

GLYCAN-INTERACTING COMPOUNDS AND METHODS OF USE

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims priority to United States Provisional Application Number 62/466,766 filed on March 3, 2017 entitled GLYCAN-INTERACTING COMPOUNDS AND METHODS OF USE, United States Provisional Application Number 62/480,126 filed on March 31, 2017 entitled GLYCAN-INTERACTING COMPOUNDS AND METHODS OF USE, United States Provisional Application Number 62/486,826 filed on April 18, 2017 entitled GLYCAN-INTERACTING COMPOUNDS AND METHODS OF USE, United States Provisional Application Number 62/563,718 filed on September 27, 2017 entitled GLYCAN-INTERACTING COMPOUNDS AND METHODS OF USE, and United States Provisional Application Number 62/577,830 filed on October 27, 2017 entitled GLYCAN-INTERACTING COMPOUNDS AND METHODS OF USE, the contents of each of which are herein incorporated by reference in their entirety.

SEQUENCE LISTING

[0002] The instant application contains a Sequence Listing which has been submitted electronically in ASCII format and is hereby incorporated by reference in its entirety. Said ASCII copy, created on March 2, 2018, is named 2033_1030PCT_SL.txt and is 24,876 bytes in size.

BACKGROUND

[0003] Aberrant glycosylation accompanies some of the other mutations commonly observed in carcinomas. It has been estimated that about 80% of all carcinomas express the truncated glycans, the Tn Antigen and the sialylated form, Sialyl Tn (STn). With few exceptions, Tn and STn are not expressed in normal, healthy tissues. Furthermore, the non-human immunogenic sialic acid, N-glycolylneuraminic acid (Neu5Gc), seems to be differentially expressed on carcinomas such as breast cancer in the form of Neu5Gc-STn (GcSTn).

[0004] Multiple aberrant glycosylation forms have been described in human cancers, identifying specific glycans as a class of cell surface molecules suitable for specific tumor targeting (Cheever, M.A. et al., Clin Cancer Res. 2009 Sep 1;15(17):5323-37). For example, various human cancer types (such as bladder, breast, cervical, colon, lung, and ovarian cancer among others) show high expression of STn antigen, which is rare in normal human tissues (Karlen, P. et al., Gastroenterology. 1998 Dec;115(6):1395-404; Ohno, S. et al, Anticancer

Res. 2006 Nov-Dec;26(6A):4047-53). In addition, the presence of STn on tumor-associated mucins relates to cancer with poor prognosis and is therewith considered an attractive epitope for cancer detection and targeted therapy (Cao, Y. et al., *Virchows Arch.* 1997 Sep;431(3):159-66; Julien, S. et al., *Br J Cancer.* 2009 Jun 2;100(11):1746-54; Itzkowitz, S.H. et al., *Cancer.* 1990 Nov 1;66(9):1960-6; Motoo, Y. et al., *Oncology.* 1991;48(4):321-6; Kobayashi, H. et al., *J Clin Oncol.* 1992 Jan;10(1):95-101). Tn and STn formation is associated with somatic mutations in the gene *Cosmc* that encodes a molecular chaperon required for the formation of the activate T-synthase (Ju, T. et al., *Nature.* 2005 Oct 27;437(7063):1252; Ju, T. et al., *Cancer Res.* 2008 Mar 15;68(6):1636-46). It can also result from increased expression of the sialyl transferase, ST6GalNAc I (Ikehara, Y. et al., *Glycobiology.* 1999 Nov;9(11):1213-24; Brockhausen, I. et al., *Biol Chem.* 2001 Feb;382(2):219-32). De-novo expression of STn can modulate carcinoma cells, change the malignant phenotype, and lead to more aggressive cell behaviors (Pinho, S. et al., *Cancer Lett.* 2007 May 8;249(2):157-70). Although STn is highly expressed in malignant tissues, low levels are also found on healthy human cells (Jass, J.R. et al., *J Pathol.* 1995 Jun;176(2):143-9; Kirkeby, S. et al., *Arch Oral Biol.* 2010 Nov;55(11):830-41). STn alone has attracted attention as a target for cancer detection and therapy (Cheever, M.A. et al., *Clin Cancer Res.* 2009 Sep 1;15(17):5323-37). STn is also present in mucins associated with cancer stem cells (Engelmann et al., *Cancer research*, 2008, 68, 2419-2426) and STn is implicated in immune suppression (Carrascal, M.A., et al., *Molecular Oncology.* 2014. 8(3): 753-65).

[0005] In addition to the presence of STn, other glycosylation changes have been described in cancer. One of them involves Neu5Gc. *N*-acetylneuraminic acid (Neu5Ac) and Neu5Gc are the two major sialic acids on mammalian cell surfaces. Neu5Ac and Neu5Gc differ only in that Neu5Gc includes an additional oxygen atom associated with chemical group attached to carbon 5. Due to the loss of a functional gene, humans can only synthesize sialic acid in the form of Neu5Ac, but not Neu5Gc. However, Neu5Gc can be metabolically incorporated into humans from animal-derived dietary sources such as red meats (Tangvoranuntakul, P. et al., *Proc Natl Acad Sci U S A.* 2003 Oct 14; 100(21):12045-50; Nguyen, D.H. et al., *J Immunol.* 2005 Jul 1; 175(1):228-36; US7,682,794, US8,084,219, US2012/0142903, WO2010030666 and WO2010030666). Neu5Gc is significantly abundant among human tumors (Higashi, H. et al., *Cancer Res.* 1985 Aug; 45(8):3796-802; Miyoshi I. et al., *Mol Immunol.* 1986. 23: 631-638; Hirabayashi, Y. et al., *Jpn J Cancer Res.* 1987. 78: 614-620; Kawachi, S. et al., *Int Arch Allergy Appl Immunol.* 1988. 85: 381-383; Devine,

et al., *Cancer Res.* 1991. 51: 5826-5836; Malykh, Y.N. et al, *Biochimie.* 2001. 83: 623-634 and Inoue, S. et al., 2010. *Glycobiology.* 20(6): 752-762) and remarkably low in normal human tissues, which had been overlooked for several decades (Diaz, S.L. et al., *PLoS One.* 2009. 4: e4241; Tangvoranuntakul, P. et al., *Proc Natl Acad Sci U S A.* 2003. 100: 12045-12050; Varki, A. et al., *Glycoconj J.* 2009. 26: 231-245). The increased metabolic accumulation of diet-derived Neu5Gc in cancer tissue compared to healthy human tissues is likely explained by at least three factors: rapid growth with underproduction of competing endogenous Neu5Ac, enhanced macropinocytosis induced by growth factors (Dharmawardhane, S. et al., *Mol Biol Cell.* 2000 Oct;11(10):3341-52; Simonsen, A. et al., *Curr Opin Cell Biol.* 2001 Aug;13(4):485-92; Johannes, L. et al., *Traffic.* 2002 Jul;3(7):443-51; Amyere, M. et al., *Int J Med Microbiol.* 2002 Feb;291(6-7):487-94), and the upregulation of gene expression of the lysosomal sialic acid transporter gene sialin by hypoxia (Yin, J. et al., *Cancer Res.* 2006 Mar 15;66(6):2937-45). In addition, all humans tested to date include a polyclonal antibody reservoir against non-human Neu5Gc, which makes it the first example of a xeno-autoantigen (Padler-Karavani, V. et al., *Glycobiology.* 2008 Oct;18(10):818-30; Varki, N.M. et al., *Annu Rev Pathol.* 2011;6:365-93). The accumulation of dietary Neu5Gc in malignant tumors in the face of an anti-Neu5Gc response was shown to facilitate tumor progression by inducing a low-grade chronic inflammation (Hedlund, M. et al., *Proc Natl Acad Sci U S A.* 2008 Dec 2;105(48):18936-41). Thus, Neu5Gc containing glycan epitopes on human tumors represent a valuable possibility for drug targeting. A recent study suggests the existence of antibodies against Neu5Gc-containing STn (GcSTn), but not Neu5Ac-STn (AcSTn), in cancer patients and explores their potential as a specific biomarker for cancer detection (Padler-Karavani, V. et al., *Cancer Res.* 2011 May 1;71(9):3352-63).

[0006] There remains a need in the art for therapeutic antibodies capable of binding glycans, including glycans associated with disease and diseased cells and tissues. Further, there remains a need for better methods to develop such antibodies and methods of using these antibodies to target diseased cells and tissues. The present disclosure meets these needs by providing related compounds and methods.

SUMMARY

[0007] In some examples, the present disclosure provides a method of treating cancer in a subject by administering an antibody, wherein the antibody is administered at a dose of from about 1 mg/kg to about 10 mg/kg, wherein the antibody has a terminal half-life of from about 50 hours to about 200 hours, and wherein the antibody binds sialyl Tn antigen (STn).

The antibody may include a heavy chain variable domain (VH) with an amino acid sequence selected from the group consisting of SEQ ID NOs: 7 and 9; and a light chain variable domain (VL) with an amino acid sequence selected from the group consisting of SEQ ID NOs: 8 and 10. The antibody may be an antibody-drug conjugate. The antibody may be conjugated with monomethyl auristatin E (MMAE). The antibody may be administered intravenously.

[0007a] In a first aspect, there is provided use of an antibody-drug conjugate (ADC) in the manufacture of a medicament for treating a platinum refractory cancer in a subject, wherein the platinum refractory cancer (i) is selected from breast cancer, melanoma, colorectal cancer, liver cancer, lung cancer, bladder cancer, cervical cancer, stomach cancer, and prostate cancer, (ii) is resistant to cisplatin or oxaliplatin, or (iii) is ovarian cancer and is resistant to cisplatin, wherein said ADC comprises: a) an anti-STn antibody, wherein said anti-STn antibody comprises: 1) a heavy chain variable domain (VH) with the amino acid sequence of SEQ ID NO: 7 and a light chain variable domain (VL) with the amino acid sequence of SEQ ID NO: 8; 2) a VH with the amino acid sequence of SEQ ID NO: 9 and a VL with the amino acid sequence of SEQ ID NO: 10; or 3) a VH with the amino acid sequence of SEQ ID NO: 11 and a VL with the amino acid sequence of SEQ ID NO: 12; and b) a cytotoxic agent conjugated to the anti-STn antibody.

[0008] In some examples, the present disclosure provides a method of treating colorectal cancer by administering an anti-STn antibody to a subject with colorectal cancer, wherein the anti-STn antibody includes a heavy chain variable domain (VH) with an amino acid sequence selected from the group consisting of SEQ ID NOs: 7, 9, and 11; and a light chain variable domain (VL) with an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 10, and 12. The colorectal cancer may be resistant to treatment with at least one of cetuximab, panitumumab, bevacizumab, ramucirumab, trastuzumab, ponatinib, sorafenib, 5-fluorouracil, cisplatin, docetaxel, gemcitabine, irinotecan, paclitaxel, and oxaliplatin. The anti-STn antibody may include an antibody drug conjugate (ADC). The ADC may include at least one conjugate, wherein the at least one conjugate is selected from one or more of an auristatin, a maytansine, a tubulysin, a vinca alkaloid, a pyrrolobenzodiazepine dimer, a camptothecin, a duocarmycin, an amanitin, a phosphoinositide 3-kinase (PI3K) inhibitor, and a mitogen-activated protein kinase kinase (MEK) inhibitor. The ADC may include one or more polymer, wherein the one or more polymer connects the anti-STn antibody and the at least one conjugate. The one or more polymer may include one or more of a poly(ethylene glycol) (PEG), a poly(N-(2-hydroxypropyl)methacrylamide) (polyHPMA), a poly(α -amino acid), a carbohydrate polymer, a glycopolysaccharide, a glycolipid, a glycoconjugate, a polyglycerol, a polyvinyl alcohol, a poly(acrylic acid), a polyketal, and a polyacetal. The one or more polymer may include a poly(1-hydroxymethylethylene hydroxymethylformal) (PHF). The anti-STn

antibody may kill cells expressing STn with a half maximal inhibitory concentration of from about 0.1 nM to about 50 nM.

[0008a] In one example, there is provided use of an anti-STn antibody in the manufacture of a medicament for treating colorectal cancer in a subject with colorectal cancer, wherein said anti-STn antibody comprises: a heavy chain variable domain (VH) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 7, 9, and 11; and a light chain variable domain (VL) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 10, and 12.

[0009] Administering the anti-STn antibody may be carried out in combination with at least one other therapeutic treatment. The other therapeutic treatment may include a standard of care therapeutic treatment. The other therapeutic treatment may be selected from one or more of cetuximab, panitumumab, bevacizumab, ramucirumab, trastuzumab, ponatinib, sorafenib, 5-fluorouracil, cisplatin, docetaxel, gemcitabine, irinotecan, paclitaxel, and oxaliplatin. The other therapeutic treatment may also include administration of at least one cell cycle inhibitor. The cell cycle inhibitor may be a cyclin-dependent kinase (CDK)

inhibitor. The CDK inhibitor may inhibit CDK4 and/or CDK6. The CDK inhibitor may be selected from palbociclib, ribociclib, and abemaciclib. The anti-STn antibody may be treated concurrently with the other therapeutic treatment. The anti-STn antibody may be treated sequentially with the other therapeutic treatment.

[0010] In some examples, the present disclosure provides a method of treating cancer, the method comprising administering an anti-STn antibody to a subject with cancer, wherein the anti-STn antibody includes a heavy chain variable domain (VH) with an amino acid sequence selected from the group consisting of SEQ ID NOs: 7, 9, and 11; and a light chain variable domain (VL) with an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 10, and 12, wherein administering the anti-STn antibody is carried out in combination with at least one cell cycle inhibitor. The cell cycle inhibitor may be a cyclin-dependent kinase (CDK) inhibitor. The CDK inhibitor may inhibit CDK4 and/or CDK6. The CDK inhibitor may be selected from palbociclib, ribociclib, and abemaciclib. The anti-STn antibody may be administered concurrently with the cell cycle inhibitor. The anti-STn antibody may be administered sequentially with the cell cycle inhibitor.

[0010a] In one example, there is provided use of an anti-STn antibody in the manufacture of a medicament for treating cancer in a subject with cancer, wherein said anti-STn antibody comprises: a heavy chain variable domain (VH) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 7, 9, and 11; and a light chain variable domain (VL) comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 10, and 12, wherein said anti-STn antibody is to be administered in combination with at least one cell cycle inhibitor.

[0011] In some examples, the present disclosure provides an ADC that includes an anti-STn antibody with a VH with an amino acid sequence selected from SEQ ID NOs: 7, 9, and 11; a VL with an amino acid sequence selected from SEQ ID NOs: 8, 10, and 12; and one or more cytotoxic agent. The cytotoxic agent may be selected from at least one of an auristatin, a maytansine, a tubulysin, a vinca alkaloid, a pyrrolobenzodiazepine dimer, a camptothecin, a duocarmycin, an amanitin, a PI3K inhibitor, and a MEK inhibitor. The anti-STn antibody may be conjugated with the one or more cytotoxic agent via a linker. The ADC may include one or more polymer connecting the anti-STn antibody and the one or more cytotoxic agent. The one or more polymer may include one or more of a PEG, a polyHPMA, a poly(α -amino acid), a carbohydrate polymer, a glycopolysaccharide, a glycolipid, a glycoconjugate, a polyglycerol, a polyvinyl alcohol, a poly(acrylic acid), a polyketal, and a polyacetal. The one or more polymer may include a PHF. The one or more polymer may be

attached to the anti-STn antibody via a linker. The one or more cytotoxic agent may be attached to the one or more polymer via a linker. The linker may be a cleavable linker.

[0011a] In one example, there is provided an antibody-drug conjugate (ADC) comprising:

- a. an anti-STn antibody, wherein said anti-STn antibody comprises:
 - a VH comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 7, 9, and 11; and
 - a VL comprising an amino acid sequence selected from the group consisting of SEQ ID NOs: 8, 10, and 12; and
- b. one or more cytotoxic agents, wherein said one or more cytotoxic agents is selected from at least one of a pyrrolobenzodiazepine dimer, a camptothecin, an amanitin, a PI3K inhibitor, and a MEK inhibitor.

[0012] In some examples, the present disclosure provides a method of treating cancer in a subject, wherein the subject has at least one cancer cell expressing STn, the method including administering an anti-STn antibody drug conjugate described herein. The at least one cancer cell may be an ovarian cancer cell. The at least one cancer cell may be resistant to treatment with at least one chemotherapeutic agent. The chemotherapeutic agent may be

cisplatin. The anti-STn antibody drug conjugate may be administered at a dose of from about 0.1 mg/kg to about 25 mg/kg. Administration may be by intravenous injection. The anti-STn antibody drug conjugate may be administered daily, weekly, or monthly.

[0012a] In one example, there is provided use of the ADC disclosed herein in the manufacture of a medicament for treating cancer in a subject, wherein the subject has at least one cancer cell expressing STn.

[0013] Some methods of the present disclosure include methods of treating cancer by administering an ADC to a subject, the ADC including: a VH including SEQ ID NO: 7; a VL including SEQ ID NO: 8; at least one human IgG constant region; and a cytotoxic conjugate, wherein the cytotoxic conjugate is conjugated to the at least one human IgG constant region via a linker. The at least one human IgG constant region may be selected from one or more of SEQ ID NOs: 15 and 16. The cytotoxic conjugate may include MMAE. The ADC may be administered at a dose of from about 1 mg/kg to about 6 mg/kg. The ADC may be administered by intravenous bolus injection. The ADC may be administered as part of a composition, wherein the composition includes at least one excipient. The composition may be administered at a volume of from about 0.1 ml/kg to about 10 ml/kg. The composition may be administered at a volume of about 1.2 ml/kg. The composition may include an ADC concentration of from about 0.5 mg/ml to about 10 mg/ml. The ADC may exhibit an apparent terminal elimination half-life of from about 2 days to about 8 days. The ADC may exhibit an apparent clearance rate of from about 10 ml/kg/day to about 20 ml/kg/day. The ADC may exhibit an apparent volume of distribution at steady state of from about 50 ml/kg to about 100 ml/kg. The ADC may exhibit a maximum observed concentration of from about 10 µg/ml to about 200 µg/ml. The ADC may exhibit an area under the concentration versus time curve (AUC) from the start of administration to the last observed quantifiable concentration of from about 50 days*µg/ml to about 500 days*µg/ml.

BRIEF DESCRIPTION OF THE FIGURES

[0014] The foregoing and other objects, features and advantages will be apparent from the following description of particular embodiments of the invention, as illustrated in the accompanying drawings in which like reference characters refer to the same parts throughout the different views. The drawings are not necessarily to scale; emphasis instead being placed upon illustrating the principles of various embodiments of the invention.

[0015] Fig. 1A is a schematic depicting α2,6-sialylated N-acetylgalactosamine (STn) with the largest ellipse indicating the specific region of STn recognized by Group 1 antibodies.

[0016] Fig. 1B is a schematic depicting α2,6-sialylated N-acetylgalactosamine (STn) with the largest ellipse indicating the specific region of STn recognized by Group 2 antibodies.

[0017] Fig. 1C is a schematic depicting α 2,6-sialylated N-acetylgalactosamine (STn) with the largest ellipse indicating the specific region of STn recognized by Group 3 antibodies.

[0018] Fig. 1D is a schematic depicting α 2,6-sialylated N-acetylgalactosamine (STn) with the largest ellipse indicating the specific region of STn recognized by Group 4 antibodies.

[0019] Fig. 2 is a graph showing mean serum antibody concentrations over time following an initial dose of hSIA101-MMAE in *Cynomolgus* monkeys.

[0020] Fig. 3 is a graph showing mean serum antibody concentrations over time following a second dose (on Day 22 of treatment) of hSIA101-MMAE in *Cynomolgus* monkeys.

[0021] Fig. 4 is a set of graphs showing patient-derived xenograft tumor volumes over the course of treatment with hSIA101-ADC in comparison with other treatments. Tumors in the top panel were initiated with ovarian tumors from a chemo-naïve patient and tumors in the bottom panel were initiated with ovarian tumors from a patient who had previously been treated with chemotherapeutic agents.

DETAILED DESCRIPTION

Introduction

[0022] According to the present invention are antibodies specific for or which interact with epitopes that include carbohydrate groups referred to herein as glycans. Some glycan-interacting antibodies described herein may be used as biotherapeutics. Other embodiments provide methods for generating such glycan-interacting antibodies.

[0023] In nature, STns may be sialylated with N-acetylneuraminic acid (Neu5Ac) or N-glycolylneuraminic acid (Neu5Gc). Glycan-interacting antibodies according to the present invention may be directed to glycans having any STns (pan-STn antibodies), glycans having STns that include Neu5Ac specifically (AcSTn) or glycans having STns that include Neu5Gc specifically (GcSTn). In some embodiments, glycan-interacting antibodies of the present invention target cancer-related glycan antigens, such as α 2,6-sialylated N-acetylgalactosamine (STn).

[0024] In some embodiments, the present disclosure provides methods of producing glycan-interacting antibodies. Such methods may include the use of mice for generating an immune response to one or more antigens, including STn (e.g. AcSTn and/or GcSTn). As described herein, a number of methods may be utilized in order to manipulate the resulting antibodies produced through mouse immunization. Such methods may include varying the strain and/or gender of the mice being immunized, varying the antigen used, varying the type

and dose of adjuvant included in antigen administration and time course of immunization before initiation of hybridoma fusion.

[0025] In some embodiments, the present disclosure provides methods for treating cancer using glycan-interacting antibodies. Such methods may include the use of antibody drug conjugates (ADCs). The ADCs may include cytotoxic conjugates attached to glycan-interacting antibodies directly or via a linker. The glycan-interacting antibodies may bind STn. In some embodiments, the methods for treating cancer include eliminating cancer stem cells. In some aspects, glycan-interacting antibodies may be used alone. In other aspects, glycan-interacting antibodies are used in combination with chemotherapeutic agents. The glycan-interacting antibodies may be prepared as compositions including one or more excipients for administration to subjects. The compositions and routes of administration may provide glycan-interacting antibodies at concentrations and doses suitable for achieving bioavailability, therapeutic window, and/or volume of distribution necessary for effective treatment.

[0026] Further provided are optimized, humanized and conjugated forms of glycan-interacting antibodies disclosed herein. Additionally, kits, assays and reagents including antibodies and/or methods of the present invention are presented.

Definitions

[0027] *Adjacent*: As used herein, the term “adjacent” refers to something that is adjoining, neighboring or next to a given entity. In some embodiments, “adjacent residues” are sugar residues within a glycan chain that are linked to one another. In some embodiments, “adjacent glycans” are glycan chains that next to each other either in direct contact or within close proximity and without another glycan in between the two.

[0028] *Administered in combination*: As used herein, the term “administered in combination” or “combined administration” means that a subject is simultaneously exposed to two or more agents administered at the same time or within an interval of time such that the subject is at some point in time simultaneously exposed to both and/or such that there may be an overlap in the effect of each agent on the patient. In some embodiments, at least one dose of one or more agents is administered within about 24 hours, 12 hours, 6 hours, 3 hours, 1 hour, 30 minutes, 15 minutes, 10 minutes, 5 minutes, or 1 minute of at least one dose of one or more other agents. In some embodiments, administration occurs in overlapping dosage regimens. As used herein, the term “dosage regimen” refers to a plurality of doses spaced apart in time. Such doses may occur at regular intervals or may include one or more

hiatus in administration. In some embodiments, the administration of individual doses of one or more glycan-interacting antibodies, as described herein, are spaced sufficiently closely together such that a combinatorial (*e.g.*, a synergistic) effect is achieved.

[0029] *Amino acid*: As used herein, the terms "amino acid" and "amino acids" refer to all naturally occurring L-alpha-amino acids as well as non-naturally occurring amino acids. Amino acids are identified by either the one-letter or three-letter designations as follows: aspartic acid (Asp:D), isoleucine (Ile:I), threonine (Thr:T), leucine (Leu:L), serine (Ser:S), tyrosine (Tyr:Y), glutamic acid (Glu:E), phenylalanine (Phe:F), proline (Pro:P), histidine (His:H), glycine (Gly:G), lysine (Lys:K), alanine (Ala:A), arginine (Arg:R), cysteine (Cys:C), tryptophan (Trp:W), valine (Val:V), glutamine (Gln:Q) methionine (Met:M), asparagine (Asn:N), where the amino acid is listed first followed parenthetically by the three and one letter codes, respectively.

[0030] *Animal*: As used herein, the term "animal" refers to any member of the animal kingdom. In some embodiments, "animal" refers to humans at any stage of development. In some embodiments, "animal" refers to non-human animals at any stage of development. In certain embodiments, the non-human animal is a mammal (*e.g.*, a rodent, a mouse, a rat, a rabbit, a monkey, a dog, a cat, a sheep, cattle, a primate, or a pig). In some embodiments, animals include, but are not limited to, mammals, birds, reptiles, amphibians, fish, and worms. In some embodiments, the animal is a transgenic animal, genetically-engineered animal, or a clone.

[0031] *Antibody*: As used herein, the term "antibody" is used in the broadest sense and specifically covers various embodiments including, but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (*e.g.* bispecific antibodies formed from at least two intact antibodies), and antibody fragments such as diabodies so long as they exhibit a desired biological activity. Antibodies are primarily amino-acid based molecules but may also include one or more modifications such as with sugar moieties.

[0032] *Antibody fragment*: As used herein, the term "antibody fragment" refers to a portion of an intact antibody, preferably including an antigen binding region thereof. Examples of antibody fragments include Fab, Fab', F(ab')₂, and Fv fragments; diabodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site. Also produced is a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab')₂ fragment that has two antigen-binding sites and is still capable of cross-

linking antigen. Glycan-interacting antibodies may include one or more of these fragments. For the purposes herein, an antibody may include a heavy and light variable domain as well as an Fc region.

[0033] *Antigen-binding region:* As used herein, the term “antigen-binding region” refers to the portion of an antibody, antibody fragment, or related molecule that directly interacts with a target molecule or epitope. Antigen-binding regions typically include a variable domain pair, as in the Fab region of an antibody or as linked together in a scFv.

[0034] *Approximately:* As used herein, the term “approximately” or “about,” as applied to one or more values of interest, refers to a value that is similar to a stated reference value. In certain embodiments, the term “approximately” or “about” refers to a range of values that fall within 25%, 20%, 19%, 18%, 17%, 16%, 15%, 14%, 13%, 12%, 11%, 10%, 9%, 8%, 7%, 6%, 5%, 4%, 3%, 2%, 1%, or less in either direction (greater than or less than) of the stated reference value unless otherwise stated or otherwise evident from the context (except where such number would exceed 100% of a possible value).

[0035] *Associated with:* As used herein, the terms “associated with,” “conjugated,” “linked,” “attached,” and “tethered,” when used with respect to two or more moieties, means that the moieties are physically associated or connected with one another, either directly or via one or more additional moieties that serves as a linking agent, to form a structure that is sufficiently stable so that the moieties remain physically associated under the conditions in which the structure is used, *e.g.*, physiological conditions. An “association” need not be strictly through direct covalent chemical bonding. It may also suggest ionic or hydrogen bonding or a hybridization based connectivity sufficiently stable such that the “associated” entities remain physically associated.

[0036] *Bifunctional:* As used herein, the term “bifunctional” refers to any substance, molecule or moiety which is capable of or maintains at least two functions. The functions may affect the same outcome or a different outcome. The structure that produces the function may be the same or different.

[0037] *Biomolecule:* As used herein, the term “biomolecule” is any natural molecule which is amino acid-based, nucleic acid-based, carbohydrate-based or lipid-based, and the like.

[0038] *Bispecific antibody:* As used herein, the term “bispecific antibody” refers to an antibody capable of binding two different antigens. Such antibodies typically include regions from at least two different antibodies. Bispecific antibodies may include any of those described in Riethmuller, G. 2012. Cancer Immunity. 12:12-18, Marvin, J.S. et al., 2005.

Acta Pharmacologica Sinica. 26(6):649-58 and Schaefer, W. et al., 2011. PNAS. 108(27):11187-92, the contents of each of which are herein incorporated by reference in their entirety.

[0039] *Branch*: As used herein, the term “branch” refers to an entity, moiety or appendage that is linked or extends out from a main entity or source. In some embodiments, a “branch chain” or “branching chain” includes one or more residues (including, but not limited to sugar residues) that extend from a parent chain. As used herein, a “parent chain” is used to refer to a chain of residues (including, but not limited to sugar residues) from which a branching chain is linked. In the case of a glycan with multiple branches, the parent chain may also refer to the source chain from which all such branches are directly or indirectly attached. In the case of a polysaccharide having a chain of hexose residues, parent chain linkages typically occur between carbons 1 and 4 of adjacent residues while branching chains are attached to a parent chain through a linkage between carbon 1 of the branching residue and carbon 3 of the parent residue from which the branch extends. As used herein, the term “branching residue” refers to the residue attached to the parent chain in a branching chain.

[0040] *Cancer stem cells*: As used herein, cancer stem cells (CSCs) refer to a subset of tumor cells that have the ability to self-renew. CSCs may be able to regenerate diverse cell types. In some cases, these cells are difficult or impossible to remove through surgical or chemical treatment of a tumor.

[0041] *Compound*: As used herein, the term “compound,” refers to a distinct chemical entity. In some embodiments, a particular compound may exist in one or more isomeric or isotopic forms (including, but not limited to stereoisomers, geometric isomers and isotopes). In some embodiments, a compound is provided or utilized in only a single such form. In some embodiments, a compound is provided or utilized as a mixture of two or more such forms (including, but not limited to a racemic mixture of stereoisomers). Those of skill in the art appreciate that some compounds exist in different such forms, show different properties and/or activities (including, but not limited to biological activities). In such cases it is within the ordinary skill of those in the art to select or avoid particular forms of the compound for use in accordance with the present invention. For example, compounds that contain asymmetrically substituted carbon atoms can be isolated in optically active or racemic forms. Methods on how to prepare optically active forms from optically active starting materials are known in the art, such as by resolution of racemic mixtures or by stereoselective synthesis.

[0042] *Cyclic or Cyclized:* As used herein, the term “cyclic” refers to the presence of a continuous loop. Cyclic molecules need not be circular, only joined to form an unbroken chain of subunits.

[0043] *Cytidine monophosphate-N-acetylneuraminic acid hydroxylase:* As used herein, the term “cytidine monophosphate-N-acetylneuraminic acid hydroxylase” or “CMAH” refers to an enzyme, absent in humans, but present in most other mammals (including, but not limited to mice, pigs and chimpanzees) that catalyzes the formation of N-glycolylneuraminic acid from N-acetylneuraminic acid. The absence of the enzyme in humans is due to a frameshift mutation resulting in the premature termination of the CMAH transcript and the production of a non-functional protein.

[0044] *Cytotoxic:* As used herein, the term “cytotoxic” is used to refer to an agent that kills or causes injurious, toxic, or deadly effects on a cell (*e.g.*, a mammalian cell (*e.g.*, a human cell)), bacterium, virus, fungus, protozoan, parasite, prion, or a combination thereof.

[0045] *Delivery:* As used herein, “delivery” refers to the act or manner of transporting a compound, substance, entity, moiety, cargo or payload to an intended destination.

[0046] *Delivery Agent:* As used herein, “delivery agent” refers to any substance which facilitates, at least in part, the *in vivo* delivery of a compound, substance, entity, moiety, cargo or payload.

[0047] *Detectable label:* As used herein, “detectable label” refers to one or more markers, signals, or moieties which are attached, incorporated or associated with another entity, which markers, signals or moieties are readily detected by methods known in the art including radiography, fluorescence, chemiluminescence, enzymatic activity, absorbance and the like. Detectable labels include radioisotopes, fluorophores, chromophores, enzymes, dyes, metal ions, ligands such as biotin, avidin, streptavidin and haptens, quantum dots, and the like. Detectable labels may be located at any position in the entity with which they are attached, incorporated or associated. For example, when attached, incorporated in or associated with a peptide or protein, they may be within the amino acids, the peptides, or proteins, or located at the N- or C- termini.

[0048] *Display library:* As used herein, the term “display library” refers to a tool used in scientific discovery to identify biomolecular interactions. Different variations of display libraries exist that include the utilization of bacteriophages, yeast and ribosomes. In each case, proteins within a given library (also referred to herein as “library members”) are linked (physically or through association with a host) to the nucleic acid which encodes the protein. When a target molecule is incubated with the members of a display library, any library

members that bind to the target may be isolated and the sequences encoding the bound protein may be determined through analysis of the linked nucleic acid. In some embodiments, display libraries are “phage display libraries” wherein the display library is made up of bacteriophage viral particles (also referred to herein as “phage particles”) wherein nucleic acids have been incorporated into the phage genome resulting in the production of viral coat proteins that are fused to proteins encoded by the nucleic acids that have been introduced. Such fused proteins are “displayed” on the outer surface of the assembled phage particles where they may interact with a given target.

[0049] *Distal*: As used herein, the term “distal” means situated away from the center or away from a point or region of interest.

[0050] *Engineered*: As used herein, embodiments of the invention are “engineered” when they are designed to have a feature or property, whether structural or chemical, that varies from a starting point, wild type or native molecule. Thus, engineered agents or entities are those whose design and/or production include an act of the hand of man.

[0051] *Epitope*: As used herein, an “epitope” refers to a surface or region on a molecule that is capable of interacting with components of the immune system, including, but not limited to antibodies. In some embodiments, an epitope may include a target site. Epitopes may include a region on an antigen or between two or more antigens that is specifically recognized and bound by a corresponding antibody. Some epitopes may include one or more sugar residues along one or more glycan. Such epitopes may include 1, 2, 3, 4, 5, 6, 7, 8, 9 or at least 10 sugar residues. Epitopes may also include one or more regions of interaction between entities. In some embodiments, epitopes may include a junction between two sugar residues, between a branching chain and a parent chain or between a glycan and a protein.

[0052] *Ether bond*: As used herein, an “ether bond” refers to a chemical bond that includes an oxygen bonded between two carbon atoms. In some embodiments, ether bonds link sugar residues to other entities, including, but not limited to other sugar residues to form a glycan chain. Such bonds are also referred to as “glycosidic bonds” or “glycosidic linkages”. In the context of at least one sugar residue, the terms “link” and/or “linkage” are also used herein when referring to a glycosidic linkage. In some embodiments, linkages may link glycans to other entities, including, but not limited to proteins, lipids, phospholipids and sphingolipids. In some embodiments, sugar residues may be linked to protein, typically forming a link between a sugar residue and an amino acid residue. Such amino acid residues include serine and threonine. In some embodiments, ether bonds link glycans to a glycan array through a carbohydrate linker that participates in bond formation. Glycosidic linkages

may differ in their stereochemical properties. In some embodiments, alpha oriented glycosidic linkages (also referred to herein as “alpha linkages”) result in an axial orientation between the bonded oxygen of the ether bond and the cyclohexane ring of the sugar residue. In some embodiments, beta oriented glycosidic linkages (also referred to herein as “beta linkages”) result in an equatorial orientation between the bonded oxygen of the ether bond and the cyclohexane ring of the sugar residue.

[0053] *Expression:* As used herein, “expression” of a nucleic acid sequence refers to one or more of the following events: (1) production of an RNA template from a DNA sequence (e.g., by transcription); (2) processing of an RNA transcript (e.g., by splicing, editing, 5' cap formation, and/or 3' end processing); (3) translation of an RNA into a polypeptide or protein; (4) folding of a polypeptide or protein; and (5) post-translational modification of a polypeptide or protein.

[0054] *Feature:* As used herein, a “feature” refers to a characteristic, a property, or a distinctive element.

[0055] *Formulation:* As used herein, a “formulation” refers to a material or mixture prepared according to a formula and which may include at least one antibody, compound, substance, entity, moiety, cargo or payload and a delivery agent, carrier or excipient.

[0056] *Functional:* As used herein, a “functional” biological molecule is a biological entity with a structure and in a form in which it exhibits a property and/or activity by which it is characterized. As used herein, a “functional group” or “chemical group” refers to a characteristic group of atoms or chemical bonds that are part of a larger molecule. In some embodiments, functional groups may be associated with different molecules, but may participate in similar chemical reactions regardless of the molecule of which they are a part. Common functional groups include, but are not limited to carboxyl groups (-COOH), acetyl groups (-COH), amino groups (-NH₂), methyl groups (-CH₃), sulfate groups (-SO₃H) and acyl groups. In some embodiments, the addition of one or more functional group to a molecule may be conveyed using terms that modify the name of the functional group with the ending “-ylated”, e.g., acetylated, methylated and sulfated.

[0057] *Glycan:* As used herein, the terms “glycan”, “oligosaccharide” and “polysaccharide” are used interchangeably and refer to polymers made up of sugar monomers, typically joined by glycosidic bonds also referred to herein as linkages. In some embodiments, the terms “glycan”, “oligosaccharide” and “polysaccharide” may be used to refer to the carbohydrate portion of a glycoconjugate (e.g., glycoprotein, glycolipid or proteoglycan).

[0058] *Glycan chain*: As used herein, the term “glycan chain” refers to a sugar polymer that includes two or more sugars. In some embodiments, glycan chains are covalently linked to proteins through serine or threonine residues on the protein.

[0059] *Glycan-rich composition*: As used herein, the term “glycan-rich composition” refers to a mixture that includes a large percentage of glycans. In some embodiments, glycans within a glycan-rich composition may make up from about 1% to about 10%, from about 5% to about 15%, from about 20% to about 40%, from about 30% to about 50%, from about 60% to about 80%, from about 70% to about 90% or at least 100% of the total weight of the composition.

[0060] *Glycosidic bond*: As used herein, the term “glycosidic bond” refers to a covalent bond formed between a carbohydrate and another chemical group. In some embodiments, glycosidic bonds are formed between the reducing end of one sugar molecule and the non-reducing end of a second sugar molecule or polysaccharide chain. Such glycosidic bonds are also known as O-glycosidic bonds due to the oxygen (or ether bond) between the joined sugars. In some embodiments, a glycosidic bond between two sugars or between a sugar and a linker may also be referred to as a “linkage”.

[0061] *In vitro*: As used herein, the term “*in vitro*” refers to events that occur in an artificial environment, *e.g.*, in a test tube or reaction vessel, in cell culture, in a Petri dish, *etc.*, rather than within an organism (*e.g.*, animal, plant, or microbe).

[0062] *In vivo*: As used herein, the term “*in vivo*” refers to events that occur within an organism (*e.g.*, animal, plant, or microbe or cell or tissue thereof).

[0063] *Isolated*: As used herein, the term “isolated” is synonymous with “separated”, but carries with it the inference separation was carried out by the hand of man. In one embodiment, an isolated substance or entity is one that has been separated from at least some of the components with which it was previously associated (whether in nature or in an experimental setting). Isolated substances may have varying levels of purity in reference to the substances from which they have been associated. Isolated substances and/or entities may be separated from at least about 10%, about 20%, about 30%, about 40%, about 50%, about 60%, about 70%, about 80%, about 90%, or more of the other components with which they were initially associated. In some embodiments, isolated agents are more than about 80%, about 85%, about 90%, about 91%, about 92%, about 93%, about 94%, about 95%, about 96%, about 97%, about 98%, about 99%, or more than about 99% pure. As used herein, a substance is “pure” if it is substantially free of other components.

[0064] *Kit*: As used herein, the term “kit” refers to a set that includes one or more components adapted for a cooperative purpose and instructions for use thereof.

[0065] *Knockout*: As used herein, the term “knockout” refers to an organism wherein an existing gene has been inactivated through a process that typically involves the hand of man. In a knockout organism, a gene that has been inactivated is said to have been “knocked out”. In some embodiments, the knocked out gene may be inactivated through the insertion of a nucleotide sequence into the gene or through replacement of the gene entirely.

[0066] *Linker*: As used herein, a “linker” refers to a moiety that connects two or more domains, moieties or entities. In one embodiment, a linker may include 10, 11, 12, 13, 14, 15 or more atoms. In a further embodiment, a linker may include a group of atoms, e.g., 10-1,000 atoms. Such atoms or groups thereof may include, but are not limited to, carbon, amino, alkylamino, oxygen, sulfur, sulfoxide, sulfonyl, carbonyl, and imine. In some embodiments, the linker may include an amino acid, peptide, polypeptide or protein. In some embodiments, a moiety bound by a linker may include, but is not limited to an atom, a chemical group, a nucleoside, a nucleotide, a nucleobase, a sugar, a nucleic acid, an amino acid, a peptide, a polypeptide, a protein, a protein complex, a payload (e.g., a therapeutic agent) or a marker (including, but not limited to a chemical, fluorescent, radioactive or bioluminescent marker). The linker can be used for any useful purpose, such as to form multimers or conjugates, as well as to administer a payload, as described herein. Examples of chemical groups that can be incorporated into the linker include, but are not limited to, alkyl, alkenyl, alkynyl, amido, amino, ether, thioether, ester, alkylene, heteroalkylene, aryl, or heterocyclyl, each of which can be optionally substituted, as described herein. Examples of linkers include, but are not limited to, unsaturated alkanes, polyethylene glycols (e.g., ethylene or propylene glycol monomeric units, e.g., diethylene glycol, dipropylene glycol, triethylene glycol, tripropylene glycol, tetraethylene glycol, or tetraethylene glycol), and dextran polymers. Other examples include, but are not limited to, cleavable moieties within the linker, such as, for example, a disulfide bond (-S-S-) or an azo bond (-N=N-), which can be cleaved using a reducing agent or photolysis. Non-limiting examples of a selectively cleavable bonds include an amido bond which may be cleaved for example by the use of tris(2-carboxyethyl)phosphine (TCEP), or other reducing agents, and/or photolysis, as well as an ester bond which may be cleaved for example by acidic or basic hydrolysis. In some embodiments, a linker is a carbohydrate moiety used to link glycans to a substrate, such as in a glycan array. Such carbohydrate linkers include, but are not limited to $-\text{O}(\text{CH}_2)_2\text{CH}_2\text{NH}_2$ and $-\text{O}(\text{CH}_2)_3\text{NHCOCH}_2(\text{OCH}_2\text{CH}_2)_6\text{NH}_2$.

[0067] *mRNA*: As used herein, the term “mRNA” refers to messenger RNA produced as a result of gene transcription and processing of the generated transcript. In some embodiments, mRNA that has left the nucleus of the cell may be extracted from a cell or set of cells and analyzed to determine which genes have undergone transcription at a given time or under a given set of circumstances.

[0068] *Mucin*: As used herein, the term “mucin” refers to a family of proteins that are heavily glycosylated. In some embodiments mucins are produced by the submaxillary glands and are found in saliva and mucous.

[0069] *Negative selection*: As used herein, the term “negative selection” refers to the selection of library members from a display library based on their ability to bind entities and/or components of a composition that do not include a target antigen. In some embodiments, negative selection is used prior to positive selection to remove elements that might bind non-specifically to the target.

[0070] *Off-target*: As used herein, “off target” refers to any unintended effect on any one or more target, gene, or cellular transcript.

[0071] *Patient*: As used herein, “patient” refers to a subject who may seek or be in need of treatment, requires treatment, is receiving treatment, will receive treatment, or a subject who is under care by a trained (e.g., licensed) professional for a particular disease or condition.

[0072] *Peptide*: As used herein, “peptide” is a protein or polypeptide which is less than or equal to 50 amino acids long, e.g., about 5, 10, 15, 20, 25, 30, 35, 40, 45, or 50 amino acids long.

[0073] *Pharmaceutically acceptable*: The phrase “pharmaceutically acceptable” is employed herein to refer to those compounds, materials, compositions, and/or dosage forms which are, within the scope of sound medical judgment, suitable for use in contact with the tissues of human beings and animals without excessive toxicity, irritation, allergic response, or other problem or complication, commensurate with a reasonable benefit/risk ratio.

[0074] *Pharmaceutically acceptable excipients*: The phrase “pharmaceutically acceptable excipient,” as used herein, refers any ingredient other than active agents (e.g., as described herein) present in a pharmaceutical composition and having the properties of being substantially nontoxic and non-inflammatory in a patient. In some embodiments, a pharmaceutically acceptable excipient is a vehicle capable of suspending or dissolving the active agent. Excipients may include, for example: antiadherents, antioxidants, binders, coatings, compression aids, disintegrants, dyes (colors), emollients, emulsifiers, fillers (diluents), film formers or coatings, flavors, fragrances, glidants (flow enhancers), lubricants,

preservatives, printing inks, sorbents, suspending or dispersing agents, sweeteners, and waters of hydration. Exemplary excipients include, but are not limited to: butylated hydroxytoluene (BHT), calcium carbonate, calcium phosphate (dibasic), calcium stearate, croscarmellose, crosslinked polyvinyl pyrrolidone, citric acid, crospovidone, cysteine, ethylcellulose, gelatin, hydroxypropyl cellulose, hydroxypropyl methylcellulose, lactose, magnesium stearate, maltitol, mannitol, methionine, methylcellulose, methyl paraben, microcrystalline cellulose, polyethylene glycol, polyvinyl pyrrolidone, povidone, pregelatinized starch, propyl paraben, retinyl palmitate, shellac, silicon dioxide, sodium carboxymethyl cellulose, sodium citrate, sodium starch glycolate, sorbitol, starch (corn), stearic acid, sucrose, talc, titanium dioxide, vitamin A, vitamin E, vitamin C, and xylitol.

[0075] *Pharmaceutically acceptable salts:* Pharmaceutically acceptable salts of the compounds described herein are forms of the disclosed compounds wherein the acid or base moiety is in its salt form (e.g., as generated by reacting a free base group with a suitable organic acid). Examples of pharmaceutically acceptable salts include, but are not limited to, mineral or organic acid salts of basic residues such as amines; alkali or organic salts of acidic residues such as carboxylic acids; and the like. Representative acid addition salts include acetate, adipate, alginate, ascorbate, aspartate, benzenesulfonate, benzoate, bisulfate, borate, butyrate, camphorate, camphorsulfonate, citrate, cyclopentanepropionate, digluconate, dodecylsulfate, ethanesulfonate, fumarate, glucoheptonate, glycerophosphate, hemisulfate, heptonate, hexanoate, hydrobromide, hydrochloride, hydroiodide, 2-hydroxy-ethanesulfonate, lactobionate, lactate, laurate, lauryl sulfate, malate, maleate, malonate, methanesulfonate, 2-naphthalenesulfonate, nicotinate, nitrate, oleate, oxalate, palmitate, pamoate, pectinate, persulfate, 3-phenylpropionate, phosphate, picrate, pivalate, propionate, stearate, succinate, sulfate, tartrate, thiocyanate, toluenesulfonate, undecanoate, valerate salts, and the like. Representative alkali or alkaline earth metal salts include sodium, lithium, potassium, calcium, magnesium, and the like, as well as nontoxic ammonium, quaternary ammonium, and amine cations, including, but not limited to ammonium, tetramethylammonium, tetraethylammonium, methylamine, dimethylamine, trimethylamine, triethylamine, ethylamine, and the like. Pharmaceutically acceptable salts include the conventional nontoxic salts, for example, from non-toxic inorganic or organic acids. In some embodiments a pharmaceutically acceptable salt is prepared from a parent compound which contains a basic or acidic moiety by conventional chemical methods. Generally, such salts can be prepared by reacting the free acid or base forms of these compounds with a stoichiometric amount of the appropriate base or acid in water or in an organic solvent, or in a mixture of the two;

generally, nonaqueous media like ether, ethyl acetate, ethanol, isopropanol, or acetonitrile are preferred. Lists of suitable salts are found in *Remington's Pharmaceutical Sciences*, 17th ed., Mack Publishing Company, Easton, Pa., 1985, p. 1418, *Pharmaceutical Salts: Properties, Selection, and Use*, P.H. Stahl and C.G. Wermuth (eds.), Wiley-VCH, 2008, and Berge et al., *Journal of Pharmaceutical Science*, 66, 1-19 (1977), each of which is incorporated herein by reference in its entirety. *Pharmaceutically acceptable solvate*: The term "pharmaceutically acceptable solvate," as used herein, refers to a crystalline form of a compound wherein molecules of a suitable solvent are incorporated in the crystal lattice. For example, solvates may be prepared by crystallization, recrystallization, or precipitation from a solution that includes organic solvents, water, or a mixture thereof. Examples of suitable solvents are ethanol, water (for example, mono-, di-, and tri-hydrates), *N*-methylpyrrolidinone (NMP), dimethyl sulfoxide (DMSO), *N,N'*-dimethylformamide (DMF), *N,N'*-dimethylacetamide (DMAC), 1,3-dimethyl-2-imidazolidinone (DMEU), 1,3-dimethyl-3,4,5,6-tetrahydro-2-(1H)-pyrimidinone (DMPU), acetonitrile (ACN), propylene glycol, ethyl acetate, benzyl alcohol, 2-pyrrolidone, benzyl benzoate, and the like. When water is the solvent, the solvate is referred to as a "hydrate." In some embodiments, the solvent incorporated into a solvate is of a type or at a level that is physiologically tolerable to an organism to which the solvate is administered (e.g., in a unit dosage form of a pharmaceutical composition).

[0076] *Pharmacokinetic*: As used herein, "pharmacokinetic" refers to any one or more properties of a molecule or compound as it relates to the determination of the fate of substances administered to a living organism. Pharmacokinetics is divided into several areas including the extent and rate of absorption, distribution, metabolism and excretion. This is commonly referred to as ADME where: (A) Absorption is the process of a substance entering the blood circulation; (D) Distribution is the dispersion or dissemination of substances throughout the fluids and tissues of the body; (M) Metabolism (or Biotransformation) is the irreversible transformation of parent compounds into daughter metabolites; and (E) Excretion (or Elimination) refers to the elimination of the substances from the body. In rare cases, some drugs irreversibly accumulate in body tissue.

[0077] *Physicochemical*: As used herein, "physicochemical" means of or relating to a physical and/or chemical property.

[0078] *Positive selection*: As used herein, the term "positive selection" refers to the selection of a given entity from a group of unique entities. Such entities and groups thereof may be, for example antibodies. In some cases they may be antibody fragments or antibody fragments expressed in association with an agent capable of expressing such fragments (e.g.

library members from a display library). Selection may be based on the ability of selected entities to bind to a desired target or epitope. In some embodiments, positive selection may be used with phage display libraries to identify phage particles expressing scFvs that bind to the desired target. In other embodiments, positive selection may refer to the selection of antibody candidates from among a pool of antibodies. In other cases, entities may be cells, cell lines or clones as in the selection of clones during hybridoma selection. In such cases, positive selection may refer to clonal selection based on one or more features of antibodies (e.g. specificity for one or more desired epitopes) produced by such clones. In some cases, desired epitopes in positive selection methods may include STn (e.g. AcSTn and/or GcSTn).

[0079] Conversely, “negative selection,” as used herein, included the same principles and examples described for positive selection, but with the distinguishing characteristic that it is used for *removal* of undesired entities from a group of unique entities.

[0080] *Preventing*: As used herein, the term “preventing” refers to partially or completely delaying onset of an infection, disease, disorder and/or condition; partially or completely delaying onset of one or more symptoms, features, or clinical manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delaying onset of one or more symptoms, features, or manifestations of a particular infection, disease, disorder, and/or condition; partially or completely delaying progression from an infection, a particular disease, disorder and/or condition; and/or decreasing the risk of developing pathology associated with the infection, the disease, disorder, and/or condition.

[0081] *Prodrug*: The present disclosure also includes prodrugs of the compounds described herein. As used herein, “prodrugs” refer to any substance, molecule or entity which is in a form predicate for that substance, molecule or entity to act as a therapeutic upon chemical or physical alteration. Prodrugs may be covalently bonded or sequestered in some way and which release or are converted into the active drug moiety prior to, upon or after administered to a mammalian subject. Prodrugs can be prepared by modifying functional groups present in the compounds in such a way that the modifications are cleaved, either in routine manipulation or *in vivo*, to the parent compounds. Prodrugs include compounds wherein hydroxyl, amino, sulfhydryl, or carboxyl groups are bonded to any group that, when administered to a mammalian subject, cleaves to form a free hydroxyl, amino, sulfhydryl, or carboxyl group respectively. Preparation and use of prodrugs is discussed in T. Higuchi and V. Stella, “Pro-drugs as Novel Delivery Systems,” Vol. 14 of the A.C.S. Symposium Series, and in *Bioreversible Carriers in Drug Design*, ed. Edward B. Roche, American

Pharmaceutical Association and Pergamon Press, 1987, both of which are hereby incorporated by reference in their entirety.

[0082] *Proximal*: As used herein, the term “proximal” means situated nearer to the center or to a point or region of interest.

[0083] *Region of interaction*: As used herein, the term “region of interaction” refers to a region along any of two or more entities where such entities interact or overlap. In some embodiments, a region of interaction may include one or more sugar residues along a glycan chain that contacts a second glycan chain. In some embodiments, the glycan chains are branching chains from the same parent chain. In some embodiments, a region of interaction may occur between two glycan chains wherein one chain is a branching chain and the second chain is a parent chain. In the case of glycan chains, regions of interaction may include 1, 2, 3, 4, 5, 6, 7, 8, 9 or at least 10 sugar residues. In some embodiments, regions of interaction may also occur between glycans and proteins or between glycans and lipids.

[0084] *Residue*: As used herein, the term “residue” refers to a monomer associated with or capable of associating with a polymer. In some embodiments, residues include sugar molecules including, but not limited to glucose, galactose, N-acetylglucosamine, N-acetylgalactosamine, sialic acids. In some embodiments, residues include amino acids.

[0085] *Sample*: As used herein, the term “sample” refers to an aliquot or portion taken from a source and/or provided for analysis or processing. In some embodiments, a sample is from a biological source (also referred to herein as a “biological sample”) such as a tissue, cell or component part (e.g. a body fluid, including but not limited to blood, plasma, serum, mucus, lymphatic fluid, synovial fluid, cerebrospinal fluid, saliva, amniotic fluid, amniotic cord blood, urine, vaginal fluid and semen). In some embodiments, a sample may be or include a homogenate, lysate or extract prepared from a whole organism or a subset of its tissues, cells or component parts, or a fraction or portion thereof, including but not limited to, for example, plasma, serum, spinal fluid, lymph fluid, the external sections of the skin, respiratory, intestinal, and genitourinary tracts, tears, saliva, milk, blood cells, tumors, organs. In some embodiments, a sample includes a medium, such as a nutrient broth or gel, which may contain cellular components, such as proteins or nucleic acid molecule. In some embodiments, a “primary” sample is an aliquot of the source. In some embodiments, a primary sample is subjected to one or more processing (e.g., separation, purification, etc.) steps to prepare a sample for analysis or other use.

[0086] *Sialyl*: As used herein, the prefix “sialyl” as well as the term “sialylated” describe compounds including sialic acid.

[0087] *Single unit dose*: As used herein, a “single unit dose” is a dose of any therapeutic administered in one dose/at one time/single route/single point of contact, i.e., single administration event. In some embodiments, a single unit dose is provided as a discrete dosage form (e.g., a tablet, capsule, patch, loaded syringe, vial, etc).

[0088] *Split dose*: As used herein, a “split dose” is the division of single unit dose or total daily dose into two or more doses.

[0089] *Stable*: As used herein “stable” refers to a compound or entity that is sufficiently robust to survive isolation to a useful degree of purity from a reaction mixture, and preferably capable of formulation into an efficacious therapeutic agent.

[0090] *Stabilized*: As used herein, the term “stabilize”, “stabilized,” “stabilized region” means to make or become stable. In some embodiments, stability is measured relative to an absolute value. In some embodiments, stability is measured relative to a reference compound or entity.

[0091] *Standard of care*: As used herein, the phrase “standard of care” refers to methods of therapeutic treatment that align with methods practiced by a majority of those skilled in providing such treatment.

[0092] *Subject*: As used herein, the term “subject” or “patient” refers to any organism to which a composition in accordance with the invention may be administered, e.g., for experimental, diagnostic, prophylactic, and/or therapeutic purposes. Typical subjects include animals (e.g., mammals such as mice, rats, rabbits, non-human primates, and humans) and/or plants.

[0093] *Submaxillary glands*: As used herein, the term “submaxillary glands” or “submandibular glands” refers to mucous producing glands located beneath the mouth floor. These glands are capable of producing mucins and in some embodiments, may be extracted from mammals as a source of mucin.

[0094] *Suffering from*: An individual who is “suffering from” a disease, disorder, and/or condition has been diagnosed with or displays one or more symptoms of a disease, disorder, and/or condition.

[0095] *Susceptible to*: An individual who is “susceptible to” a disease, disorder, and/or condition has not been diagnosed with and/or may not exhibit symptoms of the disease, disorder, and/or condition but harbors a propensity to develop a disease or its symptoms. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition (for example, cancer) may be characterized by one or more of the following: (1) a genetic mutation associated with development of the disease, disorder, and/or condition; (2) a genetic

polymorphism associated with development of the disease, disorder, and/or condition; (3) increased and/or decreased expression and/or activity of a protein and/or nucleic acid associated with the disease, disorder, and/or condition; (4) habits and/or lifestyles associated with development of the disease, disorder, and/or condition; (5) a family history of the disease, disorder, and/or condition; and (6) exposure to and/or infection with a microbe associated with development of the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will develop the disease, disorder, and/or condition. In some embodiments, an individual who is susceptible to a disease, disorder, and/or condition will not develop the disease, disorder, and/or condition.

[0096] *Synthetic*: The term “synthetic” means produced, prepared, and/or manufactured by the hand of man. Synthesis of polynucleotides or polypeptides or other molecules of the present invention may be chemical or enzymatic.

[0097] *Target*: As used herein, the term “target” refers to an object or entity to be affected by an action. In some embodiments, targets refer to antigens to be used for the development of antibodies that specifically bind the antigens.

[0098] *Target screening*: As used herein, the term “target screening” refers to the use of a target substance to identify binding partners for that substance.

[0099] *Target site*: As used herein, the term “target site” refers to a region on or within one or more glycans, glycoproteins, biomolecules and/or biostructures on or within a cell, the extracellular space, a tissue, an organ and/or an organism that is recognized by a binding agent or effector molecule (e.g., an antibody). In some embodiments, glycan target sites may reside exclusively on one sugar residue, may be formed by two or more residues, or may include both glycan and non-glycan components. In some embodiments, target sites are formed between two or more glycans or glycoproteins. In some embodiments, target sites are formed between branching chains of the same glycan or between one or more branching chains and a parent chain.

[00100] *Targeted Cells*: As used herein, “targeted cells” refers to any of one or more cells of interest. The cells may be found *in vitro*, *in vivo*, *in situ* or in the tissue or organ of any organism. The organism may be an animal, a mammal, or a human (e.g., a human patient).

[00101] *Terminal residue*: As used herein, the term “terminal residue” refers to the last residue in a polymeric chain. In some embodiments, terminal residues are sugar residues located at the non-reducing end of a polysaccharide chain.

[00102] *Therapeutic*: As used herein, the term “therapeutic” refers to any substance or procedure that relates to healing of a disease, disorder, or condition. For example, therapeutic treatment refers to any treatment that relates to healing of a disease, disorder, or condition.

[00103] *Therapeutic agent*: The term “therapeutic agent” refers to any substance that, when administered to a subject, has a therapeutic, diagnostic, and/or prophylactic effect and/or elicits a desired biological and/or pharmacological effect.

[00104] *Therapeutically effective amount*: As used herein, the term “therapeutically effective amount” means an amount of an agent to be delivered (*e.g.*, nucleic acid, drug, therapeutic agent, diagnostic agent, prophylactic agent, *etc.*) that is sufficient, when administered to a subject suffering from or susceptible to an infection, disease, disorder, and/or condition, to treat, improve symptoms of, diagnose, prevent, and/or delay the onset of the infection, disease, disorder, and/or condition. In some embodiments, a therapeutically effective amount is provided in a single dose. In some embodiments, a therapeutically effective amount is administered in a dosage regimen that includes a plurality of doses. Those skilled in the art will appreciate that in some embodiments, a unit dosage form may be considered to include a therapeutically effective amount of a particular agent or entity if it includes an amount that is effective when administered as part of such a dosage regimen.

[00105] *Therapeutically effective outcome*: As used herein, the term “therapeutically effective outcome” means an outcome that is sufficient in a subject suffering from or susceptible to an infection, disease, disorder, and/or condition, to treat, improve symptoms of, diagnose, prevent, and/or delay the onset of the infection, disease, disorder, and/or condition.

[00106] *Total daily dose*: As used herein, a “total daily dose” is an amount given or prescribed in 24 hr period. It may be administered as a single unit dose.

[00107] *Transgenic*: As used herein, the term “transgenic” refers to an organism that includes one or more genes incorporated within the organisms genome that are not naturally found in that organism.

[00108] *Treating*: As used herein, the term “treating” refers to partially or completely alleviating, healing, ameliorating, improving, relieving, delaying onset of, inhibiting progression of, reducing severity of, and/or reducing incidence of one or more symptoms or features of a particular infection, disease, disorder, and/or condition. For example, “treating” cancer may refer to inhibiting survival, growth, and/or spread of a tumor. Treatment may be administered to a subject who does not exhibit signs of a disease, disorder, and/or condition and/or to a subject who exhibits only early signs of a disease, disorder, and/or condition for

the purpose of decreasing the risk of developing pathology associated with the disease, disorder, and/or condition.

[00109] *Variable region*: As used herein, the term “variable region” or “variable domain” refers to specific antibody domains that differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen.

[00110] *Whole IgG*: As used herein, the term “whole IgG” refers to a complete IgG molecule. In some embodiments, whole IgG molecules include regions found naturally in two or more other organisms.

[00111] *Wild type*: As used herein, the term “wild type” refers to an organism that includes a natural genome (free from genes derived from other organisms).

I. Compounds and compositions

[00112] In some embodiments, the present invention provides compounds as well as compositions that include at least one glycan-interacting antibody. Within a glycan, monosaccharide monomers may all be the same or they may differ. Common monomers include, but are not limited to trioses, tetroses, pentoses, glucose, fructose, galactose, xylose, arabinose, lyxose, allose, altrose, mannose, gulose, iodose, ribose, mannoheptulose, sedoheptulose and talose. Amino sugars may also be monomers within a glycan. Glycans including such sugars are herein referred to as aminoglycans. Amino sugars, as used herein, are sugar molecules that include an amine group in place of a hydroxyl group, or in some embodiments, a sugar derived from such a sugar. Examples of amino sugars include, but are not limited to glucosamine, galactosamine, N-acetylglucosamine, N-acetylgalactosamine, sialic acids (including, but not limited to, N-acetylneuraminic acid and N-glycolylneuraminic acid) and L-daunosamine.

[00113] As used herein the term “glycan-interacting antibody” refers to an antibody that can interact with a glycan moiety. Such antibodies may bind to a glycan moiety alone, to multiple glycan moieties, or to epitopes that include both glycan and non-glycan components. Non-glycan components may include, but are not limited to proteins, protein-associated moieties (such post-translational modifications), cells, and cell-associated molecules/structures. Glycan-interacting antibodies may function to bind to, alter, activate, inhibit, stabilize, degrade and/or modulate a glycan or a glycan-associated molecule or entity. In so doing, glycan-interacting antibodies may function as a therapeutic, whether palliative, prophylactic or as an ongoing treatment composition. In some embodiments, glycan-interacting antibodies may include conjugates or combinations with other molecules. In some

embodiments, glycan-interacting antibodies are directed toward glycans having one or more amino sugar. In a further embodiment, one or more amino sugars is a sialic acid. In a further embodiment, one or more sialic acids is N-acetylneuraminic acid and/or N-glycolylneuraminic acid.

Antibodies

[00114] Glycan-interacting antibodies may include entire antibodies or fragments thereof. As used herein, the term “antibody” is used in the broadest sense and embraces various formats including, but not limited to monoclonal antibodies, polyclonal antibodies, multispecific antibodies (e.g., bispecific antibodies formed from at least two intact antibodies), antibody conjugates (including, but not limited to antibody-drug conjugates), antibody variants [including, but not limited to antibody mimetics, chimeric antibodies (e.g. antibodies with amino acid sequences derived from more than one species), and synthetic variants], and antibody fragments, so long as they exhibit a desired biological activity (e.g., binding, activating, inhibiting, stabilizing, degrading, and/or modulating one or more targets). Antibodies are primarily amino-acid based molecules but may include one or more post-translational or synthetic modifications. Post-translational modifications may include glycosylation.

[00115] As used herein, the term “antibody fragment” refers to a portion of an intact antibody or fusion-protein thereof, in some cases including at least one antigen binding region. Examples of antibody fragments include Fab, Fab', F(ab')₂, Fv fragments, single-chain variable fragments (scFvs); diabodies; tri(a)bodies; linear antibodies; single-chain antibody molecules; and multispecific antibodies formed from antibody fragments. Papain digestion of antibodies produces two identical antigen-binding fragments, called "Fab" fragments, each with a single antigen-binding site. Also produced is a residual "Fc" fragment, whose name reflects its ability to crystallize readily. Pepsin treatment yields an F(ab')₂ fragment that has two antigen-binding sites and is still capable of cross-linking antigen. Glycan-interacting antibodies may include one or more of these fragments and may, for example, be generated through enzymatic digestion of whole antibodies or through recombinant expression.

[00116] "Native antibodies" are usually heterotetrameric glycoproteins of about 150,000 daltons, composed of two identical light (L) chains and two identical heavy (H) chains. Genes encoding antibody heavy and light chains are known and segments making up each have been well characterized and described (Matsuda, F. et al., 1998. The Journal of Experimental Medicine. 188(11); 2151-62 and Li, A. et al., 2004. Blood. 103(12): 4602-9, the content of

each of which are herein incorporated by reference in their entirety). Each light chain is linked to a heavy chain by one covalent disulfide bond, while the number of disulfide linkages varies among the heavy chains of different immunoglobulin isotypes. Each heavy and light chain also has regularly spaced intrachain disulfide bridges. Each heavy chain has at one end a variable domain (V_H) followed by a number of constant domains. Each light chain has a variable domain at one end (V_L) and a constant domain at its other end; the constant domain of the light chain is aligned with the first constant domain of the heavy chain, and the light chain variable domain is aligned with the variable domain of the heavy chain.

[00117] As used herein, the term "variable domain" refers to specific antibody domains found on both the antibody heavy and light chains that differ extensively in sequence among antibodies and are used in the binding and specificity of each particular antibody for its particular antigen. Variable domains include hypervariable regions. As used herein, the term "hypervariable region" refers to a region within a variable domain that includes amino acid residues responsible for antigen binding. The amino acids present within the hypervariable regions determine the structure of the complementarity determining regions (CDRs) that become part of the antigen-binding site of the antibody. As used herein, the term "CDR" refers to a region of an antibody that includes a structure that is complimentary to its target antigen or epitope. Other portions of the variable domain, not interacting with the antigen, are referred to as framework (FW) regions. The antigen-binding site (also known as the antigen combining site or paratope) includes the amino acid residues necessary to interact with a particular antigen. The exact residues making up the antigen-binding site are typically elucidated by co-crystallography with bound antigen, however computational assessments can also be used based on comparisons with other antibodies (Strohl, W.R. *Therapeutic Antibody Engineering*. Woodhead Publishing, Philadelphia PA. 2012. Ch. 3, p47-54, the contents of which are herein incorporated by reference in their entirety). Determining residues making up CDRs may include the use of numbering schemes including, but not limited to, those taught by Kabat [Wu, T.T. et al., 1970, JEM, 132(2):211-50 and Johnson, G. et al., 2000, Nucleic Acids Res. 28(1): 214-8, the contents of each of which are herein incorporated by reference in their entirety], Chothia [Chothia and Lesk, J. Mol. Biol. 196, 901 (1987), Chothia et al., Nature 342, 877 (1989) and Al-Lazikani, B. et al., 1997, J. Mol. Biol. 273(4):927-48, the contents of each of which are herein incorporated by reference in their entirety], Lefranc (Lefranc, M.P. et al., 2005, Immunome Res. 1:3) and Honegger (Honegger, A. and Pluckthun, A. 2001. J. Mol. Biol. 309(3):657-70, the contents of which are herein incorporated by reference in their entirety).

[00118] VH and VL domains have three CDRs each. VL CDRs are referred to herein as CDR-L1, CDR-L2 and CDR-L3, in order of occurrence when moving from N- to C- terminus along the variable domain polypeptide. VH CDRs are referred to herein as CDR-H1, CDR-H2 and CDR-H3, in order of occurrence when moving from N- to C- terminus along the variable domain polypeptide. Each of CDRs have favored canonical structures with the exception of the CDR-H3, which includes amino acid sequences that may be highly variable in sequence and length between antibodies resulting in a variety of three-dimensional structures in antigen-binding domains (Nikoloudis, D. et al., 2014. PeerJ. 2:e456). In some cases, CDR-H3s may be analyzed among a panel of related antibodies to assess antibody diversity. Various methods of determining CDR sequences are known in the art and may be applied to known antibody sequences (Strohl, W.R. Therapeutic Antibody Engineering. Woodhead Publishing, Philadelphia PA. 2012. Ch. 3, p47-54, the contents of which are herein incorporated by reference in their entirety).

[00119] As used herein, the term "Fv" refers to an antibody fragment that includes the minimum fragment on an antibody needed to form a complete antigen-binding site. These regions consist of a dimer of one heavy chain and one light chain variable domain in tight, non-covalent association. Fv fragments can be generated by proteolytic cleavage, but are largely unstable. Recombinant methods are known in the art for generating stable Fv fragments, typically through insertion of a flexible linker between the light chain variable domain and the heavy chain variable domain [to form a single chain Fv (scFv)] or through the introduction of a disulfide bridge between heavy and light chain variable domains (Strohl, W.R. Therapeutic Antibody Engineering. Woodhead Publishing, Philadelphia PA. 2012. Ch. 3, p46-47, the contents of which are herein incorporated by reference in their entirety).

[00120] Antibody "light chains" from any vertebrate species can be assigned to one of two clearly distinct types, called kappa and lambda based on amino acid sequences of their constant domains. Depending on the amino acid sequence of the constant domain of their heavy chains, antibodies can be assigned to different classes. There are five major classes of intact antibodies: IgA, IgD, IgE, IgG, and IgM, and several of these may be further divided into subclasses (isotypes), e.g., IgG1, IgG2a, IgG2b, IgG2c, IgG3, IgG4, IgA, and IgA2.

[00121] As used herein, the term "single chain Fv" or "scFv" refers to a fusion protein of VH and VL antibody domains, wherein these domains are linked together into a single polypeptide chain by a flexible peptide linker. In some embodiments, the Fv polypeptide linker enables the scFv to form the desired structure for antigen binding. In some embodiments, scFvs are utilized in conjunction with phage display, yeast display or other

display methods where they may be expressed in association with a surface member (e.g. phage coat protein) and used in the identification of high affinity peptides for a given antigen.

[00122] The term "diabodies" refers to small antibody fragments with two antigen-binding sites, which fragments include a heavy chain variable domain V_H connected to a light chain variable domain V_L in the same polypeptide chain. By using a linker that is too short to allow pairing between the two domains on the same chain, the domains are forced to pair with the complementary domains of another chain and create two antigen-binding sites. Diabodies are described more fully in, for example, EP 404,097; WO 93/11161; and Hollinger et al., Proc. Natl. Acad. Sci. USA, 90:6444-6448 (1993), the contents of each of which are incorporated herein by reference in their entirety.

[00123] The term "intrabody" refers to a form of antibody that is not secreted from a cell in which it is produced, but instead target one or more intracellular protein. Intrabodies may be used to affect a multitude of cellular processes including, but not limited to intracellular trafficking, transcription, translation, metabolic processes, proliferative signaling and cell division. In some embodiments, methods of the present invention may include intrabody-based therapies. In some such embodiments, variable domain sequences and/or CDR sequences disclosed herein may be incorporated into one or more construct for intrabody-based therapy. In some cases, intrabodies of the invention may target one or more glycosylated intracellular protein or may modulate the interaction between one or more glycosylated intracellular protein and an alternative protein.

[00124] The term "chimeric antigen receptor" or "CAR" as used herein, refers to artificial receptors that are engineered to be expressed on the surface of immune effector cells resulting in specific targeting of such immune effector cells to cells expressing entities that bind with high affinity to the artificial receptors. CARs may be designed to include one or more segments of an antibody, antibody variable domain and/or antibody CDR, such that when such CARs are expressed on immune effector cells, the immune effector cells bind and clear any cells that are recognized by the antibody portions of the CARs. In some cases, CARs are designed to specifically bind cancer cells, leading to immune-regulated clearance of the cancer cells.

[00125] The term "monoclonal antibody" as used herein refers to an antibody obtained from a population of substantially homogeneous cells (or clones), i.e., the individual antibodies making up the population are identical and/or bind the same epitope, except for possible variants that may arise during production of the monoclonal antibody, such variants generally being present in minor amounts. In contrast to polyclonal antibody preparations that

typically include different antibodies directed against different determinants (epitopes), each monoclonal antibody is directed against a single determinant on the antigen

[00126] The modifier "monoclonal" indicates the character of the antibody as being obtained from a substantially homogeneous population of antibodies, and is not to be construed as requiring production of the antibody by any particular method. The monoclonal antibodies herein include "chimeric" antibodies (immunoglobulins) in which a portion of the heavy and/or light chain is identical with or homologous to corresponding sequences in antibodies derived from a particular species or belonging to a particular antibody class or subclass, while the remainder of the chain(s) is identical with or homologous to corresponding sequences in antibodies derived from another species or belonging to another antibody class or subclass, as well as fragments of such antibodies.

[00127] "Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequences derived from non-human immunoglobulins. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from the hypervariable region from an antibody of the recipient are replaced by residues from the hypervariable region from an antibody of a non-human species (donor antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity. Humanized antibodies may include one or more back-mutation that include the reversion of one or more amino acids back to amino acids found in a donor antibody. Conversely, residues from donor antibodies included in humanized antibodies may be mutated to match residues present in human recipient antibodies.

[00128] In some embodiments, glycan-interacting antibodies of the present invention may be antibody mimetics. The term "antibody mimetic" refers to any molecule which mimics the function or effect of an antibody and which binds specifically and with high affinity to their molecular targets. In some embodiments, antibody mimetics may be monobodies, designed to incorporate the fibronectin type III domain (Fn3) as a protein scaffold (US 6,673,901; US 6,348,584). In some embodiments, antibody mimetics may be those known in the art including, but are not limited to affibody molecules, affilins, affitins, anticalins, avimers, DARPins, Fynomers and Kunitz and domain peptides. In other embodiments, antibody mimetics may include one or more non-peptide region.

[00129] As used herein, the term "antibody variant" refers to a biomolecule resembling an antibody in structure, sequence and/or function, but including some differences in their amino acid sequence, composition or structure as compared to another antibody or a native antibody.

Antibody development

[00130] Glycan-interacting antibodies of the present invention are developed to bind antigens such as those described herein. As used herein, an “antigen” is an entity which induces or evokes an immune response in an organism. An immune response is characterized by the reaction of the cells, tissues and/or organs of an organism to the presence of a foreign entity. Such an immune response typically leads to the production by the organism of one or more antibodies against the foreign entity, e.g., antigen or a portion of the antigen. In some cases, methods of immunization may be altered based on one or more desired immunization outcomes. As used here, the term “immunization outcome” refers to one or more desired effects of immunization. Examples include high antibody titers and/or increased antibody specificity for a target of interest.

[00131] Antigens of the invention may include glycans, glycoconjugates (including, but not limited to glycoproteins and glycolipids), peptides, polypeptides, fusion proteins, or any of the foregoing and may be conjugated or complexed to one or more separate adjuvants or heterologous proteins. In some embodiments, antigens used according to methods of the present invention may include sialylated glycans, such as STn. Antigens having STn may include mucins. Mucins are a family of proteins that are heavily glycosylated. They are a component of many tumors originating from epithelial cells (Ishida, A. et al., 2008. *Proteomics*. 8: 3342-9, the contents of which are herein incorporated by reference in their entirety). They are highly expressed by submaxillary glands and can be found at high levels in saliva and mucous. Animal-derived submaxillary mucins may be used as antigens to generate anti-STn antibodies in immunogenic hosts. Submaxillary mucin from different species differ in their STn content with regard to AcSTn versus GcSTn forms. Porcine submaxillary mucin (PSM) is particularly rich in GcSTn, which makes up about 90% of total STn. STn from bovine submaxillary mucin (BSM) includes roughly equal percentages of GcSTn and AcSTn. Ovine submaxillary mucin (OSM) is particularly rich in AcSTn, which makes up about 90% of total STn. In some cases, solutions prepared for immunization may be modified to include one or more of PSM, BSM and OSM depending on the desired target of antibodies resulting from such immunization. PSM may be used in immunizations to generate antibodies in immunogenic hosts that are more likely to be specific for GcSTn. PSM is rich in Neu5Gc-containing mucin-type, glycoproteins that are decorated with GcSTn. Among the currently known sources of high Neu5Gc content is red meat; especially submaxillary glands were previously described as a rich source of Neu5Gc due to the high

expression of the CMAH enzyme, which catalyzes the reaction to produce the Neu5Gc precursor, CMP-Neu5Ac. In some cases, PSM may be used to prevent a pan-anti-Neu5Gc response and induce a more specific immune response against GcSTn. OSM may be used in immunizations to generate antibodies in immunogenic hosts that are more likely to be specific for AcSTn.

[00132] In one embodiment, the present invention provides a glycan-interacting antibody that is GcSTn-specific. The antibody has little cross-reactivity to Neu5Ac-STn or Tn. The antibody can bind GcSTn but has reduced affinity for AcSTn.

[00133] In some embodiments, antigens may be subjected to enzymatic digestion prior to immunization to modulate the resulting immune response in immunogenic hosts. In one example, submaxillary mucins may be treated with trypsin or proteinase K enzymes prior to immunization. The activity of such enzymes may help to cleave off and thereby reduce the percentage and variability of non-STn epitopes. Glycan moieties may shield regions of the peptide where they are attached from enzymatic proteolysis and thereby remain intact.

[00134] Antibody titers resulting from immunizations may have different antibody levels depending on the type and amount of antigen used in such immunizations. In some cases, certain antigens may be selected for use in immunizations based on the expected titer.

[00135] As used herein, an “adjuvant” is a pharmacological or immunological agent that modifies the effect of other agents. Adjuvants according to the present invention include, but are not limited to chemical compositions, biomolecules, therapeutics, and/or therapeutic regimens. Adjuvants may include Freund’s adjuvant (complete and/or incomplete), immunostimulatory oligonucleotides [e.g. CpG oligodeoxynucleotides (ODNs)], mineral-containing compositions, bacterial ADP-ribosylating toxins, bioadhesives, mucoadhesives, microparticles, lipids, liposomes, muramyl peptides, N-oxidized polyethylene-piperazine derivatives, saponins and/or immune stimulating complexes (ISCOs). In some embodiments, adjuvants may include oil-in-water emulsions (e.g. sub-micron oil-in-water emulsions). Adjuvants according to the present invention may also include any of those disclosed in US Patent Publication No. US20120027813 and/or US Patent No. US8506966, the contents of each of which are herein incorporated by reference in their entirety.

[00136] Antibodies of the present invention may be polyclonal or monoclonal or recombinant, produced by methods known in the art or as described in this application. In some embodiments, the antibodies of the present invention may be labeled for purposes of detection with a detectable label known by one of skill in the art. The label can be a radioisotope, fluorescent compound, chemiluminescent compound, enzyme, or enzyme co-

factor, or any other labels known in the art. In some aspects, the antibody that binds to a desired antigen is not labeled, but may be detected by binding of a labeled secondary antibody that specifically binds to the primary antibody.

[00137] Antibodies of the present invention (e.g., glycan-interacting antibodies) include, but are not limited to, polyclonal, monoclonal, multispecific, human, humanized or chimeric antibodies, single chain antibodies, Fab fragments, F(ab') fragments, fragments produced by a Fab expression library, anti-idiotypic (anti-Id) antibodies (including, e.g., anti-Id antibodies to antibodies of the invention), intracellularly made antibodies (i.e., intrabodies), and epitope-binding fragments of any of the above. Antibodies of the present invention (e.g., glycan-interacting antibodies) can be from any animal origin including birds and mammals. Preferably, such antibodies are of human, murine (e.g., mouse and rat), donkey, sheep, rabbit, goat, guinea pig, camel, horse, or chicken origin. The antibodies of the present invention can be monospecific or multispecific (e.g., bispecific, trispecific, or of greater multispecificity). Multispecific antibodies can be specific for different epitopes of a target antigen of the present invention, or can be specific for both a target antigen of the present invention, and a heterologous epitope, such as a heterologous glycan, peptide or solid support material. (See, e.g., WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, A. et al., *Trispecific F(ab')₃ derivatives that use cooperative signaling via the TCR/CD3 complex and CD2 to activate and redirect resting cytotoxic T cells*. J Immunol. 1991 Jul 1;147(1):60-9; U.S. Pat. Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; and Kostelny, S.A. et al., *Formation of a bispecific antibody by the use of leucine zippers*. J Immunol. 1992 Mar 1;148(5):1547-53).

[00138] Glycan-interacting antibodies of the present disclosure may be prepared using well-established methods known in the art for developing monoclonal antibodies. In one embodiment, the monoclonal antibodies are prepared using hybridoma technology (Kohler, G. et al., *Continuous cultures of fused cells secreting antibody of predefined specificity*. Nature. 1975 Aug 7;256(5517):495-7). For hybridoma formations, first, a mouse, hamster, or other appropriate host animal, is typically immunized with an immunizing agent (e.g., a target antigen of the invention) to elicit lymphocytes that produce or are capable of producing antibodies that will specifically bind to the immunizing agent. Alternatively, the lymphocytes may be immunized in vitro. The lymphocytes are then fused with an immortalized cell line using a suitable fusing agent, such as polyethylene glycol, to form a hybridoma cell (Goding, J.W., *Monoclonal Antibodies: Principles and Practice*. Academic Press. 1986; 59-1031). Immortalized cell lines are usually transformed mammalian cells, particularly myeloma cells

of rodent, rabbit, bovine and human origin. Usually, rat or mouse myeloma cell lines are employed. The hybridoma cells may be cultured in a suitable culture medium that preferably contains one or more substances that inhibit the growth or survival of the unfused, immortalized cells. For example, if the parental cells lack the enzyme hypoxanthine guanine phosphoribosyl transferase (HGPRT or HPRT), the culture medium for the hybridomas typically will include hypoxanthine, aminopterin, and thymidine ("HAT medium"), which substances prevent the growth of HGPRT-deficient cells.

[00139] Preferred immortalized cell lines are those that fuse efficiently, support stable high level expression of antibody by the selected antibody-producing cells, and are sensitive to a medium such as HAT medium. More preferred immortalized cell lines are murine myeloma lines, which can be obtained, for instance, from the Salk Institute Cell Distribution Center, San Diego, Calif. and the American Type Culture Collection, Manassas, Va. Human myeloma and mouse-human heteromyeloma cell lines also have been described for the production of human monoclonal antibodies (Kozbor, D. et al., *A human hybrid myeloma for production of human monoclonal antibodies*. J Immunol. 1984 Dec;133(6):3001-5; Brodeur, B. et al., *Monoclonal Antibody Production Techniques and Applications*. Marcel Dekker, Inc., New York. 1987; 33:51-63).

[00140] In some embodiments, myeloma cells may be subjected to genetic manipulation. Such manipulation may be carried out using zinc-finger nuclease (ZFN) mutagenesis as described herein. Alternatively, transfection methods known in the art may be used. NS0 myeloma cells or other mouse myeloma cell lines may be used. For example, Sp2/0-Ag14 can be an alternative cell line for hybridoma development.

[00141] Transcription Activator-Like Effector Nucleases (TALENs)-induced gene editing provides an alternative gene knock out method. TALENs are artificial restriction enzymes generated by fusing the TAL effector DNA binding domain to a DNA cleavage domain. Similar to ZFNs, TALENs induce double-strand breaks at desired loci that can be repaired by error-prone NHEJ to yield insertions/deletions at the break sites (Wood, A.J. et al., Targeted genome editing across species using ZFNs and TALENs. Science. 2011 Jul 15;333(6040):307). Collectis BioResearch (Cambridge, MA) provides the service of TALEN design and plasmid construction. The culture medium in which the hybridoma cells are cultured can then be assayed for the presence of monoclonal antibodies. Preferably, the binding specificity (i.e., specific immunoreactivity) of monoclonal antibodies produced by the hybridoma cells is determined by immunoprecipitation or by an in vitro binding assay, such as radioimmunoassay (RIA) or enzyme-linked immunosorbent assay (ELISA). Such

techniques and assays are known by those skilled in the art. The binding specificity of the monoclonal antibody can, for example, be determined by Scatchard analysis (Munson, P.J. et al., *Ligand: a versatile computerized approach for characterization of ligand-binding systems*. Anal Biochem. 1980 Sep 1;107(1):220-39). In some cases, antibody specificity for regions of a given antigen may be characterized by chemically modifying the antigens prior to assaying for antibody binding. In one example, periodate treatment may be used to destroy the C6 side chain of sialic acids. Assays may be conducted with and without periodate treatment to reveal whether or not binding in untreated samples is sialic acid-specific. In some cases, antigens having 9-O-acetylated sialic acid may be subjected to mild base treatment (e.g. with 0.1 M NaOH) to destroy 9-O-acetyl groups. Assays may be conducted with and without mild base treatment to reveal whether or not binding in untreated samples depends on 9-O-acetylation of sialic acid.

[00142] After the desired hybridoma cells are identified, the clones may be subcloned by limiting dilution procedures and grown by standard methods. Suitable culture media for this purpose include, for example, Dulbecco's Modified Eagle's Medium or RPMI-1640 medium. Alternatively, the hybridoma cells may be grown in vivo as ascites in a mammal.

[00143] Alternative methods to clone hybridomas may include those provided by kits from STEMCELL Technologies (Vancouver, BC, Canada), e.g. ClonaCell™-HY kit, containing methylcellulose-based semi-solid medium and other media and reagents, to support the selection and growth of hybridoma clones. However, the media in this kit contain FCS, which provides an exogenous source for Neu5Gc incorporation. Though the machinery for endogenous Neu5Gc synthesis is destroyed in *Cmah*^{-/-} hybridoma, Neu5Gc incorporated from the culture media may also pose a problem in some cases (Bardor, M. et al., Mechanism of uptake and incorporation of the non-human sialic acid N-glycolylneuraminic acid into human cells. J Biol Chem. 2005. 280: 4228-4237). In such instances, The culture media may be supplemented with Neu5Ac to eliminate Neu5Gc incorporation by metabolic competition (Ghaderi, D. et al., Implications of the presence of N-glycolylneuraminic acid in recombinant therapeutic glycoproteins. Nat Biotechnol. 2010. 28: 863-867).

[00144] The monoclonal antibodies secreted by the subclones may be isolated or purified from the culture medium or ascites fluid by conventional immunoglobulin purification procedures such as, for example, protein A-Sepharose, hydroxylapatite chromatography, gel electrophoresis, dialysis, or affinity chromatography.

[00145] In another embodiment, the monoclonal antibodies of the present invention can also be made by recombinant DNA methods, such as those described in U.S. Pat. No.

4,816,567, which is hereby incorporated by reference in its entirety. DNA encoding the monoclonal antibodies of the invention can be readily isolated and sequenced using conventional procedures (e.g., by using oligonucleotide probes that are capable of binding specifically to genes encoding the heavy and light chains of murine antibodies). The hybridoma cells of the invention serve as a preferred source of DNA. Once isolated, the DNA can be placed into expression vectors, which are then transfected into host cells. Host cells may include, but are not limited to HEK293 cells, HEK293T cells, simian COS cells, Chinese hamster ovary (CHO) cells, and myeloma cells that do not otherwise produce immunoglobulin protein, to obtain the synthesis of monoclonal antibodies in the recombinant host cells. The DNA also can be modified, for example, by substituting the coding sequence for human heavy and light chain constant domains in place of the homologous murine sequences (U.S. Pat. No. 4,816,567) or by covalently joining to the immunoglobulin coding sequence all or part of the coding sequence for a non-immunoglobulin polypeptide. Such a non-immunoglobulin polypeptide can be substituted for the constant domains of an antibody of the invention, or can be substituted for the variable domains of one antigen-combining site of an antibody of the invention to create a chimeric bivalent antibody.

[00146] In some embodiments, antibodies of the present invention (e.g., glycan-interacting antibodies) may be produced by various procedures known by those skilled in the art. For the production of polyclonal antibodies in vivo, host animals, such as rabbits, rats, mice, cows, horses, donkeys, chickens, monkeys, sheep or goats, are immunized with either free or carrier-coupled antigens, for example, by intraperitoneal and/or intradermal injection. In some embodiments, injection material may be an emulsion containing about 100 µg of antigen or carrier protein. In some embodiments, injection materials may include a glycan-rich composition such as non-human mammalian submaxillary mucin in solution. Various adjuvants can also be used to increase the immunological response, depending on the host species. Adjuvants include, but are not limited to, Freund's (complete and incomplete), mineral gels such as aluminum hydroxide, surface active substances such as lysolecithin, pluronic polyols, polyanions, peptides, oil emulsions, TITERMAX® (CytRx Corp, Los Angeles, CA), keyhole limpet hemocyanins, dinitrophenol, and potentially useful human adjuvants such as BCG (bacille Calmette-Guerin) and corynebacterium parvum. Such adjuvants are also well known in the art. Several booster injections may be needed, for instance, at intervals of about two weeks, to provide a useful titer of antibody which can be detected, for example, by ELISA assay using glycans and/or free peptide adsorbed to a solid surface. The titer of antibodies in serum from an immunized animal can be increased by

selection of antibodies, e.g., by adsorption of antigens onto a solid support and elution of the selected antibodies according to methods well known in the art.

[00147] Glycan-interacting antibodies, variants and fragments thereof may be selected and produced using high throughput methods of discovery. In one embodiment, glycan-interacting antibodies that include synthetic antibodies, variants and fragments thereof are produced through the use of display libraries. The term “display” as used herein, refers to the expression or “display” of proteins or peptides on the surface of a given host. The term “library” as used herein, refers to a collection of unique cDNA sequences and/or the proteins that are encoded by them. A library may contain from as little as two unique cDNAs to hundreds of billions of unique cDNAs. In some embodiments, glycan-interacting antibodies that are synthetic antibodies are produced using antibody display libraries or antibody fragment display libraries. The term “antibody fragment display library” as used herein, refers to a display library wherein each member encodes an antibody fragment containing at least one variable region of an antibody. Such antibody fragments are preferably Fab fragments, but other antibody fragments such as single-chain variable fragments (scFvs) are contemplated as well. In an Fab antibody fragment library, each Fab encoded may be identical except for the amino acid sequence contained within the variable loops of the complementarity determining regions (CDRs) of the Fab fragment. In an alternative or additional embodiment, amino acid sequences within the individual V_H and/or V_L regions may differ as well.

[00148] Display libraries may be expressed in a number of possible hosts including, but not limited to yeast, bacteriophage, bacteria and retroviruses. Additional display technologies that may be used include ribosome-display, microbead-display and protein-DNA linkage techniques. In a preferred embodiment, Fab display libraries are expressed in yeast or in bacteriophages (also referred to herein as “phages” or “phage particles”). When expressed, the Fabs decorate the surface of the phage or yeast where they can interact with a given antigen. An antigen that includes a glycan or other antigen from a desired target may be used to select phage particles or yeast cells expressing antibody fragments with the highest affinity for that antigen. The DNA sequence encoding the CDR of the bound antibody fragment can then be determined through sequencing using the bound particle or cell. In one embodiment, positive selection is used in the development of antibodies. In some embodiments, negative selection is utilized in the development of antibodies. In some embodiments, both positive and negative selection methods are utilized during multiple rounds of selection in the development of antibodies using display libraries.

[00149] In yeast display, cDNA encoding different antibody fragments are introduced into yeast cells where they are expressed and the antibody fragments are “displayed” on the cell surface as described by Chao et al. (Chao, G. et al., *Isolating and engineering human antibodies using yeast surface display*. Nat Protoc. 2006;1(2):755-68). In yeast surface display, expressed antibody fragments may contain an additional domain that includes the yeast agglutinin protein, Aga2p. This domain allows the antibody fragment fusion protein to attach to the outer surface of the yeast cell through the formation of disulphide bonds with surface-expressed Aga1p. The result is a yeast cell, coated in a particular antibody fragment. Display libraries of cDNA encoding these antibody fragments are utilized initially in which the antibody fragments each have a unique sequence. These fusion proteins are expressed on the cell surface of millions of yeast cells where they can interact with a desired antigenic target antigen, incubated with the cells. Target antigens may be covalently or otherwise modified with a chemical or magnetic group to allow for efficient cell sorting after successful binding with a suitable antibody fragment takes place. Recovery may be by way of magnetic-activated cell sorting (MACS), fluorescence-activated cell sorting (FACS) or other cell sorting methods known in the art. Once a subpopulation of yeast cells is selected, the corresponding plasmids may be analyzed to determine the CDR sequence.

[00150] Bacteriophage display technology typically utilizes filamentous phage including, but not limited to fd, F1 and M13 virions. Such strains are non-lytic, allowing for continued propagation of the host and increased viral titres. Examples of phage display methods that can be used to make the antibodies of the present invention include those disclosed in Miersch et al. (Miersch, S. et al., *Synthetic antibodies: Concepts, potential and practical considerations*. Methods. 2012 Aug; 57(4):486-98), Bradbury et al. (Bradbury, A.R. et al., *Beyond natural antibodies: the power of in vitro display technologies*. Nat Biotechnol. 2011 Mar;29(3):245-54), Brinkman et al. (Brinkmann, U. et al., *Phage display of disulfide-stabilized Fv fragments*. J Immunol Methods. 1995 May 11; 182(1):41-50); Ames et al. (Ames, R.S. et al., *Conversion of murine Fabs isolated from a combinatorial phage display library to full length immunoglobulins*. J Immunol Methods. 1995 Aug 18;184(2):177-86); Kettleborough et al. (Kettleborough, C.A. et al., *Isolation of tumor cell-specific single-chain Fv from immunized mice using phage-antibody libraries and the re-construction of whole antibodies from these antibody fragments*. Eur J Immunol. 1994 Apr; 24(4):952-8); Persic et al. (Persic, L. et al., *An integrated vector system for the eukaryotic expression of antibodies or their fragments after selection from phage display libraries*. Gene. 1997 Mar 10; 187(1):9-18); PCT application No. PCT/GB91/01134; PCT publications WO 90/02809; WO 91/10737;

WO 92/01047; WO 92/18619; WO 93/11236; WO 95/15982; WO 95/20401; and U.S. Pat. Nos. 5,698,426; 5,223,409; 5,403,484; 5,580,717; 5,427,908; 5,750,753; 5,821,047; 5,571,698; 5,427,908; 5,516,637; 5,780,225; 5,658,727; 5,733,743 and 5,969,108, each of which is incorporated herein by reference in its entirety. Antibody fragment expression on bacteriophages may be carried out by inserting the cDNA encoding the fragment into the gene expressing a viral coat protein. The viral coat of filamentous bacteriophages is made up of five coat proteins, encoded by a single-stranded genome. Coat protein pIII is the preferred protein for antibody fragment expression, typically at the N-terminus. If antibody fragment expression compromises the function of pIII, viral function may be restored through coexpression of a wild type pIII, although such expression will reduce the number of antibody fragments expressed on the viral coat, but may enhance access to the antibody fragment by the target antigen. Expression of viral as well as antibody fragment proteins may alternatively be encoded on multiple plasmids. This method may be used to reduce the overall size of infective plasmids and enhance the transformation efficiency.

[00151] As described above, after selection of a host expressing a high affinity antibody or antibody fragment, (e.g., glycan-interacting antibodies) the coding regions from the antibody or antibody fragment can be isolated and used to generate whole antibodies, including human antibodies, or any other desired antigen binding fragment, and expressed in any desired host, including mammalian cells, insect cells, plant cells, yeast, and bacteria, e.g., as described in detail below.

[00152] The DNA sequence encoding a high affinity antibody can be mutated for additional rounds of selection in a process known as affinity maturation. The term “affinity maturation”, as used herein, refers to a method whereby antibodies are produced with increasing affinity for a given antigen through successive rounds of mutation and selection of antibody- or antibody fragment-encoding cDNA sequences. In some cases, this process is carried out in vitro. To accomplish this, amplification of CDR coding sequences may be carried out using error-prone PCR to produce millions of copies containing mutations including, but not limited to point mutations, regional mutations, insertional mutations and deletional mutations. As used herein, the term “point mutation” refers to a nucleic acid mutation in which one nucleotide within a nucleotide sequence is changed to a different nucleotide. As used herein, the term “regional mutation” refers to a nucleic acid mutation in which two or more consecutive nucleotides are changed to different nucleotides. As used herein, the term “insertional mutation” refers to a nucleic acid mutation in which one or more nucleotides are inserted into a nucleotide sequence. As used herein, the term “deletional

mutation” refers to a nucleic acid mutation in which one or more nucleotides are removed from a nucleotide sequence. Insertional or deletional mutations may include the complete replacement of an entire codon or the change of one codon to another by altering one or two nucleotides of the starting codon.

[00153] Mutagenesis may be carried out on CDR-encoding cDNA sequences to create millions of mutants with singular mutations in CDR heavy and light chain regions. In another approach, random mutations are introduced only at CDR residues most likely to improve affinity. These newly generated mutagenic libraries can be used to repeat the process to screen for clones that encode antibody fragments with even higher affinity for the target antigen. Continued rounds of mutation and selection promote the synthesis of clones with greater and greater affinity (Chao, G. et al., *Isolating and engineering human antibodies using yeast surface display*. Nat Protoc. 2006;1(2):755-68).

[00154] Examples of techniques that can be used to produce antibodies and antibody fragments, such as Fabs and scFvs, include those described in U.S. Pat. Nos. 4,946,778 and 5,258, 498; Miersch et al. (Miersch, S. et al., *Synthetic antibodies: Concepts, potential and practical considerations*. Methods. 2012 Aug;57(4):486-98), Chao et al. (Chao, G. et al., *Isolating and engineering human antibodies using yeast surface display*. Nat Protoc. 2006;1(2):755-68), Huston et al. (Huston, J.S. et al., *Protein engineering of single-chain Fv analogs and fusion proteins*. Methods Enzymol. 1991;203:46-88); Shu et al. (Shu, L. et al., *Secretion of a single-gene-encoded immunoglobulin from myeloma cells*. Proc Natl Acad Sci U S A. 1993 Sep 1;90(17):7995-9); and Skerra et al. (Skerra, A. et al., *Assembly of a functional immunoglobulin Fv fragment in Escherichia coli*. Science. 1988 May 20;240(4855):1038-41), each of which is incorporated herein by reference in its entirety.

[00155] For some uses, including the *in vivo* use of antibodies (e.g., glycan-interacting antibodies) in humans and *in vitro* detection assays, it may be preferable to use chimeric, humanized, or human antibodies. A chimeric antibody is a molecule in which different portions of the antibody are derived from different animal species, such as antibodies having a variable region derived from a murine monoclonal immunoglobulin and a human immunoglobulin constant region. Methods for producing chimeric antibodies are known in the art. (Morrison, S.L., *Transfectomas provide novel chimeric antibodies*. Science. 1985 Sep 20;229(4719):1202-7; Gillies, S.D. et al., *High-level expression of chimeric antibodies using adapted cDNA variable region cassettes*. J Immunol Methods. 1989 Dec 20;125(1-2):191-202.; and U.S. Pat. Nos. 5,807, 715; 4,816,567; and 4,816,397, which are incorporated herein by reference in their entirety).

[00156] Humanized antibodies are antibody molecules from non-human species that bind to the desired antigen and have one or more complementarity determining regions (CDRs) from the nonhuman species and framework regions from a human immunoglobulin molecule. Often, framework residues in the human framework regions are substituted with corresponding residues from the CDR and framework regions of the donor antibody to alter, preferably improve, antigen binding. These framework substitutions are identified by methods well known in the art, e.g., by modeling of the interactions of the CDR and framework residues to identify framework residues important for antigen binding, and by sequence comparison to identify unusual framework residues at particular positions. (U.S. Pat. Nos. 5,693,762 and 5,585,089; Riechmann, L. et al., *Reshaping human antibodies for therapy*. Nature. 1988 Mar 24;332(6162):323-7, which are incorporated herein by reference in their entireties). Antibodies can be humanized using a variety of techniques known in the art, including, for example, CDR-grafting (EP 239,400; PCT publication WO 91/09967; U.S. Pat. Nos. 5,225,539; 5,530,101; and 5,585,089); veneering or resurfacing (EP 592,106; EP 519,596; Padlan, E.A., *A possible procedure for reducing the immunogenicity of antibody variable domains while preserving their ligand-binding properties*. Mol Immunol. 1991 Apr-May;28(4-5):489-98; Studnicka, G.M. et al., *Human-engineered monoclonal antibodies retain full specific binding activity by preserving non-CDR complementarity-modulating residues*. Protein Eng. 1994 Jun;7(6):805-14; Roguska, M.A. et al., *Humanization of murine monoclonal antibodies through variable domain resurfacing*. Proc Natl Acad Sci U S A. 1994 Feb 1;91(3):969-73); and chain shuffling (U.S. Pat. No. 5,565,332); each of which is incorporated herein by reference in their entirety. Humanized antibodies of the present invention may be developed for desired binding specificity, complement-dependent cytotoxicity, and antibody-dependent cellular-mediated cytotoxicity, etc.

[00157] In some cases, human frameworks are selected by alignment of donor antibody sequences with human framework sequences to find human framework candidates with the highest level of homology. In some cases, framework regions may be selected from more than one human framework candidate (e.g., framework regions 1-3 may be selected from one candidate and framework region 4 may be selected from an alternative candidate). In some cases, framework regions may be selected from human consensus sequences to avoid the risk of including immunogenic epitopes created by somatic mutations. Consensus sequences are sequences formed by comparing many sequences and adopting most commonly occurring residues at each position. In some cases, human frameworks may be selected from human

germline sequences. These may be identified through database searching (e.g., using the NCBI protein database or other databases).

[00158] Light and heavy chain human frameworks may be selected from the same or from different clones. Light and heavy chains derived from the same clone have a greater likelihood of associating to form binding sites that are functional; however, the conserved nature of the interface between heavy and light chains typically allows light and heavy chains from different clones to associate and be functional. Frequency of pairing between human light and heavy chain frameworks can be reviewed, for example, in Tiller et al., 2013. MAbs. 5(3): 445-70, the contents of which are herein incorporated by reference in their entirety.

[00159] Residues in humanized antibody sequences may be considered for “back-mutation” to improve or restore antibody affinity lost during humanization. Back-mutation involves changing residues altered during humanization back to those present in the original non-human antibody sequence. Residues that are candidates for back-mutation may be identified, for example, by comparison to standard conformations found in canonical antibody structures (see Al-Lazikani, et al., 1997. J. Mol. Biol. 273: 927-48, the contents of which are herein incorporated by reference in their entirety). Unusual canonical residues may be identified and targeted for back-mutation. In some cases, residues that are candidates for back-mutation may be “Vernier residues”, a term used to refer to residues in contact with CDRs. These residues have a higher likelihood of impacting CDR positioning and conformation, and therefore antibody affinity and/or specificity (Strohl, W.R. Therapeutic Antibody Engineering. Woodhead Publishing, Philadelphia PA. 2012. Ch. 6, p117). In some cases, human framework regions are kept constant and CDRs from donor antibodies are back-mutated to fit human CDR regions while maintaining binding through empirical methods.

[00160] Completely human antibodies (e.g., glycan-interacting antibodies) are particularly desirable for therapeutic treatment of human patients, so as to avoid or alleviate immune reaction to foreign protein. Human antibodies can be made by a variety of methods known in the art, including the antibody display methods described above, using antibody libraries derived from human immunoglobulin sequences. See also, U.S. Pat. Nos. 4,444,887 and 4,716,111; and PCT publications WO 98/46645, WO 98/50433, WO 98/24893, WO 98/16654, WO 96/34096, WO 96/33735, and WO 91/10741; each of which is incorporated herein by reference in its entirety.

[00161] Human antibodies (e.g., glycan-interacting antibodies) can also be produced using transgenic mice which are incapable of expressing functional endogenous immunoglobulins, but which can express human immunoglobulin polynucleotides. For example, the human

heavy and light chain immunoglobulin polynucleotide complexes can be introduced randomly, or by homologous recombination, into mouse embryonic stem cells. Alternatively, the human variable region, constant region, and diversity region may be introduced into mouse embryonic stem cells, in addition to the human heavy and light chain polynucleotides. The mouse heavy and light chain immunoglobulin polynucleotides can be rendered nonfunctional separately or simultaneously with the introduction of human immunoglobulin loci by homologous recombination. In particular, homozygous deletion of the J_H region prevents endogenous antibody production. The modified embryonic stem cells are expanded and microinjected into blastocysts to produce chimeric mice. The chimeric mice are then bred to produce homozygous offspring which express human antibodies. The transgenic mice are immunized in the normal fashion with a selected antigen, e.g., all or a portion of a glycan, glycoconjugate and/or polypeptide of the invention.

[00162] Thus, using such a technique, it is possible to produce useful human IgG, IgA, IgM, IgD and IgE antibodies. For an overview of the technology for producing human antibodies, see Lonberg and Huszar (Lonberg, N. et al., *Human antibodies from transgenic mice*. Int Rev Immunol. 1995;13(1):65-93). For a detailed discussion of the technology for producing human antibodies and human monoclonal antibodies and protocols for producing such antibodies, see, e.g., PCT publications WO 98/24893; WO 92/01047; WO 96/34096; WO 96/33735; U.S. Pat. Nos. 5,413,923; 5,625,126; 5,633,425; 5,569,825; 5,661,016; 5,545,806; 5,814,318; 5,885,793; 5,916,771; 5,939,598; 6,075,181; and 6,114,598, each of which are incorporated by reference herein in their entirety. In addition, companies such as Abgenix, Inc. (Fremont, Calif.), Protein Design Labs, Inc. (Mountain View, Calif.) and Genpharm (San Jose, Calif.) can be engaged to provide human antibodies directed against a selected antigen using technology similar to the above described technologies.

[00163] Once an antibody molecule of the present invention has been produced by an animal, a cell line, chemically synthesized, or recombinantly expressed, it can be purified (i.e., isolated) by any method known in the art for the purification of an immunoglobulin or polypeptide molecule, for example, by chromatography (e.g., ion exchange, affinity, particularly by affinity for the specific antigen, Protein A, and sizing column chromatography), centrifugation, differential solubility, or by any other standard technique for the purification of proteins. In addition, the antibodies of the present invention or fragments thereof can be fused to heterologous polypeptide sequences described herein or otherwise known in the art, to facilitate purification.

[00164] The affinity between an antibody and a target or ligand (such as an antigen used to generate a given antibody) may be measured in terms of K_D using one or more binding assays as described herein. Depending on the desired application for a given antibody, varying K_D values may be desirable. High affinity antibodies typically form ligand bonds with a K_D of about 10^{-5} M or less, e.g. about 10^{-6} M or less, about 10^{-7} M or less, about 10^{-8} M or less, about 10^{-9} M or less, about 10^{-10} M or less, about 10^{-11} M or less or about 10^{-12} M or less.

[00165] In some embodiments, antibodies of the invention may be characterized according to their half maximal effective or inhibitory concentration (EC_{50} or IC_{50} , respectively). In some cases, this value may represent the concentration of antibody necessary to inhibit cells expressing STn (e.g. kill, reduce proliferation and/or reduce one or more cell function) at a level equal to half of the maximum inhibition observed with the highest concentrations of antibody. Such IC_{50} values may be from about 0.001 nM to about 0.01 nM, from about 0.005 nM to about 0.05 nM, from about 0.01 nM to about 1 nM, from about 0.05 nM to about 5 nM, from about 0.1 nM to about 10 nM, from about 0.5 nM to about 25 nM, from about 1 nM to about 50 nM, from about 5 nM to about 75 nM, from about 10 nM to about 100 nM, from about 25 nM to about 250 nM, from about 200 nM to about 1000 nM or more than 1000 nM.

[00166] In some embodiments, antibodies taught in the present disclosure may be tested for their ability to target patient-derived cancer cells and/or cancer stem cells (CSCs). According to such embodiments, patient-derived cancer cells may be cultured in vitro and antibodies of the present disclosure may be used to target such cells.

[00167] In other embodiments, patient-derived tumor cells or tumor fragments may be used to produce patient-derived xenograft (PDX) tumors. In some cases, pieces of primary or metastatic solid tumors maintained as tissue structures may be collected by surgery or biopsy procedures. In some cases, fluid drained from malignant ascites or pleural effusions may be used. Tumors may be implanted as pieces or single cell suspensions, either alone or in some studies coated with MATRIGEL® (Corning Life Sciences, Corning, NY) or mixed with human fibroblasts or mesenchymal stem cells. Sites of implantation may include the dorsal region of mice (subcutaneous implantation), although implantation in the same organ as the original tumor may be an option (orthotopic implantation, i.e. pancreas, oral cavity, ovary, mammary fat pad, brain, etc.). In addition, independently of the tumor origin, some approaches may include implanting primary tumors in the renal capsule in an effort to increase engraftment success rates. A variety of mouse strains having different degrees of immunosuppression may be used in such studies. For hormone sensitive tumors, some studies may use hormone supplementation with the intent of increasing engraftment rates. In some

embodiments, PDX tumors may be generated in non-obese diabetic/severe combined immunodeficiency (NOD/SCID) mice. Antibodies may be administered to mice with PDX tumors and the effect on tumor volume may be analyzed. In some cases, PDX tumors may be dissected, subjected to cellular dissociation, and the resulting cells grown in culture. The ability of antibodies of the present disclosure to target these cells may be assessed *in vitro*.

[00168] The preparation of antibodies, whether monoclonal or polyclonal, is known in the art. Techniques for the production of antibodies are well known in the art and described, e.g. in Harlow and Lane "Antibodies, A Laboratory Manual", Cold Spring Harbor Laboratory Press, 1988 and Harlow and Lane "Using Antibodies: A Laboratory Manual" Cold Spring Harbor Laboratory Press, 1999.

Targets

[00169] Glycan-interacting antibodies of the present invention may exert their effects via binding (reversibly or irreversibly) to one or more glycan or glycan-associated or glycan-related targets. In some embodiments, glycan-interacting antibodies can be prepared from any region of the targets taught herein. In some embodiments, targets of the present invention include glycans. Glycans used for generating antibodies may include a chain of sugars having at least 2, at least 3, at least 4, at least 5, at least 6, at least 7, at least 8, at least 9, at least 10, at least 11, at least 12, at least 13, at least 14, at least 15, at least 16, at least 17, at least 18, at least 19 or at least 20 residues. Some glycans used for generating antibodies may include from about 2 residue to about 5 residues.

[00170] In some embodiments, glycan-interacting antibody target antigens include sialic acids. N-acetylneuraminic acid (Neu5Ac) and N-glycolylneuraminic acid (Neu5Gc) are the major sialic acids on mammalian cell surfaces. Of these, Neu5Ac is naturally produced in humans. Neu5Gc is naturally produced in most mammals with the exception of humans due to a mutation in the cytidine monophosphate (CMP)-N-acetylneuraminic acid hydroxylase (CMAH) gene responsible for CMP-Neu5Gc production from CMP-Neu5Ac. Neu5Gc in humans is in fact immunogenic with nearly all humans expressing anti-Neu5Gc antibodies. Despite a lack of production, most human systems include some level of Neu5Gc due to dietary intake. These foreign products are subsequently incorporated into human glycoproteins. Such glycoproteins are contemplated as targets of the invention.

[00171] Glycan target antigens of the present invention may include, but are not limited to those listed in Table 1. Abbreviations used include: Glc – glucose, Gal – galactose, GlcNAc – N-acetylglucosamine, GalNAc – N-acetylgalactosamine, GlcNAc6S – 6-Sulfo-N-

acetylglucosamine, KDN – 2-keto-3-deoxy-D-glycero-D-galactonononic acid, Neu5,9Ac2 – N-acetyl-9-O-acetylneuraminic acid, Fuc – fucose and Neu5GcOMe – 2-O-methyl-N-glycolylneuraminic acid. O-glycosidic bonds are present between each residue in the glycans listed with α and β indicating the relative stoichiometry between the two residues joined by the bond, wherein α indicates an axial orientation and β indicates an equatorial orientation. The numbers following α and/or β , in the format x,x , indicate the carbon number of each of the carbons from each of the adjoined residues that participate in bond formation. While the glycans listed represent individual glycan target antigens contemplated, the present invention also includes embodiments wherein the above presented glycans include different combinations of α and β -oriented O-glycosidic bonds than the ones presented. “R” represents an entity that the glycan may be coupled with. In some embodiments, R is a protein wherein the glycan is linked typically to a serine or threonine residue. In some embodiments, R is a linker molecule used to join the glycan to a substrate, such as in a glycan array. In some embodiments, R may be a linker with the formula of $-(CH_2)_2CH_2NH_2$ or $-(CH_2)_3NHCOCH_2(OCH_2CH_2)_6NH_2$. In some embodiments, R may be biotin, albumin, $ProNH_2$, $-CH-$, $-OH$, $-OCH_3$, $-OCH_2CH_3$, $-H$, hydrido, hydroxy, alkoxy, oxygen, carbon, sulfur, nitrogen, polyacrylamide, phosphorus, NH_2 , $ProNH_2=O(CH_2)_2CH_2NH_2$, $(OCH_2CH_2)_6NH_2$, $O(CH_2)_3NHCOCH_2(OCH_2CH_2)_6NH_2$, the fluorescent labels 2-aminobenzamide (AB) and/or 2-aminobenzoid acid (AA), 2-aminobenzamide analog that contains an alkyl amine (AEAB), aminoxy- groups, methylaminoxy groups, hydrazide groups, amino lipid 1,2-dihexadecyl-sn-glycero-3-phosphoethanolamine (DHPE), aminoxy (AO) functionalized DHPE and glycosylphosphatidylinositol (GPI). Without intending to limit the source or nature of R, this may include structures that affect the physical spacing of glycan residue. In some embodiments, the R group may include a combination of the R groups presented herein, e.g. a biotinylated polyacrylamide. In some embodiments, the R group in combination with underlying substrates may affect glycan residue spacing.

Table 1. Glycan target antigens

Glycan
GalNAc α -R
Gal α 1,3Gal β 1,4GlcNAc β -R
Gal β 1,3GalNAc β -R
Gal β 1,3GlcNAc α -R
Gal β 1,3GlcNAc β 1,3Gal β 1,4Glc β -R

Gal β 1,3GlcNAc β -R
Gal β 1,4GlcNAc6S β -R
Gal β 1,4GlcNAc β -R
Gal β 1,4Glc β -R
KDN α 2,8Neu5Ac α 2,3Gal β 1,4Glc β -R
KDN α 2,8Neu5Gc α 2,3Gal β 1,4Glc β -R
Neu5,9Ac2 α 2,3Gal β 1,3GalNAc α -R
Neu5,9Ac2 α 2,3Gal β 1,3GalNAc β -R
Neu5,9Ac2 α 2,3Gal β 1,3GlcNAc β -R
Neu5,9Ac2 α 2,3Gal β 1,4GlcNAc β -R
Neu5,9Ac2 α 2,3Gal β 1,4Glc β -R
Neu5,9Ac2 α 2,3Gal β -R
Neu5,9Ac2 α 2,6GalNAc α -R
Neu5,9Ac2 α 2,6Gal β 1,4GlcNAc β -R
Neu5,9Ac2 α 2,6Gal β 1,4Glc β -R
Neu5,9Ac2 α 2,6Gal β -R
Neu5Ac α 2,3Gal β 1,3GalNAc α -R
Neu5Ac α 2,3Gal β 1,3GalNAc β -R
Neu5Ac α 2,3Gal β 1,3GlcNAc β 1,3Gal β 1,4Glc β -R
Neu5Ac α 2,3Gal β 1,3GlcNAc β -R
Neu5Ac α 2,3Gal β 1,4(Fuc α 1,3)GlcNAc6S β -R
Neu5Ac α 2,3Gal β 1,4(Fuc α 1,3)GlcNAc β -R
Neu5Ac α 2,3Gal β 1,4GlcNAc6S β -R
Neu5Ac α 2,3Gal β 1,4GlcNAc β -R
Neu5Ac α 2,3Gal β 1,4Glc β -R
Neu5Ac α 2,3Gal β -R
Neu5Ac α 2,6(KDN α 2,3)Gal β 1,4Glc β -R
Neu5Ac α 2,6(Neu5Ac α 2,3)Gal β 1,4Glc β -R
Neu5Ac α 2,6(Neu5Gc α 2,3)Gal β 1,4Glc β -R
Neu5Ac α 2,6GalNAc α -R
Neu5Ac α 2,6Gal β 1,4GlcNAc β -R
Neu5Ac α 2,6Gal β 1,4Glc β -R
Neu5Ac α 2,6Gal β -R
Neu5Ac α 2,8KDN α 2,6Gal β 1,4Glc β -R
Neu5Ac α 2,8Neu5Ac α 2,3Gal β 1,4Glc β -R

Neu5Ac α 2,8Neu5Ac α 2,3Gal β 1,4Glc β -R
Neu5Ac α 2,8Neu5Ac α 2,6Gal β 1,4Glc β -R
Neu5Ac α 2,8Neu5Ac α 2,8Neu5Ac α 2,3Gal β 1,4Glc β -R
Neu5Ac α 2,8Neu5Ac α 2,8Neu5Ac α 2,3Gal β 1,4Glc β -R
Neu5Ac α 2,8Neu5Gc α 2,3Gal β 1,4Glc β -R
Neu5Ac α 2,8Neu5Gc α 2,6Gal β 1,4Glc β -R
Neu5Gc9Ac α 2,3Gal β 1,4Glc β -R
Neu5Gc9Ac α 2,6Gal β 1,4Glc β -R
Neu5Gc9Ac α 2,3Gal β 1,3GalNAc α -R
Neu5Gc9Ac α 2,3Gal β 1,3GalNAc β -R
Neu5Gc9Ac α 2,3Gal β 1,3GlcNAc β -R
Neu5Gc9Ac α 2,3Gal β 1,4GlcNAc β -R
Neu5Gc9Ac α 2,3Gal β -R
Neu5Gc9Ac α 2,6GalNAc α -R
Neu5Gc9Ac α 2,6Gal β 1,4GlcNAc β -R
Neu5Gc9Ac α 2,6Gal β -R
Neu5GcOMe α 2,8Neu5Ac α 2,3Gal β 1,4Glc β -R
Neu5Gc α 2,3Gal β 1,3GalNAc α -R
Neu5Gc α 2,3Gal β 1,3GalNAc β -R
Neu5Gc α 2,3Gal β 1,3GlcNAc β 1,3Gal β 1,4Glc β -R
Neu5Gc α 2,3Gal β 1,3GlcNAc β -R
Neu5Gc α 2,3Gal β 1,4(Fuc α 1,3)GlcNAc6S β -R
Neu5Gc α 2,3Gal β 1,4(Fuc α 1,3)GlcNAc β -R
Neu5Gc α 2,3Gal β 1,4GlcNAc6S β -R
Neu5Gc α 2,3Gal β 1,4GlcNAc β -R
Neu5Gc α 2,3Gal β 1,4Glc β -R
Neu5Gc α 2,3Gal β -R
Neu5Gc α 2,6GalNAc α -R
Neu5Gc α 2,6Gal β 1,4GlcNAc β -R
Neu5Gc α 2,6Gal β 1,4Glc β -R
Neu5Gc α 2,6Gal β -R
Neu5Gc α 2,8Neu5Ac α 2,3Gal β 1,4Glc β -R
Neu5Gc α 2,8Neu5Gc α 2,3Gal β 1,4Glc β -R

[00172] Glycan targets of the present invention may include one or more regions of antibody recognition. As used herein, the term “region of antibody recognition” refers to a segment located on any part of the molecule, an attached group or located on a region of interaction between the glycan and another molecule, including, but not limited to another glycan, protein, membrane, cell surface structure, or extracellular matrix component. In some embodiments, regions of antibody recognition are located at interchain target sites, wherein the term “interchain” means within the present polymeric chain. Intergain target sites may include regions of antibody recognition having 1, 2, 3, 4, 5, 6, 7, 8, 9 or at least 10 residues, bonds between residues or combinations of residues and bonds. In some embodiments, regions of antibody recognition are located at regions of interaction between one or more glycan chains. Such regions may be between 2, 3, 4 or at least 5 glycan chains.

[00173] In some embodiments, regions of antibody recognition are located at regions of interaction between glycan branch chains connected to a common parent chain. In some embodiments, regions of antibody recognition are located at regions of interaction between a glycan branch chain and a parent chain. In some embodiments, regions of antibody recognition are located at regions of interaction between glycans and proteins. Such regions of interaction may include chemical bonds between the glycan and the protein, including, but not limited to covalent bonds, ionic bonds, hydrostatic bonds, hydrophobic bonds and hydrogen bonds. In some embodiments, regions of antibody recognition are located at regions of interaction between glycans and other biomolecules including, but not limited to lipids and nucleic acids. Such regions of interaction may include chemical bonds between the glycan and the biomolecule, including, but not limited to covalent bonds, ionic bonds, hydrostatic bonds, hydrophobic bonds and hydrogen bonds.

[00174] In some embodiments, glycan targets of the present invention are components of glycoconjugates. As used herein, the term “glycoconjugate” refers to any entity joined with a glycan moiety. In some embodiments, glycoconjugates are glycolipids. As used herein, the term “glycolipid” refers to a class of lipids wherein a carbohydrate moiety is covalently attached. In some embodiments, carbohydrate moieties present on glycolipids may be glycans. In some embodiments, lipid components of glycolipids include ceramide moieties. Examples of glycolipids contemplated as targets of the present invention include, but are not limited to glyceroglycolipids (including, but not limited to galactolipids and sulfolipids), glycosphingolipids (including, but not limited to cerebroside (e.g., galactocerebroside, glucocerebroside and sulfatide), gangliosides, globosides and glycosphosphosphingolipids) and glycosylphosphatidylinositols. When located within cell membranes, glycan moieties of

glycolipids are located on the extracellular side of the membrane where they may interact with other cells as well as cell signaling ligands (Maccioni, H.J. et al., *Organization of the synthesis of glycolipid oligosaccharides in the Golgi complex*. FEBS Lett. 2011 Jun 6;585(11):1691-8).

[00175] In some embodiments, glycoconjugate targets of the present invention are glycoprotein and/or proteoglycans. Glycoproteins refer to any proteins that are covalently bonded with glycans. Proteoglycans are a class of proteins that are heavily glycosylated with glycans that often carry a negative charge. This property makes them very hydrophilic and important components of connective tissue.

Cancer-related targets

[00176] In some embodiments, targets of the present invention are cancer-related antigens or epitopes. As used herein, the term “cancer-related” is used to describe entities that may be in some way associated with cancer, cancerous cells and/or cancerous tissues. Many cancer-related antigens or epitopes that include glycans have been identified that are expressed in correlation with tumor cells (Heimburg-Molinaro, J. et al., *Cancer vaccines and carbohydrate epitopes*. Vaccine. 2011 Nov 8;29(48):8802-26). These are referred to herein as “tumor-associated carbohydrate antigens” or “TACAs.” TACAs include, but are not limited to mucin-related antigens [including, but not limited to Tn, Sialyl Tn (STn) and Thomsen-Friedenreich antigen], blood group Lewis related antigens [including, but not limited to Lewis^Y (Le^Y), Lewis^X (Le^X), Sialyl Lewis^X (SLe^X) and Sialyl Lewis^A (SLe^A)], glycosphingolipid-related antigens [including, but not limited to Globo H, stage-specific embryonic antigen-3 (SSEA-3) and glycosphingolipids that include sialic acid], ganglioside-related antigens [including, but not limited to gangliosides GD2, GD3, GM2, fucosyl GM1 and Neu5GcGM3] and polysialic acid-related antigens. Many of such antigens are described in International Publication No. WO2015054600, the contents of which are herein incorporated by reference in their entirety.

[00177] In some embodiments, TACA targets of the present invention include Lewis blood group antigens. Lewis blood group antigens include a fucose residue linked to GlcNAc by an α 1-3 linkage or an α 1-4 linkage. They may be found on both glycolipids and glycoproteins. Lewis blood group antigens may be found in the body fluid of individuals that are secretors of these antigens. Their appearance on red cells is due to absorption of Lewis antigens from the serum by the red cells.

[00178] In some embodiments, TACA targets of the present invention include Le^Y. Le^Y (also known as CD174) is made up of Galβ1,4GlcNAc having α1,2- as well as α1,3-linked fucose residues yielding the Fuca(1,2)Galβ(1,4)Fuca(1,3)GlcNAc epitope. It is synthesized from the H antigen by α1,3 fucosyltransferases which attach the α1,3 fucose to the GlcNAc residue of the parent chain. Le^Y may be expressed in a variety of cancers including, but not limited to ovarian, breast, prostate, colon, lung and epithelial. Due to its low expression level in normal tissues and elevated expression level in many cancers, the Le^Y antigen is an attractive target for therapeutic antibodies.

[00179] In some embodiments, TACA targets of the present invention include Le^X. Le^X includes the epitope Galβ1-4(Fuca1-3)GlcNAcβ-R. It is also known as CD15 and stage-specific embryonic antigen-1 (SSEA-1). This antigen was first recognized as being immunoreactive with sera taken from a mouse subjected to immunization with F9 teratocarcinoma cells. Le^X was also found to correlate with embryonic development at specific stages. It is also expressed in a variety of tissues both in the presence and absence of cancer, but can also be found in breast and ovarian cancers where it is only expressed by cancerous cells.

[00180] In some embodiments, TACA targets of the present invention include SLe^A and/or SLe^X. SLe^A and SLe^X are made up of structures Neu5Acα2-3Galβ1-3(Fuca1-4)GlcNAcβ-R and Neu5Acα2-3Galβ1-4(Fuca1-3)GlcNAcβ-R, respectively. Their expression is upregulated in cancer cells. The presence of these antigens in serum correlates with malignancy and poor prognosis. SLe^X is mostly found as a mucin terminal epitope. It is expressed in a number of different cancers including breast, ovarian, melanoma, colon, liver, lung and prostate. In some embodiments of the present invention, SLe^A and SLe^X targets include Neu5Gc (referred to herein as GcSLe^A and GcSLe^X, respectively).

[00181] In some cases, cancer-related targets of the invention may include mucins. *Ishida et al* demonstrate that interaction of MUC2 with dendritic cells (with anti-tumor activity) leads to dendritic cell apoptosis (Ishida, A. et al., 2008. *Proteomics*. 8: 3342-9, the contents of which are herein incorporated by reference in their entirety). In some aspects, the present invention provided anti-mucin antibodies to prevent dendritic cell apoptosis and support anti-tumor activity.

[00182] In some embodiments, TACA targets of the present invention include glycolipids and/or epitopes present on glycolipids, including, but not limited to glycosphingolipids. Glycosphingolipids include the lipid ceramide linked to a glycan by the ceramide hydroxyl group. On the cell membrane, glycosphingolipids form clusters referred to as “lipid rafts”.

[00183] In some embodiments, TACA targets of the present invention include Globo H. Globo H is a cancer-related glycosphingolipid first identified in breast cancer cells. The glycan portion of Globo H includes $\text{Fuc}\alpha(1-2)\text{Gal}\beta(1-3)\text{GalNAc}\beta(1-3)\text{Gal}\alpha(1-4)\text{Gal}\beta(1-4)\text{Glc}\beta(1)$. Although found in a number of normal epithelial tissues, Globo H has been identified in association with many tumor tissues including, but not limited to, small cell lung, breast, prostate, lung, pancreatic, gastric, ovarian and endometrial tumors.

[00184] In some embodiments, cancer-related glycosphingolipid targets of the present invention include gangliosides. Gangliosides are glycosphingolipids having one or more sialic acid. According to ganglioside nomenclature, G is used as an abbreviation for ganglioside. This abbreviation is followed by the letters M, D or T referring to the number of sialic acid residues attached (1, 2 or 3 respectively). Finally the numbers 1, 2 or 3 are used to refer to the order of the distance each migrates when analyzed by thin layer chromatography (wherein 3 travels the greatest distance, followed by 2, and then 1). Gangliosides are known to be involved in cancer-related growth and metastasis and may be expressed on the cell surface of tumor cells. Gangliosides expressed on tumor cells may include, but are not limited to GD2, GD3, GM2 and fucosyl GM1 (also referred to herein as Fuc-GM1). In some embodiments of the present invention, glycan-interacting antibodies are directed toward GD3. GD3 is a regulator of cell growth. In some embodiments, GD3-directed antibodies are used to modulate cell growth and/or angiogenesis. In some embodiments, GD3-directed antibodies are used to modulate cell attachment. GD3 associated with some tumor cells may include 9-O-acetylated sialic acid residues (Mukherjee, K. et al., 2008. *J Cell Biochem.* 105: 724-34 and Mukherjee, K. et al., 2009. *Biol Chem.* 390: 325-35, the contents of each of which are herein incorporated by reference in their entirety). In some cases, antibodies of the invention are selective for 9-O-acetylated sialic acid residues. Some antibodies may be specific for 9-O-acetylated GD3s. Such antibodies may be used to target tumor cells expressing 9-O-acetylated GD3. In some embodiments of the present invention, glycan interacting antibodies are directed toward GM2. In some embodiments, GM2-directed antibodies are used to modulate cell to cell contact. In some embodiments, ganglioside targets of the present invention include Neu5Gc. In some embodiments, such targets may include a GM3 variant having Neu5Gc (referred to herein as GcGM3). The glycan component of GcGM3 is $\text{Neu5Gc}\alpha2-3\text{Gal}\beta1-4\text{Glc}$. GcGM3 is a known component of tumor cells (Casadesus, A.V. et al., 2013. *Glycoconj J.* 30(7):687-99, the contents of which are herein incorporated by reference in their entirety).

[00185] In some embodiments, TACAs of the present disclosure include at least one Neu5Gc residue.

Recombinant antibodies

[00186] Recombinant antibodies (e.g., glycan-interacting antibodies) of the invention may be generated using standard techniques known in the art. In some embodiments, recombinant antibodies may be anti-glycan antibodies. Further antibodies may be anti-STn antibodies (e.g. anti-GcSTn or anti-AcSTn antibodies). Recombinant antibodies of the invention may be produced using variable domains obtained from hybridoma cell-derived antibodies produced according to methods described herein. Heavy and light chain variable region cDNA sequences of antibodies may be determined using standard biochemical techniques. Total RNA may be extracted from antibody-producing hybridoma cells and converted to cDNA by reverse transcriptase (RT) polymerase chain reaction (PCR). PCR amplification may be carried out on resulting cDNA to amplify variable region genes. Such amplification may include the use of primers specific for amplification of heavy and light chain sequences. In other embodiments, recombinant antibodies may be produced using variable domains obtained from other sources. This includes the use of variable domains selected from one or more antibody fragment library, such as an scFv library used in antigen panning. Resulting PCR products may then be subcloned into plasmids for sequence analysis. Once sequenced, antibody coding sequences may be placed into expression vectors. For humanization, coding sequences for human heavy and light chain constant domains may be used to substitute for homologous murine sequences. The resulting constructs may then be transfected into mammalian cells for large scale translation.

Anti-Tn antibodies

[00187] In some embodiments, recombinant antibodies of the invention (e.g., glycan-interacting antibodies) may be anti-Tn antibodies. Such antibodies may bind to targets having Tn. Anti-Tn antibodies may be specific for Tn or may bind other modified forms of Tn, such as Tn linked to other moieties, including, but not limited to additional carbohydrate residues. In some cases anti-Tn antibodies may be anti-sialyl-Tn antibodies. Such antibodies may bind to sialylated Tn that includes Neu5Ac and/or sialylated Tn that include Neu5Gc. Some anti-Tn antibodies may bind specifically to clusters of Tn antigen.

Anti-STn antibodies

[00188] In some embodiments, antibodies of the invention (e.g., glycan-interacting antibodies) may specifically bind to STn. Anti-STn antibodies of the invention may be categorized by their binding to specific portions of STn antigens and/or by their specificity for AcSTn versus GcSTn. In some cases, anti-STn antibodies of the invention are Group 1 antibodies. “Group 1” antibodies according to the invention are antibodies capable of binding AcSTn and GcSTn. Such antibodies may also be referred to herein as pan-STn antibodies due to their ability to associate with a wider range of STn structures. In some embodiments, Group 1 antibodies may associate with the portion of STn indicated by the largest ellipse in Fig. 1A. In some cases, anti-STn antibodies of the invention are Group 2 antibodies. “Group 2” antibodies, according to the invention, are antibodies capable of binding STn as well as some related structures that include an O-linkage to serine or threonine. In some embodiments, Group 2 antibodies may associate with glycans that include a sialylated galactose residue. In some cases, Group 2 antibodies may associate with the portion of STn indicated by the largest ellipse in Fig. 1B. Some Group 2 antibodies preferably bind to structures with AcSTn over structures with GcSTn. Further anti-STn antibodies may be Group 3 antibodies. As referred to herein, “Group 3” antibodies are antibodies capable of binding STn, but may also bind a broader set of related structures. Unlike Group 2 antibodies, Group 3 antibodies do not require that such structures have an O-linkage to serine or threonine. In some embodiments, Group 3 antibodies may associate with the portion of STn indicated by the largest ellipse in Fig. 1C. Finally, some anti-STn antibodies of the invention may be Group 4 antibodies. As referred to herein, “Group 4” antibodies are capable of binding to both AcSTn and GcSTn as well as the un-sialylated Tn antigen, and therefore have broader specificity. In some embodiments, Group 4 antibodies may associate with the portion of STn indicated by the largest ellipse in Fig. 1D.

[00189] In some cases, anti-STn antibodies of the invention may bind specifically to clusters of STn on a particular antigen or cell surface. Some such antibodies may recognize epitopes formed by the clustering of STn, including epitopes that include areas of contact between neighboring STn structures. Such epitopes may be formed by the clustering of 2, 3, 4, 5, 6, 7, 8, 9, 10 or more STn structures.

[00190] In some embodiments, anti-STn antibodies of the present disclosure may be used bind cellular proteins carrying STn. Such antibodies may be useful for targeting cellular proteins associated with cancer cells that are distinguishable from similar proteins in non-cancerous cells by STn expression. In some cases, such proteins may include cell surface proteins. Cancer cell surface proteins carrying STn may be targeted by anti-STn antibodies

during cancer treatment and/or diagnosis. Cell surface proteins carrying STn may be identified using mass spectrometry and/or using immunological methods (e.g., FACS analysis, immunoprecipitation, immunoblotting, ELISA, etc.). In some cases, cellular proteins carrying STn may include cancer cell markers, cancer stem cell markers, and/or cancer stem cell signaling proteins. In some embodiments, cellular proteins carrying STn may include, but are not limited to CD44, CD133, CD117, integrins, Notch, and Hedgehog.

Antibody components

[00191] In some cases, antibodies or antigen binding fragments thereof of the invention may include variable domain and/or CDR amino acid sequences provided herein. In some cases, antibodies may include any of the antibody or antibody fragment sequences presented in International Publication Number WO2017083582 (the entire content of which is herein incorporated by reference), including: any of the variable domain sequences presented in Table 2 therein; any of the CDR sequences presented in Table 3 therein; any of the VH CDR sequence groups presented in Table 4 therein; any of the VL CDR sequence groups presented in Table 5 therein; any of the variable domain nucleotide sequences presented in Table 6 therein; or any of the humanized variable domain sequences presented in Table 11 therein. Some antibodies or antigen binding fragments may include different combinations of such sequences or variants with at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or at least 99.5% sequence identity. In some cases, antibodies or antigen binding fragments of the invention may include one or more of the variable domain sequences listed below in Table 2. Light chain variable domains presented may be expressed with or without a C-terminal arginine residue. This residue typically links light chain variable domains with light chain constant domains and may be expressed as part of the light chain constant domain instead of the light chain variable domain. In some cases, antibodies or antigen binding fragments thereof may include an amino acid sequence with from about 50% to about 99.9% sequence identity (e.g. from about 50% to about 60%, from about 55% to about 65%, from about 60% to about 70%, from about 65% to about 75%, from about 70% to about 80%, from about 75% to about 85%, from about 80% to about 90%, from about 85% to about 95%, from about 90% to about 99.9%, from about 95% to about 99.9%, about 97%, about 97.5%, about 98%, about 98.5%, about 99%, about 99.5%, about 99.6%, about 99.7% or about 99.8%) with one or more of the variable domain sequences listed in

Table 2. In some cases, antibodies or antigen binding fragments thereof of the invention may include an amino acid sequence having one or more fragments of any of the sequences listed.

Table 2. Variable domain sequences

Domain	Sequence	SEQ ID NO
mSIA101 VH domain	QVQLQQSDAELVKPGASVKISCKASGYTFTDHAHWWVKQKPEQGLEWIGYFS PGNDDIKYNEKFRGKATLTADKSSSTAYMQLNSLSSDDSAVYFCKRSLSTPY WGQGTLVTVSA	1
mSIA101 VL domain	DIVMTQSPSSLTVTAGEKVTMSCKSSQSLNLRGNHKNYLTWYRQKPLPPKL LIYWASTRESGVPDRFTGSGSGTDFALTISSVQAEDLAVYYCQNDYTYPTYTF GGGTKLEIKR	2
mSIA102 VH domain	QVQLQQSDAELVKPGASVKISCKASGYTFTDHAHWWVKQKPEQGLEWIGYFS PGNDDIKYNEKFKVKATLTADKSSSTAYMQLTSLTSEDSAVYFCKRSYYGD WGQGTTTLTVSS	3
mSIA102 VL domain	DIQMTQSPASLSVSVGETVTITCRASENIYSHLAWYQQKQKSPQLLVYGAT NLADGVPSRFSGSGSGTQFSLKIHSLSQSEDFGSYYCQHFHWGAPFTFGSGTKLE IK	4
mSIA103 VH domain	QVQLQQSDAELVKPGASVKISCKASGYTFTDHAHWWVKQKPEQGLDWIGYIS PGNGDIKYNEKFKDKVTLTADKSSSTACMHLNSLTSEDSAVYFCKRSLALD YWQGTTTLTVSS	5
mSIA103 VL domain	DIVMTQSHKFMSTSVGDRVSITCKASQDVGTNIAWYQQKPGRSPKVLIYSAS TRHTGVPDRFTGSGSGTDFTLTISNVQSEDLTDYFCQQYSSFPLTFGVGKLE LK	6
hSIA101 VH domain	EVQLVQSGAEVKKPGASVKVSCASGYTFTDHAHWWVRQAPQGQLEWMGY FSPGNDDIKYNEKFRGRVTMTADKSSSTAYMELRSLRSDDTAVYFCKRSLST PYWGQGTLVTVSS	7
hSIA101 VL domain	DIVMTQSPDSLAVSLGERATINCKSSQSLNLRGNHKNYLTWYQQKPGQPPL LIYWASTRESGVPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNDYTYPTYTFG QGKVEIK	8
hSIA102 VH domain	QVQLVQSGAEVKKPGASVKISCKASGYTFTDHAHWWVRQAPQGQLEWIGYF SPGNDDIKYNEKFKVRATLTADKSSSTAYMELRSLRSDDTAVYFCKRSYYGD WGQGTLVTVSS	9
hSIA102 VL domain	DIQMTQSPSSLSASVGDRTVTITCRASENIYSHLAWYQQKPGKAPKLLVYGAT NLASGVPSRFSGSGSGTQFTLTISLQPEDFATYYCQHFHWGAPFTFGQGKVEI K	10
hSIA103 VH domain	QVQLVQSGAEVKKPGASVKVSCASGYTFTDHAHWWVRQAPQGQLEWMGY ISPGNGDIKYNEKFKDRVTMTADKSSSTAYMQLRSLRSDDTAVYFCKRSLLA LDYWQGQTLVTVSS	11
hSIA103 VL domain	DIQMTQSPSSLSASVGDRTVTITCKASQDVGTNIAWYQQKPGKAPKVLIYSAST RHTGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCQQYSSFPLTFGQGKVEIK	12

[00192] In some cases, antibodies or antigen binding fragments of the invention may include any of the IgG sequences presented in Table 3. In some cases, antibodies or fragments thereof may include an amino acid sequence with from about 50% to about 99.9% sequence identity (e.g. from about 50% to about 60%, from about 55% to about 65%, from about 60% to about 70%, from about 65% to about 75%, from about 70% to about 80%, from about 75% to about 85%, from about 80% to about 90%, from about 85% to about 95%, from about 90% to about 99.9%, from about 95% to about 99.9%, about 97%, about 97.5%, about 98%, about 98.5%, about 99%, about 99.5%, about 99.6%, about 99.7% or about 99.8%) with

one or more of the constant domain sequences listed. In some cases, antibodies or fragments thereof of the invention may include an amino acid sequence having one or more fragments of any of the sequences listed.

Table 3. IgG Constant domain sequences

Domain	Sequence	SEQ ID NO
Murine IgG2a heavy chain constant domain regions	AKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHTFPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKPCPPCKCPAPNLLGGPSVFIFPPKIKDVLMLSPIVTCVVVDVSEDDPDVQISWVFVNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQQDWMSGKEFKCKVNNKDLPAPIERTISKPKGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDMPEDIYVEWTNNGKTELNYKNTEPVLDSGYSFYMSKLRVEKKNWVERNYSYSCSVVHEGLHNHHTTKSFSRTPGK	13
Murine IgG2a kappa light chain constant region	RADAAPTVISIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSRQNGVLNSWTDQDSKDSTYSMSSTLTLTKDEYERHNSYTCEATHKTSTSPIVKSFNRNEC	14
Human IgG1 heavy chain constant regions	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVTPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTTPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSPGK	15
Human IgG1 light chain constant regions	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKDSTYSLSSTLTLTKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	16

[00193] In some embodiments, the disclosure includes antibody fragments produced using one or more of the antibody sequences or related variants described above. Such antibody fragments may include scFvs, Fab fragments, or any other antibody fragments, including any of those described herein.

Humanized antibodies

[00194] "Humanized" forms of non-human (e.g., murine) antibodies are chimeric antibodies that contain minimal sequences derived from non-human immunoglobulin. For the most part, humanized antibodies are human immunoglobulins (recipient antibody) in which residues from the hypervariable region from an antibody of the recipient are replaced by residues from the hypervariable region from an antibody of a non-human species (donor

antibody) such as mouse, rat, rabbit or nonhuman primate having the desired specificity, affinity, and capacity.

[00195] For construction of expression plasmids encoding fully humanized antibodies with human constant regions, DNA sequences encoding antibody variable region may be inserted into expression vectors (e.g., mammalian expression vectors) between an upstream promoter/enhancer, for example, cytomegalovirus immediate/early promoter/enhancer (CMV IE), plus the immunoglobulin signal sequence and a downstream immunoglobulin constant region gene. DNA samples may then be prepared for transfection into mammalian cells.

[00196] For generation of cell lines and selection of fully humanized antibodies, heavy and light chain plasmid DNA pairs may be transfected into cells for expression. In some embodiments, mammalian NS0 cells may be used. Cell lines producing humanized antibodies may be expanded for expression antibodies that may be harvested and purified from cell culture media.

[00197] In some embodiments, the humanized antibodies may have cross-reactivity with non-human species. Species cross-reactivity may allow the antibodies to be used in different animals for various purposes. For example, cross-reactive antibodies may be used in pre-clinical animal studies to provide information about antibody efficacy and/or toxicity. Non-human species may include mouse, rat, rabbit, dog, pig, goat, sheep, or nonhuman primate such as cynomolgus monkey.

IgG synthesis

[00198] IgG antibodies (e.g. IgG1, IgG2, IgG3 or IgG4) including one or more variable domain and/or CDR amino acid sequences presented herein (or fragment or variants thereof) may be synthesized for further testing and/or product development. Such antibodies may be produced by insertion of one or more segments of cDNA encoding desired amino acid sequences into expression vectors suited for IgG production. Expression vectors may include mammalian expression vectors suitable for IgG expression in mammalian cells. Mammalian expression of IgGs may be carried out to ensure that antibodies produced include modifications (e.g. glycosylation) characteristic of mammalian proteins and/or to ensure that antibody preparations lack endotoxin and/or other contaminants that may be present in protein preparations from bacterial expression systems.

Immunogenic hosts

[00199] In some embodiments, glycan-interacting antibodies of the present invention may be developed through the use of non-human animals as hosts for immunization, referred to herein as “immunogenic hosts”. In some embodiments, immunogenic hosts are mammals. In some embodiments, immunogenic hosts are transgenic knockout mice. Antigens having target sites and/or epitope targets of glycan-interacting antibodies may be used to contact immunogenic hosts in order to stimulate an immune response and produce antibodies in the immunogenic host that specifically bind the target sites and/or epitope targets present on the antigens introduced.

[00200] Antibodies produced through immunization may be isolated from serum of the immunogenic hosts. Antibody producing cells from the immunogenic hosts may also be used to generate cell lines that produce the desired antibody. In some embodiments, screening for antibodies and/or antibody producing cells from the immunogenic host may be carried out through the use of enzyme-linked immunosorbent assays (ELISAs) and/or glycan arrays.

Antibody sequence and structural analysis and optimization

[00201] In some embodiments, antibodies of the present invention may be subjected to sequence analysis and/or structural analysis wherein they are analyzed for characteristics that may affect antibody chemistry, affinity, specificity, protein folding, stability, manufacturing, expression, and/or immunogenicity (i.e., immune reactions in subjects being treated with such antibodies). Such analysis may include comparisons between antibodies binding to the same or similar epitopes.

[00202] Antibodies sequences of antibodies binding to the same epitope may be analyzed for variation in light and/or heavy chain sequences. Such analysis may include germline sequences and/or CDR sequences. Information obtained from such analysis may be used to identify (and optionally to modify, delete, replace or repair) conserved amino acid residues; conserved segments of amino acids; amino acid positions with conserved side chain characteristics; conserved CDR lengths; and other features conserved among antibodies binding to the same epitope. This information may be used to design variants or to inform antibody optimization procedures to improve antibody affinity, specificity, protein folding, stability, manufacturing, expression and/or immunogenicity.

[00203] Sequence analysis may include aligning two or more antibodies that bind to the same or similar epitopes to identify similarities. Such analysis may compare the sequence and/or length of antibody regions (e.g., CDRs, variable domains, germline segments). Amino

acid insertions, amino acid deletions, and substitutions may be identified and assessed. Sequence differences may be compared against antibody affinity and/or specificity.

[00204] In some cases, sequence analyses are conducted to identify (and optionally to modify, delete, replace or repair) one or more of unpaired cysteines or irregular disulfides; glycosylation sites (e.g., N-linked NXS/T sites); acid cleavage sites, amino acid oxidation sites, conformity with mouse germline sequences; asparagine deamidation sites; aspartate isomerization sites; N-terminal pyroglutamate formation sites; and aggregation-prone patches in CDRs.

[00205] In some cases, the present invention provides sequence analysis-informed variants of antibodies presented herein. As used herein, the term “sequence analysis-informed variant” refers to an antibody variant that has been modified based on one or more conclusions derived from antibody sequence analysis. In some cases, antibodies of the invention may be modified to produce antibody variants that include modifications to one or more of antibody affinity, specificity, protein folding, stability, manufacturing, expression and/or immunogenicity.

[00206] Some sequence analysis-informed variants include one or more CDR length modification. CDR length modified antibodies may include one or more added or deleted amino acids in one or more CDRs relative to an original antibody sequence. In some cases, sequence analysis-informed variants may include a substitution of one or more CDRs with one or more CDRs derived from another antibody (e.g., an antibody binding to the same or similar epitope). In some cases, sequence analysis-informed variants may include a substitution of a heavy or light chain variable domain from another antibody (e.g., an antibody binding to the same or similar epitope). Sequence analysis-informed variants may include modifications to one or more germline genes that the antibody is expressed from. Such modifications may include point mutations, regional mutations, insertional mutations or deletional mutations. In some case, germline gene modifications are carried out to move CDRs from one known germline gene to another. Sequence analysis-informed variants may include other variants described herein, including, but not limited to scFvs, monobodies, diabodies, intrabodies, CARs, antibody mimetics, etc.

[00207] In some embodiments, sequence and/or structural analysis may be used to inform the construction of antibody fragment display libraries (including, but not limited to scFv libraries, phage display libraries, and yeast display libraries). In one example, sequence alignment may be carried out to align two or more antibodies with a common antigen or epitope and amino acid residues may be identified that are conserved among the aligned

antibodies or that are variable among the aligned antibodies. In such cases, antibody fragment display libraries may be constructed such that variability among library members is primarily limited to the variable amino acids identified in the sequence analysis. In some cases, such libraries may be used to identify variants with altered affinity and/or specificity for a target antigen (e.g., STn) or a specific epitope of the target antigen (e.g., the epitopes recognized by Group 1, 2, 3 and 4 antibodies as described in Example 1, herein below).

[00208] In some embodiments, antibodies of the invention may be modified to remove, replace or otherwise eliminate one or more unpaired cysteine residues. In some cases, unpaired cysteine residues may be reactive and in some cases may affect antibody affinity and/or specificity. Accordingly, some antibodies of the invention have been modified to eliminate unpaired cysteine residues. In some cases, such variants may have modified epitope specificity and/or affinity. In some cases, modification of unpaired cysteine residues may alter antibody folding. In some cases, these variants include a substitution or deletion of one or more cysteine residues. In some cases, these variants include one or more additional amino acid residues (including, but not limited to, the addition of one or more cysteine residues) to prevent or reduce undesired effects from unpaired cysteine residues. In some cases, cysteine residues are replaced with an amino acid having a hydrophobic side chain (e.g., tyrosine, alanine, valine, isoleucine, leucine, methionine, phenylalanine or tryptophan).

Antibody testing and characterization

[00209] Antibodies described herein may be tested and/or characterized using a variety of methods. Such methods may be used to determine a variety of characteristics that may include, but are not limited to, antibody affinity; specificity; and activity (e.g., activation or inhibition of cellular signaling pathways or other cellular or biological activities). Antibody testing may further include testing *in vivo* (e.g., in animal and/or human studies) for one or more of toxicity, therapeutic effect, pharmacodynamics, pharmacokinetics, absorption, deposition, metabolism, and excretion. Testing in animals may include, but is not limited to, testing in mice, rats, rabbits, guinea pigs, pigs, primates (e.g., cynomolgus monkeys), sheep, goats, horses, and cattle.

Cell-based assays

[00210] In some embodiments, antibodies of the present invention may be tested or characterized through the use of one or more cell-based assays. Such cell-based assays may be carried out *in vitro* with cells in culture. In some cases, cell-based assays may be carried

out *in vivo*. Examples of cell-based *in vivo* assays include tumor models in which tumor cells are injected or otherwise introduced into a host.

[00211] In some cases, cells used in cell-based assays may express one or more target glycans recognized by one or more antibodies of the invention. Such glycans may be naturally expressed by such cells or, alternatively, cells may be induced to express one or more glycans desired for purposes of a particular assay. Induced expression may be through one or more treatments that upregulate expression of glycosylated proteins or enzymes that regulate glycosylation. In other cases, induced expression may include transfection, transduction, or other form of introduction of one or more genes or transcripts for the endogenous expression of one or more glycosylated proteins or enzymes involved in regulation of glycosylation.

[00212] In some cases, cell-based assays used herein may include the use of cancer cells. Many cancer cell lines are available for experiments to test antibodies of the invention. Such cells may express target glycan or may be induced to express target glycans. Additionally, cancer cell lines may be used to test antibodies of the invention, where the cancer cell lines are representative of cancer stem cells. Cancer stem cell (CSC) cell lines may be isolated or differentiated from cancer cells grown in culture (e.g., through sorting based on markers specific for cancer stem cells). Cell lines used in cell-based assays may include, but are not limited to breast, colon, ovary, lymphocyte, bone marrow, and skin cell lines. Specific cell lines may include, but are not limited to SNU-16 cells, LS-174T cells, MC38 cells, TOV-112D cells, TOV-21G cells, Jurkat E6.1 cells, K-562 cells, B16-F0 cells, B16-F10 cells, LS180 cells, COLO205 cells, TB4 cells, HT29 cells, Panc1 cells, HPAC cells, HPAFII cells, RKO cells, SW480 cells, and SNU-C2A cells.

[00213] In some embodiments, ovarian cancer cell lines may be used. Such cell lines may include, but are not limited to SKOV3, OVCAR3, OV90 and A2870 cell lines. In some cases, CSC cells may be isolated from these cell lines by isolating cells expressing CD44 and/or CD133 cell markers.

[00214] OVCAR3 cells were first established using malignant ascites obtained from a patient suffering from progressive ovarian adenocarcinoma (Hamilton, T.C. et al., 1983. Cancer Res. 43: 5379-89). Cancer stem cell populations may be isolated from OVCAR3 cell cultures through selection based on specific cell surface markers such as CD44 (involved in cell adhesion and migration), CD133 and CD117 (Liang, D. et al., 2012. BMC Cancer. 12: 201, the contents of which are herein incorporated by reference in their entirety). OV90 cells are epithelial ovarian cancer cells that were similarly derived from human ascites (see US

Patent No. 5,710,038). OV-90 cells may also express CD44 when activated (Meunier, L. et al., 2010. Transl Oncol. 3(4): 230-8).

Glycan arrays

[00215] In some embodiments, glycan-interacting antibodies of the present invention may be developed through the use of glycan arrays. As used herein, the term “glycan array” refers to a tool used to identify agents that interact with any of a number of different glycans linked to the array substrate. In some embodiments, glycan arrays include a number of chemically-synthesized glycans, referred to herein as “glycan probes”. In some embodiments, glycan arrays include at least 2, at least 5, at least 10, at least 20, at least 30, at least 40, at least 50, at least 60, at least 70, at least 80, at least 90, at least 100, at least 150, at least 350, at least 1000 or at least 1500 glycan probes. In some embodiments, glycan arrays may be customized to present a desired set of glycan probes. In some embodiments, glycan probes may be attached to the array substrate by a linker molecule. Such linkers may include molecules including, but not limited to $-\text{O}(\text{CH}_2)_2\text{CH}_2\text{NH}_2$ and $\text{O}(\text{CH}_2)_3\text{NHCOCH}_2(\text{OCH}_2\text{CH}_2)_6\text{NH}_2$.

[00216] In some embodiments, a glycan array has more than 70 chemically-synthesized glycans, most of which are presented as Neu5Ac and Neu5Gc-containing glycan pairs. Some examples of glycan probes may include: Neu5Ac- α -2-6-GalNAc (AcSTn); Neu5Gc- α -2-6-GalNAc (GcSTn); Neu5,9Ac2- α -2,6-GalNAc; Neu9Ac5Gc- α -2,6-GalNAc, and GalNAc (Tn). The antibody binding specificity to AcSTn vs. GcSTn can be determined using the array or other methods of determining specificity known in the art. In addition, the binding profile of antibodies to O-acetylated STn can be determined. The loss of O-acetylation on STn is relevant to cancer as cancer-associated expression correlates with increased STn recognition by antibodies (Ogata, S. et al., Tumor-associated sialylated antigens are constitutively expressed in normal human colonic mucosa. Cancer Res. 1995 May 1;55(9):1869-74) In some cases, glycan arrays may be used to determine recognition of STn vs. Tn.

Antibody fragment display library screening techniques

[00217] In some embodiments, antibodies of the present invention may be produced and/or optimized using high throughput methods of discovery. Such methods may include any of the display techniques (e.g. display library screening techniques) disclosed in International Patent Application No. WO2014074532, the contents of which are herein incorporated by reference in their entirety. In some embodiments, synthetic antibodies may be designed, selected or

optimized by screening target antigens using display technologies (e.g. phage display technologies). Phage display libraries may include millions to billions of phage particles, each expressing unique antibody fragments on their viral coats. Such libraries may provide richly diverse resources that may be used to select potentially hundreds of antibody fragments with diverse levels of affinity for one or more antigens of interest (McCafferty, et al., 1990. Nature. 348:552-4; Edwards, B.M. et al., 2003. JMB. 334: 103-18; Schofield, D. et al., 2007. Genome Biol. 8, R254 and Pershad, K. et al., 2010. Protein Engineering Design and Selection. 23:279-88; the contents of each of which are herein incorporated by reference in their entirety). Often, the antibody fragments present in such libraries include scFv antibody fragments that include a fusion protein of V_H and V_L antibody domains joined by a flexible linker. In some cases, scFvs may contain the same sequence with the exception of unique sequences encoding variable loops of the complementarity determining regions (CDRs). In some cases, scFvs are expressed as fusion proteins, linked to viral coat proteins (e.g. the N-terminus of the viral pIII coat protein). V_L chains may be expressed separately for assembly with V_H chains in the periplasm prior to complex incorporation into viral coats. Precipitated library members may be sequenced from the bound phage to obtain cDNA encoding desired scFvs. Such sequences may be directly incorporated into antibody sequences for recombinant antibody production, or mutated and utilized for further optimization through in vitro affinity maturation.

Development of cytotoxic antibodies

[00218] In some embodiments, antibodies of the present invention may be capable of inducing antibody-dependent cell-mediated cytotoxicity (ADCC) and/or antibody-dependent cell phagocytosis (ADCP). ADCC is an immune mechanism whereby cells are lysed as a result of immune cell attack. Such immune cells may include CD56⁺ cells, CD3⁺ natural killer (NK) cells, monocytes and neutrophils (Strohl, W.R. Therapeutic Antibody Engineering. Woodhead Publishing, Philadelphia PA. 2012. Ch. 8, p186, the contents of which are herein incorporated by reference in their entirety).

[00219] In some cases, antibodies of the present invention may be engineered to include a given isotype depending on whether or not ADCC or ADCP is desired upon antibody binding. Such antibodies, for example, may be engineered according to any of the methods disclosed by Alderson, K.L. et al., J Biomed Biotechnol. 2011. 2011:379123). In the case of mouse antibodies, different isotypes of antibodies are more effective at promoting ADCC. IgG2a, for example, is more effective at inducing ADCC than is IgG2b. Some antibodies of

the present invention, including mouse IgG2b antibodies may be reengineered to be IgG2a antibodies. Such reengineered antibodies may be more effective at inducing ADCC upon binding cell-associated antigens. In some embodiments, antibodies are reengineered by modifying or introducing one or more post-translational modifications to improve ADCC and/or complement-dependent cytotoxicity (CDC) biological activity.

[00220] In some embodiments, genes encoding variable regions of antibodies developed according to methods of the present invention may be cloned into mammalian expression vectors encoding human Fc regions. Such Fc regions may be Fc regions from human IgG1k. IgG1k Fc regions may include amino acid mutations known to enhance Fc-receptor binding and ADCC.

Antibody drug conjugates

[00221] In some embodiments, antibodies of the invention may be developed for antibody drug conjugate (ADC) therapeutic applications. ADCs are antibodies in which one or more cargo (e.g., therapeutic agents) are attached [e.g. directly or via linker (e.g. a cleavable linker or a non-cleavable linker)]. ADCs are useful for delivery of therapeutic agents (e.g., drugs or cytotoxic agents) to one or more target cells or tissues (Panowski, S. et al., 2014. mAbs 6:1, 34-45). In some cases, ADCs may be designed to bind to a surface antigen on a targeted cell. Upon binding, the entire antibody-antigen complex may be internalized and directed to a cellular lysosome. ADCs may then be degraded, releasing the bound cargo. Where the cargo is a cytotoxic agent, the target cell will be killed or otherwise disabled. Cytotoxic agents may include, but are not limited to cytoskeletal inhibitors [e.g., tubulin polymerization inhibitors, and kinesin spindle protein (KSP) inhibitors], DNA damaging agents (e.g., calicheamicins, duocarmycins, and pyrrolobenzodiazepine dimers such as talirine and tesirine), topoisomerase inhibitors [e.g., camptothecin compounds or derivatives such as 7-ethyl-10-hydroxycamptothecin (SN-38) and exatecan derivative DXd], transcription inhibitors (e.g., RNA polymerase inhibitors such as amanitin), and kinase inhibitors [e.g., phosphoinositide 3-kinase (PI3K) inhibitors or mitogen-activated protein kinase kinase (MEK) inhibitors].

[00222] Tubulin polymerization inhibitors may include, but are not limited to, maytansines (e.g., emtansine [DM1] and ravtansine [DM4]), auristatins, tubulysins, and vinca alkaloids or derivatives thereof. Exemplary auristatins include auristatin E (also known as a derivative of dolastatin-10), auristatin EB (AEB), auristatin EFP (AEFP), monomethyl auristatin E (MMAE), monomethyl auristatin F (MMAF), auristatin F and dolastatin. Exemplary tubulysin compounds include naturally occurring tubulysins A, B, C, D, E, F, G, H, I, U, and

V, and tubulysin analogs such as pretubulysin D (PTb-D43) and N¹⁴-desacetoxytubulysin H (Tb1). Exemplary vinca alkaloids include vincristine, vinblastine, vindesine, and navelbine (vinorelbine). In some embodiments, cytotoxic agents may include auristatin derivatives [e.g. 1-aminopropan-2-yl-auristatin F, auristatin F-hydroxypropylamide, auristatin F-propylamide, auristatin F phenylenediamine (AFP)]; tubulysin derivatives; vinca alkaloid derivatives [e.g. N-(3-hydroxypropyl)vindesine (HPV)], and any of those described in U.S. Pat. Nos. 8,524,214; 8,685,383; 8,808,679; and 9,254,339; US Patent Application Publications US20150314008A1, US20160220696A1 and US20160022829A1; the contents of each of which are herein incorporated by reference in their entirety.

[00223] In some embodiments, antibody-drug conjugates (ADCs) of the invention may further comprise one or more polymeric carrier connecting the antibody and the therapeutic agents (e.g., antibody-polymer-drug conjugates). As used herein, the term “polymeric carrier” refers to a polymer or a modified polymer, which may be covalently attached to one or more therapeutic agents and/or antibodies. Polymeric carriers may provide additional conjugation sites for therapeutic agents, increasing the drug-to-antibody ratio and enhancing therapeutic effects of ADCs. In some embodiments, polymeric carriers used in this invention may be water soluble and/or biodegradable. Such polymeric carriers may include, but are not limited to poly(ethylene glycol) (PEG), poly(N-(2-hydroxypropyl)methacrylamide) (polyHPMA), poly(α -amino acids) [e.g., poly(L-lysine), poly(L-glutamic acid), and poly((N-hydroxyalkyl)glutamine)], carbohydrate polymers [e.g., dextrans, hydroxyethylstarch (HES), and polysialic acid], glycopolysaccharides (e.g., homopolysaccharide such as cellulose, amylose, dextran, levan, fucoidan, carrageenan, inulin, pectin, amylopectin, glycogen and lichenan; or homopolysaccharide such as agarose, hyaluronan, chondroitinsulfate, dermatansulfate, keratansulfate, alginic acid and heparin), glycolipids, glycoconjugates, polyglycerols, polyvinyl alcohols, poly(acrylic acid), polyketal and polyacetal [e.g., poly(1-hydroxymethylethylene hydroxymethylformal), also known as PHF or FLEXIMER®, described in U.S. Pat. Nos. 5,811,510; 5,863,990; and 5,958,398; the contents of each of which are herein incorporated by reference in their entirety], and derivatives, dendrimers, copolymers and mixtures thereof. For example, the polymeric carrier may include a copolymer of a polyacetal/polyketal (e.g., PHF) and a hydrophilic polymer such as polyacrylates, polyvinyl polymers, polyesters, polyorthoesters, polyamides, polypeptides, and derivatives thereof.

[00224] In some embodiments, therapeutic agents are attached (e.g., covalently bonded) to antibodies of the invention directly or via linkers. In some embodiments, therapeutic agents

are attached to polymeric carriers directly or via linkers, and the polymeric carriers are attached to the antibodies directly or via linkers. In some embodiments, linkers may comprise an oxalic, malonic, succinic, glutaric, adipic, pimelic, suberic, azelaic, sebacic, phthalic, isophthalic, terephthalic, diglycolic acid, tartaric, glutamic, fumaric, or aspartic moiety, including amide, imide, or cyclic-imide derivatives of each thereof, and each optionally substituted. Exemplary linkers may include any of those disclosed in U.S. Pat. No. 8,524,214; 8,685,383; 8,808,679; 9,254,339; and/or 9,555,112 the contents of each of which are herein incorporated by reference in their entirety.

[00225] In some embodiments, linkers may be cleavable linkers. Cleavable linkers may break down under certain conditions (such as changes in pH, temperature, or reduction) or cleaved by enzymes (e.g., proteases and glucuronidases) to allow release of therapeutic agents from ADCs. Such linkers may include a labile bond such as an ester bond, amide bond, or disulfide bond. Non-limiting cleavable linkers may include pH-sensitive linkers (e.g., hydrazone, semicarbazone, thiosemicarbazone, cis-aconitic amide, thioether, orthoester, acetal, or ketal); reduction-sensitive linkers [e.g., N-succinimidyl 3-(2-pyridyldithio)propionate (SPDP), N-succinimidyl 4-(2-pyridyldithio)butanoate (SPDB), N-succinimidyl 4-(2-pyridyldithio)pentanoate (SPP), N-succinimidyl-S-acetylthioacetate (SATA) and N-succinimidyl-oxycarbonyl-alpha-methyl-alpha-(2-pyridyl-dithio)toluene or 2,5-dioxypyrrolidin-1-yl 4-(1-(pyridin-2-yl)disulfanyl)ethyl)benzoate (SMPT)]; photosensitive linkers; and enzymatically cleavable linkers [e.g., peptide linkers such as valine-citrulline, valine-citrulline-p-aminobenzoyloxycarbonyl (vc-PAB), maleimidocaproyl-valine-citrulline-p-aminobenzoyloxycarbonyl (MC-vc-PAB), linkers cleavable by glucuronidases, such as glucuronide-MABC, or linkers cleavable by esterases].

[00226] In other embodiments, linkers may be non-cleavable linkers. Non-cleavable linkers may increase plasma stability of the ADCs compared to cleavable linkers. Exemplary non-cleavable linkers include maleimide alkane and maleimide cyclohexane (MCC).

[00227] Antibody-drug conjugates (ADCs) of the invention may be prepared using any method known in the art. For example, therapeutic agents may be modified to contain a functional group that can react with a functional group on the antibody. Antibody-drug conjugates (ADCs) may be prepared by reacting the two functional groups to form a conjugate. In some cases, polymeric carriers may be modified to contain functional groups that can react with the functional group on the therapeutic agents and the functional group on the antibody under different chemical conditions. Antibodies, polymeric carriers, and therapeutic agents may be linked to form the antibody-polymer-drug conjugates through

sequential chemical reactions. Conjugation to antibodies may employ a lysine or a cysteine residue as the conjugation site. In some embodiments, antibodies may be engineered to have additional lysine or cysteine residues. Such approaches may avoid disruption of antibody structure (e.g., interchain disulfide bonds) and maintain antibody stability and/or activity.

[00228] As described herein, drug-to-antibody ratio (DAR) is the average number of therapeutic agents (e.g., drugs or cytotoxic agents) conjugated to the antibodies. In some embodiments, drug-to-antibody ratio of an ADC of the invention is at least 1:1, at least 2:1, at least 4:1, at least 6:1, at least 8:1, at least 10:1, at least 12:1, at least 15:1, at least 20:1 or at least 25:1.

[00229] In some embodiments, antibodies of the invention may be tested for their ability to promote cell death when developed as ADCs. Cell viability assays may be performed in the presence and absence of secondary antibody-drug conjugates. Antibodies with potent cell growth inhibition may then be used to design direct antibody-drug conjugates (ADCs). The use of such secondary antibody-drug conjugates in cell-based cytotoxic assays may allow for quick pre-screening of many ADC candidates. Based on such assays, an unconjugated antibody candidate is directly added to cells in the presence of a secondary antibody that is conjugated to one or more cytotoxic agents (referred to herein as a 2^oADC). Internalization of the antibody/2^oADC complex into cells that express a high density of the targeted antigen can achieve a dose-dependent drug release within the cells, causing a cytotoxic effect to kill the cells (e.g., tumor cells), while cells expressing a low density of the targeted antigen are not affected (e.g., normal cells).

[00230] ADCs of the invention may be designed to target cancer cells. Such ADCs may include antibodies directed to one or more tumor-associated carbohydrate antigen (TACA). In some cases, ADCs of the invention are anti-STn antibodies. In some embodiments, ADCs include one or more of the variable domains presented in Table 2. Such ADC may also include at least one human IgG constant region, including, but not limited to any of those presented in Table 3.

Development of chimeric antigen receptors

[00231] In some embodiments, antibody sequences of the invention may be used to develop a chimeric antigen receptor (CAR). CARs are transmembrane receptors expressed on immune cells that facilitate recognition and killing of target cells (e.g. tumor cells). CARs typically include three basic parts. These include an ectodomain (also known as the recognition domain), a transmembrane domain and an intracellular (signaling) domain.

Ectodomains facilitate binding to cellular antigens on target cells, while intracellular domains typically include cell signaling functions to promote the killing of bound target cells. Further, they may have an extracellular domain with one or more antibody variable domains described herein or fragments thereof. CARs of the invention also include a transmembrane domain and cytoplasmic tail. CARs may be designed to include one or more segments of an antibody, antibody variable domain and/or antibody CDR, such that when such CARs are expressed on immune effector cells, the immune effector cells bind and clear any cells that are recognized by the antibody portions of the CARs.

[00232] Characteristics of CARs include their ability to redirect T-cell specificity and reactivity toward a selected target in a non-MHC -restricted manner, exploiting the antigen-binding properties of monoclonal antibodies. The non-MHC-restricted antigen recognition gives T cells expressing CARs the ability to recognize antigen independent of antigen processing, thus bypassing a major mechanism of tumor escape. Moreover, when expressed in T-cells, CARs advantageously do not dimerize with endogenous T cell receptor (TCR) alpha and beta chains.

[00233] CARs engineered to target tumors may have specificity for one or more tumor associated carbohydrate antigens (TACAs). In some embodiments, ectodomains of these CARs may include one or more antibody variable domains or a fragment thereof. In some embodiments, CARs are expressed in T cells, and may be referred to as “CAR-engineered T cells” or “CAR-Ts”. CAR-Ts may be engineered with CAR ectodomains having one or more antibody variable domains.

Structural features of chimeric antigen receptors

[00234] With gene-transfer technology, T cells can be engineered to stably express antibodies on their surface, conferring a desired antigen specificity. Chimeric antigen receptors (CARs) combine an antigen-recognition domain of a specific antibody with an intracellular domain of the CD3-zeta chain or FcγRI protein having T cell activating properties into a single chimeric fusion protein. CAR technology provides MHC-unrestricted recognition of target cells by T cells. Removal of the MHC restriction of T cells facilitates the use of these molecules in any patient, and also, in both CD8⁺ and CD4⁺ T cells, usually restricted to MHC class I or II epitopes, respectively. The use of Ab-binding regions allows T cells to respond to epitopes formed not only by protein, but also carbohydrate and lipid. This chimeric receptor approach is especially suited to immunotherapy of cancer, being able to bypass many of the mechanisms by which tumors avoid immunorecognition, such as MHC

down-regulation, lack of expression of costimulatory molecules, CTL resistance, and induction of T cell suppression, and where the use of both CD8⁺ CTL and CD4⁺ T cells are best combined for optimum antitumor efficacy. This approach has been demonstrated to be applicable to a wide range of tumor antigens, in addition to viruses such as HIV (Finney, et al., *J. Immunology*, 2004, 172:104-113).

[00235] Although chimeric antigen receptors can trigger T-cell activation in a manner similar to that of endogenous T-cell receptors, in practice, the clinical application of CAR technology has been impeded by inadequate *in vivo* expansion of chimeric antigen receptor T cells. For example, first generation CARs included as their signaling domain the cytoplasmic region of the CD3 ζ or Fc receptor γ chain. These first generation CARs were tested in phase I clinical studies in patients with ovarian cancer, renal cancer, lymphoma, and neuroblastoma, and were found to induce modest responses, effectively redirecting T cell cytotoxicity but failing to enable T cell proliferation and survival upon repeated antigen exposure. The prototypes for second generation CARs involved receptors encompassing both CD28 and CD3 ζ , and second generation CARs have been tested for treatment of B cell malignancies and other cancers (Sadelain, et al., (2009) *Current Opinion in Immunology*, 21(2):215-223). Thus, CARs have rapidly expanded into a diverse array of receptors with different functional properties.

[00236] More recently, it was discovered that CAR-mediated T-cell responses can be enhanced with the addition of a costimulatory domain. In preclinical models, the inclusion of the CD137 (4-1BB) signaling domain was found to significantly increase antitumor activity and *in vivo* persistence of chimeric antigen receptors as compared with inclusion of the CD3-zeta chain alone (Porter, et al., *N. Engl. J. Med.* 2011, 365:725-733).

[00237] Thus, in some embodiments of the present disclosure, antibody sequences of the invention may be used to develop a chimeric antigen receptor (CAR). In some embodiments, CARs are transmembrane receptors expressed on immune cells that facilitate recognition and killing of target cells (e.g. tumor cells).

[00238] In many cancers, tumor-specific antigens for targeting have not been defined, but in B-cell neoplasms, CD19 is an attractive target. Expression of CD19 is restricted to normal and malignant B cells and B-cell precursors. A pilot clinical trial of treatment with autologous T cells expressing an anti-CD19 chimeric antigen receptor (CART19) was performed in patients with advanced, p53-deficient chronic lymphoid leukemia (CLL). The generation of a CD19-specific immune response in bone marrow was demonstrated by temporal release of cytokines and ablation of leukemia cells that coincided with peak

infiltration of chimeric antigen receptor T cells. (Porter, *et al.*, *N. Engl. J. Med.* 2011, 365:725-733).

[00239] Further structural features of CARs may include any of those disclosed in several PCT Publications assigned to City of Hope and having the common inventor Michael Jensen. For example, PCT Publication WO 00/23573 describes genetically engineered, CD20-specific redirected T cells expressing a cell surface protein having an extracellular domain that includes a receptor specific for CD20, an intracellular signaling domain, and a transmembrane domain. Use of such cells for cellular immunotherapy of CD20⁺ malignancies and for abrogating any untoward B cell function. In one embodiment, the cell surface protein is a single chain FvFc:ζ receptor where Fv designates the VH and VL chains of a single chain monoclonal antibody to CD20 linked by peptide, Fc represents a hinge-CH2-CH3 region of a human IgG1, and ζ represents the intracellular signaling domain of the zeta chain of human CD3. A method of making a redirected T cell expressing a chimeric T cell receptor by electroporation using naked DNA encoding the receptor. Similarly, PCT Publication WO 02/077029 describes genetically engineered, CD19-specific redirected immune cells expressing a cell surface protein having an extracellular domain that includes a receptor which is specific for CD19, an intracellular signaling domain, and a transmembrane domain. Use of such cells for cellular immunotherapy of CD19⁺ malignancies and for abrogating any untoward B cell function. In one embodiment, the immune cell is a T cell and the cell surface protein is a scFvFc:ζ receptor where Fv designates the VH and VL chains of a single chain monoclonal antibody to CD19, Fc represents at least part of a constant region of an IgG1, and ζ represents the intracellular signaling domain of the T cell antigen receptor complex zeta chain (zeta chain of human CD3). The extracellular domain scFvFc and the intracellular domain zeta are linked by a transmembrane domain such as the transmembrane domain of CD4. A method of making a redirected T cell expressing a chimeric T cell receptor by electroporation using naked DNA encoding the receptor. These chimeric antigen receptors have the ability, when expressed in T cells, to redirect antigen recognition based on the monoclonal antibody's specificity. The design of scFvFc: receptors with target specificities for tumor cell-surface epitopes is a conceptually attractive strategy to generate antitumor immune effector cells for adoptive therapy as it does not rely on pre-existing anti-tumor immunity. These receptors are "universal" in that they bind antigen in a MHC independent fashion, thus, one receptor construct can be used to treat a population of patients with antigen positive tumors. City of Hope PCT Publications WO 02/088334, WO 2007/059298 and WO

2010/065818 describe “zetakines” made up of an extracellular domain that includes a soluble receptor ligand linked to a support region capable of tethering the extracellular domain to a cell surface, a transmembrane region and an intracellular signaling domain. Zetakines, when expressed on the surface of T lymphocytes, direct T cell activity to those specific cells expressing a receptor for which the soluble receptor ligand is specific.

[00240] Additional features of CARs may include any of those disclosed in two PCT Publications assigned to University of Texas and having a common inventor Lawrence Cooper. PCT Publication No. WO 2009/091826 describes compositions that include a human CD19-specific chimeric T cell receptor (or chimeric antigen receptor, CAR) polypeptide (designated hCD19CAR) that includes an intracellular signaling domain, a transmembrane domain and an extracellular domain, the extracellular domain including a human CD19 binding region. In another aspect, the CD19 binding region is an F(ab')₂, Fab', Fab, Fv or scFv. The intracellular domain may include an intracellular signaling domain of human CD3ζ and may further include human CD28 intracellular segment. In certain aspects the transmembrane domain is a CD28 transmembrane domain. PCT Publication No. WO 2013/074916 describes methods and compositions for immunotherapy employing CAR⁺ T cells genetically modified to eliminate expression of T cell receptor and/or HLA. In particular embodiments, the T cell receptor-negative and/or HLA-negative T cells are generated using zinc finger nucleases, for example. The CAR⁺ T cells from allogeneic healthy donors can be administered to any patient without causing graft versus host disease (GVHD), acting as universal reagents for off-the-shelf treatment of medical conditions such as cancer, autoimmunity, and infection.

[00241] PCT Publication WO 2011/041093 assigned to the U.S. Department of Health and Human Services describes anti-vascular endothelial growth factor receptor-2 chimeric antigen receptors that include an antigen binding domain of a KDR-1121 or DC101 antibody, an extracellular hinge domain, a T cell receptor transmembrane domain, and an intracellular T cell receptor signaling domain, and their use in the treatment of cancer.

[00242] PCT Publications WO 2012/079000 and WO 2013/040557, the contents of each of which are herein incorporated by reference in their entirety, are assigned to University of Pennsylvania and share the common inventor Carl H. June; these publications describe CARs comprising an antigen binding domain, a transmembrane domain, a costimulatory signaling region, and a CD3 zeta signaling domain, and methods for generating RNA CAR transfected T cells, respectively.

[00243] PCT Publication WO2013/126712, also assigned to University of Pennsylvania and sharing the common inventor Carl H. June, describes compositions and methods for generating a persisting population of T cells exhibiting prolonged exponential expansion in culture that is ligand independent and independent of the addition of exogenous cytokines or feeder cells, which are useful for the treatment of cancer. In some embodiments, the antigen binding domain is an anti-cMet binding domain. In some embodiments, the antigen binding domain is an anti-mesothelin binding domain. In some embodiments, the antigen binding domain is an anti-CD19 binding domain. The hinge domain is IgG4, the transmembrane domain is a CD28 transmembrane domain. In some embodiments, the costimulatory signaling region is a CD28 signaling region. Also provided is a vector comprising a nucleic acid sequence encoding a chimeric antigen receptor (CAR), and the CAR comprising an antigen binding domain, a hinge domain, a transmembrane domain, a costimulatory signaling region, and a CD3 zeta signaling domain.

[00244] PCT Publication WO 2014/039513 assigned to University of Pennsylvania describes compositions and methods for inhibiting one or more diacylglycerol kinase (DGK) isoform in a cell in order to enhance the cytolytic activity of the cell. The cells may be used in adoptive T cell transfer in which, the cell is modified to express a chimeric antigen receptor (CAR). Inhibition of DGK in T cells used in adoptive T cell transfer increases cytolytic activity of the T cells and thus may be used in the treatment of a variety of conditions, including cancer, infection, and immune disorders.

[00245] PCT Publication WO 2014/055771 assigned to University of Pennsylvania describes compositions and methods for treating ovarian cancer. Specifically, the invention relates to administering a genetically modified T cell having alpha-folate receptor (FR-alpha) binding domain and CD27 costimulatory domain to treat ovarian cancer. In one embodiment, the FR-alpha binding domain is said to be fully human, thereby preventing a host immune response.

[00246] In some embodiments, CARs of the invention may be engineered to target tumors. Such CARs may have specificity for one or more TACAs. In some case, ectodomains of these CARs may comprise one or more antibody variable domain presented herein or a fragment thereof. In some embodiments, CARs of the invention are expressed in T cells, referred to herein as “CAR-engineered T cells” or “CAR-Ts”. CAR-Ts may be engineered with CAR ectodomains having one or more antibody variable domain presented herein.

Multispecific antibodies

[00247] In some embodiments, antibodies of the present invention may bind more than one epitope. As used herein, the terms “multibody” or “multispecific antibody” refer to an antibody wherein two or more variable regions bind to different epitopes. The epitopes may be on the same or different targets. In certain embodiments, a multi-specific antibody is a “bispecific antibody,” which recognizes two different epitopes on the same or different antigens.

Bispecific antibodies

[00248] Bispecific antibodies are capable of binding two different antigens. Such antibodies typically comprise antigen-binding regions from at least two different antibodies. For example, a bispecific monoclonal antibody (BsMAb, BsAb) is an artificial protein composed of fragments of two different monoclonal antibodies, thus allowing the BsAb to bind to two different types of antigen. One common application for this technology is in cancer immunotherapy, where BsMAbs are engineered to simultaneously bind to a cytotoxic cell (using a receptor like CD3) and a target like a tumor cell to be destroyed.

[00249] Bispecific antibodies may include any of those described in Riethmuller, G., 2012. *Cancer Immunity*. 12:12-18; Marvin, J.S. *et al.*, 2005. *Acta Pharmacologica Sinica*. 26(6):649-58; and Schaefer, W. *et al.*, 2011. *PNAS*. 108(27):11187-92, the contents of each of which are herein incorporated by reference in their entirety.

[00250] New generations of BsMAb, called “trifunctional bispecific” antibodies, have been developed. These consist of two heavy and two light chains, one each from two different antibodies, where the two Fab regions (the arms) are directed against two antigens, and the Fc region (the foot) comprises the two heavy chains and forms the third binding site.

[00251] Of the two paratopes that form the tops of the variable domains of a bispecific antibody, one can be directed against a target antigen and the other against a T-lymphocyte antigen like CD3. In the case of trifunctional antibodies, the Fc region may additionally binds to a cell that expresses Fc receptors, like a macrophage, a natural killer (NK) cell or a dendritic cell. In sum, the targeted cell is connected to one or two cells of the immune system, which subsequently destroy it.

[00252] Other types of bispecific antibodies have been designed to overcome certain problems, such as short half-life, immunogenicity and side-effects caused by cytokine liberation. They include chemically linked Fabs, consisting only of the Fab regions, and various types of bivalent and trivalent single-chain variable fragments (scFvs), fusion proteins mimicking the variable domains of two antibodies. The furthest developed of these

newer formats are the bi-specific T-cell engagers (BiTEs) and mAb2's, antibodies engineered to contain an Fcab antigen-binding fragment instead of the Fc constant region.

[00253] A bispecific, single-chain antibody Fv fragment (Bs-scFv) was successfully used to kill cancer cells. Some human cancers are caused by functional defects in p53 that are restored by gene therapy with wild-type p53. Weisbart, *et al.*, describe the construction and expression of a bispecific single-chain antibody that penetrates living colon cancer cells, binds intracellular p53, and targets and restores its wild type function (Weisbart, *et al.*, *Int. J. Oncol.* 2004 Oct;25(4):1113-8; and Weisbart, *et al.*, *Int. J. Oncol.* 2004 Dec;25(6):1867-73). In these studies, a bispecific, single-chain antibody Fv fragment (Bs-scFv) was constructed from (i) a single-chain Fv fragment of mAb 3E10 that penetrates living cells and localizes in the nucleus, and (ii) a single-chain Fv fragment of a non-penetrating antibody, mAb PAb421 that binds the C-terminal of p53. PAb421 binding restores wild-type functions of some p53 mutants, including those of SW480 human colon cancer cells. The Bs-scFv penetrated SW480 cells and was cytotoxic, suggesting an ability to restore activity to mutant p53. COS-7 cells (monkey kidney cells with wild-type p53) served as a control since they are unresponsive to PAb421 due to the presence of SV40 large T antigen that inhibits binding of PAb421 to p53. Bs-scFv penetrated COS-7 cells but was not cytotoxic, thereby eliminating non-specific toxicity of Bs-scFv unrelated to binding p53. Fv fragments alone were not cytotoxic, indicating that killing was due to transduction of p53. A single mutation in CDR1 of PAb421 VH eliminated binding of the Bs-scFv to p53 and abrogated cytotoxicity for SW480 cells without altering cellular penetration, further supporting the requirement of PAb421 binding to p53 for cytotoxicity (Weisbart, *et al.*, *Int. J. Oncol.* 2004 Oct;25(4):1113-8; and Weisbart, *et al.*, *Int. J. Oncol.* 2004 Dec;25(6):1867-73).

[00254] In some embodiments, antibodies of the present invention may be diabodies. Diabodies are functional bispecific single-chain antibodies (bscAb). These bivalent antigen-binding molecules are composed of non-covalent dimers of scFvs, and can be produced in mammalian cells using recombinant methods. (See, e.g., Mack *et al*, *Proc. Natl. Acad. Sci.*, 92: 7021-7025, 1995). Few diabodies have entered clinical development. An iodine-123-labeled diabody version of the anti-CEA chimeric antibody cT84.66 has been evaluated for pre-surgical immunoscintigraphic detection of colorectal cancer in a study sponsored by the Beckman Research Institute of the City of Hope (Clinicaltrials.gov NCT00647153) (Nelson, A. L., *MAbs*.2010. Jan-Feb; 2(1):77-83).

[00255] Using molecular genetics, two scFvs can be engineered in tandem into a single polypeptide, separated by a linker domain, called a "tandem scFv" (tascFv). TascFvs have

been found to be poorly soluble and require refolding when produced in bacteria, or they may be manufactured in mammalian cell culture systems, which avoids refolding requirements but may result in poor yields. Construction of a tascFv with genes for two different scFvs yields a “bispecific single-chain variable fragments” (bis-scFvs). Only two tascFvs have been developed clinically by commercial firms; both are bispecific agents in active early phase development by Micromet for oncologic indications, and are described as “Bispecific T-cell Engagers (BiTE).” Blinatumomab is an anti-CD19/anti-CD3 bispecific tascFv that potentiates T-cell responses to B-cell non-Hodgkin lymphoma in Phase 2. MT110 is an anti-EP-CAM/anti-CD3 bispecific tascFv that potentiates T-cell responses to solid tumors in Phase 1. Bispecific, tetravalent “TandAbs” are also being researched by Affimed (Nelson, A. L., *MAbs*.2010. Jan-Feb; 2(1):77–83).

[00256] Also included are maxibodies (bivalent scFV fused to the amino terminus of the Fc (CH2-CH3 domains) of IgG).

[00257] Bispecific T-cell-engager (BiTE) antibodies are designed to transiently engage cytotoxic T-cells for lysis of selected target cells. These typically include two scFvs (one binding to CD3 on Tcells and one binding to a target antigen on the surface of a cell being targeted for destruction). In some embodiments, the two scFvs are joined by a linker. In other embodiments, the two scFvs are different regions on an antibody. The clinical activity of BiTE antibodies corroborates findings that *ex vivo* expanded, autologous T-cells derived from tumor tissue, or transfected with specific T-cell receptors, have shown therapeutic potential in the treatment of solid tumors. While these personalized approaches prove that T-cells alone can have considerable therapeutic activity, even in late-stage cancer, they are cumbersome to perform on a broad basis. This is different for cytotoxic T-lymphocyte antigen 4 (CTLA-4) antibodies, which facilitate generation of tumor-specific T-cell clones, and also for bi- and tri-specific antibodies that directly engage a large proportion of patients' T-cells for cancer cell lysis. The potential of global T-cell engagement for human cancer therapy by T-cell-engaging antibodies is under active investigation (Baeuerle PA, *et al.*, *Current Opinion in Molecular Therapeutics*. 2009, 11(1):22-30 and Baeuerle PA and Reinhardt C, *Cancer Res*. 2009, 69(12): 4941-4, the contents of each of which are herein incorporated by reference in their entirety).

[00258] Third generation molecules include “miniaturized” antibodies. Among the best examples of mAb miniaturization are the small modular immunopharmaceuticals (SMIPs) from Trubion Pharmaceuticals. These molecules, which can be monovalent or bivalent, are recombinant single-chain molecules containing one V_L, one V_H antigen-binding domain, and

one or two constant “effector” domains, all connected by linker domains. Presumably, such a molecule might offer the advantages of increased tissue or tumor penetration claimed by fragments while retaining the immune effector functions conferred by constant domains. At least three “miniaturized” SMIPs have entered clinical development. TRU-015, an anti-CD20 SMIP developed in collaboration with Wyeth, is the most advanced project, having progressed to Phase 2 for rheumatoid arthritis (RA). Earlier attempts in systemic lupus erythematosus (SLE) and B cell lymphomas were ultimately discontinued. Trubion and Facet Biotechnology are collaborating in the development of TRU-016, an anti-CD37 SMIP, for the treatment of CLL and other lymphoid neoplasias, a project that has reached Phase 2. Wyeth has licensed the anti-CD20 SMIP SBI-087 for the treatment of autoimmune diseases, including RA, SLE and possibly multiple sclerosis, although these projects remain in the earliest stages of clinical testing. (Nelson, A. L., *MAbs*.2010. Jan-Feb; 2(1):77–83).

[00259] Genmab is researching application of their “Unibody” technology, in which the hinge region has been removed from IgG4 molecules. While IgG4 molecules are unstable and can exchange light-heavy chain heterodimers with one another, deletion of the hinge region prevents heavy chain-heavy chain pairing entirely, leaving highly specific monovalent light/heavy heterodimers, while retaining the Fc region to ensure stability and extended half-life in vivo. This configuration may minimize the risk of immune activation or oncogenic growth, as IgG4 interacts poorly with FcRs and monovalent unibodies fail to promote intracellular signaling complex formation. These contentions are, however, largely supported by laboratory, rather than clinical, evidence. Biotechnol is also developing a “miniaturized” mAb, CAB051, which is a “compacted” 100 kDa anti-HER2 antibody in preclinical research (Nelson, A. L., *MAbs*.2010. Jan-Feb; 2(1):77–83).

[00260] Recombinant therapeutics composed of single antigen-binding domains have also been developed, although they currently account for only 4% of the clinical pipeline. These molecules are extremely small, with molecular weights approximately one-tenth of those observed for full-sized mAbs. Arana and Domantis engineer molecules composed of antigen-binding domains of human immunoglobulin light or heavy chains, although only Arana has a candidate in clinical testing, ART-621, an anti-TNF α molecule in Phase 2 study for the treatment of psoriasis and rheumatoid arthritis. Ablynx produces “nanobodies” derived from the antigen-binding variable heavy chain regions (V_{HHS}) of heavy chain antibodies found in camels and llamas, which lack light chains. Two Ablynx anti-von Willebrand Factor nanobodies have advanced to clinical development, including ALX-0081, in Phase 2 development as an intravenous therapy to prevent thrombosis in patients undergoing

percutaneous coronary intervention for acute coronary syndrome, and ALX-0681, a Phase 1 molecule for subcutaneous administration intended for both patients with acute coronary syndrome and thrombotic thrombocytopenic purpura (Nelson, A. L., *MAbs*.2010. Jan-Feb; 2(1):77–83).

Development of multispecific antibodies

[00261] In some embodiments, antibody sequences of the invention may be used to develop multispecific antibodies (*e.g.*, bispecific, trispecific, or of greater multispecificity). Multispecific antibodies can be specific for different epitopes of a target antigen of the present invention, or can be specific for both a target antigen of the present invention, and a heterologous epitope, such as a heterologous glycan, peptide or solid support material. (*See, e.g.*, WO 93/17715; WO 92/08802; WO 91/00360; WO 92/05793; Tutt, A. *et al.*, *Trispecific F(ab')₃ derivatives that use cooperative signaling via the TCR/CD3 complex and CD2 to activate and redirect resting cytotoxic T cells*. *J. Immunol.* 1991 Jul 1;147(1):60-9; U.S. Pat. Nos. 4,474,893; 4,714,681; 4,925,648; 5,573,920; 5,601,819; and Kostelny, S.A. *et al.*, *Formation of a bispecific antibody by the use of leucine zippers*. *J. Immunol.* 1992 Mar 1;148(5):1547-53); U.S. Pat. No. 5,932,448.

[00262] Disclosed and claimed in PCT Publication WO2014144573 to Memorial Sloan-Kettering Cancer Center are multimerization technologies for making dimeric multispecific binding agents (*e.g.*, fusion proteins comprising antibody components) with improved properties over multispecific binding agents without the capability of dimerization.

[00263] Disclosed and claimed in PCT Publication WO2014144357 to Merck Patent GMBH are tetravalent bispecific antibodies (TetBiAbs), and methods of making and methods of using TetBiAbs for diagnostics and for the treatment of cancer or immune disorders. TetBiAbs feature a second pair of Fab fragments with a second antigen specificity attached to the C-terminus of an antibody, thus providing a molecule that is bivalent for each of the two antigen specificities. The tetravalent antibody is produced by genetic engineering methods, by linking an antibody heavy chain covalently to a Fab light chain, which associates with its cognate, co-expressed Fab heavy chain.

[00264] Disclosed and claimed in PCT Publication WO2014028560 to IBC Pharmaceuticals, Inc. are T cell redirecting bispecific antibodies (bsAb), with at least one binding site for a T-cell antigen and at least one binding site for an antigen on a diseased cell or pathogen, for treatment of disease. Preferably, this bsAb is an anti-CD3 x anti-CD19 bispecific antibody, although antibodies against other T-cell antigens and/or disease-

associated antigens may be used. The complex is capable of targeting effector T cells to induce T-cell-mediated cytotoxicity of cells associated with a disease, such as cancer, autoimmune disease or infectious disease. The cytotoxic immune response is enhanced by co-administration of interferon-based agents that comprise interferon- α , interferon- β ; interferon- λ 1, interferon- λ 2 or interferon- λ 3.

[00265] Disclosed and claimed in PCT Publication WO2013092001 to Synimmune GMBH is a bispecific antibody molecule, as well as a method for producing the same, its use and a nucleic acid molecule encoding the bispecific antibody molecule. In particular is provided an antibody molecule that is capable of mediating target cell restricted activation of immune cells.

[00266] Disclosed and claimed in PCT Publication WO2012007167 is a multispecific modular antibody specifically binding to at least a glycoepitope and a receptor of the erbB class on the surface of a tumor cell, thereby crosslinking the glycoepitope and the receptor, which antibody has apoptotic activity effecting cytolysis independent of NK cells.

[00267] Disclosed and claimed in PCT Publications WO2012048332 and WO2013055404 are mediotopes, mediotope-binding antibodies, mediotope delivery systems, as well as a monoclonal antibody framework binding interface for mediotopes, and methods for their use. Specifically, two antibody binding peptides, C-QFDLSTRRLK-C ("cQFD"; sequence identification number 1 therein; SEQ ID NO: 17 herein) and C-QYNLSSRALK-C ("cQYN"; sequence identification number 2 therein; SEQ ID NO: 18 herein) were shown to have novel mAb binding properties. Also called "mediotopes," cQFD and cQYN were shown to bind to a region of the Fab framework of the anti-EGFR mAb cetuximab and not to bind the complementarity determining regions (CDRs) that bind antigen. The binding region on the Fab framework is distinct from other framework-binding antigens, such as the superantigens *Staphylococcal* protein A (SpA) (Graille *et al.*, 2000) and *Peptostreptococcus magnus* protein L (PpL) (Graille *et al.*, 2001). Accordingly, one embodiment disclosed is a framework binding interface comprising a framework region of a unique murine-human antibody or functional fragment thereof that binds a cyclic mediotope.

[00268] Exemplary patents and patent publications of interest are: U.S. Patent Nos. 5,585,089; 5,693,761; and 5,693,762, all filed Jun 7, 1995 and U.S. Patent No. 6,180,370, all assigned to Protein Design Labs, Inc., describe methods for producing, and compositions of, humanized immunoglobulins having one or more complementarity determining regions (CDR's) and possible additional amino acids from a donor immunoglobulin and a framework region from an accepting human immunoglobulin. Each humanized immunoglobulin chain is

said to usually comprise, in addition to the CDR's, amino acids from the donor immunoglobulin framework that are, *e.g.*, capable of interacting with the CDRs to effect binding affinity, such as one or more amino acids which are immediately adjacent to a CDR in the donor immunoglobulin or those within about 3 Å as predicted by molecular modeling. The heavy and light chains may each be designed by using any one or all of various position criteria. When combined into an intact antibody, the humanized immunoglobulins of the present invention is said to be substantially non-immunogenic in humans and retain substantially the same affinity as the donor immunoglobulin to the antigen, such as a protein or other compound containing an epitope.

[00269] U.S. Patent No. 5,951,983, assigned to Universite Catholique De Louvain and Bio Transplant, Inc., describes a humanized antibody against T-lymphocytes. Framework regions from a human V kappa gene designated as HUM5400 (EMBL accession X55400) and from the human antibody clone Amu 5-3 (GenBank accession number U00562) are set forth therein.

[00270] U.S. Patent No. 5,091,513, to Creative Biomolecules, Inc., describes a family of synthetic proteins having affinity for a preselected antigen. The proteins are characterized by one or more sequences of amino acids constituting a region which behaves as a biosynthetic antibody binding site (BABS). The sites comprise 1) non-covalently associated or disulfide bonded synthetic V_H and V_L dimers, 2) V_H-V_L or V_L-V_H single chains wherein the V_H and V_L are attached by a polypeptide linker, or 3) individuals V_H or V_L domains. The binding domains comprise linked CDR and FR regions, which may be derived from separate immunoglobulins. The proteins may also include other polypeptide sequences which function, *e.g.*, as an enzyme, toxin, binding site, or site of attachment to an immobilization media or radioactive atom. Methods are disclosed for producing the proteins, for designing BABS having any specificity that can be elicited by in vivo generation of antibody, and for producing analogs thereof.

[00271] U.S. Patent No. 8,399,625, to ESBATech, an Alcon Biomedical Research Unit, LLC, describes antibody acceptor frameworks and methods for grafting non-human antibodies, *e.g.*, rabbit antibodies, using a particularly well suited antibody acceptor framework.

Intrabodies

[00272] In some embodiments, antibodies of the present invention may be intrabodies. Intrabodies are a form of antibody that is not secreted from a cell in which it is produced, but

instead targets one or more intracellular proteins. Intrabodies are expressed and function intracellularly, and may be used to affect a multitude of cellular processes including, but not limited to intracellular trafficking, transcription, translation, metabolic processes, proliferative signaling and cell division. In some embodiments, methods described herein include intrabody-based therapies. In some such embodiments, variable domain sequences and/or CDR sequences disclosed herein are incorporated into one or more constructs for intrabody-based therapy. For example, intrabodies may target one or more glycosylated intracellular proteins or may modulate the interaction between one or more glycosylated intracellular proteins and an alternative protein.

[00273] More than two decades ago, intracellular antibodies against intracellular targets were first described (Biocca, Neuberger and Cattaneo *EMBO J.* 9: 101-108, 1990). The intracellular expression of intrabodies in different compartments of mammalian cells allows blocking or modulation of the function of endogenous molecules (Biocca, *et al.*, *EMBO J.* 9: 101-108, 1990; Colby *et al.*, *Proc. Natl. Acad. Sci. U.S.A.* 101: 17616-21, 2004). Intrabodies can alter protein folding, protein-protein, protein-DNA, protein-RNA interactions and protein modification. They can induce a phenotypic knockout and work as neutralizing agents by direct binding to the target antigen, by diverting its intracellular traffic or by inhibiting its association with binding partners. They have been largely employed as research tools and are emerging as therapeutic molecules for the treatment of human diseases as viral pathologies, cancer and misfolding diseases. The fast growing bio-market of recombinant antibodies provides intrabodies with enhanced binding specificity, stability and solubility, together with lower immunogenicity, for their use in therapy (Biocca, abstract in *Antibody Expression and Production Cell Engineering* Volume 7, 2011, pp. 179-195).

[00274] In some embodiments, intrabodies have advantages over interfering RNA (iRNA); for example, iRNA has been shown to exert multiple non-specific effects, whereas intrabodies have been shown to have high specificity and affinity of to target antigens. Furthermore, as proteins, intrabodies possess a much longer active half-life than iRNA. Thus, when the active half-life of the intracellular target molecule is long, gene silencing through iRNA may be slow to yield an effect, whereas the effects of intrabody expression can be almost instantaneous. Lastly, it is possible to design intrabodies to block certain binding interactions of a particular target molecule, while sparing others.

Development of intrabodies

[00275] Intrabodies are often single chain variable fragments (scFvs) expressed from a recombinant nucleic acid molecule and engineered to be retained intracellularly (*e.g.*, retained in the cytoplasm, endoplasmic reticulum, or periplasm). Intrabodies may be used, for example, to ablate the function of a protein to which the intrabody binds. The expression of intrabodies may also be regulated through the use of inducible promoters in the nucleic acid expression vector comprising the intrabody. Intrabodies may be produced using methods known in the art, such as those disclosed and reviewed in: (Marasco *et al.*, 1993 *Proc. Natl. Acad. Sci. USA*, 90: 7889-7893; Chen *et al.*, 1994, *Hum. Gene Ther.* 5:595-601; Chen *et al.*, 1994, *Proc. Natl. Acad. Sci. USA*, 91: 5932-5936; Maciejewski *et al.*, 1995, *Nature Med.*, 1: 667-673; Marasco, 1995, *Immunotech*, 1: 1-19; Mhashilkar, *et al.*, 1995, *EMBO J.* 14: 1542-51; Chen *et al.*, 1996, *Hum. Gene Therap.*, 7: 1515-1525; Marasco, *Gene Ther.* 4:11-15, 1997; Rondon and Marasco, 1997, *Annu. Rev. Microbiol.* 51:257-283; Cohen, *et al.*, 1998, *Oncogene* 17:2445-56; Proba *et al.*, 1998, *J. Mol. Biol.* 275:245-253; Cohen *et al.*, 1998, *Oncogene* 17:2445-2456; Hassanzadeh, *et al.*, 1998, *FEBS Lett.* 437:81-6; Richardson *et al.*, 1998, *Gene Ther.* 5:635-44; Ohage and Steipe, 1999, *J. Mol. Biol.* 291:1119-1128; Ohage *et al.*, 1999, *J. Mol. Biol.* 291:1129-1134; Wirtz and Steipe, 1999, *Protein Sci.* 8:2245-2250; Zhu *et al.*, 1999, *J. Immunol. Methods* 231:207-222; Arafat *et al.*, 2000, *Cancer Gene Ther.* 7:1250-6; der Maur *et al.*, 2002, *J. Biol. Chem.* 277:45075-85; Mhashilkar *et al.*, 2002, *Gene Ther.* 9:307-19; and Wheeler *et al.*, 2003, *FASEB J.* 17: 1733-5; and references cited therein). In particular, a CCR5 intrabody has been produced by Steinberger *et al.*, 2000, *Proc. Natl. Acad. Sci. USA* 97:805-810). See generally Marasco, WA, 1998, "Intrabodies: Basic Research and Clinical Gene Therapy Applications" Springer:New York; and for a review of scFvs, see Pluckthun in "The Pharmacology of Monoclonal Antibodies," 1994, vol. 113, Rosenberg and Moore *eds.* Springer-Verlag, New York, pp. 269-315.

[00276] In some embodiments, antibody sequences are used to develop intrabodies. Intrabodies are often recombinantly expressed as single domain fragments such as isolated VH and VL domains or as a single chain variable fragment (scFv) antibody within the cell. For example, intrabodies are often expressed as a single polypeptide to form a single chain antibody comprising the variable domains of the heavy and light chain joined by a flexible linker polypeptide. Intrabodies typically lack disulfide bonds and are capable of modulating the expression or activity of target genes through their specific binding activity. Single chain antibodies can also be expressed as a single chain variable region fragment joined to the light chain constant region.

[00277] As is known in the art, an intrabody can be engineered into recombinant polynucleotide vectors to encode sub-cellular trafficking signals at its N or C terminus to allow expression at high concentrations in the sub-cellular compartments where a target protein is located. For example, intrabodies targeted to the endoplasmic reticulum (ER) are engineered to incorporate a leader peptide and, optionally, a C-terminal ER retention signal, such as the KDEL (SEQ ID NO: 23) amino acid motif. Intrabodies intended to exert activity in the nucleus are engineered to include a nuclear localization signal. Lipid moieties are joined to intrabodies in order to tether the intrabody to the cytosolic side of the plasma membrane. Intrabodies can also be targeted to exert function in the cytosol. For example, cytosolic intrabodies are used to sequester factors within the cytosol, thereby preventing them from being transported to their natural cellular destination.

[00278] There are certain technical challenges with intrabody expression. In particular, protein conformational folding and structural stability of the newly-synthesized intrabody within the cell is affected by reducing conditions of the intracellular environment. In human clinical therapy, there are safety concerns surrounding the application of transfected recombinant DNA, which is used to achieve intrabody expression within the cell. Of particular concern are the various viral-based vectors commonly-used in genetic manipulation. Thus, one approach to circumvent these problems is to fuse protein transduction domains (PTD) to scFv antibodies, to create a 'cell-permeable' antibody or 'Transbody.' Transbodies are cell-permeable antibodies in which a protein transduction domain (PTD) is fused with single chain variable fragment (scFv) antibodies (Heng and Cao, 2005, *Med Hypotheses*. 64:1105-8).

[00279] Upon interaction with a target gene, an intrabody modulates target protein function and/or achieves phenotypic/functional knockout by mechanisms such as accelerating target protein degradation and sequestering the target protein in a non-physiological sub-cellular compartment. Other mechanisms of intrabody-mediated gene inactivation can depend on the epitope to which the intrabody is directed, such as binding to the catalytic site on a target protein or to epitopes that are involved in protein-protein, protein-DNA, or protein-RNA interactions.

[00280] In one embodiment, intrabodies are used to capture a target in the nucleus, thereby preventing its activity within the nucleus. Nuclear targeting signals are engineered into such intrabodies in order to achieve the desired targeting. Such intrabodies are designed to bind specifically to a particular target domain. In another embodiment, cytosolic intrabodies that specifically bind to a target protein are used to prevent the target from gaining access to the

nucleus, thereby preventing it from exerting any biological activity within the nucleus (e.g., preventing the target from forming transcription complexes with other factors).

[00281] In order to specifically direct the expression of such intrabodies to particular cells, the transcription of the intrabody is placed under the regulatory control of an appropriate tumor-specific promoter and/or enhancer. In order to target intrabody expression specifically to prostate, for example, the PSA promoter and/or promoter/enhancer can be utilized (See, for example, U.S. Patent No. 5,919,652 issued 6 July 1999).

[00282] Protein transduction domains (PTDs) are short peptide sequences that enable proteins to translocate across the cell membrane and be internalized within the cytosol, through atypical secretory and internalization pathways. There are a number of distinct advantages that a 'Transbody' would possess over conventional intrabodies expressed within the cell. For a start, 'correct' conformational folding and disulfide bond formation can take place prior to introduction into the target cell. More importantly, the use of cell-permeable antibodies or 'Transbodies' would avoid the overwhelming safety and ethical concerns surrounding the direct application of recombinant DNA technology in human clinical therapy, which is required for intrabody expression within the cell. 'Transbodies' introduced into the cell would possess only a limited active half-life, without resulting in any permanent genetic alteration. This would allay any safety concerns with regards to their application in human clinical therapy (Heng and Cao 2005, *Med Hypotheses*. 64:1105-8).

[00283] Intrabodies are promising therapeutic agents for the treatment of misfolding diseases, including Alzheimer's, Parkinson's, Huntington's and prion diseases, because of their virtually infinite ability to specifically recognize the different conformations of a protein, including pathological isoforms, and because they can be targeted to the potential sites of aggregation (both intra- and extracellular sites). These molecules can work as neutralizing agents against amyloidogenic proteins by preventing their aggregation, and/or as molecular shunters of intracellular traffic by rerouting the protein from its potential aggregation site (Cardinale, and Biocca, *Curr. Mol. Med.* 2008, 8:2-11).

[00284] Exemplary Patent Publications describing intracellular antibodies or intrabodies are set forth herein below, each of which is incorporated by reference in its entirety.

[00285] PCT Publication WO03014960 and US Patent 7,608,453 granted to Cattaneo, *et al.*, describe an intracellular antibody capture technology method of identifying at least one consensus sequence for an intracellular antibody (ICS) comprising the steps of: creating a database comprising sequences of validated intracellular antibodies (VIDA database) and aligning the sequences of validated intracellular antibodies according to Kabat; determining

the frequency with which a particular amino acid occurs in each of the positions of the aligned antibodies; selecting a frequency threshold value (LP or consensus threshold) in the range from 70% to 100%; identifying the positions of the alignment at which the frequency of a particular amino acid is greater than or equal to the LP value; and identifying the most frequent amino acid, in the position of said alignment.

[00286] PCT Publications WO0054057; WO03077945; WO2004046185; WO2004046186; WO2004046187; WO2004046188; WO2004046189; US Patent Application Publications US2005272107; US2005276800; US2005288492; US2010143939; granted US Patents 7,569,390 and 7,897,347 and granted European Patents EP1560853; and EP1166121 all assigned to the Medical Research Council and including inventors Cattaneo, *et al.*, describe intracellular single domain immunoglobulins, and a method for determining the ability of a immunoglobulin single domain to bind to a target in an intracellular environment, as well as methods for generating intracellular antibodies.

[00287] PCT Publication WO0235237; US Patent Application Publication 2003235850 and granted European Patent EP1328814 naming Cattaneo as an inventor and assigned to S.I.S.S.A. Scuola Internazionale Superiore describe a method for the *in vivo* identification of epitopes of an intracellular antigen.

[00288] PCT Publication WO2004046192 and European Patent EP1565558 assigned to Lay Line Genomics SPA and naming Cattaneo as an inventor describe a method for isolating intracellular antibodies that disrupt and neutralize an interaction between a protein ligand x and a protein ligand y inside a cell. Also disclosed are a method to identify a protein ligand x able to bind to a known y ligand using intracellular antibodies able to the interaction between x and y; and a method for the isolation of a set of antibody fragments against a significant proportion of the protein-protein interactions of a given cell (interactome) or against the protein interactions that constitute an intracellular pathway or network.

[00289] US Patent Application Publication 2006034834 and PCT Publication WO9914353 entitled "Intrabody-mediated control of immune reactions" and assigned to Dana Farber Cancer Institute Inc. name inventors Marasco and Mhashilkar are directed to methods of altering the regulation of the immune system, *e.g.*, by selectively targeting individual or classes of immunomodulatory receptor molecules (IRMs) on cells comprising transducing the cells with an intracellularly expressed antibody, or intrabody, against the IRMs. In a preferred embodiment the intrabody comprises a single chain antibody against an IRM, *e.g.*, MHC-1 molecules.

[00290] PCT Publication WO2013033420 assigned to Dana Farber Cancer Institute Inc. and Whitehead Biomedical Institute, and naming inventors Bradner, Rahl and Young describes methods and compositions useful for inhibiting interaction between a bromodomain protein and an immunoglobulin (Ig) regulatory element and downregulating expression of an oncogene translocated with an Ig locus, as well as for treating a cancer (*e.g.*, hematological malignancy) characterized by increased expression of an oncogene which is translocated with an Ig locus. Intrabodies are generally described.

[00291] PCT Publication WO02086096 and US Patent Application Publication 2003104402 entitled “Methods of producing or identifying intrabodies in eukaryotic cells,” assigned to University of Rochester Medical Center and naming inventors Zauderer, Wei and Smith describe a high efficiency method of expressing intracellular immunoglobulin molecules and intracellular immunoglobulin libraries in eukaryotic cells using a trimolecular recombination method. Further provided are methods of selecting and screening for intracellular immunoglobulin molecules and fragments thereof, and kits for producing, screening and selecting intracellular immunoglobulin molecules, as well as the intracellular immunoglobulin molecules and fragments produced using these methods.

[00292] PCT Publication WO2013023251 assigned to Affinity Biosciences PTY LTD and naming inventors Beasley, Niven and Kiefel describes polypeptides, such as antibody molecules and polynucleotides encoding such polypeptides, and libraries thereof, wherein the expressed polypeptides that demonstrate high stability and solubility. In particular, polypeptides comprising paired VL and VH domains that demonstrate soluble expression and folding in a reducing or intracellular environment are described, wherein a human scFv library was screened, resulting in the isolation of soluble scFv genes that have identical framework regions to the human germline sequence as well as remarkable thermostability and tolerance of CDR3 grafting onto the scFv scaffold.

[00293] European Patent Application EP2314622 and PCT Publications WO03008451 and WO03097697 assigned to Esbatech AG and University of Zuerich and naming inventors Ewert, Huber, Honneger and Plueckthun describe the modification of human variable domains and provide compositions useful as frameworks for the creation of very stable and soluble single-chain Fv (scFv) antibody fragments. These frameworks have been selected for intracellular performance and are thus ideally suited for the creation of scFv antibody fragments or scFv antibody libraries for applications where stability and solubility are limiting factors for the performance of antibody fragments, such as in the reducing

environment of a cell. Such frameworks can also be used to identify highly conserved residues and consensus sequences which demonstrate enhanced solubility and stability.

[00294] PCT Publication WO02067849 and US Patent Application Publication 2004047891 entitled "Systems devices and methods for intrabody targeted delivery and reloading of therapeutic agents" describe systems, devices and methods for intrabody targeted delivery of molecules. More particularly, some embodiments relate to a reloadable drug delivery system, which enables targeted delivery of therapeutic agents to a tissue region of a subject, in a localized and timely manner.

[00295] PCT Publication WO2005063817 and US Patent 7,884,054 assigned to Amgen Inc. and naming inventors Zhou, Shen and Martin describe methods for identifying functional antibodies, including intrabodies. In particular, a homodimeric intrabody is described, wherein each polypeptide chain of the homodimer comprises an Fc region, an scFv, and an intracellular localization sequence. The intracellular localization sequence may cause the intrabody to be localized to the ER or the Golgi. Optionally, each polypeptide chain comprises not more than one scFv.

[00296] PCT Publication WO2013138795 by Vogan, *et al.* and assigned to Permeon Biologics Inc. describes cell penetrating compositions for delivery of intracellular antibodies and antibody-like moieties and methods for delivering them (referred to herein as "AAM moieties" or "an AAM moiety") into a cell. Without being bound by theory, the present disclosure is based, at least in part, on the discovery that an AAM moiety can be delivered into a cell by complexing the AAM moiety with a cell penetrating polypeptide having surface positive charge (referred to herein as a "Surf+ Penetrating Polypeptide"). Examples of some applications of intraphilin technology are also provided

[00297] PCT Publication WO2010004432 assigned to the Pasteur Institute describes immunoglobulins from camelidae (camels, dromedaries, llamas and alpacas), about 50% of which are antibodies devoid of light chain. These heavy-chain antibodies interact with the antigen by the virtue of only one single variable domain, referred to as VHH(s), VHH domain(s) or VHH antibody (ies). Despite the absence of light chain, these homodimeric antibodies exhibit a broad antigen-binding repertoire by enlarging their hypervariable regions, and can act as a transbody and/or intrabody *in vitro* as well as *in vivo*, when the VHH domain is directed against an intracellular target.

[00298] PCT Publication WO2014106639 describes a method for identifying a cellular target involved in a cell phenotype by identifying an intrabody that can modify a cell phenotype and identifying a direct or indirect cellular target of the intrabody. In particular,

intrabodies 3H2-1, 3H2-VH and 5H4 are capable of inhibiting the degranulation reaction in mast cells triggered by an allergic stimulus; furthermore, intrabodies 3H2-1 and 5H4 directly or indirectly targeted a protein of the ABCF1 family and C120RF4 family, respectively. These ABCF1 and C120RF4 inhibitors are said to be useful in therapy, in particular for treating allergic and/or inflammatory conditions.

[00299] PCT Publication WO0140276 assigned to Urogenesis Inc. generally describes the possibility of inhibition of STEAP (Six Transmembrane Epithelial Antigen of the Prostate) proteins using intracellular antibodies (intrabodies).

[00300] PCT Publication WO02086505 assigned to University of Manchester and US Patent Application Publication US2004115740 naming inventors Simon and Benton describe a method for the intracellular analysis of a target molecule, wherein intrabodies are said to be preferred. In one embodiment, a vector (designated pScFv-ECFP) capable of expressing an anti-MUC1 intrabody coupled to CFP is described.

[00301] PCT Publication WO03095641 and WO0143778 assigned to Gene Therapy Systems Inc. describe compositions and methods for intracellular protein delivery, and intrabodies are generally described.

[00302] PCT Publication WO03086276 assigned to Selective Genetics Inc. describes a platform technology for the treatment of intracellular infections. Compositions and methods described therein include non-target specific vectors that target infectable cells via linked ligands that bind and internalize through cell surface receptors/moieties associated with infection. The vectors comprise exogenous nucleic acid sequences that are expressed upon internalization into a target cell. Vector associated ligands and nucleic acid molecules may be altered to target different infectious agents. In addition, the invention provides methods of identifying epitopes and ligands capable of directing internalization of a vector and capable of blocking viral entry.

[00303] PCT Publication WO03062415 assigned to Erasmus University describes a transgenic organism comprising a polynucleotide construct encoding an intracellular antibody which disrupts the catalysis of the production of the xenoantigen galactose α 1,3 galactose and/or a polynucleotide construct which encodes an intracellular antibody which binds specifically to a retrovirus protein, such as a PERV particle protein. Cells, tissues and organs of the transgenic organism may be used in xenotransplantation.

[00304] PCT Publication WO2004099775 entitled "Means for detecting protein conformation and applications thereof" describes the use of scFv fragments as conformation-specific antibodies for specifically detecting a conformational protein state, said to have

applications as sensors for following in living cells, upon intracellular expression, the behavior of endogenous proteins.

[00305] PCT Publication WO2008070363 assigned to Imclone Systems Inc. describes a single domain intrabody that binds to an intracellular protein or to an intracellular domain of an intracellular protein, such as Etk, the endothelial and epithelial tyrosine kinase, which is a member of the Tec family of non-receptor tyrosine kinases. Also provided is a method of inhibiting an intracellular enzyme, and treating a tumor in a patient by administering the intrabody or a nucleic acid expressing the intrabody.

[00306] PCT Publication WO2009018438 assigned to Cornell Research Foundation Inc. describes a method of identifying a protein that binds to a target molecule and has intracellular functionality, by providing a construct comprising a DNA molecule encoding the protein which binds to the target molecule, with the DNA molecule being coupled to a stall sequence. A host cell is transformed with the construct and then cultured under conditions effective to form, within the host cell, a complex of the protein whose translation has been stalled, the mRNA encoding the protein, and ribosomes. The protein in the complex is in a properly folded, active form and the complex is recovered from the cell. This method can be carried out with a cell-free extract preparation containing ribosomes instead of a host cell. The present invention also relates to a construct which includes a DNA molecule encoding a protein that binds to a target molecule and an SecM stalling sequence coupled to the DNA molecule. The DNA molecule and the SecM stalling sequence are coupled with sufficient distance between them to permit expression of their encoded protein, within the cell, in a properly folded, active form. The use of intrabodies is generally described.

[00307] PCT Publication WO2014030780 assigned to Mogam Biotech Research Institute describes a method named Tat-associated protein engineering (TAPE), for screening a target protein having higher solubility and excellent thermostability, in particular, an immunoglobulin variable domain (VH or VL) derived from human germ cells, by preparing a gene construct where the target protein and an antibiotic -resistant protein are linked to a Tat signal sequence, and then expressing this within *E. coli*. Also disclosed are human or engineered VH and VL domain antibodies and human or engineered VH and VL domain antibody scaffolds having solubility and excellent thermostability, which are screened by the TAPE method. Also provided is a library including random CDR sequences in the human or engineered VH or VL domain antibody scaffold screened by the TAPE method, a preparing method thereof, a VH or VL domain antibody having binding ability to the target protein

screened by using the library, and a pharmaceutical composition including the domain antibody.

[00308] European Patent Application EP2422811 describes an antibody that binds to an intracellular epitope; such intrabodies comprise at least a portion of an antibody that is capable of specifically binding an antigen and preferably does not contain operable sequences coding for its secretion and thus remains within the cell. In one embodiment, the intrabody comprises a scFv. 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. Also described is a specific embodiment in which the intrabody binds to the cytoplasmic domain of an Eph receptor and prevents its signaling (*e.g.*, autophosphorylation). In another specific embodiment, an intrabody binds to the cytoplasmic domain of a B-type Ephrin (*e.g.*, EphrinB1, EphrinB2 or EphrinB3).

[00309] PCT Publication WO2011003896 and European Patent Application EP2275442 describe intracellular functional PCNA-Chromobodies made using nucleic acid molecule encoding a polypeptide specifically binding to proliferating cell nuclear antigen (PCNA). Examples of such polypeptides comprising conservative substitutions of one or more amino acids in one or two framework regions include

MANVQLNESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSDISPS
GAVKAYSDSVKGRFTISRDNNAKRLYLQMNSLTPEDTGEYFCTKVQSPRTRIPAPSS
QGTQVTVSS (SEQ ID NO: 19) and

MANVQLNESGGGLVQPGGSLRLSCAASGFTFSSYAMSWVRQAPGKGLEWVSEISPS
GAVKAYSDSVKGRFTISRDNNAKRLYLQMNSLTPEDTGEYFCTKVQSPRTRIPAPSS
QGTQVTVSS (SEQ ID NO: 20), including the framework regions of the polypeptides. In the examples, the framework regions as well as the CDR regions involved in the binding of PCNA have been determined.

[00310] European Patent Application EP2703485 describes a method for selecting plasma cells or plasmablasts, as well as for producing target antigen specific antibodies, and novel monoclonal antibodies. In one embodiment, cells expressing intracellular immunoglobulin were identified.

Antibody-coated agents

[00311] In some embodiments, antibodies or antibody fragments described herein may be used to prepare a composition that includes an antibody-coated agent. As used herein, the term “antibody-coated agent” refers to any particle, nanoparticle, molecule, protein, fusion-

protein, lipid, liposome, cell membrane, cell, or other structure that includes one or more surface-associated antibodies or antibody fragments. Antibody-coated agents may target one or more glycans, proteins, cells, tissues, and/or organs based on the specificity of the antibody or antibody fragments used for coating.

[00312] Antibody-coated agents may include associated, enclosed, or embedded cargo. The cargo may be a detectable label. Some cargo may include one or more therapeutic agent. Such therapeutic agents may include, but are not limited to drugs, chemotherapeutic agents, and cytotoxic agents. Cytotoxic agents may be used to kill or otherwise disable a cell. Cytotoxic agents may include, but are not limited to cytoskeletal inhibitors [e.g., tubulin polymerization inhibitors such as maytansines or auristatins (e.g. monomethyl auristatin E [MMAE] and monomethyl auristatin F [MMAF]), and kinesin spindle protein (KSP) inhibitors], DNA damaging agents (e.g., calicheamicins, duocarmycins, and pyrrolobenzodiazepine dimers such as talirine and tesirine), topoisomerase inhibitors [e.g., camptothecin compounds or derivatives such as 7-ethyl-10-hydroxycamptothecin (SN-38) and exatecan derivative DXd], transcription inhibitors (e.g., RNA polymerase inhibitors such as amanitin), and kinase inhibitors [e.g., phosphoinositide 3-kinase (PI3K) inhibitors or mitogen-activated protein kinase kinase (MEK) inhibitors].

[00313] In some embodiments, antibody-coated agents may include nanoparticles coated with one or more antibodies or antibody fragments described herein. Such antibody-coated agents may target one or more glycan, including, but not limited to cell-associated glycans. Some such antibody-coated agents include one or more cytotoxic agents.

Proteins and Variants

[00314] Glycan-interacting antibodies of the present invention may exist as a whole polypeptide, a plurality of polypeptides or fragments of polypeptides, which independently may be encoded by one or more nucleic acids, a plurality of nucleic acids, fragments of nucleic acids or variants of any of the aforementioned. As used herein, “polypeptide” means a polymer of amino acid residues (natural or unnatural) linked together most often by peptide bonds. The term, as used herein, refers to proteins, polypeptides, and peptides of any size, structure, or function. In some instances the polypeptide encoded is smaller than about 50 amino acids and the polypeptide is then termed a peptide. If the polypeptide is a peptide, it will be at least about 2, 3, 4, or at least 5 amino acid residues long. Thus, polypeptides include gene products, naturally occurring polypeptides, synthetic polypeptides, homologs, orthologs, paralogs, fragments and other equivalents, variants, and analogs of the

foregoing. A polypeptide may be a single molecule or may be a multi-molecular complex such as a dimer, trimer or tetramer. They may also include single chain or multichain polypeptides and may be associated or linked. The term polypeptide may also apply to amino acid polymers in which one or more amino acid residues are an artificial chemical analogue of a corresponding naturally occurring amino acid.

[00315] The term “polypeptide variant” refers to molecules which differ in their amino acid sequence from a native or reference sequence. The amino acid sequence variants may possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence, as compared to a native or reference sequence. Ordinarily, variants will possess at least about 50% identity (homology) to a native or reference sequence, and preferably, they will be at least about 80%, more preferably at least about 90% identical (homologous) to a native or reference sequence.

[00316] In some embodiments “variant mimics” are provided. As used herein, the term “variant mimic” is one which contains one or more amino acids which would mimic an activated sequence. For example, glutamate may serve as a mimic for phospho-threonine and/or phospho-serine. Alternatively, variant mimics may result in deactivation or in an inactivated product containing the mimic, e.g., phenylalanine may act as an inactivating substitution for tyrosine; or alanine may act as an inactivating substitution for serine. The amino acid sequences of the glycan-interacting antibodies of the invention may include naturally occurring amino acids and as such may be considered to be proteins, peptides, polypeptides, or fragments thereof.

Alternatively, the glycan-interacting antibodies may include both naturally and non-naturally occurring amino acids.

[00317] The term “amino acid sequence variant” refers to molecules with some differences in their amino acid sequences as compared to a native or starting sequence. The amino acid sequence variants may possess substitutions, deletions, and/or insertions at certain positions within the amino acid sequence. “Native” or “starting” sequence should not be confused with a wild type sequence. As used herein, a native or starting sequence is a relative term referring to an original molecule against which a comparison may be made. “Native” or “starting” sequences or molecules may represent the wild-type (that sequence found in nature) but do not have to be the wild-type sequence.

[00318] Ordinarily, variants will possess at least 70%, at least 75%, at least 80%, at least 85%, at least 90%, at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, at least 99.5% at least 99.8%, or at least 99.9% sequence identity as compared to a native sequence.

"Sequence identity" as it applies to amino acid sequences or nucleotide sequences is defined as the percentage of residues in the candidate sequence that are identical with the residues in the second sequence after aligning the sequences and taking gaps and fragments into consideration, if necessary, to achieve the maximum percent sequence identity. Calculation of the percent identity of two polymeric sequences, for example, can be performed by aligning the two sequences for optimal comparison purposes (*e.g.*, gaps can be introduced in one or both of a first and a second polymeric sequence for optimal alignment and non-identical sequences can be disregarded for comparison purposes). In certain embodiments, the length of a sequence aligned for comparison purposes is at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 90%, at least 95%, or 100% of the length of the reference sequence. The residues at corresponding positions are then compared. When a position in the first sequence is occupied by the same residue 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 needs to be introduced for optimal alignment of the two sequences. The comparison of sequences and determination of percent identity between two sequences can be accomplished using a mathematical algorithm. For example, the percent identity between two nucleotide sequences can be determined using methods such as those described in Computational Molecular Biology, Lesk, A. M., ed., Oxford University Press, New York, 1988; Biocomputing: Informatics and Genome Projects, Smith, D. W., ed., Academic Press, New York, 1993; Sequence Analysis in Molecular Biology, von Heinje, G., Academic Press, 1987; Computer Analysis of Sequence Data, Part I, Griffin, A. M., and Griffin, H. G., eds., Humana Press, New Jersey, 1994; and Sequence Analysis Primer, Gribskov, M. and Devereux, J., eds., M Stockton Press, New York, 1991; each of which is incorporated herein by reference. For example, the percent identity between two nucleotide sequences can be determined using the algorithm of Meyers and Miller (CABIOS, 1989, 4:11-17), which has been incorporated into the ALIGN program (version 2.0) using a PAM120 weight residue table, a gap length penalty of 12 and a gap penalty of 4. The percent identity between two nucleotide sequences can, alternatively, be determined using the GAP program in the GCG software package using an NWSgapdna.CMP matrix. Methods commonly employed to determine percent identity between sequences include, but are not limited to those disclosed in Carillo, H., and Lipman, D., SIAM J Applied Math., 48:1073 (1988); incorporated herein by reference. Techniques for determining identity are codified in publicly available computer programs. Exemplary

computer software to determine homology between two sequences include, but are not limited to, GCG program package, Devereux, J., *et al.*, *Nucleic Acids Research*, 12(1), 387 (1984)), BLASTP, BLASTN, and FASTA Altschul, S. F. *et al.*, *J. Molec. Biol.*, 215, 403 (1990)).

[00319] By "homologs" as it applies to amino acid sequences is meant the corresponding sequence of other species having substantial identity to a second sequence of a second species.

[00320] "Analog" is meant to include polypeptide variants which differ by one or more amino acid alterations, e.g., substitutions, additions or deletions of amino acid residues that still maintain the properties of the parent polypeptide.

[00321] The present invention contemplates several types of glycan-interacting antibodies which are amino acid based including variants and derivatives. These include substitutional, insertional, deletion and covalent variants and derivatives. As such, included within the scope of this invention are glycan-interacting antibody molecules containing substitutions, insertions and/or additions, deletions and covalently modifications. For example, sequence tags or amino acids, such as one or more lysines, can be added to the peptide sequences of the invention (e.g., at the N-terminal or C-terminal ends). Sequence tags can be used for peptide purification or localization. Lysines can be used to increase peptide solubility or to allow for biotinylation. Alternatively, amino acid residues located at the carboxy and amino terminal regions of the amino acid sequence of a peptide or protein may optionally be deleted providing for truncated sequences. Certain amino acids (e.g., C-terminal or N-terminal residues) may alternatively be deleted depending on the use of the sequence, as for example, expression of the sequence as part of a larger sequence which is soluble, or linked to a solid support.

[00322] "Substitutional variants" when referring to proteins are those that have at least one amino acid residue in a native or starting sequence removed and a different amino acid inserted in its place at the same position. The substitutions may be single, where only one amino acid in the molecule has been substituted, or they may be multiple, where two or more amino acids have been substituted in the same molecule.

[00323] As used herein the term "conservative amino acid substitution" refers to the substitution of an amino acid that is normally present in the sequence with a different amino acid of similar size, charge, or polarity. Examples of conservative substitutions include the substitution of a non-polar (hydrophobic) residue such as isoleucine, valine and leucine for another non-polar residue. Likewise, examples of conservative substitutions include the

substitution of one polar (hydrophilic) residue for another such as between arginine and lysine, between glutamine and asparagine, and between glycine and serine. Additionally, the substitution of a basic residue such as lysine, arginine or histidine for another, or the substitution of one acidic residue such as aspartic acid or glutamic acid for another acidic residue are additional examples of conservative substitutions. Examples of non-conservative substitutions include the substitution of a non-polar (hydrophobic) amino acid residue such as isoleucine, valine, leucine, alanine, methionine for a polar (hydrophilic) residue such as cysteine, glutamine, glutamic acid or lysine and/or a polar residue for a non-polar residue.

[00324] "Insertional variants" when referring to proteins are those with one or more amino acids inserted immediately adjacent to an amino acid at a particular position in a native or starting sequence. "Immediately adjacent" to an amino acid means connected to either the alpha-carboxy or alpha-amino functional group of the amino acid.

[00325] "Deletional variants" when referring to proteins, are those with one or more amino acids in the native or starting amino acid sequence removed. Ordinarily, deletional variants will have one or more amino acids deleted in a particular region of the molecule.

[00326] As used herein, the term "derivative" is used synonymously with the term "variant" and refers to a molecule that has been modified or changed in any way relative to a reference molecule or starting molecule. In some embodiments, derivatives include native or starting proteins that have been modified with an organic proteinaceous or non-proteinaceous derivatizing agent, and post-translational modifications. Covalent modifications are traditionally introduced by reacting targeted amino acid residues of the protein with an organic derivatizing agent that is capable of reacting with selected side-chains or terminal residues, or by harnessing mechanisms of post-translational modifications that function in selected recombinant host cells. The resultant covalent derivatives are useful in programs directed at identifying residues important for biological activity, for immunoassays, or for the preparation of anti-protein antibodies for immunoaffinity purification of the recombinant glycoprotein. Such modifications are within the ordinary skill in the art and are performed without undue experimentation.

[00327] Certain post-translational modifications are the result of the action of recombinant host cells on the expressed polypeptide. Glutaminyl and asparaginyl residues are frequently post-translationally deamidated to the corresponding glutamyl and aspartyl residues. Alternatively, these residues are deamidated under mildly acidic conditions. Either form of these residues may be present in the proteins used in accordance with the present invention.

[00328] Other post-translational modifications include hydroxylation of proline and lysine, phosphorylation of hydroxyl groups of seryl or threonyl residues, methylation of the alpha-amino groups of lysine, arginine, and histidine side chains (T. E. Creighton, *Proteins: Structure and Molecular Properties*, W.H. Freeman & Co., San Francisco, pp. 79-86 (1983)).

[00329] Covalent derivatives specifically include fusion molecules in which proteins of the invention are covalently bonded to a non-proteinaceous polymer. The non-proteinaceous polymer ordinarily is a hydrophilic synthetic polymer, i.e. a polymer not otherwise found in nature. However, polymers which exist in nature and are produced by recombinant or in vitro methods are useful, as are polymers which are isolated from nature. Hydrophilic polyvinyl polymers fall within the scope of this invention, e.g. polyvinylalcohol and polyvinylpyrrolidone. Particularly useful are polyvinylalkylene ethers such as polyethylene glycol, polypropylene glycol. The proteins may be linked to various non-proteinaceous polymers, such as polyethylene glycol, polypropylene glycol or polyoxyalkylenes, in the manner set forth in U.S. Pat. No. 4,640,835; 4,496,689; 4,301,144; 4,670,417; 4,791,192 or 4,179,337.

[00330] "Features" when referring to proteins are defined as distinct amino acid sequence-based components of a molecule. Features of the proteins of the present invention include surface manifestations, local conformational shape, folds, loops, half-loops, domains, half-domains, sites, termini or any combination thereof.

[00331] As used herein when referring to proteins the term "surface manifestation" refers to a polypeptide based component of a protein appearing on an outermost surface.

[00332] As used herein when referring to proteins the term "local conformational shape" means a polypeptide based structural manifestation of a protein which is located within a definable space of the protein.

[00333] As used herein when referring to proteins the term "fold" means the resultant conformation of an amino acid sequence upon energy minimization. A fold may occur at the secondary or tertiary level of the folding process. Examples of secondary level folds include beta sheets and alpha helices. Examples of tertiary folds include domains and regions formed due to aggregation or separation of energetic forces. Regions formed in this way include hydrophobic and hydrophilic pockets, and the like.

[00334] As used herein the term "turn" as it relates to protein conformation means a bend which alters the direction of the backbone of a peptide or polypeptide and may involve one, two, three or more amino acid residues.

[00335] As used herein when referring to proteins the term "loop" refers to a structural feature of a peptide or polypeptide which reverses the direction of the backbone of a peptide or polypeptide and includes four or more amino acid residues. Oliva et al. have identified at least 5 classes of protein loops (J. Mol Biol 266 (4): 814-830; 1997).

[00336] As used herein when referring to proteins the term "half-loop" refers to a portion of an identified loop having at least half the number of amino acid residues as the loop from which it is derived. It is understood that loops may not always contain an even number of amino acid residues. Therefore, in those cases where a loop contains or is identified to include an odd number of amino acids, a half-loop of the odd-numbered loop will include the whole number portion or next whole number portion of the loop (number of amino acids of the loop/2+/-0.5 amino acids). For example, a loop identified as a 7 amino acid loop could produce half-loops of 3 amino acids or 4 amino acids ($7/2=3.5\pm0.5$ being 3 or 4).

[00337] As used herein when referring to proteins the term "domain" refers to a motif of a polypeptide having one or more identifiable structural or functional characteristics or properties (e.g., binding capacity, serving as a site for protein-protein interactions).

[00338] As used herein when referring to proteins the term "half-domain" means portion of an identified domain having at least half the number of amino acid residues as the domain from which it is derived. It is understood that domains may not always contain an even number of amino acid residues. Therefore, in those cases where a domain contains or is identified to include an odd number of amino acids, a half-domain of the odd-numbered domain will include the whole number portion or next whole number portion of the domain (number of amino acids of the domain/2+/-0.5 amino acids). For example, a domain identified as a 7 amino acid domain could produce half-domains of 3 amino acids or 4 amino acids ($7/2=3.5\pm0.5$ being 3 or 4). It is also understood that sub-domains may be identified within domains or half-domains, these subdomains possessing less than all of the structural or functional properties identified in the domains or half domains from which they were derived. It is also understood that the amino acids of any of the domain types herein need not be contiguous along the backbone of the polypeptide (i.e., nonadjacent amino acids may fold structurally to produce a domain, half-domain or subdomain).

[00339] As used herein when referring to proteins the terms "site" as it pertains to amino acid based embodiments is used synonymous with "amino acid residue" and "amino acid side chain". A site represents a position within a peptide or polypeptide that may be modified, manipulated, altered, derivatized or varied within the polypeptide based molecules of the present invention.

[00340] As used herein the terms "termini or terminus" when referring to proteins refers to an extremity of a peptide or polypeptide. Such extremity is not limited only to the first or final site of the peptide or polypeptide but may include additional amino acids in the terminal regions. The polypeptide based molecules of the present invention may be characterized as having both an N-terminus (terminated by an amino acid with a free amino group (NH₂)) and a C-terminus (terminated by an amino acid with a free carboxyl group (COOH)). Proteins of the invention are in some cases made up of multiple polypeptide chains brought together by disulfide bonds or by non-covalent forces (multimers, oligomers). These sorts of proteins will have multiple N- and C-termini. Alternatively, the termini of the polypeptides may be modified such that they begin or end, as the case may be, with a non-polypeptide based moiety such as an organic conjugate.

[00341] Once any of the features have been identified or defined as a component of a molecule of the invention, any of several manipulations and/or modifications of these features may be performed by moving, swapping, inverting, deleting, randomizing or duplicating. Furthermore, it is understood that manipulation of features may result in the same outcome as a modification to the molecules of the invention. For example, a manipulation which involved deleting a domain would result in the alteration of the length of a molecule just as modification of a nucleic acid to encode less than a full-length molecule would.

[00342] Modifications and manipulations can be accomplished by methods known in the art such as site directed mutagenesis. The resulting modified molecules may then be tested for activity using in vitro or in vivo assays such as those described herein or any other suitable screening assay known in the art.

Isotopic variations

[00343] The glycan-interacting antibodies of the present invention may contain one or more atoms that are isotopes. As used herein, the term "isotope" refers to a chemical element that has one or more additional neutron. In one embodiment, compounds of the present invention may be deuterated. As used herein, the term "deuterated" refers to a substance that has had one or more hydrogen atoms replaced by deuterium isotopes. Deuterium isotopes are isotopes of hydrogen. The nucleus of hydrogen contains one proton while deuterium nuclei contain both a proton and a neutron. The glycan-interacting antibodies may be deuterated in order to change a physical property of the compound, such as stability, or to allow the compounds to be used in diagnostic and experimental applications.

Conjugates and Combinations

[00344] It is contemplated by the present invention that the glycan-interacting antibodies of the present invention may be complexed, conjugated or combined with one or more homologous or heterologous molecules. As used herein, “homologous molecule” means a molecule which is similar in at least one of structure or function relative to a starting molecule while a “heterologous molecule” is one that differs in at least one of structure or function relative to a starting molecule. Structural homologs are therefore molecules which are substantially structurally similar. They can be identical. Functional homologs are molecules which are substantially functionally similar. They can be identical.

[00345] Glycan-interacting antibodies of the invention may include conjugates. Such conjugates of the invention may include a naturally occurring substance or ligand, such as a protein (e.g., human serum albumin (HSA), low-density lipoprotein (LDL), high-density lipoprotein (HDL), or globulin); a carbohydrate (e.g., a dextran, pullulan, chitin, chitosan, inulin, cyclodextrin or hyaluronic acid); or a lipid. The ligand may also be a recombinant or synthetic molecule, such as a synthetic polymer, e.g., a synthetic polyamino acid, an oligonucleotide (e.g. an aptamer). Examples of polyamino acids include polyamino acid is a polylysine (PLL), poly L-aspartic acid, poly L-glutamic acid, styrene-maleic acid anhydride copolymer, poly(L-lactide-co-glycolide) copolymer, divinyl ether-maleic anhydride copolymer, N-(2-hydroxypropyl)methacrylamide copolymer (HMPA), polyethylene glycol (PEG), polyvinyl alcohol (PVA), polyurethane, poly(2-ethylacrylic acid), N-isopropylacrylamide polymers, or polyphosphazine. Example of polyamines include: polyethylenimine, polylysine (PLL), spermine, spermidine, polyamine, pseudopeptide-polyamine, peptidomimetic polyamine, dendrimer polyamine, arginine, amidine, protamine, cationic lipid, cationic porphyrin, quaternary salt of a polyamine, or an alpha helical peptide.

[00346] The conjugates can also include targeting groups, e.g., a cell or tissue targeting agent or group, e.g., a lectin, glycoprotein, lipid or protein, e.g., an antibody, that binds to a specified cell type such as a kidney cell. A targeting group can be a thyrotropin, melanotropin, lectin, glycoprotein, surfactant protein A, mucin carbohydrate, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-gulucosamine multivalent mannose, multivalent fucose, glycosylated polyaminoacids, multivalent galactose, transferrin, bisphosphonate, polyglutamate, polyaspartate, a lipid, cholesterol, a steroid, bile acid, folate, vitamin B12, biotin, an RGD peptide, an RGD peptide mimetic or an aptamer.

[00347] Targeting groups can be proteins, e.g., glycoproteins, or peptides, e.g., molecules having a specific affinity for a co-ligand, or antibodies e.g., an antibody, that binds to a specified cell type such as a cancer cell, endothelial cell, or bone cell. Targeting groups may also include hormones and hormone receptors. They can also include non-peptidic species, such as lipids, lectins, carbohydrates, vitamins, cofactors, multivalent lactose, multivalent galactose, N-acetyl-galactosamine, N-acetyl-galucosamine multivalent mannose, multivalent fucose, or aptamers.

[00348] The targeting group can be any ligand that is capable of targeting a specific receptor. Examples include, without limitation, folate, GalNAc, galactose, mannose, mannose-6P, aptamers, integrin receptor ligands, chemokine receptor ligands, transferrin, biotin, serotonin receptor ligands, PSMA, endothelin, GCPII, somatostatin, LDL, and HDL ligands. In particular embodiments, the targeting group is an aptamer. The aptamer can be unmodified or have any combination of modifications disclosed herein.

[00349] In still other embodiments, glycan-interacting antibodies are covalently conjugated to a cell penetrating polypeptide. The cell-penetrating peptide may also include a signal sequence. The conjugates of the invention can be designed to have increased stability; increased cell transfection; and/or altered biodistribution (e.g., targeted to specific tissues or cell types).

[00350] Conjugating moieties may be added to glycan-interacting antibodies such that they allow labeling or flagging targets for clearance. Such tagging/flagging molecules include, but are not limited to ubiquitin, fluorescent molecules, human influenza hemagglutinin (HA), c-myc [a 10 amino acid segment of the human protooncogene myc with sequence EQKLISEEDL (SEQ ID NO: 21)], histidine (His), flag [a short peptide of sequence DYKDDDDK (SEQ ID NO: 22)], glutathione S-transferase (GST), V5 (a paramyxovirus of simian virus 5 epitope), biotin, avidin, streptavidin, horse radish peroxidase (HRP) and digoxigenin.

[00351] In some embodiments, glycan-interacting antibodies may be combined with one another or other molecule in the treatment of a disease or condition.

Nucleic acids

[00352] The present invention embraces nucleic acid molecules. In some embodiments, nucleic acids encode antibodies of the invention (including, but not limited to antibodies, antibody fragments, intrabodies and chimeric receptor antigens). Such nucleic acid molecules include, without limitation, DNA molecules, RNA molecules, polynucleotides,

oligonucleotides, mRNA molecules, vectors, plasmids and other constructs. As used herein, the term "construct" refers to any recombinant nucleic acid molecule including, but not limited to plasmids, cosmids, autonomously replicating polynucleotide molecules or linear or circular single-stranded or double-stranded DNA or RNA polynucleotide molecules. The present invention also embraces cells programmed or generated to express nucleic acid molecules encoding glycan-interacting antibodies. Such cells may be generated through the use of transfection, electroporation, viral delivery and the like. Viruses engineered with constructs of the invention may include, but are not limited to lentiviruses, adenoviruses, adeno-associated viruses and phages. In some cases, nucleic acids of the invention include codon-optimized nucleic acids. Methods of generating codon-optimized nucleic acids are known in the art and may include, but are not limited to those described in US Patent Nos. 5,786,464 and 6,114,148, the contents of each of which are herein incorporated by reference in their entirety. In some embodiments, nucleic acid sequence are codon optimized to improve protein expression or to remove cryptic splice sites.

II. Methods and uses

Therapeutics

[00353] Methods of the present disclosure include, but are not limited to, methods of utilizing one or more glycan-interacting antibody for therapeutic, diagnostic, quantitative, bioprocessing, experimental, and/or investigative purposes. Such glycan-interacting antibodies may include anti-STn antibodies.

Cancer-related applications

[00354] Aberrant glycosylation is a hallmark of cancer cell transformation. Multiple aberrant glycosylation forms have been described in human cancers, identifying specific tumor-associated carbohydrate antigens (TACAs) as a class of cell surface molecules suitable for specific tumor targeting (Cheever, M.A. et al., Clin Cancer Res. 2009 Sep 1;15(17):5323-37). TACA antigen expression has been found in epithelial cancers including, but not limited to, breast, colon, lung, bladder, cervical, ovarian, stomach, prostate, and liver. TACA antigen expression has been found in embryonal cancers including, but not limited to, yolk sac tumors and seminomas. In addition, TACA antigen expression has been found in many melanomas, carcinomas, and leukemias of various tissues (Heimburg-Molinaro et al., Vaccine. 2011 Nov 8; 29(48):8802-8826). Antibodies of the present invention that target one or more TACA are referred to herein as "anti-TACA antibodies."

[00355] It has been estimated that about 80% of all carcinomas express a truncated glycan, the Tn Antigen. With few exceptions, Tn and the sialylated form Sialyl Tn (STn), are not expressed in normal, healthy tissues. Furthermore, the non-human immunogenic sialic acid, N-glycolylneuraminic acid (Neu5Gc), seems to be differentially expressed on carcinomas such as breast cancer in the form of Neu5Gc-STn (GcSTn).

[00356] Multiple aberrant glycosylation forms have been described in human cancers, identifying specific glycans as a class of cell surface molecules suitable for specific tumor targeting (Cheever, M.A. et al., Clin Cancer Res. 2009 Sep 1;15(17):5323-37). For example, various human cancer types (such as bladder, breast, cervical, colon, lung, and ovarian cancer among others) show high expression of STn antigen, which is rare in normal human tissues (Karlen, P. et al., Gastroenterology. 1998 Dec;115(6):1395-404; Ohno, S. et al, Anticancer Res. 2006 Nov-Dec;26(6A):4047-53). In addition, the presence of STn on tumor-associated mucins relates to cancer with poor prognosis and is therewith considered an attractive epitope for cancer detection and targeted therapy (Cao, Y. et al., Virchows Arch. 1997 Sep;431(3):159-66; Julien, S. et al., Br J Cancer. 2009 Jun 2;100(11):1746-54; Itzkowitz, S.H. et al., Cancer. 1990 Nov 1;66(9):1960-6; Motoo, Y. et al., Oncology. 1991;48(4):321-6; Kobayashi, H. et al., J Clin Oncol. 1992 Jan;10(1):95-101). Tn and STn formation is associated with somatic mutations in the gene Cosmc that encodes a molecular chaperon required for the formation of the activate T-synthase (Ju, T. et al., Nature. 2005 Oct 27;437(7063):1252; Ju, T. et al., Cancer Res. 2008 Mar 15;68(6):1636-46). It can also result from increased expression of the sialyl transferase, ST6GalNAc I (Ikehara, Y. et al., Glycobiology. 1999 Nov;9(11):1213-24; Brockhausen, I. et al., Biol Chem. 2001 Feb;382(2):219-32). *De novo* expression of STn can modulate carcinoma cells, change the malignant phenotype, and lead to more aggressive cell behaviors (Pinho, S. et al., Cancer Lett. 2007 May 8;249(2):157-70). Although STn is highly expressed in malignant tissues, low levels are also found on healthy human cells (Jass, J.R. et al., J Pathol. 1995 Jun;176(2):143-9; Kirkeby, S. et al., Arch Oral Biol. 2010 Nov;55(11):830-41). STn alone has attracted attention as a target for cancer detection and therapy (Cheever, M.A. et al., Clin Cancer Res. 2009 Sep 1;15(17):5323-37).

[00357] In addition to the presence of STn, other glycosylation changes have been described in cancer. One of them involves Neu5Gc. N-acetylneuraminic acid (Neu5Ac) and Neu5Gc are the two major sialic acids on mammalian cell surfaces. Neu5Ac and Neu5Gc differ only in that Neu5Gc comprises an additional oxygen atom associated with chemical group attached to carbon 5. Due to the loss of a functional gene, humans can only synthesize

sialic acid in the form of Neu5Ac, but not Neu5Gc. However, Neu5Gc can be metabolically incorporated into humans from animal-derived dietary sources such as red meats (Tangvoranuntakul, P. et al., *Proc Natl Acad Sci U S A.* 2003 Oct 14; 100(21):12045-50; Nguyen, D.H. et al., *J Immunol.* 2005 Jul 1; 175(1):228-36; US7,682,794, US8,084,219, US2012/0142903, WO2010030666 and WO2010030666, herein incorporated by reference in their entirety). Neu5Gc is significantly abundant among human tumors (Higashi, H. et al., *Cancer Res.* 1985 Aug; 45(8):3796-802; Miyoshi I. et al., *Mol Immunol.* 1986. 23: 631-638; Hirabayashi, Y. et al., *Jpn J Cancer Res.* 1987. 78: 614-620; Kawachi, S. et al., *Int Arch Allergy Appl Immunol.* 1988. 85: 381-383; Devine, P.L. et al., *Cancer Res.* 1991. 51: 5826-5836; Malykh, Y.N. et al., *Biochimie.* 2001. 83: 623-634 and Inoue, S. et al., 2010. *Glycobiology.* 20(6): 752-762) and remarkably low in normal human tissues, which had been overlooked for several decades (Diaz, S.L. et al., *PLoS One.* 2009. 4: e4241; Tangvoranuntakul, P. et al., *Proc Natl Acad Sci U S A.* 2003. 100: 12045-12050; Varki, A. et al., *Glycoconj J.* 2009. 26: 231-245). The increased metabolic accumulation of diet-derived Neu5Gc in cancer tissue compared to healthy human tissues is likely explained by at least three factors: rapid growth with underproduction of competing endogenous Neu5Ac, enhanced macropinocytosis induced by growth factors (Dharmawardhane, S. et al., *Mol Biol Cell.* 2000 Oct;11(10):3341-52; Simonsen, A. et al., *Curr Opin Cell Biol.* 2001 Aug;13(4):485-92; Johannes, L. et al., *Traffic.* 2002 Jul;3(7):443-51; Amyere, M. et al., *Int J Med Microbiol.* 2002 Feb;291(6-7):487-94), and the upregulation of gene expression of the lysosomal sialic acid transporter gene sialin by hypoxia (Yin, J. et al., *Cancer Res.* 2006 Mar 15;66(6):2937-45). In addition, all humans tested to date comprise a polyclonal antibody reservoir against non-human Neu5Gc, which makes it the first example of a xeno-autoantigen (Padler-Karavani, V. et al., *Glycobiology.* 2008 Oct;18(10):818-30; Varki, N.M. et al., *Annu Rev Pathol.* 2011;6:365-93). The accumulation of dietary Neu5Gc in malignant tumors in the face of an anti-Neu5Gc response was shown to facilitate tumor progression by inducing a low-grade chronic inflammation (Hedlund, M. et al., *Proc Natl Acad Sci U S A.* 2008 Dec 2;105(48):18936-41). Thus, Neu5Gc containing glycan epitopes on human tumors represent a valuable possibility for drug targeting. A recent study suggests the existence of antibodies against Neu5Gc-containing STn (GcSTn), but not Neu5Ac-STn (AcSTn), in cancer patients and explores their potential as a specific biomarker for cancer detection (Padler-Karavani, V. et al., *Cancer Res.* 2011 May 1;71(9):3352-63).

[00358] MUC1 is a key cell surface glycoprotein that is normally extensively glycosylated but is underglycosylated in tumor cells. Sparse glycosylation of MUC1 leads to exposure of

immunogenic antigens. These may be along the MUC1 core peptide sequence or along core carbohydrate residues. These TACAs include, but are not limited to N-acetylgalactosamine (Tn), sialyl(α 2,6)N-acetylgalactosamine (STn) and galactose(β 1-3)N-acetylgalactosamine (also known as Thomsen-Friedenreich antigen or TF). It has been estimated that about 80% of all carcinomas express Tn among the core carbohydrates of MUC1 with STn being strongly expressed on human carcinoma cells and linked to cancer progression and metastasis. With few exceptions, Tn and STn are not expressed in normal healthy tissues. Sialic acid forms a prominent epitope on STn. The invention takes advantage of the fact that aberrant Neu5Gc-STn (GcSTn) glycan expression appears to be highly specific to various carcinomas.

[00359] In the case of MUC1, Neu5Gc incorporation into STn yields a tumor-specific target, a site that is an attractive target for antibody-based therapies to treat tumor tissue. In some embodiments of the present invention, glycan-interacting antibodies target MUC1 expressing cancer cells comprising Neu5Gc. To date, Neu5Gc has been detected in glycoconjugates from a number of human cancer tissues including, but not limited to colon cancer, retinoblastoma tissue, melanoma, breast cancer and yolk sac tumor tissue. In some embodiments of the present invention, methods are contemplated for glycan-interacting antibody treatment of these forms of cancer as well as other forms of cancer, not specifically listed here, characterized by the presence of cancer cells comprising Neu5Gc.

[00360] Additional antigens comprising glycans have been identified that are expressed in correlation with cancer (Heimburg-Molinaro, J. et al., Cancer vaccines and carbohydrate epitopes. Vaccine. 2011 Nov 8;29(48):8802-26). These tumor-associated carbohydrate antigens include, but are not limited to blood group Lewis related antigens [including, but not limited to Lewis^Y (Le^Y), Lewis^X (Le^X), Sialyl Lewis^X (SLe^X) and Sialyl Lewis^A (SLe^A)], glycosphingolipid-related antigens [including, but not limited to Globo H, stage-specific embryonic antigen-3 (SSEA-3) and glycosphingolipids comprising sialic acid], ganglioside-related antigens [including, but not limited to gangliosides GD2, GD3, GM2, fucosyl GM1 and Neu5GcGM3] and polysialic acid-related antigens.

[00361] In some embodiments, therapeutics of the present invention may be directed toward Lewis blood group antigens. Lewis blood group antigens comprise a fucose residue linked to GlcNAc by an α 1-3 linkage or an α 1-4 linkage. They may be found on both glycolipids and glycoproteins. Lewis blood group antigens may be found in the body fluid of individuals that are secretors of these antigens. Their appearance on red cells is due to absorption of Lewis antigens from the serum by the red cells.

[00362] In some embodiments, therapeutics of the present invention may be directed toward Le^Y. Le^Y (also known as CD174) is made up of Galβ1,4GlcNAc comprising α1,2- as well as α1,3-linked fucose residues yielding the Fuca(1,2)Galβ(1,4)Fuca(1,3)GlcNAc epitope. It is synthesized from the H antigen by α1,3 fucosyltransferases which attach the α1,3 fucose to the GlcNAc residue of the parent chain. Le^Y may be expressed in a variety of cancers including, but not limited to ovarian, breast, prostate, colon, lung and epithelial. Due to its low expression level in normal tissues and elevated expression level in many cancers, the Le^Y antigen is an attractive target for therapeutic antibodies.

[00363] In some embodiments, therapeutics of the present invention may be directed toward Le^X. Le^X comprises the epitope Galβ1-4(Fuca1-3)GlcNAcβ-R. It is also known as CD15 and stage-specific embryonic antigen-1 (SSEA-1). This antigen was first recognized as being immunoreactive with sera taken from a mouse subjected to immunization with F9 teratocarcinoma cells. Le^X was also found to correlate with embryonic development at specific stages. It is also expressed in a variety of tissues both in the presence and absence of cancer, but can also be found in breast and ovarian cancers where it is only expressed by cancerous cells.

[00364] In some embodiments, therapeutics of the present invention may be directed toward SLe^A and/or SLe^X. SLe^A and SLe^X comprise the structures [Neu5Acα2-3Galβ1-3(Fuca1-4)GlcNAcβ-R] and [Neu5Acα2-3Galβ1-4(Fuca1-3)GlcNAcβ-R] respectively. Their expression is upregulated in cancer cells. The presence of these antigens in serum correlates with malignancy and poor prognosis. SLe^X is mostly found as a mucin terminal epitope. It is expressed in a number of different cancers including breast, ovarian, melanoma, colon, liver, lung and prostate. In some embodiments of the present invention, SLe^A and SLe^X targets comprise Neu5Gc (referred to herein as GcSLe^A and GcSLe^X, respectively).

[00365] In some embodiments, therapeutics of the present invention may be directed toward glycolipids and/or epitopes present on glycolipids, including, but not limited to glycosphingolipids. Glycosphingolipids comprise the lipid ceramide linked to a glycan by the ceramide hydroxyl group. On the cell membrane, glycosphingolipids form clusters referred to as “lipid rafts”.

[00366] In some embodiments, therapeutics of the present invention may be directed toward Globo H. Globo H is a cancer-related glycosphingolipid first identified in breast cancer cells. The glycan portion of Globo H comprises Fuca(1-2)Galβ(1-3)GalNAcβ(1-3)Galα(1-4)Galβ(1-4)Glcβ(1). Although found in a number of normal epithelial tissues,

Globo H has been identified in association with many tumor tissues including, but not limited to, small cell lung, breast, prostate, lung, pancreatic, gastric, ovarian and endometrial tumors.

[00367] In some embodiments, therapeutics of the present invention may be directed toward gangliosides. Gangliosides are glycosphingolipids comprising sialic acid. According to ganglioside nomenclature, G is used as an abbreviation for ganglioside. This abbreviation is followed by the letters M, D or T referring to the number of sialic acid residues attached (1, 2 or 3 respectively). Finally, the numbers 1, 2 or 3 are used to refer to the order of the distance each migrates when analyzed by thin layer chromatography (wherein 3 travels the greatest distance, followed by 2 and then 1). Gangliosides are known to be involved in cancer-related growth and metastasis and are expressed on the cell surface of tumor cells. Gangliosides expressed on tumor cells include, but are not limited to GD2, GD3, GM2 and fucosyl GM1 (also referred to herein as Fuc-GM1). In some embodiments of the present invention, glycan-interacting antibodies are directed toward GD3. GD3 is a regulator of cell growth. In some embodiments, GD3-directed antibodies are used to modulate cell growth and/or angiogenesis. In some embodiments, GD3-directed antibodies are used to modulate cell attachment. In some embodiments of the present invention, glycan interacting antibodies are directed toward GM2. In some embodiments, GM2-directed antibodies are used to modulate cell to cell contact. In some embodiments, ganglioside targets of the present invention comprise Neu5Gc. In some embodiments, such targets may include a GM3 variant comprising Neu5Gc (referred to herein as GcGM3). The glycan component of GcGM3 is Neu5Gc α 2-3Gal β 1-4Glc. GcGM3 is a known component of tumor cells.

[00368] In some embodiments, TACAs targeted by anti-TACA antibodies of the present invention may include, but are not limited to any of those listed in US Publication Nos. US2013/0236486A1, US2013/0108624A1, US2010/0178292A1, US2010/0104572A1, US2012/0039984A1, US2009/0196916A1, and US2009/0041836A1, the contents of each of which are herein incorporated by reference in their entirety.

[00369] Method of the present disclosure include methods of treating cancer with one or more of the antibodies described herein. Such antibodies may include one or more of the variable domains presented in Table 2. Antibodies may further include one or more of the IgG constant domains presented in Table 3. The antibodies may be humanized antibodies. The antibodies may be antibody drug conjugates that include a therapeutic agent, including, but not limited to any of those presented herein. The therapeutic agent may be a cytotoxic agent, including, but not limited to any of those presented herein. The cytotoxic agent may be MMAE. The cytotoxic agent may be joined to the antibody by a linker.

STn in Cancer

[00370] The immune system has multiple mechanisms for promoting anti-tumor cell immune activity including both innate and adaptive immune activity. As used herein, the term “anti-tumor cell immune activity” refers to any activity of the immune system that kills or prevents growth and/or proliferation of tumor cells. In some cases, anti-tumor immune activity includes recognition and tumor cell killing by natural killer (NK) cells and phagocytosis by macrophages. Adaptive anti-tumor immune responses include tumor antigen uptake and presentation by antigen presenting cells (APCs,) such as dendritic cells (DCs,) leading to modulation of T cell anti-tumor activity and/or expansion of B cells with secretion of tumor-specific antibodies. The binding of tumor-specific antibodies to tumors can lead to antibody-dependent cellular cytotoxicity (ADCC) and complement-dependent cytotoxicity (CDC) mechanisms of tumor cell death.

[00371] As used herein, the term “immune-resistant tumor cell” refers to a tumor cell that reduces or evades anti-tumor cell immune activity. Some studies indicate that the expression of STn (a known TACA) on tumor cell surfaces or secreted into the tumor cell microenvironment can promote tumor cell evasion of anti-tumor immune activity. As used herein, the term “tumor cell microenvironment” refers to any area adjacent to or surrounding a tumor cell. Such areas include, but are not limited to areas between tumor cells, between tumor and non-tumor cells, surrounding fluids and surrounding components of the extracellular matrix.

[00372] Sialylated mucins comprising STn were demonstrated by Ogata et al to reduce NK cell targeting of tumor cells (Ogata, S. et al., 1992, *Canc. Res.* 52:4741-6, the contents of which are herein incorporated by reference in their entirety). This study found that the presence of ovine, bovine and porcine submaxillary mucin (OSM, BSM and PSM, respectively) led to nearly one hundred percent inhibition of cytotoxicity (see Table 2 of Ogata et al). Further studies by Jandus et al, demonstrate that some tumor cells can evade NK destruction due to the expression of sialoglycan ligands that can interact with NK cell siglec receptors, leading to NK inhibition (Jandus, C. et al., 2014, *JCI*. pii: 65899, the contents of which are herein incorporated by reference in their entirety).

[00373] Studies by Toda et al., demonstrate that STn may bind CD22 receptors on B cells, leading to decreased signal transduction and reduced B cell activation (Toda, M. et al., 2008, *Biochem Biophys Res Commun.* 372(1):45-50, the contents of which are herein incorporated by reference in their entirety). Dendritic cells (DCs) can affect adaptive immune activity by

modulating T cell activity. Studies by Carrascal et al found that STn expression by bladder cancer cells induced tolerance in DCs, reducing their ability to induce anti-tumor cell immune activity in T cells (Carrascal, MA et al., 2014. *Mol Oncol.* pii: S1574-7891(14)00047-7, the contents of which are herein incorporated by reference in their entirety). These studies revealed that DCs coming into contact with STn-positive bladder cancer cells displayed a tolerogenic expression profile with low expression of CD80, CD86, IL-12 and TNF- α . Further, DCs were found to modulate regulatory T cells such that the T cells had low expression of IFN γ and high expression of FoxP3. Other studies by van Vliet and others, indicate that DC surface expression of macrophage galactose-type lectin (MGL) can lead to targeting of those cells to tumor tissues (van Vliet, SJ., 2007. Amsterdam: Vrije Universiteit. p1-232 and van Vliet, SJ. et al., 2008. *J Immunol.* 181(5):3148-55, Nollau, P. et al., 2013. *J Histochem Cytochem.* 61(3):199-205, the contents of each of which are herein incorporated by reference in their entirety). DCs arriving at tissues due to MGL interactions may influence T helper (Th) cells in one of three ways. DCs can induce T cell tolerance, T cell immune activity or downregulation of effector T cells. MGL has been shown to bind to both AcSTn and GcSTn and the affinity has been analyzed in depth (Mortezai, N. et al., 2013. *Glycobiology.* 23(7):844-52, the contents of which are herein incorporated by reference in their entirety). Interestingly, MUC1 expression on tumors has been shown to lead to T cell tolerance, protecting tumor cells from immune eradication.

[00374] In some embodiments, glycan-interacting antibodies (including, but not limited to anti-STn antibodies) of the present invention may be used to treat subjects comprising one or more tumor cells expressing one or more TACAs. In some cases, glycan-interacting antibodies (including, but not limited to anti-STn antibodies) of the invention may be used to increase anti-tumor cell immune activity toward tumor cells expressing STn. Such antibodies may increase the adaptive immune response and/or the innate immune response toward immune-resistant tumor cells. Some glycan-interacting antibodies may be used to increase NK anti-tumor cell activity. Such glycan-interacting antibodies may, in some cases, block the interaction between glycan receptors expressed on NK cells and STn glycans on cancer cells or in surrounding tissues.

[00375] In some embodiments, glycan-interacting antibodies (including, but not limited to anti-STn antibodies) of the invention may be used to increase B cell anti-tumor cell activity. Such antibodies may reduce the interaction between CD22 receptors on B cells and STn glycans on cancer cells or in surrounding tissues. A study by Sjoberg et al. demonstrates that 9-O-acetylation of α 2,6-linked sialic acids on glycoproteins also reduced interaction between

B cell CD22 receptors and such glycoproteins (Sjoberg, E.R. et al. 1994. JCB. 126(2): 549-562). Another study by Shi et al. reveals that higher levels of 9-O-acetylated sialic acid residues on murine erythroleukemia cells makes these cells more susceptible to complement-mediated lysis (Shi, W-X. et al., 1996. J of Biol Chem. 271(49): 31526-32, the contents of which are herein incorporated by reference in their entirety). In some embodiments, anti-STn antibodies of the invention are capable of selectively binding non-9-O-acetylated STn, reducing overall STn binding, but reducing tumor cell growth and/or proliferation. (e.g. through increased B cell anti-tumor activity and increased complement-mediated tumor cell destruction). In some embodiments, glycan-interacting antibodies (including, but not limited to anti-STn antibodies) of the invention may be used to increase DC anti-tumor activity. Such antibodies may be used to reduce DC tolerance to tumor cells. Reduced DC tolerance may comprise increasing DC expression of CD80, CD86, IL-12 and/or TNF- α . In some cases, DC anti-tumor cell activity may comprise promotion of T cell anti-tumor cell activity. Such antibodies may prevent binding between DC MGL and glycans expressed on or around cancer cells.

[00376] A study by Ibrahim et al. suggests that high levels of anti-STn antibodies along with endocrine therapy may increase overall survival and time to progression (TTP) in women with metastatic breast cancer (Ibrahim, N.K. et al., 2013. 4(7): 577-584, the contents of which are herein incorporated by reference in their entirety). In this study, anti-STn antibody levels were elevated after vaccination with STn linked to keyhole-limpet Hemocyanin (KLH). In some embodiments, anti-STn antibodies of the invention may be used in combination with endocrine therapy (e.g. tamoxifen and/or an aromatase inhibitor).

[00377] STn expression has been implicated in contributing to the metastatic potential of ovarian tumor cells. According to some methods of the disclosure, anti-STn antibodies may be used to reduce ovarian tumor cell metastasis. Such methods may include the reduction of metastasis by from about 1% to about 15%, from about 5% to about 25%, from about 10% to about 50%, from about 20% to about 60%, from about 30% to about 70%, from about 40% to about 80%, from about 50% to about 90%, from about 75% to about 95%, or at least 95%.

[00378] Some methods of the present disclosure include methods of treating cancer in a subject with one or more of the antibodies described herein, wherein the subject has at least one cancer cell expressing STn. The antibodies may bind STn. Such antibodies may include one or more of the variable domains presented in Table 2. Some antibodies or antigen binding fragments may include different combinations of antibody sequences described herein. Such antibodies may further include one or more of the IgG constant domains presented in Table 3.

The antibodies may be humanized antibodies. The antibodies may be antibody drug conjugates that include a therapeutic agent, including, but not limited to any of those presented herein. The therapeutic agent may be a cytotoxic agent, including, but not limited to any of those presented herein. The cytotoxic agent may be MMAE. The cytotoxic agent may be joined to the antibody by a linker.

[00379] In some embodiments, the present disclosure provides methods for treating cancer in a subject, wherein the subject has at least one cancer cell expressing STn and wherein the subject has platinum refractory disease. Platinum refractory disease is a resistance to platinum based treatment experienced by a percentage of the total population of subjects treated for cancer. The subject may be treated by administering an anti-STn antibody to the subject. The at least one cancer cell may be an ovarian cancer cell. The at least one cancer cell may be resistant to cisplatin. The at least one cancer cell may be part of a tumor.

[00380] Anti-STn antibodies used to treat cancer in subjects with platinum refractory disease may be ADCs. The ADCs may be conjugated with a cytotoxic agent, including any of the formats presented herein. The anti-STn antibodies may be administered at a dose of from about 0.1 mg/kg to about 25 mg/kg. Administration may be by intravenous injection. Administration may include, but is not limited to, daily administration, weekly administration, or monthly administration.

[00381] In some embodiments, anti-STn antibody treatments may reduce detectable levels of STn in subject body fluids and/or tissues. The STn may be associated with proteins or other carriers. In some cases, levels of STn in serum are reduced.

Cancer stem cells as therapy targets

[00382] Cancer stem cells or CSCs (also called tumor initiating cells) are a subset of cells within a heterogeneous cancerous tissue or tumor cell population that drive the initiation, growth, dissemination, and recurrence of primary and metastatic tumors (Karsten and Goletz, SpringerPlus, 2013, 2, 301), which can occur in varying proportions of the total population depending on tumor type. CSCs are distinguished from terminally differentiated cells by their capacity to self-renew and give rise to non-CSC, differentiated progeny (Gupta et al., Nature medicine, 2009, 15, 1010-1012). These properties are akin to those of normal stem cells. Such distinctions between normal stem cells and CSCs have important implications for therapy.

[00383] An increasing number of cell-surface biomarkers have been identified that purport to differentiate CSCs from their non-CSC counterparts (Medema et al., Nature cell biology,

2013, 15, 338-344; Zoller, *Cancer*, 2011, 11, 254-267). These may include, but are not limited to CD44, CD133, CD117, and aldehyde dehydrogenase isoform 1 (ALDH1). Although some of these derive from studies of mouse tumors and human cell lines, others have been validated using primary human tumor samples. One of these, the membrane-spanning CD44 glycoprotein, or hyaluronan receptor, which is a well-known constituent of a variety of tumor types, has also more recently found acceptance as a bona fide CSC marker in human cancers, and in fact is the one most frequently observed (Lobo et al., 2007, 23, 675-699).

[00384] CD44 exists in several variant isoforms generated by alternative splicing events occurring among the 20 exons and 19 introns of the full-length CD44 gene (Williams et al, *Experimental biology and medicine*, 2013, 238, 324-338). Growing experimental evidence points to the supporting role of CD44 and its variants in contributing to the innate metastatic and drug resistant phenotype of CSCs (Negi et al., *Journal of drug targeting*, 2012, 20, 561-573), in part due to modulation of intracellular signal transduction pathways (Williams et al, *Experimental biology and medicine*, 2013, 238, 324-338). Additionally, patients with triple negative breast cancer, along with several other cancer types, that display high levels of CD44 cells are known to have a poor prognosis and higher mortality (Negi et al., *Journal of drug targeting*, 2012, 20, 561-573). These observations support the notion that targeting CD44 offers a means of treating cancer through inhibition or elimination of CSCs, in addition to mature cancer cells. Indeed, numerous approaches to targeting CD44 have been attempted experimentally with varying degrees of success. These comprise a wide range of technologies that include the use of conjugated and unconjugated antibodies, nano-carrier drug systems, and hyaluronan-conjugated drugs (Negi et al., *Journal of drug targeting*, 2012, 20, 561-573). In several instances, however, toxic effects were observed in in vivo studies; these untoward side effects may be attributable to the widespread occurrence of CD44 and variants on the membranes of most vertebrate cells (Naor et al., *Seminars in cancer biology*, 2008, 18, 260-267), in addition to its presence on the surface of the targeted CSCs and mature tumor cells. Targeting CD44 protein, which is a constituent of normal human stem cells (Williams et al, *Experimental biology and medicine*, 2013, 238, 324-338), can also harm normal stem cell function (Leth-Larsen et al., *Molecular medicine*, 2012, 18, 1109-1121). Although a large body of research points to the desirability of targeting CD44 protein on CSCs, as well as on mature tumor cells, the intrinsic problem with this approach remains the present difficulty in designing inhibitors that will spare normal tissue as well as normal stem cells.

[00385] Another well-known tumor antigen with implications to CSC biology is the epithelial mucin MUC1, a membrane tethered glycoprotein that is differentially expressed at high levels on the majority of adenocarcinomas but at low levels or not at all on normal epithelial cells. MUC1 has recently been identified as a CSC biomarker on a variety of neoplasias including breast (Engelmann et al., Cancer research,2008, 68, 2419-2426), and pancreatic cancers, where its expression is correlated with high metastasis and poor prognosis. As a constituent of CSCs, MUC1 has been shown to function in cell adhesion, proliferation, survival, and signaling (Engelmann et al., Cancer research,2008, 68, 2419-2426) and may also be co-expressed with CD44 (Leth-Larsen et al., Molecular medicine,2012, 18, 1109-1121). Immunotherapeutic approaches for targeting MUC1 in cancer are being pursued using vaccines as well as other approaches, but primarily in the context of mature cancer cell therapy (Julien et al., Biomolecules,2012, 2, 435-466; Acres et al., Expert review of vaccines,2005, 4, 493-502).

[00386] Cancer stem cells have been hypothesized to be generated through the epithelial-to-mesenchymal (EMT) transition (Gupta et al., Nature medicine, 2009, 15, 1010-1012), and /or reversely the mesenchymal-to-epithelial (MET) transition that occurs at the site of metastasis (Leth-Larsen et al., Molecular medicine,2012, 18, 1109-1121) (also called CSCs plasticity where non-CSCs can give rise to CSCs). This discovery further underscores the need to eliminate both CSCs and non-CSCs in a cancerous tissue or tumor cell population.

[00387] Recent studies with enriched CSC populations has revealed that these cells, unlike the bulk of the tumor, are relatively quiescent and are preferentially resistant to many types of current therapies, including chemotherapy and radiation (Leth-Larsen et al., Molecular medicine,2012, 18, 1109-1121). Thus current therapeutic strategies target non-CSC components of the tumor, leaving CSCs largely unaffected only to re-emerge after appropriate cues to reform recurrent primary tumors at the initial site or to disseminate to distant sites, colonize, and create metastatic disease, the major cause of cancer mortality.

[00388] Current understanding of the properties of cancer stem cells clearly emphasized the need not only to target the bulk of cells present in tumors, as is current practice, but also the CSC compartment in order to potentially effect complete cures.

[00389] As discussed above, strategies that have been developed based on tumor (including CSCs) associated biomarkers face a challenge that most cancer biomarkers are also present in normal cells including normal stem cells. A therapy that targets a protein biomarker to eliminate CSCs, may also target normal stem cells, causing elimination of normal cells.

Tumor-specific glycans in CSCs

[00390] Aberrant forms of glycosylation, including appearance of the Thomsen-nouveau (Tn) antigen (GalNAc-O-Ser/Thr), have been described in numerous human cancers, identifying glycans as an entirely novel class of tumor-associated carbohydrate antigens suitable for specific tumor targeting (Rabu et al., *Future oncology*, 2012, 8, 943-960). The formation of the sialyl derivative of Tn (STn) is mediated by the sialyl transferase ST6GalNAc I which adds sialic acid in an α 2,6 linkage to the Tn antigen. The sialylation of Tn prevents further sugar additions, thus truncating further glycan extensions (Schultz et al., *Cancer metastasis reviews*, 2012, 31, 501-518).

[00391] While the presence of STn in normal adult human tissues is rare, STn occurs in various human cancers, including ovarian, bladder, breast, cervical, colon, and lung cancer, among others (Ferreira et al., *Molecular oncology*, 2013, 7, 719-731; Kinney et al., *Cancer*, 1997, 80, 2240-2249). Further, the presence of STn in tumors is associated with metastatic disease, poor prognosis, and reduced overall survival (Ferreira et al., *Molecular oncology*, 2013, 7, 719-731; Kinney et al., *Cancer*, 1997, 80, 2240-2249); therefore, STn is considered a highly attractive target for cancer detection and therapy. There are two distinct forms of sialic acid – Neu5Ac and Neu5Gc – located at the terminal position of STn. The Neu5Ac-sialylated form is predominant in humans since humans cannot synthesize Neu5Gc due to an inactive CMP-Neu5Ac hydroxylase (CMAH) gene. However, consumption of Neu5Gc-rich foods leads to foreign Neu5Gc incorporation into human cells, especially in carcinomas. Previous studies have shown that solid tumors take up and express the Neu5Gc form of sialic acid (Inoue et al., *Glycobiology*, 2010, 20, 752-762; Malykh et al., *Biochimie*, 2001, 83, 623-634; Padler-Karavani et al., *Cancer research*, 2011, 71, 3352-3363). mAbs that bind to both glyco-isoforms of STn [Neu5Ac-STn (AcSTn) and Neu5Gc-STn (GcSTn)] that are potential cancer targets are designated as pan-STn antibodies.

[00392] STn accumulation is associated with specific somatic mutations observed repeatedly in solid tumors and with the inactivation of the gene that encodes the molecular chaperone Core 1 Beta3-Galactosyltransferase-Specific Molecular Chaperone (COSMC), which is required for the formation of active T-synthase (Ju et al., *Nature*, 2005, 437, 125). T-synthase competes with ST6GalNAc I for the GalNAc substrate and therefore when inactivated by mutation results in elevated STn synthesis. Additionally, STn accumulation can also result from increased expression of ST6GalNAc I, which is often observed (Brockhausen et al., *Biological chemistry*, 2001, 382, 219-232; Ikehara et al.,

Glycobiology, 1999, 9, 1213-1224). De novo expression of STn can modulate carcinoma cells, change the malignant phenotype, and lead to more aggressive cell behaviors (Pinho et al., Cancer letters, 2007, 249, 157-170). As such, STn is not only an interesting cancer biomarker and therapeutic target, but interfering with STn function offers the intriguing potential to have significant functional, anti-metastatic therapeutic benefits.

[00393] Although it is well-known that glycosylation of cellular glycoproteins is altered in cancer, it appears that aberrant glycosylation is selective with respect to both the glycoprotein and glycan in question. In fact, in human tumor CSCs only CD44 and MUC1 are major carriers of the STn antigen (Cazet et al., Breast cancer research: BCR, 2010, 12, 204; Julien et al., Glycobiology, 2006, 16, 54-64), immediately suggesting a selective approach for targeting not only mature tumor cells but also CSCs. Whereas MUC1 is a normal surface constituent of some epithelial cells where it serves a barrier function. Tumor-associated MUC1 is characterized by hypoglycosylation and increased sialylation on CSCs in the same fashion as observed in mature cancer cells, with STn appearing as a specific marker for both CSCs and mature tumor cells (Curry et al., Journal of surgical oncology, 2013, 107, 713-722). The aberrant oligosaccharide profile of MUC1 gives rise to the expression of neomarkers such as sialyl-Le^a (used in the CA19-9 test), sialyl-Le^x, and sialyl-Tn (TAG-72), as well as the cryptic epitopes such as Tn in cancer cells (e.g., CSCs). In addition, because of underglycosylation, the peptide core of the mucin becomes exposed such that epitopes within the core (not accessible within normal tissue-derived MUC1) may serve as potential antigens.

[00394] Clinical approaches targeting STn have thus far consisted solely of STn vaccines. The most advanced clinical candidate is Theratope, a therapeutic vaccine consisting of STn coupled to keyhole limpet hemocyanin. In in vivo mouse studies Theratope immunization induced a potent antibody response that was shown to mediate a delay in the growth of injected STn-expressing mammary carcinoma cells (Julien et al., British journal of cancer, 2009, 100, 1746-1751). However, Theratope failed to meet its primary endpoint in a phase III clinical trial in metastatic breast cancer. A leading hypothesis for why the Theratope trial missed its primary endpoint is that the patient population was not evaluated for STn expression prior to enrollment. Since STn expression in breast cancer is highly heterogeneous between patients, ranging from 25%-80% depending on the study and detection method, lack of ability to correlate STn expression with response may have masked any benefit from Theratope. Importantly, a subset of patients receiving hormonal therapy showed a significant 7.5 month increase in median overall survival when treated with Theratope compared to hormone therapy alone (Ibrahim et al., Journal of clinical oncology: official journal of the

American Society of Clinical Oncology, 2004, 22, 2547; and Miles et al., The oncologist, 2011, 16, 1092-1100), validating the therapeutic potential of targeting STn in particular patient populations. Additionally, since the immune response often varies considerably between vaccinated patients, vaccine approaches lack the ability to control or modulate antibody titer, resulting in wide ranges of therapeutic antibody exposure among patients. Nonetheless, Theratope was well tolerated with minimal toxicity, demonstrating the safety of targeting STn for cancer therapy.

[00395] The growing understanding of the molecular basis of STn expression in cancer cells strongly suggests that cells that express STn on any cell surface protein will also express STn on many (if not all) other O-glycosylated cell surface proteins, rendering it an excellent widely-distributed cancer-associated therapeutic target. Thus, STn positive cancer cell populations may be enriched for CSCs. In addition, recent data demonstrate that abrogation of STn expression renders cancers less aggressive with significant reductions in metastatic behavior (Gill et al., Proceedings of the National Academy of Sciences of the United States of America 2013, 110, E3152-3161).

Anti-STn antibodies targeting CSCs as cancer treatment

[00396] Several anti-STn antibodies have been described in the field, but some demonstrate low specificity towards the STn antigen or sialylated isoforms. For example, the commercial B72.3 anti-STn antibody has been shown to bind not only to STn but also to the Tn antigen (Bapat, S. A. (2010) Human ovarian cancer stem cells. Reproduction 140, 33-41). The availability of monoclonal antibodies (mAbs) targeting STn, engineered to induce antibody-dependent cellular cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC), or conjugated with a cytotoxic payload [e.g. antibody drug conjugate (ADC)], offers the potential of a significant therapeutic benefit for cancer patients with STn-expressing tumors. In addition, such antibodies would also allow for the development of a companion diagnostic to pre-select patients most likely to respond to therapy.

[00397] STn is often present on one or more of CSC surface antigens, and together they serve to promote the stemness and chemoresistance properties associated with CSCs. Thus, anti-STn antibodies offer a CSC-associated cancer targeting agent with the potential not only to directly kill CSCs via direct engagement and/or ADCC, but also offer a unique opportunity to bind to a wide array of cell-surface proteins and interfere with their associated functions essential for CSC viability, self-renewal, and replication.

[00398] As discuss herein, the rationale and advantages of targeting STn on CSCs may include: (1) many tumor-specific truncated glycoproteins carry STn in cancer; (2) STn is a unique glycan target expressed preferentially on CD44, MUC1, and potentially other important cell-surface markers, on both CSCs and mature tumor cells, irrespective of proliferation status, allowing for targeting of both of these tumor components by a single therapeutic agent.; (3) STn is also a component of CA-125, a biomarker of ovarian cancer and others; (4) STn is a component of the ovarian CSC marker CD44. Therefore, the use of pan-STn murine mAbs, targeting an epitope that encompasses both the Neu5Ac and Neu5Gc forms of sialic acid linked to Tn, will bind to and kill or impair the function of CSCs and, by virtue of the common epitope, non-CSC tumor cells.

[00399] In some embodiments, the present invention provides anti-pan STn mAb(s) for specific elimination of human CSCs as well as mature tumor cells. In one aspect of the present invention, the anti-STn antibody will target the validated STn glycan itself – not a particular glycopeptide or carrier protein, which should offer the broad potential of binding to CD44, MUC1, or other STn-glycosylated markers on both CSC and non-CSC tumor populations.

[00400] Given the exceptional specificity in targeting tumor-associated STn, the present invention may spare normal tissues, including normal adult stem cells, thereby allowing for an excellent therapeutic window.

[00401] In accordance with the present invention, provided herein is a unique immunotherapeutic solution aimed at eradicating human neoplasias by eliminating both cancer stem cells (CSCs) and mature cancer cells contained within cancerous tissues and/or tumor cell populations. The elimination is specifically conferred through targeting cell-surface sialylated Tn antigen (STn) structures that are uniquely present in cancerous tissues and/or tumor cell populations, including such structures associated with cancer stem cells.

Colorectal cancer

[00402] Colorectal cancer (CRC) has the 4th largest incidence, and is currently the third leading cause of cancer-related death in the US. Currently, 20% of patients are diagnosed with metastatic disease and roughly 50% of patients with CRC will eventually develop metastases. For those diagnosed with metastatic disease, the 5-year survival rate is 13.1%. In patients with metastatic colon cancer (mCRC), there is precedence for use of therapeutic antibodies (e.g., monoclonal antibodies), such as anti-epidermal growth factor receptor (EGFR) monoclonal antibodies (e.g., cetuximab and panitumumab) and anti-vascular

endothelial growth factor (VEGF) monoclonal antibodies (e.g. bevacizumab and ramucirumab).

[00403] Expression of STn is reported to be present in 83.4% of CRC patient samples and is correlated with increased malignancy and poor prognosis. STn antigen is present in adult normal colonocytes but is detectable only after removal of O-acetyl groups by saponification, a process that does not occur naturally *in vivo* (Julien et al., Biomolecules, 2012, 2, 435-466). Thus, STn may be used as a therapeutic target for treating CRC.

[00404] In some embodiments, glycan-interacting antibodies of the present disclosure may be used to treat CRC and/or mCRC. In some cases, such glycan-interacting antibodies are anti-STn antibodies, including, but not limited to any of those described herein. Glycan-interacting antibodies used to treat CRC and/or mCRC may be conjugated with a cytotoxic agent (e.g., MMAE and MMAF). Glycan-interacting antibodies may be used in combination with other therapies such as therapies with a chemotherapeutic agent (e.g., fluoropyrimidine, oxaliplatin, and/or irinotecan) and/or with a therapeutic antibody (e.g., cetuximab, panitumumab, bevacizumab and/or ramucirumab). In some cases, glycan-interacting antibodies may be used to treat colorectal cancers that are resistant to one or more other therapeutic treatments.

[00405] According to some embodiments, glycan-interacting antibodies used to treat colorectal cancer may be administered at a dose of from about 0.5 mg/kg to about 20 mg/kg. For example, antibodies may be administered at doses of from about 0.5 mg/kg to about 2 mg/kg, from about 1 mg/kg to about 5 mg/kg, from about 2.5 mg/kg to about 10 mg/kg, or from about 5 mg/kg to about 20 mg/kg.

Ovarian cancer

[00406] In some embodiments, methods of the present disclosure include methods of treating ovarian cancer. Ovarian cancer is the leading gynecological cancer effecting women in the U.S. During 2013. It is estimated that 22,240 women will be diagnosed with and 14,030 will die of this disease, making it the fifth leading cause of female-related cancer deaths and the most lethal gynecologic malignancy in the U.S. (Siegel et al., Cancer statistics, 2013. CA: a cancer journal for clinicians 63, 11-30). This high mortality can be ascribed to non-symptomatic onset, late-stage initial diagnosis, aggressiveness of this type of cancer, and a general lack of therapeutically targetable genetic changes. The current standard of care is tumor debulking followed by taxane and platinum based chemotherapy. While this initial treatment results in ~70% of patients achieving an initial complete clinical response, a

majority of these patients will unfortunately relapse with chemoresistant disease (Foster et al., Cancer letters, 2013, 338, 147-157; and McCann et al., PloS one, 2011, 6, e28077). In part, recurrent disease has been attributable, as with other cancer types, to the presence of CSCs within the total tumor population. Indeed, ovarian CSCs have been identified and shown to be resistant to chemo- and radiotherapy (Burgos-Ojeda et al., Cancer letters, 2012, 322, 1-7). Thus, again as the case with other forms of cancer, eliminating CSCs along with mature cells in the cancerous tissues and/or tumor cell population offers the best hope to manage recurrent disease and ideally effect cures.

[00407] In some embodiments of the present invention, methods are provided for treating ovarian cancer using anti-STn antibodies. Methods include administering anti-STn antibodies to subjects having ovarian cancer or suspected of having ovarian cancer. In some embodiments, ovarian CSCs may be targeted for ovarian cancer treatment, including, but not limited to those present in cancerous tissues and/or tumor cell populations. Although CD133 is the most widely studied of putative ovarian CSC markers, it is recognized that CD44, a known carrier of STn as discussed above, is associated with ovarian cancer and is included in the set of markers that identify ovarian CSCs (Zhang et al., Cancer research, 2008, 68, 4311-4320; Foster et al., Cancer letters, 2013, 338, 147-157; and Zoller, Cancer, 2011, 11, 254-267). Further, STn is expressed on the well-known ovarian cancer biomarker CA-125 (MUC16), as well as on MUC1, where the levels of these STn-associated mucins in serum have been used recently as further differentiators of cancerous versus benign ovarian disease. Elevated serum levels of STn occur in ~50% of ovarian cancer patients and correlate with a lower 5-year survival rate (Kobayashi et al., Journal of clinical oncology: official journal of the American Society of Clinical Oncology, 1991, 9, 983-987; Kobayashi et al., Journal of clinical oncology: official journal of the American Society of Clinical Oncology, 1992, 10, 95-101; and Chen et al., Journal of proteome research, 2013, 12, 1408-1418). Finally, Vathipadiekal et al. in a study of differential gene expression between human primary ovarian carcinoma CSCs and non-CSC populations found that the expression of STn-generating sialyl transferase ST6GalNAc I did not differ among cells from the two compartments.

[00408] In some embodiments, administering anti-STn antibodies to a subject having ovarian cancer or suspected of having ovarian cancer, according to the methods described herein, leads to the reduction of STn-positive cells in such subjects and/or the reduction of STn-positive cells in one or more ovarian cancerous tissues or tumor cell populations present in such subjects. In some embodiments, the reduction may include a decrease in STn-positive cells of from about 10% to about greater than 90% (for example, at least 10%, at least 20%,

at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, or at least 90%).

[00409] In some embodiments, the present invention provides antibodies for targeting CSCs to prevent control or cure cancer related to CSCs. Such antibodies may include anti-STn antibodies, including, but not limited to any of those described herein. Further anti-STn antibodies may include antibody 3F1 (SBH Sciences, Natick, MA) or derivatives thereof, including recombinant antibodies with CDRs from 3F1 and/or humanized derivatives.

[00410] In some embodiments, anti-STn antibodies of the invention may be used to target ovarian cancer stem cells that are resistant to other forms of treatment. Such treatments may include chemotherapy. As used herein, the term, “chemotherapy” refers to a form of treatment using chemical substances. Such chemical substances are referred to herein as “chemotherapeutic agents.” In the treatment of cancer, chemotherapeutic agents are agents that slow or prohibit the proliferation of cancer cells. As used herein, the term “chemotherapy-resistant” or “chemoresistant” is used to refer to cells that are unaffected by or that have limited susceptibility to chemotherapy treatment. Such chemotherapy treatments may include treatment with olaparib, carboplatin, and/or paclitaxel. Methods of targeting chemotherapy-resistant ovarian cancer stem cells may take advantage of changes in STn expression in ovarian cancer stem cells occurring after chemotherapy treatment. In some cases, chemotherapy-resistant ovarian cancer stem cells express STn before and/or after chemotherapy treatment. In some cases, cell surface STn expression in chemotherapy-resistant ovarian cancer stem cells may be increased following chemotherapy treatment. After chemotherapy treatments with olaparib, carboplatin, and/or paclitaxel, some ovarian cancer stem cells may proliferate resulting in a population of STn-expressing cancer cells that are olaparib-, carboplatin-, and/or paclitaxel-resistant. In some embodiments, anti-STn antibodies may be used to target olaparib-, carboplatin-, and/or paclitaxel-resistant cells. In some cases, these resistant cells are cancer stem cells. In some embodiments, anti-STn antibody treatment of a subject may be carried out after treatment of the subject with olaparib, carboplatin, and/or paclitaxel.

[00411] Accordingly, methods of the invention may include methods of treating cancer by administering an anti-STn antibody to a subject with ovarian cancer. Anti-STn antibodies may be administered before, during, or after treatment with chemotherapeutic agents (e.g., olaparib, carboplatin, and/or paclitaxel). Anti-STn antibodies may target STn-expressing ovarian cancer stem cells present before, during, and/or after administration of chemotherapeutic agents (e.g., olaparib, carboplatin, and/or paclitaxel). Anti-STn antibodies

may include a variable domain with an amino acid sequence selected from one or more of SEQ ID NOs: 1-12. In some embodiments, anti-STn antibodies are antibody-drug conjugates. Such antibody drug conjugates may include a cytotoxic agent (e.g., monomethyl auristatin E). Cancerous tissues in subjects treated with anti-STn antibodies may experience a reduction in STn-positive cells. In some embodiments, the reduction may include a decrease in STn-positive cells of from about 10% to about greater than 90% (for example, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, or at least 90%). Antibodies may target CSCs

[00412] In some embodiments, subjects having one or more chemotherapy-resistant ovarian cancer stem cells may be treated with anti-STn antibodies of the invention after treatment with one or more chemotherapeutic agent (e.g., olaparib, carboplatin, and/or paclitaxel). Anti-STn antibody treatment after chemotherapeutic agent treatment may prevent tumor resurgence. Tumor resurgence is the development of one or more tumor cells or tumors after levels of one or more tumor cells or tumors have been reduced (e.g., due to prior or current therapies).

[00413] In some methods of treating ovarian cancer, anti-STn antibodies of the present disclosure are administered in combination with modulators of cell signaling that are attributed to stemness and/or differentiation. Such modulators may include modulators of Notch and/or Hedgehog signaling.

[00414] Methods of the present disclosure include methods of treating ovarian cancer by obtaining a sample from a subject having or suspected of having ovarian cancer and detecting STn in the sample, wherein if STn is detected, an anti-STn antibody is administered to the subject. In some embodiments, the sample is a cellular sample (e.g., a cancerous tissue sample or a tumor sample). Cellular samples may include BRCA1 mutant cells or non-BRCA1 mutant cells.

[00415] STn detection in subject samples may be carried out by any methods known in the art for detection of molecular compounds. Such methods may include the use of one or more STn-detection antibodies. STn-detection antibodies may include any antibody capable of binding STn. Some methods of STn detection may include, but are not limited to, mass spectrometry, Western blotting, flow cytometry, immunoprecipitation, and enzyme-linked immunosorbent assay (ELISA). In some embodiments, protein-associated STn is detected.

[00416] In some embodiments, the STn detected may be associated with ovarian cancer stem cell-related proteins. As used herein, the term “ovarian cancer stem cell-related protein” refers to any protein that is associated with one or more ovarian cancer stem cells. Such

proteins may include, but are not limited to, cell surface proteins, markers, intracellular proteins, transcription factors, and proteins involved in cellular signaling that affect ovarian cancer stem cell survival, growth, replication, and/or maintenance. Ovarian cancer stem cell-related proteins may include, but are not limited to, Notch, Hedgehog, MUC1, CD44, CD117, CD133, and integrin.

[00417] In some embodiments, the present disclosure provides methods of treating ovarian cancer comprising providing a combined treatment with olaparib and an anti-STn antibody. Such methods may include treating a subject with olaparib and later treating the subject with an anti-STn antibody. In some cases, methods of treating ovarian cancer include identifying a subject that is not fully responsive to treatment with olaparib and administering an anti-STn antibody to the subject.

[00418] Methods of the present disclosure may include methods of consolidation cancer treatment. Consolidation treatment is treatment that is carried out following chemotherapy to achieve sustained remission. Typically, consolidation treatment involves lower doses of chemotherapy to prevent tumor resurgence while keeping toxicity levels low. Methods of the present disclosure for consolidation cancer treatment may include reducing the number of cancer cells in a subject by administering at least one chemotherapeutic agent and maintaining the reduced number (or further reducing the number) of cancer cells in the subject or in one or more cancerous tissue in the subject by administering an anti-STn antibody. In some embodiments, the cancer is ovarian cancer. The chemotherapeutic agent may be olaparib, carboplatin, and/or paclitaxel. In some embodiments, the anti-STn antibody is an antibody drug conjugate (ADC). The ADC may include monomethyl auristatin E (MMAE).

[00419] In some embodiments, methods of the disclosure include completely eradicating ovarian tumor cells to induce durable initial remission through administration of one or more anti-STn antibodies. Other methods include inhibition of ovarian tumor resurgence for a period of time through administration of one or more anti-STn antibodies, in some cases without excessive toxicity. Such periods of time may be from about 1 month to about 18 months, from about 1 year to about 5 years, from about 2 years to about 10 years, or greater than 10 years.

[00420] In some embodiments, the present disclosure provides methods of treating cancer that include isolating one or more ovarian tumor cells from a subject, generating a xenograft tumor in a host (e.g., a mouse, rat, rabbit, pig, or primate) with the one or more ovarian tumor cells, administering one or more anti-STn antibodies to the host, and selecting at least one of

the one or more anti-STn antibodies tested to use as a therapeutic antibody to treat the subject. The anti-STn antibody may be selected based on the ability of that antibody to reduce tumor volumes in the host.

Immune-related targets

[00421] In some embodiments, glycan-interacting antibodies of the invention may be immunomodulatory antibodies. As used herein, an immunomodulatory antibody is an antibody that enhances or suppresses one or more immune function or pathway.

[00422] Many bacterial glycans are known to comprise sialic acid. In some cases, such glycans allow bacteria to evade the innate immune system of hosts, including, but not limited to humans. In one example, bacterial glycans inhibit alternate complement pathway activation through factor H recognition. In another example, bacterial glycans mask underlying residues that may be antigenic. Some bacterial glycans participate in cell signaling events through activation of inhibitory sialic acid binding Ig-like lectins (Siglecs) that dampen the immune response to entities comprising certain sialylated moieties (Chen, X. et al., Advances in the biology and chemistry of sialic acids. ACS Chem Biol. 2010 Feb 19;5(2):163-76). In some embodiments, glycan-interacting antibodies of the present invention may be used to treat immune complications related to bacterial glycans.

[00423] Due to the foreign nature of Neu5Gc as described herein, some Neu5Gc glycans are immunogenic resulting in immune related destruction of cells and other entities where these glycans may be expressed. Such autoimmune destruction may be pathogenic. In some embodiments, glycan-interacting antibodies may be used to treat patients suffering from autoimmune disorders related to Neu5Gc glycans.

[00424] In some embodiments, immunomodulatory antibodies of the invention may be used to promote or suppress T cell-mediated immunity. Such antibodies may interact with one or more glycans present on T cells, T cell-related proteins and/or on one or more other cell types that interact with T cells. Immunomodulatory antibodies that enhance T cell mediated immunity may be used to stimulate T cell mediated targeting of cancer cells.

[00425] In some tumors, infiltration by tumor-associated macrophages (TAMs) may lead to immunosuppression promoting tumor cell viability and growth. This is thought to be due to immunosuppressive cell signaling that occurs through interactions between myeloid C-type lectin receptors (CLRs) present on TAMs and tumor-associated mucins (Allavena, P. et al., Clin Dev Immunol. 2010;2010:547179). In some embodiments, binding of

immunomodulatory antibodies of the invention to one or more tumor-associated mucin or TACA prevents immunosuppressive cell signaling in TAMs.

Veterinary applications

[00426] It is contemplated that glycan-interacting antibodies of the invention will find utility in the area of veterinary care including the care and treatment of non-human vertebrates. As described herein, the term “non-human vertebrate” includes all vertebrates with the exception of *Homo sapiens*, including wild and domesticated species such as companion animals and livestock. Non-human vertebrates include mammals, such as alpaca, banteng, bison, camel, cat, cattle, deer, dog, donkey, gayal, goat, guinea pig, horse, llama, mule, pig, rabbit, reindeer, sheep water buffalo, and yak. Livestock includes domesticated animals raised in an agricultural setting to produce materials such as food, labor, and derived products such as fiber and chemicals. Generally, livestock includes all mammals, avians and fish having potential agricultural significance. In particular, four-legged slaughter animals include steers, heifers, cows, calves, bulls, cattle, swine and sheep.

Bioprocessing

[00427] In some embodiments of the invention are methods for producing biological products in host cells by contacting the cells with one or more glycan-interacting antibody (such as an antibody or fusion protein) capable of modulating gene expression, or altering levels and/or types of glycans produced wherein such modulation or alteration enhances production of biological products. According to the present invention, bioprocessing methods may be improved by using one or more of the glycan-interacting antibodies of the present invention. They may also be improved by supplementing, replacing or adding one or more glycan-interacting antibodies.

Diagnostics

[00428] In some embodiments, compounds and compositions of the invention may be used as diagnostics. In some cases, antibodies of the invention may be used to identify, label or stain cells, tissues, organs, etc. expressing target antigens. In further embodiments, antibodies of the invention may be used to identify STn present in tissue sections (i.e., histological tissue sections), including tissue known or suspected of having cancerous cells. Such methods of using antibodies of the invention may in some cases be used to identify cancerous cells or tumors in tissue sections. Tissue sections may be from any tissue or organ including, but not

limited to breast, colon, pancreatic, ovarian, brain, liver, kidney, spleen, lung, skin, stomach, intestine, esophagous, or bone.

[00429] In some embodiments, diagnostic methods of the invention may include the analysis of one or more cells or tissues using immunohistochemical techniques. Such methods may include the use of one or more of any of the glycan-interacting antibodies described herein. Immunohistochemical methods of the invention may include staining tissue sections to determine the presence and/or level of one or more glycosylated proteins or other markers. Tissue sections may be derived from subject tumors (e.g., patient tumors and animal tumors such as animal model tumors). Tissue sections may come from formalin-fixed or unfixed fresh frozen tissues. In some case, tissue section come from formalin fixed paraffin-embedded (FFPE) tissues. Glycan-interacting antibodies described herein may be used as primary antibodies. Primary antibodies are used to contact tissue sections directly and bind to target epitopes. Primary antibodies may be directly conjugated with a detectable label or may be detected through the use of a detection agent such as a secondary antibody. In some embodiments, primary antibodies or detection agents include an enzyme that can be used to react with a substrate to generate a visible product (e.g., precipitate). Such enzymes may include, but are not limited to horse radish peroxidase, alkaline phosphatase, beta-galactosidase, and catalase.

[00430] Anti-STn antibodies described herein may be used according to immunohistochemical methods of the present disclosure to detect STn-glycosylated proteins in tissues or cells. In some cases, these antibodies are used to detect and/or determine the level of STn in tumor tissues. Such tumor tissues may include tumor tissues included in tumor microarrays. Suitable tumor types include, but are not limited to breast, colon, ovarian, pancreatic, skin, intestinal, lung, and brain tumors. Levels of anti-STn antibodies used in immunohistochemical staining techniques may be varied to increase visible staining or to decrease background levels of staining. In some embodiments, antibody concentrations of from about 0.01 µg/ml to about 50 µg/ml are used. For example, antibody concentrations of from about 0.01 µg/ml to about 1 µg/ml, from about 0.05 µg/ml to about 5 µg/ml, from about 0.1 µg/ml to about 3 µg/ml, from about 1 µg/ml to about 10 µg/ml, from about 2 µg/ml to about 20 µg/ml, from about 3 µg/ml to about 25 µg/ml, from about 4 µg/ml to about 30 µg/ml, or from about 5 µg/ml to about 50 µg/ml may be used.

[00431] In some embodiments, diagnostic methods of the invention include methods of generating an STn-linked glycoprotein profile. As used herein the term “STn-linked glycoprotein profile” refers to a set of information indicating the level and/or identity of STn-

linked glycoproteins in a sample or subject. Methods of generating an STn-linked glycoprotein profile may be carried out on a sample obtained from a subject. Such samples may be biological samples including, but not limited to, any of those described herein. Biological samples may be cellular samples. In some cases, cellular samples may include at least one tumor cell. In some embodiments, tumor cell samples may include BRCA1 mutant or non-BRCA1 mutant tumor cells.

[00432] Glycoproteins included in STn-linked glycoprotein profiles may include, but are not limited to, cancer cell markers, stem cell markers, cancer stem cell markers, and stem cell-related proteins. In some embodiments, glycoproteins identified and/or quantitated as part of a STn-linked glycoprotein profile may include, but are not limited to CD44, CD133, CD117, integrin, Notch, and Hedgehog.

[00433] Levels and/or identities of STn-linked glycoproteins in STn-linked glycoprotein profiles may be determined according to any methods known in the art for identifying proteins and/or quantitating protein levels. In some embodiments, such methods may include, but are not limited to mass spectrometry, array analysis (e.g., antibody array or protein array), Western blotting, flow cytometry, immunoprecipitation, and ELISA. STn-linked glycoproteins may in some cases be immunoprecipitated from a sample prior to analysis. Such immunoprecipitation may be carried out using an anti-STn antibody. Anti-STn antibodies used for immunoprecipitation of STn-linked glycoproteins may include any of those known in the art or described herein. In some embodiments, STn-glycoproteins are immunoprecipitated from biological samples using an anti-STn antibody and then identified and/or quantitated using mass spectrometry.

[00434] In some embodiments, cancer treatments are informed by STn-linked glycoprotein profile information. Accordingly, the present disclosure provides methods of treating cancer that include obtaining a sample from a subject in need of cancer treatment, generating an STn-linked glycoprotein profile from the sample, selecting a glycan-interacting antibody that binds to an STn-glycosylated protein from the STn-linked glycoprotein profile, and administering the glycan-interacting antibody to the subject. Glycan-interacting antibodies administered according to such methods may include one or more CDRs or variable domains taught herein.

[00435] In some embodiments, methods of the present disclosure may be used as companion diagnostics. As used herein, the term “companion diagnostic” refers to an assay, the results of which aid in the diagnosis or treatment of subjects. Companion diagnostics may be useful for stratifying patient disease, disorder or condition severity levels, allowing

for modulation of treatment regimen and dose to reduce costs, shorten the duration of clinical trial, increase safety and/or increase effectiveness. Companion diagnostics may be used to predict the development of a disease, disorder or condition and aid in the prescription of preventative therapies. Some companion diagnostics may be used to select subjects for one or more clinical trials. In some cases, companion diagnostic assays may go hand-in-hand with a specific treatment to facilitate treatment optimization.

[00436] In some embodiments, methods of the present disclosure may be useful as companion diagnostics for diseases, disorders and/or conditions related to cancer. Some companion diagnostics of the present invention may be useful for predicting and/or determining the severity of one or more forms of cancer. Some companion diagnostics of the present invention may be used to stratify subjects by risk of developing one or more forms of cancer. Some companion diagnostics of the present invention may be used to facilitate and expedite drug development for cancer therapeutics.

[00437] In some embodiments, the present disclosure provides methods of detecting and/or quantifying STn in a sample through the use of a capture antibody and a detection antibody. As used herein, a “capture antibody” is an antibody that binds an analyte in a way that it may be detected. Capture antibodies may be associated with surfaces or other carriers. Detection antibodies are antibodies that facilitate observation of the presence or absence of an analyte. In some embodiments, both capture antibody and detection antibody bind to STn. According to such embodiments, the capture antibody and the detection antibody may be derived from different species. This may allow for the use of secondary antibodies that recognize only the detection antibody and are not influenced by the presence of the capture antibody. In some embodiments, the capture antibody may bind to STn and the detection antibody may bind to a protein or carrier of the bound STn. Capture antibodies and detection antibodies used for STn detection in samples may be selected from commercially available anti-STn antibodies as well as any of the anti-STn antibodies presented herein.

STn expression-modified cells

[00438] In some embodiments, the present disclosure provides modified cells having altered STn levels. Such cells may be used for various purposes (e.g., experimental, therapeutic, antibody testing etc.). In some cases, methods of the present disclosure include methods of enhancing the expression of ST6GalNAc I in one or more cells or tissues. This may result in the generation of one or more cells having increased expression of cellular STn (e.g., surface-expressed STn). Expression of ST6GalNAc I may be enhanced, for example, by

introducing one or more vectors carrying a ST6GalNAc I expression construct. Such expression constructs may be designed with the natural ST6GalNAc I promoter or with a promoter to enhance gene expression. Promoters configured for enhancement of gene expression may have constitutively or overly active promoter elements. In some cases, promoters may be configured for inducible gene expression. Such promoters may become active or have elevated activity when contacted with factors that activate inducible elements of the promoter. STn expression constructs may include hST6GalNAc I pRc-CMV as described in Julien, S. et al., 2001. Glycoconj J, 18: 883-93, the contents of which are herein incorporated by reference in their entirety. In some embodiments, expression constructs may encode other factors involved in STn synthesis and/or expression. Such factors may include, but are not limited to, T-synthase, and Core 1 Beta3-Galactosyltransferase-Specific Molecular Chaperone (COSMC). In some embodiments, cells with minimal STn expression are converted to STn-expressing cells. Such cells may include, but are not limited to, SKOV3 cells, BRCA1 mutant cells, and non-mutant BRCA1 cells.

[00439] Also provided are modified cells having decreased STn expression relative to unmodified cells. Accordingly, methods of the present disclosure include methods of repressing STn expression. Such methods may include reducing ST6GalNAc I expression. In some embodiments, such methods may include the administration of one or more nucleic acid molecules that repress ST6GalNAc I expression. Such nucleic acid molecules may include, but are not limited to inhibitory RNA (e.g., RNAi or silencer siRNA). In some embodiments, other factors involved in STn synthesis and/or expression may be reduced. Such factors may include, but are not limited to T-synthase and COSMC. In some embodiments, cells naturally expressing STn are converted to STn-deficient cells. Such cells may include, but are not limited to, OVCAR3 cells and OVCAR4 cells.

III. Pharmaceutical compositions

[00440] In some embodiments, the present disclosure includes pharmaceutical compositions. Such pharmaceutical compositions may include antibodies of the present disclosure and/or fragments, peptides, or proteins derived from such antibodies. Pharmaceutical compositions may be characterized by one or more of bioavailability, therapeutic window and/or volume of distribution.

Bioavailability

[00441] Glycan-interacting antibodies, when formulated into a composition with a delivery/formulation agent or vehicle as described herein, can exhibit an increase in bioavailability as compared to a composition lacking a delivery agent as described herein. As used herein, the term “bioavailability” refers to the systemic availability of a given amount of glycan-interacting antibodies administered to a mammal. Bioavailability can be assessed by measuring the area under the curve (AUC) or the maximum serum or plasma concentration ($C_{\max}$) of the unchanged form of a compound following administration of the compound to a mammal. AUC is a determination of the area under the curve plotting the serum or plasma concentration of a compound along the ordinate (Y-axis) against time along the abscissa (X-axis). Generally, the AUC for a particular compound can be calculated using methods known to those of ordinary skill in the art and as described in G. S. Banker, *Modern Pharmaceutics, Drugs and the Pharmaceutical Sciences*, v. 72, Marcel Dekker, New York, Inc., 1996, herein incorporated by reference. In some embodiments, the AUC is calculated using the linear trapezoidal method with linear/linear interpolation. AUC may be expressed in units of time multiplied by concentration (i.e., unit of time*unit of mass/unit of volume). For example, the AUC may be expressed in units of days* $\mu\text{g/ml}$. The terminal elimination phase of each concentration versus time curve may be identified using one or more final concentration values observed. Terminal elimination phase slope may be determined using log linear regression on unweighted concentration data.

[00442] The $C_{\max}$ value is the maximum concentration of the compound achieved in the serum or plasma of a subject following administration. The $C_{\max}$ value of a particular compound can be measured using methods known to those of ordinary skill in the art. The phrases “increasing bioavailability” or “improving the pharmacokinetics,” as used herein mean that the systemic availability of a glycan-interacting antibody, measured as AUC, $C_{\max}$, or $C_{\min}$ in a mammal is greater, when co-administered with a delivery agent as described herein, than when such co-administration does not take place. In some embodiments, the bioavailability of the glycan-interacting antibody can increase by at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100%.

[00443] In some embodiments, the bioavailability of anti-STn antibodies may be determined after pharmaceutical composition administration. The anti-STn antibodies may be

antibody drug conjugates that include a therapeutic agent, including, but not limited to any of those presented herein. The therapeutic agent may be a cytotoxic agent, including, but not limited to any of those presented herein. The cytotoxic agent may be MMAE. The cytotoxic agent may be joined to the antibody by a linker. Pharmaceutical compositions of the present disclosure may be used to provide the anti-STn antibodies to a subject, wherein the anti-STn antibodies exhibit a C_{max} of from about 1 $\mu\text{g/ml}$ to about 5 $\mu\text{g/ml}$, from about 2 $\mu\text{g/ml}$ to about 10 $\mu\text{g/ml}$, from about 3 $\mu\text{g/ml}$ to about 15 $\mu\text{g/ml}$, from about 4 $\mu\text{g/ml}$ to about 20 $\mu\text{g/ml}$, from about 5 $\mu\text{g/ml}$ to about 50 $\mu\text{g/ml}$, from about 20 $\mu\text{g/ml}$ to about 100 $\mu\text{g/ml}$, from about 50 $\mu\text{g/ml}$ to about 200 $\mu\text{g/ml}$, from about 75 $\mu\text{g/ml}$ to about 150 $\mu\text{g/ml}$, or from about 100 $\mu\text{g/ml}$ to about 500 $\mu\text{g/ml}$. Pharmaceutical compositions of the present disclosure may be used to provide the anti-STn antibodies to a subject, wherein the anti-STn antibodies exhibit an AUC (from the start of administration to the last observed quantifiable concentration) of from about 1 day* $\mu\text{g/ml}$ to about 5 days* $\mu\text{g/ml}$, from about 2 days* $\mu\text{g/ml}$ to about 10 days* $\mu\text{g/ml}$, from about 5 days* $\mu\text{g/ml}$ to about 50 days* $\mu\text{g/ml}$, from about 20 days* $\mu\text{g/ml}$ to about 200 days* $\mu\text{g/ml}$, from about 100 days* $\mu\text{g/ml}$ to about 500 days* $\mu\text{g/ml}$, or from about 250 days* $\mu\text{g/ml}$ to about 1000 days* $\mu\text{g/ml}$.

Therapeutic window

[00444] Glycan-interacting antibodies, when formulated into a composition with a delivery agent as described herein, can exhibit an increase in the therapeutic window of the administered glycan-interacting antibody composition as compared to the therapeutic window of the administered glycan-interacting antibody composition lacking a delivery agent as described herein. As used herein “therapeutic window” refers to the range of plasma concentrations, or the range of levels of therapeutically active substance at the site of action, with a high probability of eliciting a therapeutic effect. In some embodiments, the therapeutic window of the glycan-interacting antibody when co-administered with a delivery agent as described herein can increase by at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%, at least about 75%, at least about 80%, at least about 85%, at least about 90%, at least about 95%, or about 100%.

[00445] In some embodiments, compound half-life and/or clearance rate may be monitored as an indicator of therapeutic window. As used herein, the term “half-life” or “ $t_{1/2}$ ” refers to the time it takes for a given process or compound concentration to reach half of a final value.

The “terminal elimination half-life” or “terminal half-life” refers to the time needed for the plasma concentration of a factor to be reduced by half after the concentration of the factor has reached a pseudo-equilibrium. Where the decline in concentration may be influenced by one or more factors independent of elimination (e.g., absorption rate or distribution rate), the half-life observed is referred to as “apparent” half-life. As used herein, the term “clearance rate” refers to the velocity at which a particular compound is cleared from a biological system or fluid. Where the rate may be influenced by one or more factors independent of clearance, the clearance rate is referred to as the “apparent” clearance rate.

[00446] In some embodiments, the therapeutic window of anti-STn antibodies may be determined after pharmaceutical composition administration. The anti-STn antibodies may be antibody drug conjugates that include a therapeutic agent, including, but not limited to any of those presented herein. The therapeutic agent may be a cytotoxic agent, including, but not limited to any of those presented herein. The cytotoxic agent may be MMAE. The cytotoxic agent may be joined to the antibody by a linker. Pharmaceutical compositions of the present disclosure may be used to provide the anti-STn antibodies to a subject, wherein the anti-STn antibodies exhibit an apparent terminal elimination half-life of from about 1 hour to about 10 hours, from about 2 hours to about 12 hours, from about 4 hours to about 24 hours, from about 20 hours to about 30 hours, from about 1 day to about 5 days, from about 2 days to about 14 days, from about 4 days to about 21 days, from about 8 days to about 28 days, or greater than 28 days. In some embodiments, pharmaceutical compositions of the present disclosure may be used to provide anti-STn antibodies to a subject, wherein the anti-STn antibodies exhibit an apparent clearance rate of from about 1 ml/kg/day to about 10 ml/kg/day, from about 5 ml/kg/day to about 20 ml/kg/day, from about 15 ml/kg/day to about 50 ml/kg/day, or greater than 50 ml/kg/day.

Volume of distribution

[00447] Glycan-interacting antibodies, when formulated into a composition with a delivery agent as described herein, can exhibit an improved volume of distribution (V_{dist}), e.g., reduced or targeted, relative to a composition lacking a delivery agent as described herein. The volume of distribution (V_{dist}) relates the amount of the drug in the body to the concentration of the drug in the blood or plasma. As used herein, the term “volume of distribution” refers to the fluid volume that would be required to contain the total amount of the drug in the body at the same concentration as in the blood or plasma: V_{dist} equals the amount of drug in the body/concentration of drug in blood or plasma. For example, for a 10

mg dose and a plasma concentration of 10 mg/L, the volume of distribution would be 1 liter. The volume of distribution reflects the extent to which the drug is present in the extravascular tissue. A large volume of distribution reflects the tendency of a compound to bind to the tissue components compared with plasma protein binding. In a clinical setting, V_{dist} can be used to determine a loading dose to achieve a steady state concentration. V_{ss} refers to the apparent volume of distribution at steady state. In some embodiments, the volume of distribution of the glycan-interacting antibody when co-administered with a delivery agent as described herein can decrease at least about 2%, at least about 5%, at least about 10%, at least about 15%, at least about 20%, at least about 25%, at least about 30%, at least about 35%, at least about 40%, at least about 45%, at least about 50%, at least about 55%, at least about 60%, at least about 65%, at least about 70%.

[00448] In some embodiments, the volume of distribution of anti-STn antibodies in a subject may be determined after pharmaceutical composition administration. The anti-STn antibodies may be antibody drug conjugates that include a therapeutic agent, including, but not limited to any of those presented herein. The therapeutic agent may be a cytotoxic agent, including, but not limited to any of those presented herein. The cytotoxic agent may be MMAE. The cytotoxic agent may be joined to the antibody by a linker. Pharmaceutical compositions of the present disclosure may be used to provide the anti-STn antibodies to a subject, wherein the anti-STn antibodies exhibit an apparent V_{ss} of from about 1 ml/kg to about 10 ml/kg, from about 5 ml/kg to about 50 ml/kg, from about 20 ml/kg to about 100 ml/kg, from about 75 ml/kg to about 150 ml/kg, or greater than 150 ml/kg.

[00449] In some embodiments, glycan-interacting antibodies comprise compositions and/or complexes in combination with one or more pharmaceutically acceptable excipients. Pharmaceutical compositions may optionally comprise one or more additional active substances, e.g. therapeutically and/or prophylactically active substances. General considerations in the formulation and/or manufacture of pharmaceutical agents may be found, for example, in *Remington: The Science and Practice of Pharmacy* 21st ed., Lippincott Williams & Wilkins, 2005 (incorporated herein by reference).

[00450] In some embodiments, compositions are administered to humans, human patients or subjects. For the purposes of the present disclosure, the phrase “active ingredient” generally refers to glycan-interacting antibodies to be delivered as described herein.

[00451] Although the descriptions of pharmaceutical compositions provided herein are principally directed to pharmaceutical compositions which are suitable for administration to humans, it will be understood by the skilled artisan that such compositions are generally

suitable for administration to any other animal, *e.g.*, to non-human animals, *e.g.* non-human mammals. Modification of pharmaceutical compositions suitable for administration to humans in order to render the compositions suitable for administration to various animals is well understood, and the ordinarily skilled veterinary pharmacologist can design and/or perform such modification with merely ordinary, if any, experimentation. Subjects to which administration of the pharmaceutical compositions is contemplated include, but are not limited to, humans and/or other primates; mammals, including commercially relevant mammals such as cattle, pigs, horses, sheep, cats, dogs, mice, and/or rats; and/or birds, including commercially relevant birds such as poultry, chickens, ducks, geese, and/or turkeys.

[00452] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of bringing the active ingredient into association with an excipient and/or one or more other accessory ingredients, and then, if necessary and/or desirable, dividing, shaping and/or packaging the product into a desired single- or multi-dose unit.

[00453] A pharmaceutical composition in accordance with the invention may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses. As used herein, a “unit dose” is discrete amount of the pharmaceutical composition comprising a predetermined amount of the active ingredient. The amount of the active ingredient is generally equal to the dosage of the active ingredient which would be administered to a subject and/or a convenient fraction of such a dosage such as, for example, one-half or one-third of such a dosage.

[00454] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the invention will vary, depending upon the identity, size, and/or condition of the subject treated and further depending upon the route by which the composition is to be administered. By way of example, the composition may comprise between 0.1% and 100%, *e.g.*, between .5 and 50%, between 1-30%, between 5-80%, or at least 80% (w/w) active ingredient. In one embodiment, active ingredients are antibodies directed toward cancer cells.

Formulation

[00455] Glycan-interacting antibodies of the can be formulated using one or more excipients to: (1) increase stability; (2) increase cell permeability; (3) permit the sustained or delayed release (*e.g.*, from a formulation of the glycan-interacting antibody); and/or (4) alter

the biodistribution (e.g., target the glycan-interacting antibody to specific tissues or cell types). In addition to traditional excipients such as any and all solvents, dispersion media, diluents, or other liquid vehicles, dispersion or suspension aids, surface active agents, isotonic agents, thickening or emulsifying agents, preservatives, formulations of the present invention can include, without limitation, liposomes, lipid nanoparticles, polymers, lipoplexes, core-shell nanoparticles, peptides, proteins, cells transfected with the glycan-interacting antibodies (e.g., for transplantation into a subject) and combinations thereof.

Excipients

[00456] As used herein, the term “excipient” refers to any substance combined with a compound and/or composition of the invention before use. In some embodiments, excipients are inactive and used primarily as a carrier, diluent or vehicle for a compound and/or composition of the present invention. Various excipients for formulating pharmaceutical compositions and techniques for preparing the composition are known in the art (see Remington: The Science and Practice of Pharmacy, 21st Edition, A. R. Gennaro, Lippincott, Williams & Wilkins, Baltimore, MD, 2006; incorporated herein by reference).

[00457] The use of a conventional excipient medium is contemplated within the scope of the present disclosure, except insofar as any conventional excipient medium may be incompatible with a substance or its derivatives, such as by producing any undesirable biological effect or otherwise interacting in a deleterious manner with any other component(s) of the pharmaceutical composition.

[00458] Formulations of the pharmaceutical compositions described herein may be prepared by any method known or hereafter developed in the art of pharmacology. In general, such preparatory methods include the step of associating the active ingredient with an excipient and/or one or more other accessory ingredients.

[00459] A pharmaceutical composition in accordance with the present disclosure may be prepared, packaged, and/or sold in bulk, as a single unit dose, and/or as a plurality of single unit doses.

[00460] Relative amounts of the active ingredient, the pharmaceutically acceptable excipient, and/or any additional ingredients in a pharmaceutical composition in accordance with the present disclosure may vary, depending upon the identity, size, and/or condition of the subject being treated and further depending upon the route by which the composition is to be administered.

[00461] In some embodiments, a pharmaceutically acceptable excipient is at least 95%, at least 96%, at least 97%, at least 98%, at least 99%, or 100% pure. In some embodiments, an excipient is approved for use in humans and for veterinary use. In some embodiments, an excipient is approved by United States Food and Drug Administration. In some embodiments, an excipient is pharmaceutical grade. In some embodiments, an excipient meets the standards of the United States Pharmacopoeia (USP), the European Pharmacopoeia (EP), the British Pharmacopoeia, and/or the International Pharmacopoeia.

[00462] Pharmaceutically acceptable excipients used in the manufacture of pharmaceutical compositions include, but are not limited to, inert diluents, dispersing and/or granulating agents, surface active agents and/or emulsifiers, disintegrating agents, binding agents, preservatives, buffering agents, lubricating agents, and/or oils. Such excipients may optionally be included in pharmaceutical compositions.

[00463] Exemplary diluents include, but are not limited to, calcium carbonate, sodium carbonate, calcium phosphate, dicalcium phosphate, calcium sulfate, calcium hydrogen phosphate, sodium phosphate lactose, sucrose, cellulose, microcrystalline cellulose, kaolin, mannitol, sorbitol, inositol, sodium chloride, dry starch, cornstarch, powdered sugar, *etc.*, and/or combinations thereof.

[00464] Exemplary granulating and/or dispersing agents include, but are not limited to, potato starch, corn starch, tapioca starch, sodium starch glycolate, clays, alginic acid, guar gum, citrus pulp, agar, bentonite, cellulose and wood products, natural sponge, cation-exchange resins, calcium carbonate, silicates, sodium carbonate, cross-linked poly(vinylpyrrolidone) (crospovidone), sodium carboxymethyl starch (sodium starch glycolate), carboxymethyl cellulose, cross-linked sodium carboxymethyl cellulose (croscarmellose), methylcellulose, pregelatinized starch (starch 1500), microcrystalline starch, water insoluble starch, calcium carboxymethyl cellulose, magnesium aluminum silicate (VEEGUM[®]), sodium lauryl sulfate, quaternary ammonium compounds, *etc.*, and/or combinations thereof.

[00465] Exemplary surface active agents and/or emulsifiers include, but are not limited to, natural emulsifiers (e.g. acacia, agar, alginic acid, sodium alginate, tragacanth, chondrux, cholesterol, xanthan, pectin, gelatin, egg yolk, casein, wool fat, cholesterol, wax, and lecithin), colloidal clays (e.g. bentonite [aluminum silicate] and VEEGUM[®] [magnesium aluminum silicate]), long chain amino acid derivatives, high molecular weight alcohols (e.g. stearyl alcohol, cetyl alcohol, oleyl alcohol, triacetin monostearate, ethylene glycol distearate, glyceryl monostearate, and propylene glycol monostearate, polyvinyl alcohol), carbomers (e.g. carboxy polymethylene, polyacrylic acid, acrylic acid polymer, and carboxyvinyl

polymer), carrageenan, cellulosic derivatives (e.g. carboxymethylcellulose sodium, powdered cellulose, hydroxymethyl cellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, methylcellulose), sorbitan fatty acid esters (e.g. polyoxyethylene sorbitan monolaurate [TWEEN[®]20], polyoxyethylene sorbitan [TWEEN[®]60], polyoxyethylene sorbitan monooleate [TWEEN[®]80], sorbitan monopalmitate [SPAN[®]40], sorbitan monostearate [Span[®]60], sorbitan tristearate [Span[®]65], glyceryl monooleate, sorbitan monooleate [SPAN[®]80]), polyoxyethylene esters (e.g. polyoxyethylene monostearate [MYRJ[®]45], polyoxyethylene hydrogenated castor oil, polyethoxylated castor oil, polyoxymethylene stearate, and SOLUTOL[®]), sucrose fatty acid esters, polyethylene glycol fatty acid esters (e.g. CREMOPHOR[®]), polyoxyethylene ethers, (e.g. polyoxyethylene lauryl ether [BRIJ[®]30]), poly(vinyl-pyrrolidone), diethylene glycol monolaurate, triethanolamine oleate, sodium oleate, potassium oleate, ethyl oleate, oleic acid, ethyl laurate, sodium lauryl sulfate, PLUORINC[®]F 68, POLOXAMER[®]188, cetrimonium bromide, cetylpyridinium chloride, benzalkonium chloride, docusate sodium, etc. and/or combinations thereof.

[00466] Exemplary binding agents include, but are not limited to, starch (*e.g.* cornstarch and starch paste); gelatin; sugars (*e.g.* sucrose, glucose, dextrose, dextrin, molasses, lactose, lactitol, mannitol,); natural and synthetic gums (*e.g.* acacia, sodium alginate, extract of Irish moss, panwar gum, ghatti gum, mucilage of isapol husks, carboxymethylcellulose, methylcellulose, ethylcellulose, hydroxyethylcellulose, hydroxypropyl cellulose, hydroxypropyl methylcellulose, microcrystalline cellulose, cellulose acetate, poly(vinyl-pyrrolidone), magnesium aluminum silicate (Veegum[®]), and larch arabogalactan); alginates; polyethylene oxide; polyethylene glycol; inorganic calcium salts; silicic acid; polymethacrylates; waxes; water; alcohol; *etc.*; and combinations thereof.

[00467] Exemplary preservatives may include, but are not limited to, antioxidants, chelating agents, antimicrobial preservatives, antifungal preservatives, alcohol preservatives, acidic preservatives, and/or other preservatives. Exemplary antioxidants include, but are not limited to, alpha tocopherol, ascorbic acid, acorbyl palmitate, butylated hydroxyanisole, butylated hydroxytoluene, monothioglycerol, potassium metabisulfite, propionic acid, propyl gallate, sodium ascorbate, sodium bisulfite, sodium metabisulfite, and/or sodium sulfite. Exemplary chelating agents include ethylenediaminetetraacetic acid (EDTA), citric acid monohydrate, disodium edetate, dipotassium edetate, edetic acid, fumaric acid, malic acid, phosphoric acid, sodium edetate, tartaric acid, and/or trisodium edetate. Exemplary antimicrobial preservatives include, but are not limited to, benzalkonium chloride, benzethonium chloride, benzyl alcohol, bronopol, cetrimide, cetylpyridinium chloride,

chlorhexidine, chlorobutanol, chlorocresol, chloroxylenol, cresol, ethyl alcohol, glycerin, hexetidine, imidurea, phenol, phenoxyethanol, phenylethyl alcohol, phenylmercuric nitrate, propylene glycol, and/or thimerosal. Exemplary antifungal preservatives include, but are not limited to, butyl paraben, methyl paraben, ethyl paraben, propyl paraben, benzoic acid, hydroxybenzoic acid, potassium benzoate, potassium sorbate, sodium benzoate, sodium propionate, and/or sorbic acid. Exemplary alcohol preservatives include, but are not limited to, ethanol, polyethylene glycol, phenol, phenolic compounds, bisphenol, chlorobutanol, hydroxybenzoate, and/or phenylethyl alcohol. Exemplary acidic preservatives include, but are not limited to, vitamin A, vitamin C, vitamin E, beta-carotene, citric acid, acetic acid, dehydroacetic acid, ascorbic acid, sorbic acid, and/or phytic acid. Other preservatives include, but are not limited to, tocopherol, tocopherol acetate, deteroxime mesylate, cetrimide, butylated hydroxyanisole (BHA), butylated hydroxytoluene (BHT), ethylenediamine, sodium lauryl sulfate (SLS), sodium lauryl ether sulfate (SLES), sodium bisulfite, sodium metabisulfite, potassium sulfite, potassium metabisulfite, GLYDANT PLUS[®], PHENONIP[®], methylparaben, GERMALL[®] 115, GERMABEN[®] II, NEOLONE[™], KATHON[™], and/or EUXYL[®].

[00468] Exemplary buffering agents include, but are not limited to, citrate buffer solutions, acetate buffer solutions, phosphate buffer solutions, ammonium chloride, calcium carbonate, calcium chloride, calcium citrate, calcium gluconate, calcium gluceptate, calcium gluconate, D-gluconic acid, calcium glycerophosphate, calcium lactate, propanoic acid, calcium levulinate, pentanoic acid, dibasic calcium phosphate, phosphoric acid, tribasic calcium phosphate, calcium hydroxide phosphate, potassium acetate, potassium chloride, potassium gluconate, potassium mixtures, dibasic potassium phosphate, monobasic potassium phosphate, potassium phosphate mixtures, sodium acetate, sodium bicarbonate, sodium chloride, sodium citrate, sodium lactate, dibasic sodium phosphate, monobasic sodium phosphate, sodium phosphate mixtures, tromethamine, magnesium hydroxide, aluminum hydroxide, alginic acid, pyrogen-free water, isotonic saline, Ringer's solution, ethyl alcohol, *etc.*, and/or combinations thereof.

[00469] Exemplary lubricating agents include, but are not limited to, magnesium stearate, calcium stearate, stearic acid, silica, talc, malt, glyceryl behenate, hydrogenated vegetable oils, polyethylene glycol, sodium benzoate, sodium acetate, sodium chloride, leucine, magnesium lauryl sulfate, sodium lauryl sulfate, *etc.*, and combinations thereof.

[00470] Exemplary oils include, but are not limited to, almond, apricot kernel, avocado, babassu, bergamot, black current seed, borage, cade, camomile, canola, caraway, carnauba,

castor, cinnamon, cocoa butter, coconut, cod liver, coffee, corn, cotton seed, emu, eucalyptus, evening primrose, fish, flaxseed, geraniol, gourd, grape seed, hazel nut, hyssop, isopropyl myristate, jojoba, kukui nut, lavandin, lavender, lemon, litsea cubeba, macademia nut, mallow, mango seed, meadowfoam seed, mint, nutmeg, olive, orange, orange roughy, palm, palm kernel, peach kernel, peanut, poppy seed, pumpkin seed, rapeseed, rice bran, rosemary, safflower, sandalwood, sasquana, savoury, sea buckthorn, sesame, shea butter, silicone, soybean, sunflower, tea tree, thistle, tsubaki, vetiver, walnut, and wheat germ oils. Exemplary oils include, but are not limited to, butyl stearate, caprylic triglyceride, capric triglyceride, cyclomethicone, diethyl sebacate, dimethicone 360, isopropyl myristate, mineral oil, octyldodecanol, oleyl alcohol, silicone oil, and/or combinations thereof.

[00471] Excipients such as cocoa butter and suppository waxes, coloring agents, coating agents, sweetening, flavoring, and/or perfuming agents can be present in the composition, according to the judgment of the formulator.

[00472] In some embodiments, antibodies of the present disclosure are formulated with at least one excipient. The antibodies may be anti-STn antibodies. The antibodies may be antibody drug conjugates that include a therapeutic agent, including, but not limited to any of those presented herein. The therapeutic agent may be a cytotoxic agent, including, but not limited to any of those presented herein. The cytotoxic agent may be MMAE. The cytotoxic agent may be joined to the antibody by a linker.

Vehicles

Liposomes, lipoplexes and lipid nanoparticles

[00473] Glycan-interacting antibodies of the present invention may be formulated using one or more liposomes, lipoplexes, or lipid nanoparticles. In one embodiment, pharmaceutical compositions comprising glycan-interacting antibodies further comprise liposomes. Liposomes are artificially-prepared vesicles which may primarily comprise one or more lipid bilayers and may be used as a delivery vehicle for the administration of nutrients and pharmaceutical formulations. Liposomes can be of different sizes such as, but not limited to, a multilamellar vesicle (MLV) which may be hundreds of nanometers in diameter and may contain a series of concentric bilayers separated by narrow aqueous compartments, a small unicellular vesicle (SUV) which may be smaller than 50 nm in diameter, and a large unilamellar vesicle (LUV) which may be between 50 and 500 nm in diameter. Liposome design may include, but is not limited to, opsonins or ligands in order to improve the attachment of liposomes to unhealthy tissue or to activate events such as, but not limited to,

endocytosis. Liposomes may contain a low or a high pH in order to improve the delivery of the pharmaceutical formulations.

[00474] The formation of liposomes may depend on the physicochemical characteristics such as, but not limited to, the pharmaceutical formulation entrapped and the liposomal ingredients, the nature of the medium in which the lipid vesicles are dispersed, the effective concentration of the entrapped substance and its potential toxicity, any additional processes involved during the application and/or delivery of the vesicles, the optimization size, polydispersity and the shelf-life of the vesicles for the intended application, and the batch-to-batch reproducibility and possibility of large-scale production of safe and efficient liposomal products.

[00475] In one embodiment such formulations may also be constructed or compositions altered such that they passively or actively are directed to different cell types *in vivo*.

[00476] Formulations can also be selectively targeted through expression of different ligands on their surface as exemplified by, but not limited by, folate, transferrin, N-acetylgalactosamine (GalNAc), and antibody targeted approaches.

[00477] Liposomes, lipoplexes, or lipid nanoparticles may be used to improve the efficacy of glycan-interacting antibody function as these formulations may be able to increase cell transfection with glycan-interacting antibodies. The liposomes, lipoplexes, or lipid nanoparticles may also be used to increase the stability of glycan-interacting antibodies.

[00478] Liposomes that are specifically formulated for antibody cargo are prepared according to techniques known in the art, such as described by Eppstein et al. (Eppstein, D.A. et al., *Biological activity of liposome-encapsulated murine interferon gamma is mediated by a cell membrane receptor*. Proc Natl Acad Sci U S A. 1985 Jun;82(11):3688-92); Hwang et al. (Hwang, K.J. et al., *Hepatic uptake and degradation of unilamellar sphingomyelin/cholesterol liposomes: a kinetic study*. Proc Natl Acad Sci U S A. 1980 Jul;77(7):4030-4); US 4,485,045 and US 4,544,545. Production of liposomes with sustained circulation time is also described in US 5,013,556.

[00479] Liposomes comprising glycan-interacting antibodies of the present invention may be generated using reverse phase evaporation utilizing lipids such as phosphatidylcholine, cholesterol as well as phosphatidylethanolamine that has been polyethylene glycol-derivatized. Filters with defined pore size are used to extrude liposomes of the desired diameter. In another embodiment, glycan-interacting antibodies of the present invention can be conjugated to the external surface of liposomes by disulfide interchange reaction as is described by Martin et al. (Martin, F.J. et al., *Irreversible coupling of immunoglobulin*

fragments to preformed vesicles. An improved method for liposome targeting. J Biol Chem. 1982 Jan 10;257(1):286-8).

Polymers and nanoparticles

[00480] Glycan-interacting antibodies of the invention can be formulated using natural and/or synthetic polymers. Non-limiting examples of polymers which may be used for delivery include, but are not limited to DMRI/DOPE, poloxamer, chitosan, cyclodextrin, and poly(lactic-co-glycolic acid) (PLGA) polymers. These may be biodegradable.

[00481] The polymer formulation can permit the sustained or delayed release of glycan-interacting antibodies (e.g., following intramuscular or subcutaneous injection). The altered release profile for glycan-interacting antibodies can result in, for example, release of the glycan-interacting antibodies over an extended period of time. The polymer formulation may also be used to increase the stability of glycan-interacting antibodies.

[00482] Polymer formulations can also be selectively targeted through expression of different ligands as exemplified by, but not limited by, folate, transferrin, and N-acetylgalactosamine (GalNAc) (Benoit et al., Biomacromolecules. 2011 12:2708-2714; Rozema et al., Proc Natl Acad Sci U S A. 2007 104:12982-12887; Davis, Mol Pharm. 2009 6:659-668; Davis, Nature 2010 464:1067-1070; herein incorporated by reference in its entirety).

[00483] Glycan-interacting antibodies of the invention can also be formulated as nanoparticles using a combination of polymers, lipids, and/or other biodegradable agents, such as, but not limited to, calcium phosphate. Components may be combined in a core-shell, hybrid, and/or layer-by-layer architecture, to allow for fine-tuning of the nanoparticle so delivery of glycan-interacting antibodies may be enhanced. For glycan-interacting antibodies, systems based on poly(2-(methacryloyloxy)ethyl phosphorylcholine)-block-(2-(diisopropylamino)ethyl methacrylate), (PMPC-PDPA), a pH sensitive diblock copolymer that self-assembles to form nanometer-sized vesicles, also known as polymersomes, at physiological pH may be used. These polymersomes have been shown to successfully deliver relatively high antibody payloads within live cells. (Massignani, et al, Cellular delivery of antibodies: effective targeted subcellular imaging and new therapeutic tool. Nature Proceedings, May, 2010).

[00484] In one embodiment, a PEG-charge-conversional polymer (Pitella et al., Biomaterials. 2011 32:3106-3114) may be used to form a nanoparticle to deliver glycan-interacting antibodies of the present invention. The PEG-charge-conversional polymer may

improve upon the PEG-polyanion block copolymers by being cleaved into a polycation at acidic pH, thus enhancing endosomal escape.

[00485] The use of core-shell nanoparticles has additionally focused on a high-throughput approach to synthesize cationic cross-linked nanogel cores and various shells (Siegwart et al., Proc Natl Acad Sci U S A. 2011 108:12996-13001). The complexation, delivery, and internalization of the polymeric nanoparticles can be precisely controlled by altering the chemical composition in both the core and shell components of the nanoparticle.

[00486] In one embodiment, matrices of poly(ethylene-co-vinyl acetate), are used to deliver glycan-interacting antibodies of the invention. Such matrices are described in Nature Biotechnology 10, 1446 - 1449 (1992).

Antibody formulations

[00487] Glycan-interacting antibodies of the invention may be formulated for intravenous administration or extravascular administration (Daugherty, et al., *Formulation and delivery issues for monoclonal antibody therapeutics*. Adv Drug Deliv Rev. 2006 Aug 7;58(5-6):686-706, US patent publication number 2011/0135570, all of which are incorporated herein in their entirety). Extravascular administration routes may include, but are not limited to subcutaneous administration, intraperitoneal administration, intracerebral administration, intraocular administration, intralesional administration, topical administration and intramuscular administration.

[00488] Antibody structures may be modified to improve their effectiveness as therapeutics. Improvements may include, but are not limited to improved thermodynamic stability, reduced Fc receptor binding properties and improved folding efficiency. Modifications may include, but are not limited to amino acid substitutions, glycosylation, palmitoylation and protein conjugation.

[00489] Glycan-interacting antibodies may be formulated with antioxidants to reduce antibody oxidation. glycan-interacting antibodies may also be formulated with additives to reduce protein aggregation. Such additives may include, but are not limited to albumin, amino acids, sugars, urea, guanidinium chloride, polyalcohols, polymers (such as polyethylene glycol and dextrans), surfactants (including, but not limited to polysorbate 20 and polysorbate 80) or even other antibodies.

[00490] Glycan-interacting antibodies of the present invention may be formulated to reduce the impact of water on antibody structure and function. Antibody preparations in such formulations may be lyophilized. Formulations subject to lyophilization may include

carbohydrates or polyol compounds to protect and stabilize antibody structure. Such compounds include, but are not limited to sucrose, trehalose and mannitol.

[00491] Glycan-interacting antibodies of the present invention may be formulated with polymers. In one embodiment, polymer formulations may contain hydrophobic polymers. Such polymers may be microspheres formulated with polylactide-co-glycolide through a solid-in-oil-in-water encapsulation method. Microspheres comprising ethylene-vinyl acetate copolymer are also contemplated for antibody delivery and may be used to extend the time course of antibody release at the site of delivery. In another embodiment, polymers may be aqueous gels. Such gels may, for example, comprise carboxymethylcellulose. Aqueous gels may also comprise hyaluronic acid hydrogel. Antibodies may be covalently linked to such gels through a hydrazone linkage that allows for sustained delivery in tissues, including but not limited to the tissues of the central nervous system.

Peptide and protein formulations

[00492] Glycan-interacting antibodies of the invention may be formulated with peptides and/or proteins. In one embodiment, peptides such as, but not limited to, cell penetrating peptides and proteins and peptides that enable intracellular delivery may be used to deliver pharmaceutical formulations. A non-limiting example of a cell penetrating peptide which may be used with the pharmaceutical formulations of the present invention includes a cell-penetrating peptide sequence attached to polycations that facilitates delivery to the intracellular space, e.g., HIV-derived TAT peptide, penetratins, transportans, or hCT derived cell-penetrating peptides (see, e.g., Caron et al., Mol. Ther. 3(3):310-8 (2001); Langel, Cell-Penetrating Peptides: Processes and Applications (CRC Press, Boca Raton FL, 2002); El-Andaloussi et al., Curr. Pharm. Des. 11(28):3597-611 (2003); and Deshayes et al., Cell. Mol. Life Sci. 62(16):1839-49 (2005), all of which are incorporated herein by reference). The compositions can also be formulated to include a cell penetrating agent, e.g., liposomes, which enhance delivery of the compositions to the intracellular space. Glycan-interacting antibodies of the invention may be complexed to peptides and/or proteins such as, but not limited to, peptides and/or proteins from Aileron Therapeutics (Cambridge, MA) and Permeon Biologics (Cambridge, MA) in order to enable intracellular delivery (Cronican et al., ACS Chem. Biol. 2010 5:747-752; McNaughton et al., Proc. Natl. Acad. Sci. USA 2009 106:6111-6116; Sawyer, Chem Biol Drug Des. 2009 73:3-6; Verdine and Hilinski, Methods Enzymol. 2012; 503:3-33; all of which are herein incorporated by reference in their entirety).

[00493] In one embodiment, the cell-penetrating polypeptide may comprise a first domain and a second domain. The first domain may comprise a supercharged polypeptide. The second domain may comprise a protein-binding partner. As used herein, “protein-binding partner” includes, but are not limited to, antibodies and functional fragments thereof, scaffold proteins, or peptides. The cell-penetrating polypeptide may further comprise an intracellular binding partner for the protein-binding partner. The cell-penetrating polypeptide may be capable of being secreted from a cell where glycan-interacting antibodies may be introduced.

[00494] In formulations of the present invention, peptides or proteins may be incorporated to increase cell transfection by glycan-interacting antibodies or alter the biodistribution of glycan-interacting antibodies (e.g., by targeting specific tissues or cell types).

Cell formulations

[00495] Cell-based formulations of glycan-interacting antibody compositions of the invention may be used to ensure cell transfection (e.g., in the cellular carrier) or alter the biodistribution of the compositions (e.g., by targeting the cell carrier to specific tissues or cell types).

Cell transfer methods

[00496] A variety of methods are known in the art and are suitable for introduction of nucleic acids or proteins, such as glycan-interacting antibodies, into a cell, including viral and non-viral mediated techniques. Examples of typical non-viral mediated techniques include, but are not limited to, electroporation, calcium phosphate mediated transfer, nucleofection, sonoporation, heat shock, magnetofection, liposome mediated transfer, microinjection, microprojectile mediated transfer (nanoparticles), cationic polymer mediated transfer (DEAE-dextran, polyethylenimine, polyethylene glycol (PEG) and the like) or cell fusion.

[00497] The technique of sonoporation, or cellular sonication, is the use of sound (e.g., ultrasonic frequencies) for modifying the permeability of the cell plasma membrane.

Sonoporation methods are known to those in the art and are used to deliver nucleic acids *in vivo* (Yoon and Park, *Expert Opin Drug Deliv.* 2010 7:321-330; Postema and Gilja, *Curr Pharm Biotechnol.* 2007 8:355-361; Newman and Bettinger, *Gene Ther.* 2007 14:465-475; all herein incorporated by reference in their entirety). Sonoporation methods are known in the art and are also taught for example as it relates to bacteria in US Patent Publication 20100196983 and as it relates to other cell types in, for example, US Patent Publication 20100009424, each of which are incorporated herein by reference in their entirety.

[00498] Electroporation techniques are also well known in the art and are used to deliver nucleic acids *in vivo* and clinically (Andre et al., Curr Gene Ther. 2010 10:267-280; Chiarella et al., Curr Gene Ther. 2010 10:281-286; Hojman, Curr Gene Ther. 2010 10:128-138; all herein incorporated by reference in their entirety). In one embodiment, glycan-interacting antibodies may be delivered by electroporation.

Administration and delivery

[00499] The compositions of the present disclosure may be administered by any of the standard methods or routes known in the art.

[00500] Glycan-interacting antibodies of the present disclosure may be administered by any route which results in a therapeutically effective outcome. These include, but are not limited to enteral, gastrointestinal, epidural, oral, transdermal, epidural (peridural), intracerebral (into the cerebrum), intracerebroventricular (into the cerebral ventricles), epicutaneous (application onto the skin), intradermal, (into the skin itself), subcutaneous (under the skin), nasal administration (through the nose), intravenous (into a vein), intraarterial (into an artery), intramuscular (into a muscle), intracardiac (into the heart), intraosseous infusion (into the bone marrow), intrathecal (into the spinal canal), intraperitoneal, (infusion or injection into the peritoneum), intravesical infusion, intravitreal, (through the eye), intracavernous injection, (into the base of the penis), intravaginal administration, intrauterine, extra-amniotic administration, transdermal (diffusion through the intact skin for systemic distribution), transmucosal (diffusion through a mucous membrane), insufflation (snorting), sublingual, sublabial, enema, eye drops (onto the conjunctiva), or in ear drops. In specific embodiments, compositions may be administered in a way which allows them cross the blood-brain barrier, vascular barrier, or other epithelial barrier. Non-limiting routes of administration for glycan-interacting antibodies of the present invention are described below.

Parenteral and injectable administration

[00501] Liquid dosage forms for oral and parenteral administration include, but are not limited to, pharmaceutically acceptable emulsions, microemulsions, solutions, suspensions, syrups, and/or elixirs. In addition to active ingredients, liquid dosage forms may comprise inert diluents commonly used in the art such as, for example, water or other solvents, solubilizing agents and emulsifiers such as ethyl alcohol, isopropyl alcohol, ethyl carbonate, ethyl acetate, benzyl alcohol, benzyl benzoate, propylene glycol, 1,3-butylene glycol, dimethylformamide, oils (in particular, cottonseed, groundnut, corn, germ, olive, castor, and

sesame oils), glycerol, tetrahydrofurfuryl alcohol, polyethylene glycols and fatty acid esters of sorbitan, and mixtures thereof. Besides inert diluents, oral compositions can include adjuvants such as wetting agents, emulsifying and suspending agents, sweetening, flavoring, and/or perfuming agents. In certain embodiments for parenteral administration, compositions are mixed with solubilizing agents such as CREMOPHOR[®], alcohols, oils, modified oils, glycols, polysorbates, cyclodextrins, polymers, and/or combinations thereof. In other embodiments, surfactants are included such as hydroxypropylcellulose.

[00502] Injectable preparations, for example, sterile injectable aqueous or oleaginous suspensions may be formulated according to the known art using suitable dispersing agents, wetting agents, and/or suspending agents. Sterile injectable preparations may be sterile injectable solutions, suspensions, and/or emulsions in nontoxic parenterally acceptable diluents and/or solvents, for example, as a solution in 1,3-butanediol. Among the acceptable vehicles and solvents that may be employed are water, Ringer's solution, U.S.P., and isotonic sodium chloride solution. Sterile, fixed oils are conventionally employed as a solvent or suspending medium. For this purpose, any bland fixed oil can be employed including synthetic mono- or diglycerides. Fatty acids such as oleic acid can be used in the preparation of injectables.

[00503] Injectable formulations can be sterilized, for example, by filtration through a bacterial-retaining filter, and/or by incorporating sterilizing agents in the form of sterile solid compositions which can be dissolved or dispersed in sterile water or other sterile injectable medium prior to use.

[00504] In order to prolong the effect of an active ingredient, it is often desirable to slow the absorption of the active ingredient from subcutaneous or intramuscular injection. This may be accomplished by the use of a liquid suspension of crystalline or amorphous material with poor water solubility. The rate of absorption of the drug then depends upon its rate of dissolution which, in turn, may depend upon crystal size and crystalline form. Alternatively, delayed absorption of a parenterally administered drug form is accomplished by dissolving or suspending the drug in an oil vehicle. Injectable depot forms are made by forming microencapsule matrices of the drug in biodegradable polymers such as polylactide-polyglycolide. Depending upon the ratio of drug to polymer and the nature of the particular polymer employed, the rate of drug release can be controlled. Examples of other biodegradable polymers include poly(orthoesters) and poly(anhydrides). Depot injectable formulations are prepared by entrapping the drug in liposomes or microemulsions which are compatible with body tissues.

[00505] In some embodiments, compositions of the present disclosure may be administered intravenously. The administration may be by intravenous bolus injection.

Rectal and vaginal administration

[00506] Compositions for rectal or vaginal administration are typically suppositories which can be prepared by mixing compositions with suitable non-irritating excipients such as cocoa butter, polyethylene glycol or a suppository wax which are solid at ambient temperature but liquid at body temperature and therefore melt in the rectum or vaginal cavity and release the active ingredient.

Oral administration

[00507] Solid dosage forms for oral administration include capsules, tablets, pills, powders, and granules. In such solid dosage forms, an active ingredient is mixed with at least one inert, pharmaceutically acceptable excipient such as sodium citrate or dicalcium phosphate and/or fillers or extenders (e.g. starches, lactose, sucrose, glucose, mannitol, and silicic acid), binders (e.g. carboxymethylcellulose, alginates, gelatin, polyvinylpyrrolidinone, sucrose, and acacia), humectants (e.g. glycerol), disintegrating agents (e.g. agar, calcium carbonate, potato or tapioca starch, alginic acid, certain silicates, and sodium carbonate), solution retarding agents (e.g. paraffin), absorption accelerators (e.g. quaternary ammonium compounds), wetting agents (e.g. cetyl alcohol and glycerol monostearate), absorbents (e.g. kaolin and bentonite clay), and lubricants (e.g. talc, calcium stearate, magnesium stearate, solid polyethylene glycols, sodium lauryl sulfate), and mixtures thereof. In the case of capsules, tablets and pills, the dosage form may comprise buffering agents.

Topical or transdermal administration

[00508] As described herein, compositions containing glycan-interacting antibodies of the invention may be formulated for administration topically. The skin may be an ideal target site for delivery as it is readily accessible. Gene expression may be restricted not only to the skin, potentially avoiding nonspecific toxicity, but also to specific layers and cell types within the skin.

[00509] The site of cutaneous expression of the delivered compositions will depend on the route of nucleic acid delivery. Three routes are commonly considered to deliver glycan-interacting antibodies to the skin: (i) topical application (e.g. for local/regional treatment and/or cosmetic applications); (ii) intradermal injection (e.g. for local/regional treatment and/or cosmetic applications); and (iii) systemic delivery (e.g. for treatment of dermatologic

diseases that affect both cutaneous and extracutaneous regions). glycan-interacting antibodies can be delivered to the skin by several different approaches known in the art.

[00510] In one embodiment, the invention provides for a variety of dressings (e.g., wound dressings) or bandages (e.g., adhesive bandages) for conveniently and/or effectively carrying out methods of the present invention. Typically dressing or bandages may comprise sufficient amounts of pharmaceutical compositions and/or glycan-interacting antibodies described herein to allow a user to perform multiple treatments of a subject(s).

[00511] In one embodiment, the invention provides for compositions comprising glycan-interacting antibodies to be delivered in more than one injection.

[00512] Dosage forms for topical and/or transdermal administration of a composition may include ointments, pastes, creams, lotions, gels, powders, solutions, sprays, inhalants and/or patches. Generally, an active ingredient is admixed under sterile conditions with a pharmaceutically acceptable excipient and/or any needed preservatives and/or buffers as may be required.

[00513] Additionally, the present invention contemplates the use of transdermal patches, which often have the added advantage of providing controlled delivery of a compound to the body. Such dosage forms may be prepared, for example, by dissolving and/or dispensing the compound in the proper medium. Alternatively, or additionally, rate may be controlled by either providing a rate controlling membrane and/or by dispersing the compound in a polymer matrix and/or gel.

[00514] Formulations suitable for topical administration include, but are not limited to, liquid and/or semi liquid preparations such as liniments, lotions, oil in water and/or water in oil emulsions such as creams, ointments and/or pastes, and/or solutions and/or suspensions.

[00515] Topically-administrable formulations may, for example, comprise from about 1% to about 10% (w/w) active ingredient, although the concentration of active ingredient may be as high as the solubility limit of the active ingredient in the solvent. Formulations for topical administration may further comprise one or more of the additional ingredients described herein.

Depot administration

[00516] As described herein, in some embodiments, compositions of the present invention are formulated in depots for extended release. Generally, a specific organ or tissue (a “target tissue”) is targeted for administration.

[00517] In some aspects of the invention, glycan-interacting antibodies are spatially retained within or proximal to a target tissue. Provided are methods of providing compositions to one or more target tissue of a mammalian subject by contacting the one or more target tissue (comprising one or more target cells) with compositions under conditions such that the compositions, in particular glycan-interacting antibody component(s) of the compositions, are substantially retained in the target tissue, meaning that at least 10, 20, 30, 40, 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.99 or greater than 99.99% of the composition is retained in the target tissue. Advantageously, retention is determined by measuring the level of glycan-interacting antibodies present in the compositions entering the target tissues and/or cells. For example, at least 1, 5, 10, 20, 30, 40, 50, 60, 70, 80, 85, 90, 95, 96, 97, 98, 99, 99.9, 99.99 or greater than 99.99% of glycan-interacting antibodies administered to the subject are present intracellularly at a period of time following administration. For example, intramuscular injection to a mammalian subject is performed using an aqueous composition comprising one or more glycan-interacting antibody and a transfection reagent, and retention of the composition is determined by measuring the level of glycan-interacting antibodies present in the muscle cells.

[00518] Certain aspects of the invention are directed to methods of providing compositions to target tissues of mammalian subjects, by contacting the target tissues (containing one or more target cells) with compositions under conditions such that the compositions are substantially retained in the target tissue. Compositions contain an effective amount of glycan-interacting antibodies such that the effect of interest is produced in at least one target cell. Compositions generally contain cell penetration agents and a pharmaceutically acceptable carrier, although “naked” glycan-interacting antibodies (such as glycan-interacting antibodies without cell penetration agents or other agents) are also contemplated.

[00519] In some embodiments, compositions include a plurality of different glycan-interacting antibodies, where one or more than one of the glycan-interacting antibodies targets a glycan of interest. Optionally, compositions also contain cell penetration agents to assist in the intracellular delivery of compositions. A determination is made of the composition dose required to target glycans of interest in a substantial percentage of cells contained within a predetermined volume of the target tissue (generally, without targeting glycans in tissue adjacent to the predetermined volume, or distally to target tissues). Subsequent to this determination, the determined dose may be introduced directly into the tissue of the mammalian subject.

[00520] In one embodiment, the invention provides for glycan-interacting antibodies to be delivered in more than one injection or by split dose injections.

Pulmonary administration

[00521] Pharmaceutical compositions may be prepared, packaged, and/or sold in formulations suitable for pulmonary administration via the buccal cavity. Such formulations may comprise dry particles further comprising active ingredients and having a diameter in the range from about 0.5 nm to about 7 nm or from about 1 nm to about 6 nm. Such compositions are suitably in the form of dry powders for administration using a device comprising a dry powder reservoir to which a stream of propellant may be directed to disperse the powder and/or using a self-propelling solvent/powder dispensing container such as a device comprising the active ingredient dissolved and/or suspended in a low-boiling propellant in a sealed container. Such powders comprise particles wherein at least 98% of the particles by weight have a diameter greater than 0.5 nm and at least 95% of the particles by number have a diameter less than 7 nm. Alternatively, at least 95% of the particles by weight have a diameter greater than 1 nm and at least 90% of the particles by number have a diameter less than 6 nm. Dry powder compositions may include a solid fine powder diluent such as sugar and are conveniently provided in a unit dose form.

[00522] Low boiling propellants generally include liquid propellants having a boiling point of below 65 °F at atmospheric pressure. Generally, the propellant may constitute 50% to 99.9% (w/w) of the composition, and active ingredient may constitute 0.1% to 20% (w/w) of the composition. A propellant may further comprise additional ingredients such as a liquid non-ionic and/or solid anionic surfactant and/or a solid diluent (which may have a particle size of the same order as particles comprising the active ingredient).

[00523] Pharmaceutical compositions formulated for pulmonary delivery may provide an active ingredient in the form of droplets of a solution and/or suspension. Such formulations may be prepared, packaged, and/or sold as aqueous and/or dilute alcoholic solutions and/or suspensions, optionally sterile, comprising active ingredient, and may conveniently be administered using any nebulization and/or atomization device. Such formulations may further comprise one or more additional ingredients including, but not limited to, a flavoring agent such as saccharin sodium, a volatile oil, a buffering agent, a surface active agent, and/or a preservative such as methylhydroxybenzoate. Droplets provided by this route of administration may have an average diameter in the range from about 0.1 nm to about 200 nm.

Intranasal, nasal and buccal administration

[00524] Formulations described herein as being useful for pulmonary delivery are useful for intranasal delivery of a pharmaceutical composition. Another formulation suitable for intranasal administration is a coarse powder comprising the active ingredient and having an average particle from about 0.2 μm to 500 μm . Such a formulation is administered in the manner in which snuff is taken, *i.e.* by rapid inhalation through the nasal passage from a container of the powder held close to the nose.

[00525] Formulations suitable for nasal administration may, for example, comprise from about as little as 0.1% (w/w) and as much as 100% (w/w) of active ingredient, and may comprise one or more of the additional ingredients described herein. A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for buccal administration. Such formulations may, for example, be in the form of tablets and/or lozenges made using conventional methods, and may, for example, 0.1% to 20% (w/w) active ingredient, the balance comprising an orally dissolvable and/or degradable composition and, optionally, one or more of the additional ingredients described herein. Alternately, formulations suitable for buccal administration may comprise a powder and/or an aerosolized and/or atomized solution and/or suspension comprising active ingredient. Such powdered, aerosolized, and/or aerosolized formulations, when dispersed, may have an average particle and/or droplet size in the range from about 0.1 nm to about 200 nm, and may further comprise one or more of any additional ingredients described herein.

Ophthalmic or otic administration

[00526] A pharmaceutical composition may be prepared, packaged, and/or sold in a formulation suitable for ophthalmic or otic administration. Such formulations may, for example, be in the form of eye or ear drops including, for example, a 0.1/1.0% (w/w) solution and/or suspension of the active ingredient in an aqueous or oily liquid excipient. Such drops may further comprise buffering agents, salts, and/or one or more other of any additional ingredients described herein. Other ophthalmically-administrable formulations which are useful include those which comprise the active ingredient in microcrystalline form and/or in a liposomal preparation. Subretinal inserts may also be used as a form of administration.

Payload administration

[00527] Glycan-interacting antibodies described herein may be used in a number of different scenarios in which delivery of a substance (the “payload”) to a biological target is

desired, for example delivery of detectable substances for detection of the target, or delivery of a therapeutic or diagnostic agent. Detection methods can include, but are not limited to, both imaging *in vitro* and *in vivo* imaging methods, *e.g.*, immunohistochemistry, bioluminescence imaging (BLI), Magnetic Resonance Imaging (MRI), positron emission tomography (PET), electron microscopy, X-ray computed tomography, Raman imaging, optical coherence tomography, absorption imaging, thermal imaging, fluorescence reflectance imaging, fluorescence microscopy, fluorescence molecular tomographic imaging, nuclear magnetic resonance imaging, X-ray imaging, ultrasound imaging, photoacoustic imaging, lab assays, or in any situation where tagging/staining/imaging is required.

[00528] Glycan-interacting antibodies can be designed to include both a linker and a payload in any useful orientation. For example, a linker having two ends is used to attach one end to the payload and the other end to the glycan-interacting antibody. The glycan-interacting antibodies of the invention can include more than one payload as well as a cleavable linker. In another example, a drug that may be attached to glycan-interacting antibodies via a linker and may be fluorescently labeled can be used to track the drug *in vivo*, *e.g.* intracellularly.

[00529] Other examples include, but are not limited to, the use of glycan-interacting antibodies in reversible drug delivery into cells.

[00530] Glycan-interacting antibodies described herein can be used in intracellular targeting of a payload, *e.g.*, detectable or therapeutic agents, to specific organelles. In addition, glycan-interacting antibodies described herein may be used to deliver therapeutic agents to cells or tissues, *e.g.*, in living animals. For example, glycan-interacting antibodies described herein may be used to deliver chemotherapeutic agents to kill cancer cells. glycan-interacting antibodies attached to therapeutic agents through linkers can facilitate member permeation allowing the therapeutic agent to travel into a cell to reach an intracellular target.

[00531] In some embodiments, the payload may be a therapeutic agent such as a cytotoxin, radioactive ion, chemotherapeutic, or other therapeutic agent. A cytotoxin or cytotoxic agent includes any agent that may be detrimental to cells. Examples include, but are not limited to, taxol, cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, teniposide, vincristine, vinblastine, colchicine, doxorubicin, daunorubicin, dihydroxyanthracenedione, mitoxantrone, mithramycin, actinomycin D, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, maytansinoids, *e.g.*, maytansinol (see U.S. Pat. No. 5,208,020 incorporated herein in its entirety), rachelmycin (CC-1065, see U.S. Pat. Nos. 5,475,092, 5,585,499, and 5,846,545, all

of which are incorporated herein by reference), and analogs or homologs thereof. Radioactive ions include, but are not limited to iodine (*e.g.*, iodine 125 or iodine 131), strontium 89, phosphorous, palladium, cesium, iridium, phosphate, cobalt, yttrium 90, samarium 153, and praseodymium. Other therapeutic agents include, but are not limited to, antimetabolites (*e.g.*, methotrexate, 6-mercaptopurine, 6-thioguanine, cytarabine, 5-fluorouracil decarbazine), alkylating agents (*e.g.*, mechlorethamine, thiotepa chlorambucil, rachelmycin (CC-1065), melphalan, carmustine (BSNU), lomustine (CCNU), cyclophosphamide, busulfan, dibromomannitol, streptozotocin, mitomycin C, and cis-dichlorodiamine platinum (II) (DDP) cisplatin), anthracyclines (*e.g.*, daunorubicin (formerly daunomycin) and doxorubicin), antibiotics (*e.g.*, dactinomycin (formerly actinomycin), bleomycin, mithramycin, and anthramycin (AMC)), and anti-mitotic agents (*e.g.*, vincristine, vinblastine, taxol and maytansinoids). In the case of anti-STn antibodies of the present invention, tumor killing may be boosted by the conjugation of a toxin to such anti-STn antibodies.

[00532] In some embodiments, the payload may be a detectable agent, such as various organic small molecules, inorganic compounds, nanoparticles, enzymes or enzyme substrates, fluorescent materials, luminescent materials (*e.g.*, luminol), bioluminescent materials (*e.g.*, luciferase, luciferin, and aequorin), chemiluminescent materials, radioactive materials (*e.g.*, ^{18}F , ^{67}Ga , $^{81\text{m}}\text{Kr}$, ^{82}Rb , ^{111}In , ^{123}I , ^{133}Xe , ^{201}Tl , ^{125}I , ^{35}S , ^{14}C , ^3H , or $^{99\text{m}}\text{Tc}$ (*e.g.*, as pertechnetate (technetate(VII), TcO_4^-)), and contrast agents (*e.g.*, gold (*e.g.*, gold nanoparticles), gadolinium (*e.g.*, chelated Gd), iron oxides (*e.g.*, superparamagnetic iron oxide (SPIO), monocrystalline iron oxide nanoparticles (MIONs), and ultrasmall superparamagnetic iron oxide (USPIO)), manganese chelates (*e.g.*, Mn-DPDP), barium sulfate, iodinated contrast media (iohexol), microbubbles, or perfluorocarbons). Such optically-detectable labels include for example, without limitation, 4-acetamido-4'-isothiocyanatostilbene-2,2'-disulfonic acid; acridine and derivatives (*e.g.*, acridine and acridine isothiocyanate); 5-(2'-aminoethyl)aminonaphthalene-1-sulfonic acid (EDANS); 4-amino-N-[3-vinylsulfonyl]phenyl]naphthalimide-3,5 disulfonate; N-(4-anilino-1-naphthyl)maleimide; anthranilamide; BODIPY; Brilliant Yellow; coumarin and derivatives (*e.g.*, coumarin, 7-amino-4-methylcoumarin (AMC, Coumarin 120), and 7-amino-4-trifluoromethylcoumarin (Coumarin 151)); cyanine dyes; cyanosine; 4',6-diaminidino-2-phenylindole (DAPI); 5' 5"-dibromopyrogallol-sulfonaphthalein (Bromopyrogallol Red); 7-diethylamino-3-(4'-isothiocyanatophenyl)-4-methylcoumarin; diethylenetriamine pentaacetate; 4,4'-diisothiocyanatodihydro-stilbene-2,2'-disulfonic acid; 4,4'-diisothiocyanatostilbene-2,2'-disulfonic acid; 5-[dimethylamino]-naphthalene-1-sulfonyl chloride (DNS, dansylchloride);

4-dimethylaminophenylazophenyl-4'-isothiocyanate (DABITC); eosin and derivatives (e.g., eosin and eosin isothiocyanate); erythrosin and derivatives (e.g., erythrosin B and erythrosin isothiocyanate); ethidium; fluorescein and derivatives (e.g., 5-carboxyfluorescein (FAM), 5-(4,6-dichlorotriazin-2-yl)aminofluorescein (DTAF), 2',7'-dimethoxy-4'5'-dichloro-6-carboxyfluorescein, fluorescein, fluorescein isothiocyanate, X-rhodamine-5-(and-6)-isothiocyanate (QFITC or XRITC), and fluorescamine); 2-[2-[3-[[1,3-dihydro-1,1-dimethyl-3-(3-sulfopropyl)-2H-benz[e]indol-2-ylidene]ethylidene]-2-[4-(ethoxycarbonyl)-1-piperazinyl]-1-cyclopenten-1-yl]ethenyl]-1,1-dimethyl-3-(3-sulfopropyl)-1H-benz[e]indolium hydroxide, inner salt, compound with n,n-diethylethanamine(1:1) (IR144); 5-chloro-2-[2-[3-[(5-chloro-3-ethyl-2(3H)-benzothiazol-ylidene)ethylidene]-2-(diphenylamino)-1-cyclopenten-1-yl]ethenyl]-3-ethyl benzothiazolium perchlorate (IR140); Malachite Green isothiocyanate; 4-methylumbelliferone orthocresolphthalein; nitrotyrosine; pararosaniline; Phenol Red; B-phycoerythrin; o-phthalaldehyde; pyrene and derivatives(e.g., pyrene, pyrene butyrate, and succinimidyl 1-pyrene); butyrate quantum dots; Reactive Red 4 (CIBACRON™ Brilliant Red 3B-A); rhodamine and derivatives (e.g., 6-carboxy-X-rhodamine (ROX), 6-carboxyrhodamine (R6G), lissamine rhodamine B sulfonyl chloride rhodamine (Rhod), rhodamine B, rhodamine 123, rhodamine X isothiocyanate, sulforhodamine B, sulforhodamine 101, sulfonyl chloride derivative of sulforhodamine 101 (Texas Red), N,N,N',N' tetramethyl-6-carboxyrhodamine (TAMRA) tetramethyl rhodamine, and tetramethyl rhodamine isothiocyanate (TRITC)); riboflavin; rosolic acid; terbium chelate derivatives; Cyanine-3 (Cy3); Cyanine-5 (Cy5); cyanine-5.5 (Cy5.5), Cyanine-7 (Cy7); IRD 700; IRD 800; Alexa 647; La Jolla Blue; phthalocyanine; and naphthalocyanine.

[00533] In some embodiments, the detectable agent may be a non-detectable precursor that becomes detectable upon activation (e.g., fluorogenic tetrazine-fluorophore constructs (e.g., tetrazine-BODIPY FL, tetrazine-Oregon Green 488, or tetrazine-BODIPY TMR-X) or enzyme activatable fluorogenic agents [e.g., PROSENSE® (VisEn Medical)]. In vitro assays in which the enzyme labeled compositions can be used include, but are not limited to, enzyme linked immunosorbent assays (ELISAs), immunoprecipitation assays, immunofluorescence, enzyme immunoassays (EIA), radioimmunoassays (RIA), and Western blot analysis.

Combinations

[00534] Glycan-interacting antibodies may be used in combination with one or more other therapeutic, prophylactic, diagnostic, or imaging agents. By “in combination with,” it is not

intended to imply that the agents must be administered at the same time and/or formulated for delivery together, although these methods of delivery are within the scope of the present disclosure. Compositions can be administered concurrently with, prior to, or subsequent to, one or more other desired therapeutics or medical procedures. In general, each agent will be administered at a dose and/or on a time schedule determined for that agent. In some embodiments, the present disclosure encompasses the delivery of pharmaceutical, prophylactic, diagnostic, and/or imaging compositions in combination with agents that may improve their bioavailability, reduce and/or modify their metabolism, inhibit their excretion, and/or modify their distribution within the body.

[00535] In some embodiments, the glycan-interacting antibodies may be used in combination with one or more cancer therapeutic, such as a chemotherapeutic agent, a therapeutic antibody, and/or a cell cycle inhibitor. Combining glycan-interacting antibody treatment with such cancer therapeutics may provide beneficial therapeutic properties, e.g. synergistic anti-tumor activity, and may be used for the treatment of cancer. In some cases, such methods may be used to target cancer cells that are resistant to the chemotherapy, antibody therapy and/or cell cycle inhibitor treatment. Methods of targeting drug-resistant cancer cells may take advantage of changes in STn expression in cancer cells occurring after chemotherapy, antibody therapy and/or cell cycle inhibitor treatment. In some cases, drug-resistant cancer cells express STn before and/or after treatment. In some cases, cell surface STn expression in drug-resistant cancer cells may be increased following treatment. After treatments, some cancer cells may proliferate resulting in a population of STn-expressing cancer cells that are resistant to the cancer therapeutic(s) used. In some cases, drug-resistant cancer cells are cancer stem cells.

[00536] Accordingly, methods of the invention may include methods of administering an anti-STn antibody to a subject to target STn-expressing cancer cells present after administration of one or more cancer therapeutic. Such anti-STn antibodies may include a variable domain with an amino acid sequence selected from any of SEQ ID NOs: 1-12. Subjects and/or cancerous tissues in subjects treated with anti-STn antibodies may experience a reduction in STn-positive cells. In some embodiments, the reduction may include a decrease in STn-positive cells of from about 10% to about greater than 90% (for example, at least 10%, at least 20%, at least 30%, at least 40%, at least 50%, at least 60%, at least 70%, at least 80%, at least 85%, or at least 90%).

[00537] In some embodiments, chemotherapeutic agents used in combination with anti-STn antibodies may include, but are not limited to, taxanes (e.g. paclitaxel and docetaxel),

platinum-based agents (e.g. cisplatin, oxaliplatin, and carboplatin), topoisomerase inhibitors (e.g. irinotecan), nucleotide analogs (e.g. 5-fluorouracil and gemcitabine), kinase inhibitors [e.g. ponatinib (ICLUSIG®) and sorafenib (NEXAVAR®)], and PARP inhibitors [e.g. olaparib (LYNPARZA™)]. Chemotherapeutic agents may cause cell death or prevent cell growth through mechanisms that include, but are not limited to, inhibiting microtubule function, enzymatic function, or DNA synthesis.

[00538] In some embodiments, therapeutic antibodies used in combination with anti-STn antibodies may include antibodies that target cancer cell surface receptors. Such surface receptors may be involved in cell signaling pathways important for cell proliferation and/or migration. In some cases, the surface receptor may be an epidermal growth factor receptor (EGFR), a vascular endothelial growth factor receptor (VEGFR), or a human epidermal growth factor receptor (HER). Antibodies targeting these receptors or associated factors may inhibit cancer cell growth and/or migration. Exemplary anti-EGFR antibodies include cetuximab (ERBITUX®) and panitumumab (VECTIBIX®). Exemplary anti-VEGF antibodies include bevacizumab (AVASTIN®) and ramucirumab (CYRAMZA®). Exemplary anti-HER2 antibodies include trastuzumab (HERCEPTIN®). Among them, cetuximab, panitumumab, bevacizumab and ramucirumab are FDA-approved antibodies for treating colorectal cancer.

[00539] In some embodiments, cell cycle inhibitors may be used in combination with anti-STn antibody treatments. Cell cycle inhibitors may include, but are not limited to, cyclin-dependent kinase (CDK) inhibitors, checkpoint kinase inhibitors, Polo-like kinase (PLK) inhibitors, and Aurora kinase inhibitors. As used herein, the term “cell cycle inhibitor” refers to any agent that slows or halts cell cycle progression. Cell cycle inhibitors may induce cell cycle arrest at different stages, and may lead to cell death and/or growth inhibition.

[00540] In some embodiments, cell cycle inhibitors may be CDK inhibitors. Cyclin-dependent kinases (CDKs) are a group of serine/threonine kinases. CDKs and their cyclin partners are key regulatory enzymes that drive cell cycle transitions. Among them, CDK4 and CDK6 are key drivers of the transition from G0 or G1 phase into S phase. CDK4 and CDK6 (referred to herein as CDK4/6) are close homologs that are expressed in a tissue-specific manner. CDK4/6 forms a complex with cyclin D and phosphorylates the retinoblastoma (Rb) protein. Rb phosphorylation attenuates its suppression of E2F transcription factors, which leads to transcription of genes encoding proteins necessary for DNA replication. This may allow cell entry into S phase. Progression through S phase is controlled by cyclin E-CDK2 and cyclin A-CDK2 complexes. Transition from G2 to M phase is regulated by CDK1 through interactions with cyclin partners, cyclin A2 and cyclin B. Therapeutics targeting

CDKs (generally or specific CDKs) may impede cell cycle progression and prevent cell proliferation. See Otto and Sicinski, *Nat Rev Cancer*. 2017;17(2):93-115; and Asghar et al., *Nat Rev Drug Discov*. 2015; 14(2): 130–146; the contents of which are herein incorporated by reference in their entirety.

[00541] In some cases, CDK inhibitors may be selective CDK4/6 inhibitors. Due to the role of CDK4/6 in G1 to S transition, inhibition of CDK4/6 causes cell cycle arrest in G1 phase. Exemplary CDK4/6 inhibitors include, but are not limited to, palbociclib (Pfizer), ribociclib (Novartis), and abemaciclib (Eli Lilly). Palbociclib (PD-0332991, IBRANCE®) is an approved drug for the treatment of advanced (metastatic) breast cancer (Finn et al., *Breast Cancer Res*. 2009;11(5):R77; Rocca et al., *Expert Opin Pharmacother*. 2014;15(3):407-20; U.S. Pat. Nos. 6,936,612; 7,863,278; 7,208,489; and 7,456,168, the contents of which are herein incorporated by reference in their entirety). Palbociclib may be prepared and characterized according to the methods disclosed in U.S. Pat. No. 7,345,171, the contents of which are herein incorporated by reference in their entirety. Similarly, ribociclib (LEE011) also selectively inhibits CDK4/6 with high potency. Ribociclib and pharmaceutically acceptable salts of ribociclib may be prepared according to the methods detailed in U.S. Pat. Nos. 8,685,980 and 9,193,732, the contents of which are herein incorporated by reference in their entirety. Abemaciclib (LY-2835219) inhibits not only CDK4 and CDK6 but also several other kinases including PIM1 (U.S. Pat. No. 7,855,211, the contents of which are herein incorporated by reference in their entirety). Early activity of abemaciclib was reported in lung cancer, ovarian cancer, and melanoma patients (Shapiro et al., ASCO Meeting abstracts 2013; 31:2500).

[00542] In some cases, CDK inhibitors may be pan-CDK inhibitors. Such inhibitors may block several CDKs and lead to cell cycle arrest at different stages, such as G1 arrest, G2 arrest, and/or G2/M arrest. Exemplary pan-CDK inhibitors include, but are not limited to, flavopiridol (alvociclib), dinaciclib (MK-7965), R-roscovitine, AT7519, milciclib, TG02, CYC065 and RGB-286638. For example, pan-CDK inhibitors may be semi-synthetic flavopiridol (e.g., see U.S. Pat. No. 4,900,727) or analogs of flavopiridol (e.g., see U.S. Pat. Nos. 5,733,920 and 5,849,733, the contents of which are herein incorporated by reference in their entirety). As another example, pan-CDK inhibitors may be dinaciclib, or a pharmaceutically acceptable salt thereof, as disclosed in U.S. Pat. No. 7,119,200, the contents of which are herein incorporated by reference in their entirety.

[00543] In some embodiments, cell cycle inhibitors used in combination with glycan-interacting antibodies may be checkpoint kinase inhibitors. Inhibition of checkpoint kinases,

for example CHK1, CHK2, or WEE1, may compromise cell cycle checkpoints (e.g., S checkpoint, G2/M checkpoint or the mitotic spindle assembly checkpoint), allowing for cell cycle progression even in the presence of DNA damage. This can trigger cell death during mitosis via a mechanism known as “mitotic catastrophe.” Exemplary checkpoint kinase inhibitors include, but are not limited to, MK-8776, prexasertib (LY2606368), AZD1775, GDC-0575, and those described in Visconti et al. *J Exp Clin Cancer Res.* 2016; 35:153, the contents of which are herein incorporated by reference in their entirety.

[00544] In some embodiments, cell cycle inhibitors may be Polo-like kinase (PLK) inhibitors. Polo-like kinases (PLKs) are regulatory serine/threonine kinases of the cell cycle. PLK1 is the most characterized member of PLK family and it is a crucial mitotic protein kinase that is involved in many regulatory events such as G2/M transition, spindle assembly maturation, chromosome separation, and mitotic exit. Exemplary PLK1 inhibitors include, but are not limited to, rigosertib (ON 01910.Na), volasertib (BI 6727), BI 2536, HMN-176, TKM-080301, NMS-P937, DAP-81, Cyclopalin1, ZK-Thiazolidinone (TAL), SBE13, COM-36, LFM-A13, scytonemin, wortmannin, and GSK461364A (Kumar and Kim, *Biomed Res Int.* 2015;2015:705745, the contents of which are herein incorporated by reference in their entirety).

[00545] In some embodiments, cell cycle inhibitors may be Aurora kinase inhibitors. Aurora kinases include a family of highly conserved serine/threonine kinases that are important for faithful transition through mitosis. Aurora A plays a critical role in various mitotic events including centrosome maturation, chromosomal alignment, spindle assembly, meiotic maturation, and cytokinesis. Aurora B is a component of the chromosomal passenger complex and is involved in chromosome condensation and orientation as well as proper execution of cytokinesis. Exemplary Aurora kinase inhibitors include, barasertib (AZD1152), alisertib (MLN8237), danusertib (PHA-739358), ENMD-2076, AT9283, PF-03814735, and AMG 900 (Bavetsias and Linardopoulos, *Front Oncol.* 2015; 5: 278, the contents of which are herein incorporated by reference in their entirety).

Dosage

[00546] The present disclosure encompasses delivery of glycan-interacting antibodies for any of therapeutic, pharmaceutical, diagnostic or imaging by any appropriate route taking into consideration likely advances in the sciences of drug delivery. Delivery may be naked or formulated.

Naked delivery

[00547] Glycan-interacting antibodies of the present invention may be delivered to cells, tissues, organs or organisms in naked form. As used herein in, the term “naked” refers to glycan-interacting antibodies delivered free from agents or modifications which promote transfection or permeability. Naked glycan-interacting antibodies may be delivered to cells, tissues, organs and/or organisms using routes of administration known in the art and described herein. Naked delivery may include formulation in a simple buffer such as saline or PBS.

Formulated delivery

[00548] Glycan-interacting antibodies of the present invention may be formulated, using methods described herein. Formulations may comprise glycan-interacting antibodies which may be modified and/or unmodified. Formulations may further include, but are not limited to, cell penetration agents, pharmaceutically acceptable carriers, delivery agents, bioerodible or biocompatible polymers, solvents, and sustained-release delivery depots. Formulated glycan-interacting antibodies may be delivered to cells using routes of administration known in the art and described herein.

[00549] Compositions may also be formulated for direct delivery to organs or tissues in any of several ways in the art including, but not limited to, direct soaking or bathing, via a catheter, by gels, powder, ointments, creams, gels, lotions, and/or drops, by using substrates such as fabric or biodegradable materials coated or impregnated with compositions, and the like.

Dosing

[00550] The present invention provides methods comprising administering one or more glycan-interacting antibodies in accordance with the invention to a subject in need thereof. Nucleic acids encoding glycan-interacting antibodies, proteins or complexes comprising glycan-interacting antibodies, or pharmaceutical, imaging, diagnostic, or prophylactic compositions thereof, may be administered to a subject using any amount and any route of administration effective for preventing, treating, diagnosing, or imaging a disease, disorder, and/or condition. The exact amount required will vary from subject to subject, depending on the species, age, and general condition of the subject, the severity of the disease, the particular composition, its mode of administration, its mode of activity, and the like. Compositions in accordance with the invention are typically formulated in dosage unit form for ease of administration and uniformity of dosage. It will be understood, however, that the

total daily usage of the compositions of the present invention will be decided by the attending physician within the scope of sound medical judgment. The specific therapeutically effective, prophylactically effective, or appropriate imaging dose level for any particular patient will depend upon a variety of factors including the disorder being treated and the severity of the disorder; the activity of the specific compound employed; the specific composition employed; the age, body weight, general health, sex and diet of the patient; the time of administration, route of administration, and rate of excretion of the specific compound employed; the duration of the treatment; drugs used in combination or coincidental with the specific compound employed; and like factors well known in the medical arts.

[00551] In some embodiments, compounds of the present disclosure (e.g., anti-STn antibodies) may be administered as part of a composition, wherein the composition includes a concentration of such compounds of from about 0.01 mg/ml to about 1 mg/ml, from about 0.1 mg/ml to about 5 mg/ml, from about 0.5 mg/ml to about 10 mg/ml, from about 2 mg/ml to about 20 mg/ml or from about 5 mg/ml to about 50 mg/ml.

[00552] In some embodiments, compositions may be administered at a volume per subject weight of from about 0.01 ml/kg to about 1 ml/kg, from about 0.2 ml/kg to about 1.2 ml/kg, from about 0.05 ml/kg to about 2 ml/kg, from about 0.1 ml/kg to about 3 ml/kg, from about 0.5 ml/kg to about 5 ml/kg, from about 2 ml/kg to about 10 ml/kg, or from about 5 ml/kg to about 20 ml/kg. The administration may be by intravenous administration (e.g., intravenous bolus injection).

[00553] In certain embodiments, compounds or compositions of the present disclosure may be administered at dosage levels sufficient to deliver an amount of active compound (e.g., anti-STn antibody or chemotherapeutic agent) per subject body weight of from about 0.0001 mg/kg to about 100 mg/kg, from about 0.01 mg/kg to about 50 mg/kg, from about 0.1 mg/kg to about 40 mg/kg, from about 0.5 mg/kg to about 30 mg/kg, from about 1 mg/kg to about 6mg/kg, from about 2 mg/kg to about 10 mg/kg, from about 5 mg/kg to about 20 mg/kg, or from about 10 mg/kg to about 200 mg/kg. Administrations may be carried out one or more times a day, to obtain the desired therapeutic, diagnostic, prophylactic, or imaging effect. The desired dosage may be delivered according to any dosage schedule, including, but not limited to three times a day, two times a day, once a day, every other day, every third day, every week, every two weeks, every three weeks, or every four weeks, every two months, every three months, every four months, every 5 months, every 6 months, or every year. In certain embodiments, the desired dosage may be delivered using multiple administrations (e.g., two,

three, four, five, six, seven, eight, nine, ten, eleven, twelve, thirteen, fourteen, or more administrations).

[00554] According to the present invention, glycan-interacting antibodies may be administered in split-dose regimens. As used herein, a “split dose” is the division of single unit dose or total daily dose into two or more doses, e.g., two or more administrations of the single unit dose. As used herein, a “single unit dose” is a dose of any therapeutic administered in one dose/at one time/single route/single point of contact, i.e., single administration event. As used herein, a “total daily dose” is an amount given or prescribed in a 24-hour period. It may be administered as a single unit dose. In one embodiment, glycan-interacting antibodies of the present invention are administered to a subject in split doses. Glycan-interacting antibodies may be formulated in buffer only or in a formulation described herein. Pharmaceutical compositions comprising glycan-interacting antibodies as described herein may be formulated into a dosage form described herein, such as a topical, intranasal, intratracheal, or injectable (e.g., intravenous, intraocular, intravitreal, intramuscular, intracardiac, intraperitoneal or subcutaneous). General considerations in the formulation and/or manufacture of pharmaceutical agents may be found, for example, in Remington: The Science and Practice of Pharmacy 21st ed., Lippincott Williams & Wilkins, 2005 (incorporated herein by reference).

Coatings or shells

[00555] Solid dosage forms of tablets, dragees, capsules, pills, and granules can be prepared with coatings and shells such as enteric coatings and other coatings well known in the pharmaceutical formulating art. They may optionally comprise opacifying agents and can be of a composition that they release the active ingredient(s) only, or preferentially, in a certain part of the intestinal tract, optionally, in a delayed manner. Examples of embedding compositions which can be used include polymeric substances and waxes. Solid compositions of a similar type may be employed as fillers in soft and hard-filled gelatin capsules using such excipients as lactose or milk sugar as well as high molecular weight polyethylene glycols and the like.

IV. Kits and devices

Kits

[00556] Any of the compositions described herein may be included in a kit. In a non-limiting example, reagents for generating glycan-interacting antibodies, including antigen

molecules are included in a kit. The kit may further include reagents or instructions for creating or synthesizing glycan-interacting antibodies. It may also include one or more buffers. Other kits of the invention may include components for making glycan-interacting antibody protein or nucleic acid arrays or libraries and thus, may include, for example, a solid support.

[00557] In some embodiments, the present disclosure includes kits for screening, monitoring, and/or diagnosis of a subject that include one or more glycan-interacting antibodies. Such kits may be used alone or in combination with one or more other methods of screening, monitoring, and/or diagnosis (e.g., as a companion diagnostic). Some kits include one or more of a buffer, a biological standard, a secondary antibody, a detection reagent, and a composition for sample pre-treatment (e.g., for antigen retrieval, blocking, etc.).

[00558] The components of the kits may be packaged either in aqueous media or in lyophilized form. The container means of the kits will generally include at least one vial, test tube, flask, bottle, syringe or other container means, into which a component may be placed, and preferably, suitably aliquoted. Where there are more than one component in the kit (labeling reagent and label may be packaged together), the kit also will generally contain a second, third or other additional container into which the additional components may be separately placed. The kits may also comprise a second container means for containing a sterile, pharmaceutically acceptable buffer and/or other diluent. However, various combinations of components may be comprised in a vial. The kits of the present invention also will typically include a means for containing the glycan-interacting antibodies, e.g., proteins, nucleic acids, and any other reagent containers in close confinement for commercial sale. Such containers may include injection or blow-molded plastic containers into which the desired vials are retained.

[00559] When the components of the kit are provided in one and/or more liquid solutions, the liquid solution is an aqueous solution, with a sterile aqueous solution being particularly preferred. However, the components of the kit may be provided as dried powder(s). When reagents and/or components are provided as a dry powder, the powder can be reconstituted by the addition of a suitable solvent. It is envisioned that the solvent may also be provided in another container means. In some embodiments, labeling dyes are provided as a dried powder. It is contemplated that 10, 20, 30, 40, 50, 60, 70, 80, 90, 100, 120, 130, 140, 150, 160, 170, 180, 190, 200, 300, 400, 500, 600, 700, 800, 900, 1000 micrograms or at least 1000 micrograms or at most 10 g of dried dye are provided in kits of the invention. The dye may then be resuspended in any suitable solvent, such as DMSO.

[00560] A kit may include instructions for employing the kit components as well the use of any other reagent not included in the kit. Instructions may include variations that can be implemented.

Devices

[00561] Any of the compositions described herein may be combined with, coated onto or embedded in a device. Devices include, but are not limited to, dental implants, stents, bone replacements, artificial joints, valves, pacemakers or other implantable therapeutic devices.

V. Equivalents and scope

[00562] Those skilled in the art will recognize or be able to ascertain using no more than routine experimentation, many equivalents to the specific embodiments in accordance with the invention described herein. The scope of the present invention is not intended to be limited to the above Description, but rather is as set forth in the appended claims.

[00563] In the claims, articles such as “a,” “an,” and “the” may mean one or more than one unless indicated to the contrary or otherwise evident from the context. Claims or descriptions that include “or” between one or more members of a group are considered satisfied if one, more than one, or all of the group members are present in, employed in, or otherwise relevant to a given product or process unless indicated to the contrary or otherwise evident from the context. The invention includes embodiments in which exactly one member of the group is present in, employed in, or otherwise relevant to a given product or process. The invention includes embodiments in which more than one, or the entire group members are present in, employed in, or otherwise relevant to a given product or process.

[00564] It is also noted that the term “comprising” is intended to be open and permits but does not require the inclusion of additional elements or steps. When the term “comprising” is used herein, the term “consisting of” is thus also encompassed and disclosed.

[00565] Where ranges are given, endpoints are included. Furthermore, it is to be understood that unless otherwise indicated or otherwise evident from the context and understanding of one of ordinary skill in the art, values that are expressed as ranges can assume any specific value or subrange within the stated ranges in different embodiments of the invention, to the tenth of the unit of the lower limit of the range, unless the context clearly dictates otherwise.

[00566] In addition, it is to be understood that any particular embodiment of the present invention that falls within the prior art may be explicitly excluded from any one or more of the claims. Since such embodiments are deemed to be known to one of ordinary skill in the

art, they may be excluded even if the exclusion is not set forth explicitly herein. Any particular embodiment of the compositions of the invention (*e.g.*, any nucleic acid or protein encoded thereby; any method of production; any method of use; *etc.*) can be excluded from any one or more claims, for any reason, whether or not related to the existence of prior art.

[00567] All cited sources, for example, references, publications, databases, database entries, and art cited herein, are incorporated into this application by reference, even if not expressly stated in the citation. In case of conflicting statements of a cited source and the instant application, the statement in the instant application shall control.

[00568] Section and table headings are not intended to be limiting.

EXAMPLES

Example 1. Glycan array analysis

[00569] Optimized glycan arrays are utilized to test antibody affinity and specificity for multiple glycans in a single experiment. Glycan arrays include 71 chemically synthesized and well-defined glycans, most of which are Neu5Ac and Neu5Gc glycan pairs. Array slides are obtained commercially (ArrayIt Corp, Sunnyvale, CA) and include the glycans listed in Table 4.

Table 4. Array glycans

Glycan ID No.	Glycan
1	Neu5,9Ac2 α 2,3Gal β 1,4GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
2	Neu5Gc9Ac α 2,3Gal β 1,4GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
3	Neu5,9Ac2 α 2,6Gal β 1,4GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
4	Neu5Gc9Ac α 2,6Gal β 1,4GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
5	Neu5Ac α 2,6GalNAc α O(CH ₂) ₂ CH ₂ NH ₂
6	Neu5Gc α 2,6GalNAc α O(CH ₂) ₂ CH ₂ NH ₂
7	Neu5,9Ac2 α 2,3Gal β 1,3GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
8	Neu5Gc9Ac α 2,3Gal β 1,3GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
9	Neu5,9Ac2 α 2,3Gal β 1,3GalNAc α O(CH ₂) ₂ CH ₂ NH ₂
10	Neu5Gc9Ac α 2,3Gal β 1,3GalNAc α O(CH ₂) ₂ CH ₂ NH ₂
11	Neu5Ac α 2,3Gal β 1,4GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
12	Neu5Gc α 2,3Gal β 1,4GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
13	Neu5Ac α 2,3Gal β 1,3GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
14	Neu5Gc α 2,3Gal β 1,3GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
15	Neu5Ac α 2,3Gal β 1,3GalNAc α O(CH ₂) ₂ CH ₂ NH ₂
16	Neu5Gc α 2,3Gal β 1,3GalNAc α O(CH ₂) ₂ CH ₂ NH ₂
17	Neu5Ac α 2,6Gal β 1,4GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
18	Neu5Gc α 2,6Gal β 1,4GlcNAc β O(CH ₂) ₂ CH ₂ NH ₂
19	Neu5Ac α 2,6Gal β 1,4Glc β O(CH ₂) ₂ CH ₂ NH ₂
20	Neu5Gc α 2,6Gal β 1,4Glc β O(CH ₂) ₂ CH ₂ NH ₂
21	Neu5Ac α 2,3Gal β 1,4Glc β O(CH ₂) ₂ CH ₂ NH ₂

22	Neu5Gca2,3Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
23	Neu5,9Ac2α2,6GalNAcaO(CH ₂) ₂ CH ₂ NH ₂
24	Neu5Gc9Aca2,6GalNAcaO(CH ₂) ₂ CH ₂ NH ₂
25	Neu5Aca2,3GalβO(CH ₂) ₂ CH ₂ NH ₂
26	Neu5Gca2,3GalβO(CH ₂) ₂ CH ₂ NH ₂
27	Neu5Aca2,6GalβO(CH ₂) ₂ CH ₂ NH ₂
28	Neu5Gca2,6GalβO(CH ₂) ₂ CH ₂ NH ₂
29	Neu5,9Ac2α2,3GalβO(CH ₂) ₂ CH ₂ NH ₂
30	Neu5Gc9Aca2,3GalβO(CH ₂) ₂ CH ₂ NH ₂
31	Neu5,9Ac2α2,6GalβO(CH ₂) ₂ CH ₂ NH ₂
32	Neu5Gc9Aca2,6GalβO(CH ₂) ₂ CH ₂ NH ₂
33	Neu5Aca2,3Galβ1,3GalNAcβO(CH ₂) ₂ CH ₂ NH ₂
34	Neu5Gca2,3Galβ1,3GalNAcβO(CH ₂) ₂ CH ₂ NH ₂
35	Neu5,9Ac2α2,3Galβ1,3GalNAcβO(CH ₂) ₂ CH ₂ NH ₂
36	Neu5Gc9Aca2,3Galβ1,3GalNAcβO(CH ₂) ₂ CH ₂ NH ₂
37	Neu5,9Ac2α2,6Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
38	Neu5Gc9Aca2,6Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
39	Neu5,9Ac2α2,3Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
40	Neu5Gc9Aca2,3Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
41	Neu5Aca2,8Neu5Aca2,3Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
42	Neu5Aca2,8Neu5Aca2,8Neu5Aca2,3Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
43	Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
45	Galβ1,4GlcNAcβO(CH ₂) ₂ CH ₂ NH ₂
47	GalNAcaO(CH ₂) ₂ CH ₂ NH ₂
51	Galβ1,3GalNAcβO(CH ₂) ₂ CH ₂ NH ₂
52	Galβ1,3GlcNAcaO(CH ₂) ₂ CH ₂ NH ₂
53	Galβ1,3GlcNAcβO(CH ₂) ₂ CH ₂ NH ₂
54	Galβ1,4GlcNAc6SβO(CH ₂) ₂ CH ₂ NH ₂
55	Neu5Aca2,3Galβ1,4(Fuca1,3)GlcNAcβO(CH ₂) ₂ CH ₂ NH ₂
56	Neu5Gca2,3Galβ1,4(Fuca1,3)GlcNAcβO(CH ₂) ₂ CH ₂ NH ₂
57	Neu5Aca2,3Galβ1,4(Fuca1,3)GlcNAc6SβO(CH ₂) ₂ CH ₂ NH ₂
58	Neu5Gca2,3Galβ1,4(Fuca1,3)GlcNAc6SβO(CH ₂) ₂ CH ₂ NH ₂
59	Galβ1,3GlcNAcβ1,3Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
60	Neu5Aca2,3Galβ1,3GlcNAcβ1,3Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
61	Neu5Gca2,3Galβ1,3GlcNAcβ1,3Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
62	Neu5Aca2,3Galβ1,4GlcNAc6SβO(CH ₂) ₂ CH ₂ NH ₂
63	Neu5Gca2,3Galβ1,4GlcNAc6SβO(CH ₂) ₂ CH ₂ NH ₂
64	Neu5Aca2,8Neu5Aca2,3Galβ1,4GlcβO(CH ₂) ₃ NHCOCH ₂ (OCH ₂ CH ₂) ₆ NH ₂
65	Neu5Aca2,8Neu5Aca2,8Neu5Aca2,3Galβ1,4GlcβO(CH ₂) ₃ NHCOCH ₂ (OCH ₂ CH ₂) ₆ NH ₂
66	Neu5Aca2,6(Neu5Aca2,3)Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
67	Neu5Aca2,6(Neu5Gca2,3)Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
68	Neu5Aca2,6(KDNα2,3)Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
69	Neu5Gca2,8Neu5Aca2,3Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
70	KDNα2,8Neu5Aca2,3Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
71	Neu5Aca2,8Kdα2,6Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
72	Neu5Aca2,8Neu5Gca2,3Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
73	Neu5Aca2,8Neu5Gca2,6Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
74	KDNα2,8Neu5Gca2,3Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
75	Neu5Gca2,8Neu5Gca2,3Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂
76	Neu5Aca2,8Neu5Aca2,6Galβ1,4GlcβO(CH ₂) ₂ CH ₂ NH ₂

[00570] 300 ml of epoxy blocking buffer is prepared by combining 15 ml of 2 M Tris buffer (pH 8) with 0.9 ml of 16.6 M ethanolamine and 284.1 ml of distilled water. The solution is brought to a final pH of 9.0 with HCl. The solution is filtered using a 0.2 μ M nitrocellulose membrane. The epoxy buffer solution as well as 1 L of distilled water are pre-warmed to 50°C. Glass slides are arranged in a slide holder and quickly submerged in a staining tub with the warmed epoxy blocking buffer. Slides are incubated in the epoxy blocking buffer for 1 hour at 50°C with periodic shaking to deactivate epoxy binding sites. Next, slides are rinsed and blocked with PBS with 1% OVA at 25°C for one hour. Serum samples with polyclonal antibodies (1:1000) or purified monoclonal antibodies (1 μ g/mL), are diluted in PBS with 1% OVA and added to the glycan array for one hour at 25°C. After extensive washing, binding of antibodies are detected by incubating glycan microarray slides with Cy3-conjugated anti-mouse IgG (Jackson ImmunoResearch, West Grove, PA) for one hour. Slides are then washed extensively, dried and scanned with a Genepix 4000B scanner (Laser at 100%; gain at 350; 10 μ m pixels). Raw data from scanned images are extracted using the Genepix software and analysis of raw data is carried out. Antibodies are considered to be highly specific for AcSTn and GcSTn if they demonstrate binding to both molecules, but not to Tn or any other glycans on the array.

[00571] Based on array analysis, antibodies are classified according to array glycan binding profile. Antibodies are classified as “Group 1” antibodies, capable of binding AcSTn and GcSTn, if they bind to glycans 5, 6, 23 and 24. Such antibodies are referred to as Pan-STn antibodies due to their ability to associate with a wider range of STn structures and the portion of STn indicated by the largest ellipse in Fig. 1A. Antibodies are classified as “Group 2” antibodies, capable of binding STn as well as some related structures that include an O-linkage to serine or threonine, if they bind to glycans 5, 6, 23, 24, 27 and 31. These antibodies are thought to associate with the portion of STn indicated by the largest ellipse in Fig. 1B. Some Group 2 antibodies preferably bind to structures with AcSTn over structures with GcSTn. Antibodies are classified as “Group 3” antibodies (capable of binding STn, but may also bind a broader set of related structures) if they bind glycans 5, 6, 23, 24, 17, 3, 19, 37, 27 and 31. Unlike Group 2 antibodies, Group 3 antibodies do not require that such structures have an O-linkage to serine or threonine. Group 3 antibodies are thought to associate with the portion of STn indicated by the largest ellipse in Fig. 1C. Finally, antibodies are “Group 4” antibodies, capable of binding to both AcSTn and GcSTn as well as the un-sialylated Tn

antigen (therefore having broader specificity) if they bind to glycans 5, 6, 23, 24 and 47.

Group 4 antibodies are thought to associate with the portion of STn indicated by the largest ellipse in Fig. 1D.

Example 2. Generation of mouse anti-STn antibodies

[00572] mSIA101 and mSIA102 antibodies were generated using variable domains from anti-STn monoclonal antibodies obtained, as described previously in US Publication Number US2016/0130356 (the contents of which are herein incorporated by reference in their entirety), through murine immunization with mucins. mSIA103 variable domain sequences were obtained from 3F1 IgG1 antibodies (SBH Biosciences, Natick, MA). IgG2a expression vector constructs (plasmid H1206 for antibody heavy chains and plasmid L1206 for antibody light chains, LakePharma, Belmont, CA) were modified to encode mSIA101, mSIA102, and mSIA103 variable domains upstream of IgG2a constant regions. Sequences of the domains expressed are presented in Table 5.

Table 5. Sequences utilized in IgG2a antibody generation

Domain	Sequence	SEQ ID NO
mSIA101 VH domain	QVQLQQSDAELVKPGASVKISCKASGYTFTDHAHVVVKQKPEQGLEWIGYFS PGNDDIKYNEKFRGKATLTADKSSSTAYMQLNSLSSDDSAVYFCKRSLSTPY WGQGTLLTVSA	1
mSIA101 VL domain	DIVMTQSPSSLTDTAGEKVTMSCKSSQSLNLRGNHKNYLTWYRQKPLPPKL LIYWASTRESGVPDRFTGSGSGTDFTLTISVQAEDLAVYYCQNDYTYPTF GGGTKLEIKR	2
mSIA102 VH domain	QVQLQQSDAELVKPGASVKISCKASGYTFTDHAHVVVKQKPEQGLEWIGYFS PGNDDIKYNEKFKVKATLTADKSSSTAYMQLTSLTSEDSAVYFCKRSYYGD WGQGTLLTVSS	3
mSIA102 VL domain	DIQMTQSPASLSVSVGETVTITCRASENIYSHLAWYQQKQKSPQLLVYGAT NLADGVPSRFSGSGSGTQFSLKIHSLSQSEDFGSYYCQHFVGAPFTFGSGTKLE IK	4
mSIA103 VH domain	QVQLQQSDAELVKPGASVKISCKASGYTFTDHAHVVVKQKPEQGLDWIGYIS PGNGDIKYNEKFKDKVTLTADKSSSTACMHLNSLTSEDSAVYFCKRSLALD YWQGTLLTVSS	5
mSIA103 VL domain	DIVMTQSHKFMSTSVGDRVSITCKASQDVGTNIAWYQQKPGRSPKVLISAS TRHTGVPDRFTGSGSGTDFTLTISNVQSEDLTDYFCQQYSSFPLTFGVGKLE LK	6
IgG2a heavy chain constant domain	AKTTAPSVYPLAPVCGDTTGSSVTLGCLVKGYFPEPVTLTWNSGSLSSGVHT FPAVLQSDLYTLSSSVTVTSSTWPSQSITCNVAHPASSTKVDKKIEPRGPTIKP CPPCKCPAPNLLGGPSVFIFPPKIKDVLMSLSPIVTCVVVDVSEDDPDVQISWF VNNVEVHTAQTQTHREDYNSTLRVVSALPIQHQQDWMSGKEFKCKVNNKDL PAPIERTISKPKGSVRAPQVYVLPPEEEMTKKQVTLTCMVTDFMPEDIYVEW TNNGKTELNYKNTPEVLSDSGSYFMYSKLRVEKKNWVERNSYSCSVVHEGL HNHHTTKSFSRTPGK	13
kappa light chain constant domain	RADAAPTIVSIFPPSSEQLTSGGASVVCFLNNFYPKDINVKWKIDGSERQNGVL NSWTDQDSKDYSTYSMSSTLTLTKEDEYERHNSYTCEATHKSTSTSPIVKSFNRE C	14

[00573] Plasmids encoding full heavy chain amino acid sequences and plasmids encoding full light chain amino acid sequences were transfected into cells and expressed to produce mature antibodies.

[00574] Chinese hamster ovary-K1 (CHO-K1) cells were transfected for the generation of stable cell lines expressing IgG2a antibodies. The cells were cultured in a humidified 5% CO₂ incubator at 37°C in chemically defined media (CD-CHO, Invitrogen, Carlsbad, CA) supplemented with L-glutamine.

[00575] Approximately 80 million suspension CHO cells, growing in log phase, were transfected by electroporation (MaxCyte) with 80 µg of total plasmid encoding the full length heavy and light chains. Twenty-four hours later, the transfected cells were placed under selection for stable integration of the antibody genes. During the selection process the cells were spun down and resuspended in fresh selection media every 2-3 days until the pool recovered its growth rate and viability. Cells were monitored for growth, titer, and stable integration of the antibody expression constructs. The doubling rate was 20 hours.

[00576] Stable cell lines were cultured for large scale production and 10 L of culture were produced. The conditioned media harvested from the stable cell pool production run was clarified by centrifugation and 0.2 µm membrane filtration. Antibody was purified using Protein A affinity chromatography, then sterilized and cleared of particulates by passing through a 0.2 µm membrane filter. After low endotoxin purification and filtration, concentration was set to 5 mg/mL and 120 mg of antibody was recovered.

[00577] To prepare antibody-drug conjugates (ADCs), antibodies were conjugated with monomethyl auristatin E (MMAE). This was carried out by contacting antibodies with maleimidocaproyl-valine-citruline-p-aminobenzyloxycarbonyl-monomethyl auristatin E (MC-vc-PAB-MMAE, referred to herein as CL-MMAE). The resulting conjugation is maleimide-cysteine based, where the antibody interchain disulfide bonds were reduced with TCEP and then linked to the maleimide moiety of the drug.

[00578] Conjugated antibodies were desalted on Sephadex G50 columns to remove residual unreactive toxins and then dialyzed in 30 mM HEPES pH 7.7 with 150 mM NaCl. Size-exclusion chromatography (SEC) and hydrophobic interaction chromatography (HIC) were used to determine the drug-to-antibody ratio (DAR).

Example 3. Characterization of mouse antibodies and ADCs

[00579] Mouse antibodies were characterized for *in vitro* and *in vivo* efficacy in breast, colon and ovarian cancer models. The antibodies were used to identify CRC cell lines that

express STn on the surface. Five CRC lines were incubated *in vitro* and grown to confluency. %STn expression was determined by flow cytometry using anti-STn antibodies. Approximately 30-70% of cultured SW403, COLO205 and LS174T cells naturally expressed STn on the surface (as detected using mSIA103), while HT29 and RKO had very little to no detectable STn (as detected using hSIA101) on their cell surface (see Table 6).

Table 6. STn expression in CRC lines

Cell line	% STn expression
COLO205	67.7
LS174T	69.9
SW403	30.0
RKO	Low to non-detectable
HT29	Low to non-detectable

[00580] Further, a CRC Tissue Microarray (TMA) (NBP2-30214; Novus Bio, Littleton, CO) was stained for STn expression. The array consists of 59 cores with and without a neuraminidase (sialidase) enzyme treatment. Neuraminidase specifically cleaves terminal sialic acids and destroys STn antigen present on the tissue. Out of the 51 neoplastic cores on the TMA, 45 demonstrated some level of positive membrane staining, and staining with anti-STn mSIA103 on all 45 was abolished with 250 mU/mL neuraminidase treatment. Staining with a vimentin-specific antibody was not affected, suggesting mSIA103 anti-STn antibody binding is sialic acid-specific. Staining on normal colon (8 cores) was restricted to crypt and surface mucosa, and on the apical side of cells which should be protected from an intravenously-administered ADC therapeutic.

[00581] A preliminary xenograft study was performed using the COLO205 cells to evaluate the *in vivo* efficacy of the mouse antibody-drug conjugates (ADCs). COLO205 subcutaneous xenograft model was generated by injecting 5×10^6 cells/mouse into the right flank of Athymic Nude mice. Treatments began when tumor reached a mean size of 180 mm³. Mice were dosed once per week at 5 mg/kg for three weeks. mSIA103 alone with no toxin, mSIA103-CL-MMAE, mSIA103 conjugated with monomethyl auristatin F (MMAF) using a non-cleavable linker, and a vehicle control were evaluated in the study. mSIA103-CL-MMAE demonstrated significant tumor growth inhibition with a 44.5% treatment-to-control (T/C) ratio (p=0.008), compared to the other three groups.

Example 4. Generation of humanized anti-STn antibodies

[00582] Humanized versions of mSIA101, mSIA102, and mSIA103 variable domains were prepared by incorporating CDRs into human germline sequences, a process referred to as “CDR grafting.” Resulting variable domains were used to prepare humanized antibodies hSIA101, hSIA102, and hSIA103, by expressing constructs encoding the humanized variable domains with human IgG1 constant domains. Sequences of expressed antibody variable domains and constant domains are presented in Table 7.

Table 7. Humanized variable domains

Antibody	Domain	Sequence	SEQ ID NO
hSIA101	VH	EVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAHWVRQAPGQGLEWMGYFSPGNDIIKYNEKFRGRVTMTADKSSSTAYMELRSLRSDDTAVYFCKRSLSTPYWGQGLTVTVSS	7
hSIA101	VL	DIVMTQSPDSLAVSLGERATINCKSSQSLNLRGNHKNYLTWYQQKPGQPPKLLIYWASTRESGVDPDRFSGSGSGTDFTLTISSLQAEDVAVYYCQNDYTYPTFGQGTKVEIK	8
hSIA102	VH	QVQLVQSGAEVKKPGASVKISCKASGYTFTDHAHWVRQAPGQGLEWIGYFSPGNDIIKYNEKFKVRATLTADKSSSTAYMELRSLRSDDTAVYFCKRSYYGDWQGLTVTVSS	9
hSIA102	VL	DIQMTQSPSSLSASVGDRTITCRASENIYSHLAWYQQKPGKAPKLLVYGATNLAGVPSRFSGSGSGTQFTLTISSLQPEDFATYYCQHFHWGAPFTFGQGTKVEIK	10
hSIA103	VH	QVQLVQSGAEVKKPGASVKVSCKASGYTFTDHAHWVRQAPGQGLEWMGYISPNGDIKYNEKFKDRVTMTADKSSSTAYMQLRSLRSDDTAVYFCKRSLALDYWGQGLTVTVSS	11
hSIA103	VL	DIQMTQSPSSLSASVGDRTITCKASQDVGNTIAWYQQKPGKAPKVLIIYSASTRHTGVPSRFSGSGSGTDFTLTISSLQPEDFATYFCQQYSSFPLTFGQGTKVEIK	12
Human IgG1	Heavy chain constant domain	ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGLYSLSSVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVDVSHEDPEVKFNWYVDGVEVHNATKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSRDELTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSPGK	15
Human IgG1	Light chain constant domain	RTVAAPSVFIFPPSDEQLKSGTASVVCLLNNFYPREAKVQWKVDNALQSGNSQESVTEQDSKSTYLSSTLTLSKADYEKHKVYACEVTHQGLSSPVTKSFNRGEC	16

[00583] Preparation of humanized antibody-drug conjugates (ADCs) with MMAE was performed utilizing the same methods as described previously with the murine antibodies.

Example 5. Characterization of humanized antibodies

[00584] Humanized IgG1 antibodies, prepared as described above, were subjected to characterization analysis including flow cytometry-based binding analysis with MDA-MB-231-STn cells; binding analysis by BSM ELISA; and glycan array analysis.

[00585] In flow cytometry-based binding studies, antibodies were screened over a concentration range of 0 to 300 nM, comparing binding to MDA-MB-231 cells with or without transfection-induced STn expression. Binding was determined using an anti-human APC conjugated secondary antibody and only live cells were considered (based on propidium iodide negative gating). 5,000 events were collected per sample on average. Data were analyzed using FlowJo software (Asland, OR) and resulting APC means and % APC were obtained. These data were log transformed then fit to a nonlinear regression model to obtain a dose response curve and half-maximal effective concentration (EC₅₀) binding information. Human isotype IgG1 antibody was used as an isotype negative control. Epidermal growth factor receptor (LA22, EMD Millipore, Billerica, MA) was used as a positive control.

[00586] For BSM ELISA analysis, antibodies were screened over a concentration range of 0 to 100 nM on bovine submaxillary mucin (BSM) coated wells. A subset of wells were treated with mild periodate solution before antibody introduction to remove the side chain on terminal sialic acid residues (destroying the STn antigen). Optical densities of periodate and non-periodate-treated wells were determined and log transformed then fit to a nonlinear regression model to obtain a dose response curve. Optical density values obtained from periodate-treated wells were subtracted from non-periodate treated wells to obtain a periodate-sensitive STn binding curve and corresponding EC₅₀ values.

[00587] Glycan array analysis was carried out as described previously and antibodies were assigned array glycan binding profiles based on these results.

[00588] Results from flow cytometry, ELISA, and glycan array analysis are presented in Table 8.

Table 8. Antibody characterization results

Clone ID	MDA-MB-231-STn cell binding [EC ₅₀ (nM)]	BSM ELISA [EC ₅₀ (nM)]	Array glycan binding profile
hSIA101	2.0	4.2	Group 1
hSIA102	0.1	Not Determined	Group 4
hSIA103	0.3	1.8	Group 1

[00589] All antibodies tested demonstrated binding to cell- and BSM-associated STn. No binding was observed with human IgG1 isotype control (Southern Biotech, Birmingham, AL). hSIA102 binding was not periodate sensitive in ELISA assays, so a reliable EC₅₀ could not be determined by BSM ELISA.

Example 6. Analysis of humanized antibody-drug conjugates

[00590] MMAE-conjugated ADC versions of hSIA101, hSIA102, and hSIA103 were assessed in an ADC cytotoxicity assay using MDA-MB-231 cells (parental or transfected for enhanced expression of STn). Parental cells were grown in Eagle's Minimum Essential Medium (EMEM) supplemented with 10% FBS, 1x Pen/Strep and 45 µg/mL gentamycin. STn positive cells were grown in the same media except with the addition of 1 mg/mL G418 for antibiotic selection. Cells were seeded separately (4,000 cells/well for parental cells or 2,000/well for STn positive cells) in 96-well plates using proper media described above. Cells were grown overnight. After 16-20 hours, cells were treated with varying concentrations of test antibodies in triplicate (50 nM to 0.012 nM) for 72 hours. Then, cells were analyzed using an ADC CELLTITER-GLO® luminescent cell viability assay kit (Promega, Madison, WI) to determine the amount of ATP present, an indicator of metabolically active cells. The assay uses a single reagent that is added directly to the cultured cells in serum-supplemented medium. The reagent lyses the cells and generates a luminescent signal proportional to the amount of ATP present. Luminescent signals were analyzed and used to calculate the half-maximal inhibitory concentration (IC₅₀) values for each antibody used based on the concentration required to kill STn positive cells at half-maximal levels (see Table 9).

Table 9. IC₅₀ values for humanized ADC antibodies

Humanized Antibody	IC ₅₀ (nM)
hSIA103	1.3
hSIA102	2.6
hSIA101	5.2

[00591] All antibodies tested demonstrated IC₅₀ values in the single nanomolar range indicating a strong capability for each to kill STn expressing cells.

[00592] Similar studies were carried out using OVCAR3 cells in culture. Doses of hSIA101-MMAE and hSIA102-MMAE from 5 pM to about 300 nM were used, yielding an IC₅₀ value of 29 nM for hSIA101-MMAE and an IC₅₀ value of 15 nM for hSIA102-MMAE.

Example 7. STn assays

[00593] ELISA assays are developed to identify and quantitate STn levels in subject samples. Bovine submaxillary mucin (BSM), which is heavily sialylated, is used to generate standard curves and to aid in assay optimization. In initial assays, commercially available mouse anti-STn antibodies [e.g., antibody 3F1 (SBH Sciences, Natick, MA), antibody B72.3 (see Colcher, D. et al., 1981. PNAS. 78(5): 3199-203), and antibody CC49 (see Muraro, R. et al., 1988. Cancer Res. 48: 4588-96)] are used to coat assay plates and capture BSM in

samples (buffered solutions or serum samples with added BSM) being tested. Humanized anti-STn antibodies (e.g., hSIA101, hSIA102, and hSIA103) are used to detect captured BSM. Antibodies used for capture and detection are tested by two methods. One is by testing for pair matching in sandwich ELISA assays. The second is by flow cytometry for competitive binding by using fluorescent antibody labels and alternating the order of exposure to STn-expressing cells. Antibody pairs that do not bind competitively are used for further assay development.

[00594] Further assays utilize anti-STn antibodies as capture antibodies, bound to assay plates, and species-specific detection antibodies to detect bound proteins. For example, human anti-STn mAb can be utilized as both the coating and detection mAb if the detection mAb is directly labeled (e.g. Alexa-488, HRP etc.) For example, murine anti-STn mAb can be utilized as both the coating and detection mAb if the detection mAb is directly labeled (e.g. Alexa-488, HRP etc.) Detection of STn in mouse samples (e.g., collected from xenograft model studies described herein) and in human samples (e.g., serum samples obtained as described herein) are assessed with both murine and human detection antibodies.

Example 8. STn assay with humanized anti-STn antibodies

[00595] An STn ELISA assay was carried out using mSIA102 as a capture antibody and hSIA102 or hSIA103 as a detection antibody, with human isotype IgG as a control detection antibody. Five micrograms per milliliter ($\mu\text{g/ml}$) solutions of capture antibody were used to coat the surface of an ELISA assay plate overnight. Plates were washed and blocked before being treated with a dilution series of bovine submaxillary mucin (BSM) ranging from 0 nM to 6×10^{-8} nM. BSM bound to the assay surface was detected using 3 $\mu\text{g/ml}$ detection antibody. Anti-human antibody with horseradish peroxidase conjugate (0.08 $\mu\text{g/ml}$) was used to bind detection antibodies and antibody-antigen complexes were detected using HRP substrate. Results demonstrated that humanized anti-STn antibodies were capable of detecting bound BSM with a half-maximal effective concentration (EC_{50}) for antibody binding of 9×10^{-9} nM for hSIA103 and 2×10^{-8} nM for hSIA101. No binding was observed with the isotype control detection antibody.

Example 9. Pharmacokinetic studies

[00596] Antibodies hSIA101 and hSIA102, both conjugated with MMAE, were administered to female athymic mice at a dose of 5 mg/kg (dosage determined gravimetrically) by intravenous (IV) tail vein injection administration to evaluate antibody

terminal elimination half-life. Each group consisted of 9 mice. Blood (serum) was collected at hours 1, 4, 8, 24, 48, 72, 168, 336 and 672 and used to calculate antibody half-life.

[00597] Pharmacokinetic (PK) parameter evaluation was performed at the end the study using WINNONLIN® software (Pharsight Corp., Mountain View, CA). Area under the curve (AUC) was evaluated from the start of the first dose administered until the time after dosing of the last quantifiable observed concentration (AUC_{last}) and from initiation of the study to infinity (AUC_{INF}). Maximum observed plasma concentration (C_{max}), observed clearance (CL), terminal elimination half-life (HL), steady state volume of distribution (V_{ss}) and terminal phase volume of distribution (V_z) were also determined. PK parameters were calculated using non-compartmental analysis with sparse sampling. Results are presented in Table 10.

Table 10. PK parameters

Antibody	C_{max} ($\mu\text{g/ml}$)	AUC_{last} (Day* $\mu\text{g/ml}$)	AUC_{INF} (Day* $\mu\text{g/ml}$)	CL (ml/Day/kg)	HL (Day)	V_z (ml/kg)	V_{ss} (ml/kg)
hSIA101-MMAE	254	1050.00	1050.00	4.77	2.55	17.51	20.07
hSIA102-MMAE	285	1075.00	1079.17	4.63	3.77	25.16	23.57

[00598] From the study, hSIA101-MMAE was determined to have a terminal elimination half-life of 2.55 days, while hSIA102-MMAE was determined to have a terminal elimination half-life of 3.77 days. These values were comparable to those reported with other antibody-drug conjugates (see, e.g., Leal, M. et al., 2015, Bioconjugate Chem, 26: 2223-32 reporting a terminal elimination half-life for an anti-5T4 antibody-drug conjugate of 3.5 days).

Example 10. Generation of cell lines over-expressing STn

[00599] Established cancer cell lines are engineered to stably express STn as described by Julien et al (Julien, S. et al., 2005. Breast Cancer Res Treat. 90(1): 77-84). Cells are transduced with lentiviral vectors delivering ST6GalNAc I expression constructs (hST6GalNAc I pRc-CMV) or control (pRc-CMV empty vector). Stable transfections are selected in medium containing geneticin 418 (G418, 1 mg/mL). Resistant cells are seeded into 96 well plates using the limit dilution strategy and subcloned three times to obtain stable clonal lines. Clonal lines demonstrate varying expression of ST6GalNAc I [as determined by quantitative polymerase chain reaction (qPCR) analysis]. OVCAR3 cell lines stably expressing additional STn (as compared to wild type cells) are referred to as “OVCAR3-

STn” cells. Successful expression of STn is verified using flow cytometry analysis using anti-STn antibodies.

Example 11. Generation of SKOV3 cell lines with enhanced ST6GalNAc I expression

[00600] SKOV3 cells were transduced with lentiviral vectors delivering ST6GalNAc I expression constructs (hST6GalNAc I pRc-CMV). Stable cell pools were generated and 6 clones with varying expression of ST6GalNAc I [as determined by quantitative polymerase chain reaction (qPCR) analysis] were selected (see Table 11).

Table 11. Expression levels of ST6GalNAc I in selected clones

Clone ID	ST6GalNAc I mRNA expression level (fold expression level over control)
Clone 7	165
Clone 8	105
Clone 10	15
Clone 13	125
Clone 15	20
Clone 16	30

[00601] Clones 7, 8, and 13 demonstrated the highest level of ST6GalNAc I mRNA when compared to levels in non-transduced cell lines.

[00602] SKOV3 clones expressing none (wild type), moderate (clone 15), and high levels (clone 13) of STn were tested for sensitivity to anti-STn antibody treatment. Cells were cultured and treated with vehicle control, hSIA101, hSIA101-MMAE, hSIA102, or hSIA102-MMAE at concentration levels of 2.5 nM, 5 nM, 10 nM, 50 nM, and 100 nM. Cell viability was subsequently measured by MTT assay (EMD Millipore, Billerica, MA). Cell viability was reduced with all doses hSIA101-MMAE and hSIA102-MMAE treatment in the cells expressing high levels of STn and at doses greater than 2.5 nM in moderate STn-expressing cells. Cell viability was reduced with all doses of hSIA101-MMAE and hSIA102-MMAE treatment in the cells expressing moderate levels of STn and at doses greater than 5 nM in moderate STn-expressing cells. In cells expressing no STn, hSIA101-MMAE and hSIA102-MMAE had little effect, except at the highest dose used. These results indicate that cell susceptibility to anti-STn treatment is dependent on expression level of STn.

Example 12. In vivo studies in OVCAR3 cell line-derived mouse xenograft

[00603] An Athymic Nude mouse subcutaneous xenograft mouse model was generated with OVCAR3 human ovarian cancer cells. Animals were treated with humanized anti-STn

ADCs (1 mg/kg, 2.5 mg/kg, 5 mg/kg), an isotype ADC (1 mg/kg, 5 mg/kg), Paclitaxel (20 mg/kg) or vehicle alone. ADCs were administrated intravenously once per week for four weeks. Paclitaxel was administrated intraperitoneally once per week for three weeks. Tumor volume and body weight were measured on the days indicated in Table 12. The humanized ADCs significantly reduced tumor volumes compared to vehicle and isotype ADC control (see Table 12), while had little effects on gross body weight. This data suggests that the anti-STn ADCs are not toxic *in vivo* and are effective in reducing serous ovarian xenograft volumes. At the end of the study, tumor tissues were collected and analyzed using immunohistochemistry for expression of STn. Tumors tissues from mice treated with anti-STn ADCs showed reduced STn expression in comparison to other treatments.

Table 12. Tumor volume

Treatment	Mean tumor volume (mm ³)									
	Day 1	Day 4	Day 9	Day 12	Day 15	Day 18	Day 23	Day 26	Day 30	Day 31
Vehicle	161.8	242.8	373.5	539.1	678.4	803.1	987.1	1039.3	1175.6	1224.0
hSIA101-MMAE, 1 mg/kg	161.7	228.9	347.7	448.2	551.5	609.0	768.4	882.7	937.1	966.8
hSIA101-MMAE, 2.5 mg/kg	161.9	213.2	275.8	302.6	313.5	299.7	234.0	195.9	169.7	169.1
hSIA101-MMAE, 5 mg/kg	161.7	203.4	211.0	193.4	165.0	131.1	77.8	59.4	39.5	39.1
hSIA102-MMAE, 1 mg/kg	161.7	219.7	335.5	465.7	567.1	707.1	871.9	897.0	957.7	984.8
hSIA102-MMAE, 2.5 mg/kg	161.8	202.9	291.8	358.5	399.5	420.8	414.3	370.2	384.0	384.7

hSIA102-MMAE, 5 mg/kg	161.7	210.7	210.6	187.4	168.7	127.2	84.0	67.3	50.5	46.8
Isotype-MMAE, 1 mg/kg	161.1	222.1	312.6	368.8	423.1	468.3	510.6	472.5	531.4	548.2
Isotype-MMAE, 5 mg/kg	161.4	196.5	255.9	241.8	205.9	176.9	127.1	94.3	78.0	74.0
Paclitaxel, 20mg/kg	161.2	210.0	370.0	482.3	620.4	767.4	971.6	1133.0	1277.8	1385.2

Example 13. STn expression in patient CRC samples

[00604] Primary patient CRC samples were probed by flow cytometry with hSIA101 and two commercial antibodies B72.3 and CC49. All 10 samples tested were STn positive. The results are presented in Table 13. In the table, %STn is expressed as % of viable CD45-/CD34- population. STn expression was often more robustly identified with hSIA101 than the commercial antibodies. Expression ranged from 3-47% positive, with a metastatic sample demonstrating the highest percent positive cells. This suggests that the anti-STn antibodies can be utilized to identify STn specific populations in tumor samples.

Table 13. STn expression in patient samples

Sample	Tissue of Origin	Antibody	%STn+
160093-2	Colon	SIA201a	22.9
		B72.3	18.9
		CC49	17.1
160101-2	Colon	SIA201a	10
		B72.3	5.8
		CC49	5
160102-2	Colon	SIA201a	12.9
		B72.3	14.7
		CC49	11.4

160115-2	Colon	SIA201a	5.4
		B72.3	13
		CC49	5.7
160127-2	Colon	SIA201a	4.2
		B72.3	14.4
		CC49	3.5
160149-1	Colon	SIA201a	22.7
		B72.3	10.5
		CC49	9.8
160151-1	Colon	SIA201a	19.6
		B72.3	6.8
		CC49	11.5
169068-1	Colon	SIA201a	10.9
		B72.3	7.4
		CC49	8.4
1601666	Colon	SIA201a	29.4
		B72.3	17.3
		CC49	20.6
160104-2	Colon Met to Liver	SIA201a	47.8
		B72.3	46.6
		CC49	39.4

Example 14. *In vitro* toxin testing in CRC lines

[00605] Based on preliminary flow cytometric analyses revealing that approximately 60-70% of the total cell population in COLO205 and LS174T lines express STn, *in vitro* testing is carried out to test anti-STn antibody-based treatments. Given preliminary results with mouse and humanized anti-STn MMAE ADCs, experiments are carried out to identify optimal toxin/linkers for an anti-STn ADC for colorectal cancer. The resistance of 8 CRC lines (COLO205, LS174T, RKO, HT-29, LS180, and SW403) as well as STn expression-modified cell lines [COLO205 STn knockdown, LS174T STn knockdown, RKO STn+ (over expressing ST6GalNAc I), and HT-29 STn+ (over expressing ST6GalNAc I)] with various levels of STn expression is examined with a panel of 10 common ADC toxins including auristatins (MMAE and MMAF), Maytansines (DM4 and DM1), pyrrolobenzodiazepine

dimers (Talirine and Tesirine) and topoisomerase inhibitors (SN-38 and DXd) and other natural agents (Duocarmycin and Amanitin). Sensitivity of the CRCs is compared to standard of care (irinotecan, also known as CPT-11) and vehicle alone to obtain half maximal inhibitory (cytotoxic) concentrations (IC₅₀). To accomplish this, unconjugated toxins are tested in CRC cell lines with varying levels of endogenous STn expression as well as derivatives of those cells lines in which STn levels are either increased or depleted.

[00606] Knockdown of STn expression in COLO205 and LS174T cells is performed with ST6GalNAc I-directed Silencer siRNA (ThermoFisher Scientific, Waltham, MA) using methods described in the literature (e.g., Gao et al., Nat Med. 2015 Nov;21(11):1318-25). In contrast, RKO and HT-29 cells (expressing little to no detectable levels of STn) are transduced with lentiviral vectors delivering ST6GalNAc I expression constructs, to obtain clonal populations expressing elevated levels of STn. The engineered cells are monitored for STn expression on cell surfaces by flow cytometric and Western blot analyses and ST6GalNAc I mRNA levels are monitored utilizing qRT-PCR. Unconjugated toxins are added to CRC lines to obtain IC₅₀ values at 24, 48, 72 and 96 hours post incubation to establish sensitivity. The CELLTITER-GLO® luminescent cell viability assay kit (Promega, Madison, WI) is used according to the manufacturer's directions to evaluate cell viability after treatment. Two toxins with desirable ADC properties are selected for antibody conjugation and further characterization.

Example 15. Evaluation of anti-STn ADCs in CRC models

[00607] Two toxins are conjugated to two selected humanized anti-STn antibodies and binding assays are utilized to ensure STn binding specificity is maintained. The binding assays (flow cytometry-based assay, ELISA and glycan array) are performed as described previously. One human isotype IgG1 antibody is separately conjugated with each of the two toxins and utilized as a negative binding control. The EGFR (clone LA22) and CEA (clone CD66e) antibodies are utilized as positive controls for binding to MDA-MB-231 and CRC lines, respectively.

[00608] The anti-STn ADCs are tested in the three CRC lines described above for *in vitro* efficacy. A total of 8 conditions are tested including: anti-STn 1+Toxin 1; anti-STn 1+Toxin2; anti-STn 2+Toxin 1; anti-STn 2+Toxin 2; isotype+Toxin 1; isotype+Toxin 2; standard of care and vehicle. The anti-STn ADCs are evaluated for their effect on cell viability and colony formation using viability assays and soft agar growth assays, respectively. Viability assays are performed according to the CELLTITER-GLO®

Luminescent Cell Viability Assay kit (Promega, Madison, WI). Relative ADC potency is calculated, according to the manufacturer's instructions, to obtain percent viability and IC₅₀ values. Colony formation assays are performed where cells are diluted into a methylcellulose-based media and grown at 37 °C for 5-14 days in the presence of the ADCs. The number of colonies are counted and compared. In both assays, a range of anti-STn ADC concentrations are tested and applicable IC₅₀ values are generated. The results are compared to the standard of care chemotherapeutic and isotype ADC controls. Two top ADCs are selected based on the IC₅₀ data and colony formation information.

[00609] A CRC xenograft TMA is evaluated for STn expression to identify two STn positive CRC models for *in vivo* studies. The array consists of 20 unique models covering various stages, subtypes, genetic backgrounds and treatment responsive/resistant models. STn expression is assessed by immunohistochemistry (IHC). The TMA is stained with the two anti-STn antibodies, an isotype control and a secondary antibody only. IHC detection is performed using a pre-complexing strategy where the antibody is incubated with anti-human IgG biotin-labeled secondary antibody and then incubated with tissue. Staining is scored to assess neoplastic specific membrane staining. Two CRC xenograft models and one anti-STn ADC are selected for *in vivo* xenograft studies.

[00610] The anti-STn ADC candidate is evaluated for *in vivo* efficacy in a single agent, multiple drug dose study using two cell lines and three dose concentrations (1, 2.5 and 5 mg/kg). Xenograft mouse models are prepared by subcutaneous injection of human CRC STn expressing cells (i.e., 5x10⁶ cells 1:1 (v/v) Matrigel) into the right flank of ICR SCID mice (~80 mice). Once 60 mice have reached mean tumor volumes of ~200 mm³, they are randomized into 6 groups (n=10 mice per group) with approximately equivalent group mean tumor sizes. The groups receive either anti-STn ADC (1, 2.5 or 5 mg/kg), isotype ADC control (5 mg/kg), SOC (irinotecan 40 mg/kg) or vehicle (DPBS). Treatments are administered via intravenous injection once a week for four weeks based upon previous anti-STn ADC MMAE PK studies. Tumor volumes and body weights are measured twice weekly and individual mice are sacrificed when tumor size reaches endpoint volume (≥1500mm³) for vehicle control group or when a mouse becomes moribund. Mice from each group are assessed for tumor STn expression via IHC and flow cytometry at the end of the study.

[00611] Based on results of these studies, the toxin/linkers are further optimized to improve *in vivo* and *in vitro* efficacy of the anti-STn ADCs.

Example 16. Selection of CRC PDX models

[00612] CRC tumor microarrays (TMAs) from patient-derived tumor (PDX) cells are assessed for STn expression using the humanized anti-STn antibodies to determine the responsive models for PDX studies. Well-characterized commercial formalin-fixed paraffin-embedded (FFPE) PDX TMAs (CrownBio, Santa Clara, CA) containing over 200 cores total are assessed for STn expression by IHC. These TMAs consist of multiple naïve and standard of care resistant PDX models including those that are resistant to AP24534 [ponatinib, (RET inhibitor)], Cetuximab, Bevacizumab (AVASTIN®), Trastuzumab (HERCEPTIN®), Sorafenib, 5-Fluorouracil, Cisplatin, Docetaxel, Gemcitabine, Irinotecan or Paclitaxel. TMAs are stained with an unconjugated anti-STn antibody, an isotype antibody or a secondary antibody only. All IHC analyzed TMAs are scored microscopically in a blinded fashion for antibody staining intensity, frequency, and localization. The correlations between STn expression and CRC progression are examined and used to inform model choices.

[00613] Based on IHC data, 10 PDXs are chosen for *in vitro* testing with the anti-STn ADC candidate. These 10 PDX models are assessed for cytotoxicity and colony formation in viability assays and colony formation assays described previously. An isotype ADC control, three SOC agents (irinotecan, cetuximab and bevacizumab, as single agents) and a vehicle control are included for analysis. Three PDX models [including 1 standard of care (SOC) resistant PDX model] are selected for *in vivo* studies based on STn expression and anti-STn ADC *in vitro* response.

Example 17. Single dose PK studies

[00614] Prior to *in vivo* studies, the anti-STn ADC candidate is evaluated for PK properties in a single dose *in vivo* mouse study. Two dose levels (2.5 and 5 mg/kg) and 10 time points (0, 1, 4, 8, 24, 48, 72, 168, 336, and 672 hours post dose) are utilized in this single dose study. A total of nine mice are included per treatment group, with three sub-groups of mice to allow for staggered blood collections. At the assigned time points, mice are bled and serum is collected into serum separator tubes. Blood is allowed to clot for a minimum of 30 minutes and then processed by centrifugation (3500 rpm for 10 min at 5°C) within 1 hour of collection. Following centrifugation, serum is separated and used in immunoassays to determine anti-STn ADC present in the serum. Terminal elimination half-life (HL), area under the curve (AUC), maximum observed plasma concentration (C_{max}), steady state volume of distribution (V_{ss}) and terminal phase volume of distribution (V_z) are determined. The PK parameters are used to determine optimal dosing regimen for *in vivo* PDX studies.

Example 18. Evaluation of *in vivo* efficacy in PDX models in mice

[00615] PDX mouse studies are conducted using the three PDX models identified through immunohistochemical analysis of PDX TMAs to evaluate the *in vivo* efficacy of anti-STn ADC candidates as a single agent therapy. The PDX mice, randomized into 6 groups (10 mice per group), are administered with anti-STn ADC at 1, 2.5 or 5 mg/kg, an isotype ADC control (5 mg/kg), a SOC (irinotecan (40 mg/kg), cetuximab (10 mg/kg) or bevacizumab (10 mg/kg)) or vehicle alone. Animals are dosed once per week for four weeks. STn expression is examined at the beginning and end of the study by IHC and flow cytometry.

Example 19. Combination treatment in PDX models in mice

[00616] Anti-STn ADC candidates are evaluated for combination therapy with standard of care (SOC) therapies in a selected PDX mouse model in either concurrent or sequential treatments.

[00617] For concurrent treatment, a total of 9 treatment arms with 10 mice per arm are tested including: vehicle only, anti-STn ADC (1 mg/kg), anti-STn ADC (5 mg/kg), SOC only, SOC + vehicle, SOC + anti-STn ADC (1 mg/kg), SOC + anti-STn ADC (5 mg/kg), SOC + unconjugated anti-STn (1 mg/kg), and SOC + unconjugated anti-STn (5 mg/kg). Animals are dosed weekly for four weeks. STn tumor expression is examined at the beginning and end of the study by IHC and flow cytometry.

[00618] For sequential treatment, mice are initially treated with SOC. Following a four-week treatment or a median tumor regression to < 25% of the original tumor volume, mice are then randomized into 6 arms as follows: anti-STn ADC (1 mg/kg), anti-STn ADC (5 mg/kg), unconjugated antibodies (1 mg/kg), unconjugated antibodies (5 mg/kg), one isotype ADC control and vehicle control. Animals are dosed weekly for four weeks. STn tumor expression is examined at the beginning and end of the study by IHC and flow cytometry.

Example 20. Pilot multiple dose toxicology study

[00619] A multiple dose study was conducted to assess pharmacokinetic characteristics and toxicity with hSIA101-MMAE administration in *Cynomolgus* monkeys. Studies included in-life assessments such as mortality/morbidity, clinical observations, body weight, food consumption, body temperature, local irritation, ophthalmology, and clinical pathology assessments. 9 male animals (2-4 years of age) were utilized for these studies, with 3 monkeys per treatment group listed in Table 14.

Table 14. Treatment groups

Group	Dose (mg/kg)	Dose volume (ml/kg)	Dose Conc. (mg/ml)	Animals per Group
1	1	1.2	0.833	3
2	3	1.2	2.5	3
3	6	1.2	5	3

[00620] All groups were dosed 2 times total by intravenous bolus injection, once on Day 1 and again on Day 22. Blood samples were collected for pharmacokinetic studies on days 1 and 22 using the following schedule: prior to dosing (0) and then 1, 3, 6, 24, 48, 72, 96, and 168 hours post dosing. Additional blood samples were collected at 240, 336, and 504 hours post dosing for day 1 dosing only. Blood samples were collected for clinical pathology evaluations (clinical chemistry, hematology, and coagulation) using the following schedule: prior to dosing (pretest), Day 8, Day 22 (pre-second dose) and on Day 29 before euthanasia. The clinical chemistry evaluations included evaluation of sodium creatinine, total protein, potassium, alkaline phosphatase, triglycerides, chloride, alanine aminotransferase, total bilirubin, calcium aspartate aminotransferase, albumin, inorganic phosphorus, glucose, globulin, urea nitrogen, cholesterol, and albumin/globulin ratio. The hematology evaluations included evaluation of hematocrit, mean corpuscular hemoglobin concentration, hemoglobin, reticulocyte count (absolute and relative), platelet count, erythrocyte count, mean platelet volume, total white blood cell count, mean corpuscular hemoglobin, differential white blood cell count (absolute & relative), mean corpuscular volume, and red blood cell distribution width. K3-EDTA was used as an anti-coagulant. The coagulation evaluations included evaluation of prothrombin time and activated partial thromboplastin time. All groups were euthanized on Day 29 and organs collected for necropsy.

[00621] Serum from subject blood samples was used for pharmacokinetic (PK) evaluation of human IgG levels. PK parameters were estimated using Phoenix pharmacokinetic software (Certara, USA) using a non-compartmental approach consistent with the intravenous bolus route of administration. All parameters were generated from individual human IgG concentrations in serum from Days 1 and 22. Parameters were estimated using nominal sampling times relative to the end of each dose administration. The concentration at time zero on day 1 was back extrapolated based on the first two observed serum concentrations for the purpose of parameter estimation. Concentration values reported as not quantifiable were treated as absent samples.

[00622] Serum antibody concentration values obtained in relation to the first dose are presented in Fig. 2 and values obtained in relation to the second dose are presented in Fig. 3. The area under the concentration vs. time curve (AUC) was calculated using the linear

trapezoidal method with linear/linear interpolation. The AUC was not calculated for PK profiles with less than 3 quantifiable concentrations of test item at separate time points. When practical, the terminal elimination phase of each concentration versus time curve was identified using at least the final three observed concentration values. The slope of the terminal elimination phase was determined using log linear regression on the unweighted concentration data. Mean values with standard deviation (SD; in parentheses) are presented in Table 15 and Table 16. $C_{\max}$ refers to the maximum observed concentration measured after dosing. AUC_{last} refers to the area under the concentration versus time curve from the start of dose administration to the last observed quantifiable concentration using the linear or linear/log trapezoidal method. $AUC_{0-7\text{Day}}$ refers to the same, but where the area beyond Day 7 is not included. AUC_{INF} refers to the area under the concentration versus time curve from the start of dose administration extrapolated to infinite time. CL refers to the apparent clearance rate of the antibody from the analyzed matrix. HL refers to the apparent terminal elimination half-life. V_{ss} refers to the apparent volume of distribution at steady state.

Table 15. PK parameters obtained post first dose

Dose (mg/kg)	$C_{\max}$ (µg/ml); Mean (SD)	AUC_{last} (Day*µg/ml); Mean (SD)	$AUC_{0-7\text{Day}}$ (Day*µg/ml); Mean (SD)	AUC_{INF} (Day*µg/ml); Mean (SD)	CL (ml/kg/day); Mean (SD)	HL (Day); Mean (SD)	V_{ss} (ml/kg); Mean (SD)
1	19.13 (1.78)	57.31 (7.5)	42.6 (0.96)	62.62 (7.81)	16.13 (1.96)	4.66 (1.43)	96.83 (17.29)
3	81.24 (11.17)	228.39 (25.02)	157.34 (4.94)	267.39 (56.39)	11.57 (2.52)	7.47 (3.91)	96.16 (30.64)
6	151.37 (20.97)	419.05 (50.18)	342.22 (32.58)	440.63 (28.26)	13.65 (0.86)	3.53 (0.51)	61.62 (10.98)

Table 16. PK parameters obtained post second dose

Dose (mg/kg)	$C_{\max}$ (µg/ml); Mean (SD)	AUC_{last} (Day*µg/ml); Mean (SD)
1	22.16 (0.01)	41.59 (4.68)
3	75.27 (5.18)	145.48 (51.93)
6	117.23 (45.7)	172.36 (141.18)

[00623] On Day 1, the increase in systemic exposure to human IgG was linear and dose-proportional between 1 and 6 mg/kg/dose, with a slight trend to be more than dose-

proportional. On day 22, the increase in systemic exposure was non-linear between 1 and 6 mg/kg/dose. A decrease in systemic exposure to human IgG, in terms of $AUC_{(0-t)}$, was observed when comparing Day 1 and Day 22, increasing with the dose level increase.

[00624] Animals surviving to Day 29 were euthanized and submitted for necropsy. Tissues required for microscopic evaluation were trimmed, processed routinely, embedded in paraffin, and stained with hematoxylin and eosin prior to evaluation by light microscopy.

[00625] No test article-related gross findings were noted. The gross findings observed were considered incidental, of the nature commonly observed in this strain and age of *Cynomolgus* monkeys, and/or were of similar incidence in control and treated animals and, therefore, were considered unrelated to antibody administration. No body weight loss or deaths occurred among study animals and no gross pathologic changes were observed across all organs assessed. Histopathological changes were limited to the bone marrow, which is an effect associated with MMAE. Minimal to mild decreased cellularity and mild decrease in myeloid to erythroid ratio were observed. Changes in hematology parameters (i.e., modest neutropenia) were consistent with other MMAE antibody drug conjugates and not considered target related. Finally, all clinical chemistry results remained normal throughout the study.

Example 21. Cell Line Generation

[00626] In order to produce clinical grade antibodies, a stable Chinese Hamster Ovary (CHO) cell line is generated according to the GIBCO® FREEDOM® CHO-S® Kit (Thermo Fisher Scientific, Waltham, MA). CHO-S cells are transfected with a construct containing the genes that encode the humanized anti-STn antibody. Stable clones are selected based on a two-phase selection scheme. Clones with high production yields are selected for further characterization. The stable cell line is transferred to a Good Manufacturing Practice (GMP) facility for production of antibodies for GLP toxicology testing and phase I clinical trial supply.

Example 22. Tissue cross-reactivity studies

[00627] Tissue cross-reactivity studies were conducted to assess the binding profile (both on- and potential off-target binding) to human and relevant species used in nonclinical safety testing. Humanized anti-STn antibodies were examined for binding to a panel of normal human, rat, mouse, and cynomolgus monkey tissues. The study was conducted on 10 normal frozen tissue sections at 1-3 µg/ml: heart, brain, kidney, stomach, lung, small intestines, colon, liver, pancreas, spleen and bone marrow. The positive control was a human pancreatic

neoplasm that contained STn positive cancer cells and the negative control was normal stromal cells contained within the same specimen. Staining across all normal tissues was restricted to the cytoplasm. Additional analyses were conducted without primary antibody or using isotype control IgG as negative controls.

[00628] Among the three tested antibodies, hSIA101 has the lowest cross-reactivity with normal human tissues. Moderate binding activity was seen with tissues of heart vessels, stomach epithelial cells, small intestine epithelial cells and spleen vessels. Antibodies were also found to cross react with similar types of tissue from *Cynomolgus* monkey (see Table 17) and rat (not shown). In the Table: 1+ = weak staining; 2+ = moderate staining; 3+ = strong staining; 4+ = intense staining; Neg = negative; freq = frequent (staining in >75%-100% of cells); occ = occasional (staining in >25%-50% of cells); and rare refers to staining in 1-5% of cells.

Table 17. Tissue cross reactivity study results

Tissue	Human	Cyno
Heart Vessel	2+ rare (cytoplasm)	Neg
Stomach Epithelial Cells	2-3+ freq (cytoplasm)	2-3+ occ (cytoplasm)
Stomach Vessel	Neg	2+ occ (cytoplasm)
Lung Epithelium	Neg	2-3+ freq (cytoplasm)
Small Intestine Epithelial Cells	2-4+ freq (cytoplasm)	3-4+ freq (cytoplasm)
Colon Epithelial Cells	1+ freq (cytoplasm)	Neg
Colon Vessel	Neg	4+ freq (cytoplasm)
Pancreas Vessel	Neg	2-3+ freq (cytoplasm)
Spleen Vessel	2-3+ freq (cytoplasm)	Neg

[00629] No staining was observed on cell membranes in any tissue. Cerebrum, cerebellum, kidney and liver did not stain. Isotype and secondary-only control analyses demonstrated no staining, while only positive control tissue (pancreatic neoplasm) demonstrated strong membrane staining with hSIA101.

Example 23. Comparison between anti-STn antibody treatment and cisplatin treatment in an ovarian PDX tumor model

[00630] Anti-STn ADC candidates were compared with standard of care (SOC) therapies in a xenograft tumor model using human patient-derived ovarian cancer tumors from two different patients. Patient samples were previously confirmed to express STn by immunohistochemical staining and tumors from one of the patients not previously treated with chemotherapy (chemo-naïve) and those from a patient previously treated with chemotherapy were implanted in athymic nude immune-deficient mice. Mice with successful tumor growth were selected and administered antibodies (5 mg/kg dose) by weekly IV

injection; cisplatin (3 mg/kg dose) by weekly intraperitoneal injection (limited to 3 doses); or vehicle control. Antibodies administered included hSIA101, hSIA101-ADC (conjugated with MMAE), or IgG isotype control conjugated with MMAE (isotype-ADC). Mice with chemo-naïve tumors received antibody treatments for 4 weeks, while mice with chemo-resistant tumors received antibody treatments for 6 weeks. Tumor volumes in mice treated with hSIA101-ADC or isotype-ADC were monitored for 4-5 weeks after the end of treatment to assess tumor regression.

[00631] Cisplatin-sensitive (Fig. 4, top panel) and cisplatin-resistant (Fig. 4, bottom panel) tumor volumes were measured over time. hSIA101-ADC treatment yielded the most effective reduction in tumor volume in comparison to other treatments. Tumors generated with cisplatin-resistant cells were significantly reduced by treatment with hSIA101-ADC treatment in comparison to cisplatin treatment (Fig. 4, bottom panel). Accordingly, hSIA101-ADC treatment may be used to effectively treat cisplatin-sensitive and cisplatin-resistant tumors. Interestingly, 75% of chemo-naïve mice treated with hSIA101-ADC were completely tumor free four weeks after the end of treatment, while greater than 50% tumor regression was observed in 75% of mice with chemo-resistant tumors 5 weeks after the end of hSIA101-ADC treatment.

CLAIMS

What is claimed is:

1. Use of an antibody-drug conjugate (ADC) in the manufacture of a medicament for treating a platinum refractory cancer in a subject, wherein the platinum refractory cancer (i) is selected from breast cancer, melanoma, colorectal cancer, liver cancer, lung cancer, bladder cancer, cervical cancer, stomach cancer, and prostate cancer, (ii) is resistant to cisplatin or oxaliplatin, or (iii) is ovarian cancer and is resistant to cisplatin, wherein said ADC comprises:
 - a) an anti-STn antibody, wherein said anti-STn antibody comprises:
 - 1) a heavy chain variable domain (VH) with the amino acid sequence of SEQ ID NO: 7 and
 - a light chain variable domain (VL) with the amino acid sequence of SEQ ID NO: 8;
 - 2) a VH with the amino acid sequence of SEQ ID NO: 9 and a VL with the amino acid sequence of SEQ ID NO: 10; or
 - 3) a VH with the amino acid sequence of SEQ ID NO: 11 and a VL with the amino acid sequence of SEQ ID NO: 12; and
 - b) a cytotoxic agent conjugated to the anti-STn antibody.
2. The use of claim 1, wherein the cytotoxic agent is selected from an auristatin, a maytansine, a tubulysin, a vinca alkaloid, a pyrrolobenzodiazepine dimer, a camptothecin, a duocarmycin, an amanitin, a phosphoinositide 3-kinase (PI3K) inhibitor, and a mitogen-activated protein kinase kinase (MEK) inhibitor.
3. The use of claim 1 or 2, wherein the cytotoxic agent is conjugated to the anti-STn antibody via a linker.
4. The use of claim 3, wherein the linker is a cleavable linker.
5. The use of claim 3 or 4, wherein the linker comprises one or more polymers selected from a poly(ethylene glycol) (PEG), a poly(N-(2-hydroxypropyl)methacrylamide) (polyHPMA), a poly(α -amino acid), a carbohydrate polymer, a glycopolysaccharide, a glycolipid, a glycoconjugate, a polyglycerol, a polyvinyl alcohol, a poly(acrylic acid), a polyketal, a polyacetal, and a poly(1-hydroxymethylethylene hydroxymethylformal) (PHF).
6. The use of any one of claims 3-5, wherein the linker comprises valine-citrulline.

7. The use of any one of claims 1-6, wherein the anti-STn antibody comprises a VH with the amino acid sequence of SEQ ID NO: 7 and a VL with the amino acid sequence of SEQ ID NO: 8.
8. The use of any one of claims 1-7, wherein the platinum refractory cancer is ovarian cancer and is resistant to cisplatin.
9. The use of any one of claims 1-7, wherein the platinum refractory cancer is colorectal cancer.
10. The use of claim 9, wherein said colorectal cancer is resistant to treatment with at least one of cetuximab, panitumumab, bevacizumab, ramucirumab, trastuzumab, ponatinib, sorafenib, 5-fluorouracil, docetaxel, gemcitabine, irinotecan, or paclitaxel.
11. The use of any one of claims 1-10, wherein the cytotoxic agent comprises MMAE.
12. The use of any one of claims 1-11, wherein the the anti-STn antibody comprises a VH with the amino acid sequence of SEQ ID NO: 7 and a VL with the amino acid sequence of SEQ ID NO: 8 and the cytotoxic agent comprises MMAE.
13. The use of any one of claims 1-12, wherein the ADC is to be administered at a dose of from 1 mg/kg to 6 mg/kg.