

CONJUGATES OF ANTI-SLC34A2 ANTIBODY AND USES THEREOF**CROSS REFERENCE TO RELATED APPLICATIONS**

[0001] This application claims the benefit of International Application No. PCT/CN2023/140633, filed December 21, 2023, the content of which is hereby incorporated by reference in its entirety.

REFERENCE TO AN ELECTRONIC SEQUENCE LISTING

[0002] The content of the electronic sequence listing (371464.xml; Size: 16,805 bytes; and Date of Creation: December 19, 2024) is herein incorporated by reference in its entirety.

BACKGROUND

[0003] Cell membrane transporter proteins such as transporters belonging to glucose transporter GLUT, ATP-binding cassette transporter ABC, and solute carrier transporter SLC families are frequently upregulated on cancer cells, compared to adjacent normal cells. High levels of transporters are found in a wide range of solid tumors, correlating with poor survival. One of the potential molecular tumor markers may be the sodium-dependent phosphate transporter NaPi2b encoded by the SLC34A2 (Solute carrier 34 A2) gene.

[0004] SLC34A2 encodes the type II Na/Pi co-transporter (NaPi2b) which is a multi-transmembrane sodium-dependent phosphate transporter responsible for transcellular inorganic phosphate absorption. NaPi2b is highly abundant in the brush-border membrane of the small intestine, where it is involved in the transcellular flux of inorganic phosphates via the apical membrane of epithelial cells. An altered expression of sodium-dependent phosphate transporter NaPi2b has been reported in ovarian cancer, lung cancer, gastric cancer, thyroid cancer, and other cancers. Currently, NaPi2b is a target for therapeutic antibodies XMT-1536 and RG-7599, which are in clinical trials for the treatment of ovarian and lung cancers.

[0005] Antibody-drug conjugates (ADC) are one of the fast-growing anticancer drugs. This approach comprises a mAb conjugated to the cytotoxic payload via a chemical linker that directed toward a target antigen expressed on the cancer cell surface, reducing systemic exposure and therefore toxicity. ADCs are complex molecules that require careful attention to various components. Selection of an appropriate target, an mAb, cytotoxic payload, and the

manner in which the antibody is linked to the payload are key determinants of the safety and efficacy of ADCs.

[0006] There is a need to develop more efficacious and safer clinical candidates targeting the SLC34A2 protein with potent cytotoxic payloads.

SUMMARY

[0007] It is found that the antibody-drug conjugates of anti-SLC34A2 antibodies provided herein have strong killing activities toward human NaPi2b expressing cell lines. The ADCs also show potent *in vivo* efficacy in inhibiting tumor growth at various concentrations in different tumor models.

[0008] Accordingly, one embodiment of the present disclosure provides an antibody-drug conjugate comprising an antibody or antigen-binding fragment thereof conjugated to a drug moiety, wherein the antibody or antigen-binding fragment thereof has binding specificity to a human solute carrier 34 A2 (SLC34A2) protein and comprises a heavy chain variable region comprising heavy chain complementarity determining regions VH CDR1, VH CDR2, and VH CDR3, and a light chain variable region comprising light chain complementarity determining regions VL CDR1, VL CDR2, and VL CDR3, wherein the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 3, the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 4 or 9, the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 5, the VL CDR1 comprises the amino acid sequence of SEQ ID NO: 6, the VL CDR2 comprises the amino acid sequence of SEQ ID NO: 7, and the VL CDR3 comprises the amino acid sequence of SEQ ID NO: 8.

[0009] In some embodiments, the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 3, the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 4, the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 5, the VL CDR1 comprises the amino acid sequence of SEQ ID NO: 6, the VL CDR2 comprises the amino acid sequence of SEQ ID NO: 7, and the VL CDR3 comprises the amino acid sequence of SEQ ID NO: 8.

[0010] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and 10-13, and the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 2 and 14-17. In some embodiments, the heavy chain variable region comprises the

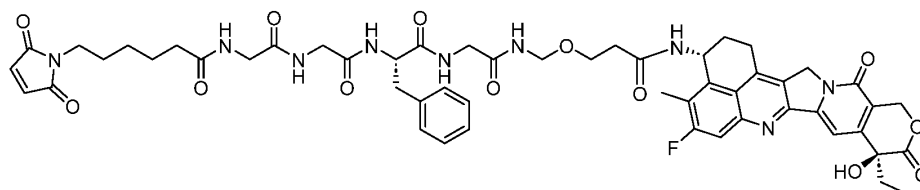
amino acid sequence of SEQ ID NO:11, and the light chain variable region comprises the amino acid sequence of SEQ ID NO:14.

[0011] In some embodiments, the antibody or antigen-binding fragment thereof further comprises a heavy chain constant region, a light chain constant region, an Fc region, or the combination thereof. In some embodiments, the antibody or antigen-binding fragment thereof is of an isotype of IgG1, IgG2, IgG3 or IgG4. In some embodiments, the antibody or antigen-binding fragment thereof is of an isotype of human IgG1.

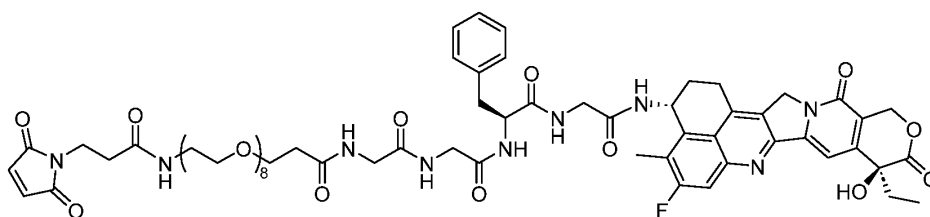
[0012] In some embodiments, the drug moiety is a cytotoxic or cytostatic agent, an immunosuppressive agent, a radioisotope, or a toxin. In some embodiments, the drug moiety is selected from the group consisting of auristatins, maytansinoids, macrocyclic ketone analogues, topoisomerase inhibitors, benzodiazepines, tubulysins, duocarmycin, camptothecin, calicheamicins, exatecans, irinotecans (SN38), doxorubicin, anthracycline, the pyrrolobenzodiazepenes (PBD), TLR agonist, STING agonists, pseudomonas aeruginosa exotoxin PE38, diphtheria toxin, staphylococcus aureus enterotoxin A/E-120, antibacterial antibiotic, shigatoxin, ricin, and urease.

[0013] In some embodiments, the drug moiety is a cytotoxin selected from the group consisting of monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), maytansine, mertansine (DM1), ravtansine (DM4), tublysin A, DXd, 7-ethyl-10-hydroxycamptothecin (SN-38), DGN462, Amberstatin269, anthramycin, SG3199/SCX, IRDye®700DX, TLR7/8 agonist, diABZI STING agonist-2, or any derivative thereof. In some embodiments, the drug moiety comprises MMAE, MMAF, or DXd.

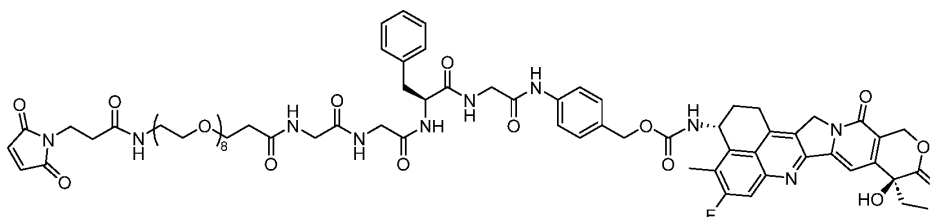
[0014] In some embodiments, the drug moiety comprises a compound selected from the group consisting of:



(LM-D01),



(LM-D02), and



(LM-D03).

[0015] In some embodiments, the antibody or antigen-binding fragment thereof is conjugated to the drug moiety via a linker. In some embodiments, the linker is a cleavable linker or a non-cleavable linker. In some embodiments, the linker is a cleavable linker.

[0016] In some embodiments, the linker comprises succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), sulfo-SMCC, p-carboxycyclohexylmethylmaleimide, maleimide-caproyl (MC)-Valine-citrulline (VC)-para-aminobenzyloxycarbonyl (PABC), CL2A, maleimide-caproyl (MC), MC-glycine-glycine-phenylalanine-glycine (GGFG), MC-PEG8-GGFG, MC-PEG8-GGFG-PAB, or maleimide propoyl (MP)-PEG8-Valine-alanine (VA)-PABC.

[0017] In some embodiments, antibody-drug conjugate comprises ozogamicin, vedotin, mafodotin, emtansine, deruxtecan, govitecan, or tesirine.

[0018] In some embodiments, wherein the drug-antibody ratio (DAR) is 1-20. In some embodiments, the drug-antibody ratio (DAR) is 4-8.

[0019] Also provided, in some embodiments, are compositions comprising the ADCs and a pharmaceutically acceptable carrier.

[0020] Methods and uses for the treatment of diseases and conditions are also provided. In one embodiment, provided is a method of treating cancer in a patient in need thereof, comprising administering to the patient the ADCs of the present disclosure.

BRIEF DESCRIPTION OF THE DRAWINGS

[0021] FIG. 1 shows the effects of ADCs in the inhibition of proliferation of NaPi2b Expressing cells.

[0022] FIG. 2 shows the effects of ADCs in reducing tumors in OVCAR-3 Model.

[0023] FIG. 3 shows the effects of ADCs in reducing tumors in NCI-H1975/H_NaPi2b Model.

DETAILED DESCRIPTION

Definitions

[0024] It is to be noted that the term “a” or “an” entity refers to one or more of that entity; for example, “an antibody,” is understood to represent one or more antibodies. As such, the terms “a” (or “an”), “one or more,” and “at least one” can be used interchangeably herein.

[0025] As used herein, the term “polypeptide” is intended to encompass a singular “polypeptide” as well as plural “polypeptides,” and refers to a molecule composed of monomers (amino acids) linearly linked by amide bonds (also known as peptide bonds). The term “polypeptide” refers to any chain or chains of two or more amino acids, and does not refer to a specific length of the product. Thus, peptides, dipeptides, tripeptides, oligopeptides, “protein,” “amino acid chain,” or any other term used to refer to a chain or chains of two or more amino acids, are included within the definition of “polypeptide,” and the term “polypeptide” may be used instead of, or interchangeably with any of these terms. The term “polypeptide” is also intended to refer to the products of post-expression modifications of the polypeptide, including without limitation glycosylation, acetylation, phosphorylation, amidation, derivatization by known protecting/blocking groups, proteolytic cleavage, or modification by non- naturally occurring amino acids. A polypeptide may be derived from a natural biological source or produced by recombinant technology, but is not necessarily translated from a designated nucleic acid sequence. It may be generated in any manner, including by chemical synthesis.

[0026] “Homology” or “identity” or “similarity” refers to sequence similarity between two peptides or between two nucleic acid molecules. Homology can be determined by comparing a position in each sequence which may be aligned for purposes of comparison. When a position in the compared sequence is occupied by the same base or amino acid, then the molecules are homologous at that position. A degree of homology between sequences is a function of the number of matching or homologous positions shared by the sequences. An “unrelated” or “non-homologous” sequence shares less than 40% identity, though preferably less than 25% identity, with one of the sequences of the present disclosure.

[0027] A polynucleotide or polynucleotide region (or a polypeptide or polypeptide region) has a certain percentage (for example, 60 %, 65 %, 70 %, 75 %, 80 %, 85 %, 90 %, 95 %, 98 % or 99 %) of “sequence identity” to another sequence means that, when aligned, that percentage of bases (or amino acids) are the same in comparing the two sequences.

[0028] The term “an equivalent nucleic acid or polynucleotide” refers to a nucleic acid having a nucleotide sequence having a certain degree of homology, or sequence identity, with the nucleotide sequence of the nucleic acid or complement thereof. A homolog of a double stranded nucleic acid is intended to include nucleic acids having a nucleotide sequence which has a certain degree of homology with or with the complement thereof. In one aspect, homologs of nucleic acids are capable of hybridizing to the nucleic acid or complement thereof. Likewise, “an equivalent polypeptide” refers to a polypeptide having a certain degree of homology, or sequence identity, with the amino acid sequence of a reference polypeptide. In some aspects, the sequence identity is at least about 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99%. In some aspects, the equivalent polypeptide or polynucleotide has one, two, three, four or five addition, deletion, substitution and their combinations thereof as compared to the reference polypeptide or polynucleotide. In some aspects, the equivalent sequence retains the activity (*e.g.*, epitope-binding) or structure (*e.g.*, salt-bridge) of the reference sequence.

[0029] As used herein, an “antibody” or “antigen-binding polypeptide” refers to a polypeptide or a polypeptide complex that specifically recognizes and binds to an antigen. An antibody can be a whole antibody and any antigen binding fragment or a single chain thereof. Thus the term “antibody” includes any protein or peptide containing molecule that comprises at least a portion of an immunoglobulin molecule having biological activity of binding to the antigen. Examples of such include, but are not limited to a complementarity determining region (CDR) of a heavy or light chain or a ligand binding portion thereof, a heavy chain or light chain variable region,

a heavy chain or light chain constant region, a framework (FR) region, or any portion thereof, or at least one portion of a binding protein.

[0030] The terms “antibody fragment” or “antigen-binding fragment”, as used herein, is a portion of an antibody such as F(ab')₂, F(ab)₂, Fab', Fab, Fv, scFv and the like. Regardless of structure, an antibody fragment binds with the same antigen that is recognized by the intact antibody. The term “antibody fragment” includes aptamers, spiegelmers, and diabodies. The term “antibody fragment” also includes any synthetic or genetically engineered protein that acts like an antibody by binding to a specific antigen to form a complex.

[0031] A “single-chain variable fragment” or “scFv” refers to a fusion protein of the variable regions of the heavy (V_H) and light chains (V_L) of immunoglobulins. In some aspects, the regions are connected with a short linker peptide of ten to about 25 amino acids. The linker can be rich in glycine for flexibility, as well as serine or threonine for solubility, and can either connect the N-terminus of the V_H with the C-terminus of the V_L, or vice versa. This protein retains the specificity of the original immunoglobulin, despite removal of the constant regions and the introduction of the linker. ScFv molecules are known in the art and are described, *e.g.*, in US patent 5,892,019.

[0032] The term antibody encompasses various broad classes of polypeptides that can be distinguished biochemically. Those skilled in the art will appreciate that heavy chains are classified as gamma, mu, alpha, delta, or epsilon (γ , μ , α , δ , ϵ) with some subclasses among them (*e.g.*, γ 1- γ 4). It is the nature of this chain that determines the “class” of the antibody as IgG, IgM, IgA, IgG, or IgE, respectively. The immunoglobulin subclasses (isotypes) *e.g.*, IgG₁, IgG₂, IgG₃, IgG₄, IgG₅, etc. are well characterized and are known to confer functional specialization. Modified versions of each of these classes and isotypes are readily discernable to the skilled artisan in view of the instant disclosure and, accordingly, are within the scope of the instant disclosure. All immunoglobulin classes are clearly within the scope of the present disclosure, the following discussion will generally be directed to the IgG class of immunoglobulin molecules. With regard to IgG, a standard immunoglobulin molecule comprises two identical light chain polypeptides of molecular weight approximately 23,000 Daltons, and two identical heavy chain polypeptides of molecular weight 53,000-70,000. The four chains are typically joined by disulfide bonds in a “Y” configuration wherein the light chains bracket the heavy chains starting at the mouth of the “Y” and continuing through the variable region.

[0033] Antibodies, antigen-binding polypeptides, variants, or derivatives thereof of the disclosure include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized, primatized, or chimeric antibodies, single chain antibodies, epitope-binding fragments, *e.g.*, Fab, Fab' and F(ab')₂, Fd, Fvs, single-chain Fvs (scFv), single-chain antibodies, disulfide-linked Fvs (sdFv), fragments comprising either a VK or VH domain, fragments produced by a Fab expression library, and anti- idiotypic (anti-Id) antibodies (including, *e.g.*, anti-Id antibodies to LIGHT antibodies disclosed herein). Immunoglobulin or antibody molecules of the disclosure 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 of immunoglobulin molecule.

[0034] Light chains are classified as either kappa or lambda (K, λ). Each heavy chain class may be bound with either a kappa or lambda light chain. In general, the light and heavy chains are covalently bonded to each other, and the “tail” portions of the two heavy chains are bonded to each other by covalent disulfide linkages or non-covalent linkages when the immunoglobulins are generated either by hybridomas, B cells or genetically engineered host cells. In the heavy chain, the amino acid sequences run from an N-terminus at the forked ends of the Y configuration to the C-terminus at the bottom of each chain.

[0035] Both the light and heavy chains are divided into regions of structural and functional homology. The terms “constant” and “variable” are used functionally. In this regard, it will be appreciated that the variable domains of both the light (VK) and heavy (VH) chain portions determine antigen recognition and specificity. Conversely, the constant domains of the light chain (CK) and the heavy chain (CH1, CH2 or CH3) confer important biological properties such as secretion, transplacental mobility, Fc receptor binding, complement binding, and the like. By convention the numbering of the constant region domains increases as they become more distal from the antigen-binding site or amino- terminus of the antibody. The N-terminal portion is a variable region and at the C-terminal portion is a constant region; the CH3 and CK domains actually comprise the carboxy-terminus of the heavy and light chain, respectively.

[0036] As indicated above, the variable region allows the antibody to selectively recognize and specifically bind epitopes on antigens. That is, the VK domain and VH domain, or subset of the complementarity determining regions (CDRs), of an antibody combine to form the variable region that defines a three-dimensional antigen-binding site. This quaternary antibody structure forms the antigen-binding site present at the end of each arm of the Y. More specifically, the antigen-binding site is defined by three CDRs on each of the VH and VK

chains (*i.e.* CDR-H1, CDR-H2, CDR-H3, CDR-L1, CDR-L2 and CDR-L3). In some instances, *e.g.*, certain immunoglobulin molecules derived from camelid species or engineered based on camelid immunoglobulins, a complete immunoglobulin molecule may consist of heavy chains only, with no light chains. *See, e.g.*, Hamers-Casterman *et al.*, *Nature* 363:446-448 (1993).

[0037] In naturally occurring antibodies, the six “complementarity determining regions” or “CDRs” present in each antigen-binding domain are short, non-contiguous sequences of amino acids that are specifically positioned to form the antigen-binding domain as the antibody assumes its three dimensional configuration in an aqueous environment. The remainder of the amino acids in the antigen-binding domains, referred to as “framework” regions, show less inter-molecular variability. The framework regions largely adopt a β -sheet conformation and the CDRs form loops which connect, and in some cases form part of, the β -sheet structure. Thus, framework regions act to form a scaffold that provides for positioning the CDRs in correct orientation by inter-chain, non-covalent interactions. The antigen-binding domain formed by the positioned CDRs defines a surface complementary to the epitope on the immunoreactive antigen. This complementary surface promotes the non-covalent binding of the antibody to its cognate epitope. The amino acids comprising the CDRs and the framework regions, respectively, can be readily identified for any given heavy or light chain variable region by one of ordinary skill in the art, since they have been precisely defined (*see* “Sequences of Proteins of Immunological Interest,” Kabat, E., *et al.*, U.S. Department of Health and Human Services, (1983); and Chothia and Lesk, *J. Mol. Biol.*, 196:901-917 (1987)).

[0038] In the case where there are two or more definitions of a term which is used and/or accepted within the art, the definition of the term as used herein is intended to include all such meanings unless explicitly stated to the contrary. A specific example is the use of the term “complementarity determining region” (“CDR”) to describe the non-contiguous antigen combining sites found within the variable region of both heavy and light chain polypeptides. This particular region has been described by Kabat *et al.*, U.S. Dept. of Health and Human Services, “Sequences of Proteins of Immunological Interest” (1983) and by Chothia *et al.*, *J. Mol. Biol.* 196:901-917 (1987), which are incorporated herein by reference in their entireties. The CDR definitions according to Kabat and Chothia include overlapping or subsets of amino acid residues when compared against each other. Nevertheless, application of either definition to refer to a CDR of an antibody or variants thereof is intended to be within the scope of the term as defined and used herein. The appropriate amino acid residues which encompass the

CDRs as defined by each of the above cited references are set forth in the table below as a comparison. The exact residue numbers which encompass a particular CDR will vary depending on the sequence and size of the CDR. Those skilled in the art can routinely determine which residues comprise a particular CDR given the variable region amino acid sequence of the antibody.

	Kabat	Chothia
CDR-H1	31-35	26-32
CDR-H2	50-65	52-58
CDR-H3	95-102	95-102
CDR-L1	24-34	26-32
CDR-L2	50-56	50-52
CDR-L3	89-97	91-96

[0039] Kabat *et al.* also defined a numbering system for variable domain sequences that is applicable to any antibody. One of ordinary skill in the art can unambiguously assign this system of “Kabat numbering” to any variable domain sequence, without reliance on any experimental data beyond the sequence itself. As used herein, “Kabat numbering” refers to the numbering system set forth by Kabat *et al.*, U.S. Dept. of Health and Human Services, “Sequence of Proteins of Immunological Interest” (1983).

[0040] In addition to table above, the Kabat number system describes the CDR regions as follows: CDR-H1 begins at approximately amino acid 31 (*i.e.*, approximately 9 residues after the first cysteine residue), includes approximately 5-7 amino acids, and ends at the next tryptophan residue. CDR-H2 begins at the fifteenth residue after the end of CDR-H1, includes approximately 16-19 amino acids, and ends at the next arginine or lysine residue. CDR-H3 begins at approximately the thirty third amino acid residue after the end of CDR-H2; includes 3-25 amino acids; and ends at the sequence W-G-X-G, where X is any amino acid. CDR-L1 begins at approximately residue 24 (*i.e.*, following a cysteine residue); includes approximately 10-17 residues; and ends at the next tryptophan residue. CDR-L2 begins at approximately the sixteenth residue after the end of CDR-L1 and includes approximately 7 residues. CDR-L3 begins at approximately the thirty third residue after the end of CDR-L2 (*i.e.*, following a cysteine residue); includes approximately 7-11 residues and ends at the sequence F or W-G-X-G, where X is any amino acid.

[0041] Antibodies disclosed herein may be from any animal origin including birds and mammals. Preferably, the antibodies are human, murine, donkey, rabbit, goat, guinea pig,

camel, llama, horse, or chicken antibodies. In another embodiment, the variable region may be condrictoid in origin (*e.g.*, from sharks).

[0042] As used herein, the term “heavy chain constant region” includes amino acid sequences derived from an immunoglobulin heavy chain. A polypeptide comprising a heavy chain constant region comprises at least one of: a CH1 domain, a hinge (*e.g.*, upper, middle, and/or lower hinge region) domain, a CH2 domain, a CH3 domain, or a variant or fragment thereof. For example, an antigen-binding polypeptide for use in the disclosure may comprise a polypeptide chain comprising a CH1 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH2 domain; a polypeptide chain comprising a CH1 domain and a CH3 domain; a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, and a CH3 domain, or a polypeptide chain comprising a CH1 domain, at least a portion of a hinge domain, a CH2 domain, and a CH3 domain. In another embodiment, a polypeptide of the disclosure comprises a polypeptide chain comprising a CH3 domain. Further, an antibody for use in the disclosure may lack at least a portion of a CH2 domain (*e.g.*, all or part of a CH2 domain). As set forth above, it will be understood by one of ordinary skill in the art that the heavy chain constant region may be modified such that they vary in amino acid sequence from the naturally occurring immunoglobulin molecule.

[0043] The heavy chain constant region of an antibody disclosed herein may be derived from different immunoglobulin molecules. For example, a heavy chain constant region of a polypeptide may comprise a CH1 domain derived from an IgG₁ molecule and a hinge region derived from an IgG₃ molecule. In another example, a heavy chain constant region can comprise a hinge region derived, in part, from an IgG₁ molecule and, in part, from an IgG₃ molecule. In another example, a heavy chain portion can comprise a chimeric hinge derived, in part, from an IgG₁ molecule and, in part, from an IgG₄ molecule.

[0044] As used herein, the term “light chain constant region” includes amino acid sequences derived from antibody light chain. Preferably, the light chain constant region comprises at least one of a constant kappa domain or constant lambda domain.

[0045] A “light chain-heavy chain pair” refers to the collection of a light chain and heavy chain that can form a dimer through a disulfide bond between the CL domain of the light chain and the CH1 domain of the heavy chain.

[0046] As previously indicated, the subunit structures and three-dimensional configuration of the constant regions of the various immunoglobulin classes are well known. As used herein, the term “VH domain” includes the amino terminal variable domain of an immunoglobulin heavy chain and the term “CH1 domain” includes the first (most amino terminal) constant region domain of an immunoglobulin heavy chain. The CH1 domain is adjacent to the VH domain and is amino terminal to the hinge region of an immunoglobulin heavy chain molecule.

[0047] As used herein the term “CH2 domain” includes the portion of a heavy chain molecule that extends, *e.g.*, from about residue 244 to residue 360 of an antibody using conventional numbering schemes (residues 244 to 360, Kabat numbering system; and residues 231-340, EU numbering system; *see* Kabat *et al.*, U.S. Dept. of Health and Human Services, “Sequences of Proteins of Immunological Interest” (1983). The CH2 domain is unique in that it is not closely paired with another domain. Rather, two N-linked branched carbohydrate chains are interposed between the two CH2 domains of an intact native IgG molecule. It is also well documented that the CH3 domain extends from the CH2 domain to the C-terminal of the IgG molecule and comprises approximately 108 residues.

[0048] As used herein, the term “hinge region” includes the portion of a heavy chain molecule that joins the CH1 domain to the CH2 domain. This hinge region comprises approximately 25 residues and is flexible, thus allowing the two N-terminal antigen-binding regions to move independently. Hinge regions can be subdivided into three distinct domains: upper, middle, and lower hinge domains (Roux *et al.*, *J. Immunol* 161:4083 (1998)).

[0049] As used herein the term “disulfide bond” includes the covalent bond formed between two sulfur atoms. The amino acid cysteine comprises a thiol group that can form a disulfide bond or bridge with a second thiol group. In most naturally occurring IgG molecules, the CH1 and CK regions are linked by a disulfide bond and the two heavy chains are linked by two disulfide bonds at positions corresponding to 239 and 242 using the Kabat numbering system (position 226 or 229, EU numbering system).

[0050] As used herein, the term “chimeric antibody” will be held to mean any antibody wherein the immunoreactive region or site is obtained or derived from a first species and the constant region (which may be intact, partial or modified in accordance with the instant disclosure) is obtained from a second species. In certain embodiments the target binding region or site will be from a non-human source (*e.g.* mouse or primate) and the constant region is human.

[0051] As used herein, “percent humanization” is calculated by determining the number of framework amino acid differences (*i.e.*, non-CDR difference) between the humanized domain and the germline domain, subtracting that number from the total number of amino acids, and then dividing that by the total number of amino acids and multiplying by 100.

[0052] By “specifically binds” or “has specificity to,” it is generally meant that an antibody binds to an epitope via its antigen-binding domain, and that the binding entails some complementarity between the antigen-binding domain and the epitope. According to this definition, an antibody is said to “specifically bind” to an epitope when it binds to that epitope, via its antigen-binding domain more readily than it would bind to a random, unrelated epitope. The term “specificity” is used herein to qualify the relative affinity by which a certain antibody binds to a certain epitope. For example, antibody “A” may be deemed to have a higher specificity for a given epitope than antibody “B,” or antibody “A” may be said to bind to epitope “C” with a higher specificity than it has for related epitope “D.”

[0053] As used herein, the terms “treat” or “treatment” refer to both therapeutic treatment and prophylactic or preventative measures, wherein the object is to prevent or slow down (lessen) an undesired physiological change or disorder, such as the progression of cancer. Beneficial or desired clinical results include, but are not limited to, alleviation of symptoms, diminishment of extent of disease, stabilized (*i.e.*, not worsening) state of disease, delay or slowing of disease progression, amelioration or palliation of the disease state, and remission (whether partial or total), whether detectable or undetectable. “Treatment” can also mean prolonging survival as compared to expected survival if not receiving treatment. Those in need of treatment include those already with the condition or disorder as well as those prone to have the condition or disorder or those in which the condition or disorder is to be prevented.

[0054] By “subject” or “individual” or “animal” or “patient” or “mammal,” is meant any subject, particularly a mammalian subject, for whom diagnosis, prognosis, or therapy is desired. Mammalian subjects include humans, domestic animals, farm animals, and zoo, sport, or pet animals such as dogs, cats, guinea pigs, rabbits, rats, mice, horses, cattle, cows, and so on.

[0055] As used herein, phrases such as “to a patient in need of treatment” or “a subject in need of treatment” includes subjects, such as mammalian subjects, that would benefit from administration of an antibody or composition of the present disclosure used, *e.g.*, for detection, for a diagnostic procedure and/or for treatment.

Anti-SLC34A2 Antibody

[0056] In accordance with one embodiment of the present disclosure, therefore, provided are ADCs comprising antibodies and antigen-binding fragments thereof that are able to bind to SLC34A2. Example antibodies include those murine antibody of 30H9B8F9 as well as humanized ones of **Table 2**. Also included are those that include the same CDRs as illustrated herein. In some embodiments, the disclosed antibodies and fragments include those that bind to the same epitope as those illustrated here, and those that compete with the instantly disclosed in binding to SLC34A2.

[0057] In accordance with one embodiment of the present disclosure, provided is an antibody or antigen-binding fragments thereof that includes the heavy chain and light chain variable domains with the CDR regions disclosed herein, as well as their biological equivalents.

[0058] In one embodiment, the CDRs are those of 30H9B8F9 or its humanized counterparts, as exemplified in **Table 2A**. In one embodiment, the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 3 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 4 or 9 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 5 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VL CDR1 comprises the amino acid sequence of SEQ ID NO: 6 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, the VL CDR2 comprises the amino acid sequence of SEQ ID NO: 7 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof, and the VL CDR3 comprises the amino acid sequence of SEQ ID NO: 8 or a variant thereof having one, two, or three deletions, additions, substitutions or the combinations thereof.

[0059] As shown in the experimental examples, the VH CDR2 (SEQ ID NO: 9) of 30H9B8F9 includes a NG dipeptide which is likely subject to post-translational modification (PTM). Accordingly, a N=>S mutation was made to prevent such PTM, and the mutated VH CDR2 is referred to a PTM-derisked CDR. Experimental data, however, have demonstrated that the PTM-derisked version was as effective as the original one.

[0060] In one embodiment, the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 3, the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 4, the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 5, the VL CDR1 comprises the amino acid sequence of SEQ ID NO: 6, the VL CDR2 comprises the amino acid sequence of SEQ ID NO: 7, and the VL CDR3 comprises the amino acid sequence of SEQ ID NO: 8. In one embodiment, the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 3, the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 9, the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 5, the VL CDR1 comprises the amino acid sequence of SEQ ID NO: 6, the VL CDR2 comprises the amino acid sequence of SEQ ID NO: 7, and the VL CDR3 comprises the amino acid sequence of SEQ ID NO: 8.

[0061] Also provided, in some embodiments, are those that include the same CDRs as 30H9B8F9 or its humanized counterparts. In some embodiments, the disclosed antibodies and fragments include those that bind to the same epitope as 30H9B8F9 or its humanized counterparts, and those that compete with any of them in binding to SLC34A2.

[0062] In some embodiments, the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:1 (mouse or chimeric) and 10-13 (humanized), or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO:1 (mouse or chimeric) and 10-13 (humanized).

[0063] In some embodiments, the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO:2 (mouse or chimeric) and 14-17 (humanized), or a peptide having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NO: 2 (mouse or chimeric) and 14-17 (humanized).

[0064] In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:11 and the light chain variable region comprises the amino acid sequence of any one of SEQ ID NO: 14-17. In some embodiments, the heavy chain variable region comprises the amino acid sequence of any one of SEQ ID NO: 10-13 and the light chain variable region comprises the amino acid sequence of SEQ ID NO: 14. In some embodiments, the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:11 and the light chain variable region comprises the amino acid sequence of SEQ ID NO:14

[0065] It is appreciated that CDRs can be modified to include those having one, two or three amino acid addition, deletion and/or substitutions. In some embodiments, the substitutions can be conservative substitutions.

[0066] A “conservative amino acid substitution” is one in which the amino acid residue is replaced with an amino acid residue having a similar side chain. Families of amino acid residues having similar side chains have been defined in the art, including basic side chains (*e.g.*, lysine, arginine, histidine), acidic side chains (*e.g.*, aspartic acid, glutamic acid), uncharged polar side chains (*e.g.*, glycine, asparagine, glutamine, serine, threonine, tyrosine, cysteine), nonpolar side chains (*e.g.*, alanine, valine, leucine, isoleucine, proline, phenylalanine, methionine, tryptophan), beta-branched side chains (*e.g.*, threonine, valine, isoleucine) and aromatic side chains (*e.g.*, tyrosine, phenylalanine, tryptophan, histidine). Thus, a nonessential amino acid residue in an immunoglobulin polypeptide is preferably replaced with another amino acid residue from the same side chain family. In another embodiment, a string of amino acids can be replaced with a structurally similar string that differs in order and/or composition of side chain family members.

[0067] It will also be understood by one of ordinary skill in the art that antibodies as disclosed herein may be modified such that they vary in amino acid sequence from the naturally occurring binding polypeptide from which they were derived. For example, a polypeptide or amino acid sequence derived from a designated protein may be similar, *e.g.*, have a certain percent identity to the starting sequence, *e.g.*, it may be 60%, 70%, 75%, 80%, 85%, 90%, 95%, 98%, or 99% identical to the starting sequence.

[0068] According to specific embodiments, the antibody is a humanized antibody. Humanized forms of non-human (*e.g.*, murine) antibodies are chimeric molecules of immunoglobulins, immunoglobulin chains or fragments thereof (such as Fv, Fab, Fab', F(ab')₂ or other antigen-binding subsequences of antibodies) which contain minimal sequence derived from non-human immunoglobulin. Humanized antibodies include human immunoglobulins (recipient antibody) in which residues form a complementary determining region (CDR) of the recipient are replaced by residues from a CDR of a non-human species (donor antibody) such as mouse, rat or rabbit having the desired specificity, affinity and capacity. In some instances, Fv framework residues of the human immunoglobulin are replaced by corresponding non-human residues. Humanized antibodies may also comprise residues which are found neither in the recipient antibody nor in the imported CDR or framework sequences. In general, the humanized antibody

will comprise substantially all of at least one, and typically two, variable domains, in which all or substantially all of the CDR regions correspond to those of a non-human immunoglobulin and all or substantially all of the FR regions are those of a human immunoglobulin consensus sequence. The humanized antibody optimally also will comprise at least a portion of an immunoglobulin constant region (Fc), typically that of a human immunoglobulin (Jones et al., *Nature*, 321: 522-525 (1986); Riechmann et al., *Nature*, 332: 323-329 (1988); and Presta, *Curr. Op. Struct. Biol.*, 2: 593-596 (1992)).

[0069] In certain embodiments, the antibody provided herein further comprises a heavy chain constant region, a light chain constant region, an Fc region, or the combination thereof.

[0070] The Fc region can be engineered to enhance effector function. IgG antibodies can induce direct anti-tumor effects by way of indirect anti-tumor effects via the Fc-mediated effector functions that engage other immune cells or killer mechanisms. “Effector functions” or “antibody effector functions” as used herein refer to biological activities attributable to the binding of Fc region of an antibody to its effectors such as C1 complex and Fc receptor (FcγRIIa or FcγRIIIa). Exemplary effector functions include: complement dependent cytotoxicity (CDC) induced by interaction of antibodies and C1q on the C1 complex; antibody-dependent cell-mediated cytotoxicity (ADCC) induced by binding of Fc region of an antibody to Fc receptor on an effector cell; and antibody dependent cell mediated phagocytosis (ADCP), where nonspecific cytotoxic cells that express FcγRs recognize bound antibody on a target cell and subsequently cause phagocytosis of the target cell. Among the four IgG subclasses, IgG1 and IgG3 induce the strongest Fc-effector functions. However, since IgG1 has the longest half-life and is more stable than IgG3, most therapeutic antibodies with Fc-mediated functions are of IgG1 isotype.

[0071] In certain embodiments, the antibody or antigen-binding fragment thereof provided herein is of an isotype of IgG1, IgG2, IgG3 or IgG4. In certain embodiments, the antibody or antigen-binding fragment thereof is of an isotype of IgG1. In certain embodiments, the antibody or antigen-binding fragment thereof is of human IgG1.

[0072] In certain embodiments, the Fc region of the antibodies provided herein is engineered to enhance the effector function, such as ADCC or CDC. Various methods (mainly Fc mutations) can be found to enhance the Fc-mediated effector function, such as those described in the PCT publications WO2007024249A2 and WO2011044368A1, which are incorporated

herein by reference in their entireties. In certain embodiments, the Fc region is engineered to include S298A/E333A/K334A mutations in human IgG1. Such Fc mutation combination is reported to enhance binding to FcγRIIIa, thus enhancing ADCC (See Shields R.L. et al, J. Biol. Chem 276:6591-6604 (2001)).

Antibody-Drug Conjugates

[0073] The present disclosure provides anti-SLC34A2 antibody-drug conjugates that have binding specificity to the human SLC34A2 protein and significantly inhibit the proliferation of NaPi2b expressing cells. Such ADCs (e.g., 30H9B8F9.p1.z5-MMAE, 30H9B8F9.p1.z5-Dxd and 30H9B8F9.p1.z5-LM-D01) are potent in *in vivo* anti-tumor effect in various tumor models.

[0074] The antibody-drug conjugate comprises the antibody or the antigen-binding fragment thereof conjugated to a drug (or payload) via a linker. The drugs can be connected to the antibodies via a cysteine residue or a lysine residue on the antibodies. The ADC can be constructed via chemical approach such as stochastic conjugation on pre-existing lysine or cysteine residues via appropriate coupling reactions, such as amide coupling (of lysine) and sulfhydryl coupling (of cysteine). An active carboxylic acid ester (when available in the linker) is used to connect payloads to lysine residues on the antibody. The primary amine in Lys easily reacts with N-hydroxysuccinimide (NHS) esters introduced into the linker, forming a stable amide. A typical IgG1 antibody molecule has roughly 90 Lys residues, of which approximately 30 can be modified for conjugation, implying that between 1 and 30 payloads can be covalently coupled to the antibody. As for cysteine, after reduction, the disulfide bond could transform to expose free cysteine residues which are accessible for coupling reactions, such as Michael additions, disulfide formation, and α-halo carbonyl alkylations.

[0075] The ADC can also be constructed via enzymatic approach such as site-specific conjugation. The site-specific conjugation includes introduction of engineered reactive cysteine residues, disulfide re-bridging, unnatural amino acids, enzyme-assisted ligation, or glycan remodeling, glycoconjugation or click chemistry. Details of the methods of conjugation can be found in the art, such as Fu et al., Signal Transduction and Targeted Therapy 7:93 (2022).

[0076] The drug-antibody ratio (DAR) for an ADC can be more than 2, more than 4, more than 6, more than 8, more than 10, more than 16, more than 20, or more than 30. In certain embodiments, the drug-antibody ratio (DAR) can be from 1-30, from 1-20, from 1-16, from 1-

10, from 1-8, such as, 4-8, or 2-4. In certain embodiments, the drug-antibody ratio (DAR) can be 1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 15, 16, 20, or 30. In certain embodiments, the DAR is homogenous.

[0077] The linker contained in the drug-linker compound can be non-cleavable linker and cleavable linker. The cleavable linker mainly includes enzyme-cleavable linkers and chemically sensitive linkers.

[0078] Non-cleavable linkers consist of stable bonds that resist proteolytic degradation and ensure greater plasma stability. The mechanism of action of non-cleavable linkers is based on the internalization of the ADC complex followed by degradation of the mAb component in the lysosome, resulting in the release of a cytotoxic drug that kills tumor cells. They do not unleash cytotoxic agents at off-target sites and thus do not harm healthy cells. Non-cleavable linkers are divided into two groups, namely thioether or maleimidocaproyl (MC). Examples of the non-cleavable linkers include, but not limited to, 4-maleimidomethyl cyclohexane-1-carboxylate (MCC), succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), maleimidecaproyl (MC), and p-carboxycyclo hexylmethylmaleimide.

[0079] Cleavable linkers mainly include chemically sensitive linkers those are usually cleaved by environmental differences (such as redox potential, pH) and enzyme-cleavable linkers which are cleaved by specific enzymes in response to extracellular and intracellular environments.

[0080] Chemically sensitive linkers include, but not limited to, types of pH-sensitive linkers, and glutathione-sensitive disulfide linkers. (Khongorzul et al., Mol Cancer Res; 18(1) (2020)).

[0081] PH-sensitive linkers are a group of linkers that are sensitive to the acidic environment but are stable in the alkaline environment such as systemic circulation, such as hydrozone based linker. One successful example of ADC design using pH-sensitive linker is the IMMU-110 which is composed of a humanized anti-CD74 mAb conjugated to doxorubicin via acid-labile hydrazone.

[0082] Glutathione-sensitive disulfide linkers utilize difference in reduction potential in the cytoplasm in contrast to plasma. A high concentration of glutathione can be found in cancer cells than normal cells. Glutathione-sensitive linkers are stable in the blood flow and particularly cleaved by the elevated intracellular concentration of glutathione in the tumor cell, releasing the active drugs at the tumor sites from the nontoxic prodrugs (see *supra*).

[0083] Enzyme-cleavable linkers include, but not limited to, peptide based linkers, β -glucuronide based linkers and phosphate based linkers.

[0084] Peptide based linkers, also known as protease-sensitive linkers, are the most commonly used ADC linkers. These linkers can be cleaved by specific proteases extracellularly and/or intracellularly. For intracellular cleavage, as tumor cells exhibit high expression of lysosomal proteases like cathepsin B compared with the normal cells, therefore, proteases-sensitive peptide linker ADCs are selectively bound to and transformed into cancerous cells through receptor mediated endocytosis. The peptide linkers are stable in the systemic circulation and only unleash the drug in the target cells (see *supra*). Examples are Valine-citrulline (VC or Val-Cit), Valine-alanine (VA), phenylalanine-lysine (PL), and glycine-glycine-phenylalanine-glycine (GGFG).

[0085] β -glucuronide based linkers are recognized and hydrolyzed by β -glucuronidase or β -galactosidase for the drug release, while the β -glucuronidase and β -galactosidase are enriched in lysosomes and tumor necrotic regions. β -glucuronidase is inactive at physiologic pH (blood circulation) and active at lysosomal pH. Such selective site of action allows for the cleavage of the glycosidic linkage of the β -glucuronidase-sensitive β -glucuronide linker, thereby enabling the selective release of cytotoxic payloads (see *supra*).

[0086] Phosphate based linkers are a class of enzyme-cleavable linkers expressed exclusively to target enzymes in the lysosomal compartment. These linkers target pyrophosphatase and acid phosphatase enzymes, which hydrolyze pyrophosphates and terminal monophosphates into their respective alcohols.

[0087] Moieties that can be introduced as part of the linkers to facilitate the drug-linker connection or antibody-linker connection include maleimidocaproyl, maleimide-caproyl (MC) moiety, maleimide-methylene-cyclohexyl carbonyl moiety spacer, maleimide propoyl (MP), para-aminobenzylcarbamate (PAB) spacer, para-aminobenzylloxycarbonyl (PABC) spacer, aminomethoxy methylenecarbonyl (-NH-CH₂-O-CH₂-CO-) spacer, 4-(4'-acetylphenoxy) butanoic acid moiety, acylhydrazide (-CO-NH-NH₂) and thiol (-SH) moieties, Lys-PABC, PEG chain and PEG8.

[0088] Examples of the linkers include, but not limited to succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), sulfo-SMCC, MC-VC-PABC, CL2A, MC-GGFG, MC, MC-PEG8-GGFG, MC-PEG8-GGFG-PAB, or MP-PEG8-VA-PABC.

[0089] The drug or payload can be a cytotoxin, a therapeutic peptide or polypeptide.

[0090] The peptide or polypeptide may be any peptide or polypeptide which has therapeutic properties, for example antinociceptive, antidiabetes, antitumor or antiviral activity. Additionally, or alternatively, the drug preferably comprises an amine group, a thiol group, or a carboxylic acid group, as these types of groups provide ideal sites for conjugation of the drug with the linker of the present disclosure. Examples of biologics drug are pseudomonas aeruginosa exotoxin PE38, diphtheria toxin, staphylococcus aureus enterotoxin A/E-120, shigatoxin, ricin, and urease.

[0091] The cytotoxin are activated after release from ADC inside the cytoplasm of tumor cells and are able to destroy the tumor cells. There are two main classes of cytotoxins that can be used in the ADC designs: microtubule-disrupting agents (such as auristatins and maytansinoids) and DNA-damaging agents (such as calicheamicins, duocarmycin, and doxorubicin). Examples included, but not limited to includes auristatins, maytansinoids, benzodiazepines, tubulysins, duocarmycin, calicheamicins, exatecans, irinotecans (SN38), doxorubicin, anthracycline, pyrrolobenzodiazepenes (PBD), TLR agonist, and STING agonists.

[0092] Auristatins are synthetic antineoplastic agents derived from the natural product dolastatin 10. They block the tubulin polymerization process resulting in cell-cycle arrest and apoptosis. Examples include monomethyl auristatin E (MMAE) and monomethyl auristatin F (MMAF).

[0093] Maytansinoids are isolated from the maytansine, a benzoansamacrolide. These drugs inhibit tubulin polymerization. Examples include DM1 and DM4.

[0094] Calicheamicins are a class of enediyne antitumor antibiotics derived from the bacterium *Micromonospora echinospora*. Calicheamicin recognizes the minor groove of DNA and halts DNA replication resulting in mitotic arrest and cell death. One example is N-acetyl-calicheamicin, a derivative of calicheamicin.

[0095] Duocarmycin is a natural product derivative extracted from the bacteria *Streptomyces* strains. Duocarmycins are another class of DNA minor groove-binding alkylating agents. This class of drugs shows its action by binding to the minor groove of DNA and subsequently cause irreparable alkylation of DNA that disrupts the nucleic acid architecture and structural integrity.

[0096] Doxorubicin shows its action by intercalation of DNA that inhibits DNA synthesis. One example is IMMU-110.

[0097] Exatecans are synthetic derivatives of the natural cytotoxin, camptothecin, isolated from the Chinese tree *Camptotheca acuminata*. Like camptothecin, exatecan binds to the topoisomerase 1-DNA complex, preventing DNA re-ligation which results in the accumulation of DNA strand breaks and ultimately leads to cell death. Examples of exatecans are DX-8951f and DXd. 7-ethyl-10-hydroxycamptothecin (SN-38) is also a derivative of camptothecin.

[0098] Further examples of the cytotoxin includes, without limitation, macrocyclic ketone analogue, topoisomerase inhibitors, tublysin A, camptothecin, DGN462, Amberstatin269, anthramycin, SG3199/SCX, IRI-700DX, TLR7/8 agonist, diABZI STING agonist-2.

[0099] The cytotoxin may be a chemotherapeutic agent which may be categorized by their mechanism of action into, for example, the following groups:

- anti-metabolites/anti-cancer agents such as pyrimidine analogs floxuridine, capecitabine, and cytarabine;
- purine analogs, folate antagonists, and related inhibitors;
- antiproliferative/antimitotic agents including natural products such as vinca alkaloid (vinblastine, vincristine) and microtubule such as taxane (paclitaxel, docetaxel), vinblastin, nocodazole, epothilones, vinorelbine (NAVELBINE[®]), and epipodophyllotoxins (etoposide, teniposide);
- DNA damaging agents such as actinomycin, amsacrine, busulfan, carboplatin, chlorambucil, cisplatin, cyclophosphamide (CYTOXAN[®]), dactinomycin, daunorubicin, doxorubicin, epirubicin, ifosfamide, melphalan, mechlorethamine, mitomycin, mitoxantrone, nitrosourea, procarbazine, taxol, taxotere, teniposide, etoposide, and triethylenethiophosphoramide;
- antibiotics such as dactinomycin, daunorubicin, doxorubicin, idarubicin, anthracyclines, mitoxantrone, bleomycins, plicamycin (mithramycin), and mitomycin;
- enzymes such as L-asparaginase which systemically metabolizes L-asparagine and deprives cells which do not have the capacity to synthesize their own asparagine;

- antiplatelet agents;
- antiproliferative/antimitotic alkylating agents such as nitrogen mustards cyclophosphamide and analogs (melphalan, chlorambucil, hexamethylmelamine, and thiotepa), alkyl nitrosoureas (carmustine) and analogs, streptozocin, and triazenes (dacarbazine);
- antiproliferative/antimitotic antimetabolites such as folic acid analogs (methotrexate);
- platinum coordination complexes (cisplatin, oxiloplatinim, and carboplatin), procarbazine, hydroxyurea, mitotane, and aminoglutethimide;
- hormones, hormone analogs (estrogen, tamoxifen, goserelin, bicalutamide, and nilutamide), and aromatase inhibitors (letrozole and anastrozole);
- anticoagulants such as heparin, synthetic heparin salts, and other inhibitors of thrombin;
- fibrinolytic agents such as tissue plasminogen activator, streptokinase, urokinase, aspirin, dipyridamole, ticlopidine, and clopidogrel;
- antimigratory agents;
- antisecretory agents (breveldin);
- immunosuppressives tacrolimus, sirolimus, azathioprine, and mycophenolate;
- compounds (TNP-470, genistein) and growth factor inhibitors (vascular endothelial growth factor inhibitors and fibroblast growth factor inhibitors);
- angiotensin receptor blockers, nitric oxide donors;
- anti-sense oligonucleotides;
- antibodies such as trastuzumab and rituximab;
- cell cycle inhibitors and differentiation inducers such as tretinoin;
- inhibitors, topoisomerase inhibitors (doxorubicin, daunorubicin, dactinomycin, eniposide, epirubicin, etoposide, idarubicin, irinotecan, mitoxantrone, topotecan, and irinotecan), and corticosteroids (cortisone, dexamethasone, hydrocortisone, methylprednisolone, prednisone, and prednisolone);
- growth factor signal transduction kinase inhibitors;

- dysfunction inducers;
- toxins such as Cholera toxin, ricin, Pseudomonas exotoxin, Bordetella pertussis adenylate cyclase toxin, diphtheria toxin, and caspase activators;
- and chromatin.

[0100] Further examples of chemotherapeutic agents include:

- alkylating agents such as thiotepa and cyclophosphamide (CYTOXAN[®]);
- alkyl sulfonates such as busulfan, improsulfan, and piposulfan;
- aziridines such as benzodopa, carboquone, meturedopa, and uredopa;
- emylerumines and memylamelamines including alfretamine, triemylenemelamine, triethylenephosphoramidate, triethylenethiophosphoramidate, and trimemylolomelamine;
- acetogenins, especially bullatacin and bullatacinone;
- a camptothecin, including synthetic analog topotecan;
- bryostatin;
- callystatin;
- CC-1065, including its adozelesin, carzelesin, and bizelesin synthetic analogs;
- cryptophycins, particularly cryptophycin 1 and cryptophycin 8;
- dolastatin;
- duocarmycin, including the synthetic analogs KW-2189 and CBI-TMI;
- eleutherobin;
- pancratistatin;
- a sarcodictyin;
- spongistatin;
- nitrogen mustards such as chlorambucil, chlornaphazine, cyclophosphamide, estramustine, ifosfamide, mechlorethamine, mechlorethamine oxide hydrochloride, melphalan, novembichin, phenesterine, prednimustine, trofosfamide, and uracil mustard;
- nitrosoureas such as carmustine, chlorozotocin, foremustine, lomustine, nimustine, and ranimustine;

- antibiotics such as the enediyne antibiotics (*e.g.*, calicheamicin, especially calicheamicin gammaII and calicheamicin phiII), dynemicin including dynemicin A, bisphosphonates such as clodronate, an esperamicin, neocarzinostatin chromophore and related chromoprotein enediyne antibiotic chromomophores, aclacinomycins, actinomycin, authramycin, azaserine, bleomycins, cactinomycin, carabycin, carminomycin, carzinophilin, chromomycins, dactinomycin, daunorubicin, detorubicin, 6-diazo-5-oxo-L-norleucine, doxorubicin (including morpholino-doxorubicin, cyanomorpholino-doxorubicin, 2-pyrrolino-doxorubicin, and deoxydoxorubicin), epirubicin, esorubicin, idarubicin, marcellomycin, mitomycins such as mitomycin C, mycophenolic acid, nogalamycin, olivomycins, peplomycin, porfiromycin, puromycin, quelamycin, rodorubicin, streptonigrin, streptozocin, tubercidin, ubenimex, zinostatin, and zorubicin;
- anti-metabolites such as methotrexate and 5-fluorouracil (5-FU);
- folic acid analogs such as demopterin, methotrexate, pteropterin, and trimetrexate;
- purine analogs such as fludarabine, 6-mercaptopurine, thiamiprine, and thioguanine;
- pyrimidine analogs such as ancitabine, azacitidine, 6-azauridine, carmofur, cytarabine, dideoxyuridine, doxifluridine, enocitabine, and floxuridine;
- androgens such as calusterone, dromostanolone propionate, epitiostanol, mepitiothane, and testolactone;
- anti-adrenals such as aminoglutethimide, mitotane, and trilostane;
- folic acid replinishers such as frolinic acid;
- trichothecenes, especially T-2 toxin, verracurin A, roridin A, and anguidine;
- taxoids such as paclitaxel (TAXOL[®]) and docetaxel (TAXOTERE[®]);
- platinum analogs such as cisplatin and carboplatin;
- aceglatone; aldophosphamide glycoside; aminolevulinic acid; eniluracil; amsacrine; hestrabucil; bisantrene; edatraxate; defofamine; demecolcine; diaziquone; elformthine; elliptinium acetate; an epothilone; etoglucid; gallium nitrate; hydroxyurea; lentinan; leucovorin; lonidamine; maytansinoids such as maytansine and ansamitocins; mitoguazone; mitoxantrone; mopidamol; nitracrine; pentostatin; phenamet; pirarubicin; losoxantrone;

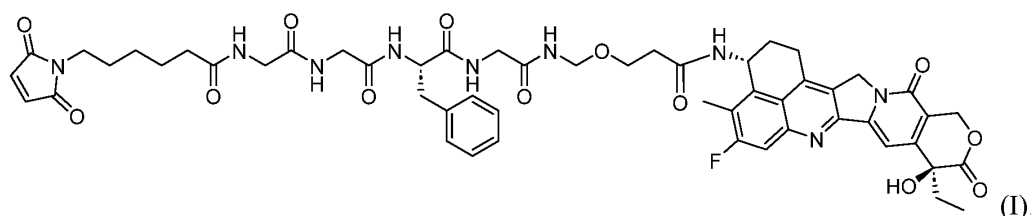
fluoropyrimidine; folinic acid; podophyllinic acid; 2-ethylhydrazide; procarbazine; polysaccharide-K (PSK); razoxane; rhizoxin; sizofiran; spirogermanium; tenuazonic acid; triaziquone; 2,2',2''-trichloro-2,2',2''-triethylamine; urethane; vindesine; dacarbazine; mannomustine; mitobronitol; mitolactol; pipobroman; gacytosine; arabinoside ("Ara-C"); cyclophosphamide; thiopeta; chlorambucil; gemcitabine (GEMZAR[®]); 6-thioguanine; mercaptopurine; methotrexate; vinblastine; platinum; etoposide (VP-16); ifosfamide; mitroxantrone; vancristine; vinorelbine (NAVELBINE[®]); novantrone; teniposide; edatrexate; daunomycin; aminopterin; xeoloda; ibandronate; CPT-11; topoisomerase inhibitor RFS 2000; difluoromethylornithine (DFMO); retinoids such as retinoic acid; capecitabine; FOLFIRI (fluorouracil, leucovorin, and irinotecan);

- and pharmaceutically acceptable salts, acids, or derivatives of any of the above.

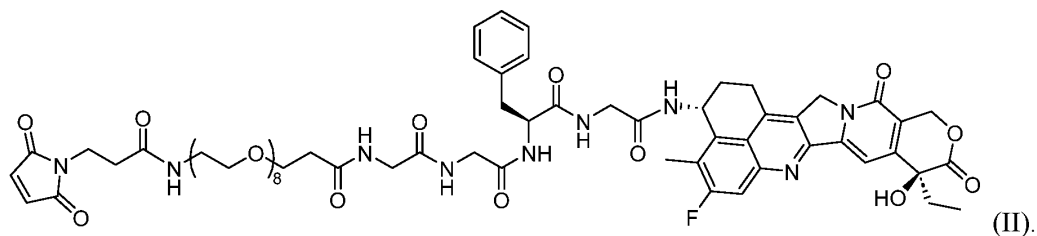
[0101] In certain embodiments of the present disclosure, the drug and the linker form a drug-linker compound. In certain embodiments, the drug-linker compound can be ozogamicin (derivatives of calicheamicin, see U.S. Pat. No. 5773001), vedotin (MC-VC-PABC-MMAE, see U.S. Pat. No. 7659241), mafodotin (MC-MMAF, see U.S. Pat. No. 7498298), emtansine (SMCC-DM1, U.S. Pat. No. 5208020), deruxtecan (MC-GGFG-DXd, see U.S. Pat. No. 10195288), govitecan (CL2A-SN38, see U.S. Pat. No. 8420086), or tesirine (MP-PEG8-VA-PABC-SG3199/SCX, see U.S. Pat. No. 9889207), which are hereby incorporated by reference in their entirety.

[0102] Further examples of the drug-linker compound includes pasudotox (PE38), soravtansine (DM4), ravtansine (DM4), and mertansine (DM1).

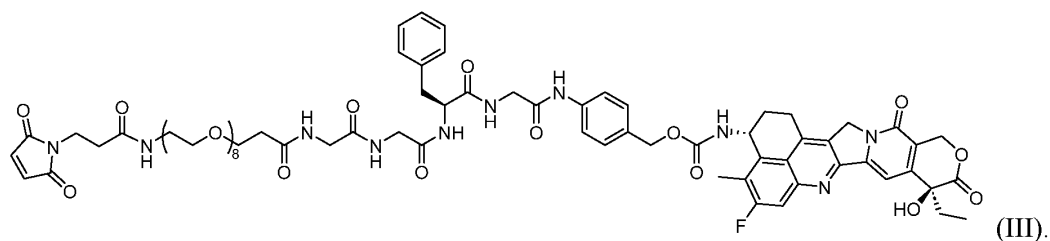
[0103] In certain embodiments of the present disclosure, the drug-linker compound includes LM-D01 having Formula I



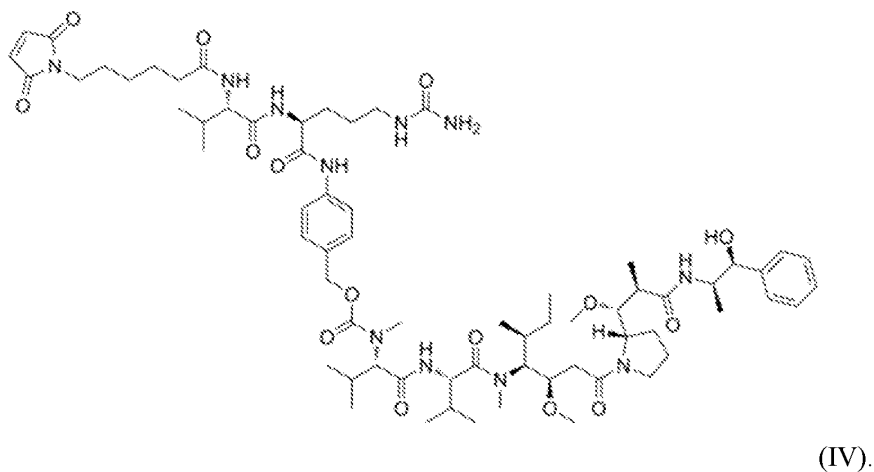
[0104] In certain embodiments of the present disclosure, the drug-linker compound includes LM-D02 having Formula II



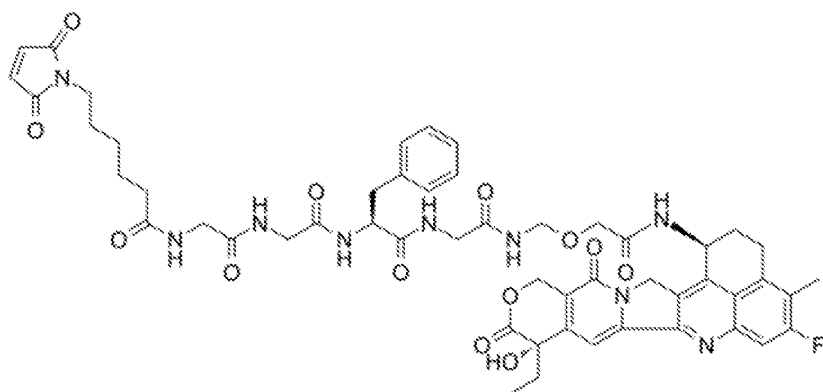
[0105] In certain embodiments of the present disclosure, the drug-linker compound includes LM-D03 having Formula III



[0106] In certain embodiments of the present disclosure, the drug-linker compound includes vedotin having Formula IV

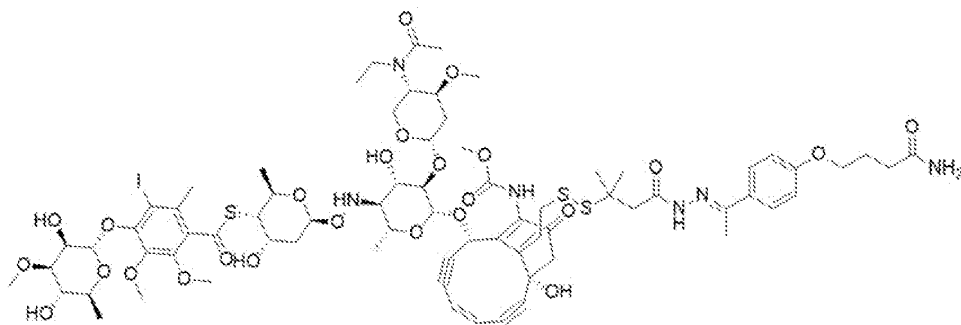


[0107] In certain embodiments of the present disclosure, the drug-linker compound includes deruxtecan having Formula V



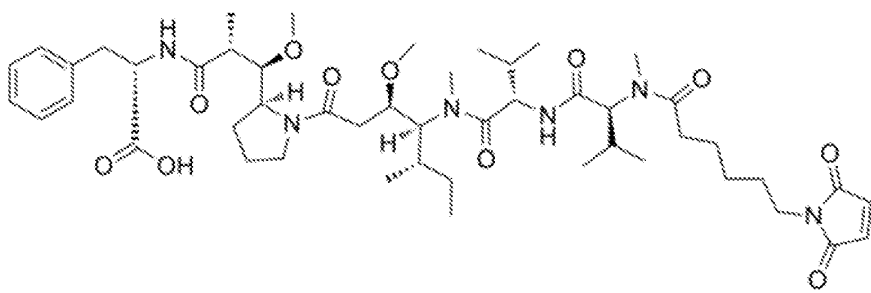
(V).

[0108] In certain embodiments of the present disclosure, the drug-linker compound includes ozogamicin having Formula VI



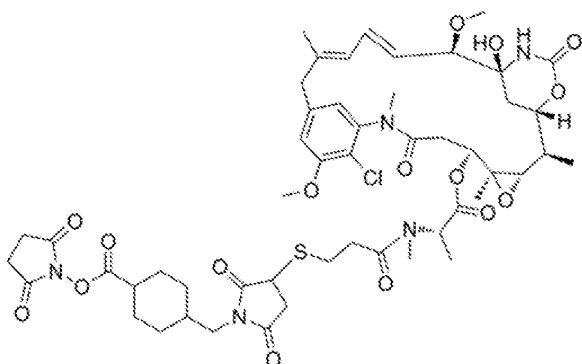
(VI).

[0109] In certain embodiments of the present disclosure, the drug-linker compound includes mafodotin having Formula VII



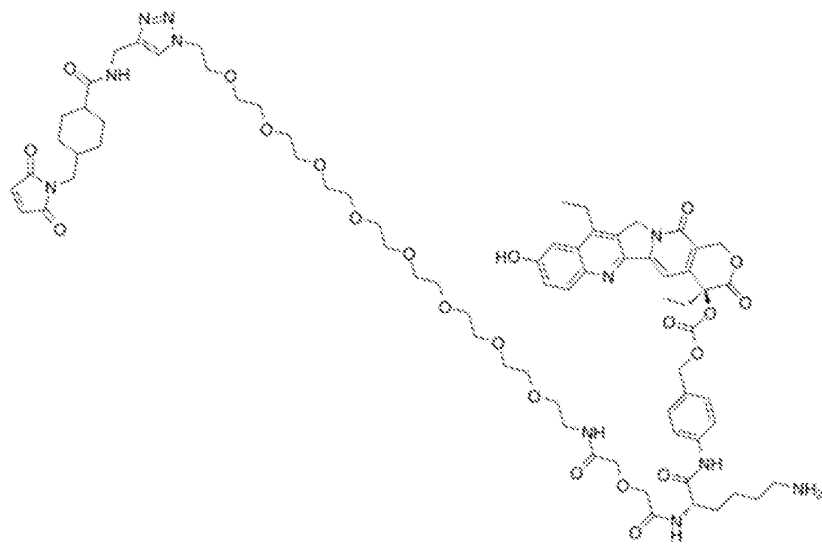
(VII).

[0110] In certain embodiments of the present disclosure, the drug-linker compound includes emtansine having Formula VIII



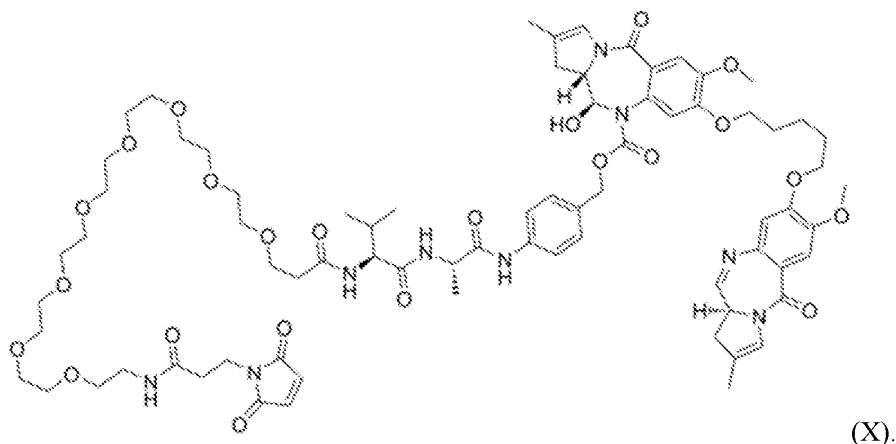
(VIII).

[0111] In certain embodiments of the present disclosure, the drug-linker compound includes
govitecan having Formula IX



(IX).

[0112] In certain embodiments of the present disclosure, the drug-linker compound includes
 tesirine having Formula X



Treatment Methods

[0113] As described herein, the antibody-drug conjugates of the present disclosure may be used in certain treatment and diagnostic methods.

[0114] The present disclosure is further directed to antibody-based therapies which involve administering the ADCs of the disclosure to a patient such as an animal, a mammal, and a human for treating one or more of the disorders or conditions described herein.

[0115] The ADCs of the disclosure can also be used to treat or inhibit cancer. In some embodiments, the cancer cells in the patient express or overexpress SLC34A2/NaPi2b. As provided above, NaPi2b can be overexpressed in tumor cells, in particular gastric, pancreatic, esophageal, ovarian, and lung tumors. Inhibition of SLC34A2/NaPi2b has been shown to be useful for treating the tumors.

[0116] Accordingly, in some embodiments, provided are methods for treating a cancer in a patient in need thereof. The method, in one embodiment, entails administering to the patient an effective amount of an antibody of the present disclosure. In some embodiments, at least one of the cancer cells (e.g., stromal cells) in the patient over-express SLC34A2/NaPi2b.

[0117] In some embodiments, the cell was isolated from the cancer patient him- or her-self. In some embodiments, the cell was provided by a donor or from a cell bank. When the cell is isolated from the cancer patient, undesired immune reactions can be minimized.

[0118] Non-limiting examples of cancers include bladder cancer, breast cancer, colorectal cancer, endometrial cancer, esophageal cancer, head and neck cancer, kidney cancer, leukemia, liver cancer, lung cancer, lymphoma, melanoma, pancreatic cancer, prostate cancer, and thyroid cancer. In some embodiments, the cancer is one or more of gastric, pancreatic, esophageal, ovarian, and lung cancers.

[0119] Additional diseases or conditions associated with increased cell survival, that may be treated, prevented, diagnosed and/or prognosed with the antibodies or variants, or derivatives thereof of the disclosure include, but are not limited to, progression, and/or metastases of malignancies and related disorders such as leukemia (including acute leukemias (*e.g.*, acute lymphocytic leukemia, acute myelocytic leukemia (including myeloblastic, promyelocytic, myelomonocytic, monocytic, and erythroleukemia)) and chronic leukemias (*e.g.*, chronic myelocytic (granulocytic) leukemia and chronic lymphocytic leukemia)), polycythemia vera, lymphomas (*e.g.*, Hodgkin's disease and non-Hodgkin's disease), multiple myeloma, Waldenstrom's macroglobulinemia, heavy chain disease, and solid tumors including, but not limited to, sarcomas and carcinomas such as fibrosarcoma, myxosarcoma, liposarcoma, chondrosarcoma, osteogenic sarcoma, chordoma, angiosarcoma, endotheliosarcoma, lymphangiosarcoma, lymphangioendotheliosarcoma, synovioma, mesothelioma, Ewing's tumor, leiomyosarcoma, rhabdomyo sarcoma, colon carcinoma, pancreatic cancer, breast cancer, ovarian cancer, prostate cancer, squamous cell carcinoma, basal cell carcinoma, adenocarcinoma, sweat gland carcinoma, sebaceous gland carcinoma, papillary carcinoma, papillary adenocarcinomas, cystadenocarcinoma, medullary carcinoma, bronchogenic carcinoma, renal cell carcinoma, hepatoma, bile duct carcinoma, choriocarcinoma, seminoma, embryonal carcinoma, Wilm's tumor, cervical cancer, testicular tumor, lung carcinoma, small cell lung carcinoma, bladder carcinoma, epithelial carcinoma, glioma, astrocytoma, medulloblastoma, craniopharyngioma, ependymoma, pinealoma, hemangioblastoma, acoustic neuroma, oligodendroglioma, menangioma, melanoma, neuroblastoma and retinoblastoma.

[0120] A specific dosage and treatment regimen for any particular patient will depend upon a variety of factors, including the particular ADCs used, the patient's age, body weight, general health, sex, and diet, and the time of administration, rate of excretion, drug combination, and the severity of the particular disease being treated. Judgment of such factors by medical caregivers is within the ordinary skill in the art. The amount will also depend on the individual patient to be treated, the route of administration, the type of formulation, the characteristics of

the compound used, the severity of the disease, and the desired effect. The amount used can be determined by pharmacological and pharmacokinetic principles well known in the art.

[0121] Methods of administration of the ADCs include but are not limited to intradermal, intramuscular, intraperitoneal, intravenous, subcutaneous, intranasal, epidural, and oral routes. The antigen-binding polypeptides or compositions may be administered by any convenient route, for example by infusion or bolus injection, by absorption through epithelial or mucocutaneous linings (*e.g.*, oral mucosa, rectal and intestinal mucosa, etc.) and may be administered together with other biologically active agents. Thus, pharmaceutical compositions containing the antigen-binding polypeptides of the disclosure may be administered orally, rectally, parenterally, intracisternally, intravaginally, intraperitoneally, topically (as by powders, ointments, drops or transdermal patch), buccally, or as an oral or nasal spray.

[0122] The term “parenteral” as used herein refers to modes of administration which include intravenous, intramuscular, intraperitoneal, intrasternal, subcutaneous and intra-articular injection and infusion.

[0123] Administration can be systemic or local. In addition, it may be desirable to introduce the antibodies of the disclosure into the central nervous system by any suitable route, including intraventricular and intrathecal injection; intraventricular injection may be facilitated by an intraventricular catheter, for example, attached to a reservoir, such as an Ommaya reservoir. Pulmonary administration can also be employed, *e.g.*, by use of an inhaler or nebulizer, and formulation with an aerosolizing agent.

[0124] It may be desirable to administer the ADCs or compositions of the disclosure locally to the area in need of treatment; this may be achieved by, for example, and not by way of limitation, local infusion during surgery, topical application, *e.g.*, in conjunction, with a wound dressing after surgery, by injection, by means of a catheter, by means of a suppository, or by means of an implant, said implant being of a porous, non-porous, or gelatinous material, including membranes, such as sialastic membranes, or fibers. Preferably, when administering a protein, including an antibody, of the disclosure, care must be taken to use materials to which the protein does not absorb.

[0125] The amount of the ADCs of the disclosure which will be effective in the treatment, inhibition and prevention of an immune or malignant disease, disorder or condition can be

determined by standard clinical techniques. In addition, *in vitro* assays may optionally be employed to help identify optimal dosage ranges. The precise dose to be employed in the formulation will also depend on the route of administration, and the seriousness of the disease, disorder or condition, and should be decided according to the judgment of the practitioner and each patient's circumstances. Effective doses may be extrapolated from dose-response curves derived from *in vitro* or animal model test systems.

[0126] As a general proposition, the dosage administered to a patient of the ADCs of the present disclosure is typically 0.1 mg/kg to 100 mg/kg of the patient's body weight, between 0.1 mg/kg and 20 mg/kg of the patient's body weight, or 1 mg/kg to 10 mg/kg of the patient's body weight. Generally, human antibodies have a longer half-life within the human body than antibodies from other species due to the immune response to the foreign polypeptides. Thus, lower dosages of human antibodies and less frequent administration is often possible. Further, the dosage and frequency of administration of ADCs of the disclosure may be reduced by enhancing uptake and tissue penetration (*e.g.*, into the brain) of the ADCs by modifications such as, for example, lipidation.

[0127] In an additional embodiment, the compositions of the disclosure are administered in combination with cytokines. Cytokines that may be administered with the compositions of the disclosure include, but are not limited to, IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-10, IL-12, IL-13, IL-15, anti-CD40, CD40L, and TNF- α .

[0128] In additional embodiments, the compositions of the disclosure are administered in combination with other therapeutic or prophylactic regimens, such as, for example, radiation therapy.

Compositions

[0129] The present disclosure also provides pharmaceutical compositions. Such compositions comprise an effective amount of an ADC, and an acceptable carrier. In some embodiments, the composition further includes a second anticancer agent (*e.g.*, an immune checkpoint inhibitor).

[0130] In a specific embodiment, the term “pharmaceutically acceptable” means approved by a regulatory agency of the Federal or a state government or listed in the U.S. Pharmacopeia or other generally recognized pharmacopeia for use in animals, and more particularly in humans.

Further, a “pharmaceutically acceptable carrier” will generally be a non-toxic solid, semisolid or liquid filler, diluent, encapsulating material or formulation auxiliary of any type.

[0131] The term “carrier” refers to a diluent, adjuvant, excipient, or vehicle with which the therapeutic is administered. Such pharmaceutical carriers can be sterile liquids, such as water and oils, including those of petroleum, animal, vegetable or synthetic origin, such as peanut oil, soybean oil, mineral oil, sesame oil and the like. Water is a preferred carrier when the pharmaceutical composition is administered intravenously. Saline solutions and aqueous dextrose and glycerol solutions can also be employed as liquid carriers, particularly for injectable solutions. Suitable pharmaceutical excipients include starch, glucose, lactose, sucrose, gelatin, malt, rice, flour, chalk, silica gel, sodium stearate, glycerol monostearate, talc, sodium chloride, dried skim milk, glycerol, propylene, glycol, water, ethanol and the like. The composition, if desired, can also contain minor amounts of wetting or emulsifying agents, or pH buffering agents such as acetates, citrates or phosphates. Antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as ethylenediaminetetraacetic acid; and agents for the adjustment of tonicity such as sodium chloride or dextrose are also envisioned. These compositions can take the form of solutions, suspensions, emulsion, tablets, pills, capsules, powders, sustained-release formulations and the like. The composition can be formulated as a suppository, with traditional binders and carriers such as triglycerides. Oral formulation can include standard carriers such as pharmaceutical grades of mannitol, lactose, starch, magnesium stearate, sodium saccharine, cellulose, magnesium carbonate, etc. Examples of suitable pharmaceutical carriers are described in Remington's Pharmaceutical Sciences by E. W. Martin, incorporated herein by reference. Such compositions will contain a therapeutically effective amount of the antigen-binding polypeptide, preferably in purified form, together with a suitable amount of carrier so as to provide the form for proper administration to the patient. The formulation should suit the mode of administration. The parental preparation can be enclosed in ampoules, disposable syringes or multiple dose vials made of glass or plastic.

[0132] In an embodiment, the composition is formulated in accordance with routine procedures as a pharmaceutical composition adapted for intravenous administration to human beings. Typically, compositions for intravenous administration are solutions in sterile isotonic aqueous buffer. Where necessary, the composition may also include a solubilizing agent and a local anesthetic such as lignocaine to ease pain at the site of the injection. Generally, the ingredients

are supplied either separately or mixed together in unit dosage form, for example, as a dry lyophilized powder or water free concentrate in a hermetically sealed container such as an ampoule or sachette indicating the quantity of active agent. Where the composition is to be administered by infusion, it can be dispensed with an infusion bottle containing sterile pharmaceutical grade water or saline. Where the composition is administered by injection, an ampoule of sterile water for injection or saline can be provided so that the ingredients may be mixed prior to administration.

[0133] The ADCs of the disclosure can be formulated as neutral or salt forms. Pharmaceutically acceptable salts include those formed with anions such as those derived from hydrochloric, phosphoric, acetic, oxalic, tartaric acids, etc., and those formed with cations such as those derived from sodium, potassium, ammonium, calcium, ferric hydroxides, isopropylamine, triethylamine, 2-ethylamino ethanol, histidine, procaine, etc.

EXAMPLES

Example 1: Monoclonal antibodies against human SLC34A2

[0134] The VH/VL sequences of a cloned murine antibody are provided in Table 1 below.

Table 1. VH/VL sequence of murine antibody 30H9B8F9

Antibody chain	Sequence (CDRs are underlined)	SEQ ID NO:
30H9B8F9 VH	QVQLQQPGAELVKPGASVKMSCKTSGYTFT <u>TNNMH</u> WVKQTPGQGLEWIG <u>AIY</u> <u>PGNGATAYNQKFKG</u> KATLTADKSSSTAYMQLSSLTSEASAVYYCAR <u>GMYGHG</u> <u>AMDY</u> WGQGTSVIVSS	1
30H9B8F9 VL	DIVMTQSHKFMSRSVGDRVRI TC <u>KASQDVGTAV</u> AWYQQKPGQSPKLLIY <u>WAT</u> <u>TRHS</u> GVPDRFTGSGSGTDFIFTISNVQSEDLADYFC <u>QQYSSNPLT</u> FGAGTKL ELK	2

[0135] The amino acid sequences of the VH and VL of 30H9B8F9 were compared against the available database of human Ig gene sequences to find the overall best-matching human germline Ig gene sequences. The CDRs of the murine antibodies were then grafted into the matched human sequences. The cDNA was synthesized and used to produce the humanized antibodies. Certain back mutations from the murine antibodies were then introduced back to the humanized antibodies.

[0136] The amino acid sequences of the humanized and PTM-derisked antibodies are provided in **Table 2** below.

Table 2A. Humanized Sequences

Antibody chain	Sequence (bold residues are back mutations; PTM-derisked ones are boxed)	SEQ ID NO:
30H9B8F9 VH	QVQLQQPGAELVKPGASVKMSCKTSGYTFT TNNMH WVKQTPGQGLEWIG AI Y PGNGATAYNQKFKG KATLTADKSSSTAYMQLSSLTSEASAVYYCARG MYG HGAMDY WGQGTSVIVSS	1
30H9B8F9.p1 VH_1	QVQLVQSGAEVKKPGASVKVSKASGYTFTTNNMHWVRQAPGQGLEWMGAI Y PG SG ATAYNQKFKGRVTMT RD TSTSTVYME LS SLRSEDTAVYYCARGMYG HGAMDYWGQGT LV TVSS	10
30H9B8F9.p1 VH_2	QVQLVQSGAEVKKPGASVKVSK T SGYTFTTNNMHWVRQAPGQGLEW I GAI Y PG SG ATAYNQKFKGRVTMT AD TSTSTVYME LS SLRSEDTAVYYCARGMYG HGAMDYWGQGT LV TVSS	11
30H9B8F9.p1 VH_3	QVQLVQSGAEV V KPGASVKVSK T SGYTFTTNNMHWVRQAPGQGLEW I GAI Y PG SG ATAYNQKFKGRVT LTADK STST AY ME LS SLRSE AT AVYYCARGMYG HGAMDYWGQGT LV TVSS	12
30H9B8F9.p1 VH_4	QVQLVQSGAEV V KPGASVK MSCK TSGYTFTTNNMHWV K QAPGQGLEW I GAI Y PG SG ATAYNQKFKGR ATLTADK STST AY ME LS SLRSE AT AVYYCARGMYG HGAMDYWGQGT SV TVSS	13
30H9B8F9 VL	DIVMTQSHKFMSRSVGDVRVIT C KASQDVGTAVA WYQQKPGQSPKLLI YWA TTRHS GV PD RF TG SGSG TDF IFTISNVQSEDLADY FCQQYSSNPLT FGAGT KLEIK	2
30H9B8F9 VL_1	DIQLTQSPSFLSASVGDRTITCKASQDVGTAVAWYQQKPGKAPKLLI YWA TTRHSGVPSRFSGSGSGTEFTLTIS SLQ PEDFATYYCQQYSSNPLTFGQGT KLEIK	14
30H9B8F9 VL_2	DIQLTQSPSFLSASVGDRTITCKASQDVGTAVAWYQQKPGK SP KLLI YWA TTRHSGV PD RFSGSGSGTEFT FT IS SLQ PEDFATY FC QQYSSNPLTFGQGT KLEIK	15
30H9B8F9 VL_3	DI Q MT QSPSFLSASVGDRTITCKASQDVGTAVAWYQQKPGK SP KLLI YWA TTRHSGV PD RFSGSGSGTEF IFT IS VQ PEDFATY FC QQYSSNPLTF G AGT KLEIK	16
30H9B8F9 VL_4	DIV MT QSPSFLSASVGDRTITCKASQDVGTAVAWYQQKPGK SP KLLI YWA TTRHSGV PD RF TG SGSG TDFIFT IS VQ PEDFATY FC QQYSSNPLTF G AGT KLEIK	17

Table 2B. CDR Sequences

Region	Sequence	SEQ ID NO:
VH CDR1	TNNMH	3
VH CDR2	AIY PG NGATAYNQKFKG	9
	AIY PG SG ATAYNQKFKG	4
VH CDR3	GM YGH AMDY	5
VL CDR1	KASQDVGTAVA	6
VL CDR2	WAT TRHS	7
VL CDR3	QQYSSN PLT	8

Table 2C. Humanized Antibodies

30H9B8F9.p1	VH_1	VH_2	VH_3	VH_4
VL_1	Z1	Z5	Z9	Z13
VL_2	Z2	Z6	Z10	Z14
VL_3	Z3	Z7	Z11	Z15
VL_4	Z4	Z8	Z12	Z16

[0137] The humanized/PTM-derived Abs can bind to human SLC34A2 expressing cells, such as HEK293/H_SLC34A2, OVCAR3 and RMG-1 cells with high affinity. In a further assay, these antibodies were evaluated for their binding affinity to Rhesus/rat/mouse SLC34A2, and the results showed that these humanized/PTM-derived antibodies retained the high affinity. Another assay confirmed the potent ADCC induction activity of these humanized/PTM-derived antibodies. In addition, 30H9B8F9.p1.z5 outperformed the benchmarks BMK-01(XMT-1536) or BMK-02 (RG-7599) significantly in many aspects, such as binding to human SLC34A2 on different cell types, cross-reactivity to the SLC34A2 protein from different species and ADCC induction activities.

Example 2: Inhibition of ADCs Towards Human NaPi2b Expressing Cells

[0138] 30H9B8F9.p1.z5-MMAE, 30H9B8F9.p1.z5-Dxd and 30H9B8F9.p1.z5-LM-D01 are derived antibody-drug conjugates (ADC) that are conjugated with MMAE, Dxd and a self-developed payload LM-D01, respectively. XMT-1536-MMAE, an ADC developed by Mersana was used as reference. The human NaPi2b expressing cell lines (human ovarian cancer cell lines OVCAR-3, RMG-1, and engineered cell lines NCI-H1975/H_NaPi2b.#5, NCI-H441/H_NaPi2b.#5) were seeded to a 96-well plate, and treated with the ADCs at respective concentrations for 6 days. The cell viability was measured by CellTiter-Glo reagent. The luciferase activity was detected by Envision.

[0139] The results in **FIG. 1A-1D** show that these ADCs have strong killing activities toward human NaPi2b expressing cell lines, and the 30H9B8F9.p1.z5-MMAE has higher inhibition effect than the XMT-1536-MMAE.

Example 3. ADCs Reduce Tumors in OVCAR-3 Tumor Model

[0140] Test ADCs are 30H9B8F9.p1.z5 derived antibody-drug conjugates (ADC) that are conjugated with MMAE, Dxd and a self-developed payload LM-D01, respectively. CB17-

SCID mice bearing OVCAR-3 tumor cells were randomized into groups (5 mouse/group) and administered intravenously (i.v) at day 0, day 7, day 14 after tumor size reached 80~100mm³. Dose-dependent anti-tumor activity was exhibited in OVCAR-3-CDX model after treatment with ADCs.

[0141] All ADCs effectively inhibit the tumor growth in the OVCAR-3 model at various administration doses. The 30H9B8F9.p1.z5-LM-D01 was observed to have superior tumor inhibition activity as compared with 30H9B8F9.p1.z5-Dxd, and 3mg/kg and 10mg/kg of 30H9B8F9.p1.z5-LM-D01 showed complete eradication of tumor in CDX model (**FIG. 2A**). The body weight fluctuation shown all treatments were well tolerated (**FIG. 2B**).

Example 4. ADCs Reduce Tumors in NCI-H1975/H₂NaPi2b Tumor Model

[0142] Test ADCs are 30H9B8F9.p1.z5 derived antibody-drug conjugate (ADC) that are conjugated with MMAE, Dxd and a self-developed payload LM-D01, respectively. BALB/c nude mice bearing NCI-H1975/H₂NaPi2b cell lines were randomized into groups (5 mouse/group) and administered intravenously (i.v) at day 0, day 7, day 14 after tumor size reached 80~100mm³. Dose-dependent anti-tumor activity was exhibited in NCI-H1975/H₂NaPi2b-CDX model after treatment with ADCs.

[0143] The 30H9B8F9.p1.z5-MMAE was observed to have superior tumor inhibition activity as compared with the rest ADCs, and 3mg/kg of 30H9B8F9.p1.z5-MMAE and 30H9B8F9.p1.z5-LM-D01 showed complete eradication of tumor in the CDX model (**FIG. 3A**). Body weight fluctuation shown all treatments were well tolerated (**FIG. 3B**).

* * *

[0144] The present disclosure is not to be limited in scope by the specific embodiments described which are intended as single illustrations of individual aspects of the disclosure, and any compositions or methods which are functionally equivalent are within the scope of this disclosure. It will be apparent to those skilled in the art that various modifications and variations can be made in the methods and compositions of the present disclosure without departing from the spirit or scope of the disclosure. Thus, it is intended that the present disclosure cover the modifications and variations of this disclosure provided they come within the scope of the appended claims and their equivalents.

[0145] All publications and patent applications mentioned in this specification are herein incorporated by reference to the same extent as if each individual publication or patent application was specifically and individually indicated to be incorporated by reference.

CLAIMS

What is claimed is:

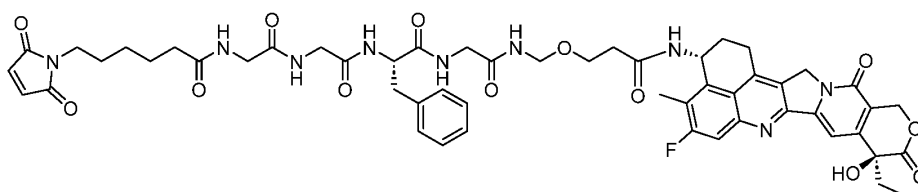
1. An antibody-drug conjugate comprising an antibody or antigen-binding fragment thereof conjugated to a drug moiety, wherein the antibody or antigen-binding fragment thereof has binding specificity to a human solute carrier 34 A2 (SLC34A2) protein and comprises a heavy chain variable region comprising heavy chain complementarity determining regions VH CDR1, VH CDR2, and VH CDR3, and a light chain variable region comprising light chain complementarity determining regions VL CDR1, VL CDR2, and VL CDR3, wherein
 - the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 3,
 - the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 4 or 9,
 - the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 5,
 - the VL CDR1 comprises the amino acid sequence of SEQ ID NO: 6,
 - the VL CDR2 comprises the amino acid sequence of SEQ ID NO: 7, and
 - the VL CDR3 comprises the amino acid sequence of SEQ ID NO: 8.
2. The antibody-drug conjugate of claim 1, wherein the VH CDR1 comprises the amino acid sequence of SEQ ID NO: 3, the VH CDR2 comprises the amino acid sequence of SEQ ID NO: 4, the VH CDR3 comprises the amino acid sequence of SEQ ID NO: 5, the VL CDR1 comprises the amino acid sequence of SEQ ID NO: 6, the VL CDR2 comprises the amino acid sequence of SEQ ID NO: 7, and the VL CDR3 comprises the amino acid sequence of SEQ ID NO: 8.
3. The antibody-drug conjugate of claim 2, wherein the heavy chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 1 and 10-13, and the light chain variable region comprises an amino acid sequence selected from the group consisting of SEQ ID NO: 2 and 14-17.

4. The antibody-drug conjugate of claim 2, wherein the heavy chain variable region comprises the amino acid sequence of SEQ ID NO:11, and the light chain variable region comprises the amino acid sequence of SEQ ID NO:14.
5. The antibody-drug conjugate of any one of claims 1-4, which is humanized.
6. The antibody-drug conjugate of any one of claims 1-5, wherein the antibody or antigen-binding fragment thereof further comprises a heavy chain constant region, a light chain constant region, an Fc region, or the combination thereof.
7. The antibody-drug conjugate of any one of claims 1-6, wherein the antibody or antigen-binding fragment thereof is of an isotype of IgG1, IgG2, IgG3 or IgG4.
8. The antibody-drug conjugate of claim 7, wherein the antibody or antigen-binding fragment thereof is of an isotype of human IgG1.
9. The antibody-drug conjugate of any one of claims 1-8, wherein the drug moiety is a cytotoxic or cytostatic agent, an immunosuppressive agent, a radioisotope, or a toxin.
10. The antibody-drug conjugate of claim 9, wherein the drug moiety is selected from the group consisting of auristatins, maytansinoids, macrocyclic ketone analogues, topoisomerase inhibitors, benzodiazepines, tubulysins, duocarmycin, camptothecin, calicheamicins, exatecans, irinotecans (SN38), doxorubicin, anthracycline, the pyrrolobenzodiazepenes (PBD), TLR agonist, STING agonists, pseudomonas aeruginosa exotoxin PE38, diphtheria toxin, staphylococcus aureus enterotoxin A/E-120, antibacterial antibiotic, shigatoxin, ricin, and urease.
11. The antibody-drug conjugate of claim 9, wherein the drug moiety is a cytotoxin selected from the group consisting of monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), maytansine, mertansine (DM1), ravtansine (DM4), tublysin A, DXd, 7-ethyl-10-hydroxycamptothecin (SN-38), DGN462, Amberstatin269, anthramycin,

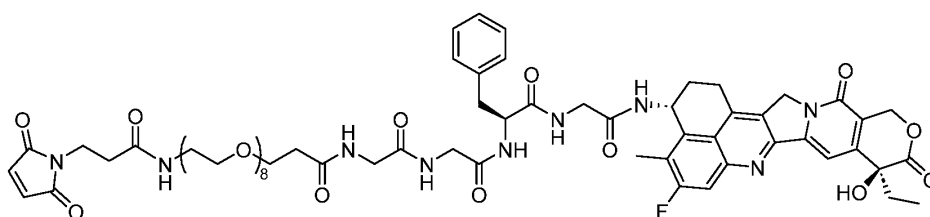
SG3199/SCX, IRDye®700DX, TLR7/8 agonist, diABZI STING agonist-2, or any derivative thereof.

12. The antibody-drug conjugate of any one of claims 9-11, wherein the drug moiety comprises MMAE, MMAF, or DXd.

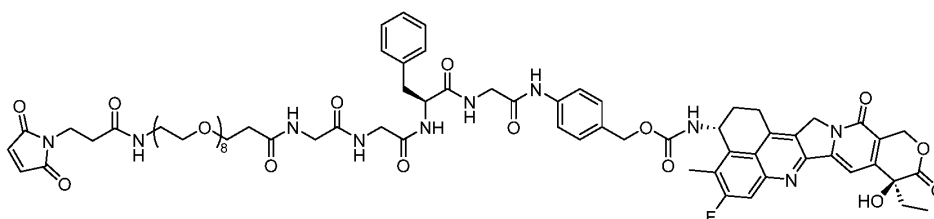
13. The antibody-drug conjugate of claim 9, wherein the drug moiety comprises a compound selected from the group consisting of:



(LM-D01),



(LM-D02), and



(LM-D03).

14. The antibody-drug conjugate of any one of claims 1-13, wherein the antibody or antigen-binding fragment thereof is conjugated to the drug moiety via a linker.

15. The antibody-drug conjugate of claim 14, wherein the linker is a cleavable linker or a non-cleavable linker.
16. The antibody-drug conjugate of claim 15, wherein the linker is a cleavable linker.
17. The antibody-drug conjugate of any one of claims 14-16, wherein the linker comprises succinimidyl-4-(N-maleimidomethyl) cyclohexane-1-carboxylate (SMCC), sulfo-SMCC, p-carboxycyclohexylmethylmaleimide, maleimide-caproyl (MC)-Valine-citrulline (VC)-para-aminobenzyloxycarbonyl (PABC), CL2A, maleimide-caproyl (MC), MC-glycine-glycine-phenylalanine-glycine (GGFG), MC-PEG8-GGFG, MC-PEG8-GGFG-PAB, or maleimide propoyl (MP)-PEG8-Valine-alanine (VA)-PABC.
18. The antibody-drug conjugate of any one of the preceding claims, comprising ozogamicin, vedotin, mafodotin, emtansine, deruxtecan, govitecan, or tesirine.
19. The antibody-drug conjugate of any one of claims 1-18, wherein the drug-antibody ratio (DAR) is 1-20.
20. The antibody-drug conjugate of any one of claims 1-18, wherein the drug-antibody ratio (DAR) is 4-8.
21. A composition comprising the antibody-drug conjugate of any one of claims 1-20 and a pharmaceutically acceptable carrier.
22. A method of treating cancer in a patient in need thereof, comprising administering to the patient the antibody-drug conjugate of any one of claims 1-20 or the composition of claim 21.
23. Use of the antibody-drug conjugate of any one of claims 1-20 or the composition of claim 21 for the preparation of a medicament for treating cancer.
24. The antibody-drug conjugate of any one of claims 1-20 or the composition of claim 21 for use in the treatment of cancer in a patient in need thereof.

25. The method of claim 22 or the use of claim 23 or the antibody-drug conjugate or composition for use of claim 24, wherein the cancer is selected from the group consisting of bladder cancer, liver cancer, colon cancer, rectal cancer, endometrial cancer, leukemia, lymphoma, pancreatic cancer, small cell lung cancer, non-small cell lung cancer, breast cancer, urethral cancer, head and neck cancer, gastrointestinal cancer, stomach cancer, oesophageal cancer, ovarian cancer, renal cancer, melanoma, prostate cancer and thyroid cancer.