 4-6, respectively, according to Chothia definition.

In some embodiments, the antibody or antigen-binding fragment thereof specifically binds to human, monkey, mouse, and/or rat GPC1.

In some embodiments, the antibody or antigen-binding fragment thereof is a human antibody or antigen-binding fragment thereof, or a single-chain variable fragment (scFv).

In some embodiments, the antibody or antigen-binding fragment thereof is a human IgG1 antibody or antigen-binding fragment thereof or a human IgG4 antibody or antigen-binding fragment thereof.

In one aspect, the disclosure is related to a nucleic acid comprising a polynucleotide encoding the antibody or antigen-binding fragment thereof.

In some embodiments, the nucleic acid is cDNA.

In one aspect, the disclosure is related to a vector comprising one or more of the nucleic acids described herein.

In one aspect, the disclosure is related to a cell comprising the vector described herein. In one embodiment, the cell is an isolated cell, such as an isolated host cell. In some embodiments, the cell is a CHO cell.

In one aspect, the disclosure is related to a cell comprising one or more of the nucleic acids described herein.

In one aspect, the disclosure is related to a method of producing an antibody or an antigen-binding fragment thereof, the method comprising culturing the cell described herein under conditions sufficient for the cell to produce the antibody or the antigen-binding fragment; and collecting the antibody or the antigen-binding fragment produced by the cell.

In one aspect, the disclosure is related to an antibody or antigen-binding fragment thereof that binds to GPC1 comprising a heavy chain variable region (VH) comprising an amino acid sequence of SEQ ID NO: 10, and a light chain variable region (VL) comprising an amino acid sequence of SEQ ID NO: 11.

In some embodiments, the antibody or antigen-binding fragment thereof specifically binds to human, monkey, mouse, and/or rat GPC1.

In some embodiments, the antibody or antigen-binding fragment thereof is a human antibody or antigen-binding fragment thereof, or a single-chain variable fragment (scFv).

In some embodiments, the antibody or antigen-binding fragment is a human IgG1 antibody or antigen-binding fragment thereof, or a human IgG4 antibody or antigen-binding fragment thereof.

In one aspect, the disclosure is related to a method of treating a subject having cancer, the method comprising administering a therapeutically effective amount of a composition comprising the antibody or antigen-binding fragment thereof described herein to the subject.

In some embodiments, the cancer is gastric cancer, breast cancer, bladder cancer, pancreatic cancer, lung cancer, or colorectal cancer.

In one aspect, the disclosure is related to a method of decreasing the rate of tumor growth, the method comprising contacting a tumor cell with an effective amount of a composition comprising an antibody or antigen-binding fragment thereof described herein.

In one aspect, the disclosure is related to a method of killing a tumor cell, the method comprising contacting a tumor cell with an effective amount of a composition comprising the antibody or antigen-binding fragment thereof described herein.

In one aspect, the disclosure is related to a pharmaceutical composition comprising the
5 antibody or antigen-binding fragment thereof described herein, and a pharmaceutically acceptable carrier.

As used herein, the term “cancer” refers to cells having the capacity for autonomous growth. Examples of such cells include cells having an abnormal state or condition characterized by rapidly proliferating cell growth. The term is meant to include cancerous growths, e.g.,
10 tumors; oncogenic processes, metastatic tissues, and malignantly transformed cells, tissues, or organs, irrespective of histopathologic type or stage of invasiveness. Also included are malignancies of the various organ systems, such as respiratory, cardiovascular, renal, reproductive, hematological, neurological, hepatic, gastrointestinal, and endocrine systems; as well as adenocarcinomas which include malignancies such as most colon cancers, renal-cell
15 carcinoma, prostate cancer and/or testicular tumors, non-small cell carcinoma of the lung, and cancer of the small intestine. Cancer that is “naturally arising” includes any cancer that is not experimentally induced by implantation of cancer cells into a subject, and includes, for example, spontaneously arising cancer, cancer caused by exposure of a patient to a carcinogen(s), cancer resulting from insertion of a transgenic oncogene or knockout of a tumor suppressor gene, and
20 cancer caused by infections, e.g., viral infections. The term “carcinoma” is art recognized and refers to malignancies of epithelial or endocrine tissues. The term also includes carcinosarcomas, which include malignant tumors composed of carcinomatous and sarcomatous tissues. An “adenocarcinoma” refers to a carcinoma derived from glandular tissue or in which the tumor cells form recognizable glandular structures. The term “sarcoma” is art recognized and refers to
25 malignant tumors of mesenchymal derivation. The term “hematopoietic neoplastic disorders” includes diseases involving hyperplastic/neoplastic cells of hematopoietic origin. A hematopoietic neoplastic disorder can arise from myeloid, lymphoid or erythroid lineages, or precursor cells thereof.

As used herein, the term “antibody” refers to any antigen-binding molecule that contains
30 at least one (e.g., one, two, three, four, five, or six) complementary determining region (CDR) (e.g., any of the three CDRs from an immunoglobulin light chain or any of the three CDRs from

an immunoglobulin heavy chain) and is capable of specifically binding to an epitope. Non-limiting examples of antibodies include: monoclonal antibodies, polyclonal antibodies, single-chain antibodies, chimeric antibodies, human antibodies, and humanized antibodies. In some embodiments, an antibody can contain an Fc region of a human antibody. The term antibody also
5 includes derivatives, e.g., single-chain antibodies, diabodies, linear antibodies formed from antibody fragments.

As used herein, the term “antigen-binding fragment” refers to a portion of a full-length antibody, wherein the portion of the antibody is capable of specifically binding to an antigen. In some embodiments, the antigen-binding fragment contains at least one variable domain (e.g., a
10 variable domain of a heavy chain or a variable domain of light chain). Non-limiting examples of antibody fragments include, e.g., Fab, Fab', F(ab')₂, and Fv fragments.

As used herein, the term “human antibody” refers to an antibody that is encoded by an endogenous nucleic acid (e.g., rearranged human immunoglobulin heavy or light chain locus) present in a human. In some embodiments, a human antibody is collected from a human or
15 produced in a human cell culture (e.g., human hybridoma cells). In some embodiments, a human antibody is produced in a non-human cell (e.g., a mouse or hamster cell line). In some embodiments, a human antibody is produced in a bacterial or yeast cell. In some embodiments, a human antibody is produced in a transgenic non-human animal (e.g., a bovine) containing an unrearranged or rearranged human immunoglobulin locus (e.g., heavy or light chain human
20 immunoglobulin locus).

As used herein, the term “chimeric antibody” refers to an antibody that contains a sequence present in at least two different antibodies (e.g., antibodies from two different mammalian species such as a human and a mouse antibody). A non-limiting example of a chimeric antibody is an antibody containing the variable domain sequences (e.g., all or part of a
25 light chain and/or heavy chain variable domain sequence) of a non-human (e.g., mouse) antibody and the constant domains of a human antibody. Additional examples of chimeric antibodies are described herein and are known in the art.

As used herein, the term “humanized antibody” refers to a non-human antibody which contains minimal sequence derived from a non-human (e.g., mouse) immunoglobulin and
30 contains sequences derived from a human immunoglobulin. In non-limiting examples, humanized antibodies are human antibodies (recipient antibody) in which hypervariable (e.g.,

CDR) region residues of the recipient antibody are replaced by hypervariable (e.g., CDR) region residues from a non-human antibody (e.g., a donor antibody), e.g., a mouse, rat, or rabbit antibody, having the desired specificity, affinity, and capacity. In some embodiments, the Fv framework residues of the human immunoglobulin are replaced by corresponding non-human (e.g., mouse) immunoglobulin residues. In some embodiments, humanized antibodies may contain residues which are not found in the recipient antibody or in the donor antibody. These modifications can be made to further refine antibody performance. In some embodiments, the humanized antibody contains substantially all of at least one, and typically two, variable domains, in which all or substantially all of the hypervariable loops (CDRs) correspond to those of a non-human (e.g., mouse) immunoglobulin and all or substantially all of the framework regions are those of a human immunoglobulin. The humanized antibody can also contain at least a portion of an immunoglobulin constant region (Fc), typically, that of a human immunoglobulin. Humanized antibodies can be produced using molecular biology methods known in the art. Non-limiting examples of methods for generating humanized antibodies are described herein.

As used herein, the term “single-chain antibody” refers to a single polypeptide that contains at least two immunoglobulin variable domains (e.g., a variable domain of a mammalian immunoglobulin heavy chain or light chain) that is capable of specifically binding to an antigen. Non-limiting examples of single-chain antibodies are described herein.

As used herein, the term “multimeric antibody” refers to an antibody that contains four or more (e.g., six, eight, or ten) immunoglobulin variable domains. In some embodiments, the multimeric antibody is able to crosslink one target molecule (e.g., GPC1) to at least one second target molecule on the surface of a mammalian cell (e.g., a human T-cell).

As used herein, the terms “subject” and “patient” are used interchangeably throughout the specification and describe an animal, human or non-human, to whom treatment according to the methods of the present invention is provided. Veterinary and non-veterinary applications are contemplated by the present invention. Human patients can be adult humans or juvenile humans (e.g., humans below the age of 18 years old). In addition to humans, patients include but are not limited to mice, rats, hamsters, guinea-pigs, rabbits, ferrets, cats, dogs, and primates. Included are, for example, non-human primates (e.g., monkey, chimpanzee, gorilla, and the like), rodents (e.g., rats, mice, gerbils, hamsters, ferrets, rabbits), lagomorphs, swine (e.g., pig, miniature pig), equine, canine, feline, bovine, and other domestic, farm, and zoo animals.

As used herein, when referring to an antibody, the phrases “specifically binding” and “specifically binds” mean that the antibody interacts with its target molecule (e.g., GPC1) preferably to other molecules, because the interaction is dependent upon the presence of a particular structure (i.e., the antigenic determinant or epitope) on the target molecule; in other words, the reagent is recognizing and binding to molecules that include a specific structure rather than to all molecules in general. An antibody that specifically binds to the target molecule may be referred to as a target-specific antibody. For example, an antibody that specifically binds to a GPC1 molecule may be referred to as a GPC1-specific antibody or an anti-GPC1 antibody.

As used herein, the terms “polypeptide,” “peptide,” and “protein” are used interchangeably to refer to polymers of amino acids of any length of at least two amino acids.

As used herein, the terms “polynucleotide,” “nucleic acid molecule,” and “nucleic acid sequence” are used interchangeably herein to refer to polymers of nucleotides of any length of at least two nucleotides, and include, without limitation, DNA, RNA, DNA/RNA hybrids, and modifications thereof.

Unless otherwise defined, all technical and scientific terms used herein have the same meaning as commonly understood by one of ordinary skill in the art to which this invention belongs. Methods and materials are described herein for use in the present invention; other, suitable methods and materials known in the art can also be used. The materials, methods, and examples are illustrative only and not intended to be limiting. All publications, patent applications, patents, sequences, database entries, and other references mentioned herein are incorporated by reference in their entirety. In case of conflict, the present specification, including definitions, will control.

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

DETAILED DESCRIPTION

The present disclosure provides examples of antibodies, antigen-binding fragment thereof, that bind to GPC1.

GPC1

Heparan sulfate proteoglycans (HSPGs) are glycoproteins consisting of a core protein covalently bound to several heparan sulfate (HS) glycosaminoglycan (GAG) chains. HSPGs are expressed ubiquitously on the cell surface and in the extracellular matrix where they interact with a wide range of ligands to mediate various cellular functions. HSPGs are categorized into three groups based on their location: membrane HSPGs (e.g., syndecans and glypicans), secreted extracellular matrix HSPGs (agrin, perlecan, type XVIII collagen), and secretory vesicle proteoglycans (serglycin).

Glypicans, of the HSPGs family, are attached to the outer surface of the plasma membrane by a glycosyl-phosphatidylinositol (GPI) anchor. Six human glypican family members (GPC1 to GPC6) have been identified and fall into two broad subfamilies, glypicans 1/2/4/6 and glypicans 3/5, both sharing ~25% amino acid identity. The three-dimensional structure of glypicans might be similar across the family, as the localization of 14 cysteine residues is conserved in all family members. All glypicans share attachment sites for HS chains, in particular, the two close to the cell membrane, and a hydrophobic sequence necessary for the GPI anchor in the C-terminal tail.

GPC1 contains 558 amino acids and is composed of a secretory signal peptide, an N-terminal core protein, a C-terminal HS chain attachment region, and a GPI anchor attached to cell membrane. GPC1 protein is substituted with a cluster of three HS chains at positions Ser-486, Ser-488, and Ser-490 and is also decorated with two N-linked glycans at Asn-79 and Asn-116 that affect the GPC1 expression level, as well as HS substitution. The structure of GPC1 with the HS chains is related to its biological function by modulating various signaling pathways through specific interactions with ligands and receptors in cell surface signaling complexes. The actual orientation and possible rotation of GPC1 (and other glypicans) relative to the cell surface is unknown. The impact of these surface signaling complexes, either activating or inhibitory, is dependent upon specific GPC1 domain interaction with signaling components under specific physiological conditions.

FGFs are secreted glycoproteins that are readily sequestered by the extracellular matrix and the cell surface HSPGs, including GPC1. GPC1 has been proposed to act as a coreceptor for FGFs that enhances the binding of FGF to its receptor, subsequently promoting FGF-FGFR activation and signaling. The binding of FGF to its receptor leads to receptor dimerization and

transphosphorylation of tyrosine kinase domains, resulting in subsequent activation of various signaling pathways, including Ras-MAPK, PI3K-AKT-mTOR, and DAG-PKC. All signaling cascades ultimately result in enhanced growth, survival, and angiogenesis. FGF2 is one of the heparin-binding growth factors (HBGFs) that can drive tumor cell proliferation upon binding to its receptors, and also functions as an angiogenic growth factor in angiogenesis. Mounting evidence indicates that changes in GPC1 expression have profound consequences on FGF2-induced cell proliferation or angiogenesis in tumors. For example, in breast cancer, GPC1 is upregulated and promotes tumor mitogenic signaling by modulating heparin-binding growth factors, including FGF2. GPC1 has also been shown to cooperate with type V collagen to concentrate FGF2 at the extracellular cell matrix (ECM) interface, thereby affecting ECM stability and breast tumor cell proliferation.

Similar to FGF2, VEGF-A is also an angiogenic growth factor and plays an essential role in angiogenesis. GPC1 acts as a coreceptor of VEGF-A, and this interaction is mediated by its HS chains, as HS removal by heparinase treatment abolished the ability of GPC1 to bind to VEGF. Cell binding assays using ECs also demonstrated that the addition of exogenous GPC1 could potentiate the VEGF-A/VEGFR binding, suggesting the potential role of GPC1 in the control of angiogenesis

The transforming growth factor- β (TGF- β) belongs to a superfamily of cytokines that activate protein kinase receptors on the plasma membrane to regulate cell growth, death, differentiation, immune response, angiogenesis, and inflammation. Dysregulation of this pathway contributes to a broad variety of pathologies, including cancer. However, TGF- β signaling is considered a challenging target due to its dual functions and pleiotropic nature. In healthy cells and early-stage cancer cells, TGF- β acts as a tumor suppressor, whereas in the late stage of cancer, it drives tumor progression and metastasis. GPC1 has been shown to interact with TGF- β and its receptors to stabilize their assembly for enhanced Smad signaling.

Bone morphogenetic proteins (BMPs) play substantial roles in cell-cell communication during animal development and are potent growth factors promoting bone formation. Glypicans have been shown to regulate BMP activity. GPC1 protein is mainly expressed in the skeletal system in humans, and Gpc1 expression was also identified in the developing murine calvarium and skeletal structures. Thus, it may be inferred that GPC1 is also involved in the regulation of the BMP signaling pathway. Based on currently available evidence, GPC1 seems to act as an

inhibitor in BMP signaling regulation in osteogenesis. GPC1 tethered at the cell surface or soluble GPC1 may compete with BMP receptors for interaction with BMP ligands, leading to reduced amounts of BMPs available for binding to their receptors and consequently attenuated Smad-dependent or -independent BMP signaling.

5 GPC1 has been reported to be present in two forms, a membrane bound core protein and secreted soluble forms, which can be detected in serum-free media harvested from prostate cancer cells DU-145 by immunoprecipitation, probably due to GPC1 shedding or proteolytic cleavage at GPI anchor that was also found in GPC3. However, the specific cleavage mechanism or site has been unknown.

10 Human GPC1 is expressed not only in the central nervous system (CNS) and skeletal system during development but also in other tissues in the adult. GPC1 is overexpressed in multiple types of cancers, including breast cancer, esophageal squamous cell carcinoma (ESCC), glioma, and pancreatic cancer. Its high expression is correlated with poorer prognosis, making it a potential target for cancer therapy.

15 A detailed review of GPC1 and its functions can be found in Pan J, Ho M. Role of glypican-1 in regulating multiple cellular signaling pathways. Am J Physiol Cell Physiol. 2021 Nov 1;321(5):C846-C858, which is incorporated by reference in its entirety.

The present disclosure provides anti-GPC1 antibodies, antigen-binding fragments thereof, and methods of using these anti-GPC1 antibodies and antigen-binding fragments to inhibit tumor growth and treat cancers.

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Antibodies and Antigen Binding Fragments

The present disclosure provides anti-GPC1 antibodies and antigen-binding fragments thereof. In general, antibodies (also called immunoglobulins) are made up of two classes of polypeptide chains, light chains and heavy chains. A non-limiting antibody of the present disclosure can be an intact, four immunoglobulin chain antibody comprising two heavy chains and two light chains. The heavy chain of the antibody can be of any isotype including IgM, IgG, IgE, IgA, or IgD or sub-isotype including IgG1, IgG2, IgG2a, IgG2b, IgG3, IgG4, IgE1, IgE2, etc. The light chain can be a kappa light chain or a lambda light chain. An antibody can comprise two identical copies of a light chain and two identical copies of a heavy chain. The heavy chains, which each contain one variable domain (or variable region, V_H) and multiple constant domains

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(or constant regions), bind to one another via disulfide bonding within their constant domains to form the “stem” of the antibody. The light chains, which each contain one variable domain (or variable region, V_L) and one constant domain (or constant region), each bind to one heavy chain via disulfide binding. The variable region of each light chain is aligned with the variable region of the heavy chain to which it is bound. The variable regions of both the light chains and heavy chains contain three hypervariable regions sandwiched between more conserved framework regions (FR).

These hypervariable regions, known as the complementary determining regions (CDRs), form loops that comprise the principle antigen binding surface of the antibody. The four framework regions largely adopt a beta-sheet conformation and the CDRs form loops connecting, and in some cases forming part of, the beta-sheet structure. The CDRs in each chain are held in close proximity by the framework regions and, with the CDRs from the other chain, contribute to the formation of the antigen-binding region.

Methods for identifying the CDR regions of an antibody by analyzing the amino acid sequence of the antibody are well known, and a number of definitions of the CDRs are commonly used. The Kabat definition is based on sequence variability, and the Chothia definition is based on the location of the structural loop regions. These methods and definitions are described in, *e.g.*, Martin, "Protein sequence and structure analysis of antibody variable domains," Antibody engineering, Springer Berlin Heidelberg, 2001. 422-439; Abhinandan, et al. "Analysis and improvements to Kabat and structurally correct numbering of antibody variable domains," Molecular immunology 45.14 (2008): 3832-3839; Wu, T.T. and Kabat, E.A. (1970) J. Exp. Med. 132: 211-250; Martin et al., Methods Enzymol. 203:121-53 (1991); Morea et al., Biophys Chem. 68(1-3):9-16 (Oct. 1997); Morea et al., J Mol Biol. 275(2):269-94 (Jan .1998); Chothia et al., Nature 342(6252):877-83 (Dec. 1989); Ponomarenko and Bourne, BMC Structural Biology 7:64 (2007); each of which is incorporated herein by reference in its entirety.

The CDRs are important for recognizing an epitope of an antigen. As used herein, an “epitope” is the smallest portion of a target molecule capable of being specifically bound by the antigen binding domain of an antibody. The minimal size of an epitope may be about three, four, five, six, or seven amino acids, but these amino acids need not be in a consecutive linear sequence of the antigen’s primary structure, as the epitope may depend on an antigen’s three-dimensional configuration based on the antigen’s secondary and tertiary structure.

In some embodiments, the antibody is an intact immunoglobulin molecule (e.g., IgG1, IgG2a, IgG2b, IgG3, IgM, IgD, IgE, IgA). The IgG subclasses (IgG1, IgG2, IgG3, and IgG4) are highly conserved, differ in their constant region, particularly in their hinges and upper CH2 domains. The sequences and differences of the IgG subclasses are known in the art, and are described, e.g., in Vidarsson, et al, "IgG subclasses and allotypes: from structure to effector functions." *Frontiers in immunology* 5 (2014); Irani, et al. "Molecular properties of human IgG subclasses and their implications for designing therapeutic monoclonal antibodies against infectious diseases." *Molecular immunology* 67.2 (2015): 171-182; Shakib, Farouk, ed. *The human IgG subclasses: molecular analysis of structure, function and regulation*. Elsevier, 2016; each of which is incorporated herein by reference in its entirety.

The antibody can also be an immunoglobulin molecule that is derived from any species (e.g., human, rodent, mouse, camelid). Antibodies disclosed herein also include, but are not limited to, polyclonal, monoclonal, monospecific, polyspecific antibodies, and chimeric antibodies that include an immunoglobulin binding domain fused to another polypeptide. The term "antigen binding domain" or "antigen binding fragment" is a portion of an antibody that retains specific binding activity of the intact antibody, i.e., any portion of an antibody that is capable of specific binding to an epitope on the intact antibody's target molecule. It includes, e.g., Fab, Fab', F(ab')₂, and variants of these fragments. Thus, in some embodiments, an antibody or an antigen binding fragment thereof can be, e.g., a scFv, a Fv, a Fd, a dAb, a bispecific antibody, a bispecific scFv, a diabody, a linear antibody, a single-chain antibody molecule, a multi-specific antibody formed from antibody fragments, and any polypeptide that includes a binding domain which is, or is homologous to, an antibody binding domain. Non-limiting examples of antigen binding domains include, e.g., the heavy chain and/or light chain CDRs of an intact antibody, the heavy and/or light chain variable regions of an intact antibody, full length heavy or light chains of an intact antibody, or an individual CDR from either the heavy chain or the light chain of an intact antibody.

Anti-GPC1 Antibodies and Antigen-Binding Fragments

The disclosure provides antibodies and antigen-binding fragments thereof that specifically bind to GPC1 (e.g., human GPC1). The antibodies and antigen-binding fragments described herein are capable of binding to GPC1. These antibodies can be agonists or

antagonists. In some embodiments, these antibodies can increase immune response. In some embodiments, these antibodies can block GPC1 activity.

The disclosure provides e.g., anti-GPC1 antibody 3G1, the chimeric antibodies thereof, and the human or humanized antibodies thereof.

5 The CDR sequences for 3G1, and 3G1 derived antibodies (e.g., human or humanized antibodies) include CDRs of the heavy chain variable domain and CDRs of light chain variable domain shown in the sequence overview herein as defined by Kabat definition and Chothia definition.

10 The amino acid sequence for the heavy chain variable region and light chain variable region of 3G1 are shown in the sequence overview herein.

In some embodiments, the antibodies or antigen-binding fragments thereof described herein can contain one, two, or three heavy chain variable region CDRs selected from the group of SEQ ID NOs: 1-3 and SEQ ID NOs: 7-9, and/or one, two, or three light chain variable region CDRs selected from the group of SEQ ID NOs: 4-6.

15 In some embodiments, the antibodies can have a heavy chain variable region (VH) comprising complementarity determining regions (CDRs) 1, 2, 3, wherein the CDR1 region comprises or consists of an amino acid sequence that is at least 85%, 90%, 95%, or 100% identical to a selected VH CDR1 amino acid sequence, the CDR2 region comprises or consists of an amino acid sequence that is at least 85%, 90%, 95%, or 100% identical to a selected VH
20 CDR2 amino acid sequence, and the CDR3 region comprises or consists of an amino acid sequence that is at least 85%, 90%, 95%, or 100% identical to a selected VH CDR3 amino acid sequence. In some embodiments, the antibody can have a light chain variable region (VL) comprising CDRs 1, 2, 3, wherein the CDR1 region comprises or consists of an amino acid sequence that is at least 85%, 90%, 95%, or 100% identical to a selected VL CDR1 amino acid
25 sequence, the CDR2 region comprises or consists of an amino acid sequence that is at least 85%, 90%, 95%, or 100% identical to a selected VL CDR2 amino acid sequence, and the CDR3 region comprises or consists of an amino acid sequence that is at least 85%, 90%, 95%, or 100% identical to a selected VL CDR3 amino acid sequence. The selected VH CDRs 1, 2, 3 amino acid sequences and the selected VL CDRs, 1, 2, 3 amino acid sequences are shown in the sequence
30 overview herein.

The disclosure also provides antibodies or antigen-binding fragments thereof that bind to GPC1. The antibodies or antigen-binding fragments thereof contain a heavy chain variable region (VH) comprising or consisting of an amino acid sequence of SEQ ID NO: 10, and a light chain variable region (VL) comprising or consisting of an amino acid sequence of SEQ ID NO:11.

To determine the percent identity of two amino acid sequences, or of two nucleic acid sequences, the sequences are aligned for optimal comparison purposes (e.g., gaps can be introduced in one or both of a first and a second amino acid or nucleic acid sequence for optimal alignment and non-homologous sequences can be disregarded for comparison purposes). The amino acid residues or nucleotides at corresponding amino acid positions or nucleotide positions are then compared. When a position in the first sequence is occupied by the same amino acid residue or nucleotide as the corresponding position in the second sequence, then the molecules are identical at that position. The percent identity between the two sequences is a function of the number of identical positions shared by the sequences, taking into account the number of gaps, and the length of each gap, which need to be introduced for optimal alignment of the two sequences. For example, the comparison of sequences and determination of percent identity between two sequences can be accomplished using a Blossum 62 scoring matrix with a gap penalty of 12, a gap extend penalty of 4, and a frameshift gap penalty of 5.

The disclosure also provides a nucleic acid comprising a polynucleotide encoding a polypeptide comprising an immunoglobulin heavy chain or an immunoglobulin light chain. The immunoglobulin heavy chain or immunoglobulin light chain comprises CDRs or have sequences as shown in the sequence overview herein. When the polypeptides are paired with a corresponding polypeptide (e.g., a corresponding heavy chain variable region or a corresponding light chain variable region), the paired polypeptides bind to GPC1 (e.g., human GPC1).

The anti-GPC1 antibodies and antigen-binding fragments can also be antibody variants of antibodies or antibody fragments. Additional antibodies provided herein are polyclonal, monoclonal, human antibodies, chimeric antibodies (e.g., human-mouse chimera), single-chain antibodies, intracellularly-made antibodies (i.e., intrabodies), and antigen-binding fragments thereof. The antibodies or antigen-binding fragments thereof can be of any type (e.g., IgG, IgE, IgM, IgD, IgA, and IgY), class (e.g., IgG1, IgG2, IgG3, IgG4, IgA1, and IgA2), or subclass. In

some embodiments, the antibody or antigen-binding fragment thereof is an IgG antibody or antigen-binding fragment thereof.

Fragments of antibodies are suitable for use in the methods provided so long as they retain the desired affinity and specificity of the full-length antibody. Thus, a fragment of an antibody that binds to GPC1 will retain an ability to bind to GPC1. An Fv fragment is an antibody fragment which contains a complete antigen recognition and binding site. This region consists of a dimer of one heavy and one light chain variable domain in tight association, which can be covalent in nature, for example in scFv. It is in this configuration that the three CDRs of each variable domain interact to define an antigen binding site on the surface of the VH-VL dimer. Collectively, the six CDRs or a subset thereof confer antigen binding specificity to the antibody. However, even a single variable domain (or half of an Fv comprising only three CDRs specific for an antigen) can have the ability to recognize and bind antigen, although usually at a lower affinity than the entire binding site.

Single-chain Fv or (scFv) antibody fragments comprise the VH and VL domains (or regions) of antibody, wherein these domains are present in a single polypeptide chain. Generally, the scFv polypeptide further comprises a polypeptide linker between the VH and VL domains, which enables the scFv to form the desired structure for antigen binding.

The Fab fragment contains a variable and constant domain of the light chain and a variable domain and the first constant domain (CH1) of the heavy chain. F(ab')₂ antibody fragments comprise a pair of Fab fragments which are generally covalently linked near their carboxy termini by hinge cysteines between them. Other chemical couplings of antibody fragments are also known in the art.

Linear antibodies comprise a pair of tandem Fd segments (VH-CH1-VH-CH1) which, together with complementary light chain polypeptides, form a pair of antigen binding regions. Linear antibodies can be bispecific or monospecific.

Antibodies and antibody fragments of the present disclosure can be modified in the Fc region to provide desired effector functions or serum half-life. In some embodiments, the Fc region can be modified to silence or decrease complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC). In some embodiments, the Fc region can be modified to increase complement-dependent cytotoxicity (CDC) or antibody-dependent cellular cytotoxicity (ADCC).

Multimerization of antibodies may be accomplished through natural aggregation of antibodies or through chemical or recombinant linking techniques known in the art. For example, some percentage of purified antibody preparations (e.g., purified IgG1 molecules) spontaneously form protein aggregates containing antibody homodimers and other higher-order antibody multimers.

Alternatively, antibody homodimers may be formed through chemical linkage techniques known in the art. For example, heterobifunctional crosslinking agents including, but not limited to SMCC (succinimidyl 4-(maleimidomethyl)cyclohexane-1-carboxylate) and SATA (N-succinimidyl S-acetylthioacetate) can be used to form antibody multimers. An exemplary protocol for the formation of antibody homodimers is described in Ghetie et al. (*Proc. Natl. Acad. Sci. U.S.A.* 94: 7509-7514, 1997). Antibody homodimers can be converted to Fab'₂ homodimers through digestion with pepsin. Another way to form antibody homodimers is through the use of the autophilic T15 peptide described in Zhao et al. (*J. Immunol.* 25:396-404, 2002).

Any of the antibodies or antigen-binding fragments described herein may be conjugated to a stabilizing molecule (e.g., a molecule that increases the half-life of the antibody or antigen-binding fragment thereof in a subject or in solution). Non-limiting examples of stabilizing molecules include: a polymer (e.g., a polyethylene glycol) or a protein (e.g., serum albumin, such as human serum albumin). The conjugation of a stabilizing molecule can increase the half-life or extend the biological activity of an antibody or an antigen-binding fragment *in vitro* (e.g., in tissue culture or when stored as a pharmaceutical composition) or *in vivo* (e.g., in a human).

The antibody or antigen-binding fragment thereof of the present disclosure characterized by binding GPC1 may in one or more embodiments of the present disclosure, be characterized by being at least one of the following:

- a. an anti-GPC1 antibody or antigen binding fragment thereof as defined herein, comprising
 - i) an immunoglobulin light chain variable region (VL) comprising as VL complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 4, as VL CDR2 an amino acid sequence of SEQ ID NO: 5, and as VL CDR3 an amino acid sequence of SEQ ID NO: 6, and/or

- ii) an immunoglobulin heavy chain variable region (VH) comprising as VH complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 1, as VH CDR2 an amino acid sequence of SEQ ID NO: 2, and as VH CDR3 an amino acid sequence of SEQ ID NO: 3; or
- 5 b. an anti-GPC1 antibody or antigen binding fragment thereof as defined herein, comprising
- i) an immunoglobulin light chain variable region (VL) comprising as VL complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 4, as VL CDR2 an amino acid sequence of SEQ ID NO: 5, and as VL
- 10 CDR3 an amino acid sequence of SEQ ID NO: 6, and/or
- ii) an immunoglobulin heavy chain variable region (VH) comprising as VH complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 7, as VH CDR2 an amino acid sequence of SEQ ID NO: 8, and as VH CDR3 an amino acid sequence of SEQ ID NO: 9; or
- 15 c. an anti-GPC1 antibody or antigen binding fragment thereof as defined herein, comprising
- i) an immunoglobulin light chain variable region (VL) comprising or consisting of the amino acid sequence of SEQ ID NO: 11 or a sequence having at least 85% sequence identity thereto; and/or
- 20 ii) an immunoglobulin heavy chain variable region (VH) comprising or consisting of the amino acid sequence of SEQ ID NO: 10 or a sequence having at least 85% sequence identity thereto; or
- d. an anti-GPC1 antibody or antigen binding fragment thereof as defined herein, comprising
- 25 i) an immunoglobulin light chain comprising or consisting of the amino acid sequences of SEQ ID NO: 11 or a sequence having at least 85% sequence identity to SEQ ID NO: 11, and SEQ ID NO: 15 or a sequence having at least 85% sequence identity to SEQ ID NO: 15; and/or
- 30 ii) an immunoglobulin heavy chain comprising or consisting of the amino acid sequence of SEQ ID NO: 10 or a sequence having at least 85% sequence identity

- to SEQ ID NO: 10, and SEQ ID NO: 14 or a sequence having at least 85% sequence identity to SEQ ID NO: 14; or
- e. an antibody which competes for binding with the antibody or antigen-binding fragment as defined in any one of a to d.

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Antibody Characteristics

The antibodies or antigen-binding fragments thereof as described herein can be GPC1 agonist or antagonist. In some embodiments, by binding to GPC1, the antibody can inhibit GPC1 activity. In some embodiments, the antibody can upregulate immune response or downregulate immune response.

In some implementations, the antibody or antigen-binding fragments thereof specifically binds to GPC1 (e.g., human GPC1, monkey GPC1 (e.g., rhesus macaques, *Macaca fascicularis*), mouse GPC1, and/or rat GPC1) with a dissociation rate (koff) of less than 0.1 s^{-1} , less than 0.01 s^{-1} , less than 0.001 s^{-1} , less than 0.0001 s^{-1} , less than 0.00001 s^{-1} , less than 0.000001 s^{-1} or less than 0.0000001 s^{-1} . In some embodiments, the dissociation rate (koff) is greater than 0.01 s^{-1} , greater than 0.001 s^{-1} , greater than 0.0001 s^{-1} , greater than 0.00001 s^{-1} , greater than 0.000001 s^{-1} , greater than 0.0000001 s^{-1} or greater than $0.00000001 \text{ s}^{-1}$.

In some embodiments, kinetic association rates (kon) is greater than $1 \times 10^2/\text{Ms}$, greater than $1 \times 10^3/\text{Ms}$, greater than $1 \times 10^4/\text{Ms}$, greater than $1 \times 10^5/\text{Ms}$, or greater than $1 \times 10^6/\text{Ms}$. In some embodiments, kinetic association rates (kon) is less than $1 \times 10^5/\text{Ms}$, less than $1 \times 10^6/\text{Ms}$, or less than $1 \times 10^7/\text{Ms}$.

Affinities can be deduced from the quotient of the kinetic rate constants ($KD=koff/kon$). In some embodiments, KD is less than $1 \times 10^{-6} \text{ M}$, less than $1 \times 10^{-7} \text{ M}$, less than $1 \times 10^{-8} \text{ M}$, less than $1 \times 10^{-9} \text{ M}$, less than $1 \times 10^{-10} \text{ M}$, less than $1 \times 10^{-11} \text{ M}$, less than $1 \times 10^{-12} \text{ M}$, less than $1 \times 10^{-13} \text{ M}$ or less than $1 \times 10^{-14} \text{ M}$. In some embodiments, the KD is less than 50 nM, 30 nM, 20 nM, 15 nM, 10 nM, 9 nM, 8 nM, 7 nM, 6 nM, 5 nM, 4 nM, 3 nM, 2 nM, or 1 nM. In some embodiments, KD is greater than $1 \times 10^{-7} \text{ M}$, greater than $1 \times 10^{-8} \text{ M}$, greater than $1 \times 10^{-9} \text{ M}$, greater than $1 \times 10^{-10} \text{ M}$, greater than $1 \times 10^{-11} \text{ M}$, greater than $1 \times 10^{-12} \text{ M}$, greater than $1 \times 10^{-13} \text{ M}$, greater than $1 \times 10^{-14} \text{ M}$.

General techniques for measuring the affinity of an antibody for an antigen include, e.g., ELISA, RIA, and surface plasmon resonance (SPR).

In some embodiments, the antibody or antigen-binding fragment thereof described herein has a tumor growth inhibition percentage (TGI%) that is greater than 10%, 20%, 30%, 40%, 50%, 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, or 200%. In some embodiments, the antibody or antigen-binding fragment thereof described herein has a tumor growth inhibition percentage that is less than 60%, 70%, 80%, 90%, 100%, 110%, 120%, 130%, 140%, 150%, 160%, 170%, 180%, 190%, or 200%. The TGI% can be determined, e.g., at 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, or 30 days after the treatment starts, or 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, or 12 months after the treatment starts. As used herein, the tumor growth inhibition percentage (TGI%) is calculated using the following formula:

$$\text{TGI (\%)} = [1 - (T_i - T_0) / (V_i - V_0)] \times 100\%$$

T_i is the average tumor volume in the treatment group on day i . T_0 is the average tumor volume in the treatment group on day zero. V_i is the average tumor volume in the control group on day i . V_0 is the average tumor volume in the control group on day zero.

In some embodiments, the antibodies or antigen-binding fragments thereof described herein are GPC1 antagonist. In some embodiments, the antibodies or antigen binding fragments described herein decrease GPC1 signal transduction in a target cell that expresses GPC1.

In some embodiments, the antibodies or antigen binding fragments described herein can bind to tumor cells that express GPC1. In some embodiments, the antibody or antigen binding fragment described herein binds to tumor associated cells that express GPC1. In some embodiments, the antibodies or antigen binding fragments described herein can induce complement-dependent cytotoxicity (CDC) and/or antibody dependent cellular cytotoxicity (ADCC), and kill the tumor cell.

In some embodiments, the antibodies or antigen binding fragments described herein have a functional Fc region. In some embodiments, effector function of a functional Fc region is antibody-dependent cell-mediated cytotoxicity (ADCC). In some embodiments, effector function of a functional Fc region is phagocytosis. In some embodiments, effector function of a functional Fc region is ADCC and phagocytosis.

In some embodiments, the antibodies or antigen binding fragments described herein can induce complement complement-dependent cytotoxicity (CDC).

In some embodiments, the Fc region is human IgG1, human IgG2, human IgG3, or human IgG4. In some embodiments, the antibody is a human IgG1 antibody, optionally with SI mutations, LALA mutations, N297A mutation, YTE mutations, and/or FLAA mutations. In some embodiments, the antibody is a human IgG4 antibody, optionally with SI mutations, LALA mutations, N297A mutation, YTE mutations, and/or FLAA mutations.

In some embodiments, the antibodies or antigen binding fragments described herein do not have a functional Fc region. For example, the antibodies or antigen binding fragments are Fab, Fab', F(ab')₂, and Fv fragments. In some embodiments, the Fc region has LALA mutations (L234A and L235A mutations according to EU numbering), or LALA-PG mutations (L234A, L235A, P329G mutations according to EU numbering). In some embodiments, the Fc region has FLAA mutations (F234A and L235A according to EU numbering). In some embodiments, the Fc has SI mutations (S239D and I332E mutations according to EU numbering). In some embodiments, the Fc has N297A mutation according to EU numbering. In some embodiments, the Fc has YTE mutations (M252Y, S254T and T256E according to EU numbering).

The antibodies or antigen binding fragments described herein internalize effectively into cells expressing GPC1. Internalization of 3G1 was evaluated in Example 5 described herein. Compared to a total of 22 other anti-GPC1 antibodies prepared according to Example 1, 3G1 was found to have the best internalization properties in GPC1 expressing cells. This property makes 3G1 an excellent candidate for antibody-drug conjugates (ADCs).

That is to say in one or more embodiments, the antibodies or antigen binding fragments described herein exhibit good endocytosis rate in GPC1 expressing cells. Endocytosis as used herein refers to a cellular process in which substances are brought into the cell. Endocytosis as used herein may in some embodiments refer to different forms of endocytosis classified by the mechanistic pathway - examples including Clathrin-mediated endocytosis and Caveolae-mediated endocytosis. For a more general description, endocytosis as used herein may also simply be referred to as receptor-mediated endocytosis.

Anti-GPC1 Antibody-Drug Conjugates (Anti-GPC1 ADCs)

Antibodies demonstrating good receptor-mediated endocytosis rates for cancer relevant receptors and/or antigens are suitable candidates for development of antibody-drug conjugates (ADCs). ADCs are a class of potent biopharmaceutical drugs designed as a targeted therapy,

intended in particular for the treatment of cancer. ADCs are complex molecules composed of an antibody (a whole mAb or an antibody fragment) linked, via a stable, chemical, linker that may possess labile bonds (such as enzyme-cleavable bonds or self-immolative moieties), to an active agent, such as a biologically active drug, radiopharmaceutical or cytotoxic compound. By combining the unique targeting capabilities of antibodies with the cell-killing ability of cytotoxic drugs, antibody-drug conjugates allow sensitive discrimination between healthy and affected tissues, based on expression of the antibody antigen. This means that, in contrast to traditional non-targeted chemotherapeutic agents like cisplatin, ADCs actively target and attack cancer cells, so that healthy cells with little or no antigen expression are less severely affected.

In one embodiment, the present disclosure relates to an antibody-drug conjugate directed against GPC1 comprising the antibodies or antigen binding fragments described herein and a payload such as a cytotoxic drug, optionally linked together via a linker.

The antibody or antigen-binding fragment thereof of the present disclosure characterized by binding GPC1 may in one or more embodiments of the present disclosure, such as when incorporated into an ADC, be characterized by being at least one of the following:

a. an anti-GPC1 antibody or antigen binding fragment thereof as defined herein, comprising

i) an immunoglobulin light chain variable region (VL) comprising as VL complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 4, as VL CDR2 an amino acid sequence of SEQ ID NO: 5, and as VL CDR3 an amino acid sequence of SEQ ID NO: 6, and/or

ii) an immunoglobulin heavy chain variable region (VH) comprising as VH complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 1, as VH CDR2 an amino acid sequence of SEQ ID NO: 2, and as VH CDR3 an amino acid sequence of SEQ ID NO: 3; or

b. an anti-GPC1 antibody or antigen binding fragment thereof as defined herein, comprising

i) an immunoglobulin light chain variable region (VL) comprising as VL complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 4, as VL CDR2 an amino acid sequence of SEQ ID NO: 5, and as VL CDR3 an amino acid sequence of SEQ ID NO: 6, and/or

ii) an immunoglobulin heavy chain variable region (VH) comprising as VH complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 7, as VH CDR2 an amino acid sequence of SEQ ID NO: 8, and as VH CDR3 an amino acid sequence of SEQ ID NO: 9; or

5 c. an anti-GPC1 antibody or antigen binding fragment thereof as defined herein, comprising

i) an immunoglobulin light chain variable region (VL) comprising or consisting of the amino acid sequence of SEQ ID NO: 11 or a sequence having at least 85% sequence identity thereto; and/or

10 ii) an immunoglobulin heavy chain variable region (VH) comprising or consisting of the amino acid sequence of SEQ ID NO: 10 or a sequence having at least 85% sequence identity thereto; or

d. an anti-GPC1 antibody or antigen binding fragment thereof as defined herein, comprising

15 i) an immunoglobulin light chain comprising or consisting of the amino acid sequences of SEQ ID NO: 11 or a sequence having at least 85% sequence identity to SEQ ID NO: 11, and SEQ ID NO: 15 or a sequence having at least 85% sequence identity to SEQ ID NO: 15; and/or

20 ii) an immunoglobulin heavy chain comprising or consisting of the amino acid sequence of SEQ ID NO: 10 or a sequence having at least 85% sequence identity to SEQ ID NO: 10, and SEQ ID NO: 14 or a sequence having at least 85% sequence identity to SEQ ID NO: 14; or

e. an antibody which competes for binding with the antibody or antigen-binding fragment as defined in any one of a to d.

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In one or more embodiments of the present disclosure, the anti-GPC1 antibody or antigen binding fragment thereof comprising

30 i) an immunoglobulin light chain comprising or consisting of the amino acid sequences of SEQ ID NO: 11 and SEQ ID NO: 15 or a sequence having at least 85% sequence identity thereto; and/or

ii) an immunoglobulin heavy chain comprising or consisting of the amino acid sequence of SEQ ID NO: 10 and SEQ ID NO: 14 or a sequence having at least 85% sequence identity thereto,

is to be construed as referring to an anti-GPC1 antibody or antigen-binding fragment thereof, comprising an immunoglobulin light chain comprising or consisting of an amino acid sequence which shares at least 85% sequence identity with the cumulative (i.e. combined) length of SEQ ID NOs: 11 and 15, such as 90% or more, such as 95%, such as 96%, 97%, 98%, 99% or more; and/or (similarly) comprising an immunoglobulin heavy chain comprising or consisting of an amino acid sequence which shares at least 85% sequence identity with the cumulative (i.e. combined) length of SEQ ID NOs: 10 and 14, such as 90% or more, such as 95%, such as 96%, 97%, 98%, 99% or more.

In one embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

- a. the anti-GPC1 antibody or antigen binding fragment thereof as defined herein, comprising
 - i) an immunoglobulin light chain variable region (VL) comprising VL complementarity determining regions (CDRs) 1, 2 and 3, wherein the VL CDR1 region comprises an amino acid sequence that is at least 85% identical to a selected VL CDR1 amino acid sequence, the VL CDR2 region comprises an amino acid sequence that is at least 85% identical to a selected VL CDR2 amino acid sequence, and the VL CDR3 region comprises an amino acid sequence that is at least 85% identical to a selected VL CDR3 amino acid sequence, and
 - ii) an immunoglobulin heavy chain variable region (VH) comprising VH complementarity determining regions (CDRs) 1, 2 and 3, wherein the VH CDR1 region comprises an amino acid sequence that is at least 85% identical to a selected VH CDR1 amino acid sequence, the VH CDR2 region comprises an amino acid sequence that is at least 85% identical to a selected VH CDR2 amino acid sequence, and the VH CDR3 region comprises an amino acid sequence that is at least 85% identical to a selected VH CDR3 amino acid sequence, wherein the selected VH CDRs 1, 2 and 3 amino acid sequences and the selected VL CDRs 1, 2 and 3 amino acid sequences are one of:

(1) the selected VH CDRs 1, 2, 3 amino acid sequences are set forth in SEQ ID NOs: 1-3, respectively, and the selected VL CDRs 1, 2, 3 amino acid sequences are set forth in SEQ ID NOs: 4-6, respectively; and
(2) the selected VH CDRs 1, 2, 3 amino acid sequences are set forth in SEQ ID NOs: 7-9, respectively, and the selected VL CDRs 1, 2, 3 amino acid sequences are set forth in SEQ ID NOs: 4-6, respectively;

- b. an active agent; and
- c. optionally a linker which links a) to b).

In one embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

- a. the anti-GPC1 antibody or antigen binding fragment thereof as defined herein, comprising

- i) an immunoglobulin light chain variable region (VL) comprising as VL complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 4 or a sequence having at least 85% sequence identity thereto, as VL CDR2 an amino acid sequence of SEQ ID NO: 5 or a sequence having at least 85% sequence identity thereto, and as VL CDR3 an amino acid sequence of SEQ ID NO: 6 or a sequence having at least 85% sequence identity thereto, and/or
- ii) an immunoglobulin heavy chain variable region (VH) comprising as VH complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 1 or a sequence having at least 85% sequence identity thereto, as VH CDR2 an amino acid sequence of SEQ ID NO: 2 or a sequence having at least 85% sequence identity thereto, and as VH CDR3 an amino acid sequence of SEQ ID NO: 3 or a sequence having at least 85% sequence identity thereto;

- b. an active agent; and
- c. optionally a linker which links a) to b).

In one embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

a. the anti-GPC1 antibody or antigen binding fragment thereof as defined herein,
comprising:

- 5 i) an immunoglobulin light chain variable region (VL) comprising as VL complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 4, as VL CDR2 an amino acid sequence of SEQ ID NO: 5, and as VL CDR3 an amino acid sequence of SEQ ID NO: 6, and/or
- 10 ii) an immunoglobulin heavy chain variable region (VH) comprising as VH complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 1, as VH CDR2 an amino acid sequence of SEQ ID NO: 2, and as VH CDR3 an amino acid sequence of SEQ ID NO: 3;

b. an active agent; and

c. optionally a linker which links a) to b).

15 In one embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

a. the anti-GPC1 antibody or antigen binding fragment thereof as defined herein,
comprising

- 20 i) an immunoglobulin light chain variable region (VL) comprising as VL complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 4 or a sequence having at least 85% sequence identity thereto, as VL CDR2 an amino acid sequence of SEQ ID NO: 5 or a sequence having at least 85% sequence identity thereto, and as VL CDR3 an amino acid sequence of SEQ ID NO: 6 or a sequence having at least 85% sequence identity thereto, and/or
- 25 ii) an immunoglobulin heavy chain variable region (VH) comprising as VH complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 7 or a sequence having at least 85% sequence identity thereto, as VH CDR2 an amino acid sequence of SEQ ID NO: 8 or a sequence having at least 85% sequence identity thereto, and as VH CDR3 an amino acid sequence of SEQ ID NO: 9 or a sequence having at least 85% sequence identity thereto;

30 b. an active agent; and

c. optionally a linker which links a) to b).

In one embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

- a. the anti-GPC1 antibody or antigen binding fragment thereof as defined herein,
 - 5 comprising
 - i) an immunoglobulin light chain variable region (VL) comprising as VL complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 4, as VL CDR2 an amino acid sequence of SEQ ID NO: 5, and as VL CDR3 an amino acid sequence of SEQ ID NO: 6, and/or
 - 10 ii) an immunoglobulin heavy chain variable region (VH) comprising as VH complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 7, as VH CDR2 an amino acid sequence of SEQ ID NO: 8, and as VH CDR3 an amino acid sequence of SEQ ID NO: 9;
 - b. an active agent; and
 - 15 c. optionally a linker which links a) to b).

In one or more embodiments, the antibodies or antigen binding fragments thereof as described herein may comprise a heavy chain variable region (VH) comprising VH complementarity determining regions (CDRs) 1, 2, 3, wherein the VH CDR1 region comprises
20 or consists of an amino acid sequence that is at least 85%, 90%, 95%, 98%, or 100% identical to SEQ ID NO: 1 if according to Kabat definition or to SEQ ID NO: 7 if according to Chotia definition. In one or more embodiments, the antibodies or antigen binding fragments thereof as described herein may comprise a heavy chain variable region (VH) comprising VH complementarity determining regions (CDRs) 1, 2, 3, wherein the VH CDR2 region comprises
25 or consists of an amino acid sequence that is at least 85%, 90%, 95%, 98%, or 100% identical to SEQ ID NO: 2 if according to Kabat definition or to SEQ ID NO: 8 if according to Chotia definition. In one or more embodiments, the antibodies or antigen binding fragments thereof as described herein may comprise a heavy chain variable region (VH) comprising VH complementarity determining regions (CDRs) 1, 2, 3, wherein the VH CDR3 region comprises
30 or consists of an amino acid sequence that is at least 85%, 90%, 95%, 98%, or 100% identical to

SEQ ID NO: 3 if according to Kabat definition or to SEQ ID NO: 9 if according to Chotia definition.

In one or more embodiments, the antibodies or antigen binding fragments thereof as described herein may comprise a light chain variable region (VL) comprising VL
5 complementarity determining regions (CDRs) 1, 2, 3, wherein the VL CDR1 region comprises or consists of an amino acid sequence that is at least 85%, 90%, 95%, 98%, or 100% identical to SEQ ID NO: 4. In one or more embodiments, the antibodies or antigen binding fragments thereof as described herein may comprise a light chain variable region (VL) comprising VL
10 complementarity determining regions (CDRs) 1, 2, 3, wherein the VL CDR2 region comprises or consists of an amino acid sequence that is at least 85%, 90%, 95%, 98%, or 100% identical to SEQ ID NO: 5. In one or more embodiments, the antibodies or antigen binding fragments thereof as described herein may comprise a light chain variable region (VL) comprising VL
15 complementarity determining regions (CDRs) 1, 2, 3, wherein the VL CDR3 region comprises or consists of an amino acid sequence that is at least 85%, 90%, 95%, 98%, or 100% identical to SEQ ID NO: 6.

In one embodiment of the present disclosure, the amino acid sequences of SEQ ID NOs: 1-3, and SEQ ID NOs: 4-6, are according to Kabat definition. In one embodiment of the present disclosure, the amino acid sequences of SEQ ID NOs: 7-9, and SEQ ID NOs: 4-6, are according to Chothia definition.

20 In one embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

- a. the anti-GPC1 antibody or antigen binding fragment thereof as defined herein, comprising
 - 25 i) an immunoglobulin light chain variable region (VL) comprising or consisting of the amino acid sequence of SEQ ID NO: 11 or a sequence having at least 85% sequence identity thereto; and/or
 - ii) an immunoglobulin heavy chain variable region (VH) comprising or consisting of the amino acid sequence of SEQ ID NO: 10 or a sequence having at least 85% sequence identity thereto;
- 30 b. an active agent; and
- c. optionally a linker which links a) to b).

In one or more embodiments of the present disclosure, the antibodies or antigen binding fragments thereof as described herein may comprise a light chain variable region (VL), wherein the VL comprises or consists of an amino acid sequence that is at least 85%, 90%, 95%, 98%, or 100% identical to SEQ ID NO: 11, and/or may comprise a heavy chain variable region (VH), wherein the VH comprises or consists of an amino acid sequence that is at least 85%, 90%, 95%, 98%, or 100% identical to SEQ ID NO: 10.

It is well accepted and understood within the field that the CDRs are important for and largely responsible for antigen recognition. Therefore, in a preferred embodiment, any variation on sequence identity within the heavy chain variable region (VH) or within the light chain variable region (VL) is located outside the CDRs. All variant antibodies and antigen binding fragments disclosed herein retain the capability to efficiently bind to one or more of human GPC1 (SEQ ID NO: 16), monkey GPC1 (SEQ ID NO: 17), mouse GPC1 (SEQ ID NO: 18), and rat GPC1 (SEQ ID NO: 19).

In one embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

- a. the anti-GPC1 antibody or antigen binding fragment thereof as defined herein, comprising
 - i) an immunoglobulin light chain variable region (VL) comprising or consisting of the amino acid sequence of SEQ ID NO: 11; and/or
 - ii) an immunoglobulin heavy chain variable region (VH) comprising or consisting of the amino acid sequence of SEQ ID NO: 10;
- b. an active agent; and
- c. optionally a linker which links a) to b).

In one embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

- a. the anti-GPC1 antibody or antigen binding fragment thereof as defined herein, comprising
 - i) an immunoglobulin light chain variable region (VL) consisting of the amino acid sequence of SEQ ID NO: 11; and/or

- ii) an immunoglobulin heavy chain variable region (VH) consisting of the amino acid sequence of SEQ ID NO: 10;
- b. an active agent; and
- c. optionally a linker which links a) to b).

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In one embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

- a. the antibody as defined herein, comprising
 - i) an immunoglobulin light chain constant region comprising or consisting of the amino acid sequence of SEQ ID NO: 15; and/or
 - ii) an immunoglobulin heavy chain constant region comprising or consisting of the amino acid sequence of SEQ ID NO: 14;
- b. an active agent; and
- c. optionally a linker which links a) to b).

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In one embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

- a. the anti-GPC1 antibody or antigen binding fragment thereof as defined herein, comprising
 - i) an immunoglobulin light chain comprising or consisting of the amino acid sequences of SEQ ID NO: 11 and SEQ ID NO: 15 or a sequence having at least 85% sequence identity thereto; and/or
 - ii) an immunoglobulin heavy chain comprising or consisting of the amino acid sequence of SEQ ID NO: 10 and SEQ ID NO: 14 or a sequence having at least 85% sequence identity thereto;
- b. an active agent; and
- c. optionally a linker which links a) to b).

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In one embodiment of the present disclosure, the antibody-drug conjugate (ADC) as defined herein comprises:

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a. the anti-GPC1 antibody or antigen binding fragment thereof as defined herein, comprising

- i) an immunoglobulin light chain comprising or consisting of the amino acid sequences of SEQ ID NO: 11 and SEQ ID NO: 15; and/or
- 5 ii) an immunoglobulin heavy chain comprising or consisting of the amino acid sequence of SEQ ID NO: 10 and SEQ ID NO: 14;
- b. an active agent; and
- c. optionally a linker which links a) to b).

10 Active agent

The ADCs of the present disclosure comprise an active agent, e.g. a drug, which can be delivered intracellularly to cells expressing GPC1. The active agent may e.g. be a therapeutic agent, a radioisotope or a detectable label. In a preferred embodiment the active agent is a therapeutic agent.

15 In one embodiment, the active agent may be or comprise a radioisotope. The radioisotope may serve as a radiation emitter either for treatment of affected tissues or for diagnostic purposes. In one embodiment, the radioisotope may consist of or comprise ^{60}Co , ^{89}Sr , ^{90}Y , $^{99\text{m}}\text{Tc}$, ^{131}I , ^{137}Cs , ^{153}Sm , or ^{223}Rd . In one embodiment of the present disclosure, the radioisotope may be in combination with a chelator such as DOTA or EDTA or others which are well known in the

20 art.

In one embodiment the active agent is a therapeutic agent. Classes of therapeutic agents include DNA crosslinking agents, DNA alkylating agents, DNA strand scission agents, anthracyclines, antimetabolites, anti-microtubule/anti-mitotic agents, histone deacetylase inhibitors, kinase inhibitors, metabolism inhibitors, peptide antibiotics, immune checkpoint

25 inhibitors, platinum-based antineoplastics, topoisomerase inhibitors, DNA or RNA polymerase inhibitors, nucleotide based agents, and cytotoxic antibiotics.

In a preferred embodiment the active agent is a cytotoxic agent allowing for efficient killing of the cells expressing GPC1.

In one embodiment the active agent is a chemotherapeutic agent.

30 In one embodiment, the active agent is a DNA-crosslinking agent, such as cisplatin, carboplatin, oxaliplatin, mitomycin C (MMC), pyrrolobenzodiazepines, dimeric

pyrrolobenzodiazepines or a derivative or prodrug of any of the foregoing, exemplary SGD-1882.

In one embodiment of the present disclosure, the active agent is a DNA alkylating agent, such as nitrogen mustards, tris(2-chloroethyl)amine, pyridinobenzodiazepines, 5 indolinobenzodiazepine dimers, or Duocarmycin SA, or a derivative or prodrug of any of the foregoing.

In one embodiment, the active agent is a DNA strand scission agent, such as calicheamicin or hamiltroline or a derivative of any of these.

In one embodiment the active agent is an anthracycline, such as Daunorubicin, 10 doxorubicin, epirubicin, idarubicin, or PNU-159682 or a derivative or prodrug of any of the foregoing.

In one embodiment the active agent is an antimetabolite, such as folic acid antagonists, methotrexate, purine antimetabolites, 6-mercaptopurine, 6-thioguanine, fludarabine phosphate, pentostatin, cladribine, pyrimidine antimetabolites, 5-fluorouracil, 5-fluorodeoxyuridine, 15 cytarabine, gemcitabine, or a derivative or prodrug of any of the foregoing.

In one embodiment the active agent is an anti-mitotic agent, such as auristatin, dolastatin, monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), taxanes, Paclitaxel, Docetaxel, a vinca alkaloid, Vinblastine, Vincristine, Vindesine, Vinorelbine, maytansine, Colchicine, Podophyllotoxin, or a derivative or prodrug of any of the foregoing.

20 In one embodiment, the active agent is monomethyl auristatin E (MMAE) or a derivative thereof.

Because of its high toxicity, MMAE, which inhibits cell division by blocking the polymerization of tubulin, cannot be used as a single-agent chemotherapeutic drug. However, the combination of MMAE linked to an anti-CD30 monoclonal antibody (Brentuximab Vedotin, 25 trade name AdcetrisTM) has been proven to be stable in extracellular fluid, cleavable by cathepsin and safe for therapy.

In one embodiment, the active agent is a histone deacetylase inhibitor, such as trichostatin A, vorinostat, belinostat, panabioestat, givinostat, resminostat, abexinostat, quisinostat, rocilinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, 30 entinostat, tacedinaline, 4SC202, mocetinostat, romidepsin, nicotinamide, sirtinol, cambinol, or EX-527, or a derivative or prodrug of any of the foregoing.

In one embodiment, the active agent is a kinase inhibitor, such as genistein, lavendustin C, PP1-AG1872, PP2-AG1879, SU6656, CGP77675, PD166285, imatinib, erlotinib, gefitinib, lavendustin A, cetuximab, UCS15A, herbimycin A, or radicicol, or a derivative or prodrug of
5 any of the foregoing.

In one embodiment, the active agent is a metabolism inhibitor, such as an NAMPT inhibitor. Examples of NAMPT inhibitors include without limitation APO866, GMX-1777, GMX-1778 ATG-019, or OT-82, or a derivative or prodrug of any of the foregoing.

In one embodiment, the active agent is an immune checkpoint inhibitor, such as a PD-1
10 inhibitor or a PD-L1 inhibitor. Examples of PD-1 inhibitors include without limitation Pembrolizumab, Nivolumab, Cemiplimab, JTX-4014, Spartalizumab, Camrelizumab, Sintilimab, Tislelizumab, Toripalimab, Dostarlimab, AMP-224, or AMP-514 or a derivative or any of the foregoing. Examples of PD-L1 inhibitors include without limitation Atezolizumab, Avelumab, Durvalumab, KN035, CK-301, AUNP12, CA-170, or BMS-986189, or a derivative or prodrug of
15 any of the foregoing.

In one embodiment, the active agent is a platinum-based antineoplastic, such as lipoplatin, cisplatin, carboplatin, oxaliplatin, nedaplatin, picoplatin, phenanthriplatin, satraplatin, or triplatin tetranitrate, or a derivative or prodrug of any of the foregoing.

In one embodiment, the active agent is a topoisomerase inhibitor, such as a
20 topoisomerase-I (TopoI) inhibitor. Examples of topoisomerase inhibitors include without limitation camptothecin, topotecan, belotecan, lurtotecan, irinotecan, SN-38, exatecan, Dxd, or a derivative or prodrug of any of the foregoing.

In one embodiment, the active agent is a TopoI inhibitor such as exatecan or a derivative thereof

25 In one embodiment, the active agent is a DNA- or RNA-polymerase inhibitor, such as amanitin, alpha-amanitin, actinomycin D, aphidicolin, or a derivative or prodrug of any of the foregoing.

In one embodiment, the active agent is a nucleotide-based agent, such as an RNA- or DNA-oligonucleotide, such as an siRNA or a miRNA.

30

There may be one or more units of drug per antibody molecule. The ratio between the number of drug molecules per antibody is denoted the drug-to-antibody ratio (DAR). In one embodiment, the DAR is between 1 and 10, such as between 2 and 8, for example between 2 and 6, such as 2 or 4. In one embodiment, the DAR is 2, 3, 4, 5, 6, 7, or 8.

5

Linker

A stable link between the antibody and the active agent is an important aspect of ADC technology. Linkers may e.g. be based on chemical motifs including disulfides, hydrazones or peptides (cleavable), or thioethers (noncleavable), and control the distribution and delivery of the cytotoxic agent to the target cell. Cleavable and noncleavable types of linkers have been proven to be safe in preclinical and clinical trials. For example, Brentuximab Vedotin includes an enzyme-sensitive cleavable linker that delivers the potent and highly toxic antimicrotubule agent monomethyl auristatin E (MMAE), a synthetic antineoplastic agent, to cells.

Trastuzumab Emtansine, another approved ADC, is a combination of the microtubule-formation inhibitor mertansine (DM-1), a derivative of Maytansine, and antibody Trastuzumab (HerceptinTM, Genentech/Roche), attached by a stable, non-cleavable linker.

The type of linker, cleavable or non-cleavable, lends specific properties to the delivered drug. For example, cleavable linkers can e.g. be cleaved by enzymes in the target cell, leading to efficient intracellular release of the active agent, for example a cytotoxic agent. In contrast, an ADC containing a non-cleavable linker has no mechanism for drug release, and must rely on mechanisms such as degradation of the targeting antibody, for drug release. Furthermore, as is appreciated by those skilled in the art, the linker composition may influence critical factors such as solubility and pharmacokinetic properties of the ADC as a whole.

For both types of linker, drug release is crucial for obtaining a cellular effect. Drugs which are able to freely diffuse across cell membranes may escape from the targeted cell and, in a process called “bystander killing,” also attack neighbouring cells, such as cancer cells in the vicinity of the GPC1 expressing target cell. In one embodiment of the present disclosure, the ADC described herein may comprise a payload which allows for bystander killing of neighbouring cells, such as neighbouring tumour and/or cancer cells. Such bystander killing is particularly desirable in case the neighbouring tumour and/or cancer cells are not expressing any GPC1. For other situations it is preferred that bystander killing is not observed, such as in

situations where neighbouring cells are healthy cells/tissue. Therefore in one or more embodiments of the present disclosure, the ADC as described herein comprises a payload which does not provide any significant bystander killing of neighbouring cells.

In a preferred embodiment of the present disclosure, the ADC targeting GPC1 as disclosed herein comprises a linker that links the antibody to the active agent.

In one embodiment of the present disclosure, the linker may be cleavable or non-cleavable.

Cleavable groups include a disulfide bond, an amide bond, a substituted amide bond in the form of a peptide bond, a thioamide, bond, an ester bond, a thioester bond, a vicinal diol bond, or a hemiacetal. These, or other cleavable bonds, may include enzymatically-cleavable bonds, such as peptide bonds (cleaved by peptidases), phosphate bonds (cleaved by phosphatases), nucleic acid bonds (cleaved by endonucleases), and sugar bonds (cleaved by glycosidases).

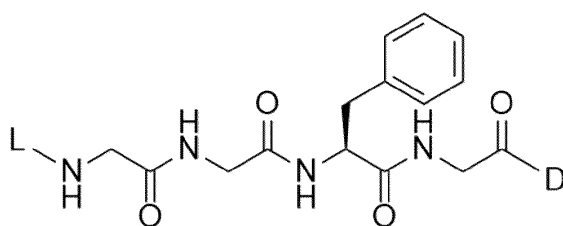
In a further embodiment of the present disclosure, the linker is a cleavable linker allowing for intracellular release of the active agent inside the target cells.

In a further embodiment the linker is a peptide linker. The choice of peptide sequence is critical to the success of the conjugate. In some embodiments the linker is stable to serum proteases, yet is cleaved by lysosomal enzymes in the target cell.

In a further embodiment the linker is an enzyme-cleavable peptide-containing linker, such as a cathepsin cleavable peptide-containing linker. Cathepsin can be one of several cathepsin types, being one of a group of lysosomal proteases.

In one or more embodiments of the present disclosure, the linker comprises or consists of a dipeptide, tripeptide, tetrapeptide or pentapeptide.

In one or more embodiments of the present disclosure, the linker comprises or consists of a tetrapeptide wherein each amino acid of the tetrapeptide is individually selected from glycine or phenylalanine. In one or more embodiments of the present disclosure, the tetrapeptide is GGFG (SEQ ID NO: 20) and has the structure of formula (I):



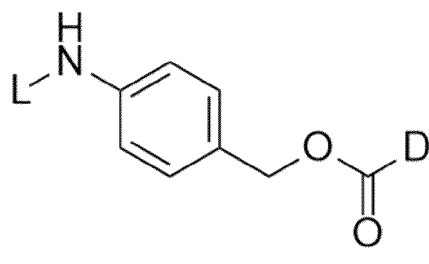
wherein 'L' represents connectivity to the remaining linker in direction of the antibody or antibody-binding attachment group of the ADC, and wherein 'D' represents connectivity to the active agent, directly or in the form of a spacer.

5 In a further embodiment of the present disclosure, the linker comprises or consists of a dipeptide, such as valine-citrulline (VC) or valine-alanine (VA).

In one embodiment the linker comprises or consists of a dipeptide, such as valine-citrulline (VC) or valine-alanine (VA), which may be further connected through an amide linkage to other structural elements. Valine-citrulline-based linkers, in which the citrulline
10 carboxyl function is modified to a substituted amide, can be cleaved by lysosomal cathepsins, whereas valine-alanine-based linkers, in which the alanine carboxyl function is modified to a substituted amide, can be cleaved by other lysosomal proteases, including other cathepsins.

In a further embodiment of the present disclosure, the antibody-drug conjugate as defined herein further comprises a spacer, such as a spacer comprising p-aminobenzyl (PAB), p-
15 aminobenzylcarbamate, p-aminobenzoyloxycabonyl (PABC), or polyethyleneglycol (PEG). Spacers such as PABC may also sometimes be referred to as self-immolative linkers. Self-immolative linkers (or spacers) are well-known in the art.

In one embodiment of the present disclosure, the antibody-drug conjugate as defined herein comprises p-aminobenzoyloxycabonyl (PABC). PABC may sometimes in the art be used
20 interchangeably with PAB. For clarity, PABC within the present disclosure is intended to be interpreted as a motif having the following structure:



wherein 'L' represents connectivity to the remaining linker in direction of the antibody of the ADC, and wherein 'D' represents connectivity to the active agent, directly or in the form of a spacer.

5 In a further embodiment of the present disclosure, the antibody-drug conjugate as defined herein further comprises an attachment group, such as an attachment group comprising or consisting of maleimide and caproic acid (MC, also sometimes referred to as maleimidocaproyl), N-hydroxysuccinimide, reactive attachment groups directed to modified or unmodified protein-bound carbohydrate, peptide sequences that are required for enzymatic reactions, azides or
10 alkynes (such as for undergoing SPAAC or CuAAC) or being derived from any of the foregoing by reaction with the antibody or a chemically or enzymatically generated derivative thereof.

In one embodiment of the present disclosure, the ADC of the present disclosure further comprises an attachment entity. The attachment entity may for example connect the antibody and the cleavable linker, where the attachment entity is the reaction product between an antibody
15 amino acid side chain and a reactive attachment group in the linker precursor. In one embodiment, this reactive attachment group comprises or consists of maleimide and caproic acid (MC), where maleimide reacts preferably with cysteine thiols during coupling to the antibody. In other embodiments, the attachment group comprises or consists of N-hydroxysuccinimide, reactive attachment groups directed to modified or unmodified protein-bound carbohydrate,
20 peptide sequences that are required for enzymatic reactions, azides or alkynes (such as for undergoing SPAAC or CuAAC) or being derived from any of the foregoing by reaction with the antibody or a chemically or enzymatically generated derivative thereof.

Within the meaning of both attachment group and attachment entity as described here above, MC refers to well-known motif in the ADC field and may also sometimes be referred to
25 as maleimido caproyl entities. The acid end of caproic acid (in the MC group) is normally connected via formation of an amide bond to a nitrogen of a subsequent group (being either the

active agent /cytotoxic drug, or a further spacer/linker of the ADC), such as the amine-end of a peptide of 2-6 residues comprised in the ADC.

In one embodiment of the present disclosure, the ADC comprises an antibody targeting GPC1 as defined herein, and the linker-drug complex Vedotin. Vedotin is a linker-drug complex comprising the cytotoxic agent MMAE, a spacer (p-aminobenzyloxycabonyl), a cathepsin-cleavable linker (Valine-citrulline dipeptide) and an attachment group consisting of caproic acid and maleimide. Vedotin is MC-VC-PABC-MMAE.

In one embodiment, the ADC of the present disclosure targeting GPC1 comprises the antibody as defined herein, and a linker-spacer-toxin unit comprising VC-PABC-MMAE.

10 In one embodiment, the ADC of the present disclosure targeting GPC1 comprises the antibody as defined herein, and a linker-spacer-toxin unit comprising VC-PABC-MMAF.

In one embodiment, the ADC of the present disclosure targeting GPC1 comprises the antibody as defined herein, and a linker-spacer-toxin unit comprising VC-PABC-exatecan.

15 In one embodiment, the ADC of the present disclosure targeting GPC1 comprises the antibody as defined herein, and a linker-spacer-toxin unit comprising VA-PABC-exatecan.

In one embodiment, the ADC of the present disclosure targeting GPC1 comprises the antibody as defined herein, and a linker-spacer-toxin unit comprising VC-PABC-Dxd.

In one embodiment, the ADC of the present disclosure targeting GPC1 comprises the antibody as defined herein, and a linker-spacer-toxin unit comprising VA-PABC-Dxd.

20 In one embodiment, the ADC of the present disclosure targeting GPC1 comprises the antibody as defined herein, and a linker-spacer-toxin unit comprising GGFG-PABC-MMAE.

In one embodiment, the ADC of the present disclosure targeting GPC1 comprises the antibody as defined herein, and a linker-spacer-toxin unit comprising GGFG-PABC-MMAF.

25 In one embodiment, the ADC of the present disclosure targeting GPC1 comprises the antibody as defined herein, and a linker-spacer-toxin unit comprising GGFG-PABC-exatecan.

In one embodiment, the ADC of the present disclosure targeting GPC1 comprises the antibody as defined herein, and a linker-spacer-toxin unit comprising GGFG-exatecan.

In one embodiment, the ADC of the present disclosure targeting GPC1 comprises the antibody as defined herein, and a linker-spacer-toxin unit comprising GGFG-PABC-Dxd.

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In one embodiment, the ADC of the present disclosure targeting GPC1 comprises the antibody as defined herein, and a linker-spacer-toxin unit comprising GGFG-Dxd.

In one embodiment, the ADC of the present disclosure targeting GPC1 comprises the antibody as defined herein, and a linker-spacer-toxin unit of formula (I), wherein D represents
5 PABC-MMAE.

In one embodiment, the ADC of the present disclosure targeting GPC1 comprises the antibody as defined herein, and a linker-spacer-toxin unit of formula (I), wherein D represents PABC-MMAF.

In one embodiment, the ADC of the present disclosure targeting GPC1 comprises the
10 antibody as defined herein, and a linker-spacer-toxin unit of formula (I), wherein D represents PABC-exatecan.

In one embodiment, the ADC of the present disclosure targeting GPC1 comprises the antibody as defined herein, and a linker-spacer-toxin of formula (I), wherein D represents exatecan.

15 In one embodiment, the ADC of the present disclosure targeting GPC1 comprises the antibody as defined herein, and a linker-spacer-toxin of formula (I), wherein D represents PABC-Dxd.

In one embodiment, the ADC of the present disclosure targeting GPC1 comprises the antibody as defined herein, and a linker-spacer-toxin of formula (I), wherein D represents Dxd.

20 In one embodiment, the ADC of the present disclosure targeting GPC1 comprises or consists of:

a. the antibody as defined herein, comprising:

- 25 i) an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 11, and/or
ii) an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 10,

b. a VC or VA dipeptide linker,

c. an MC attachment group,

d. optionally, a PAB or a PABC spacer, and

30 e. MMAE as active agent.

In one embodiment, the ADC of the present disclosure targeting GPC1 comprises or consists of:

- a. the antibody as defined herein, comprising:
 - i) an immunoglobulin light chain comprising or consisting of the amino acid sequences of SEQ ID NO: 11 and SEQ ID NO: 15, and/or
 - ii) an immunoglobulin heavy chain comprising or consisting of the amino acid sequence of SEQ ID NO: 10 and SEQ ID NO: 14,
- b. a VC or VA linker,
- c. an MC attachment group,
- d. optionally, a PAB or a PABC spacer, and
- e. MMAE as active agent.

In one embodiment, the ADC of the present disclosure targeting GPC1 comprises or consists of:

- a. the antibody as defined herein, comprising:
 - i) an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 11, and/or
 - ii) an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 10,
- b. a VC or VA dipeptide linker,
- c. an MC attachment group,
- d. optionally, a PAB or a PABC spacer, and
- e. exatecan or Dxd as active agent.

In one embodiment, the ADC of the present disclosure targeting GPC1 comprises or consists of:

- a. the antibody as defined herein, comprising:
 - i) an immunoglobulin light chain comprising or consisting of the amino acid sequences of SEQ ID NO: 11 and SEQ ID NO: 15, and/or
 - ii) an immunoglobulin heavy chain comprising or consisting of the amino acid sequence of SEQ ID NO: 10 and SEQ ID NO: 14,

- b. a VC or VA linker,
- c. an MC attachment group,
- d. optionally, a PAB or a PABC spacer, and
- e. exatecan or Dxd as active agent.

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In one embodiment, the ADC of the present disclosure targeting GPC1 comprises or consists of:

- a. the antibody as defined herein, comprising:
 - i) an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 11, and/or
 - ii) an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 10,
- b. a tetrapeptide linker, such as of formula (I),
- c. an MC attachment group,
- d. optionally, a PAB or PABC spacer group, and
- e. MMAE as active agent.

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In one embodiment, the ADC of the present disclosure targeting GPC1 comprises or consists of:

- a. the antibody as defined herein, comprising:
 - i) an immunoglobulin light chain comprising or consisting of the amino acid sequences of SEQ ID NO: 11 and SEQ ID NO: 15, and/or
 - ii) an immunoglobulin heavy chain comprising or consisting of the amino acid sequence of SEQ ID NO: 10 and SEQ ID NO: 14,
- b. a tetrapeptide linker, such as of formula (I),
- c. an MC attachment group,
- d. optionally, a PAB or PABC spacer group, and
- e. MMAE as active agent.

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In one embodiment, the ADC of the present disclosure targeting GPC1 comprises or consists of:

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- a. the antibody as defined herein, comprising:
 - i) an immunoglobulin light chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 11, and/or
 - ii) an immunoglobulin heavy chain variable region comprising or consisting of the amino acid sequence of SEQ ID NO: 10,
- b. a tetrapeptide linker, such as of formula (I),
- c. an MC attachment group,
- d. optionally, a PAB or PABC spacer group, and
- e. exatecan or Dxd as active agent.

In one embodiment, the ADC of the present disclosure targeting GPC1 comprises or consists of:

- a. the antibody as defined herein, comprising:
 - i) an immunoglobulin light chain comprising or consisting of the amino acid sequences of SEQ ID NO: 11 and SEQ ID NO: 15, and/or
 - ii) an immunoglobulin heavy chain comprising or consisting of the amino acid sequence of SEQ ID NO: 10 and SEQ ID NO: 14,
- b. a tetrapeptide linker, such as of formula (I),
- c. an MC attachment group,
- d. optionally, a PAB or PABC spacer group, and
- e. exatecan or Dxd as active agent.

Methods of Making Anti-GPC1 Antibodies

An isolated fragment of human GPC1 can be used as an immunogen to generate antibodies using standard techniques for polyclonal and monoclonal antibody preparation. Polyclonal antibodies can be raised in animals by multiple injections (e.g., subcutaneous or intraperitoneal injections) of an antigenic peptide or protein. In some embodiments, the antigenic peptide or protein is injected with at least one adjuvant. In some embodiments, the antigenic peptide or protein can be conjugated to an agent that is immunogenic in the species to be immunized. Animals can be injected with the antigenic peptide or protein more than one time (e.g., twice, three times, or four times).

The full-length polypeptide or protein can be used or, alternatively, antigenic peptide fragments thereof can be used as immunogens. The antigenic peptide of a protein comprises at least 8 (e.g., at least 10, 15, 20, or 30) amino acid residues of the amino acid sequence of GPC1 and encompasses an epitope of the protein such that an antibody raised against the peptide forms
5 a specific immune complex with the protein. As described above, the full-length sequence of human GPC1 is known in the art. In some embodiments, an Fc-tagged or His-tagged human GPC1 protein is used as the immunogen.

An immunogen typically is used to prepare antibodies by immunizing a suitable subject (e.g., human or transgenic animal expressing at least one human immunoglobulin locus). An
10 appropriate immunogenic preparation can contain, for example, a recombinantly-expressed or a chemically-synthesized polypeptide (e.g., a fragment of human GPC1). The preparation can further include an adjuvant, such as Freund's complete or incomplete adjuvant, or a similar immunostimulatory agent.

Polyclonal antibodies can be prepared as described above by immunizing a suitable
15 subject with a GPC1 polypeptide, or an antigenic peptide thereof (e.g., part of GPC1) as an immunogen. The antibody titer in the immunized subject can be monitored over time by standard techniques, such as with an enzyme-linked immunosorbent assay (ELISA) using the immobilized GPC1 polypeptide or peptide. If desired, the antibody molecules can be isolated from the mammal (e.g., from the blood) and further purified by well-known techniques, such as protein A
20 or protein G chromatography to obtain the IgG fraction. At an appropriate time after immunization, e.g., when the specific antibody titers are highest, antibody-producing cells can be obtained from the subject and used to prepare monoclonal antibodies by standard techniques, such as the hybridoma technique originally described by Kohler et al. (*Nature* 256:495-497, 1975), the human B cell hybridoma technique (Kozbor et al., *Immunol. Today* 4:72, 1983), the
25 EBV-hybridoma technique (Cole et al., *Monoclonal Antibodies and Cancer Therapy*, Alan R. Liss, Inc., pp. 77-96, 1985), or trioma techniques. The technology for producing hybridomas is well known (see, generally, *Current Protocols in Immunology*, 1994, Coligan et al. (Eds.), John Wiley & Sons, Inc., New York, NY). Hybridoma cells producing a monoclonal antibody are detected by screening the hybridoma culture supernatants for antibodies that bind the polypeptide
30 or epitope of interest, e.g., using a standard ELISA assay.

5 Variants of the antibodies or antigen-binding fragments described herein can be prepared by introducing appropriate nucleotide changes into the DNA encoding a human, humanized, or chimeric antibody, or antigen-binding fragment thereof described herein, or by peptide synthesis. Such variants include, for example, deletions, insertions, or substitutions of residues within the amino acids sequences that make-up the antigen-binding site of the antibody or an antigen-binding domain. In a population of such variants, some antibodies or antigen-binding fragments will have increased affinity for the target protein, e.g., GPC1. Any combination of deletions, insertions, and/or combinations can be made to arrive at an antibody or antigen-binding fragment thereof that has increased binding affinity for the target. The amino acid changes introduced into
10 the antibody or antigen-binding fragment can also alter or introduce new post-translational modifications into the antibody or antigen-binding fragment, such as changing (e.g., increasing or decreasing) the number of glycosylation sites, changing the type of glycosylation site (e.g., changing the amino acid sequence such that a different sugar is attached by enzymes present in a cell), or introducing new glycosylation sites.

15 Antibodies disclosed herein can be derived from any species of animal, including mammals. Non-limiting examples of native antibodies include antibodies derived from humans, primates, e.g., monkeys and apes, cows, pigs, horses, sheep, camelids (e.g., camels and llamas), chicken, goats, and rodents (e.g., rats, mice, hamsters and rabbits), including transgenic rodents genetically engineered to produce human antibodies.

20 Human and humanized antibodies include antibodies having variable and constant regions derived from (or having the same amino acid sequence as those derived from) human germline immunoglobulin sequences. Human antibodies 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), for example in the CDRs.

25 A humanized antibody, typically has a human framework (FR) grafted with non-human CDRs. Thus, a humanized antibody has one or more amino acid sequence introduced into it from a source which is non-human. These non-human amino acid residues are often referred to as “import” residues, which are typically taken from an “import” variable domain. Humanization can be essentially performed by e.g., substituting rodent CDRs or CDR sequences for the
30 corresponding sequences of a human antibody. These methods are described in e.g., Jones et al., Nature, 321:522-525 (1986); Riechmann et al., Nature, 332:323-327 (1988); Verhoeyen et al.,

Science, 239:1534-1536 (1988); each of which is incorporated by reference herein in its entirety. Accordingly, “humanized” antibodies are chimeric antibodies wherein substantially less than an intact human V domain has been substituted by the corresponding sequence from a non-human species. In practice, humanized antibodies are typically mouse antibodies in which some CDR
5 residues and some FR residues are substituted by residues from analogous sites in human antibodies.

The choice of human VH and VL domains to be used in making the humanized antibodies is very important for reducing immunogenicity. According to the so-called “best-fit” method, the sequence of the V domain of a mouse antibody is screened against the entire library
10 of known human-domain sequences. The human sequence which is closest to that of the mouse is then accepted as the human FR for the humanized antibody (Sims et al., J. Immunol., 151:2296 (1993); Chothia et al., J. Mol. Biol., 196:901 (1987)).

It is further important that antibodies be humanized with retention of high specificity and affinity for the antigen and other favorable biological properties. To achieve this goal,
15 humanized antibodies can be prepared by a process of analysis of the parental sequences and various conceptual humanized products using three-dimensional models of the parental and humanized sequences. Three-dimensional immunoglobulin models are commonly available and are familiar to those skilled in the art. Computer programs are available which illustrate and display probable three-dimensional conformational structures of selected candidate
20 immunoglobulin sequences. Inspection of these displays permits analysis of the likely role of the residues in the functioning of the candidate immunoglobulin sequence, i.e., the analysis of residues that influence the ability of the candidate immunoglobulin to bind its antigen. In this way, FR residues can be selected and combined from the recipient and import sequences so that the desired antibody characteristic, such as increased affinity for the target antigen(s), is
25 achieved.

Ordinarily, amino acid sequence variants of the human, humanized, or chimeric anti-GPC1 antibody will contain an amino acid sequence having at least 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% percent identity with a sequence present in the light or heavy chain of the original antibody.

30 In some embodiments, a mouse (e.g., RenMab™ mouse) with a humanized heavy chain immunoglobulin locus and a humanized kappa chain immunoglobulin locus is used to generate

antibodies. The heavy chain immunoglobulin locus is a region on the chromosome that contains genes for the heavy chains of antibodies. The locus can include e.g., human IGHV (variable) genes, human IGHD (diversity) genes, human IGHJ (joining) genes, and mouse heavy chain constant domain genes. The kappa chain immunoglobulin locus is a region on the chromosome
5 that contains genes that encode the light chains of antibodies (kappa chain). The kappa chain immunoglobulin locus can include e.g., human IGKV (variable) genes, human IGKJ (joining) genes, and mouse light chain constant domain genes. A detailed description regarding RenMab™ mice can be found in PCT/CN2020/075698 or US20200390073A1, which is incorporated herein by reference in its entirety.

10 In some embodiments, a mouse (e.g., RenLite™ mouse) with a humanized heavy chain immunoglobulin locus and a humanized kappa chain immunoglobulin locus is used to generate antibodies. The heavy chain immunoglobulin locus is a region on the chromosome that contains genes for the heavy chains of antibodies. The locus can include e.g., human IGHV (variable) genes, human IGHD (diversity) genes, human IGHJ (joining) genes, and mouse heavy chain
15 constant domain genes. The kappa chain immunoglobulin locus is a region on the chromosome that contains genes that encode a common light chain. The kappa chain immunoglobulin locus can include e.g., a human IGKV (variable) gene, a human IGKJ (joining) gene, and mouse light chain constant domain genes. A detailed description regarding RenLite™ mice can be found in PCT/CN2021/097652, which is incorporated herein by reference in its entirety.

20 The antibodies generated by the mice have a full human VH, a full human VL, and mouse constant regions. In some embodiments, the human VH and human VL is linked to a human IgG constant region (e.g., IgG1, IgG2, IgG3, and IgG4).

Identity or homology with respect to an original sequence is usually the percentage of amino acid residues present within the candidate sequence that are identical with a sequence
25 present within the human, humanized, or chimeric anti-GPC1 antibody or fragment, after aligning the sequences and introducing gaps, if necessary, to achieve the maximum percent sequence identity, and not considering any conservative substitutions as part of the sequence identity.

Additional modifications to the anti-GPC1 antibodies or antigen-binding fragments can
30 be made. For example, a cysteine residue(s) can be introduced into the Fc region, thereby allowing interchain disulfide bond formation in this region. The homodimeric antibody thus

generated may have any increased half-life *in vitro* and/or *in vivo*. Homodimeric antibodies with increased half-life *in vitro* and/or *in vivo* can also be prepared using heterobifunctional cross-linkers as described, for example, in Wolff et al. (*Cancer Res.* 53:2560-2565, 1993).

Alternatively, an antibody can be engineered which has dual Fc regions (see, for example, Stevenson et al., *Anti-Cancer Drug Design* 3:219-230, 1989).

In some embodiments, a covalent modification can be made to the anti-GPC1 antibody or antigen-binding fragment thereof. These covalent modifications can be made by chemical or enzymatic synthesis, or by enzymatic or chemical cleavage. Other types of covalent modifications of the antibody or antibody fragment are introduced into the molecule by reacting targeted amino acid residues of the antibody or fragment with an organic derivatization agent that is capable of reacting with selected side chains or the N- or C-terminal residues.

In some embodiments, antibody variants are provided having a carbohydrate structure that lacks fucose attached (directly or indirectly) to an Fc region. For example, the amount of fucose in such antibody may be from 1% to 80%, from 1% to 65%, from 5% to 65% or from 20% to 40%. The amount of fucose is determined by calculating the average amount of fucose within the sugar chain at Asn297, relative to the sum of all glycostructures attached to Asn 297 (e.g. complex, hybrid and high mannose structures) as measured by MALDI-TOF mass spectrometry, as described in WO 2008/077546, for example. Asn297 refers to the asparagine residue located at about position 297 in the Fc region (Eu numbering of Fc region residues; or position 314 in Kabat numbering); however, Asn297 may also be located about ± 3 amino acids upstream or downstream of position 297, i.e., between positions 294 and 300, due to minor sequence variations in antibodies. Such fucosylation variants may have improved ADCC function. In some embodiments, to reduce glycan heterogeneity, the Fc region of the antibody can be further engineered to replace the Asparagine at position 297 with Alanine (N297A).

In some embodiments, to facilitate production efficiency by avoiding Fab-arm exchange, the Fc region of the antibodies was further engineered to replace the serine at position 228 (EU numbering) of IgG4 with proline (S228P). A detailed description regarding S228 mutation is described, e.g., in Silva et al. "The S228P mutation prevents *in vivo* and *in vitro* IgG4 Fab-arm exchange as demonstrated using a combination of novel quantitative immunoassays and physiological matrix preparation." *Journal of Biological Chemistry* 290.9 (2015): 5462-5469, which is incorporated by reference in its entirety.

Recombinant Vectors

The present disclosure also provides recombinant vectors (e.g., an expression vectors) that include an isolated polynucleotide disclosed herein (e.g., a polynucleotide that encodes a polypeptide disclosed herein), host cells into which are introduced the recombinant vectors (i.e.,
5 such that the host cells contain the polynucleotide and/or a vector comprising the polynucleotide), and the production of recombinant antibody polypeptides or fragments thereof by recombinant techniques.

As used herein, a “vector” is any construct capable of delivering one or more
10 polynucleotide(s) of interest to a host cell when the vector is introduced to the host cell. An “expression vector” is capable of delivering and expressing the one or more polynucleotide(s) of interest as an encoded polypeptide in a host cell into which the expression vector has been introduced. Thus, in an expression vector, the polynucleotide of interest is positioned for expression in the vector by being operably linked with regulatory elements such as a promoter,
15 enhancer, and/or a poly-A tail, either within the vector or in the genome of the host cell at or near or flanking the integration site of the polynucleotide of interest such that the polynucleotide of interest will be translated in the host cell introduced with the expression vector.

A vector can be introduced into the host cell by methods known in the art, e.g., electroporation, chemical transfection (e.g., DEAE-dextran), transformation, transfection, and
20 infection and/or transduction (e.g., with recombinant virus). Thus, non-limiting examples of vectors include viral vectors (which can be used to generate recombinant virus), naked DNA or RNA, plasmids, cosmids, phage vectors, and DNA or RNA expression vectors associated with cationic condensing agents.

In some implementations, a polynucleotide disclosed herein (e.g., a polynucleotide that
25 encodes a polypeptide disclosed herein) is introduced using a viral expression system (e.g., vaccinia or other pox virus, retrovirus, or adenovirus), which may involve the use of a non-pathogenic (defective), replication competent virus, or may use a replication defective virus. In the latter case, viral propagation generally will occur only in complementing virus packaging cells. Suitable systems are disclosed, for example, in Fisher-Hoch et al., 1989, Proc. Natl. Acad.
30 Sci. USA 86:317-321; Flexner et al., 1989, Ann. N.Y. Acad. Sci. 569:86-103; Flexner et al., 1990, Vaccine, 8:17-21; U.S. Pat. Nos. 4,603,112, 4,769,330, and 5,017,487; WO 89/01973;

U.S. Pat. No. 4,777,127; GB 2,200,651; EP 0,345,242; WO 91/02805; Berkner-Biotechniques, 6:616-627, 1988; Rosenfeld et al., 1991, Science, 252:431-434; Kolls et al., 1994, Proc. Natl. Acad. Sci. USA, 91:215-219; Kass-Eisler et al., 1993, Proc. Natl. Acad. Sci. USA, 90:11498-11502; Guzman et al., 1993, Circulation, 88:2838-2848; and Guzman et al., 1993, Cir. Res.,
5 73:1202-1207. Techniques for incorporating DNA into such expression systems are well known to those of ordinary skill in the art. The DNA may also be "naked," as described, for example, in Ulmer et al., 1993, Science, 259:1745-1749, and Cohen, 1993, Science, 259:1691-1692. The uptake of naked DNA may be increased by coating the DNA onto biodegradable beads that are efficiently transported into the cells.

10 For expression, the DNA insert comprising an antibody-encoding or polypeptide-encoding polynucleotide disclosed herein can be operatively linked to an appropriate promoter (e.g., a heterologous promoter), such as the phage lambda PL promoter, the *E. coli* lac, trp and tac promoters, the SV40 early and late promoters and promoters of retroviral LTRs, to name a few. Other suitable promoters are known to the skilled artisan. The expression constructs can
15 further contain sites for transcription initiation, termination and, in the transcribed region, a ribosome binding site for translation. The coding portion of the mature transcripts expressed by the constructs may include a translation initiating at the beginning and a termination codon (UAA, UGA, or UAG) appropriately positioned at the end of the polypeptide to be translated.

As indicated, the expression vectors can include at least one selectable marker. Such
20 markers include dihydrofolate reductase or neomycin resistance for eukaryotic cell culture and tetracycline or ampicillin resistance genes for culturing in *E. coli* and other bacteria. Representative examples of appropriate hosts include, but are not limited to, bacterial cells, such as *E. coli*, *Streptomyces*, and *Salmonella typhimurium* cells; fungal cells, such as yeast cells; insect cells such as *Drosophila* S2 and *Spodoptera Sf9* cells; animal cells such as CHO, COS,
25 Bowes melanoma, and HK 293 cells; and plant cells. Appropriate culture mediums and conditions for the host cells described herein are known in the art.

Non-limiting vectors for use in bacteria include pQE70, pQE60 and pQE-9, available from Qiagen; pBS vectors, Phagescript vectors, Bluescript vectors, pNH8A, pNH16a, pNH18A, pNH46A, available from Stratagene; and ptrc99a, pKK223-3, pKK233-3, pDR540, pRIT5
30 available from Pharmacia. Non-limiting eukaryotic vectors include pWLNEO, pSV2CAT,

pOG44, pXT1 and pSG available from Stratagene; and pSVK3, pBPV, pMSG and pSVL available from Pharmacia. Other suitable vectors will be readily apparent to the skilled artisan.

Non-limiting bacterial promoters suitable for use include the *E. coli* lacI and lacZ promoters, the T3 and T7 promoters, the gpt promoter, the lambda PR and PL promoters and the
5 trp promoter. Suitable eukaryotic promoters include the CMV immediate early promoter, the HSV thymidine kinase promoter, the early and late SV40 promoters, the promoters of retroviral LTRs, such as those of the Rous sarcoma virus (RSV), and metallothionein promoters, such as the mouse metallothionein-I promoter.

In the yeast *Saccharomyces cerevisiae*, a number of vectors containing constitutive or
10 inducible promoters such as alpha factor, alcohol oxidase, and PGH may be used. For reviews, see Ausubel *et al.* (1989) Current Protocols in Molecular Biology, John Wiley & Sons, New York, N.Y. and Grant *et al.*, *Methods Enzymol.*, 153: 516-544 (1997).

Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-dextran mediated transfection, cationic lipid-mediated transfection,
15 electroporation, transduction, infection or other methods. Such methods are described in many standard laboratory manuals, such as Davis *et al.*, Basic Methods In Molecular Biology (1986), which is incorporated herein by reference in its entirety.

Transcription of DNA encoding an antibody of the present disclosure by higher eukaryotes may be increased by inserting an enhancer sequence into the vector. Enhancers are
20 cis-acting elements of DNA, usually about from 10 to 300 bp that act to increase transcriptional activity of a promoter in a given host cell-type. Examples of enhancers include the SV40 enhancer, which is located on the late side of the replication origin at base pairs 100 to 270, the cytomegalovirus early promoter enhancer, the polyoma enhancer on the late side of the replication origin, and adenovirus enhancers.

25 For secretion of the translated protein into the lumen of the endoplasmic reticulum, into the periplasmic space or into the extracellular environment, appropriate secretion signals may be incorporated into the expressed polypeptide. The signals may be endogenous to the polypeptide or they may be heterologous signals.

The polypeptide (e.g., antibody) can be expressed in a modified form, such as a fusion
30 protein (e.g., a GST-fusion) or with a histidine-tag, and may include not only secretion signals, but also additional heterologous functional regions. For instance, a region of additional amino

acids, particularly charged amino acids, may be added to the N-terminus of the polypeptide to improve stability and persistence in the host cell, during purification, or during subsequent handling and storage. Also, peptide moieties can be added to the polypeptide to facilitate purification. Such regions can be removed prior to final preparation of the polypeptide. The
5 addition of peptide moieties to polypeptides to engender secretion or excretion, to improve stability and to facilitate purification, among others, are familiar and routine techniques in the art.

Methods of Treatment

10 The antibodies or antigen-binding fragments thereof of the present disclosure can be used for various therapeutic purposes. For example, the antibody or antigen binding fragment or antibody-drug conjugate disclosed herein may be used to treat a disease characterized by cells expressing GPC1.

In one aspect, the disclosure provides methods for treating a cancer in a subject, methods
15 of reducing the rate of the increase of volume of a tumor in a subject over time, methods of reducing the risk of developing a metastasis, or methods of reducing the risk of developing an additional metastasis in a subject. In some embodiments, the treatment can halt, slow, retard, or inhibit progression of a cancer. In some embodiments, the treatment can result in the reduction of in the number, severity, and/or duration of one or more symptoms of the cancer in a subject.

20 In one aspect, the disclosure provides the antibody, antibody-drug conjugate or pharmaceutical composition as disclosed herein for use as a medicament.

In one aspect, the disclosure provides a use of the antibody, antibody-drug conjugate or pharmaceutical composition as disclosed herein for the manufacture of a medicament for treatment of a disease, e.g. cancer.

25 Within the scope of the present application is also provided the antibody-drug conjugate as described here above for use as a medicament.

One or more embodiments of the present disclosure is directed to the antibody-drug conjugate as described here above for use in a method of treatment of a disease or disorder characterized by cell expressing GPC1, more preferably wherein said disease or disorder is a
30 cancer characterized by expression of GPC1, such as overexpression of GPC1.

One or more embodiments of the present disclosure is directed to a method of treatment of a disease or disorder characterized by cell expressing GPC1, more preferably wherein said disease or disorder is a cancer characterized by expression of GPC1, such as overexpression of GPC1, wherein said method comprises administering the antibody-drug conjugate as described
5 here above in a therapeutically effective amount to a subject in need thereof.

One or more embodiments of the present disclosure is directed to a use of the antibody-drug conjugate as described here above for the manufacture of a medicament for treatment of a disease or disorder characterized by cell expressing GPC1, more preferably wherein said disease or disorder is a cancer characterized by expression of GPC1, such as overexpression of GPC1.

10 In one or more embodiments of the present disclosure, the disease or disorder characterized by cells expressing GPC1 is cancer. The cancer may in one or more embodiments be one or more of breast cancer, carcinoid cancer, cervical cancer, endometrial cancer, glioma, head and neck cancer, liver cancer (including hepatocellular carcinoma), lung cancer, small cell lung cancer, lymphoma, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, renal
15 cancer, colorectal cancer, gastric cancer, testicular cancer, thyroid cancer, bladder cancer, urethral cancer, or hematologic malignancy.

In one or more embodiments, the cancer is breast cancer including TNBC, HER2-positive breast cancer, ER-positive breast cancer, and PR-positive breast cancer.

In one or more embodiments, the cancer is unresectable melanoma or metastatic
20 melanoma, non-small cell lung carcinoma (NSCLC), small cell lung cancer (SCLC), bladder cancer, or metastatic hormone-refractory prostate cancer (also referred to as castrate-resistant prostate cancer).

In one or more embodiments, the cancer is NSCLC, ovarian cancer, melanoma, colorectal cancer, breast cancer, head and neck cancer, gastrointestinal cancer, bladder cancer, or bone
25 cancer.

In one or more embodiments, the cancer may be a solid tumor. In some embodiments, the cancer is esophageal squamous cell carcinoma, cervical squamous cell carcinoma, skin squamous cell carcinoma or head and neck squamous cell carcinoma. In one or more
30 embodiments, the cancer is squamous cell carcinoma of the head and neck (SCCHN, also referred to as head and neck squamous cell carcinoma, HNSCC), renal cell carcinoma (RCC), triple-negative breast cancer (TNBC), or colorectal carcinoma.

In one or more embodiments, the cancer may be triple-negative breast cancer (TNBC), gastric cancer, urothelial cancer (including upper-tract urothelial cancer), Merkel-cell carcinoma, or head and neck cancer.

In one or more embodiments, the cancer may be melanoma, pancreatic carcinoma, mesothelioma, or advanced solid tumors. In one or more embodiments, the cancer may be Hodgkin's lymphoma.

In one or more embodiments, the cancer is esophageal cancer, gastric cancer, glioblastoma, breast cancer, ovarian cancer, bladder cancer, pancreatic cancer, lung cancer, colorectal cancer, stomach cancer, prostate cancer, bone metastases from prostate cancer, kidney cancer, multiple myeloma, cholangiocarcinoma (including intrahepatic cholangiocarcinoma).

In one or more embodiments of the present disclosure, the cancer is esophageal cancer, head and neck cancer, cervical cancer, lung cancer, bladder cancer or breast cancer, including subtypes of any of the foregoing.

In one or more embodiments of the present disclosure, the cancer is sarcoma, such as stomach/gastric adenocarcinoma, NSCLC adenocarcinoma, esophageal adenocarcinoma, gastroesophageal junction adenocarcinoma, pancreatic ductal adenocarcinoma, thymoma, uterine carcinoma, osteosarcoma, or soft tissue sarcoma (STS), or subtypes of these.

In one or more embodiments of the present disclosure, the soft tissue sarcoma (STS) is selected from epithelioid sarcoma (including epithelial ovarian cancer), clear cell sarcoma, alveolar soft part sarcoma, extraskeletal myxoid chondrosarcoma, epithelioid hemangioendothelioma, inflammatory myofibroblastic tumor, undifferentiated embryonal sarcoma, alveolar soft part sarcoma (ASPS), angiosarcoma, chondrosarcoma, dermatofibrosarcoma protuberans (DFSP), desmoid sarcoma, Ewing's sarcoma, fibrosarcoma, myxofibrosarcoma, gastrointestinal stromal tumor (GIST), non-uterine leiomyosarcoma, uterine leiomyosarcoma, liposarcoma, malignant fibro histiocytoma (MFH), malignant peripheral nerve sheath tumor (MPNST), rhabdomyosarcoma, synovial sarcoma, and/or leiomyosarcoma (LMS).

In one or more embodiments of the present disclosure, the disease or disorder characterized by cells expressing GPC1 is fibrosis, such as fibrosis of the liver, lung, heart, kidney, skin, or bone including bone marrow.

In one aspect, the disclosure features methods that include administering a therapeutically effective amount of an antibody or antigen-binding fragment thereof, including in the form of an

antibody-drug conjugate, disclosed herein to a subject in need thereof (e.g., a subject having, or identified or diagnosed as having, a cancer),

In some embodiments, the compositions and methods disclosed herein can be used for treatment of patients at risk for a cancer. Patients with cancer can be identified with various
5 methods known in the art.

As used herein, by an “effective amount” is meant an amount or dosage sufficient to effect beneficial or desired results including halting, slowing, retarding, or inhibiting progression of a disease, e.g., an autoimmune disease or a cancer. An effective amount will vary depending upon, e.g., an age and a body weight of a subject to which the antibody, antigen binding
10 fragment, antibody-encoding polynucleotide, vector comprising the polynucleotide, and/or compositions thereof is to be administered, a severity of symptoms and a route of administration, and thus administration can be determined on an individual basis.

An effective amount can be administered in one or more administrations. By way of example, an effective amount of an antibody or an antigen binding fragment is an amount
15 sufficient to ameliorate, stop, stabilize, reverse, inhibit, slow and/or delay progression of an autoimmune disease or a cancer in a patient or is an amount sufficient to ameliorate, stop, stabilize, reverse, slow and/or delay proliferation of a cell (e.g., a biopsied cell, any of the cancer cells described herein, or cell line (e.g., a cancer cell line)) *in vitro*. As is understood in the art, an effective amount of an antibody or antigen binding fragment may vary, depending on, *inter*
20 *alia*, patient history as well as other factors such as the type (and/or dosage) of antibody used.

Effective amounts and schedules for administering the antibodies, antibody-encoding polynucleotides, and/or compositions disclosed herein may be determined empirically, and making such determinations is within the skill in the art. Those skilled in the art will understand that the dosage that must be administered will vary depending on, for example, the mammal that
25 will receive the antibodies, antibody-encoding polynucleotides, and/or compositions disclosed herein, the route of administration, the particular type of antibodies, antibody-encoding polynucleotides, antigen binding fragments, and/or compositions disclosed herein used and other drugs being administered to the mammal. Guidance in selecting appropriate doses for antibody or antigen binding fragment can be found in the literature on therapeutic uses of antibodies and
30 antigen binding fragments, e.g., Handbook of Monoclonal Antibodies, Ferrone et al., eds., Nokes

Publications, Park Ridge, N.J., 1985, ch. 22 and pp. 303-357; Smith et al., Antibodies in Human Diagnosis and Therapy, Haber et al., eds., Raven Press, New York, 1977, pp. 365-389.

Pharmaceutical Compositions and Routes of Administration

5 Also provided herein are pharmaceutical compositions that contain the antibodies, antigen-binding fragments thereof or antibody-drug conjugates (ADCs) directed against GPC1 as described herein. The pharmaceutical compositions may be formulated in any manner known in the art.

10 Pharmaceutical compositions are formulated to be compatible with their intended route of administration (e.g., intravenous, intraarterial, intramuscular, intradermal, subcutaneous, or intraperitoneal).

15 Compositions containing the antibodies, antigen-binding fragments thereof or antibody-drug conjugates (ADCs) directed against GPC1 as described herein can be formulated for parenteral (e.g., intravenous, intraarterial, intramuscular, intradermal, subcutaneous, or intraperitoneal) administration in dosage unit form (i.e., physically discrete units containing a predetermined quantity of active compound for ease of administration and uniformity of dosage).

20 Pharmaceutical compositions for parenteral administration are preferably sterile and substantially isotonic and manufactured under Good Manufacturing Practice (GMP) conditions. Pharmaceutical compositions can be provided in unit dosage form (i.e., the dosage for a single administration). Pharmaceutical compositions can be formulated using one or more physiologically acceptable carriers, diluents, excipients or auxiliaries.

25 Excipients intended for formulation with the antibody, antigen-binding fragment thereof or antibody-drug conjugate should be generally accepted in the pharmaceutical fields. By "pharmaceutically acceptable" it should be understood to refer to a non-toxic material that does not decrease the effectiveness of the ADC. Such pharmaceutically acceptable buffers, carriers or excipients are well-known in the art (see Remington's Pharmaceutical Sciences, 18th edition, A.R. Gennaro, Ed., Mack Publishing Company (1990) and handbook of Pharmaceutical Excipients, 3rd edition, A. Kibbe, Ed., Pharmaceutical Press (2000), the disclosures of which are incorporated herein by reference).

30 The formulation depends on the route of administration chosen. For injection, antibodies or ADCs can be formulated in aqueous solutions, preferably in physiologically-compatible

buffers to reduce discomfort at the site of injection. The solution can contain formulatory agents such as suspending, stabilizing and/or dispersing agents. Alternatively antibodies or ADCs can be in lyophilized form for constitution with a suitable vehicle, e.g., sterile pyrogen-free water, before use.

5 Toxicity and therapeutic efficacy of compositions can be determined by standard pharmaceutical procedures in cell cultures or experimental animals (e.g., monkeys). One can, for example, determine the LD50 (the dose lethal to 50% of the population) and the ED50 (the dose therapeutically effective in 50% of the population): the therapeutic index being the ratio of LD50:ED50. Agents that exhibit high therapeutic indices are preferred. Where an agent exhibits
10 an undesirable side effect, care should be taken to minimize potential damage (i.e., reduce unwanted side effects). Toxicity and therapeutic efficacy can be determined by other standard pharmaceutical procedures.

As is appreciated by those skilled in the art, the amount of a compound may vary depending on its specific activity. Suitable dosage amounts may contain a predetermined
15 quantity of active composition calculated to produce the desired therapeutic effect in association with the required diluent.

The pharmaceutical compositions can be included in a container, pack, or dispenser together with instructions for administration. The disclosure also provides methods of manufacturing the antibodies or antigen binding fragments thereof for various uses as described
20 herein.

SEQUENCES

SEQ ID NO:	COMMENT	SEQUENCE
1	3G1 VH CDR1 (Kabat)	SYGIT
2	3G1 VH CDR2 (Kabat)	WISAYTGNPTYARNLQD
3	3G1 VH CDR3 (Kabat)	GYDLWSAYPYFDY
4	3G1 VL CDR1	RASQSVSSYLA

5	3G1 VL CDR2	DASNRAT
6	3G1 VL CDR3	QQRSNWPPT
7	3G1 VH CDR1 (Chotia)	GYTFTSY
8	3G1 VH CDR2 (Chotia)	SAYTGN
9	3G1 VH CDR3 (Chotia)	GYDLWSAYPYFDY
10	3G1 VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTSYGIT WVRQAPGQGLEWMGWISAYTGNPTYARNLQDRVT MTTDTSTNTAYMDLRSLRSDDSAKYFCARGYDLWS AYPYFDYWGQGTLVTVSS
11	3G1 VL	EIVLTQSPATLSLSPGERATLSCRASQSVSSYLAWYQ QKPGQAPRLLIYDASNRATGIPARFSGSGSGTDFTLTI SSLEPEDFAVYYCQQRSNWPPTFGQGTKVEIK
12	Ref1 VH	QIQLVQSGPELKKPGETVKISCKASGYAFTDYSMNW VKQAPGKGLRWMGWINTETGEPTYTDDFKGRFAFS LETSASTAFLQINNLRNEDTATYFCARHYDYGGFPY WGQGTLVTVSS
13	Ref1 VL	DIQMTQSPASLSASVGETVTITCRASGNVHNYLAWY QQKQKGKSPQLLVYTAKTADGVPSRFSGSGSGTQYS LKINSLQPEDFGTYYCQHFWSNPWTFGGGTKLEIK
14	Human IgG1 heavy chain constant region	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPV TVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSS SLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCP PCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVV DVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNST YRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEK TISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVK GFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFL

		YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSL SLSPGK
15	Human light chain constant region	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREA KVQWKVDNALQSGNSQESVTEQDSKDSSTLSSTLT LSKADYEEKHKVYACEVTHQGLSSPVTKSFNRGEC
16	Human GPC1	DPASKSRSCGEVRQIYGAKGFSLSDVPQAEISGEHLR ICPQGYTCCTSEMEENLANRSHAELETALRDSSRVL QAMLATQLRSFDDHFQHLLNDSERTLQATFPGAFGE LYTQNARAFRDLYSELRLYYRGANLHLEETLAEFW ARLLERLFKQLHPQLLLPDDYLDCLGKQAEALRPFGE EAPRELRLRATRAFAARSFVQGLGVASDVVRKVA QVPLGPECSRAVMKLVYCAHCLGVPGARPCPDYCR NVLKGCLANQADLDAEWRNLLDSMVLITDKFWGTS GVESVIGSVHTWLAEAINALQDNRDTLTAKVIQCGG NPKVNPQGPPEEKRRRGKLAPRERPPSGTLEKLVS EAKAQLRDVQDFWISLPGTLCSEKMALSTASDDRC WNGMARGRYLPEVMGDGLANQINNPEVEVDITKPD MTIRQQIMQLKIMTNRLRSAYNGNDVDFQDASDDG SGSGSGDGCLDDLCRKVSRKSSSRTPLTHALPGLS EQEGQKTS
17	Monkey GPC1	DPASKSRSCSEVRQIYGAKGFSLSDVPQAEISGEHLRI CPQGYTCCTSEMEENLANRSRAELETALRDSSRVLQ AMLATQLRSFDDHFQHLLNDSERTLQGTFFPGAFGEL YTQNTKAFRDLYAELRLYYRGANLHLEETLAEFWA RLLERLFKQLHPQLLLPDDYLDCLGKQAEALRPFGE APRELRLRATRAFAARSFVQGLGVASDVVRKVAQ VPLGPECSRAVMKLVYCAHCLGVPGARPCPDYCRN VLKGCLANQADLDAEWRNLLDSMVLITDKFWGTSG VESVIGSVHTWLAEAINALQDNRDTLTAKVIQCGGN PKVNPQGSPEEKRRRGKLAPRERPPSGTLEKLVS AKAQLRDVQDFWISLPGTLCSEKMALSTASDDRCW

		NGMARGRYLPEVMGDGLANQINNPEVEVDITKPDM TIRQQIMQLKIMTNRLRSAYNGNDVDFQDASDDGSG SGSGDGCPDDRCGRGASRKSSGSRTPLTHALPGLSE QEGQKTS
18	Mouse GPC1	DPASKSRSCSEVRQIYGAKGFSLSVDPQAEISGEHLRI CPQGYTCCTSEMEENLANHSRMELESALHDSSRALQ ATLATQLHGIDDFQRLNDSERTLQEAFFGAFGDL YTQNTRAFRDLYAELRLYYRGANLHLEETLAEFWA RLLERLFFKQLHPQLLPDDYLDCLGKQAEALRPFGBA PRELRLRATRAFVAARSFVQGLGVASDVVRKVAQV PLAPECSRAIMKLVYCAHCRGVPGARPCPDYCRNVL KGCLANQADLDAEWRNLLDSMVLITDKFWGPGSGAE SVIGGVHVLAEAINALQDNKDTLTAKVIQACGNP KVNPHGSGPEEKRRRGKLALQEKSTGTLEKLVSEA KAQLRDIQDFWISLPGTLCSEKMAMSPASDDRCWN GISKGRYLPEVMGDGLANQINNPEVEVDITKPDMTI RQQIMQLKIMTNRLRGAYGGNDVDFQDASDDGSGS GSGGGCPDDTCGRRVSKKSSSSRTPLTHALPGLSEQE GQKTS
19	Rat GPC1	DPASKSRSCSEVRQIYGAKGFSLSVDPQAEISGEHLRI CPQGYTCCTSEMEENLANHSRMELETALHDSSRALQ ATLATQLHGIDDFQRLNDSERTLQDAFFGAFGDL YTQNTRAFRDLYAELRLYYRGANLHLEETLAEFWA RLLERLFFKQLHPQLLLPDDYLDCLGKQAEALRPFGBD APRELRLRATRAFVAARSFVQGLGVASDVVRKVAQ VPLAPECSRAVMKLVYCAHCRGVPGARPCPDYCRN VLKGCLANQADLDAEWRNLLDSMVLITDKFWGPGSG AEYVIGSVHMLAEAINALQDNKDTLTAKVIQGGCG NPKVNPHGSGPEEKRRRAKLALQEKSSSTGTLEKLVS EAKAQLRDIQDYWISLPGTLCSEKMAMSPASDDRC WNGISKGRYLPEVMGDGLANQINNPEVEVDITKPD

		MTIRQQIMQLKIMTNRLRGAYGGNDVDFQDASDDG SGSGSGGGCPDDACGRRVSKKSSSSRTPLIHALPGLS EQEGQKTS
20	linker	GGFG

EXAMPLES

The invention is further described in the following examples, which do not limit the
5 scope of the invention described in the claims.

Example 1. Generating human anti-GPC1 antibody

Human GPC1 protein (Kactus Biosystems, Cat #: GPC-HM111) or DNA encoding this protein was emulsified with adjuvants, and was used to immunize RenLite™ mice (Biocytogen,
10 complete human heavy chain variable domain combined with a common light chain substitution *in situ*). The RenLite™ mice are described, e.g., in PCT/CN2021/097652, which is incorporated herein by reference in its entirety. Before immunization, retro-orbital blood was collected as a negative control.

A total of three immunizations were performed. The first and second immunizations were
15 separated by three weeks, and the second and third immunizations were separated by two weeks. One week after the third immunization, retro-orbital blood was collected, and the antibody titer of serum was detected by fluorescence activated cell sorting (FACS). One week later, mice with high titer were further injected with GPC1 protein through intraperitoneal injection for impulse immunization.

20 Antigen-specific immune cells were isolated from the immunized mice to further obtain anti-GPC1 antibodies or to obtain the light chain and heavy chain variable region sequences of the anti-GPC1 antibodies. For example, single cell technology (e.g., Beacon® Optofluidic System, Berkeley Lights Inc.) was used to screen and find plasma cells secreting antigen-specific monoclonal antibodies, and then reverse transcription and PCR sequencing were used to obtain
25 antibody variable region sequences. The obtained variable region sequences were cloned into a vector containing a sequence encoding the human IgG1 constant region for antibody expression. Binding of the expressed antibodies to GPC1 was verified by FACS.

Exemplary antibody obtained by this method included 3G1. The VH and VL CDR 1-3 sequences of 3G1 and the VH and VL region are shown in the sequence overview herein.

Several other anti-GPC1 antibodies were obtained following the same procedure. A total of 23 antibodies were obtained and evaluated as part of a candidate selection process with 3G1 demonstrating an overall superiority to the remaining 22 comparators in terms of both GPC1 binding characteristics, internalization properties, specificity and off-target reactivity, cross-species reactivity, stability and more.

One reference antibody with specificity for GPC1, synthesized from published amino acid sequence information (WO2016168885A1), were used in the following experiments (designated Ref1). Specifically, The VH and VL sequences set forth in SEQ ID NOs: 12-13 were linked to the human IgG1 constant region to form Ref1.

Example 2. Binding specificity of anti-GPC1 antibody

The binding specificity of the anti-GPC1 antibody to his-tagged human GPC1 (hGPC1-His, ACRO Biosystems, Cat #: GP1-H52H9), his-tagged human GPC2 (hGPC2-His, ACRO Biosystems, Cat #: GP2-H52H3), his-tagged human GPC3 (hGPC3-His, ACRO Biosystems, Cat #: GP3-H52H4), or his-tagged human GPC4 (hGPC4-His, ACRO Biosystems, Cat #: GP4-H52H3) was verified using Biacore™ (Biacore, Inc., Piscataway N.J.) 8K biosensor equipped with pre-immobilized Protein A sensor chips.

Antibodies was captured on the Protein A chip for the detection. 2 µg/mL purified antibodies were loaded at 10 µL/min to bind to the recombinant his-tagged human proteins (400, 200, 100, 50, 25, 12.5, 6.25, 3.125, 1.5625, 0.78125, and 0 nM). The flow rate was 30 µL/min, the binding and dissociation time were set to 180 s and 400 s, respectively. The chip was regenerated after the last injection of each titration with a glycine solution (pH 2.0) at 30 µL/min for 30 seconds.

Kinetic association rates (k_{on}) and dissociation rates (k_{off}) were obtained simultaneously by fitting the data globally to a 1:1 Langmuir binding model (Karlsson, R. Roos, H. Fagerstam, L. Petersson, B., 1994. *Methods Enzymology* 6. 99-110) using Biacore™ 8K Evaluation Software. Affinities were deduced from the quotient of the kinetic rate constants ($KD=k_{off}/k_{on}$).

As a person of ordinary skill in the art would understand, the same method with appropriate adjustments for parameters (e.g., antibody concentration) was performed for the tested antibody.

The results are summarized in the table below, which showed that 3G1 specifically recognized human GPC1 but not other human glypican members.

Table 1

Antibody	Proteins	kon (1/Ms)	koff (1/s)	KD (M)
3G1	hGPC1-His	1.26E+05	1.93E-04	1.53E-09
	hGPC2-His	/	/	/
	hGPC3-His	/	/	/
	hGPC4-His	/	/	/

“/” means no binding.

5 Example 2b. Binding specificity of anti-GPC1 antibody (off-target screening)

The Retrogenix Cell Microarray Technology platform (Charles River) was used to screen the anti-GPC1 antibody (3G1) for specific off-target binding interactions. The anti-GPC1 antibody was screened at 20 µg/mL for binding against fixed human embryonic kidney (HEK)293 cells expressing 6105 different full-length human plasma membrane proteins, secreted or cell surface-tethered secreted proteins, and an additional 400 human heterodimers.

Compared to the comparator anti-GPC1 clones, The 3G1 antibody showed a significant specific interaction with its intended target GPC1 on both fixed and live cell microarrays. Furthermore, no other specific interactions were identified. Here a significant specific interaction is evaluated as an interaction with a signal-to-noise ratio (SNR) above 1.

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Example 2c. Binding specificity of anti-GPC1 antibody (KO cell line)

TE-8 is a well-established cell line model system for studying oesophageal carcinoma. 3G1 has been evaluated in native GPC1-positive TE-8 cell model as well as in a negative TE-8 GPC1 knock-out (KO) clone and verified by flow cytometry that 3G1 only binds to the unmodified cells, and not to GPC1 KO ones, proving the GPC1-specific binding of the antibody also in this cancer model. Furthermore, 3G1 was evaluated in GPC1-positive FaDu head and neck cancer (HNSCC) cells and GPC1-negative Burkitt’s lymphoma Raji cells and found by flow cytometry to bind the GPC1-positive FaDu cells on a nanomolar range, but not to GPC1-negative Raji cells.

25

The binding affinity of GPC1-targeting 3G1 to GPC1-positive Fadu cells and GPC1-negative Raji cells was also examined using flow cytometry. Non-targeting isotype human immunoglobulin G1 (hIgG1) (Ultra-LEAF™ Purified Human IgG1 Isotype Control Recombinant Antibody, clone QA16A12, #403502, BioLegend, San Diego, CA, USA) was used
5 as negative control. Antibodies were labeled with Alexa Fluor™ 647 (AF-647) using the GlyCLICK Fluorophore 647 kit (L1-F03, Genovis, Kävlinge, Sweden).

Human Fadu head and neck squamous carcinoma cell line (HTB-43™, lot 70044484) and Burkitt's lymphoma Raji cell line (CCL-86™, lot 70053718), were obtained from the American Type Culture Collection (ATCC, Manassas, VA, USA). The cells were respectively
10 cultured in EMEM (C0005-01, AddexBio, San Diego, CA, USA) or RPMI-1640 medium (A10491-01, Gibco®, Thermo Fisher Scientific, Waltham, MA, USA) supplemented with 10% fetal bovine serum (FBS; #S0615, Sigma) and 1% Penicillin-Streptomycin (#15070, Gibco®). The cells were maintained at 37°C in a humidified 5% CO₂ incubator.

The cells were washed with phosphate buffered saline (PBS, 10010031, Gibco®) and for
15 Fadu, detached with StemPro™ Accutase™ Cell Dissociation Reagent (A1110501, Gibco®) from the flask. The cells were collected by centrifugation (300 x g, 5 minutes, 4°C), washed in cold FACS (fluorescence-activated cell sorting) buffer (2% FBS in PBS), and then stained with the corresponding AF-647 fluorescently labeled antibodies diluted in FACS buffer on ice for 30 minutes. Finally, the cells were washed again in ice cold FACS buffer and resuspended in ice
20 cold FACS buffer containing DAPI (4',6-diamidino-2-phenylindole) (1 µg/mL, D3571, Invitrogen™) before being analyzed using a CytoFLEX S flow cytometer (Beckman Coulter, Brea, CA, USA). The analysis included a three-steps gating strategy to analyze the living cells and obtain their AF-647 mean fluorescence intensity (MFI): the selection of cells was conducted by sorting cells by the cell shape (step #1), inclusion of single cells (step #2) and alive cells by
25 DAPI staining (step #3). Binding curves were created using GraphPad Prism software version 10.0.3 (GraphPad Software Inc., La Jolla, CA, USA). Half maximal effective concentration (EC₅₀) values for each test item were determined using four-parameter logistic curve (4PL) non-linear regression analyses.

Binding in Fadu cells was evaluated in three independent experiments, the results of
30 which is shown below. In each case, the EC₅₀ of 3G1 was in the nanomolar range.

Experiment	EC50 (nM) of 3G1 in Fadu cells
#1	1.418
#2	0.1258
#3	0.3791

In summary, 3G1 is demonstrated to bind GPC-1 in a wide variety of different cancer cell line models with no interaction in GPC-1 negative cell line models. A non-targeting isotype control hIgG1 was used as negative control and did not present any binding affinity against any of the evaluated cell lines.

Example 3. Cross-species binding of anti-GPC1 antibody

CHO-hGPC1 cells, CHO-fasGPC1 cells, CHO-mGPC1 cells, or CHO-ratGPC1 cells were transferred to a 96-well plate at a density of 5×10^4 cells/well respectively. Series diluted anti-GPC1 antibodies were added to the 96-well plate, and incubated at 4°C for 30 min. Then, the cells were incubated with the secondary antibody anti-hIgG-Fc-Alex Flour 647 (RL1-H) (Jackson ImmunoResearch Laboratories, Inc., Cat#: 109-606-170) at 4°C in the dark for 15 minutes before flow cytometry analysis. EC50 was calculated.

CHO-hGPC1 cells, CHO-fasGPC1 cells, CHO-mGPC1 cells and CHO-ratGPC1 cells were obtained by transfecting CHO-S cells with vectors expressing human GPC1 (SEQ ID NO: 16), monkey (*Macaca fascicularis*) GPC1 (SEQ ID NO: 17), mouse GPC1 (SEQ ID NO: 18), and rat GPC1 (SEQ ID NO: 19), respectively.

The test results are shown in the table below. 3G1 can bind to human GPC1, monkey GPC1, mouse GPC1, and rat GPC1. Noticeably, Ref1 does not bind monkey GPC1.

Table 2

Antibody	Cells	EC50	R squared
3G1	CHO-hGPC1	0.2398	0.9917
	CHO-fasGPC1	0.8020	0.9622
	CHO-mGPC1	1.0980	0.9501
	CHO-ratGPC1	1.0500	0.9921
Ref1	CHO-hGPC1	0.1448	0.968
	CHO-fasGPC1	0.1078	0.9506
	CHO-mGPC1	/	/
	CHO-ratGPC1	2.926	0.9962

“/” means no binding.

Example 3a. Cross-species binding of anti-GPC1 antibody (flow cytometry)

Cross-species binding affinity of 3G1 was assessed in HEK293 cells transiently transfected with plasmids, resulting in the expression of human, cynomolgus monkey, rat, or mouse GPC1 proteins as well as a green fluorescent protein (GFP) reporter. Non-targeting isotype human immunoglobulin G1 (hIgG1) (Ultra-LEAF™ Purified Human IgG1 Isotype Control Recombinant Antibody, clone QA16A12, 403502, BioLegend, San Diego, CA, United States of America (USA)) was used as negative control.

Plasmids specific for species were generated from the pLenti-P2A-GFP backbone (PS100108, Origene Technologies, Inc., Rockville, MD, USA) Vectors containing either human, cynomolgus monkey, rat, or mouse GPC1 copy DNA (cDNA) incorporated in the multicloning site of the plasmid were likewise produced by Origene Technologies, Inc., Rockville, MD, USA.

HEK293 cells (CRL-1573, American Type Culture Collection (ATCC), Manassas, VA, USA) were cultured in EMEM (C0005-01, AddexBio, San Diego, CA, USA) supplemented with 10 % FBS (fetal bovine serum, F4135, Sigma-Aldrich, St. Louis, MO, USA) and 1% Penicillin-Streptomycin mix (10000 U/mL, Gibco®, Thermo Fisher Scientific, Waltham, MA, USA).

For transient transfection of human, cynomolgus monkey, rat, or mouse GPC1 and GFP encoding plasmids, the cells were transfected using FuGENE® HD Transfection Reagent (Promega, Madison, WI, USA). Plasmid DNA was mixed in Opti-MEM™ I Reduced Serum media (51985034, Gibco®) with FuGENE® HD Transfection Reagent (E2312, Promega, Madison, WI, USA) with a ratio of 4:1 (Transfection Reagent: DNA), and incubated for 15 minutes at room temperature. Subsequently, the transfection reagent/DNA mixture was added to a T75 flask (90076, TPP, Trasadingen, Switzerland) containing HEK293 cells in 20 mL of growth medium.

After the transfection mix was applied, the cells were incubated at 37°C in a humidified 5% CO₂ incubator for 72 hours. Next, the cells were washed with phosphate buffered saline (PBS, 10010031, Gibco®, Thermo Fisher Scientific) and detached with StemPro™ Accutase™ Cell Dissociation Reagent (A1110501, Gibco®) from the flask. The cells were collected by centrifugation (300 x g, 5 minutes, 4 ° C), washed in fluorescence-activated cell sorting (FACS) buffer (2% FBS in PBS), and then stained with the corresponding AF-647 fluorescently labeled antibodies (10 µg/mL in FACS buffer) on ice for 30 minutes. Finally, the cells were washed

again in ice cold FACS buffer and resuspended in ice cold FACS buffer containing 4',6-diamidino-2-phenylindole (DAPI) (1 µg/mL, D3571, Invitrogen™) before being analyzed using a CytoFLEX S flow cytometer (Beckman Coulter, Brea, CA, USA). The analysis included a four-step gating strategy to obtain the AF-647 mean fluorescence intensity (MFI) of the GFP positive cells: the selection of cells was conducted by sorting cells by the cell shape (step #1), inclusion of single cells (step #2), alive cells by DAPI staining (step #3), and highly GFP-positive cells (step #4).

Binding curves were created using GraphPad Prism software version 10.0.3 (GraphPad Software Inc., La Jolla, CA, USA). The half maximal effective concentration (EC₅₀) values for each test item were determined using four-parameter logistic curve (4PL) non-linear regression analyses.

The EC₅₀ values reported in Table 1a represent mean ± standard deviation (SD) of five independent experiments. In all experiments, the 3G1 antibody demonstrated high affinity in a nanomolar range to cells transfected with human, cynomolgus monkey, rat or mouse GPC1.

Table 1a

Species	Mean EC ₅₀ (nM) – 4PL
Human	1.3 ± 0.6
Cynomolgus monkey	1.1 ± 1.2
Rat	0.8 ± 0.2
Mouse	1.3 ± 0.7

In particular cross-species reactivity with both human and monkey (Cynomolgus) GPC1 is desirable because it allows for the effect observed in non-human animal studies in primates to be more easily extrapolated/transferred (with confidence) into an expected effect in humans due to the biological similarities between humans and monkeys. Noticeably, Ref1 which is an anti-GPC1 antibody known from WO2016168885A1, does not bind cynomolgus monkey GPC1, and 3G1 is thus superior in providing an antibody which allows for a better development candidate for a prospective medical treatment intended for humans.

Example 4. Binding activity of anti-GPC1 antibody to tumor cells

The binding activity of anti-GPC1 antibody to cancer cell lines was verified by flow cytometry. Briefly, human lung cancer cells NCI-H1792 with GPC1-overexpressing were plated in a 96-well plate at a density of 1×10^5 cells/well. Series diluted antibodies (highest concentration: 150 $\mu\text{g/mL}$, 2-fold dilution) were added to each well and was incubated at 4°C for 30 minutes. Then, after one wash with PBS, the cells were incubated with the secondary antibody Alexa Fluor® 647 anti-human IgG Fc γ (Jackson Immuno Research, Cat #:109-606-170) at 4°C for 15 minutes before flow cytometry analysis. EC50 was calculated. The results showed that 3G1 bound to NCI-H1792 with EC50 value of 0.7518 $\mu\text{g/mL}$.

Example 5. Internalization of anti-GPC1 antibody

Anti-GPC1 antibody (work concentration 2.5 $\mu\text{g/mL}$) together with Fab Fragment Goat Anti-Human IgG (Jackson Immuno Research, Cat #:109-007-008) labeled by pHAb Amine Rescive Dye (Promega, Cat #: G9845) were added to human lung cancer cells NCI-H1792, and incubated for up to 24 hours. The cells were centrifuged and washed with FACS buffer, and then measured using a flow cytometer. For isotype control (ISO), human IgG1 protein was used. Data show that 3G1 has good endocytosis rate in NCI-H1792 cells. An evaluation of 3G1 against Ref1 and the 22 other clones screened (see Example 1) illustrated that 3G1 overall exhibited the best internalization in GPC1-expressing NCI-H1792 cells.

Example 6. Epitope assays of anti-GPC1 antibody

Epitope binding assays were performed using Biacore™ to determine whether two anti-GPC1 antibodies target the same or overlapping epitopes. $1 \times \text{HBS-EP}^+$ buffer (10 mM 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid (HEPES), 150 mM NaCl, 3 mM ethylenediaminetetraacetic acid (EDTA) and 0.05% P20, pH7.4) diluted from HBS-EP+ buffer (10 $\times$) was used as the running buffer throughout the experiment. About 50 RU of hGPC1-His protein (ACRO Biosystems, Cat #: GP1-H52H9) was captured at a flow rate of 10 $\mu\text{L/min}$, and 200 nM of the tested antibody (Analyte 1) was injected at a flow rate of 30 $\mu\text{L/min}$ to bind the ligand. Another comparison antibody (Analyte 2) was injected under the same conditions to determine whether the binding of different antibodies interfered with each other. The binding time was 200 s for each antibody.

The binding value of each antibody was obtained using Biacore 8K Evaluation Software. To quantify the interference of one antibody binding to another, a binding ratio was calculated to compare each pair of antibodies. The binding ratio is defined as the binding value of the second antibody (Analyte 2), divided by the binding value of the first antibody (Analyte 1). The binding ratio of each antibody pair was summarized in the table below. More specifically, the binding ratio was less than 0.5, if analyte 1 exhibited a blocking effect to analyte 2. The binding ratio was over 0.5, if analyte 1 did not exhibit a blocking effect to analyte 2. In general, antibody pairs that interfere with each other have the same or overlapping epitopes. The results show that 3G1 and Ref1 recognize different epitopes.

Table 3

Analyte 1 Analyte 2	3G1	Ref1
3G1	0.0	1.0
Ref1	1.1	0.0

Example 7. Generation of antibody-drug conjugates (ADCs) directed against GPC1.

ADCs were prepared by a commonly employed conjugation method, described previously in the art (Doronina et al. 2003 Nature biotechnology 21(7):778-84; Francisco et al., 2003. Blood 102(4):1458-65; Hamblett et al., 2004. Clinical cancer research 10(20):7063-70), the contents of which are incorporated herein by reference.

Antibodies were subjected to mild reduction by either dithiothreitol (DTT) or tris(2-carboxyethyl)phosphine (TCEP) before conjugation by contacting with a surplus of the intended linker-payload featuring a maleimidocaproyl attachment group.

Exemplary, preparation of 3G1-MC-VC-PABC-MMAE may be prepared by subjecting 3G1 to a 10 minute incubation at 37°C in the presence of 10 mM DTT in a 50 mM sodium borate, 50 mM NaCl, pH 8.0 buffer at 5 mg/mL concentration, followed by removal of DTT by buffer exchange using 30kDa NMWL centrifugal filters to fresh PBS pH 7.4 with 1 mM EDTA, then adjusted to 2 mg/mL concentration. This was followed by immediate conjugation to a 5-10 times molar surplus of maleimidocaproyl-valine-citrulline-p-aminobenzoyloxycarbonylmonomethyl auristatin E (MC-VC-PAB-MMAE, i.e. Vedotin), dissolved in water-free DMSO to a final DMSO content of 10% v/v during conjugation for 2 hours at 37°C. The resulting ADC were purified by gel filtration on PD-10 desalting columns.

Using well-known conjugation methodologies, exemplary as outlined here above, the following ADCs were prepared.

- 3G1-MC-VC-PABC-MMAE (ADC-A)
- 5 3G1-MC-VA-PABC-MMAE (ADC-B)
- 3G1-MC-VC-PABC-MMAE (ADC-C)
- 3G1-MC-GGFG-PABC-MMAE (ADC-D)
- 3G1-MC-VA-PABC-exatecan (ADC-E)
- 3G1-MC-VC-PABC-exatecan (ADC-F)
- 10 3G1-MC-GGFG-PABC-exatecan (ADC-G)
- 3G1-MC-GGFG-exatecan (ADC-H)
- 3G1-MC-VA-PABC-Dxd (ADC-I)
- 3G1-MC-VC-PABC-Dxd (ADC-J)
- 3G1-MC-GGFG-PABC-Dxd (ADC-K)
- 15 3G1-MC-GGFG-Dxd (ADC-L)

OTHER EMBODIMENTS

- It is to be understood that while the invention has been described in conjunction with the detailed description thereof, the foregoing description is intended to illustrate and not limit the
- 20 scope of the invention, which is defined by the scope of the appended claims. Other aspects, advantages, and modifications are within the scope of the following claims.

WHAT IS CLAIMED IS:

1. An antibody or antigen-binding fragment thereof that binds to Glypican-1 (GPC1) comprising:
 - a. an anti-GPC1 antibody or antigen binding fragment thereof as defined herein, comprising
 - i) an immunoglobulin light chain variable region (VL) comprising as VL complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 4, as VL CDR2 an amino acid sequence of SEQ ID NO: 5, and as VL CDR3 an amino acid sequence of SEQ ID NO: 6, and/or
 - ii) an immunoglobulin heavy chain variable region (VH) comprising as VH complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 1, as VH CDR2 an amino acid sequence of SEQ ID NO: 2, and as VH CDR3 an amino acid sequence of SEQ ID NO: 3; or
 - b. an anti-GPC1 antibody or antigen binding fragment thereof as defined herein, comprising
 - i) an immunoglobulin light chain variable region (VL) comprising as VL complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 4, as VL CDR2 an amino acid sequence of SEQ ID NO: 5, and as VL CDR3 an amino acid sequence of SEQ ID NO: 6, and/or
 - ii) an immunoglobulin heavy chain variable region (VH) comprising as VH complementarity-determining region 1 (CDR1) an amino acid sequence of SEQ ID NO: 7, as VH CDR2 an amino acid sequence of SEQ ID NO: 8, and as VH CDR3 an amino acid sequence of SEQ ID NO: 9; or
 - c. an anti-GPC1 antibody or antigen binding fragment thereof as defined herein, comprising
 - i) an immunoglobulin light chain variable region (VL) comprising or consisting of the amino acid sequence of SEQ ID NO: 11 or a sequence having at least 85% sequence identity thereto; and/or
 - ii) an immunoglobulin heavy chain variable region (VH) comprising or consisting of the amino acid sequence of SEQ ID NO: 10 or a sequence having at least 85% sequence identity thereto; or
 - d. an anti-GPC1 antibody or antigen binding fragment thereof as defined herein, comprising
 - i) an immunoglobulin light chain comprising or consisting of the amino acid sequences of SEQ ID NO: 11 or a sequence having at least 85% sequence identity

- to SEQ ID NO: 11, and SEQ ID NO: 15 or a sequence having at least 85% sequence identity to SEQ ID NO: 15; and/or
- ii) an immunoglobulin heavy chain comprising or consisting of the amino acid sequence of SEQ ID NO: 10 or a sequence having at least 85% sequence identity to SEQ ID NO: 10, and SEQ ID NO: 14 or a sequence having at least 85% sequence identity to SEQ ID NO: 14.
2. The antibody or antigen-binding fragment thereof of claim 1, wherein the VH comprises CDRs 1, 2, 3 with the amino acid sequences set forth in SEQ ID NOs: 1-3, respectively, and the VL comprises CDRs 1, 2, 3 with the amino acid sequences set forth in SEQ ID NOs: 4-6, respectively, according to Kabat definition.
3. The antibody or antigen-binding fragment thereof of claim 1, wherein the VH comprises CDRs 1, 2, 3 with the amino acid sequences set forth in SEQ ID NOs: 7-9, respectively, and the VL comprises CDRs 1, 2, 3 with the amino acid sequences set forth in SEQ ID NOs: 4-6, respectively, according to Chothia definition.
4. The antibody or antigen-binding fragment thereof of any one of claims 1-3, characterized by comprising a heavy chain variable region (VH) comprising or consisting of an amino acid sequence of SEQ ID NO: 10, and a light chain variable region (VL) comprising or consisting of an amino acid sequence of SEQ ID NO: 11.
5. The antibody or antigen-binding fragment thereof of claim 4, comprising a heavy chain variable region (VH) comprising VH CDR1, VH CDR2, and VH CDR3 that are identical to VH CDR1, VH CDR2, and VH CDR3 of SEQ ID NO: 10; and a light chain variable region (VL) comprising VL CDR1, VL CDR2, and VL CDR3 that are identical to VL CDR1, VL CDR2, and VL CDR3 of SEQ ID NO: 11.
6. The antibody or antigen-binding fragment thereof of any one of claims 1-5, wherein the antibody or antigen-binding fragment thereof specifically binds to human, monkey, mouse, and/or rat GPC1.

7. The antibody or antigen-binding fragment thereof of any one of claims 1-6, wherein the antibody or antigen-binding fragment thereof is a human antibody or antigen-binding fragment thereof, or a single-chain variable fragment (scFv).
8. The antibody or antigen-binding fragment thereof of any one of claims 1-7, wherein the antibody or antigen-binding fragment thereof is a human IgG1 antibody or antigen-binding fragment thereof or a human IgG4 antibody or antigen-binding fragment thereof.
9. A nucleic acid comprising a polynucleotide encoding the anti-GPC1 antibody or antigen-binding fragment thereof of any one of claims 1-8.
10. The nucleic acid of claim 9, wherein the nucleic acid is cDNA.
11. A vector comprising one or more of the nucleic acids of claim 9 or claim 10.
12. A cell comprising the vector of claim 11 or the nucleic acids of claim 9 or 10.
13. The cell of claim 12, wherein the cell is a CHO cell.
14. A method of producing an antibody or an antigen-binding fragment thereof, the method comprising
 - (a) culturing the cell of any one of claims 12-13 under conditions sufficient for the cell to produce the antibody or the antigen-binding fragment; and
 - (b) collecting the antibody or the antigen-binding fragment produced by the cell.
15. An antibody-drug conjugate (ADC) directed against GPC1 comprising
 - a. The antibody or antigen-binding fragment thereof of any one of claims 1-8;
 - b. an active agent; and
 - c. optionally a linker which links a to b.

16. The antibody-drug conjugate according to claim 15, wherein the active agent is a therapeutic agent, a radioisotope or a detectable label.
17. The antibody-drug conjugate according to any one of claims 15-16, wherein the active agent is a cytotoxic agent.
18. The antibody-drug conjugate according to any one of claims 15-17, wherein the active agent is a therapeutic agent, such as a DNA crosslinking agent, DNA alkylating agent, DNA strand scission agent, anthracycline, antimetabolite, anti-microtubule/anti-mitotic agent, histone deacetylase inhibitor, kinase inhibitor, metabolism inhibitor, peptide antibiotic, immune checkpoint inhibitor, platinum-based antineoplastic, topoisomerase inhibitor, DNA or RNA polymerase inhibitor, nucleotide based agent, or cytotoxic antibiotics.
19. The antibody-drug conjugate according to any one of claims 15-18, wherein the active agent is a cytotoxic agent allowing for efficient killing of the cells expressing GPC1.
20. The antibody-drug conjugate according to any one of claims 15-19, wherein the active agent is a chemotherapeutic agent.
21. The antibody-drug conjugate according to any one of claims 15-20, wherein the active agent is a DNA-crosslinking agent.
22. The antibody-drug conjugate according to claim 21 wherein the DNA-crosslinking agent is cisplatin, carboplatin, oxaliplatin, mitomycin C (MMC), pyrrolobenzodiazepines (PDB), dimeric pyrrolobenzodiazepines or a derivative or prodrug of any of the foregoing, exemplary SGD-1882.
23. The antibody-drug conjugate according to any one of claims 15-20, wherein the active agent is a DNA alkylating agent.

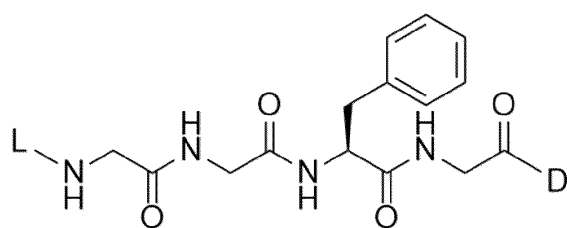
24. The antibody-drug conjugate according to claim 23, wherein the DNA alkylating agent is nitrogen mustards, tris(2-chloroethyl)amine, pyridinobenzodiazepines, indolinobenzodiazepine dimers, or Duocarmycin SA, or a derivative or prodrug of any of the foregoing.
25. The antibody-drug conjugate according to any one of claims 15-20, wherein the active agent is a DNA strand scission agent.
26. The antibody-drug conjugate according to claim 25, wherein the DNA strand scission agent is calicheamicin or hamiltroline or a derivative of any of these.
27. The antibody-drug conjugate according to any one of claims 15-20, wherein the active agent is an anthracycline.
28. The antibody-drug conjugate according to claim 27, wherein the anthracycline is Daunorubicin, doxorubicin, epirubicin, idarubicin, or PNU-159682 or a derivative or prodrug of any of the foregoing.
29. The antibody-drug conjugate according to any one of claims 15-20, wherein the active agent is an antimetabolite.
30. The antibody-drug conjugate according to claim 29, wherein the antimetabolite is folic acid antagonists, methotrexate, purine antimetabolites, 6-mercaptopurine, 6-thioguanine, fludarabine phosphate, pentostatin, cladribine, pyrimidine antimetabolites, 5-fluorouracil, 5-fluorodeoxyuridine, cytarabine, gemcitabine, or a derivative or prodrug of any of the foregoing.
31. The antibody-drug conjugate according to any one of claims 15-20, wherein the active agent is an anti-mitotic agent.

32. The antibody-drug conjugate according to claim 31, wherein the anti-mitotic agent is auristatin, dolastatin, monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), taxanes, Paclitaxel, Docetaxel, a vinca alkaloid, Vinblastine, Vincristine, Vindesine, Vinorelbine, maytansine, Colchicine, Podophyllotoxin, or a derivative or prodrug of any of the foregoing.
33. The antibody-drug conjugate according to any one of claims 15-20, wherein the active agent is a histone deacetylase inhibitor.
34. The antibody-drug conjugate according to claim 33, wherein the histone deacetylase inhibitor is trichostatin A, vorinostat, belinostat, panabioestat, givinostat, resminostat, abexinostat, quisinostat, rocilinostat, practinostat, CHR-3996, valproic acid, butyric acid, phenylbutyric acid, entinostat, tacedinaline, 4SC202, mocetinostat, romidepsin, nicotinamide, sirtinol, cambinol, or EX-527, or a derivative or prodrug of any of the foregoing.
35. The antibody-drug conjugate according to any one of claims 15-20, wherein the active agent is a kinase inhibitor.
36. The antibody-drug conjugate according to claim 35, wherein the kinase inhibitor is genistein, lavendustin C, PP1-AG1872, PP2-AG1879, SU6656, CGP77675, PD166285, imatinib, erlotinib, gefitinib, lavendustin A, cetuximab, UCS15A, herbimycin A, or radicicol, or a derivative or prodrug of any of the foregoing.
37. The antibody-drug conjugate according to any one of claims 15-20, wherein the active agent is a metabolism inhibitor.
38. The antibody-drug conjugate according to claim 37, wherein the metabolism inhibitor is a NAMPT inhibitor, APO866, GMX-1777, GMX-1778 ATG-019, or OT-82, or a derivative or prodrug of any of the foregoing.

39. The antibody-drug conjugate according to any one of claims 15-20, wherein the active agent is an immune checkpoint inhibitor.
40. The antibody-drug conjugate according to claim 39, wherein the immune checkpoint inhibitor is a PD-1 inhibitor or a PD-L1 inhibitor.
41. The antibody-drug conjugate according to claim 40, wherein the PD-1 inhibitor is Pembrolizumab, Nivolumab, Cemiplimab, JTX-4014, Spartalizumab, Camrelizumab, Sintilimab, Tislelizumab, Toripalimab, Dostarlimab, AMP-224, or AMP-514 or a derivative or any of the foregoing
42. The antibody-drug conjugate according to claim 40, wherein the immune checkpoint inhibitor is a PD-L1 inhibitor.
43. The antibody-drug conjugate according to claim 42, wherein the PD-L1 inhibitor is Atezolizumab, Avelumab, Durvalumab, KN035, CK-301, AUNP12, CA-170, or BMS-986189, or a derivative or prodrug of any of the foregoing.
44. The antibody-drug conjugate according to any one of claims 15-20, wherein the active agent is a platinum-based antineoplastic.
45. The antibody-drug conjugate according to claim 44, wherein the platinum-based antineoplastic is lipoplatin, cisplatin, carboplatin, oxaliplatin, nedaplatin, picoplatin, phenanthriplatin, satraplatin, or triplatin tetranitrate, or a derivative or prodrug of any of the foregoing.
46. The antibody-drug conjugate according to any one of claims 15-20, wherein the active agent is a topoisomerase inhibitor, such as a topoisomerase-I (Topo1) inhibitor.

47. The antibody-drug conjugate according to claim 46, wherein the topoisomerase inhibitor is camptothecin, topotecan, belotecan, lurtotecan, irinotecan, SN-38, exatecan, Dxd, or a derivative or prodrug of any of the foregoing.
48. The antibody-drug conjugate according to any one of claims 15-20, wherein the active agent is a DNA- or RNA-polymerase inhibitor.
49. The antibody-drug conjugate according to claim 48, wherein the DNA- or RNA-polymerase inhibitor is amanitin, alpha-amanitin, actinomycin D, aphidicolin, or a derivative or prodrug of any of the foregoing.
50. The antibody-drug conjugate according to any one of claims 15-49, wherein the active agent comprises a radioisotope.
51. The antibody-drug conjugate according to any one of claims 15-50, wherein the antibody-drug conjugate is characterized by a drug-to-antibody ratio (DAR) between 1 and 10, such as between 2 and 8.
52. The antibody-drug conjugate according to any one of claims 15-51, wherein the antibody-drug conjugate is characterized by a DAR of 2, 3, 4, 5, 6, 7, or 8.
53. The antibody-drug conjugate according to any one of claims 15-52, wherein the antibody-drug conjugate comprises a cleavable linker or a non-cleavable linker.
54. The antibody-drug conjugate according to any one of claims 15-53, wherein the linker comprises an attachment group for attaching to a sulfur atom on the antibody, such as a maleimide group.
55. The antibody-drug conjugate according to any one of claims 15-54, wherein the linker comprises or consists of a dipeptide, tripeptide, tetrapeptide or pentapeptide.

56. The antibody-drug conjugate according to any one of claims 15-55, wherein the linker comprises or consists of a dipeptide, such as valine-citrulline (VC) or valine-alanine (VA).
57. The antibody-drug conjugate according to any one of claims 15-55, wherein the linker comprises or consists of a tetrapeptide, wherein each amino acid is individually selected from glycine and phenylalanine, such as wherein the linker comprises a moiety of formula (I)



formula (I),

wherein 'L' represents connectivity to the remaining linker in direction of the antibody or antibody-binding attachment group of the ADC, and wherein 'D' represents connectivity to the active agent, directly or in the form of a spacer.

58. The antibody-drug conjugate according to any one of claims 15-57, wherein the antibody-drug conjugate is further characterized by comprising a spacer, such as a spacer comprising p-aminobenzyl (PAB), p-aminobenzylcarbamate, p-aminobenzylloxycarbonyl (PABC), or a polyethyleneglycol (PEG).
59. The antibody-drug conjugate according to any one of claims 15-58, wherein the antibody-drug conjugate comprises p-aminobenzylloxycarbonyl (PABC) as a spacer.
60. The antibody-drug conjugate according to any one of claims 15-59, wherein the antibody-drug conjugate comprises an attachment group for covalently bonding to the antibody, preferably wherein said attachment group comprises a maleimide moiety, more preferably wherein said attachment group comprises a maleimidocaproyl (MC) moiety.

61. A pharmaceutical composition comprising the anti-GPC1 antibody according to any one of claims 1-8, or the ADC directed against GPC1 according to any one of claims 15-60 and one or more excipients.
62. A kit of parts comprising the anti-GPC1 antibody according to any one of claims 1-8, or the ADC directed against GPC1 according to any one of claims 15-60, or the pharmaceutical composition according to claim 61.
63. The anti-GPC1 antibody according to any one of claims 1-8, or the ADC directed against GPC1 according to any one of claims 16-60 or the pharmaceutical composition according to claim 61 for use as a medicament.
64. A composition comprising the antibody or antigen-binding fragment thereof of any one of claims 1-8, or the ADC directed against GPC1 according to any one of claims 15-60, for use in a method of treating a subject having cancer.
65. The composition for use of claim 64, wherein the cancer is characterized by cells expressing GPC1, such as tumour cells expressing GPC1.
66. The composition for use according to any one of claims 64-65, wherein the cancer is breast cancer, triple-negative breast cancer, carcinoid cancer, cervical cancer, endometrial cancer, glioma, head and neck cancer, liver cancer, lung cancer, small cell lung cancer, melanoma, ovarian cancer, pancreatic cancer, prostate cancer, renal cancer, colorectal cancer, gastric cancer, testicular cancer, thyroid cancer, bladder cancer, urethral cancer, or hematologic malignancy.
67. The composition for use according to any one of claims 64-65, wherein the cancer is unresectable melanoma or metastatic melanoma, non-small cell lung carcinoma (NSCLC), small cell lung cancer (SCLC), bladder cancer, or metastatic hormone-refractory prostate cancer.

68. The composition for use according to any one of claims 64-65, wherein the cancer is oesophageal cancer, gastric cancer, breast cancer, ovarian cancer, bladder cancer, pancreatic cancer, lung cancer, colorectal cancer, stomach cancer, prostate cancer, kidney cancer, multiple myeloma, or cholangiocarcinoma.
69. The composition for use according to any one of claims 64-65, wherein the cancer is pancreatic cancer, colorectal cancer, gastric cancer, breast cancer, bladder cancer, or lung cancer.
70. A method of decreasing the rate of tumor growth, the method comprising contacting a tumor cell with an effective amount of a composition comprising the antibody or antigen-binding fragment thereof of any one of claims 1-8, or the ADC directed against GPC1 according to any one of claims 15-60.
71. A method of killing a tumor cell, the method comprising contacting a tumor cell with an effective amount of a composition comprising the antibody or antigen-binding fragment thereof of any one of claims 1-8, or the ADC directed against GPC1 according to any one of claims 15-60.