RECOMBINANT HUMAN SIALIDASES, SIALIDASE FUSION PROTEINS, AND METHODS OF USING THE SAME

CROSS REFERENCE TO RELATED APPLICATIONS

[0001] This application claims the benefit of and priority to U.S. Provisional Application No. 63/631,657, filed April 9, 2024, the entire disclosure of which is hereby incorporated by reference in its entirety for all purposes.

SEQUENCE LISTING

[0002] This application contains a Sequence Listing which has been submitted electronically in XML format and is hereby incorporated by reference in its entirety. The XML copy, created on April 4, 2025, is named PAL-047WO_SL.xml and is 51,074 bytes in size.

FIELD OF THE INVENTION

[0003] The invention relates generally to recombinant sialidases (for example, recombinant sialidases having reduced heparin binding affinity and/or increased half-life) and recombinant fusion proteins, and their use in the treatment of cancer.

BACKGROUND

[0004] A growing body of evidence supports roles for glycans, and in particular, sialoglycans, at various pathophysiological steps of tumor progression. Glycans regulate tumor proliferation, invasion, hematogenous metastasis and angiogenesis (Fuster *et al.* (2005) NAT. REV. CANCER 5(7): 526-42). The sialylation of cell surface glycoconjugates is frequently altered in cancers, resulting in the expression of sialylated tumor-associated carbohydrate antigens. The expression of sialylated glycans by tumor cells is often associated with increased aggressiveness and metastatic potential of a tumor (Julien S., Delannoy P. (2015) Sialic Acid and Cancer. In: Taniguchi N., Endo T., Hart G., Seeberger P., Wong CH. (eds) GLYCOSCIENCE: BIOLOGY AND MEDICINE, Springer, Tokyo. https://doi.org/10.1007/978-4-431-54841-6_193).

[0005] It has recently become apparent that Siglecs (sialic acid-binding immunoglobulin-like lectins), a family of sialic acid binding lectins, play a role in cancer immune suppression by binding to hypersialylated cancer cells and mediating the suppression of signals from activating NK cell receptors, thereby inhibiting NK cell-mediated killing of tumor cells (Jandus *et al.* (2014) J. CLIN. INVEST. 124: 1810-1820; Läubli *et al.* (2014) PROC. NATL.

ACAD. SCI. USA 111: 14211-14216; Hudak *et al.* (2014) NAT. CHEM. BIOL. 10: 69-75). Likewise, enzymatic removal of sialic acids by treatment with sialidase can enhance NK cell-mediated killing of tumor cells (Jandus, *supra*; Hudak, *supra;* Xiao *et al.* (2016) PROC. NATL. ACAD. SCI. USA 113(37): 10304-9).

[0006] There are numerous reports of heparin's ability to inhibit both bacterial and human sialidases (Heijlman (1974) BIOCHEM SOC TRANS 2(4): 638–639; Nagaoka *et al.* (1998) BIOL. PHARMA. BULLETIN 21(1): 1134-1138; Kopitz *et al.* (1997) EUR. J. BIOCHEM. 248, 527-534), which may lead to reduced half-life of recombinant sialidases when administered therapeutically. Despite the efforts to engineer the human sialidase Neu2 for use in therapy, there is still a need in the art to develop recombinant human Neu2 sialidase mutants with reduced heparin affinity and enhanced half-life.

SUMMARY OF THE INVENTION

[0007] The disclosure is based, in part, upon the discovery that it is possible to produce recombinant mutant forms of sialidase enzymes, including, for example, sialidase enzymes that exhibit reduced binding to heparin and have an extended half-life when administered to aa subject, *e.g.*, a human. The sialidase enzymes can be used in fusion proteins and/or antibody conjugates for removing sialic acid and/or sialic acid containing molecules from the surface of cells of interest (*e.g.*, cancer cells) and/or removing sialic acid and/or sialic acid-containing molecules from the tumor microenvironment, and/or reducing the concentration of sialic acid and/or sialic acid-containing molecules in the tumor microenvironment.

[0008] It has been discovered that certain amino acid residues in a human Neu2 sialidase enzyme contribute to a heparin binding site, which, when substituted by an amino acid having less positive charge character under biologically compatible conditions, results in a human Neu2 sialidase enzyme that exhibits reduced binding to heparin as compared to an otherwise identical human Neu2 enzyme that does not comprise the substitution. It has also been discovered that certain amino acid substitutions that reduce heparin binding affinity extend half-life of a human Neu2 sialidase enzyme.

[0009] Accordingly, in one aspect, the disclosure relates to a recombinant human Neu2 enzyme having reduced binding affinity to heparin under biologically compatible conditions. The recombinant human Neu2 enzyme includes a heparin-binding site comprising at least one amino acid selected from the group consisting of an isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 (I187); a histidine residue at a

position corresponding to position 300 of wild-type human Neu2 (H300), and an arginine residue at a position corresponding to position 314 of wild-type human Neu2 (R314), *see*, **FIG. 1**. In certain embodiments, the at least one amino acid is substituted with an amino acid having less positive charge character under biologically compatible conditions. In certain embodiments, the reduced binding affinity to heparin is relative to an otherwise identical human Neu2 enzyme that does not include the substitution of the at least one amino acid.

[0010] The disclosure also relates to a recombinant human Neu2 enzyme having an enhanced serum half-life under biologically compatible conditions. The recombinant human Neu2 enzyme includes a substitution of at least one amino acid selected from the group consisting of an isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 (I187); a histidine residue at a position corresponding to position 300 of wild-type human Neu2 (H300), and an arginine residue at a position corresponding to position 314 of wild-type human Neu2 (R314). For example, the at least one amino acid can be substituted with an amino acid with a less positive charge character under biologically compatible conditions. In certain embodiments, the enhanced serum half-life is relative to an otherwise identical human Neu2 enzyme that does not comprise the substitution of the at least one amino acid.

[0011] The recombinant human Neu2 enzyme may exhibit, for example, at least a two-fold increase in serum half-life relative to an otherwise identical human Neu2 enzyme that does not comprise the substitution of the at least one amino acid.

[0012] The disclosure also relates to a recombinant human Neu2 enzyme including a substitution of an isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 (I187) with a negatively charged amino acid residue; a substitution of a histidine residue at a position corresponding to position 300 of wild-type human Neu2 (H300) with a negatively charged amino acid residue; and/or a substitution of an arginine residue at a position corresponding to position 314 of wild-type human Neu2 (R314) with a negatively charged amino acid residue.

[0013] Disulfide bonds also can be engineered in a human Neu2 sialidase enzyme, for example, to stabilize and enhance enzyme activity and titer while facilitating a reduction in heparin binding, as described herein. Accordingly, the recombinant human Neu2 enzyme can also include a substitution of a serine residue at a position corresponding to position 155 of wild-type human Neu2 with a cysteine (S155C), and a substitution of a proline residue at a position corresponding to position 190 of wild-type human Neu2 with a cysteine (P190C). In

certain embodiments, the recombinant human Neu2 enzyme also includes a substitution of an isoleucine residue at a position corresponding to position 277 of wild-type human Neu2 with a cysteine (I277C), and a substitution of a leucine residue at a position corresponding to position 337 of wild-type human Neu2 with a cysteine (L337C).

[0014] In certain embodiments, the recombinant human Neu2 enzyme comprises a substitution of I187 with a negatively charged amino acid residue. For example, the isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 can be substituted by glutamate (E) or aspartate (D). In certain embodiments, the recombinant human Neu2 enzyme comprises the I187E substitution. In certain embodiments, the recombinant human Neu2 enzyme comprises the I187D substitution.

[0015] In certain embodiments, the histidine residue at a position corresponding to position 300 of wild-type human Neu2 can be substituted by aspartate (D) or glutamate (E). In certain embodiments, the recombinant human Neu2 enzyme comprises the H300D substitution. In certain embodiments, the recombinant human Neu2 enzyme comprises the H300E substitution.

[0016] The arginine residue at a position corresponding to position 314 of wild-type human Neu2 can be substituted by aspartate (D) or glutamate (E). In certain embodiments, the recombinant human Neu2 enzyme comprises the R314D substitution. In certain embodiments, the recombinant human Neu2 enzyme comprises the R314E substitution.

[0017] Where the recombinant human Neu2 enzyme includes a substitution of a histidine residue at a position corresponding to position 300 of wild-type human Neu2 (H300), the recombinant human Neu2 enzyme can further comprise a substitution of a tryptophan at a position corresponding to 302 of wild-type human Neu2 (W302) with an aspartic acid (D) or a glutamic acid (E). In certain embodiments, the recombinant human Neu2 enzyme comprises the W302D substitution. In certain embodiments, the recombinant human Neu2 enzyme comprises the W302E substitution.

[0018] The recombinant human Neu2 enzyme can further comprise a substitution of an arginine residue at a position corresponding to position 241 of wild-type human Neu2 (R241) with an aspartic acid (D), glutamic acid (E), or a tyrosine (Y). In certain embodiments, the recombinant human Neu2 enzyme comprises the R241D substitution. In certain embodiments, the recombinant human Neu2 enzyme comprises the R241E substitution. In

certain embodiments, the recombinant human Neu2 enzyme comprises the R241Y substitution.

[0019] The recombinant human Neu2 enzyme can further comprise a substitution of a valine residue at a position corresponding to position 276 of wild-type human Neu2 (V276) with a hydrophobic amino acid residue, such as a leucine (L). In certain embodiments, the recombinant human Neu2 enzyme comprises the V276L substitution.

[0020] The recombinant human Neu2 enzyme can also include one or more of the following substitutions: S155C, P190C, and I187E (*e.g.*, all three of the foregoing substitutions). In certain embodiments, the recombinant human Neu2 enzyme includes the following substitutions: S155C, P190C, and I187E. In certain embodiments, the recombinant human Neu2 enzyme also includes the following substitutions: M1D, V6Y, A42R, P62G, A93E, Q126Y, A242F, Q270T, and C332A.

[0021] In certain embodiments, the recombinant human Neu2 enzyme includes one or more of the following substitutions: S155C, P190C, I187E, and R314E (*e.g.*, all four of the foregoing substitutions). In certain embodiments, the recombinant human Neu2 enzyme includes the following substitutions: S155C, P190C, I187E, and R314E. In certain embodiments, the recombinant human Neu2 enzyme also includes the following substitutions: M1D, V6Y, A42R, P62G, A93E, Q126Y, A242F, Q270T, and C332A.

[0022] In certain embodiments, the recombinant human Neu2 enzyme includes one or more of the following substitutions: S155C, P190C, I187E, R314E, H300D, and W302D (*e.g.*, all six of the foregoing substitutions). In certain embodiments, the recombinant human Neu2 enzyme includes the substitutions S155C, P190C, I187E, R314E, H300D, and W302D. In certain embodiments, the recombinant human Neu2 enzyme also includes the following substitutions: M1D, V6Y, A42R, P62G, A93E, Q126Y, A242F, Q270T, and C332A.

[0023] In certain embodiments, the recombinant human Neu2 enzyme includes one or more of the following substitutions: S155C, P190C, I187E, R314E, H300D, and W302E (*e.g.*, all six of the foregoing substitutions). In certain embodiments, the recombinant human Neu2 enzyme includes the substitutions S155C, P190C, I187E, R314E, H300D, and W302E. In certain embodiments, the recombinant human Neu2 enzyme also includes the following substitutions: M1D, V6Y, A42R, P62G, A93E, Q126Y, A242F, Q270T, and C332A.

[0024] In any of the above embodiments, the recombinant human Neu2 enzyme can also include a substitution of a valine residue at a position corresponding to position 276 of wild-

type human Neu2 with a leucine (V276L). For example, a recombinant human Neu2 enzyme can include the substitutions S155C, P190C, I187E, R314E, and V276L. In certain embodiments, the recombinant human Neu2 enzyme also includes the following substitutions: M1D, V6Y, A42R, P62G, A93E, Q126Y, A242F, Q270T, and C332A.

[0025] In certain embodiments, the recombinant human Neu2 enzyme comprises an amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 8-12.

[0026] In another aspect, the disclosure provides a recombinant human Neu2 enzyme comprising a substitution of a serine residue at a position corresponding to position 155 of wild-type human Neu2 with a cysteine (S155C), and a substitution of a proline residue at a position corresponding to position 190 of wild-type human Neu2 with a cysteine (P190C). In certain embodiments, the recombinant human Neu2 enzyme further comprises a substitution of an isoleucine residue at a position corresponding to position 277 of wild-type human Neu2 with a cysteine (I277C), and a substitution of a leucine residue at a position corresponding to position 337 of wild-type human Neu2 with a cysteine (L337C).

[0027] In certain embodiments, the recombinant human Neu2 enzyme is enzymatically active to remove a terminal sialic acid residue from a glycan moiety, for example, a glycoprotein or glycolipid.

[0028] In another aspect, the disclosure provides a fusion protein comprising: a recombinant human Neu2 enzyme disclosed herein and an immunoglobulin Fc domain and/or an immunoglobulin antigen-binding domain. The fusion protein can include an immunoglobulin Fc domain, for example, an immunoglobulin Fc domain derived from a human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM Fc domain (*e.g.*, an immunoglobulin Fc domain derived from a human IgG1, IgG2, IgG3, or IgG4 Fc domain). In certain embodiments, the immunoglobulin Fc domain is derived from a human IgG1 Fc domain.

[0029] In certain embodiments, the fusion protein comprises an immunoglobulin antigen-binding domain. For example, the immunoglobulin antigen-binding domain can be associated with a second immunoglobulin antigen-binding domain to produce an antigen-binding site.

[0030] In another aspect, the disclosure provides an antibody conjugate comprising a fusion protein disclosed herein. The antibody conjugate can include, for example, a single recombinant human Neu2 enzyme or two recombinant human Neu2 enzymes. The two

recombinant human Neu2 enzymes can be identical or different. The antibody conjugate can include a single antigen-binding site or two antigen-binding sites. The two antigen-binding sites can be identical or different.

[0031] In certain embodiments, the antibody conjugate comprises: a first polypeptide comprising an immunoglobulin light chain; a second polypeptide comprising an immunoglobulin heavy chain; and a third polypeptide comprising an immunoglobulin Fc domain and a recombinant human Neu2 enzyme as disclosed herein. In certain embodiments, the first and second polypeptides are covalently linked together and the second and third polypeptides are covalently linked together, and the first polypeptide and the second polypeptide together define an antigen-binding site. In certain embodiments, the third polypeptide comprises the human Neu2 enzyme and the immunoglobulin Fc domain in an N- to C-terminal orientation.

[0032] In certain embodiments, the antibody conjugate comprises: a first polypeptide comprising a first immunoglobulin light chain; a second polypeptide comprising a first immunoglobulin heavy chain and a first recombinant human Neu2 enzyme as disclosed herein; a third polypeptide comprising a second immunoglobulin heavy chain and a second recombinant human Neu2 enzyme as disclosed herein; and a fourth polypeptide comprising a second immunoglobulin light chain. In certain embodiments, the first and second polypeptides are covalently linked together, the third and fourth polypeptides are covalently linked together, and the second and third polypeptides are covalently linked together, and the first polypeptide and the second polypeptide together define a first antigen-binding site, and the third polypeptide and the fourth polypeptide together define a second antigen-binding site. In certain embodiments, the second and third polypeptides comprise the first and second immunoglobulin heavy chain and the first and second recombinant human Neu2 enzyme, respectively, in an N- to C-terminal orientation.

[0033] In certain embodiments, the antibody conjugate comprises: a first polypeptide comprising a first recombinant human Neu2 enzyme as disclosed herein, a first immunoglobulin Fc domain, and a first single chain variable fragment (scFv); and a second polypeptide comprising a second recombinant human Neu2 enzyme as disclosed herein, a second immunoglobulin Fc domain, and a second single chain variable fragment (scFv). In certain embodiments, the first and second polypeptides are covalently linked together, and wherein the first scFv defines a first antigen-binding site, and the second scFv defines a second antigen-binding site. In certain embodiments, the first polypeptide comprises the first

recombinant human Neu2 enzyme, the first immunoglobulin Fc domain, and the first scFv in an N- to C-terminal orientation, and the second polypeptide comprises the second recombinant human Neu2 enzyme, the second immunoglobulin Fc domain, and the second scFv in an N- to C-terminal orientation.

[0034] In certain embodiments, the antibody conjugate comprises: a first polypeptide comprising an immunoglobulin light chain; a second polypeptide comprising an immunoglobulin heavy chain and a single chain variable fragment (scFv); and a third polypeptide comprising an immunoglobulin Fc domain and a recombinant human Neu2 enzyme as disclosed herein. In certain embodiments, the first and second polypeptides are covalently linked together and the second and third polypeptides are covalently linked together, and wherein the immunoglobulin light chain and immunoglobulin heavy chain together define a first antigen-binding site and the scFv defines a second antigen-binding site. In certain embodiments, the second polypeptide comprises the immunoglobulin heavy chain and the scFv in an N- to C-terminal orientation, and the third polypeptide comprises the recombinant human Neu2 enzyme and the immunoglobulin Fc domain in an N- to C-terminal orientation.

[0035] In another aspect, the disclosure provides an isolated nucleic acid comprising a nucleotide sequence encoding any of the foregoing recombinant human Neu2 enzymes, any of the foregoing fusion proteins, or at least a portion of any of the foregoing antibody conjugates.

[0036] In another aspect, the disclosure provides an expression vector comprising any of the foregoing nucleic acids.

[0037] In another aspect, the disclosure provides a host cell comprising any of the foregoing expression vectors.

[0038] In another aspect, the disclosure provides a pharmaceutical composition comprising any of the foregoing recombinant enzymes, any of the foregoing fusion proteins, or any of the foregoing antibody conjugates and a pharmaceutically acceptable carrier.

[0039] In another aspect, the disclosure provides a method of treating cancer in a subject in need thereof, the method comprising administering to the subject an effective amount of any of the foregoing recombinant enzymes, any of the foregoing fusion proteins, any of the foregoing antibody conjugates, or any of the foregoing pharmaceutical compositions. The cancer can be, *e.g.*, a solid tumor, soft tissue tumor, hematopoietic tumor, or metastatic

lesion. In certain embodiments, the solid tumor is a sarcoma, adenocarcinoma, or carcinoma. In certain embodiments, the solid tumor is a head and neck (*e.g.,* pharynx), thyroid, lung (*e.g.,* small cell or non-small cell lung carcinoma (NSCLC)), breast, lymphoid, gastrointestinal (*e.g.*, oral, esophageal, stomach, liver, pancreas, small intestine, colon and rectum, anal canal), genital or genitourinary tract (*e.g.*, renal, urothelial, bladder, ovarian, uterine, cervical, endometrial, prostate, testicular), CNS (*e.g.,* neural or glial cell, *e.g.*, neuroblastoma or glioma), or skin (*e.g.,* melanoma) tumor. In certain embodiments, the hematopoietic tumor is a leukemia, acute leukemia, acute lymphoblastic leukemia (ALL), B-cell, T-cell or FAB ALL, acute myeloid leukemia (AML), chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), *e.g.*, transformed CLL, diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, hairy cell leukemia, myelodysplastic syndrome (MDS), lymphoma, Hodgkin's disease, malignant lymphoma, non-Hodgkin's lymphoma, Burkitt's lymphoma, multiple myeloma, or Richter's Syndrome (Richter's Transformation).

[0040] These and other aspects and features of the disclosure are described in the following detailed description and claims.

DESCRIPTION OF THE DRAWINGS

[0041] The disclosure can be more completely understood with reference to the following drawings.

[0042] **FIGURE 1** depicts a 3-dimensional (3-D) space filling model of the crystal structure of human Neu2 with specific sites for modification highlighted. Also depicted is the sialidase inhibitor 2,3-dehydro-2-deoxy-N-acetylneuraminic acid (DANA) bound to the catalytic site of human Neu2.

[0043] **FIGURE 2** depicts an SEC-HPLC trace of M259 and MUT18 using a heparin resin. (*See* Examples 1 and 5 for descriptions of the respective sialidases.) Trastuzumab was used as a control for an Fc-based dimer with low affinity for heparin. M259 showed a retention time greater than 8 minutes; MUT18 showed a retention time of about 7 minutes; and trastuzumab showed a retention time under 6 minutes.

[0044] **FIGURES 3A, 3B,** and **3C** depict Western blots showing mutant human sialidases M259 (**FIGURE 3A**), MUT81 (**FIGURE 3B**), and MUT85 (**FIGURE 3C**) present in mouse plasma at 30 minutes and 2, 3, 4 and 5 days after subcutaneous injections. For each mutant sialidase, two mice were injected and evaluated.

[0045] **FIGURES 4A** and **4B** are line graphs showing the mutant sialidase levels in mouse plasma detected by ELISA. Specifically, **FIGURE 4A** shows plasma levels of M106 and M259 at 0.5, 12, 24 and 48 hours post injection. **FIGURE 4B** shows plasma levels of M259, MUT81 and MUT85 at 0.5, 48, 72, 96 and 120 hours post injection. In **FIGURE 4B**, the dotted line represents the plasma level (~5 x 10^3 ng/ml (or ~5 μg/ml)) required for the sialidases to be effective.

[0046] **FIGURES 5A** and **5B** are scans of thin layer chromatography (TLC) plates resolving sialic acid-based substrates (alpha 2,8 sialic acid dimer, alpha 2,6 sialo-lactose, and alpha 2,8 sialo-lactose) from cleavage products after incubation with mutant sialidases (M106, MUT81, and MUT85) at pH 5 (**FIGURE 5A**) or at pH 6 or pH 7.2 (**FIGURE 5B**). Negative control sample loaded with substrate but no sialidase (Sub) was included. In **FIGURE 5A**, bacterial sialidase (BiNanH2) was included as a positive control for substrate cleaving. At higher pH levels of 6 or 7.2, MUT85, and to some extent MUT81, demonstrated improved cleavage of all three tested substrates compared to M106 and M259, as evidenced by the greater reduction of each substrate (located in the same level as the band in Sub sample) and increased intensity of bands above the line separating the substrate from cleaved products (**FIGURE 5B**).

[0047] **FIGURES 6A**, **6B**, **6C**, **6D**, **6E**, **6F**, **6G**, **6H**, and **6I** depict schematic representations of certain antibody conjugate constructs containing a sialidase enzyme, *e.g.*, a human sialidase enzyme, and an antigen binding site. For each antibody conjugate construct that contains more than one (*e.g.*, two) sialidase, each sialidase may be the same or different. For each antibody conjugate construct that contains more than one (*e.g.*, two) antigen binding site, each antigen binding site may be the same or different. For each antibody conjugate construct that contains an Fc domain, it is understood that the Fc domain can be a wild-type Fc domain or can be an engineered Fc domain. For example, the Fc domain may be engineered to contain either a "knob" mutation, *e.g.*, T366Y, or a "hole" mutation, *e.g.*, Y407T, or both, to promote heterodimerization, or the Fc domain may be engineered to contain one or more modifications, *e.g.*, point mutations, to provide any other modified Fc domain functionality.

[0048] **FIGURE 7** depicts schematic representations of certain antibody conjugate constructs containing a sialidase enzyme, *e.g.*, a human sialidase enzyme, and an antigen binding site. For each antibody conjugate construct that contains more than one (*e.g.*, two) antigen binding site, each antigen binding site may be the same or different. For each antibody conjugate

construct that contains an Fc domain, it is understood that the Fc domain can be a wild-type Fc domain or can be an engineered Fc domain. For example, the Fc domain may be engineered to contain either a "knob" mutation, *e.g.,* T366Y, or a "hole" mutation, *e.g.,* Y407T, or both, to promote heterodimerization, or the Fc domain may be engineered to contain one or more modifications, *e.g.*, point mutations, to provide any other modified Fc domain functionality.

[0049] **FIGURES 8A-8E** are schematic representations of exemplary fusion protein conjugates referred to as a Raptor antibody sialidase conjugate (**FIGURE 8A**), a Janus antibody sialidase conjugate (**FIGURE 8B**), a Lobster antibody sialidase conjugate (**FIGURE 8C**), a Bunk antibody sialidase conjugate (**FIGURE 8D**), and a Lobster-Fab antibody sialidase conjugate (**FIGURE 8E**).

[0050] Various features and aspects of the disclosure are discussed in more detail below.

DETAILED DESCRIPTION

[0051] The disclosure is based, in part, upon the discovery that it is possible to produce recombinant mutant forms of sialidase enzymes, including, for example, sialidase enzymes that exhibit reduced binding to heparin and have an extended half-life when administered to an animal, *e.g.*, a human. The sialidase enzymes can be used in fusion proteins and/or antibody conjugates to remove sialic acid and/or sialic acid containing molecules from the surface of cells of interest (*e.g.*, cancer cells) and/or removing sialic acid and/or sialic acid containing molecules from the tumor microenvironment, and/or reducing the concentration of sialic acid and/or sialic acid containing molecules in the tumor microenvironment.

[0052] It has been discovered that certain amino acid residues in a human Neu2 sialidase enzyme contribute to a heparin binding site. When such an amino acid residue is substituted for an amino acid having less positive charge character under biologically compatible conditions, the resulting recombinant human Neu2 sialidase enzyme exhibits reduced binding to heparin as compared to an otherwise identical human Neu2 enzyme that does not comprise the substitution.

[0053] It has also been discovered that certain amino acid substitutions in a human Neu2 enzyme reduce heparin binding affinity and/or extend half-life of the human Neu2 sialidase enzyme. For example, disulfide bonds can be engineered in a human Neu2 sialidase enzyme to stabilize and enhance enzyme activity and titer while reducing binding to heparin, as described herein.

I. Recombinant Human Sialidases

[0054] As used herein, the term "sialidase" refers to any enzyme, or a functional fragment thereof, that cleaves a terminal sialic acid residue from a substrate, for example, a glycoprotein, glycolipid, or oligosaccharide. The term sialidase includes variants having one or more amino acid substitutions, deletions, or insertions relative to a wild-type sialidase sequence, and/or fusion proteins or conjugates including a sialidase. Sialidases are also called neuraminidases, and, unless indicated otherwise, the two terms are used interchangeably herein. In certain embodiments, a sialidase enzyme is enzymatically active to remove a terminal sialic acid residue from a glycan moiety, for example, from a glycoprotein or a glycolipid. Sialidase enzymatic activity may be assayed by any method known in the art, including, for example, by measuring the release of sialic acid from the fluorogenic substrate 4-methylumbelliferyl-N-acetylneuraminic acid (4-MU-NeuAc). Another exemplary assay is a cell-based desialylation assay, in which sialylated cells are exposed to a sialidase, stained with a sialic acid marker (*e.g.*, PNA-AF647), and the amount of sialic acid marker remaining on cells is measured and compared to untreated control cells. An exemplary assay using K562 cells is provided in Example 1.

[0055] As used herein, the term "functional fragment" of a sialidase refers to fragment of a full-length sialidase that retains, 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 90%, at least 95%, or 100% of the enzymatic activity of the corresponding full-length, naturally occurring sialidase. In certain embodiments, the functional fragment comprises at least 100, 150, 200, 250, 300, 310, 320, 330, 340, 350, 360, or 370 consecutive amino acids present in a full-length, naturally occurring sialidase. In certain embodiments, the functional fragment comprises at least 350 consecutive amino acids present in a full-length, naturally occurring sialidase, and has at least 85%, 90% or 95% activity of the enzymatic activity of the full length, naturally occurring sialidase. In certain embodiments, the functional fragment comprises at least 360, consecutive amino acids present in a full-length, naturally occurring sialidase, and has at least 85%, 90% or 95% activity of the enzymatic activity of the full length, naturally occurring sialidase. In certain embodiments, the functional fragment comprises at least 370, consecutive amino acids present in a full-length, naturally occurring sialidase, and has at least 85%, 90% or 95% activity of the enzymatic activity of the full length, naturally occurring sialidase.

[0056] Four sialidases have been found in the human genome and are referred to as Neu1, Neu2, Neu3 and Neu4. Human Neu2 is a cytosolic sialidase enzyme. The amino acid sequence of human Neu2 is depicted in SEQ ID NO: 1, and a nucleotide sequence encoding human Neu2 is depicted in SEQ ID NO: 2. Unless stated otherwise, as used herein, wild-type human Neu2 refers to human Neu2 having the amino acid sequence of SEQ ID NO: 1.

[0057] In certain embodiments, a recombinant human Neu2 enzyme has 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%, about 100%, or more than 100% of the enzymatic activity of a wild-type human Neu2 enzyme, a recombinant Neu2 enzyme having SEQ ID NO: 3, or a recombinant human Neu2 enzyme having SEQ ID NO: 4.

[0058] In certain embodiments, a recombinant human Neu2 enzyme has about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, or more than 100% of the enzymatic activity of a wild-type human Neu2 enzyme, a recombinant Neu2 enzyme having SEQ ID NO: 3, or a recombinant human Neu2 enzyme having SEQ ID NO: 4.

[0059] In certain embodiments, the recombinant human Neu2 enzyme has the same substrate specificity as the wild-type human Neu2 enzyme, a recombinant Neu2 enzyme having SEQ ID NO: 3, or a recombinant human Neu2 enzyme having SEQ ID NO: 4. In other embodiments, the human Neu2 enzyme has a different substrate specificity than the wild-type human Neu2 enzyme, a recombinant Neu2 enzyme having SEQ ID NO: 3, or a recombinant human Neu2 enzyme having SEQ ID NO: 4. For example, in certain embodiments the recombinant mutant human sialidase can cleave α2,3, α2,6, and/or α2,8 linkages. In certain embodiments the sialidase can cleave α2,3 and α2,8 linkages.

[0060] In certain embodiments, the expression yield of the recombinant human Neu2 enzyme in mammalian cells, *e.g.,* HEK293 cells, CHO cells, murine myeloma cells (*e.g.*, NS0, Sp2/0), or human fibrosarcoma cells (*e.g.*, HT-1080), is greater than about 10%, about 20%, about 50%, about 75%, about 100%, about 150%, about 200%, about 250%, about 300%, about 400%, about 500%, about 600%, about 700%, about 800%, about 900%, or about

1,000% of the expression yield of the wild-type human Neu2 enzyme, a recombinant Neu2 enzyme having SEQ ID NO: 3, or a recombinant human Neu2 enzyme having SEQ ID NO: 4.

[0061] In certain embodiments, the recombinant human Neu2 enzyme has about 5%, about 10%, about 15%, about 20%, about 25%, about 30%, about 35%, about 40%, about 45%, about 50%, about 55%, about 60%, about 65%, about 70%, about 75%, about 80%, about 85%, about 90%, about 95%, about 100%, or more than 100% of the enzymatic activity of a wild-type human Neu2 enzyme, a recombinant Neu2 enzyme having SEQ ID NO: 3, or a recombinant human Neu2 enzyme having SEQ ID NO: 4, and the expression yield of the human Neu2 enzyme in mammalian cells, *e.g.,* HEK293 cells or CHO cells, is greater than about 10%, about 20%, about 50%, about 75%, about 100%, about 150%, about 200%, about 250%, about 300%, about 400%, about 500%, about 600%, about 700%, about 800%, about 900%, or about 1,000% of the expression yield of a wild-type human Neu2 enzyme, a recombinant Neu2 enzyme having SEQ ID NO: 3, or a recombinant human Neu2 enzyme having SEQ ID NO: 4.

[0062] In certain embodiments, the amino acid sequence of the recombinant human Neu2 enzyme has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to the amino acid sequence of a wild-type human Neu2 enzyme, a recombinant Neu2 enzyme having SEQ ID NO: 3, or a recombinant human Neu2 enzyme having SEQ ID NO: 4.

a. Substitutions of Residues to Reduce Binding Affinity to Heparin and/or Extend Half-Life and/or Otherwise Improve a Recombinant Human Neu2 Enzyme

[0063] It has been discovered that certain amino acid residues in a human Neu2 enzyme contribute to a heparin binding site (*see*, **FIG. 1**). When such an amino acid residue is substituted for an amino acid having less positive charge character under biologically compatible conditions, the resulting recombinant human Neu2 enzyme exhibits reduced binding to heparin as compared to an otherwise identical human Neu2 enzyme that does not comprise the substitution of the at least one amino acid.

[0064] In addition, it has been discovered that certain amino acid substitutions in a human Neu2 enzyme reduce heparin binding affinity and/or extend half-life of the human Neu2 sialidase enzyme. For example, disulfide bonds can be engineered in a human Neu2 enzyme to reduce heparin binding affinity and/or extend half-life, as described herein.

i. Substitutions in a Heparin Binding Site

[0065] The disclosure relates in part to recombinant human Neu2 enzymes having reduced binding affinity to heparin under biologically compatible conditions. It is contemplated that reducing the heparin binding affinity of a recombinant human Neu2 enzyme will result in a recombinant human Neu2 enzyme that has an enhanced serum half-life under biologically compatible conditions. The recombinant human Neu2 enzyme can include a heparin-binding site having at least one amino acid substituted with an amino acid having less positive charge character under biologically compatible conditions. The reduced heparin binding affinity can be relative to an otherwise identical human Neu2 enzyme that does not comprise the substitution of the at least one amino acid. In certain embodiments, the reduced heparin binding affinity is relative to a wild-type human Neu2 enzyme (*e.g.*, comprising SEQ ID NO: 1). In certain embodiments, the reduced heparin binding affinity is relative to a recombinant human Neu2 enzyme comprising a mutant human Neu2 enzyme, for example, SEQ ID NO: 3 or SEQ ID NO: 4.

[0066] In certain embodiments, the recombinant human Neu2 enzyme comprising a substitution of the at least one amino acid exhibits reduced heparin binding relative to an otherwise identical human Neu2 enzyme that does not comprise the substitution of the at least one amino acid. Reduced binding affinity to heparin can be measured using any means known in the art, including, for example, by performing size exclusion high-performance liquid chromatography (SEC-HPLC) using a heparin resin, as described in Example 1. In certain embodiments, the human Neu2 enzyme, comprising a substitution of at least one amino acid, has a shorter retention time relative to an otherwise identical human Neu2 enzyme that does not comprise the substitution of the at least one amino acid.

[0067] In certain embodiments, the recombinant human Neu2 enzyme comprising a substitution of the at least one amino acid exhibits a 2-fold, 3-fold, 4-fold, or longer half-life relative to an otherwise identical human Neu2 enzyme that does not comprise the substitution of the at least one amino acid. Enhanced serum half-life can be measured using any means known in the art, including, for example, detecting the presence of the enzyme in serum by Western blot or evaluating the pharmacokinetics (PK) by ELISA, as described in Examples 1 and 14, respectively.

[0068] The at least one amino acid can be selected from the group consisting of an isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 (I187), a histidine residue at a position corresponding to position 300 of wild-type human Neu2

(H300), and an arginine residue at a position corresponding to position 314 of wild-type human Neu2 (R314). A recombinant human Neu2 enzyme that includes a substitution of a histidine residue at a position corresponding to position 300 of wild-type human Neu2 (H300), can, in some embodiments, also include a substitution of a tryptophan at a position corresponding to 302 of wild-type human Neu2 (W302). In certain embodiments, the recombinant human Neu2 enzyme can further comprise a substitution of an arginine residue at a position corresponding to position 241 of wild-type human Neu2 (R241).

[0069] The at least one amino acid can be substituted with an amino acid having less positive charge character under biologically compatible conditions. It is contemplated that reducing the positive charge character of one or more amino acids that contribute to the heparin binding site leads to reduced binding to heparin. As used herein, the term "less positive charge character" refers to a substitution of one amino acid for another amino acid having a lower positive charge (or a higher negative charge). For example, a positively charged amino acid can be substituted for an uncharged amino acid or a negatively charged amino acid. In another example, an uncharged amino acid can be substituted with a negatively charged amino acid. As used herein, the term "biologically compatible conditions" means conditions suitable for the measurement of biological activity such as heparin binding or half-life, for example, in an assay. An exemplary biologically compatible condition is isotonic buffer at pH 6.0 – 7.5 (*e.g.*, 6.0, 6.5, 7.0, 7.5).

[0070] As used herein, the term "charged amino acid" refers to an amino acid selected from arginine, histidine, lysine, aspartic acid (*i.e.*, aspartate), and glutamic acid (*i.e.*, glutamate). As used herein, the term "positively charged amino acid" refers to an amino acid selected from arginine, histidine, and lysine. As used herein, the term "negatively charged amino acid" refers to an amino acid selected from aspartic acid (*i.e.*, aspartate) and glutamic acid (*i.e.*, glutamate).

[0071] As used herein, the term "uncharged amino acid" refers to an amino acid selected from alanine, asparagine, cysteine, glutamine, glycine, isoleucine, leucine, methionine, phenylalanine, proline, serine, threonine, tryptophan, tyrosine, and valine.

[0072] In certain embodiments, the isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 is substituted by a negatively charged amino acid. In certain embodiments, the isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 is substituted by glutamate (I187E) or aspartate (I187D). In certain

embodiments, the histidine residue at a position corresponding to position 300 of wild-type human Neu2 is substituted by an uncharged amino acid or a negatively charged amino acid. In certain embodiments, the histidine residue at a position corresponding to position 300 of wild-type human Neu2 is substituted by aspartate (H300D) or glutamate (H300E). In certain embodiments, the arginine residue at a position corresponding to position 314 of wild-type human Neu2 is substituted by an uncharged amino acid or a negatively charged amino acid. In certain embodiments, the arginine residue at a position corresponding to position 314 of wild-type human Neu2 is substituted by glutamate (R314E), aspartate (R314D), alanine (R314A), or glutamine (R314Q). In certain embodiments, the tryptophan residue at a position corresponding to position 302 of wild-type human Neu2 is substituted by a negatively charged amino acid. In certain embodiments, the tryptophan residue at a position corresponding to position 302 of wild-type human Neu2 is substituted by glutamate (W302E) or aspartate (W302D). In certain embodiments, the arginine residue at a position corresponding to position 241 of wild-type human Neu2 is substituted by an uncharged amino acid or a negatively charged amino acid. In certain embodiments, the arginine residue at a position corresponding to position 241 of wild-type human Neu2 is substituted by glutamate (R241E), aspartate (R241D), tyrosine (R241Y), isoleucine (R241I), leucine (R241L), tryptophan (R241W), glutamine (R241Q), or glycine (R241G).

[0073] The disclosure also relates in part to recombinant human Neu2 enzyme including a heparin-binding site that includes a mutation that reduces the electrostatic charge of the heparin binding site under biologically compatible conditions. The reduction in electrostatic charge is relative to an otherwise identical human Neu2 enzyme that does not comprise the substitution of the at least one amino acid. As used herein, "a mutation that reduces the electrostatic charge" refers to a mutation that makes the charge less positive and more negative. In certain embodiments, a mutation that reduced the electrostatic charge is one that reduces the isoelectric point (pI) of the recombinant Neu2 enzyme as calculated using standard molecular modeling algorithms such as the Molecular Operating Environment (MOE) software platform (Chemical Consulting Group), using the Amber10:EHT forcefield.

[0074] The at least one amino acid can be selected from the group consisting of an isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 (I187), a histidine residue at a position corresponding to position 300 of wild-type human Neu2 (H300), and an arginine residue at a position corresponding to position 314 of wild-type human Neu2 (R314). A recombinant human Neu2 enzyme that includes a substitution of a

histidine residue at a position corresponding to position 300 of wild-type human Neu2 (H300), can, in some embodiments, also include a substitution of a tryptophan at a position corresponding to 302 of wild-type human Neu2 (W302). In certain embodiments, the recombinant human Neu2 enzyme can also include a substitution of an arginine residue at a position corresponding to position 241 of wild-type human Neu2 (R241).

[0075] In certain embodiments, the isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 is substituted by a negatively charged amino acid. In certain embodiments, the isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 is substituted by glutamate (I187E) or aspartate (I187D). In certain embodiments, the histidine residue at a position corresponding to position 300 of wild-type human Neu2 is substituted by an uncharged amino acid or a negatively charged amino acid. In certain embodiments, the histidine residue at a position corresponding to position 300 of wild-type human Neu2 is substituted by aspartate (H300D) or glutamate (H300E). In certain embodiments, the arginine residue at a position corresponding to position 314 of wild-type human Neu2 is substituted by an uncharged amino acid or a negatively charged amino acid. In certain embodiments, the arginine residue at a position corresponding to position 314 of wild-type human Neu2 is substituted by glutamate (R314E) or aspartate (R314D). In certain embodiments, the tryptophan residue at a position corresponding to position 302 of wild-type human Neu2 is substituted by a negatively charged amino acid. In certain embodiments, the tryptophan residue at a position corresponding to position 302 of wild-type human Neu2 is substituted by glutamate (W302E) or aspartate (W302D). In certain embodiments, the arginine residue at a position corresponding to position 241 of wild-type human Neu2 is substituted by an uncharged amino acid or a negatively charged amino acid. In certain embodiments, the arginine residue at a position corresponding to position 241 of wild-type human Neu2 is substituted by glutamate (R241E), aspartate (R241D), or tyrosine (R241Y).

[0076] The disclosure further provides a recombinant mutant human sialidase comprising an amino acid substitution at a position identified in **TABLE 1** (amino acid positions corresponding to wild-type human Neu2 (SEQ ID NO: 1). In certain embodiments, the sialidase comprises an amino acid substitution identified in **TABLE 1**. In certain embodiments, the sialidase comprises a combination of any amino acid substitutions identified in **TABLE 1**.

TABLE 1

Wild Type Human Neu2 (SEQ ID NO: 1) Amino Acid	**Exemplary Substitution(s) at Specified Position(s)**	**Exemplary Substitution(s) at Specified Position(s)**	**Exemplary Substitution(s) at Specified Position(s)**
I187	A, D, E, L, Q, T	A, D, E, L	D, E
H300	A, D, E, F, M, L, P, Q, T, V, W, Y	D	D
R314	A, D, E, F, M, L, P, Q, T, V, W, Y	A, D, E, Q	D, E
W302	A, D, E, F, I, V	A, D, E, F, I, V	D, E
R241	D, E, Q, Y	D, E, Q, Y	D, E, Y

[0077] In certain embodiments, in the sialidase, (a) the isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 is substituted by alanine (I187A), aspartic acid (I187D), glutamic acid (I187E), leucine (I187L), glutamine (I187Q), or threonine (I187T); the histidine residue at a position corresponding to position 300 of wild-type human Neu2 is substituted by alanine (H300A), aspartic acid (H300D), glutamic acid (H300E), phenylalanine (H300F), methionine (H300M), leucine (H300L), proline (H300P), glutamine (H300Q), threonine (H300T), valine (H300V), tryptophan (H300W), or tyrosine (H300Y); (c) the arginine residue at a position corresponding to position 314 of wild-type human Neu2 is substituted by alanine (R314A), aspartic acid (R314D), glutamic acid (R314E), phenylalanine (R314F), methionine (R314M), leucine (R314L), proline (R314P), glutamine (R314Q), threonine (R314T), valine (R314V), tryptophan (R314W), or tyrosine (R314Y); or (d) the sialidase comprises a combination of any of the foregoing substitutions. For example, the sialidase may comprise a substitution selected from I187D, H300D, and R314E, or a combination of any of the foregoing substitutions. In certain embodiments, in the sialidase, the tryptophan residue at position corresponding to position 302 of wild-type human Neu2 is substituted by aspartic acid (W302D) or glutamic acid (W302E). In certain embodiments, in the sialidase, the arginine residue at position corresponding to position 241 of wild-type human Neu2 is further substituted by aspartic acid (R241D), glutamic acid (R241E), glutamine (R241Q), or tyrosine (R241Y).

[0078] In certain embodiments, in the sialidase, (a) the isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 is substituted by alanine (I187A), aspartic acid (I187D), glutamic acid (I187E), or leucine (I187L); (b) the histidine residue at a

position corresponding to position 300 of wild-type human Neu2 is substituted by aspartic acid (H300D); (c) the arginine residue at a position corresponding to position 314 of wild-type human Neu2 is substituted by alanine (R314A), aspartic acid (R314D), glutamic acid (R314E), or glutamine (R314Q); or (d) the sialidase comprises a combination of any of the foregoing substitutions.

[0079] In certain embodiments, in the sialidase, the tryptophan residue at position corresponding to position 302 of wild-type human Neu2 is substituted by alanine (W302A), aspartic acid (W302D) glutamic acid (W302E), phenylalanine (W302F), isoleucine (W302I), or valine (W302V). In certain embodiments, in the sialidase, the arginine residue at position corresponding to position 241 of wild-type human Neu2 is further substituted by aspartic acid (R241D), glutamic acid (R241E), glutamine (R241Q), or tyrosine (R241Y).

[0080] In certain embodiments, in the sialidase, (a) the isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 is substituted by aspartic acid (I187D) or glutamic acid (I187E); (b) the histidine residue at a position corresponding to position 300 of wild-type human Neu2 is substituted by aspartic acid (H300D); (c) the arginine residue at a position corresponding to position 314 of wild-type human Neu2 is substituted by glutamic acid (R314E); or (d) the sialidase comprises a combination of any of the foregoing substitutions. In certain embodiments, in the sialidase, the tryptophan residue at position corresponding to position 302 of wild-type human Neu2 is substituted by aspartic acid (W302D) or glutamic acid (W302E). In certain embodiments, in the sialidase, the arginine residue at position corresponding to position 241 of wild-type human Neu2 is further substituted by aspartic acid (R241D), glutamic acid (R241E), or tyrosine (R241Y). In certain embodiments, in the sialidase, (a) the isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 is substituted by glutamic acid (I187E); (b) the histidine residue at a position corresponding to position 300 of wild-type human Neu2 is substituted by aspartic acid (H300D); and (c) the arginine residue at a position corresponding to position 314 of wild-type human Neu2 is substituted by glutamic acid (R314E), optionally wherein (d) the tryptophan residue at position corresponding to position 302 of wild-type human Neu2 is substituted by glutamic acid (W302E).

[0081] Additional exemplary sialidase mutations, and combinations of sialidase mutations, are described in U.S. Patent No. 11,965,188, including in the Detailed Description in the section entitled "I. Recombinant Human Sialidases," and in Examples 1, 2, 3, 4, 5, and 6; U.S. Patent Application Publication No. US 2022/0356457 A1, including in the Detailed

Description in the section entitled "I. Recombinant Human Sialidases," and in Examples 2, 3, 4, and 5; U.S. Patent Application Publication No. US 2022/0362351 A1, including in the Detailed Description in the section entitled "I. Recombinant Sialidases," and in Example 2; and U.S. Patent Application Publication No. US 2023/0265406 A1, including in the Detailed Description in the section entitled "I. Recombinant Human Sialidases," and in Examples 2, 3, 4, and 5.

ii. Cysteine Substitutions That Form Disulfide Bonds

[0082] The inventors have also discovered that substituting certain amino acid pairs to cysteines results in the formation of disulfide bonds in a recombinant human Neu2 enzyme that may have beneficial effects. For example, such substitutions may stabilize a recombinant human Neu2 enzyme with reduced heparin binding and/or enhanced half-life. Exemplary substitutions include:

(a) a substitution of a serine residue at a position corresponding to position 155 of wild-type human Neu2 with a cysteine (S155C), and a substitution of a proline residue at a position corresponding to position 190 of wild-type human Neu2 with a cysteine (P190C); and

(b) a substitution of an isoleucine residue at a position corresponding to position 277 of wild-type human Neu2 with a cysteine (I277C), and a substitution of a leucine residue at a position corresponding to position 337 of wild-type human Neu2 with a cysteine (L337C).

[0083] In certain embodiments, the disclosure relates to a recombinant human Neu2 enzyme comprising (a) a substitution of a serine residue at a position corresponding to position 155 of wild-type human Neu2 with a cysteine (S155C) and a substitution of a proline residue at a position corresponding to position 190 of wild-type human Neu2 with a cysteine (P190C), and (b) a substitution of an isoleucine residue at a position corresponding to position 277 of wild-type human Neu2 with a cysteine (I277C), and a substitution of a leucine residue at a position corresponding to position 337 of wild-type human Neu2 with a cysteine (L337C).

[0084] In certain embodiments, the recombinant human Neu2 enzyme comprising two cysteine amino acid substitutions exhibits reduced heparin binding relative to an otherwise identical human Neu2 enzyme that does not comprise the substitutions. Reduced binding affinity to heparin can be measured using any means known in the art, including, for example, by performing size exclusion high-performance liquid chromatography (SEC-HPLC) using a heparin resin, as described in Example 1. In certain embodiments, the human

Neu2 enzyme, comprising a substitution of at least one amino acid, has a shorter retention time relative to an otherwise identical human Neu2 enzyme that does not comprise the substitution of the at least one amino acid.

[0085] In certain embodiments, the recombinant human Neu2 enzyme comprising two cysteine amino acid substitutions exhibits an at least 2-fold, at least 3-fold, at least 4-fold, or longer half-life relative to an otherwise identical human Neu2 enzyme that does not comprise the two substitutions. Enhanced serum half-life can be measured using any means known in the art, including, for example, detecting the presence of the enzyme in serum by Western blot or evaluating the pharmacokinetics (PK) by ELISA, as described in Examples 1 and 12, respectively.

b. Combinations of Substitutions

[0086] The disclosure further provides a recombinant mutant human sialidase comprising a combination of any of the mutations contemplated herein. For example, the recombinant human Neu2 enzyme may comprise a combination of 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15 or more of the mutations contemplated herein. It is contemplated that the recombinant human Neu2 enzyme may comprise 1-15, 1-10, 1-7, 1-6, 1-5, 1-4, 1-3, 1-2, 2-15, 2-10, 2-7, 2-6, 2-5, 2-4, 2-3, 3-15, 3-10, 3-7, 3-6, 3-5, or 3-4 of the mutations contemplated herein.

[0087] For example, alternatively or in addition to the modifications to heparin binding and/or increase half-life, the recombinant human Neu2 enzyme may comprise (i) a substitution of a methionine residue at a position corresponding to position 1 of wild-type human Neu2; (ii) a substitution of a valine residue at a position corresponding to position 6 of wild-type human Neu2; (iii) a substitution of an alanine residue at a position corresponding to position 42 of wild-type human Neu2; (iv) a substitution of a proline residue at a position corresponding to position 62 of wild-type human Neu2; (v) a substitution of an alanine residue at a position corresponding to position 93 of wild-type human Neu2; (vi) a substitution of a glutamine residue at a position corresponding to position 126 of wild-type human Neu2; (vii) a substitution of an alanine residue at a position corresponding to position 242 of wild-type human Neu2; (viii) a substitution of a glutamine residue at a position corresponding to position 270 of wild-type human Neu2; (ix) a substitution of a cysteine residue at a position corresponding to position 332 of wild-type human Neu2; or any combination of the foregoing (*e.g.*, each of the foregoing). In certain embodiments, the recombinant mutant human Neu2 enzyme comprises an M1D substitution, a V6Y

substitution, an A42R substitution, a P62G substitution, an A93E substitution, a Q126Y substitution, an A242F substitution, a Q270T substitution, a C332A substitution, or any combination of the foregoing substitutions (*e.g.*, each of the nine foregoing substitutions).

[0088] In certain embodiments, in the recombinant human Neu2 enzyme (a) the isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 can be substituted by alanine (I187A), aspartic acid (I187D), glutamic acid (I187E), leucine (I187L), glutamine (I187Q), or threonine (I187T); (b) the histidine residue at a position corresponding to position 300 of wild-type human Neu2 can be substituted by aspartic acid (H300D); or (c) the arginine residue at a position corresponding to position 314 of wild-type human Neu2 can be substituted by alanine (R314A), aspartic acid (R314D), glutamic acid (R314E), or glutamine (R314Q); or (d) the sialidase can include a combination of any of the foregoing substitutions. In certain embodiments, in the recombinant human Neu2 enzyme, the tryptophan residue at position corresponding to position 302 of wild-type human Neu2 can be substituted by aspartic acid (W302D) or glutamic acid (W302E). In certain embodiments, in the recombinant human Neu2 enzyme, the arginine residue at position corresponding to position 241 of wild-type human Neu2 can be substituted by aspartic acid (R241D) or glutamic acid (R241E). For example, the sialidase may comprise a substitution selected from I187D, H300D, and R314E, and optionally W302D and/or R241E, or a combination of any of the foregoing substitutions.

[0089] In certain embodiments, the recombinant human Neu2 enzyme further comprises a substitution of a serine residue at a position corresponding to position 155 of wild-type human Neu2 with a cysteine (S155C), and a substitution of a proline residue at a position corresponding to position 190 of wild-type human Neu2 with a cysteine (P190C).

[0090] In certain embodiments, the recombinant human Neu2 enzyme comprises the following substitutions: S155C, P190C, I187E, R314E, H300D, and W302D, wherein each amino acid position corresponds to human Neu2.

[0091] In certain embodiments, the recombinant human Neu2 enzyme comprises the following substitutions: S155C, P190C, I187E, R314E, H300D, and W302E, wherein each amino acid position corresponds to human Neu2.

[0092] In certain embodiments, the recombinant human Neu2 enzyme comprises the following substitutions: S155C, P190C, and I187E, wherein each amino acid position corresponds to human Neu2.

[0093] In certain embodiments, the recombinant human Neu2 enzyme comprises the following substitutions: S155C, P190C, I187E, and R314E, wherein each amino acid position corresponds to human Neu2.

[0094] In certain embodiments, the recombinant human Neu2 enzyme further comprises a substitution of a valine residue at a position corresponding to position 276 of wild-type human Neu2 with a leucine (V276L), wherein each amino acid position corresponds to human Neu2.

[0095] In certain embodiments, the recombinant human Neu2 sialidase enzyme further comprises an M1D substitution, V6Y substitution, P62G substitution, A93E substitution, C332A substitution, or a combination of any of the foregoing.

[0096] In certain embodiments, the recombinant human Neu2 sialidase enzyme further comprises an M1D substitution, V6Y substitution, A42R substitution, P62G substitution, A93E substitution, Q126Y substitution, A242F substitution, Q270T substitution, C332A substitution, or a combination of any of the foregoing.

[0097] In certain embodiments, the recombinant human Neu2 sialidase enzyme further comprises (i) an amino acid substitution identified in **TABLE 1**, or a combination of any amino acid substitutions identified in **TABLE 1**, and (ii) an M1D substitution, V6Y substitution, P62G substitution, A93E substitution, C332A substitution, or a combination of any of the foregoing.

[0098] In certain embodiments, the recombinant human Neu2 sialidase enzyme comprises (i) an amino acid substitution identified in **TABLE 1**, or a combination of any amino acid substitutions identified in **TABLE 1**, and (ii) an M1D substitution, V6Y substitution, A42R substitution, P62G substitution, A93E substitution, Q126Y substitution, A242F substitution, Q270T substitution, C332A substitution, or a combination of any of the foregoing.

[0099] In certain embodiments, the recombinant mutant human sialidase further comprises a combination of substitutions corresponding to a combination of substitutions listed in a row of **TABLE 2** (amino acid positions corresponding to wild-type human Neu2 (SEQ ID NO: 1)).

TABLE 2

Mutant	Substitutions
MUT84	S155C, P190C, I187E, R314E, H300D, W302D

Mutant	Substitutions
MUT84 (M106 background)	M1D, V6Y, P62G, A93E, C332A, S155C, P190C, I187E, R314E, H300D, W302D
MUT84 (M259 background)	M1D, V6Y, A42R, P62G, A93E, Q126Y, A242F, Q270T, C332A, S155C, P190C, I187E, R314E, H300D, W302D
MUT85	S155, P190C, I187E, R314E, H300D, W302E
MUT85 (M106 background)	M1D, V6Y, P62G, A93E, C332A, S155C, P190C, I187E, R314E, H300D, W302E
MUT85 (M259 background)	M1D, V6Y, A42R, P62G, A93E, Q126Y, A242F, Q270T, C332A, S155C, P190C, I187E, R314E, H300D, W302E
MUT81	S155C, P190C, I187E
MUT81 (M106 background)	M1D, V6Y, P62G, A93E, C332A, S155C, P190C, I187E
MUT81 (M259 background)	M1D, V6Y, A42R, P62G, A93E, Q126Y, A242F, Q270T, C332A, S155C, P190C, I187E
MUT83	S155C, P190C, I187E, R314E
MUT83 (M106 background)	M1D, V6Y, P62G, A93E, C332A, S155C, P190C, I187E, R314E
MUT83 (M259 background)	M1D, V6Y, A42R, P62G, A93E, Q126Y, A242F, Q270T, C332A, S155C, P190C, I187E, R314E
MUT89	S155C, P190C, I187E, V276L, R314E
MUT89 (M106 background)	M1D, V6Y, P62G, A93E, C332A, S155C, P190C, I187E, V276L, R314E
MUT89 (M259 background)	M1D, V6Y, A42R, P62G, A93E, Q126Y, A242F, Q270T, C332A, S155C, P190C, I187E, V276L, R314E

[00100] In certain embodiments, the recombinant mutant human Neu2 sialidase comprises a conservative substitution relative to a recombinant mutant human Neu2 sialidase sequence disclosed herein. As used herein, the term "conservative substitution" refers to a substitution with a structurally similar amino acid. For example, conservative substitutions may include those within the following groups: Ser and Cys; Leu, Ile, and Val; Glu and Asp; Lys and Arg; Phe, Tyr, and Trp; and Gln, Asn, Glu, Asp, and His. Conservative substitutions may also be defined by the BLAST (Basic Local Alignment Search Tool) algorithm, the BLOSUM substitution matrix (*e.g.*, BLOSUM 62 matrix), or the PAM substitution:p matrix (*e.g.*, the PAM 250 matrix).

[00101] Sequence identity may be determined in various ways that are within the skill of a person skilled in the art, *e.g.*, using publicly available computer software such as BLAST, BLAST-2, ALIGN or Megalign (DNASTAR) software. BLAST (Basic Local Alignment Search Tool) analysis using the algorithm employed by the programs blastp, blastn, blastx, tblastn and tblastx (Karlin *et al.*, (1990) PROC. NATL. ACAD. SCI. USA 87:2264-2268; Altschul, (1993) J. MOL. EVOL. 36:290-300; Altschul *et al.*, (1997) Nucleic Acids Res. 25:3389-3402, incorporated by reference herein) are tailored for sequence similarity searching. For a discussion of basic issues in searching sequence databases see Altschul *et al.*, (1994) NATURE GENETICS 6:119-129, which is fully incorporated by reference herein. Those skilled in the art can determine appropriate parameters for measuring alignment, including any algorithms needed to achieve maximal alignment over the full length of the sequences being compared. The search parameters for histogram, descriptions, alignments, expect (*i.e.*, the statistical significance threshold for reporting matches against database sequences), cutoff, matrix, and filter are at the default settings. The default scoring matrix used by blastp, blastx, tblastn, and tblastx is the BLOSUM62 matrix (Henikoff *et al.*, (1992) PROC. NATL. ACAD. SCI. USA 89:10915-10919, fully incorporated by reference herein). Four blastn parameters may be adjusted as follows: Q=10 (gap creation penalty); R=10 (gap extension penalty); wink=1 (generates word hits at every wink.sup.th position along the query); and gapw=16 (sets the window width within which gapped alignments are generated). The equivalent blastp parameter settings may be Q=9; R=2; wink=1; and gapw=32. Searches may also be conducted using the NCBI (National Center for Biotechnology Information) BLAST Advanced Option parameter (*e.g.*: -G, Cost to open gap [Integer]: default = 5 for nucleotides/ 11 for proteins; -E, Cost to extend gap [Integer]: default = 2 for nucleotides/ 1 for proteins; -q, Penalty for nucleotide mismatch [Integer]: default = -3; -r, reward for nucleotide match [Integer]: default = 1; -e, expect value [Real]: default = 10; -W, wordsize [Integer]: default = 11 for nucleotides/ 28 for megablast/ 3 for proteins; -y, Dropoff (X) for blast extensions in bits: default = 20 for blastn/ 7 for others; -X, X dropoff value for gapped alignment (in bits): default = 15 for all programs, not applicable to blastn; and –Z, final X dropoff value for gapped alignment (in bits): 50 for blastn, 25 for others). ClustalW for pairwise protein alignments may also be used (default parameters may include, *e.g.*, Blosum62 matrix and Gap Opening Penalty = 10 and Gap Extension Penalty = 0.1). A Bestfit comparison between sequences, available in the GCG package version 10.0, uses DNA parameters GAP=50 (gap creation penalty) and LEN=3 (gap extension penalty). The equivalent settings in Bestfit protein comparisons are GAP=8 and LEN=2.

II. Fusion Proteins/Antibody Conjugates

[00102] To promote the selective removal of sialic acids on hypersialylated cancer cells and/or in the tumor microenvironment, it may be helpful to target a sialidase as described herein to such a cell or to such a tumor microenvironment. Additionally, in order to promote the removal of sialic acid by a sialidase in a subject, it may be helpful to extend the plasma half-life of the sialidase in the subject. These can be achieved by including the sialidase in a fusion protein and/or antibody conjugate (*e.g.*, a chemically conjugated conjugate).

[00103] Accordingly, the disclosure further provides fusion proteins comprising a sialidase enzyme, or a functional fragment thereof, and a portion or fragment of an antibody, such as an immunoglobulin Fc domain (also referred to herein as an Fc domain), or an immunoglobulin antigen-binding domain (also referred to herein as an antigen-binding domain). In certain embodiments, the sialidase and antibody or portion thereof (*e.g.*, immunoglobulin Fc domain or antigen-binding domain) are linked by a peptide bond or an amino acid linker.

[00104] As used herein, unless otherwise indicated, the term "fusion protein" is understood to refer to a single polypeptide chain comprising amino acid sequences based upon two or more separate proteins or polypeptide chains, where the two amino acid sequences may be fused together directly or via an intervening linker sequence, *e.g.*, via an intervening amino acid linker. A nucleotide sequence encoding a fusion protein can, for example, be created using conventional recombinant DNA technologies.

[00105] In certain embodiments, the fusion protein comprises a tag, such as a Strep tag (*e.g.*, a Strep II tag), a His tag (*e.g.*, a 10x His tag (SEQ ID NO: 37)), a myc tag, or a FLAG tag. The tag can be located on the C-terminus or the N-terminus of the fusion protein. In certain embodiments, a fusion protein comprises a sialidase portion joined to a polypeptide comprising an immunoglobulin heavy chain in an N- to C-terminal orientation, wherein the sialidase portion comprises an N-terminal addition of MEDLRP (SEQ ID NO: 18), and a Strep II Tag is located on the C-terminus of the immunoglobulin heavy chain or the N-terminus of the sialidase portion.

a. Sialidase Portion

[00106] The sialidase portion of the fusion protein described herein can be any sialidase or neuraminidase disclosed herein. In certain embodiments, the sialidase portion is a recombinant human Neu2 sialidase disclosed herein, or a functional fragment thereof.

[00107] In certain embodiments, the human Neu2 sialidase is engineered to include one or more modifications, for example, an amino acid substitution to decrease heparin binding and/or enhance half-life of the sialidase as disclosed herein, and may then, alternatively or in addition, include one or more of the following substitutions.

[00108] In certain embodiments, the human Neu2 sialidase portion further comprises an M1D substitution, V6Y substitution, A42R substitution, P62G substitution, A93E substitution, Q126Y substitution, I187K substitution, A242F substitution, Q270T substitution, C332A substitution, or a combination of any of the foregoing.

[00109] In certain embodiments, the human Neu2 sialidase portion comprises a group of the amino acid substitutions set forth in Table 2 and/or the human Neu2 sialidase portion comprises the amino acid sequence of any one of SEQ ID NOs: 8-12, or an amino acid sequence that has at least 85%, 90%, 95%, 96%, 97%, 98%, or 99% sequence identity to any one of SEQ ID NOs: 8-12.

b. Antibody Portion

[00110] As used herein, unless otherwise indicated, the term "antibody" is understood to mean an intact antibody (*e.g.*, an intact monoclonal antibody), or a fragment thereof, such as a Fc fragment of an antibody (*e.g.*, an Fc fragment of a monoclonal antibody), or an antigen-binding fragment of an antibody (*e.g.*, an antigen-binding fragment of a monoclonal antibody), including an intact antibody, antigen-binding fragment, or Fc fragment that has been modified, engineered, or chemically conjugated. Examples of antigen-binding fragments include Fab, Fab', $(Fab')_2$, Fv, single chain antibodies (*e.g.*, scFv), minibodies, and diabodies. Examples of antibodies that have been modified or engineered include chimeric antibodies, humanized antibodies, and multispecific antibodies (*e.g.*, bispecific antibodies). An example of a chemically conjugated antibody is an antibody conjugated to a toxin moiety.

[00111] In certain embodiments, the fusion protein comprises an immunoglobulin Fc domain. As used herein, unless otherwise indicated, the term "immunoglobulin Fc domain" refers to a fragment of an immunoglobulin heavy chain constant region which, either alone or in combination with a second immunoglobulin Fc domain, is capable of binding to an Fc receptor. An immunoglobulin Fc domain may include, *e.g.*, immunoglobulin CH2 and CH3 domains. An immunoglobulin Fc domain may include, *e.g.*, immunoglobulin CH2 and CH3 domains and an immunoglobulin hinge region. Boundaries between immunoglobulin hinge

regions, CH2, and CH3 domains are well known in the art, and can be found, *e.g.*, in the PROSITE database (available on the world wide web at prosite.expasy.org).

[00112] In certain embodiments, the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, and IgM Fc domain. A single amino acid substitution (S228P according to Kabat numbering; designated IgG4Pro) may be introduced to abolish the heterogeneity observed in recombinant IgG4 antibody. See Angal, S. *et al.* (1993) MOL. IMMUNOL. 30:105-108.

[00113] In certain embodiments, the immunoglobulin Fc domain is derived from a human IgG1 isotype or another isotype that elicits antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC). In certain embodiments, the immunoglobulin Fc domain is derived from a human IgG1 isotype (*e.g.*, SEQ ID NOs: 19-21).

[00114] The disclosure relates in part to a recombinant human Neu2 sialidase-Fc fusion protein. In certain embodiments, the amino acid sequence of the recombinant human Neu2 sialidase-Fc fusion protein has at least 50%, 55%, 60%, 65%, 70%, 75%, 80%, 85%, 90%, 95%, 96%, 97%, 98%, 99%, or 100% sequence identity to the amino acid sequence of any one of SEQ ID NOS: 13-17.

[00115] In certain embodiments, the immunoglobulin Fc domain is derived from a human IgG4 isotype or another isotype that elicits little or no antibody-dependent cell-mediated cytotoxicity (ADCC) and/or complement-dependent cytotoxicity (CDC). In certain embodiments, the immunoglobulin Fc domain is derived from a human IgG4 isotype.

[00116] The assembly of heterodimeric antibody heavy chains can be accomplished by expressing two different antibody heavy chain sequences in the same cell, which may lead to the assembly of homodimers of each antibody heavy chain as well as assembly of heterodimers. Promoting the preferential assembly of heterodimers can be accomplished by incorporating different mutations in the CH3 domain of each antibody heavy chain constant region as shown in US13/494870, US16/028850, US11/533709, US12/875015, US13/289934, US14/773418, US12/811207, US13/866756, US14/647480, and US14/830336. For example, mutations can be made in the CH3 domain based on human IgG1 and incorporating distinct pairs of amino acid substitutions within a first polypeptide and a second polypeptide that allow these two chains to selectively heterodimerize with each

other. The positions of amino acid substitutions illustrated below are all numbered according to the EU index as in Kabat.

[00117] In one scenario, an amino acid substitution in the first polypeptide replaces the original amino acid with a larger amino acid, selected from arginine (R), phenylalanine (F), tyrosine (Y) or tryptophan (W), and at least one amino acid substitution in the second polypeptide replaces the original amino acid(s) with a smaller amino acid(s), chosen from alanine (A), serine (S), threonine (T), or valine (V), such that the larger amino acid substitution (a protuberance) fits into the surface of the smaller amino acid substitutions (a cavity). For example, one polypeptide can incorporate a T366W substitution, and the other can incorporate three substitutions including T366S, L368A, and Y407V.

[00118] In certain embodiments, the immunoglobulin Fc domain comprises a heterodimerization mutations, such as a "knob" mutation, *e.g.,* T366Y, or a "hole" mutation, *e.g.,* Y407T, for heterodimerization with a second polypeptide (residue numbers according to EU numbering, Kabat, E.A., *et al.* (1991) SEQUENCES OF PROTEINS OF IMMUNOLOGICAL INTEREST, FIFTH EDITION, U.S. Department of Health and Human Services, NIH Publication No. 91-3242). For example, in certain embodiments, the immunoglobulin Fc domain is derived from a human IgG1 Fc domain and comprises a Y407T mutation (*e.g.*, the fusion protein comprises any of SEQ ID NOs: 22-25). In certain embodiments, the immunoglobulin Fc domain is derived from a human IgG1 Fc domain and comprises a T366Y mutation (*e.g.*, the fusion protein comprises any of SEQ ID NOs: 26-29).

[00119] Alternatively, amino acid substitutions could be selected from the following sets of substitutions shown in **TABLE 3**.

TABLE 3

	First Polypeptide	**Second Polypeptide**
Set 1	S364E/F405A	Y349K/T394F
Set 2	S364H/D401K	Y349T/T411E
Set 3	S364H/T394F	Y349T/F405A
Set 4	S364E/T394F	Y349K/F405A
Set 5	S364E/T411E	Y349K/D401K
Set 6	S364D/T394F	Y349K/F405A
Set 7	S364H/F405A	Y349T/T394F
Set 8	S364K/E357Q	L368D/K370S

	First Polypeptide	Second Polypeptide
Set 9	L368D/K370S	S364K
Set 10	L368E/K370S	S364K
Set 11	K360E/Q362E	D401K
Set 12	L368D/K370S	S364K/E357L
Set 13	K370S	S364K/E357Q
Set 14	F405L	K409R
Set 15	K409R	F405L

[00120] Alternatively, amino acid substitutions could be selected from the following sets of substitutions shown in **TABLE 4**.

TABLE 4

	First Polypeptide	Second Polypeptide
Set 1	K409W	D399V/F405T
Set 2	Y349S	E357W
Set 3	K360E	Q347R
Set 4	K360E/K409W	Q347R/D399V/F405T
Set 5	Q347E/K360E/K409W	Q347R/D399V/F405T
Set 6	Y349S/K409W	E357W/D399V/F405T

[00121] Alternatively, amino acid substitutions could be selected from the following set of substitutions shown in **TABLE 5**.

TABLE 5

	First Polypeptide	Second Polypeptide
Set 1	T366K/L351K	L351D/L368E
Set 2	T366K/L351K	L351D/Y349E
Set 3	T366K/L351K	L351D/Y349D
Set 4	T366K/L351K	L351D/Y349E/L368E
Set 5	T366K/L351K	L351D/Y349D/L368E
Set 6	E356K/D399K	K392D/K409D

[00122] Alternatively, at least one amino acid substitution in each polypeptide chain could be selected from **TABLE 6**.

TABLE 6

First Polypeptide	Second Polypeptide
L351Y, D399R, D399K, S400K, S400R, Y407A, Y407I, Y407V	T366V, T366I, T366L, T366M, N390D, N390E, K392L, K392M, K392V, K392F K392D, K392E, K409F, K409W, T411D and T411E

[00123] Alternatively, at least one amino acid substitutions could be selected from the following set of substitutions in **TABLE 7**, where the position(s) indicated in the First Polypeptide column is replaced by any known negatively-charged amino acid, and the position(s) indicated in the Second Polypeptide column is replaced by any known positively-charged amino acid.

TABLE 7

First Polypeptide	Second Polypeptide
K392, K370, K409, or K439	D399, E356, or E357

[00124] Alternatively, at least one amino acid substitutions could be selected from the following set of in **TABLE 8**, where the position(s) indicated in the First Polypeptide column is replaced by any known positively-charged amino acid, and the position(s) indicated in the Second Polypeptide column is replaced by any known negatively-charged amino acid.

TABLE 8

First Polypeptide	Second Polypeptide
D399, E356, or E357	K409, K439, K370, or K392

[00125] Alternatively, amino acid substitutions could be selected from the following set of in **TABLE 9**.

TABLE 9

First Polypeptide	Second Polypeptide
T350V, L351Y, F405A, and Y407V	T350V, T366L, K392L, and T394W

[00126] Alternatively, or in addition, the structural stability of heterodimeric heavy chains can be increased by introducing S354C on either of the first or second polypeptide chain, and Y349C on the opposing polypeptide chain, which forms an artificial disulfide bridge within the interface of the two polypeptides.

[00127] In certain embodiments of the present disclosure, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at position T366, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of T366, L368, and Y407.

[00128] In certain embodiments of the present disclosure, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of T366, L368, and Y407, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at position T366.

[00129] In certain embodiments of the present disclosure, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of E357, K360, Q362, S364, L368, K370, T394, D401, F405, and T411 and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of Y349, E357, S364, L368, K370, T394, D401, F405, and T411.

[00130] In certain embodiments of the present disclosure, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of Y349, E357, S364, L368, K370, T394, D401, F405, and T411 and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of E357, K360, Q362, S364, L368, K370, T394, D401, F405, and T411.

[00131] In certain embodiments of the present disclosure, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an

IgG1 constant region at one or more positions selected from the group consisting of L351, D399, S400, and Y407, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of T366, N390, K392, K409, and T411.

[00132] In certain embodiments of the present disclosure, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of T366, N390, K392, K409, and T411, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of L351, D399, S400, and Y407.

[00133] In certain embodiments of the present disclosure, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of Q347, Y349, K360, and K409, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of Q347, E357, D399, and F405.

[00134] In certain embodiments of the present disclosure, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of Q347, E357, D399, and F405, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of Y349, K360, Q347, and K409.

[00135] In certain embodiments of the present disclosure, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of K370, K392, K409, and K439, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of D356, E357, and D399.

[00136] In certain embodiments of the present disclosure, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of D356, E357, and D399, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of K370, K392, K409, and K439.

[00137] In certain embodiments of the present disclosure, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of L351, E356, T366, and D399, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of Y349, L351, L368, K392, and K409.

[00138] In certain embodiments of the present disclosure, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of Y349, L351, L368, K392, and K409, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region at one or more positions selected from the group consisting of L351, E356, T366, and D399.

[00139] In certain embodiments of the present disclosure, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by an S354C substitution, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by a Y349C substitution.

[00140] In certain embodiments of the present disclosure, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by a Y349C substitution, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by an S354C substitution.

[00141] In certain embodiments of the present disclosure, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an

IgG1 constant region by K360E and K409W substitutions, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by Q347R, D399V, and F405T substitutions.

[00142] In certain embodiments of the present disclosure, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by O347R, D399V and F405T substitutions and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by K360E and K409W substitutions.

[00143] In certain embodiments of the present disclosure, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by a T366W substitutions, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by T366S, T368A, and Y407V substitutions.

[00144] In certain embodiments of the present disclosure, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by T366S, T368A, and Y407V substitutions, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by a T366W substitution.

[00145] In certain embodiments of the present disclosure, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by T350V, L351Y, F405A, and Y407V substitutions, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by T350V, T366L, K392L, and T394W substitutions.

[00146] In certain embodiments of the present disclosure, the amino acid sequence of one polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by T350V, T366L, K392L, and T394W substitutions, and wherein the amino acid sequence of the other polypeptide chain of the antibody constant region differs from the amino acid sequence of an IgG1 constant region by T350V, L351Y, F405A, and Y407V substitutions.

[00147] In certain embodiments, the immunoglobulin Fc domain is modified to prevent glycosylation of the Fc domain. For example, in certain embodiments, the immunoglobulin

Fc domain is derived from a human IgG1 Fc domain and comprises a mutation at position N297, for example, an N297A mutation (residue numbers according to EU numbering, Kabat, E.A., *et al.*, *supra*). For example, in certain embodiments, the fusion protein comprises SEQ ID NO: 25, SEQ ID NO: 29, or SEQ ID NO: 30.

[00148] In certain embodiments, the fusion protein comprises an immunoglobulin antigen-binding domain. The inclusion of such a domain may improve targeting of a fusion protein to a sialylated cancer cell and/or to the tumor microenvironment. As used herein, unless otherwise indicated, the term "immunoglobulin antigen-binding domain" refers to a polypeptide that, alone or in combination with another immunoglobulin antigen-binding domain, defines an antigen-binding site. Exemplary immunoglobulin antigen-binding domains include, for example, immunoglobulin heavy chain variable region and an immunoglobulin light chain variable region, where the variable regions together define an antigen binding site.

[00149] The immunoglobulin antigen-binding domain and/or antigen binding site can be derived from an antibody selected from, for example, adecatumumab, ascrinvacumab, cixutumumab, conatumumab, daratumumab, drozitumab, duligotumab, durvalumab, dusigitumab, enfortumab, enoticumab, eprat uxumab, figitumumab, ganitumab, glembatumumab, intetumumab, ipilimumab, iratumumab, icrucumab, lexatumumab, lucatumumab, mapatumumab, narnatumab, necitumumab, nesvacumab, ofatumumab, olaratumab, panitumumab, patritumab, pritumumab, radretumab, ramucirumab, rilotumumab, robatumumab, seribantumab, tarextumab, teprotumumab, tovetumab, vantictumab, vesencumab, votumumab, zalutumumab, flanvotumab, altumomab, anatumomab, arcitumomab, bectumomab, blinatumomab, detumomab, ibritumomab, minretumomab, mitumomab, moxetumomab, naptumomab, nofetumomab, pemtumomab, pintumomab, racotumomab, satumomab, solitomab, taplitumomab, tenatumomab, tositumomab, tremelimumab, abagovomab, atezolizumab, durvalumab, avelumab, igovomab, oregovomab, capromab, edrecolomab, nacolomab, amatuximab, bavituximab, brentuximab, cetuximab, derlotuximab, dinutuximab, ensituximab, futuximab, girentuximab, indatuximab, isatuximab, margetuximab, rituximab, siltuximab, ublituximab, ecromeximab, abituzumab, alemtuzumab, bevacizumab, bivatuzumab, brontictuzumab, cantuzumab, cantuzumab, citatuzumab, clivatuzumab, dacetuzumab, demcizumab, dalotuzumab, denintuzumab, elotuzumab, emactuzumab, emibetuzumab, enoblituzumab, etaracizumab, farletuzumab, ficlatuzumab, gemtuzumab, imgatuzumab, inotuzumab, labetuzumab, lifastuzumab, lintuzumab, lirilumab,

lorvotuzumab, lumretuzumab, matuzumab, milatuzumab, moxetumomab, nimotuzumab, obinutuzumab, ocaratuzumab, otlertuzumab, onartuzumab, oportuzumab, parsatuzumab, pertuzumab, pidilizumab, pinatuzumab, polatuzumab, sibrotuzumab, simtuzumab, tacatuzumab, tigatuzumab, trastuzumab, tucotuzumab, urelumab, vandortuzumab, vanucizumab, veltuzumab, vorsetuzumab, sofituzumab, catumaxomab, ertumaxomab, depatuxizumab, ontuxizumab, blontuvetmab, tamtuvetmab, nivolumab, pembrolizumab, epratuzumab, MEDI9447, urelumab, utomilumab, hu3F8, hu14.18-IL-2, 3F8/OKT3BsAb, lirilumab, BMS-986016 pidilizumab, AMP-224, AMP-514, BMS-936559, atezolizumab, and avelumab. In certain embodiments, the immunoglobulin antigen-binding domain can be derived from an antibody selected from trastuzumab, daratumumab, girentuximab, ofatumumab, avelumab, and rituximab.

[00150] The immunoglobulin antigen-binding domain and/or antigen binding site can be derived from an antibody that binds a cancer antigen selected from, for example, adenosine A2a receptor (A2aR), A kinase anchor protein 4 (AKAP4), B melanoma antigen (BAGE), brother of the regulator of imprinted sites (BORIS), breakpoint cluster region Abelson tyrosine kinase (BCR/ABL), CA125, CAIX, CD19, CD20, CD22, CD30, CD33, CD52, CD73, CD137, carcinoembryonic antigen (CEA), a claudin (*e.g.* a claudin 18, *e.g.,* claudin 18.2), CS1, cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4), estrogen receptor binding site associated antigen 9 (EBAG9), epidermal growth factor (EGF), epidermal growth factor receptor (EGFR), EGF-like module receptor 2 (EMR2), epithelial cell adhesion molecule (EpCAM) (17-1A), FR-alpha, G antigen (GAGE), disialoganglioside GD2 (GD2), glycoprotein 100 (gp100), human epidermal growth factor receptor 2 (HER2), hepatocyte growth factor (HGF), human papillomavirus 16 (HPV-16), heat-shock protein 105 (HSP105), isocitrate dehydrogenase type 1 (IDH1), idiotype (NeuGcGM3), indoleamine-2,3-dioxygenase 1 (IDO1), IGF-1, IGF1R, IGG1K, killer cell immunoglobulin-like receptor (KIR), lymphocyte activation gene 3 (LAG-3), lymphocyte antigen 6 complex K (LY6K), Matrix-metalloproteinase-16 (MMP16), melanotransferrin (MFI2), melanoma antigen 3 (MAGE-A3), melanoma antigen C2 (MAGE-C2), melanoma antigen D4 (MAGE-D4), melanoma antigen recognized by T-cells 1 (Melan-A/MART-1), N-methyl-N'-nitroso-guanidine human osteosarcoma transforming gene (MET), mucin 1 (MUC1), mucin 4 (MUC4), mucin 16 (MUC16), New York esophageal squamous cell carcinoma 1 (NY-ESO-1), prostatic acid phosphatase (PAP), programmed cell death receptor 1 (PD-1), programmed cell death receptor ligand 1 (PD-L1), phosphatidylserine, preferentially expressed antigen of

melanoma (PRAME), prostate specific antigen (PSA), protein tyrosine kinase 7 (PTK7, also known as colon carcinoma kinase 4 (CCK4)), receptor tyrosine kinase orphan receptor 1 (ROR1), scatter factor receptor kinase, sialyl-Tn, sperm-associated antigen 9 (SPAG-9), synovial sarcoma X-chromosome breakpoint 1 (SSX1), survivin, telomerase, T-cell immunoglobulin domain and mucin domain-3 (TIM-3), vascular endothelial growth factor (VEGF) (*e.g.,* VEGF-A), vascular endothelial growth factor Receptor 2 (VEGFR2), V-domain immunoglobulin-containing suppressor of T-cell activation (VISTA),Wilms' Tumor-1 (WT1), X chromosome antigen 1b (XAGE-1b), 5T4, Mesothelin, Glypican 3 (GPC3), Folate Receptor α (FRα), Prostate Specific Membrane Antigen (PSMA), cMET, CD38, B Cell Maturation Antigen (BCMA), CD123, CLDN6, CLDN9, LRRC15, PRLR (Prolactin Receptor), RING finger protein 43 (RNF43), Uroplakin-1 B (UPK1 B), tumor necrosis factor superfamily member 9 (TNFSF9), tumor necrosis factor receptor superfamily member 21 (TNFSRF21), bone morphogenetic protein receptor type-1B (BMPR1B), Kringle domain-containing transmembrane protein 2 (KREMEN2), Delta-like protein 3 (DLL3), Siglec7 and Siglec9. Additional exemplary cancer antigens include those found on cancer stem cells, *e.g.,* SSEA3, SSEA4, TRA-1-60, TRA-1-81, SSEA1, CD133 (AC133), CD90 (Thy-1), CD326 (EpCAM), Cripto-1 (TDGF1), PODXL-1 (Podocalyxin-like protein 1), ABCG2, CD24, CD49f (Integrin α6), Notch2, CD146 (MCAM), CD10 (Neprilysin), CD117 (c-KIT), CD26 (DPP-4), CXCR4, CD34, CD271, CD13 (Alanine aminopeptidase), CD56 (NCAM), CD105 (Endoglin), LGR5, CD114 (CSF3R), CD54 (ICAM-1), CXCR1, 2, TIM-3 (HAVCR2), CD55 (DAF), DLL4 (Delta-like ligand 4), CD20 (MS4A1), and CD96.

[00151] The disclosure further provides antibody conjugates containing one or more of the fusion proteins disclosed herein. As used herein, unless otherwise indicated, the term "antibody conjugate" is understood to refer to an antibody, or a functional fragment thereof, that comprises antigen-binding activity and/or Fc receptor-binding activity, conjugated (*e.g.,* covalently coupled) to an additional functional moiety. In certain embodiments, the antibody or functional antibody fragment is conjugated to a recombinant human Neu2 enzyme disclosed herein. In certain embodiments, an antibody conjugate comprises a single polypeptide chain. In certain embodiments, an antibody conjugate comprises two, three, four, or more polypeptide chains that are covalently or non-covalently associated together to produce a multimeric complex, *e.g.*, a dimeric, trimeric, or tetrameric complex.

[00152] **TABLE 10** shows antibodies and antibody-drug conjugates suitable for use in accordance with the present disclosure, the antigen bound by the antibody or antibody-drug

conjugate, and, for certain antibodies, the type of cancer targeted by the antibody or antibody-drug conjugate.

TABLE 10

Antibody or antibody-drug conjugate	**Cancer Antigen**	**Cancer Type**
oregovomab	CA125	
girentuximab	CAIX	
obinutuzumab	CD20	
ofatumumab	CD20	
rituximab	CD20	
alemtuzumab	CD52	
Ipilimumab	cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4)	
tremelimumab	CTLA-4	
Cetuximab	epidermal growth factor receptor (EGFR)	
necitumumab	EGFR	
panitumumab	EGFR	
zalutumumab	EGFR	
edrecolomab	epithelial cell adhesion molecule (EpCAM) (17-1A)	
farletuzumab	FR-alpha	
Pertuzumab	human epidermal growth factor receptor 2 (HER2)	
trastuzumab	HER2	
rilotumumab	HGF	
figitumumab	IGF-1	
Ganitumab	IGF1R	
durvalumab	IGG1K	
bavituximab	Phosphatidylserine	
onartuzumab	scatter factor receptor kinase	
bevacizumab	vascular endothelial growth factor-A (VEGF-A)	
ramucirumab	vascular endothelial growth factor Receptor 2 (VEGFR2)	
blinatumomab	CD19	acute lymphoblastic leukemia (ALL)
Rituximab; ofatumumab; ibritumomab (*e.g.*, ^{90}Y-ibritumomab; tositumomab (*e.g.*, ^{131}I-tositumomab	CD20	non-Hodgkin's lymphoma (NHL), chronic lymphocytic leukemia (CLL) B-cell NHL pre-B ALL
brentuximab (*e.g.*, brentuximab vedotin	CD30	Hodgkin's lymphoma

Antibody or antibody-drug conjugate	**Cancer Antigen**	**Cancer Type**
gemtuzumab (*e.g.*, gemtuzumab ozogamicin	CD33	acute myelogenous leukemia (AML)
Alemtuzumab	CD52	CLL
Ipilimumab	cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4)	Unresectable or metastatic melanoma
cetuximab; panitumumab	epidermal growth factor receptor (EGFR)	colorectal cancer (CRC) Head and Neck
Catumaxomab	epithelial cell adhesion molecule (EpCAM)	Malignant ascites
trastuzumab; pertuzumab	human epidermal growth factor receptor 2 (HER2)	Breast
nivolumab; pembrolizumab	programmed cell death receptor 1 (PD-1)	Metastatic melanoma, non-small cell lung cancer (NSCLC)
Bevacizumab	vascular endothelial growth factor (VEGF)	Breast, Cervical CRC, NSCLC renal cell carcinoma (RCC), Ovarian Glioblastoma
Ramucirumab	vascular endothelial growth factor receptor 2 (VEGF-R2)	Gastric NSCLC
Epratuzumab; moxetumomab; inotuzumab (*e.g.*, inotuzumab ozogamicin)	CD22	acute lymphoblastic leukemia (ALL)
MEDI9447	CD73	Advanced solid tumors
Urelumab; utomilumab (PF-05082566)	CD137	Advanced solid tumors
Elotuzumab	CD2 subset 1 (CS1)	Multiple myeloma
Tremelimumab	cytotoxic T-lymphocyte-associated antigen 4 (CTLA-4)	Malignant mesothelioma
Necitumumab	epidermal growth factor receptor (EGFR)	non-small cell lung cancer (NSCLC)
dinutuximab; hu3F8; hu14.18-IL-2; 3F8/OKT3BsAb	disialoganglioside GD2 (GD2)	Neuroblastoma Retinoblastoma Melanoma other solid tumors
Racotumomab	Idiotype (NeuGcGM3)	NSCLC, Breast Melanoma
Lirilumab	killer cell immunoglobulin-like receptor (KIR)	Lymphoma
BMS-986016	lymphocyte activation gene 3 (LAG-3)	Breast, Hematological, Advanced solid tumors
Onartuzumab	N-methyl-N'-nitroso-guanidine human osteosarcoma transforming gene (MET)	NSCLC

Antibody or antibody-drug conjugate	Cancer Antigen	Cancer Type
abagovomab; oregovomab	mucin 16 (MUC16)	Ovarian
pidilizumab; AMP-224; AMP-514	programmed cell death receptor 1 (PD-1)	B-cell lymphoma Melanoma, CRC
BMS-936559; atezolizumab; durvalumab; avelumab	programmed cell death receptor ligand 1 (PD-L1)	NSCLC, renal cell carcinoma (RCC) Bladder, Breast Melanoma, squamous cell carcinoma of the head and neck (SCCHN)
naptumomab (*e.g.*, naptumomab estafenatox)	5T4	RCC, CRC Prostate

c. Linker

[00153] In certain embodiments, the sialidase portion of the fusion protein can be linked or fused directly to the antibody portion (*e.g.,* immunoglobulin Fc domain and/or immunoglobulin antigen-binding domain) of the fusion protein. In other embodiments, the sialidase portion can be covalently bound to the antibody portion by a linker.

[00154] The linker may couple, with one or more natural amino acids, the sialidase, or functional fragment thereof, and the antibody portions or fragments, where the amino acid (for example, a cysteine amino acid) may be introduced by site-directed mutagenesis. The linker may include one or more unnatural amino acids. It is contemplated that, in certain circumstances, a linker containing, for example, one or more sulfhydryl reactive groups (*e.g.*, a maleimide) may covalently link a cysteine in the sialidase portion or the antibody portion that is a naturally occurring cysteine residue or is the product of site-specific mutagenesis.

[00155] The linker may be a cleavable linker or a non-cleavable linker. Optionally or in addition, the linker may be a flexible linker or an inflexible linker.

[00156] The linker should be a length sufficiently long to allow the sialidase and the antibody portions to be linked without steric hindrance from one another and sufficiently short to retain the intended activity of the fusion protein. The linker preferably is sufficiently hydrophilic to avoid or minimize instability of the fusion protein. The linker preferably is sufficiently hydrophilic to avoid or minimize insolubility of the fusion protein. The linker should be sufficiently stable *in vivo* (*e.g.*, it is not cleaved by serum, enzymes, *etc.*) to permit the fusion protein to be operative *in vivo*.

[00157] The linker may be from about 1 angstroms (Å) to about 150 Å in length, or from about 1 Å to about 120 Å in length, or from about 5 Å to about 110 Å in length, or from about 10 Å to about 100 Å in length. The linker may be greater than about 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 16, 17, 18, 19, 20, 25, 27, 30 or greater angstroms in length and/or less than about 110, 100, 90, 85, 80, 75, 70, 65, 60, 55, 50, 45, 43, 42, 41, 40, 39, 38, 37, 36, 35, 34, 33, 32, 31, or fewer Å in length. Furthermore, the linker may be about 5, 10, 15, 20, 25, 30, 35, 40, 45, 50, 55, 60, 65, 70, 75, 80, 85, 90, 95, 100, 110, or 120 Å in length.

[00158] In certain embodiments, the linker comprises a polypeptide linker that connects or fuses the sialidase portion of the fusion protein to the antibody portion (*e.g.*, immunoglobulin Fc domain and/or immunoglobulin antigen-binding domain) of the fusion protein. For example, it is contemplated that a gene encoding a sialidase portion linked directly or indirectly (for example, via an amino acid containing linker) to an antibody portion can be created and expressed using conventional recombinant DNA technologies. For example, the amino terminus of a sialidase portion can be linked to the carboxy terminus of either the light or the heavy chain of an antibody portion. For example, for a Fab fragment, the amino terminus or carboxy terminus of the sialidase can be linked to the first constant domain of the heavy antibody chain (CH1). When a linker is employed, the linker may comprise hydrophilic amino acid residues, such as Gln, Ser, Gly, Glu, Pro, His, and Arg. In certain embodiments, the linker is a peptide containing 1-25 amino acid residues, 1-20 amino acid residues, 2-15 amino acid residues, 3-10 amino acid residues, 3-7 amino acid residues, 4-25 amino acid residues, 4-20 amino acid residues, 4-15 amino acid residues, 4-10 amino acid residues, 5-25 amino acid residues, 5-20 amino acid residues, 5-15 amino acid residues, or 5-10 amino acid residues. Exemplary linkers include glycine and serine-rich linkers, *e.g.*, $(GlyGlyPro)_n$, or $(GlyGlyGlyGlySer)_n$ (SEQ ID NO: 32), where n is 1-5. In certain embodiments, the linker comprises, consists, or consists essentially of GGGGS (SEQ ID NO: 31). In certain embodiments, the linker comprises, consists, or consists essentially of GGGGSGGGGS (SEQ ID NO: 33). In certain embodiments, the linker comprises, consists, or consists essentially of EPKSS (SEQ ID NO: 34). Additional exemplary linker sequences are disclosed, *e.g.*, in George *et al.* (2003) PROTEIN ENGINEERING 15:871–879, and U.S. Patent Nos. 5,482,858 and 5,525,491.

d. Antibody Conjugates

[00159] The disclosure further provides antibody conjugates comprising a fusion protein disclosed herein. The antibody conjugate may comprise a single polypeptide chain (*i.e.*, a

fusion protein disclosed herein), or the antibody conjugate may comprise additional polypeptide chains (*e.g.*, one, two, or three additional polypeptide chains). For example, an antibody conjugate may comprise a first polypeptide (fusion protein) comprising a recombinant mutant human sialidase enzyme and an immunoglobulin heavy chain, and a second polypeptide comprising an immunoglobulin light chain, where, for example, the immunoglobulin heavy and light chains together define a single antigen-binding site.

[00160] In certain embodiments, the antibody conjugate can include a single sialidase. In other embodiments, the antibody conjugate can include more than one (*e.g.*, two) sialidases. If more than one sialidase is included, the sialidases can be the same or different. In certain embodiments, the antibody conjugate can include a single antigen-binding site. In other embodiments, the antibody conjugate can include more than one (*e.g.*, two) antigen-binding sites. If two antigen-binding sites are used, they can be the same or different. In certain embodiments, the antibody conjugate comprises an immunoglobulin Fc fragment.

[00161] In certain embodiments, the antibody conjugate comprises one or two immunoglobulin heavy chains, or a functional fragment thereof. In certain embodiments, the antibody conjugate comprises one or two immunoglobulin light chains, or a functional fragment thereof. In certain embodiments, the antibody conjugate comprises a sialidase fused to the N- or C-terminus of an immunoglobulin heavy chain or an immunoglobulin light chain.

[00162] **FIGURES 6A-6I** depicts exemplary antibody conjugate constructs containing one or more sialidase enzymes. For example, in **FIGURE 6A**, a first antigen-binding site is depicted as 10, a second antigen-binding site is depicted as 20, a sialidase is depicted as 30, and a Fab is depicted as 40. In each of the constructs depicted in **FIGURES 6A-6I,** it is understood that the Fc may optionally be modified in some manner, *e.g.*, using a heterodimerization technology such as Knobs-into-Holes, *e.g.*, as depicted by 50 in **FIGURE 6B**. Throughout **FIGURES 6A-6I,** similar structures are depicted by similar schematic representations.

[00163] **FIGURE 6A** depicts antibody conjugate constructs comprising a first polypeptide comprising a first immunoglobulin light chain; a second polypeptide comprising a first immunoglobulin heavy chain; a third polypeptide comprising a second immunoglobulin heavy chain; and a fourth polypeptide comprising a second immunoglobulin light chain. The first and second polypeptides can be covalently linked together, the third and fourth polypeptides can be covalently linked together, and the second and third polypeptides can be

covalently linked together. The covalent linkages can be disulfide bonds. In certain embodiments, the first polypeptide and the second polypeptide together define a first antigen-binding site as depicted as 10, and the third polypeptide and the fourth polypeptide together define a second antigen-binding site as depicted as 20. A sialidase enzyme as depicted as 30 can be conjugated to the N- or C-terminus of the first and second immunoglobulin light chain or the first and second immunoglobulin heavy chain.

[00164] **FIGURE 6B** depicts antibody conjugate constructs comprising a first polypeptide comprising a first immunoglobulin light chain; a second polypeptide comprising a first immunoglobulin heavy chain; a third polypeptide comprising a second immunoglobulin heavy chain; and a fourth polypeptide comprising a second immunoglobulin light chain. The first and second polypeptides can be covalently linked together, the third and fourth polypeptides can be covalently linked together, and the second and third polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. In certain embodiments, the first polypeptide and the second polypeptide together define a first antigen-binding site, and the third polypeptide and the fourth polypeptide together define a second antigen-binding site. A sialidase enzyme can be conjugated to the N- or C-terminus of the first immunoglobulin light chain or the first immunoglobulin heavy chain.

[00165] **FIGURE 6C** depicts antibody conjugate constructs comprising a first polypeptide comprising an immunoglobulin light chain; a second polypeptide comprising an immunoglobulin heavy chain; and a third polypeptide comprising an immunoglobulin Fc domain. The first and second polypeptides can be covalently linked together and the second and third polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. In certain embodiments, the first polypeptide and the second polypeptide together define an antigen-binding site. A sialidase enzyme can be conjugated to the N- or C-terminus of the first immunoglobulin light chain or the first immunoglobulin heavy chain.

[00166] **FIGURE 6D** depicts antibody conjugate constructs comprising a first polypeptide comprising an immunoglobulin light chain; a second polypeptide comprising an immunoglobulin heavy chain; and a third polypeptide comprising an immunoglobulin Fc domain and a first sialidase enzyme. The first and second polypeptides can be covalently linked together and the second and third polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. The third polypeptide comprises the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation. In certain embodiments, the first polypeptide and the second polypeptide together define an antigen-binding site. An

optional second sialidase enzyme can be conjugated to the N- or C-terminus of the first immunoglobulin light chain or the first immunoglobulin heavy chain.

[00167] **FIGURE 6E** depicts antibody conjugate constructs comprising a first polypeptide comprising an immunoglobulin light chain; a second polypeptide comprising an immunoglobulin heavy chain; and a third polypeptide comprising an immunoglobulin Fc domain and a first sialidase enzyme. The first and second polypeptides can be covalently linked together and the second and third polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. The third polypeptide comprises the immunoglobulin Fc domain and the sialidase in an N- to C-terminal orientation. In certain embodiments, the first polypeptide and the second polypeptide together define an antigen-binding site. An optional second sialidase enzyme can be conjugated to the N- or C-terminus of the first immunoglobulin light chain or the first immunoglobulin heavy chain.

[00168] **FIGURE 6F** depicts antibody conjugate constructs comprising a first polypeptide comprising a first immunoglobulin Fc domain, and a second polypeptide comprising a second immunoglobulin Fc domain. The first and second polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. A sialidase enzyme can be conjugated to the N- or C-terminus of the first immunoglobulin Fc domain or to the N- or C-terminus of the second immunoglobulin Fc domain. An optional second sialidase enzyme can be conjugated to the N- or C-terminus of the first immunoglobulin Fc domain or to the N- or C-terminus of the second immunoglobulin Fc domain.

[00169] **FIGURE 6G** depicts antibody conjugate constructs comprising a first polypeptide comprising an immunoglobulin light chain; and a second polypeptide comprising an immunoglobulin heavy chain variable region. The first and second polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. In certain embodiments, the first polypeptide and the second polypeptide together define an antigen-binding site. The sialidase enzyme can be conjugated to the N- or C-terminus of the immunoglobulin light chain or the immunoglobulin heavy chain variable region.

[00170] **FIGURE 6H** depicts antibody conjugate constructs comprising a first polypeptide comprising a first immunoglobulin Fc domain, and a second polypeptide comprising a second immunoglobulin Fc domain. The first and second polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. A sialidase enzyme can be conjugated to the N-terminus of the first immunoglobulin Fc domain or the second

immunoglobulin Fc domain. An optional second sialidase enzyme can be conjugated to the N-terminus of the second immunoglobulin Fc domain or the first immunoglobulin Fc domain, respectively. A single chain variable fragment (scFv) can be conjugated to the C-terminus of the first immunoglobulin Fc domain or the second immunoglobulin Fc domain. An optional second single chain variable fragment (scFv) can be conjugated to the C-terminus of the first immunoglobulin Fc domain or the second immunoglobulin Fc domain, respectively.

[00171] **FIGURE 6I** depicts antibody conjugate constructs similar to those depicted in **FIGURE 6H** except that each scFv is replaced with an immunoglobulin antigen binding fragment, *e.g.*, a Fab. For example, **FIGURE 6I** depicts antibody conjugate constructs comprising a first polypeptide comprising a first immunoglobulin Fc domain, and a second polypeptide comprising a second immunoglobulin Fc domain. The first and second polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. A sialidase enzyme can be conjugated to the N-terminus of the first immunoglobulin Fc domain or the second immunoglobulin Fc domain. An optional second sialidase enzyme can be conjugated to the N-terminus of the second immunoglobulin Fc domain or the first immunoglobulin Fc domain, respectively. An antibody fragment (Fab) can be conjugated or fused to the C-terminus of the first immunoglobulin Fc domain or the second immunoglobulin Fc domain. An optional second antibody fragment (Fab) can be conjugated or fused to the C-terminus of the second immunoglobulin Fc domain or the first immunoglobulin Fc domain, respectively. In the case of a fusion, the C terminus of the Fc domain is linked (either by a bond or an amino acid linker) to a first polypeptide chain defining an immunoglobulin antigen binding fragment. In the case of antibodies that have an antigen binding site defined by a single variable region, then this may be sufficient to impart binding affinity to a target antigen. In other instances, *e.g.*, in the case of a human antibody, the first polypeptide chain defining an immunoglobulin antigen binding fragment can be conjugated (*e.g.*, covalently conjugated, *e.g.*, via a disulfide bond) to a second polypeptide chain defining an immunoglobulin antigen binding fragment, there the two antigen binding fragments together define an antigen binding site for binding the target antigen.

[00172] **FIGURE 7** depicts additional antibody conjugate constructs. For example, **FIGURE 7** depicts an antibody conjugate construct comprising a first polypeptide comprising an immunoglobulin light chain; a second polypeptide comprising an immunoglobulin heavy chain and an scFv; and a third polypeptide comprising an

immunoglobulin Fc domain and a first sialidase enzyme. The first and second polypeptides can be covalently linked together and the second and third polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. The second polypeptide comprises the heavy chain and the scFv in an N- to C-terminal orientation. The third polypeptide comprises the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation. In certain embodiments, the first polypeptide and the second polypeptide together define a first antigen-binding site. In certain embodiments, the scFv defines a second antigen-binding site. **FIGURE 7** depicts an additional antibody construct comprising a first polypeptide comprising an immunoglobulin light chain; a second polypeptide comprising an immunoglobulin heavy chain; and a third polypeptide comprising an immunoglobulin Fc domain and a first sialidase enzyme, wherein a Fab fragment is conjugated to the N-terminus of the immunoglobulin heavy chain. The first and second polypeptides can be covalently linked together and the second and third polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. The third polypeptide comprises the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation. In certain embodiments, the first polypeptide and the second polypeptide together define a first antigen-binding site. In certain embodiments, the Fab fragment defines a second antigen-binding site. In each of the constructs depicted in **FIGURE 7** it is understood that an scFv, when present, may be replaced with a Fab fragment, or a Fab fragment, when present, may be replaced with an scFv. In each of the constructs depicted in **FIGURE 7**, it is understood that the Fc may optionally be modified in some manner.

[00173] In certain embodiments, the antibody conjugate comprises a first polypeptide comprising a first immunoglobulin light chain; a second polypeptide comprising a first immunoglobulin heavy chain and a first sialidase; a third polypeptide comprising a second immunoglobulin heavy chain and a second sialidase; and a fourth polypeptide comprising a second immunoglobulin light chain. An example of this embodiment is shown in **FIGURE 8A**. The first and second polypeptides can be covalently linked together, the third and fourth polypeptides can be covalently linked together, and the second and third polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. In certain embodiments, the first polypeptide and the second polypeptide together define a first antigen-binding site, and the third polypeptide and the fourth polypeptide together define a second antigen-binding site. In certain embodiments, the second and third polypeptides comprise the

first and second immunoglobulin heavy chain and the first and second sialidase, respectively, in an N- to C-terminal orientation. In certain embodiments, the second and third polypeptides comprise the first and second sialidase and the first and second immunoglobulin heavy chain, respectively, in an N- to C-terminal orientation.

[00174] In certain embodiments, the antibody conjugate comprises a first polypeptide comprising an immunoglobulin light chain; a second polypeptide comprising an immunoglobulin heavy chain; and a third polypeptide comprising an immunoglobulin Fc domain and a sialidase. An example of this embodiment is shown in **FIGURE 8B**. The first and second polypeptides can be covalently linked together and the second and third polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. In certain embodiments, the first polypeptide and the second polypeptide together define an antigen-binding site. In certain embodiments, the third polypeptide comprises the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation, or the immunoglobulin Fc domain and the sialidase in an N- to C-terminal orientation.

[00175] In certain embodiments, the antibody conjugate comprises a first polypeptide comprising a first sialidase, a first immunoglobulin Fc domain, and a first single chain variable fragment (scFv) (it is also understood that the scFv may be replaced by a first polypeptide chain of an immunoglobulin antigen binding fragment, *e.g.*, Fab fragment); and a second polypeptide comprising a second sialidase, a second immunoglobulin Fc domain, and a second single chain variable fragment (scFv) (it is also understood that the scFv may be replaced by a second polypeptide chain of an immunoglobulin antigen binding fragment, *e.g.*, Fab fragment). An example of this embodiment is shown in **FIGURE 8C**. The first and second polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. In certain embodiments, the first scFv defines a first antigen-binding site, and the second scFv defines a second antigen-binding site. In certain embodiments, the first polypeptide comprises the first sialidase, the first immunoglobulin Fc domain, and the first scFv in an N- to C-terminal orientation. In certain embodiments, the first polypeptide comprises the first scFv, the first immunoglobulin Fc domain, and the first sialidase in an N- to C-terminal orientation. In certain embodiments, the second polypeptide comprises the second sialidase, the second immunoglobulin Fc domain, and the second scFv in an N- to C-terminal orientation. In certain embodiments, the second polypeptide comprises the second scFv, the second immunoglobulin Fc domain, and the second sialidase in an N- to C-terminal orientation.

[00176] In certain embodiments, the antibody conjugate comprises: a first polypeptide comprising an immunoglobulin light chain; a second polypeptide comprising an immunoglobulin heavy chain and a single chain variable fragment (scFv) (it is also understood that the scFv may be replaced by a first polypeptide chain of an immunoglobulin antigen binding fragment, *e.g.*, Fab fragment); and a third polypeptide comprising an immunoglobulin Fc domain and a sialidase. An example of this embodiment is shown in **FIGURE 8D**. The first and second polypeptides can be covalently linked together and the second and third polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. In certain embodiments, the first polypeptide and the second polypeptide together define a first antigen-binding site (*i.e.*, the immunoglobulin light chain and immunoglobulin heavy chain together define a first antigen-binding site). In certain embodiments, the scFv defines a second antigen-binding site. In certain embodiments, the second polypeptide comprises the immunoglobulin heavy chain and the scFv in an N- to C-terminal orientation, or the scFv and the immunoglobulin heavy chain in an N- to C-terminal orientation. In certain embodiments, the third polypeptide comprises the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation, or the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation.

[00177] In certain embodiments, the antibody conjugate comprises a first polypeptide comprising a first immunoglobulin light chain; a second polypeptide comprising a first sialidase, a first immunoglobulin Fc domain, and a first immunoglobulin heavy chain variable region; a third polypeptide comprising a second sialidase, a second immunoglobulin Fc domain, and a second immunoglobulin heavy chain variable region; and a fourth polypeptide comprising a second immunoglobulin light chain. It is also understood that an immunoglobulin light chain may be replaced by an immunoglobulin heavy chain variable region and an immunoglobulin heavy chain variable region may be replaced by an immunoglobulin light chain (*e.g.*, the antibody conjugate may comprise a first polypeptide comprising a first immunoglobulin heavy chain variable region; a second polypeptide comprising a first sialidase, a first immunoglobulin Fc domain, and a first immunoglobulin light chain; a third polypeptide comprising a second sialidase, a second immunoglobulin Fc domain, and a second immunoglobulin light chain; and a fourth polypeptide comprising a second immunoglobulin heavy chain variable region). An example of this embodiment is shown in **FIGURE 8E**. The second and third polypeptides can be covalently linked together. The covalent linkages can be disulfide bonds. In certain embodiments, the first and second

polypeptides define a first antigen-binding site, and the third and fourth polypeptides define a second antigen-binding site. In certain embodiments, the second polypeptide comprises the first sialidase, the first immunoglobulin Fc domain, and the first immunoglobulin heavy chain variable region in an N- to C-terminal orientation. In certain embodiments, the third polypeptide comprises the second sialidase, the second immunoglobulin Fc domain, and the second immunoglobulin heavy chain variable region in an N- to C-terminal orientation.

[00178] In certain embodiments, the antibody conjugate has a molecular weight from about 135 kDa to about 165 kDa, *e.g.,* about 140 kDa. In other embodiments, the antibody conjugate has a molecular weight from about 215 kDa to about 245 kDa, *e.g.,* about 230 kDa.

[00179] In certain embodiments, the antibody conjugate comprises two polypeptides that each comprise an immunoglobulin Fc domain comprising at least one heterodimerization mutation as described herein. In certain embodiments, the antibody conjugate comprises two polypeptides that each comprise an immunoglobulin Fc domain, and the first polypeptide has a heterodimerization mutation such as a "knob" mutation, *e.g.,* T366Y, or a "hole" mutation, *e.g.,* Y407T, for heterodimerization with the second polypeptide, and the second polypeptide has either a respective "knob" mutation, *e.g.,* T366Y, or a "hole" mutation, *e.g.,* Y407T, for heterodimerization with the first polypeptide (residue numbers according to EU numbering, Kabat, E.A., *et al.* (1991) *supra*). For example, in certain embodiments, the antibody comprises two polypeptides that each comprise an immunoglobulin Fc domain derived from human IgG1 Fc domain, and the first polypeptide comprises a Y407T mutation (*e.g.,* the first polypeptide comprises any of SEQ ID NOs: 22-25), and the second polypeptide comprises a T366Y mutation (*e.g.,* the second polypeptide comprises any of SEQ ID NOs: 26-29).

[00180] As used herein, the term "multispecific antibody" is understood to mean an antibody that specifically binds to at least two different antigens, *i.e.,* an antibody that comprises at least two antigen-binding sites that bind to at least two different antigens. As used herein, the term "bispecific antibody" is understood to mean an antibody that specifically binds to two different antigens, *i.e.,* an antibody that comprises two antigen-binding sites each of which bind to separate and distinct antigens. In other words, a first binding site binds a first antigen and a second binding site binds a second, different antigen. A multispecific or bispecific antibody may, for example, be a human or humanized antibody, and/or be a full length antibody or an antibody fragment (*e.g.*, a $F(ab')_2$ bispecific antibody).

[00181] The present disclosure encompasses antibody conjugates comprising antibody fragments, which may be generated by traditional means, such as enzymatic digestion, or by recombinant techniques. For a review of certain antibody fragments, see Hudson *et al.* (2003) *supra.*

[00182] In certain embodiments, the antibody conjugate or fusion protein can be covalently or non-covalently associated with a biological modifier, wherein the biological modifier can be used to enhance the solubility of the antibody, increase binding specificity, decrease immunogenicity or toxicity or modify the pharmacokinetic profile of the antibody. For example, the biological modifier can be used to increase the molecular weight of the antibody to increase its circulating half-life.

[00183] It is contemplated that the antibody conjugate or fusion protein may be covalently bound to one or more (for example, 2, 3, 4, 5, 6, 8, 9, 10 or more) biological modifiers that may comprise linear or branched polymers. Exemplary biological modifiers may include, for example, a variety of polymers, such as those described in U.S. Patent No. 7,842,789. Particularly useful are polyalkylene ethers such as polyethylene glycol (PEG) and derivatives thereof (for example, alkoxy polyethylene glycol, for example, methoxypolyethylene glycol, ethoxypolyethylene glycol and the like); block copolymers of polyoxyethylene and polyoxypropylene (Pluronics); polymethacrylates; carbomers; and branched or unbranched polysaccharides which comprise the saccharide monomers such as D-mannose, D- and L-galactose, fucose, fructose, D-xylose, L-arabinose, and D-glucuronic acid.

[00184] In other embodiments, the biological modifier can be a hydrophilic polyvinyl polymer such as polyvinyl alcohol and polyvinylpyrrolidone (PVP)-type polymers. The biological modifier can be a functionalized polyvinylpyrrolidone, for example, carboxy or amine functionalized on one (or both) ends of the polymer (as available from PolymerSource). Alternatively, the biological modifier can include Poly N-(2-hydroxypropyl)methacrylamide (HPMA), or functionalized HPMA (amine, carboxy, *etc.*), Poly(N-isopropylacrylamide) or functionalized poly(N-isopropylacrylamide). Alternatively, the biological modifier can include Poly N-(2-hydroxypropyl)methacrylamide (HPMA), or functionalized HPMA (amine, carboxy, *etc.*), Poly(N-isopropylacrylamide) or functionalized poly(N-isopropylacrylamide). The modifier prior to conjugation need not be, but preferably is, water soluble, but the final conjugate should be water soluble.

[00185] In general, the biological modifier may have a molecular weight from about 2 kDa to about 5 kDa, from about 2 kDa to about 10 kDa, from about 2 kDa to about 20 kDa, from about 2 kDa to about 30 kDa, from about 2 kDa to about 40 kDa, from about 2 kDa to about 50 kDa, from about 2 kDa to about 60 kDa, from about 2 kDa to about 70 kDa, from about 2 kDa to about 80 kDa, from about 2 kDa to about 90 kDa, from about 2 kDa to about 100 kDa, from about 2 kDa to about 150 kDa, from about 5 kDa to about 10 kDa, from about 5 kDa to about 20 kDa, from about 5 kDa to about 30 kDa, from about 5 kDa to about 40 kDa, from about 5 kDa to about 50 kDa, from about 5 kDa to about 60 kDa, from about 5 kDa to about 70 kDa, from about 5 kDa to about 80 kDa, from about 5 kDa to about 90 kDa, from about 5 kDa to about 100 kDa, from about 5 kDa to about 150 kDa, from about 10 kDa to about 20 kDa, from about 10 kDa to about 30 kDa, from about 10 kDa to about 40 kDa, from about 10 kDa to about 50 kDa, from about 10 kDa to about 60 kDa, from about 10 kDa to about 70 kDa, from about 10 kDa to about 80 kDa, from about 10 kDa to about 90 kDa, from about 10 kDa to about 100 kDa, from about 10 kDa to about 150 kDa, from about 20 kDa to about 30 kDa, from about 20 kDa to about 40 kDa, from about 20 kDa to about 50 kDa, from about 20 kDa to about 60 kDa, from about 20 kDa to about 70 kDa, from about 20 kDa to about 80 kDa, from about 20 kDa to about 90 kDa, from about 20 kDa to about 100 kDa, from about 20 kDa to about 150 kDa, from about 30 kDa to about 40 kDa, from about 30 kDa to about 50 kDa, from about 30 kDa to about 60 kDa, from about 30 kDa to about 70 kDa, from about 30 kDa to about 80 kDa, from about 30 kDa to about 90 kDa, from about 30 kDa to about 100 kDa, from about 30 kDa to about 150 kDa, from about 40 kDa to about 50 kDa, from about 40 kDa to about 60 kDa, from about 40 kDa to about 70 kDa, from about 40 kDa to about 80 kDa, from about 40 kDa to about 90 kDa, from about 40 kDa to about 100 kDa, from about 40 kDa to about 150 kDa, from about 50 kDa to about 60 kDa, from about 50 kDa to about 70 kDa, from about 50 kDa to about 80 kDa, from about 50 kDa to about 90 kDa, from about 50 kDa to about 100 kDa, from about 50 kDa to about 150 kDa, from about 60 kDa to about 70 kDa, from about 60 kDa to about 80 kDa, from about 60 kDa to about 90 kDa, from about 60 kDa to about 100 kDa, from about 60 kDa to about 150 kDa, from about 70 kDa to about 80 kDa, from about 70 kDa to about 90 kDa, from about 70 kDa to about 100 kDa, from about 70 kDa to about 150 kDa, from about 80 kDa to about 90 kDa, from about 80 kDa to about 100 kDa, from about 80 kDa to about 150 kDa, from about 90 kDa to about 100 kDa, from about 90 kDa to about 150 kDa, or from about 100 kDa to about 150 kDa.

[00186] It is contemplated that the antibody conjugate or fusion protein is attached to about 10 or fewer polymer molecules (*e.g.*, 9, 8, 7, 6, 5, 4, 3, 2, or 1), each polymer molecule having a molecular weight of at least about 20,000 D, or at least about 30,000 D, or at least about 40,000 D.

[00187] Although a variety of polymers can be used as biological modifiers, it is contemplated that the antibody conjugates or fusion proteins described herein may be attached to polyethylene glycol (PEG) polymers. In one embodiment, the antibody conjugate or fusion protein described herein is covalently attached to at least one PEG having an actual MW of at least about 20,000 D. In another embodiment, the antibody conjugate or fusion protein described herein is covalently attached to at least one PEG having an actual MW of at least about 30,000 D. In another embodiment, the antibody conjugate or fusion protein described herein is covalently attached to at least one PEG having an actual MW of at least about 40,000 D. In certain embodiments, the PEG is methoxyPEG(5000)-succinimidylpropionate (mPEG-SPA), methoxyPEG(5000)-succinimidylsuccinate (mPEG-SS). Such PEGS are commercially available from Nektar Therapeutics or SunBiowest.

[00188] Attachment sites on an antibody conjugate or fusion protein for a biological modifier include the N-terminal amino group and epsilon amino groups found on lysine residues, as well as other amino, imino, carboxyl, sulfhydryl, hydroxyl or other hydrophilic groups. The polymer may be covalently bonded directly to the antibody conjugate or fusion protein with or without the known use of a multifunctional (ordinarily bifunctional) crosslinking agent using chemistries and used in the art. For example, sulfhydryl groups can be derivatized by coupling to maleimido-substituted PEG (*e.g.*, alkoxy-PEG amine plus sulfosuccinimidyl 4-(N-maleimidomethyl)cyclohexane-1-carboxylate), or PEG-maleimide commercially available from Shearwater Polymers, Inc., Huntsville, Ala.).

III. Methods of Making a Recombinant Sialidase, Fusion Protein, or Antibody Conjugate

[00189] Methods for producing recombinant human Neu2 enzyme, fusion proteins, *e.g.*, those disclosed herein, antibodies, or antibody conjugates, *e.g.*, those disclosed herein, are known in the art. For example, DNA molecules encoding light chain variable regions and/or heavy chain variable regions can be synthesized chemically or by recombinant DNA methodologies. For example, the sequences of the antibodies can be cloned from hybridomas by conventional hybridization techniques or polymerase chain reaction (PCR) techniques,

using the appropriate synthetic nucleic acid primers. The resulting DNA molecules encoding the variable regions of interest can be ligated to other appropriate nucleotide sequences, including, for example, constant region coding sequences, and expression control sequences, to produce conventional gene expression constructs (*i.e.*, expression vectors) encoding the desired antibodies. Production of defined gene constructs is within routine skill in the art.

[00190] Nucleic acids encoding desired recombinant human sialidases, fusion proteins, and/or antibody conjugates can be incorporated (*e.g.*, ligated) into expression vectors, which can be introduced into host cells through conventional transfection or transformation techniques. Exemplary host cells are *E. coli* cells, Chinese hamster ovary (CHO) cells, human embryonic kidney 293 (HEK293) cells, HeLa cells, baby hamster kidney (BHK) cells, monkey kidney cells (COS), human hepatocellular carcinoma cells (*e.g.*, Hep G2), and myeloma cells that do not otherwise produce IgG protein. In certain embodiments, the nucleic acid is introduced into CHO cells. In other embodiments, the nucleic acid is introduced into HEK293 cells. Transformed host cells can be grown under conditions that permit the host cells to express the genes that encode the immunoglobulin light and/or heavy chain variable regions.

[00191] Specific expression and purification conditions will vary depending upon the expression system employed. For example, if a gene is to be expressed in *E. coli*, it is first cloned into an expression vector by positioning the engineered gene downstream from a suitable bacterial promoter, *e.g.*, Trp or Tac, and a prokaryotic signal sequence. The expressed protein may be secreted. The expressed protein may accumulate in refractile or inclusion bodies, which can be harvested after disruption of the cells by French press or sonication. The refractile bodies then are solubilized, and the protein may be refolded and/or cleaved by methods known in the art.

[00192] If the engineered gene is to be expressed in eukaryotic host cells, *e.g.*, CHO cells or HEK293 cells, it is first inserted into an expression vector containing a suitable eukaryotic promoter, a secretion signal, a poly A sequence, and a stop codon. Optionally, the vector or gene construct may contain enhancers and introns. In embodiments involving fusion proteins comprising an antibody or portion thereof, the expression vector optionally contains sequences encoding all or part of a constant region, enabling an entire, or a part of, a heavy or light chain to be expressed. The gene construct can be introduced into eukaryotic host cells using conventional techniques.

[00193] The host cells express a recombinant human sialidase or a fusion protein and/or antibody conjugate comprising a sialidase and V_L or V_H fragments, V_L-V_H heterodimers, V_H-V_L or V_L-V_H single chain polypeptides, complete heavy or light immunoglobulin chains, or portions thereof, each of which may be attached to a moiety having another function (*e.g.*, cytotoxicity). In some embodiments involving fusion proteins and/or antibody conjugates, a host cell is transfected with a single vector expressing a polypeptide expressing a sialidase and an entire, or part of, a heavy chain (*e.g.*, a heavy chain variable region) or a sialidase and a light chain (*e.g.*, a light chain variable region), or a polypeptide expressing an entire, or part of, a heavy chain (*e.g.*, a heavy chain variable region) or a light chain (*e.g.*, a light chain variable region). In some embodiments, a host cell is transfected with a single vector encoding (a) a polypeptide comprising a heavy chain variable region and a polypeptide comprising a light chain variable region, or (b) an entire immunoglobulin heavy chain and an entire immunoglobulin light chain, wherein in (a) or in (b), the polypeptide may also comprise a sialidase. In some embodiments, a host cell is co-transfected with more than one expression vector (*e.g.*, one expression vector expressing a polypeptide comprising an entire, or part of, a heavy chain or heavy chain variable region, optionally comprising a sialidase fused thereto, and another expression vector expressing a polypeptide comprising an entire, or part of, a light chain or light chain variable region, optionally comprising a sialidase fused thereto).

[00194] A polypeptide comprising a sialidase or a fusion protein, *e.g.*, a fusion protein comprising an immunoglobulin heavy chain variable region or light chain variable region, can be produced by growing (culturing) a host cell transfected with an expression vector encoding such a variable region, under conditions that permit expression of the polypeptide. Following expression, the polypeptide can be harvested and purified or isolated using techniques known in the art, *e.g.*, affinity tags such as glutathione-S-transferase (GST) or histidine tags.

[00195] In embodiments in which a fusion protein and/or antibody conjugate is produced, a sialidase fused to a monoclonal antibody, Fc domain, or an antigen-binding domain of the antibody, can be produced by growing (culturing) a host cell transfected with: (a) an expression vector that encodes a complete or partial immunoglobulin heavy chain, and a separate expression vector that encodes a complete or partial immunoglobulin light chain; or (b) a single expression vector that encodes both chains (*e.g.*, complete or partial heavy and light chains), under conditions that permit expression of both chains. The sialidase will be

fused to one or more of the chains. The intact fusion protein and/or antibody conjugate can be harvested and purified or isolated using techniques known in the art, *e.g.*, Protein A, Protein G, affinity tags such as glutathione-S-transferase (GST) or histidine tags. It is within ordinary skill in the art to express the heavy chain and the light chain from a single expression vector or from two separate expression vectors.

[00196] In certain embodiments, in order to express a protein, *e.g.,* a recombinant human sialidase, as a secreted protein, a native N-terminal signal sequence of the protein is replaced, *e.g.,* with MDMRVPAQLLGLLLLWLPGARC (SEQ ID NO: 35). In certain embodiments, to express a protein, *e.g.,* a recombinant human sialidase, as a secreted protein, an N-terminal signal sequence, *e.g.,* MDMRVPAQLLGLLLLWLPGARC (SEQ ID NO: 35), is added. In certain embodiments, in order to express a protein, *e.g.,* a recombinant human sialidase, as a secreted protein, a native N-terminal signal sequence of the protein is replaced, *e.g.,* with MGWSCIILFLVATATGVHS (SEQ ID NO: 5). In certain embodiments, to express a protein, *e.g.,* a recombinant human sialidase, as a secreted protein, an N-terminal signal sequence, *e.g.,* MGWSCIILFLVATATGVHS (SEQ ID NO: 5), is added. Additional exemplary N-terminal signal sequences include signal sequences from interleukin-2, CD-5, IgG kappa light chain, trypsinogen, serum albumin, and prolactin. In certain embodiments, in order to express a protein, *e.g.,* a recombinant human sialidase, as a secreted protein, a C terminal lysosomal signal motif, *e.g.,* YGTL (SEQ ID NO: 36) is removed.

[00197] Methods for reducing or eliminating the antigenicity of antibodies and antibody fragments are known in the art. When the antibodies are to be administered to a human, the antibodies preferably are "humanized" to reduce or eliminate antigenicity in humans. Preferably, each humanized antibody has the same or substantially the same affinity for the antigen as the non-humanized mouse antibody from which it was derived.

[00198] In one humanization approach, chimeric proteins are created in which mouse immunoglobulin constant regions are replaced with human immunoglobulin constant regions. See, *e.g.*, Morrison *et al.*, 1984, PROC. NAT. ACAD. SCI. 81:6851-6855, Neuberger *et al.*, 1984, NATURE 312:604-608; U.S. Patent Nos. 6,893,625 (Robinson); 5,500,362 (Robinson); and 4,816,567 (Cabilly).

[00199] In an approach known as CDR grafting, the CDRs of the light and heavy chain variable regions are grafted into frameworks from another species. For example, murine CDRs can be grafted into human FRs. In some embodiments, the CDRs of the light and

heavy chain variable regions of an antibody are grafted into human FRs or consensus human FRs. To create consensus human FRs, FRs from several human heavy chain or light chain amino acid sequences are aligned to identify a consensus amino acid sequence. CDR grafting is described in U.S. Patent Nos. 7,022,500 (Queen); 6,982,321 (Winter); 6,180,370 (Queen); 6,054,297 (Carter); 5,693,762 (Queen); 5,859,205 (Adair); 5,693,761 (Queen); 5,565,332 (Hoogenboom); 5,585,089 (Queen); 5,530,101 (Queen); Jones *et al.* (1986) NATURE 321: 522-525; Riechmann *et al.* (1988) NATURE 332: 323-327; Verhoeyen *et al.* (1988) SCIENCE 239: 1534-1536; and Winter (1998) FEBS LETT 430: 92-94.

[00200] In an approach called "SUPERHUMANIZATION™," human CDR sequences are chosen from human germline genes, based on the structural similarity of the human CDRs to those of the mouse antibody to be humanized. See, *e.g.*, U.S. Patent No. 6,881,557 (Foote); and Tan *et al.*, 2002, J. IMMUNOL. 169:1119-1125.

[00201] Other methods to reduce immunogenicity include "reshaping," "hyperchimerization," and "veneering/resurfacing." See, *e.g.*, Vaswami *et al.*, 1998, ANNALS OF ALLERGY, ASTHMA, & IMMUNOL. 81:105; Roguska *et al.*, 1996, PROT. ENGINEER 9:895-904; and U.S. Patent No. 6,072,035 (Hardman). In the veneering/resurfacing approach, the surface accessible amino acid residues in the murine antibody are replaced by amino acid residues more frequently found at the same positions in a human antibody. This type of antibody resurfacing is described, *e.g.*, in U.S. Patent No. 5,639,641 (Pedersen).

[00202] Another approach for converting a mouse antibody into a form suitable for medical use in humans is known as ACTIVMAB™ technology (Vaccinex, Inc., Rochester, NY), which involves a vaccinia virus-based vector to express antibodies in mammalian cells. High levels of combinatorial diversity of IgG heavy and light chains can be produced. See, *e.g.*, U.S. Patent Nos. 6,706,477 (Zauderer); 6,800,442 (Zauderer); and 6,872,518 (Zauderer). Another approach for converting a mouse antibody into a form suitable for use in humans is technology practiced commercially by KaloBios Pharmaceuticals, Inc. (Palo Alto, CA). This technology involves the use of a proprietary human "acceptor" library to produce an "epitope focused" library for antibody selection. Another approach for modifying a mouse antibody into a form suitable for medical use in humans is HUMAN ENGINEERING™ technology, which is practiced commercially by XOMA (US) LLC. See, *e.g.*, International (PCT) Publication No. WO 93/11794 and U.S. Patent Nos. 5,766,886 (Studnicka); 5,770,196 (Studnicka); 5,821,123 (Studnicka); and 5,869,619 (Studnicka).

[00203] Any suitable approach, including any of the above approaches, can be used to reduce or eliminate human immunogenicity of an antibody.

[00204] In addition, it is possible to create fully human antibodies in mice. Fully human mAbs lacking any non-human sequences can be prepared from human immunoglobulin transgenic mice by techniques referenced in, *e.g.*, Lonberg *et al.*, NATURE 368:856-859, 1994; Fishwild *et al.*, NATURE BIOTECHNOLOGY 14:845-851, 1996; and Mendez *et al.*, NATURE GENETICS 15:146-156, 1997. Fully human monoclonal antibodies can also be prepared and optimized from phage display libraries by techniques referenced in, *e.g.*, Knappik *et al.*, J. MOL. BIOL. 296:57-86, 2000; and Krebs *et al.*, J. IMMUNOL. METH. 254:67-84 2001).

[00205] The present disclosure encompasses fusion proteins comprising antibody fragments, which may be generated by traditional means, such as enzymatic digestion, or by recombinant techniques. For a review of certain antibody fragments, see Hudson *et al.* (2003) NAT. MED. 9:129-134.

[00206] Various techniques have been developed for the production of antibody fragments. Traditionally, these fragments were derived via proteolytic digestion of intact antibodies (see, *e.g.*, Morimoto *et al.* (1992) JOURNAL OF BIOCHEMICAL AND BIOPHYSICAL METHODS 24:107-117; and Brennan *et al.* (1985) SCIENCE 229:81). However, these fragments can now be produced directly by recombinant host cells. Fab, Fv and ScFv antibody fragments can all be expressed in and secreted from *E. coli*, thus allowing the facile production of large amounts of these fragments. Antibody fragments can be isolated from the antibody phage libraries. Alternatively, Fab'-SH fragments can be directly recovered from *E. coli* and chemically coupled to form $F(ab')_2$ fragments (Carter *et al.* (1992) BIO/TECHNOLOGY 10:163-167). According to another approach, $F(ab')_2$ fragments can be isolated directly from recombinant host cell culture. Fab and $F(ab')_2$ fragments with increased *in vivo* half-life comprising salvage receptor binding epitope residues are described in U.S. Patent No. 5,869,046. Other techniques for the production of antibody fragments will be apparent to the skilled practitioner. In certain embodiments, an antibody is a single chain Fv fragment (scFv). See U.S. Patent Nos. 5,571,894 and 5,587,458.

[00207] Methods for making bispecific antibodies are known in the art. See Milstein and Cuello (1983) NATURE 305:537, International (PCT) Publication No. WO93/08829, and Traunecker *et al.* (1991) EMBO J., 10:3655. For further details of generating bispecific

antibodies see, for example, Suresh *et al.* (1986) METHODS ENZYMOL. 121:210. Bispecific antibodies include cross-linked or "heteroconjugate" or "heterodimer" antibodies. For example, one of the antibodies in the heterodimer can be coupled to avidin, the other to biotin. Heterodimer antibodies may be made using any convenient cross-linking method. Suitable cross-linking agents are well known in the art, and are disclosed in U.S. Patent No. 4,676,980, along with a number of cross-linking techniques.

[00208] Examples of heterodimeric or asymmetric IgG-like molecules include but are not limited to those obtained with the following technologies or using the following formats: Triomab/Quadroma, Knobs-into-Holes, CrossMabs, electrostatically-matched antibodies, LUZ-Y, Strand Exchange Engineered Domain body, Biclonic and DuoBody.

[00209] Advantages of using antibody fragments (*e.g.,* F(ab) and F(ab')$_2$ fragments) include the elimination of non-specific binding between Fc portions of antibodies and Fc receptors on cells (such as macrophages, dendritic cells, neutrophils, NK cells and B cells). In addition, they may be able to penetrate tissues more efficiently due to their smaller size.

[00210] Heterodimeric antibodies, or asymmetric antibodies, allow for greater flexibility and new formats for attaching a variety of drugs to the antibody arms. One of the general formats for creating a heterodimeric antibody is the "knobs-into-holes" format. This format is specific to the heavy chain part of the constant region in antibodies. The "knobs" part is engineered by replacing a small amino acid with a larger one, which fits into a "hole", which is engineered by replacing a large amino acid with a smaller one. What connects the "knobs" to the "holes" are the disulfide bonds between each chain. The "knobs-into-holes" shape facilitates antibody dependent cell mediated cytotoxicity. Single chain variable fragments (scFv) are connected to the variable domain of the heavy and light chain via a short linker peptide. The linker is rich in glycine, which gives it more flexibility, and serine/threonine, which gives it specificity. Two different scFv fragments can be connected together, via a hinge region, to the constant domain of the heavy chain or the constant domain of the light chain. This gives the antibody bispecificity, allowing for the binding specificities of two different antigens. The "knobs-into-holes" or other heterodimerization format enhances heterodimer formation but doesn't suppress homodimer formation.

[00211] Several approaches to support heterodimerization have been described, for example in International (PCT) Publication Nos. WO96/27011, WO98/050431, WO2007/110205, WO2007/147901, WO2009/089004, WO2010/129304, WO2011/90754, WO2011/143545,

WO2012/058768, WO2013/157954, and WO2013/096291, and European Patent Publication No. EP1870459. Typically, in the approaches known in the art, the CH_3 domain of the first heavy chain and the CH_3 domain of the second heavy chain are both engineered in a complementary manner so that the heavy chain comprising one engineered CH_3 domain can no longer homodimerize with another heavy chain of the same structure (*e.g.*, a CH_3-engineered first heavy chain can no longer homodimerize with another CH_3-engineered first heavy chain; and a CH_3-engineered second heavy chain can no longer homodimerize with another CH_3-engineered second heavy chain). Thereby the heavy chain comprising one engineered CH_3 domain is forced to heterodimerize with another heavy chain comprising the CH_3 domain, which is engineered in a complementary manner. As a result, the CH_3 domain of the first heavy chain and the CH_3 domain of the second heavy chain are engineered in a complementary manner by amino acid substitutions, such that the first heavy chain and the second heavy chain are forced to heterodimerize, whereas the first heavy chain and the second heavy chain can no longer homodimerize (*e.g.*, for steric reasons).

IV. Pharmaceutical Compositions

[00212] For therapeutic use, a recombinant human Neu2 enzyme or a fusion protein and/or antibody conjugate thereof preferably is combined with a pharmaceutically acceptable carrier. The term "pharmaceutically acceptable" as used herein refers 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.

[00213] The term "pharmaceutically acceptable carrier" as used herein refers to buffers, carriers, and excipients 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. Pharmaceutically acceptable carriers include any of the standard pharmaceutical carriers, such as a phosphate buffered saline solution, water, emulsions (*e.g.*, such as an oil/water or water/oil emulsions), and various types of wetting agents. The compositions also can include stabilizers and preservatives. For examples of carriers, stabilizers and adjuvants, see, *e.g.*, Martin, Remington's Pharmaceutical Sciences, 15th Ed., Mack Publ. Co., Easton, PA [1975]. Pharmaceutically acceptable carriers include buffers, solvents, dispersion media, coatings, isotonic and absorption delaying agents, and the like, that are compatible with pharmaceutical

administration. The use of such media and agents for pharmaceutically active substances is known in the art.

[00214] In certain embodiments, a pharmaceutical composition may contain formulation materials for modifying, maintaining or preserving, for example, the pH, osmolarity, viscosity, clarity, color, isotonicity, odor, sterility, stability, rate of dissolution or release, adsorption or penetration of the composition. In such embodiments, suitable formulation materials include, but are not limited to, amino acids (such as glycine, glutamine, asparagine, arginine or lysine); antimicrobials; antioxidants (such as ascorbic acid, sodium sulfite or sodium hydrogen-sulfite); buffers (such as borate, bicarbonate, Tris-HCl, citrates, phosphates or other organic acids); bulking agents (such as mannitol or glycine); chelating agents (such as ethylenediamine tetraacetic acid (EDTA)); complexing agents (such as caffeine, polyvinylpyrrolidone, beta-cyclodextrin or hydroxypropyl-beta-cyclodextrin); fillers; monosaccharides; disaccharides; and other carbohydrates (such as glucose, mannose or dextrins); proteins (such as serum albumin, gelatin or immunoglobulins); coloring, flavoring and diluting agents; emulsifying agents; hydrophilic polymers (such as polyvinylpyrrolidone); low molecular weight polypeptides; salt-forming counterions (such as sodium); preservatives (such as benzalkonium chloride, benzoic acid, salicylic acid, thimerosal, phenethyl alcohol, methylparaben, propylparaben, chlorhexidine, sorbic acid or hydrogen peroxide); solvents (such as glycerin, propylene glycol or polyethylene glycol); sugar alcohols (such as mannitol or sorbitol); suspending agents; surfactants or wetting agents (such as pluronics, PEG, sorbitan esters, polysorbates such as polysorbate 20, polysorbate, triton, tromethamine, lecithin, cholesterol, tyloxapal); stability enhancing agents (such as sucrose or sorbitol); tonicity enhancing agents (such as alkali metal halides, preferably sodium or potassium chloride, mannitol sorbitol); delivery vehicles; diluents; excipients and/or pharmaceutical adjuvants (see, *Remington's Pharmaceutical Sciences*, 18th ed. (Mack Publishing Company, 1990).

[00215] In certain embodiments, a pharmaceutical composition may contain nanoparticles, *e.g.*, polymeric nanoparticles, liposomes, or micelles (See Anselmo *et al.* (2016) BIOENG. TRANSL. MED. 1: 10-29).

[00216] In certain embodiments, a pharmaceutical composition may contain a sustained- or controlled-delivery formulation. Techniques for formulating sustained- or controlled-delivery means, such as liposome carriers, bio-erodible microparticles or porous beads and depot injections, are also known to those skilled in the art. Sustained-release preparations

may include, *e.g.*, porous polymeric microparticles or semipermeable polymer matrices in the form of shaped articles, *e.g.,* films, or microcapsules. Sustained release matrices may include polyesters, hydrogels, polylactides, copolymers of L-glutamic acid and gamma ethyl-L-glutamate, poly (2-hydroxyethyl-inethacrylate), ethylene vinyl acetate, or poly-D(−)-3-hydroxybutyric acid. Sustained release compositions may also include liposomes that can be prepared by any of several methods known in the art.

[00217] Pharmaceutical compositions containing a recombinant human sialidase, a recombinant human sialidase fusion protein, or an antibody conjugate disclosed herein can be presented in a dosage unit form and can be prepared by any suitable method. A pharmaceutical composition should be formulated to be compatible with its intended route of administration. Examples of routes of administration are intravenous (IV), intradermal, inhalation, transdermal, topical, transmucosal, intrathecal and rectal administration. In certain embodiments, a recombinant human sialidase, a recombinant human sialidase fusion protein, or an antibody conjugate disclosed herein is administered by IV infusion. In certain embodiments, a recombinant human sialidase, a recombinant human sialidase fusion protein, or an antibody conjugate disclosed herein is administered by intratumoral injection. Useful formulations can be prepared by methods known in the pharmaceutical art. For example, see *Remington's Pharmaceutical Sciences*, 18th ed. (Mack Publishing Company, 1990). Formulation components suitable for parenteral administration include a sterile diluent such as water for injection, saline solution, fixed oils, polyethylene glycols, glycerin, propylene glycol or other synthetic solvents; antibacterial agents such as benzyl alcohol or methyl parabens; antioxidants such as ascorbic acid or sodium bisulfite; chelating agents such as EDTA; buffers such as acetates, citrates or phosphates; and agents for the adjustment of tonicity such as sodium chloride or dextrose.

[00218] For intravenous administration, suitable carriers include physiological saline, bacteriostatic water, Cremophor ELTM (BASF, Parsippany, NJ) or phosphate buffered saline (PBS). The carrier should be stable under the conditions of manufacture and storage, and should be preserved against microorganisms. The carrier can be a solvent or dispersion medium containing, for example, water, ethanol, polyol (for example, glycerol, propylene glycol, and liquid polyethylene glycol), and suitable mixtures thereof.

[00219] In certain embodiments, a pharmaceutical composition may contain a stabilizing agent. In certain embodiments, the stabilizing agent is a cation, such as a divalent cation. In

certain embodiments, the cation is calcium or magnesium. The cation can be in the form of a salt, such as calcium chloride ($CaCl_2$) or magnesium chloride ($MgCl_2$).

[00220] In certain embodiments, the stabilizing agent is present in an amount from about 0.05 mM to about 5 mM. For example, the stabilizing agent may be present in an amount of from about 0.05 mM to about 4 mM, from about 0.05 mM to about 3 mM, from about 0.05 mM to about 2 mM, from about 0.05 mM to about 1 mM, from about 0.05 mM to about 0.5 mM, from about 0.5 mM to about 4 mM, from about 0.5 mM to about 3 mM, from about 0.5 mM to about 2 mM, from about 0.5 mM to about 1 mM, from about 1 mM to about 4 mM, from about 1 mM to about 3 mM, of from about 1 mM to about 2 mM.

[00221] Pharmaceutical formulations preferably are sterile. Sterilization can be accomplished by any suitable method, *e.g.*, filtration through sterile filtration membranes. Where the composition is lyophilized, filter sterilization can be conducted prior to or following lyophilization and reconstitution.

[00222] The compositions described herein may be administered locally or systemically. Administration will generally be parenteral administration. In a preferred embodiment, the pharmaceutical composition is administered subcutaneously and in an even more preferred embodiment intravenously. Preparations for parenteral administration include sterile aqueous or non-aqueous solutions, suspensions, and emulsions.

[00223] Generally, a therapeutically effective amount of active component, for example, a recombinant human sialidase or fusion protein and/or antibody conjugate thereof, is in the range of 0.1 mg/kg to 100 mg/kg, *e.g.*, 1 mg/kg to 100 mg/kg, 1 mg/kg to 10 mg/kg. The amount administered will depend on variables such as the type and extent of disease or indication to be treated, the overall health of the patient, the *in vivo* potency of the antibody, the pharmaceutical formulation, and the route of administration. The initial dosage can be increased beyond the upper level in order to rapidly achieve the desired blood-level or tissue-level. Alternatively, the initial dosage can be smaller than the optimum, and the daily dosage may be progressively increased during the course of treatment. Human dosage can be optimized, *e.g.*, in a conventional Phase I dose escalation study designed to run from 0.5 mg/kg to 20 mg/kg. Dosing frequency can vary, depending on factors such as route of administration, dosage amount, serum half-life of the recombinant human sialidase or fusion protein and/or antibody conjugate thereof, and the disease being treated. Exemplary dosing frequencies are once per day, once per week and once every two weeks. A preferred route of

administration is parenteral, *e.g.*, intravenous infusion. In certain embodiments, a recombinant human sialidase or a fusion protein and/or antibody conjugate thereof is lyophilized, and then reconstituted in buffered saline, at the time of administration.

V. Therapeutic Uses

[00224] The compositions and methods disclosed herein can be used to treat various forms of cancer in a subject or inhibit cancer growth in a subject. The disclosure provides a method of treating a cancer in a subject. The method comprises administering to the subject an effective amount of a recombinant human sialidase or a fusion protein and/or antibody conjugate thereof, *e.g.,* a recombinant human sialidase, fusion protein, or antibody conjugate disclosed herein, either alone or in a combination with another therapeutic agent to treat the cancer in the subject. The term "effective amount" as used herein refers to the amount of an active agent (*e.g.*, recombinant human sialidase or fusion protein thereof according to the present disclosure) sufficient to effect beneficial or desired results. An effective amount can be administered in one or more administrations, applications or dosages and is not intended to be limited to a particular formulation or administration route.

[00225] As used herein, "treat", "treating" and "treatment" mean the treatment of a disease in a subject, *e.g.*, in a human. This includes: (a) inhibiting the disease, *i.e.*, arresting its development; and (b) relieving the disease, *i.e.*, causing regression of the disease state. As used herein, the terms "subject" and "patient" refer to an organism to be treated by the methods and compositions described herein. Such organisms preferably include, but are not limited to, mammals (*e.g.*, murines, simians, equines, bovines, porcines, canines, felines, and the like), and more preferably includes humans.

[00226] Examples of cancers include solid tumors, soft tissue tumors, hematopoietic tumors and metastatic lesions. Examples of hematopoietic tumors include, leukemia, acute leukemia, acute lymphoblastic leukemia (ALL), B-cell, T-cell or FAB ALL, acute myeloid leukemia (AML), chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), *e.g.*, transformed CLL, diffuse large B-cell lymphomas (DLBCL), follicular lymphoma, hairy cell leukemia, myelodyplastic syndrome (MDS), a lymphoma, Hodgkin's disease, a malignant lymphoma, non-Hodgkin's lymphoma, Burkitt's lymphoma, multiple myeloma, or Richter's Syndrome (Richter's Transformation). Examples of solid tumors include malignancies, *e.g.,* sarcomas, adenocarcinomas, and carcinomas, of the various organ systems, such as those affecting head and neck (including pharynx), thyroid, lung (small cell

or non-small cell lung carcinoma (NSCLC)), breast, lymphoid, gastrointestinal (*e.g.*, oral, esophageal, stomach, liver, pancreas, small intestine, colon and rectum, anal canal), genitals and genitourinary tract (*e.g.*, renal, urothelial, bladder, ovarian, uterine, cervical, endometrial, prostate, testicular), CNS (*e.g.*, neural or glial cells, *e.g.*, neuroblastoma or glioma), or skin (*e.g.*, melanoma).

[00227] In certain embodiments the cancer is an epithelial cancer, *e.g.*, an epithelial cancer that upregulates the expression of sialylated glycans. Exemplary epithelial cancers include, but are not limited to, endometrial cancer, colon cancer, ovarian cancer, cervical cancer, vulvar cancer, uterine cancer or fallopian tube cancer, breast cancer, prostate cancer, lung cancer, pancreatic cancer, urinary cancer, bladder cancer, head and neck cancer, oral cancer and liver cancer. Epithelial cancers also include carcinomas, for example, acinar carcinoma, acinous carcinoma, adenocystic carcinoma, adenoid cystic carcinoma, carcinoma adenomatosum, carcinoma of adrenal cortex, alveolar carcinoma, alveolar cell carcinoma, basal cell carcinoma, carcinoma basocellulare, basaloid carcinoma, baso squamous cell carcinoma, bronchioalveolar carcinoma, bronchiolar carcinoma, bronchogenic carcinoma, cerebriform carcinoma, cholangiocellular carcinoma, chorionic carcinoma, colloid carcinoma, comedo carcinoma, corpus carcinoma, cribriform carcinoma, carcinoma en cuirasse, carcinoma cutaneum, cylindrical carcinoma, cylindrical cell carcinoma, duct carcinoma, carcinoma durum, embryonal carcinoma, encephaloid carcinoma, epiermoid carcinoma, carcinoma epitheliale adenoides, exophytic carcinoma, carcinoma ex ulcere, carcinoma fibrosum, gelatiniforni carcinoma, gelatinous carcinoma, giant cell carcinoma, carcinoma gigantocellulare, glandular carcinoma, granulosa cell carcinoma, hair-matrix carcinoma, hematoid carcinoma, hepatocellular carcinoma, Hurthle cell carcinoma, hyaline carcinoma, hypemephroid carcinoma, infantile embryonal carcinoma, carcinoma in situ, intraepidermal carcinoma, intraepithelial carcinoma, Krompecher's carcinoma, Kulchitzky-cell carcinoma, large-cell carcinoma, lenticular carcinoma, carcinoma lenticulare, lipomatous carcinoma, lymphoepithelial carcinoma, carcinoma medullare, medullary carcinoma, melanotic carcinoma, carcinoma molle, mucinous carcinoma, carcinoma muciparum, carcinoma mucocellulare, mucoepidermoid carcinoma, carcinoma mucosum, mucous carcinoma, carcinoma myxomatodes, nasopharyngeal carcinoma, oat cell carcinoma, carcinoma ossificans, osteoid carcinoma, papillary carcinoma, periportal carcinoma, preinvasive carcinoma, prickle cell carcinoma, pultaceous carcinoma, renal cell carcinoma of kidney, reserve cell carcinoma, carcinoma sarcomatodes, schneiderian carcinoma, scirrhous

carcinoma, carcinoma scroti, signet-ring cell carcinoma, carcinoma simplex, small-cell carcinoma, solanoid carcinoma, spheroidal cell carcinoma, spindle cell carcinoma, carcinoma spongiosum, squamous carcinoma, squamous cell carcinoma, string carcinoma, carcinoma telangiectaticum, carcinoma telangiectodes, transitional cell carcinoma, carcinoma tuberosum, tuberous carcinoma, verrucous carcinoma, and carcinoma villosum.

[00228] In certain embodiments, the cancer is breast cancer. In certain embodiments, the cancer is an adenocarcinoma. In certain embodiments, the cancer is a metastatic cancer. In certain embodiments, the cancer is a refractory cancer.

[00229] In certain embodiments, the cancer is resistant to or non-responsive to treatment with an antibody, *e.g.*, an antibody with ADCC activity, *e.g.*, trastuzumab.

[00230] The methods and compositions described herein can be used alone or in combination with other therapeutic agents and/or modalities. The term administered "in combination," as used herein, is understood to mean that two (or more) different treatments are delivered to the subject during the course of the subject's affliction with the disorder, such that the effects of the treatments on the patient overlap at a point in time. In certain embodiments, the delivery of one treatment is still occurring when the delivery of the second begins, so that there is overlap in terms of administration. This is sometimes referred to herein as "simultaneous" or "concurrent delivery." In other embodiments, the delivery of one treatment ends before the delivery of the other treatment begins. In certain embodiments of either case, the treatment is more effective because of combined administration. For example, the second treatment is more effective, *e.g.*, an equivalent effect is seen with less of the second treatment, or the second treatment reduces symptoms to a greater extent, than would be seen if the second treatment were administered in the absence of the first treatment, or the analogous situation is seen with the first treatment. In certain embodiments, delivery is such that the reduction in a symptom, or other parameter related to the disorder is greater than what would be observed with one treatment delivered in the absence of the other. The effect of the two treatments can be partially additive, wholly additive, or greater than additive. The delivery can be such that an effect of the first treatment delivered is still detectable when the second is delivered.

[00231] In certain embodiments, a method or composition described herein, is administered in combination with one or more additional therapies, *e.g.*, surgery, radiation therapy, or administration of another therapeutic preparation. In certain embodiments, the additional

therapy may include chemotherapy, *e.g.*, a cytotoxic agent. In certain embodiments the additional therapy may include a targeted therapy, *e.g.*, a tyrosine kinase inhibitor, a proteasome inhibitor, or a protease inhibitor. In certain embodiments, the additional therapy may include an anti-inflammatory, anti-angiogenic, anti-fibrotic, or anti-proliferative compound, *e.g.*, a steroid, a biologic immunomodulator, a monoclonal antibody, an antibody fragment, an aptamer, an siRNA, an antisense molecule, a fusion protein, a cytokine, a cytokine receptor, a bronchodilator, a statin, an anti-inflammatory agent (*e.g.*, methotrexate), or an NSAID. In certain embodiments, the additional therapy may include a combination of therapeutics of different classes.

[00232] In certain embodiments, a method or composition described herein is administered in combination with a checkpoint inhibitor. The checkpoint inhibitor may, for example, be selected from a PD-1 antagonist, PD-L1 antagonist, CTLA-4 antagonist, adenosine A2A receptor antagonist, B7-H3 antagonist, B7-H4 antagonist, BTLA antagonist, KIR antagonist, LAG3 antagonist, TIM-3 antagonist, VISTA antagonist, and TIGIT antagonist.

[00233] In certain embodiments, the checkpoint inhibitor is a PD-1 or PD-L1 inhibitor. PD-1 is a receptor present on the surface of T-cells that serves as an immune system checkpoint that inhibits or otherwise modulates T-cell activity at the appropriate time to prevent an overactive immune response. Cancer cells, however, can take advantage of this checkpoint by expressing ligands, for example, PD-L1, that interact with PD-1 on the surface of T-cells to shut down or modulate T-cell activity. Exemplary PD-1/PD-L1 based immune checkpoint inhibitors include antibody based therapeutics. Exemplary treatment methods that employ PD-1/PD-L1 based immune checkpoint inhibition are described in U.S. Patent Nos. 8,728,474 and 9,073,994, and EP Patent No. 1537878B1, and, for example, include the use of anti-PD-1 antibodies. Exemplary anti-PD-1 antibodies are described, for example, in U.S. Patent Nos. 8,952,136, 8,779,105, 8,008,449, 8,741,295, 9,205,148, 9,181,342, 9,102,728, 9,102,727, 8,952,136, 8,927,697, 8,900,587, 8,735,553, and 7,488,802. Exemplary anti-PD-1 antibodies include, for example, nivolumab (Opdivo®, Bristol-Myers Squibb Co.), pembrolizumab (Keytruda®, Merck Sharp & Dohme Corp.), PDR001 (Novartis Pharmaceuticals), and pidilizumab (CT-011, Cure Tech). Exemplary anti-PD-L1 antibodies are described, for example, in U.S. Patent Nos. 9,273,135, 7,943,743, 9,175,082, 8,741,295, 8,552,154, and 8,217,149. Exemplary anti-PD-L1 antibodies include, for example, atezolizumab (Tecentriq®, Genentech), durvalumab (AstraZeneca), MEDI4736, avelumab, and BMS 936559 (Bristol Myers Squibb Co.).

[00234] In certain embodiments, a method or composition described herein is administered in combination with a CTLA-4 inhibitor. In the CTLA-4 pathway, the interaction of CTLA-4 on a T-cell with its ligands (*e.g.*, CD80, also known as B7-1, and CD86) on the surface of an antigen presenting cells (rather than cancer cells) leads to T-cell inhibition. Exemplary CTLA-4 based immune checkpoint inhibition methods are described in U.S. Patent Nos. 5,811,097, 5,855,887, 6,051,227. Exemplary anti-CTLA-4 antibodies are described in U.S. Patent Nos. 6,984,720, 6,682,736, 7,311,910; 7,307,064, 7,109,003, 7,132,281, 6,207,156, 7,807,797, 7,824,679, 8,143,379, 8,263,073, 8,318,916, 8,017,114, 8,784,815, and 8,883,984, International (PCT) Publication Nos. WO98/42752, WO00/37504, and WO01/14424, and European Patent No. EP 1212422 B1. Exemplary CTLA-4 antibodies include ipilimumab or tremelimumab.

[00235] In certain embodiments, a method or composition described herein is administered in combination with (i) a PD-1 or PD-L1 inhibitor, *e.g.*, a PD-1 or PD-L1 inhibitor disclosed herein, and (ii) CTLA-4 inhibitor, *e.g.*, a CTLA-4 inhibitor disclosed herein.

[00236] In certain embodiments, a method or composition described herein is administered in combination with an IDO inhibitor. Exemplary IDO inhibitors include 1-methyl-D-tryptophan (known as indoximod), epacadostat (INCB24360), navoximod (GDC-0919), and BMS-986205.

[00237] Exemplary cytotoxic agents that can be administered in combination with a method or composition described herein include, for example, antimicrotubule agents, topoisomerase inhibitors, antimetabolites, protein synthesis and degradation inhibitors, mitotic inhibitors, alkylating agents, platinating agents, inhibitors of nucleic acid synthesis, histone deacetylase inhibitors (HDAC inhibitors, *e.g.*, vorinostat (SAHA, MK0683), entinostat (MS-275), panobinostat (LBH589), trichostatin A (TSA), mocetinostat (MGCD0103), belinostat (PXD101), romidepsin (FK228, depsipeptide)), DNA methyltransferase inhibitors, nitrogen mustards, nitrosoureas, ethylenimines, alkyl sulfonates, triazenes, folate analogs, nucleoside analogs, ribonucleotide reductase inhibitors, vinca alkaloids, taxanes, epothilones, intercalating agents, agents capable of interfering with a signal transduction pathway, agents that promote apoptosis and radiation, or antibody molecule conjugates that bind surface proteins to deliver a toxic agent. In one embodiment, the cytotoxic agent that can be administered with a method or composition described herein is a platinum-based agent (such as cisplatin), cyclophosphamide, dacarbazine, methotrexate, fluorouracil, gemcitabine, capecitabine, hydroxyurea, topotecan, irinotecan, azacytidine, vorinostat, ixabepilone,

bortezomib, taxanes (*e.g.*, paclitaxel or docetaxel), cytochalasin B, gramicidin D, ethidium bromide, emetine, mitomycin, etoposide, tenoposide, vincristine, vinblastine, vinorelbine, colchicin, anthracyclines (*e.g.*, doxorubicin or epirubicin) daunorubicin, dihydroxy anthracin dione, mitoxantrone, mithramycin, actinomycin D, adriamycin, 1-dehydrotestosterone, glucocorticoids, procaine, tetracaine, lidocaine, propranolol, puromycin, ricin, or maytansinoids.

[00238] Also provided herein is a method of removing sialic acid from a cell or tissue. The method comprises contacting the cell or tissue with an effective amount of a sialidase, fusion protein, and/or antibody conjugate, *e.g.,* a sialidase, fusion protein, or antibody conjugate disclosed herein. Also provided is a method of removing sialic acid from a cell in a subject, the method comprising administering to the subject an effective amount of a pharmaceutical composition comprising a sialidase, fusion protein, and/or antibody conjugate, *e.g.,* a sialidase, fusion protein, or antibody conjugate disclosed herein, thereby to remove sialic acid from the cell.

[00239] Throughout the description, where compositions are described as having, including, or comprising specific components, or where processes and methods are described as having, including, or comprising specific steps, it is contemplated that, additionally, there are compositions of the present disclosure that consist essentially of, or consist of, the recited components, and that there are processes and methods according to the present disclosure that consist essentially of, or consist of, the recited processing steps.

[00240] In the application, where an element or component is said to be included in and/or selected from a list of recited elements or components, it should be understood that the element or component can be any one of the recited elements or components, or the element or component can be selected from a group consisting of two or more of the recited elements or components.

[00241] Further, it should be understood that elements and/or features of a composition or a method described herein can be combined in a variety of ways without departing from the spirit and scope of the present disclosure, whether explicit or implicit herein. For example, where reference is made to a particular compound, that compound can be used in various embodiments of compositions of the present disclosure and/or in methods of the present disclosure, unless otherwise understood from the context. In other words, within this application, embodiments have been described and depicted in a way that enables a clear and

concise application to be written and drawn, but it is intended and will be appreciated that embodiments may be variously combined or separated without parting from the present teachings and disclosure (s). For example, it will be appreciated that all features described and depicted herein can be applicable to all aspects of the disclosure (s) described and depicted herein.

[00242] It should be understood that the expression "at least one of" includes individually each of the recited objects after the expression and the various combinations of two or more of the recited objects unless otherwise understood from the context and use. The expression "and/or" in connection with three or more recited objects should be understood to have the same meaning unless otherwise understood from the context.

[00243] The use of the term "include," "includes," "including," "have," "has," "having," "contain," "contains," or "containing," including grammatical equivalents thereof, should be understood generally as open-ended and non-limiting, for example, not excluding additional unrecited elements or steps, unless otherwise specifically stated or understood from the context.

[00244] Where the use of the term "about" is before a quantitative value, the present disclosure also includes the specific quantitative value itself, unless specifically stated otherwise. As used herein, the term "about" refers to a ±10% variation from the nominal value unless otherwise indicated or inferred.

[00245] It should be understood that the order of steps or order for performing certain actions is immaterial so long as the present disclosure remain operable. Moreover, two or more steps or actions may be conducted simultaneously.

[00246] The use of any and all examples, or exemplary language herein, for example, "such as" or "including," is intended merely to illustrate better the present disclosure and does not pose a limitation on the scope of the disclosure unless claimed. No language in the specification should be construed as indicating any non-claimed element as essential to the practice of the present disclosure.

EXAMPLES

[00247] The following Examples are merely illustrative and are not intended to limit the scope or content of the disclosure in any way.

Example 1

[00248] This Example describes methods for characterizing recombinant human sialidases with mutations that decrease heparin binding and/or increase serum half-life, expression, and/or activity of the sialidases.

Construction and expression of Neu2 mutants

[00249] Mutant Neu2 sialidases were constructed by incorporating additional mutations in either (1) Neu2-M106 -Fc (SEQ ID NO: 6; human neuraminidase 2 Fc fusion with the following substitutions -- M1D, V6Y, P62G, A93E, I187K, and C332A as described in WO2021/003469 (PCT/US2020/040828)), described herein as "M106" or (2) Neu2-M259-Fc (SEQ ID NO: 7; human neuraminidase 2 Fc fusion with the following substitutions -- M1D, V6Y, A42R, P62G, A93E, Q126Y, I187K, A242F, Q270T, and C332A as described in WO2022/006492 (PCT/US2021/040240)), described herein as "M259". In certain embodiments, the I187K substitution in either M106 or M259 was substituted with other amino acids as denoted (for instance I187E, I187A, I187Q).

[00250] Recombinant human sialidases with defined mutations were expressed as human IgG1 Fc dimers in Expi293 human cells using the pCEP4 mammalian expression vector utilizing the mouse IgG heavy chain signal sequence (MGWSCIILFLVATATGVHS (SEQ ID NO: 5)), purified using a single step Protein-A purification, quantified with a UV-Vis spectrophotometer (NanoDrop), and examined by SEC-HPLC. SEC-HPLC was used to examine homogeneity of the Fc dimers. All constructs described herein exhibited a homogeneity of at least 80%. Titer is reported as either no expression; X for 5-14.9 mg/L; XX for 15-25 mg/L; or XXX for 25 and above mg/L in **TABLES 11-20**.

4-MU-NeuAc activity assay

[00251] Initial enzyme kinetics were assayed by measuring the release of sialic acid from the fluorogenic substrate 4-methylumbelliferyl-N-acetylneuraminic acid (4-MU-NeuAc). 4-MU activity is quantified as mU/mg. 4-MU activity is reported as "not active" for no activity; X for 25-249 mU/mg; XX for 250-499 mU/mg; XXX for 500–749 mU/mg; or XXXX for 750-1,000 mU/mg in **TABLES 11-20**. n/a indicates field is not available.

Heparin affinity assay

[00252] Heparin affinity was measured by subjecting recombinant human sialidases of the present disclosure using chromatography using a Cytiva HiTrap Heparin column (Cytiva

17040601) on an Agilent 1160 HPLC system. Sialidase-Fc constructs were captured in 100 mM sodium acetate buffer, pH 5 and eluted in a 4.5 column-volume 1M sodium chloride linear gradient.

[00253] **FIGURE 2** is an exemplary SEC-HPLC trace of Neu2 mutant M259 and new mutant MUT18, which contained the mutations in M259 and additional mutations. The additional mutations found in MUT18 are described in **TABLE 14**. Trastuzumab was used as a control for an Fc-based dimer with low affinity for heparin. As seen in **FIGURE 2**, M259 showed the greatest retention time (greater than 8 minutes), followed by the new mutants (retention time of around 7 minutes) and then by Trastuzumab, with a retention time below 6 minutes. Heparin affinity data is reported with respect to the retention time of the main Fc dimer peak with XXX for an 8.0 to 9.0 min retention time; XX for an 7.5-7.9 min retention time; or X for below a 7.4 min retention time in **TABLES 11-20**. n/a indicates field is not available.

Relative half-life assay

[00254] Relative half-life of the recombinant sialidases was determined by Western blot following subcutaneous injection of 10 mg/kg recombinant human sialidases into 2 mice. Plasma from injected mice was collected at 30 minutes as well as 2, 3, 4 and 5 days post dosage. To quantify the amount of sialidase present, 2 µL of plasma were mixed with 5 µL of 4x SDS buffer, 2 µL of 10X reducing agent, and 11 µL of DI water. The samples were heated to 70 °C for 10 minutes and run on SDS-PAGE at 100V for 120 minutes in an ice bath with MOPS buffer, transferred to nitrocellulose, and then stained with anti-human Fc antibody. Resulting gels were washed with PBST and imaged with Licor scanner at 800 nm.

[00255] **FIGURES 3A-3C** show an exemplary Western blot comparing M259 (**FIGURE 3A**), to new sialidase mutants MUT81 (**FIGURE 3B**) and MUT85 (**FIGURE 3C**). Relative half-life is reported as XXX for detection via western blot at or beyond 4 days; XX for detection between 2 to 3 days; and X for detection at 1 day in **TABLES 11-20**. n/a indicates field is not available.

Cell-based desialylation screening in K562 cells

[00256] Cell-based desialylation screening assay was performed using a human lymphoblast suspension cell line isolated from the bone marrow of a chronic myelogenous leukemia patient ("K562"). On day 0 of the assay, K562 cells were harvested and resuspended at 1 M/mL in complete IMDM (cIMDM-10) media, which includes 25 mM HEPES, 4 mM L-

glutamine, and 10% HI-FBS. A total of 100,000 cells in 100 µL of cIMDM-10 were plated per well. Subsequently, mutant sialidases and controls were added to a final concentration of 100 µg/mL and a final well volume of 200 µL. After sialidase treatment for 15 hours in a standard incubator, K562 cells were stained with PNA-AF647 (10 µg/mL; Invitrogen) to detect the resulting exposed galactose residues following removal of the terminal sialic acid by flow cytometry. Desialylation levels were determined by measuring the geometric mean fluorescence intensity (gMFI) of each mutant sialidase or control and comparing it to a normalized M259 gMFI value. Normalized desialylation levels determined in K562 cells are reported as XXXX for 1.25 and greater than M259; XXX for 1.0 to 1.25 relative to M259; XX for equivalent to M259; or X for less than M259 in **TABLES 11-20**. n/a again indicates the field is not available.

Example 2

[00257] This Example describes the screening for isoelectric point (pI) modifications of amino acid residues at the surface of the protein which impact the half-life of recombinant human Neu2 sialidase and modifications that impact the ability of recombinant the sialidase to interact with heparin. New mutations were introduced into M259 (SEQ ID NO: 7) by changing either surface exposed arginine amino acid residues or the I187 amino acid residue to alter the protein's predicted pI. Expression and activity levels for the resulting mutant sialidases were evaluated as described in Example 1 and are recorded in **TABLE 11**. n/a indicates the field is not available.

TABLE 11

	Additional Mutations to M259	**Titer**	**4-MU Activity**	**Heparin Affinity**	**Relative Half-Life**	**K562 Desialylation**
M259	**none**	**XX**	**XX**	**XXX**	**X**	**XX**
MUT1	R241Q	X	n/a	n/a	n/a	n/a
MUT2	R314A	XX	n/a	n/a	X	n/a
MUT3	R314E	XX	XXX	n/a	n/a	XX
MUT4	R314Q	XX	n/a	n/a	n/a	n/a
MUT5	I187A	X	n/a	n/a	n/a	n/a
MUT6	I187E	X	n/a	n/a	X	n/a

MUT21	I187D	X	n/a	X	n/a	n/a
MUT22	I187L	X	n/a	XXX	n/a	n/a

[00258] MUT3 showed similar titer and desialylation activity in K526 cells and an improved 4-MU activity relative to M259. Both MUT5 and MUT6 showed reasonable titer relative to M259, and MUT6 had a relative half-life comparable to M259. MUT22 also showed heparin affinity levels comparable to M259, while MUT21 showed a reduction in heparin affinity.

Example 3

[00259] This Example describes an initial screening to identify modifications in M259 which reduce aggregation while stabilizing the neighboring beta propeller motifs to increase activity and/or titer. The crystal structure was determined for M259-based human Neu2 using protein expressed and purified from *E coli*. Specifically, pairs of amino acids known to be in proximity to each other based on the crystal structure of human Neu2 were changed in tandem to cysteines so that a novel disulfide bond could form. Expression and activity levels for the resulting mutant sialidases were evaluated as described in Example 1 and are recorded in **TABLE 12**.

TABLE 12

	Additional Mutations to M259	**Titer**	**4-MU Activity**	**Heparin Affinity**	**K562 Desialylation**
M259	**none**	**XX**	**XX**	**XXX**	**XX**
MUT7	I277C-L337C	XXX	X	XX	X
MUT17	S155C-P190C	X	XXX	n/a	XXXX
MUT23	I277C-L337C, S155C-P190C	XX	X	n/a	n/a

[00260] As seen in **TABLE 12**, most mutants showed at least comparable titer to M259. MUT17 showed improved 4-MU activity relative to M259 and increased desialylation activity in K562 cell-based assays compared to M259.

Example 4

[00261] This Example describes an initial screening to identify modifications in M106 (a recombinant human Neu2 sialidase including the substitutions M1D, V6Y, P62G, A93E, I187K, and C332A) that improve the aggregation propensity of mutant sialidases. Specifically, hydrophobic patches identified by *in silico* modeling of the sialidase were modified by introducing negatively charged amino acid residues. Expression and activity levels for the resulting mutant sialidases were evaluated as described in Example 1 and are recorded in **TABLE 13**.

TABLE 13

	Additional Mutations to M106	**Titer**	**4-MU Activity**	**Heparin Affinity**
M106	**none**	**X**	**X**	**XXX**
MUT8	H300D, W302D	XX	X	XX
MUT9	H300D, W302E	XX	XX	XX
MUT10	R241E	X	not active	XX
MUT11	W302A	XX	X	XXX
MUT12	W302D	XX	n/a	XXX
MUT13	W302E	XX	XX	XXX
MUT14	W302F	XX	X	XXX
MUT15	W302I	XX	n/a	XXX
MUT16	W302V	XX	n/a	XXX

[00262] Several of the sialidase mutants showed improved titer and decreased affinity to heparin compared to M106. MUT8, MUT9, and MUT10 showed decreased heparin affinity compared to M106. Comparing the 4-MU activity of these 3 mutants, MUT9 showed improved activity compared to M106, while MUT8 showed comparable activity.

Example 5

[00263] This Example describes the evaluation of certain mutations evaluated in Example 4, but in a M259 mutant background instead of an M106 background. Specifically, the mutations which showed the largest impact on heparin affinity were selected to further evaluate their impact on the half-life of the mutant sialidases. Some mutants incorporated an

I187E mutation instead of the I187K mutation found in M259. Expression and activity levels for the resulting mutant sialidases were evaluated as described in Example 1 and are recorded in **TABLE 14**.

TABLE 14

	Additional Mutations to M259	**Titer**	**4-MU Activity**	**Heparin Affinity**	**Relative Half-Life**	**K562 Desialylation**
M259	**none**	**XX**	**XX**	**XXX**	**X**	**XX**
MUT18	I187E, R241D, H300D, W302D	X	XXX	X	X	not active
MUT19	I187E, R314D	X	XXX	XXX	XX	n/a
MUT20	I187E, R314E	X	XXX	XXX	n/a	n/a

[00264] MUT18, MUT19, and MUT20 exhibited reduced titer but increased 4-MU activity compared to M259. Furthermore, MUT19 showed improvements in relative half-life compared to other sialidases.

Example 6

[00265] This Example describes a screening to identify additional modifications in M259 which improve the desialylation activity of the mutant sialidases. Specifically, tandem cysteine residues described in Example 3 and modifications of hydrophobic patches described in Example 4 were combined. Some mutants incorporated an I187E mutation instead of the I187K mutation found in M259. Expression and activity levels for the resulting mutant sialidases were evaluated as described in Example 1 and are recorded in **TABLE 15**.

TABLE 15

	Additional Mutations to M259	**Titer**	**4-Mu Activity**
M259	**none**	**XX**	**XX**
MUT24	H300D, W302E, S155C-P190C	XX	XXXX
MUT25	I277C-L337C, H300D, W302E, S155C-P190C	XX	XXX
MUT26	I187E, H300D W302E, S155C-P190C	X	XXXX
MUT27	I187E, I277C-L337C, H300D, W302E, S155C-P190C	X	XXXX

[00266] All new sialidase mutants showed an improvement in 4-MU activity compared to M259. MUT24 and MUT25 also maintained comparable titer to M259.

Example 7

[00267] This Example describes further modifications to the sialidase mutants described in Examples 3 and 4. Specifically, either R241Y or R241D was also introduced to mutants containing tandem cysteine residues, modifications of hydrophobic patches, and combinations. Some mutants incorporated an I187E mutation instead of the I187K mutation found in M259. Expression and activity levels for the resulting mutant sialidases were evaluated as described in Example 1 and are recorded in **TABLE 16**.

TABLE 16

	Additional Mutations to M259	**Titer**	**4-MU Activity**	**Heparin Affinity**	**Relative Half-Life**	**K562 Desialylation**
M259	**none**	**XX**	**XX**	**XXX**	**X**	**XX**
MUT28	R241D	X	XX	n/a	n/a	not active
MUT29	R241D, I187E	X	XXX	n/a	n/a	not active
MUT30	R241D, I187E, H300D, W302E	X	XXX	n/a	n/a	not active
MUT31	R241D, I187E, H300D, W302E, S155C-P190C	X	XXXX	X	n/a	X
MUT32	R241D, I187E, H300D, W302E, S155C-P190C, I277C-L337C	X	XXXX	n/a	X	X
MUT33	R241Y	X	XX	n/a	n/a	not active
MUT34	R241Y, I187E	X	XX	n/a	X	not active
MUT35	R241Y, I187E, H300D, W302E	no expression	n/a	n/a	X	n/a

	Additional Mutations to M259	Titer	4-MU Activity	Heparin Affinity	Relative Half-Life	K562 Desialylation
MUT36	R241Y, I187E, H300D, W302E, S155C-P190C	X	XXXX	X	XX	X
MUT37	R241Y, I187E, H300D, W302E, S155C-P190C, I277C-L337C	no expression	n/a	n/a	n/a	n/a

[00268] All new mutants showed either reduction in titer compared to M259 or no expression. However, in the 4-MU activity assay, all but MUT35 and MUT37, which did not express, showed at least comparable activity to M259 in a 4-MU assay, while several mutants exhibited greater activity than M259. In addition, MUT36 showed a reduction in its heparin affinity and increased half-life compared to M259.

Example 8

[00269] This Example describes further modifications to the sialidase mutants described in Examples 3 and 4. Specifically, R241 mutations were introduced into mutants containing tandem cysteine residues, modifications of hydrophobic patches, and combinations. Some constructs incorporated an I187E or I187D mutation instead of the I187K mutation found in M259. Expression and activity levels for the resulting mutant sialidases were evaluated as described in Example 1 and are recorded in **TABLE 17**.

TABLE 17

	Additional Mutations to M259	Titer	4-MU Activity	Heparin Affinity	Relative Half-Life	K562 Desialylation
M259	**none**	**XX**	**XX**	**XXX**	**X**	**XX**
MUT38	H300D, W302E, I187E	X	XX	n/a	n/a	X

	Additional Mutations to M259	Titer	4-MU Activity	Heparin Affinity	Relative Half-Life	K562 Desialylation
MUT39	H300D, W302E, I187D	no expression	n/a	n/a	n/a	n/a
MUT40	H300D, W302E, I187E, R241E	X	XXXX	n/a	n/a	not active
MUT41	H300D, W302E, I187E, R241Y	X	XXXX	X	X	not active
MUT42	H300D, W302E, I187E, R241D	X	XXX	n/a	n/a	not active
MUT43	I277C-L337C, H300D, W302E	XX	not active	XX	n/a	not active
MUT44	I277C-L337C, H300D, W302E, I187E	no expression	n/a	n/a	n/a	n/a
MUT45	I277C-L337C, H300D, W302E, I187D	XX	XX	XX	n/a	not active
MUT46	I277C-L337C, H300D, W302E, I187E, R241E	X	X	X	n/a	not active
MUT47	I277C-L337C, H300D, W302E, I187E, R241Y	X	X	X	n/a	not active
MUT48	I277C-L337C, H300D, W302E, I187E, R241D	X	X	X	n/a	not active
MUT49	I277C-L337C, S155C-P190C, H300D, W302E, I187D	XX	XXXX	XX	XX	XX

	Additional Mutations to M259	Titer	4-MU Activity	Heparin Affinity	Relative Half-Life	K562 Desialylation
MUT50	I277C-L337C, S155C-P190C, H300D, W302E, I187E, R241E	X	XXXX	XX	X	XX
MUT51	I277C-L337C, S155C-P190C, H300D, W302E, I187E, R241Y	X	XXX	X	X	XX
MUT52	I277C-L337C, S155C-P190C, H300D, W302E, I187E, R241D	X	XXXX	XX	X	X
MUT53	S155C-P190C, H300D, W302E, V276L	X	XXXX	XXX	XX	XXX
MUT54	S155C-P190C, H300D, W302E, I187E, V276L	X	XXXX	XX	XX	X
MUT55	S155C-P190C, H300D, W302E, I187D, V276L	X	XXXX	XX	XX	X
MUT56	S155C-P190C, H300D, W302E, I187E, R241E, V276L	X	XXXX	X	XX	X
MUT57	S155C-P190C, H300D, W302E, I187E, R241Y, V276L	X	XXXX	X	XX	X

	Additional Mutations to M259	Titer	4-MU Activity	Heparin Affinity	Relative Half-Life	K562 Desialylation
MUT58	S155C-P190C, H300D, W302E, I187E, R241D, V276L	X	XXXX	X	XX	X
MUT59	S155C-P190C, H300D, W302E, I187D, R241E, V276L	no expression	n/a	n/a	n/a	n/a
MUT60	S155C-P190C, H300D, W302E, I187D, R241Y, V276L	X	XXXX	X	XX	X
MUT61	S155C-P190C, H300D, W302E, I187D, R241D, V276L	no expression	n/a	n/a	n/a	n/a

[00270] MUT40, MUT41, MUT42, MUT49, MUT50, MUT51, MUT52, MUT53, MUT54, MUT55, MUT56, MUT57, MUT58, and MUT60 showed increased 4-MU activity compared to M259. MUT41, MUT43, MUT45, MUT46, MUT47, MUT48, MUT49, MUT50, MUT51, MUT52, MUT54, MUT55, MUT56, MUT57, MUT58, and MUT60 showed reduced affinity to heparin compared to M259.

Example 9

[00271] This Example describes further evaluation and modifications to sialidases which showed reduced heparin affinity. Specifically, various combinations of the mutations screened in Examples 2, 4, 5, 7, and 8 were evaluated for their affinity to heparin and desialylation activity. Some constructs incorporated an I187E or I187D mutation instead of the I187K mutation found in M259. Expression and activity levels for the resulting mutant sialidases were evaluated as described in Example 1 and are recorded in **TABLE 18**.

TABLE 18

	Additional Mutations to M259	Titer	4-Mu Activity	Heparin Affinity	Relative Half-Life	K562 Desialylation
M259	**none**	**XX**	**XX**	**XXX**	**X**	**XX**
MUT62	I187E, R241D	X	XXX	n/a	n/a	not active
MUT63	I187E, R241E	X	XXX	X	n/a	X
MUT64	I187E, R241Y	X	XXX	XX	X	X
MUT65	I187D, R241D	XX	XXX	XX	XX	X
MUT66	I187D, R241E	X	XXXX	X	n/a	X
MUT67	I187D, R241Y	XX	XXX	XX	X	X
MUT68	I187E, H300D, W302D	XX	XXXX	XX	n/a	X
MUT69	I187E, H300D, W302E	XX	XXXX	XX	n/a	X
MUT70	I187D, H300D, W302D	XX	XXXX	XX	n/a	X
MUT71	I187D, H300D, W302E	XX	XXX	XX	n/a	X
MUT72	R241E, H300D, W302D	X	XXXX	XX	X	not active
MUT73	R241D, H300D, W302D	XX	XXX	XX	n/a	not active
MUT74	R241Y, H300D, W302D	XX	XXX	XX	X	not active
MUT75	R241E, H300D, W302E	XX	XXXX	XX	n/a	not active
MUT76	R241D, H300D, W302E	XX	XXX	XX	n/a	not active

	Additional Mutations to M259	Titer	4-Mu Activity	Heparin Affinity	Relative Half-Life	K562 Desialylation
MUT77	R241Y, H300D, W302E	XX	XXX	XX	n/a	not active

All mutants evaluated exhibited an increase in 4-MU activity, as well as a reduction in heparin affinity compared to M259. Furthermore, several of the mutants (MUT64, MUT67, MUT72, and MUT74) showed relative half-life stability comparable to M259, while MUT65 demonstrated an improvement in its half-life compared to other mutants.

Example 10

[00272] This Example describes further modifications of the sialidase mutants described in Example 4 with reduced heparin affinity. Specifically, mutations to the residue R314 were additionally introduced to the mutations described in Example 4 and **TABLE 13**. Expression and activity levels for the resulting mutant sialidases were evaluated as described in Example 1 and are recorded in **TABLE 19**.

TABLE 19

	Additional Mutations to M259	Titer	4-MU Activity	Heparin Affinity	Relative Half-Life	K562 Desialylation
M259	**none**	**XX**	**XX**	**XXX**	**X**	**XX**
MUT78	R314D	X	XX	XXX	XX	XX
MUT79	R314E, H300D, W302D	X	XXXX	XX	X	XX
MUT80	R314E, H300D, W302E	X	XXX	XX	X	XX

[00273] Mutants MUT79 and MUT80 showed an increase in 4-MU activity and reduced heparin affinity compared to M259. The half-life and desialylation activity according to the K562 cell-based assay indicated comparable levels between these three mutants.

Example 11

[00274] This Example describes the evaluation of sialidase constructs which were designed by incorporating the beneficial mutations found in the above Examples into M259. Specifically, the sialidases with the selected mutations exhibited high enzyme activity, titer, anti-heparin affinity, and extended half-life. Some constructs incorporated an I187E mutation instead of the I187K mutation found in M259. Expression and activity levels for the resulting mutant sialidases were evaluated as described in Example 1 and are recorded in **TABLE 20**.

TABLE 20

	Additional Mutations to M259	**Titer**	**4-Mu Activity**	**Heparin Affinity**	**Relative Half-Life**	**K562 Desialylation**
M259	**none**	**XX**	**XX**	**XXX**	**X**	**XX**
MUT81	S155C-P190C, I187E	X	XXXX	XX	XXX	XXX
MUT82	S155C-P190C, R314E	X	XXXX	XXX	XXX	XX
MUT83	S155C-P190C, I187E, R314E	X	XXXX	XX	XXX	XXX
MUT84	S155C-P190C, I187E, R314E, H300D-W302D	X	XXXX	X	n/a	XX
MUT85	S155C-P190C, I187E, R314E, H300D-W302E	X	XXXX	X	XXX	XX
MUT86	S155C-P190C, I187E, R314E, H300D-W302E, R241Y	X	XXXX	X	XXX	X
MUT87	S155C-P190C, I187E, V276L	X	XXXX	XX	n/a	XXX

	Additional Mutations to M259	Titer	4-Mu Activity	Heparin Affinity	Relative Half-Life	K562 Desialylation
MUT88	S155C-P190C, R314E, V276L	X	XXX	XXX	XX	XX
MUT89	S155C-P190C, I187E, R314E, V276L	X	XXXX	XX	XXX	XX
MUT90	S155C-P190C, I187E, R314E, R241Y	X	XXX	n/a	n/a	X
MUT91	S155C-P190C, I187E, R314E, R241D	X	XXXX	n/a	n/a	X

[00275] All mutants exhibited increased 4-MU activity and a reduction in titer relative to M259. Of the mutants evaluated for heparin affinity, MUT82 and MUT88 showed comparable heparin affinity to M259, while the other mutants showed a reduction in heparin affinity. Furthermore, MUT81 (SEQ ID NO: 15), MUT83 (SEQ ID NO: 16), and MUT87 showed greater desialylation activity than M259 in the K562 cell-based assay, while other mutants showed either similar (MUT82, MUT84 (SEQ ID NO: 13), MUT85 (SEQ ID NO: 14), MUT88, and MUT89 (SEQ ID NO: 17)) or reduced (MUT86, MUT90, and MUT91) activity as compared to M259.

Example 12

[00276] This Example describes the further characterization of sialidase mutants M106, M259, MUT81, and MUT85.

[00277] To determine the pharmacokinetics of the mutants *in vivo*, a sandwich ELISA-based assay was performed by evaluating the plasma of mice injected with the different mutants. Briefly, mouse plasma was drawn over the course of a 5-day PK study and a 15-day PK study. ELISA plates (Nunc) were coated overnight at 4 °C with an anti-human Neu2 sialidase antibody at 1.5 µg/mL in PBS and blocked in a protein-free blocking buffer (SciTek) for 2 hours at room temperature. Standards were prepared from purified mutant sialidases spiked

into naïve mouse plasma from a similar strain, with concentration from 10,000 ng/μL diluted 2-fold to 10 ng/μL. Samples were prepared by diluting early timepoints with naïve mouse plasma to be within the estimated linear range of the standards. Standards and samples were diluted 1:20 into a phosphate-buffered saline + Tween® (PBST) assay buffer supplemented with 1% BSA and added to assay plate for a 1 hour room temperature incubation. Samples were detected using an HRP-conjugated anti-human Fc antibody (Thermo) diluted 1:8000 in assay buffer and imaged on a SpectraMax plate reader following a 3,3',5,5'-tetramethylbenzidine (TMB)/acid stop development. Each standard and sample was run in replicate and the resulting average was used for data analysis.

[00278] **FIGURES 4A** and **4B** depict mutant sialidase levels in mouse plasma as detected by ELISA. Specifically, **FIGURE 4A** shows plasma levels of M106 and M259 at 0.5, 12, 24 and 48 hours post injection. **FIGURE 4B** shows plasma levels of M259, MUT81, and MUT85 at 0.5, 48, 72, 96, and 120 hours post injection. The dotted line in **FIGURE 4B** shows the plasma level (~5 x 10^3 ng/ml (or ~5 μg/ml)) required for the sialidase to be effective. MUT81 and MUT85 sustained a plasma level above this 5 μg/mL level for 4 or 5 days, respectively, while M106 and M259 fell below this level between 1 and 2 days.

[00279] A 15-day PK study was also conducted for M106, MUT81, and MUT85. For MUT81 and MUT85, a single injection of 10 mg/kg of either MUT81 or MUT85 was given to mice. Then, plasma from the injected mice was drawn at 0.5, 4 and 8 hours and 1, 2, 3, 5, 7, 10, and 15 days after injection to evaluate the levels of the sialidase by ELISA. For M106, a single injection of either 5 or 30 mg/kg was given, and serum was collected at 1, 4 and 8 hours and 1, 2, 3, 5, 7, 10 and 15 days after injection. The collected plasma was analyzed by ELISA to determine key PK parameters for each of the sialidase mutants and are recorded in **TABLE 21** below. Values below limit of quantification (BLQ) were excluded from analysis.

TABLE 21

Test Article	**M106**	**M259**	**MUT81**	**MUT85**
Dose (mg/kg)	5	30	10	10
T_{max} (h)	1.00	1.00	0.50	0.50
C_{max} (ng/mL)	127000	214000	62486.65	100752.61
C_0 (ng/mL)	n/a	n/a	69989.78	106750.95

AUC_{last} (h*ng/mL)	71500	966000	1000513.72	2520532.16
AUC_{inf} (h*ng/mL)	73500	970000	1000539.94	2520549.54
$t_{1/2}$ (h)	4.91	11.0	8.35	7.12
Apparent $t_{1/2}$ (h) *	4.18	4.69	22.45	25.96
MRT_{inf} (h)	n/a	n/a	32.39	37.46
CL (mL/h/kg)	68.0	30.9	9.99	323.72
V_{ss} (mL/kg)	411	209	3.97	148.64

* Apparent $t_{1/2}$ = MRTinf * Ln (2); n/a not analyzed

MRT_{inf} (h) = mean residence time

CL = clearance

V_{ss} = volume of distribution under steady state conditions

[00280] MUT85 and MUT81 persisted longer in the plasma of the mice than did M106 at both doses evaluated, as determined by the calculated area under the curve values (AUC_{last} and AUC_{inf}). Both MUT85 and MUT81 also exhibited greater apparent half-life ($t_{1/2}$) compared to M106 at 5 mg/kg. These results are consistent with the PK profiles determined for MUT85 and MUT81 compared to M106 (and by extension M259) shown in **FIGURES 4A** and **4B**.

[00281] Comparing the two new sialidase mutants, MUT85 had higher exposures (C_{max} and AUCs) and showed lower clearance and smaller volume of distribution as compared to MUT81.

Example 13

[00282] This example further evaluates the mutations described in Example 12.

3D modeling of human Neu2

[00283] **FIGURE 1** is a 3-dimensional (3-D) space filling model of the crystal structure of human Neu2. As described above, the crystal structure was determined for M259-based human Neu2 using protein expressed and purified from *E coli*. Specific amino acid residues that have been shown to be involved in heparin binding are highlighted, including H300, W302, R314, R241, P190, S155, and I187. Also depicted is the sialidase

inhibitor 2,3-dehydro-2-deoxy-N-acetylneuraminic acid (DANA) bound to the catalytic site of the sialidase. The highlighted amino acid residues are mapped on the same face of the sialidase, suggesting a large, linear heparin binding site. This predicted heparin binding site is located in close proximity to the sialic acid-bound catalytic site, a relationship that helps explain why some mutations in the heparin binding sites also affect the enzymatic activity of the sialidase as evaluated by 4-MU activity and K562 cell-based desialylation activity assays.

Evaluating desialylation activity of MUT81 and MUT85

[00284] Further examination of the enzymatic activity and specificity of MUT81 and MUT85 was carried out. In particular, the desialylation activity and substrate specificity of MUT81 and MUT85 was compared to M106 for their relative ability to cleave three different substrates: alpha 2,3 sialo-lactose, alpha 2,6 sialo-lactose, or alpha 2,8 sialic acid dimer. 300 nmoles of substrate is incubated with 2 μg of each sialidase mutant in phosphate buffer at pH 5, pH 6 or pH 7.2 with BSA for 18 h at 37 °C. The reaction mixtures were then separated by thin layer chromatography (TLC) to resolve the starting material and any cleaved sialic acid product. TLC was performed using 1-butanol/acetic acid/water (2:1:1) as a mobile phase and plates were spray-stained using DPA (diphenylamine, phosphoric acid, aniline) solution in acetone stain. For evaluations at pH 5, the broad spectrum bacterial sialidase BiNanH2 was included as a positive control. A sample with substrate (Sub) with no sialidase was also run in each TLC for reference.

[00285] **FIGURE 5A** shows exemplary scans of TLC plates resolving each substrate and products derived from cleavage activity from the sialidases M106, MUT81, MUT85, and BiNanH2 at pH 5. **FIGURE 5B** shows exemplary scans of TLC plates resolving each substrate and products derived from cleavage activity from the sialidases M106, MUT81, MUT85, and BiNanH2 at pH 6 or pH 7.2. pH 5 is the optimum pH for sialidase activity for the human Neu2 based sialidases as well as the bacterial sialidase. M106, MUT81, and MUT85 exhibited efficient cleavage of alpha 2,3 sialo-lactose at pH 5, as seen by the bands elongated towards the top of the TLC plate in the samples with the different sialidases but not in the Sub sample (**FIGURE 5A**). MUT81 and MUT85 both showed increased cleavage efficiency of alpha 2,6 sialo-lactose and alpha 2,8 sialo-lactose as compared to M106, as evidenced by the remaining substrates in samples incubated with M106 but not with MUT81, MUT85, and BiNanH2. At higher pH levels of 6 or 7.2, MUT85, and to some extent MUT81, demonstrated improved cleavage of all three tested substrates compared to M106 and M259, as evidenced by the greater reduction of each substrate (located in the same level

as the band in Sub sample) and increased intensity of bands above the line separating the substrate from cleaved products (**FIGURE 5B**). The same mutations in MUT81 and MUT85 resulting in reduced heparin binding and improved PK also improved the overall activity and substrate specificity of these enzymes.

INCORPORATION BY REFERENCE

[00286] The entire disclosure of each of the patent and scientific documents referred to herein is incorporated by reference for all purposes.

EQUIVALENTS

[00287] The invention may be embodied in other specific forms without departing from the spirit or essential characteristics thereof. The foregoing embodiments are therefore to be considered in all respects illustrative rather than limiting on the invention described herein. Scope of the invention is thus indicated by the appended claims rather than by the foregoing description, and all changes that come within the meaning and range of equivalency of the claims are intended to be embraced therein.

SEQUENCE LISTING

[00288] SEQ ID NO: 1:

MASLPVLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAP
THQVQWQAQEVVAQARLDGHRSMNPCPLYDAQTGTLFLFFIAIPGQVTEQQQLQTRANVTRL
CQVTSTDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHP
IQRPIPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRARVQAQS
TNDGLDFQESQLVKKLVEPPPQGCQGSVISFPSPRSGPGSPAQWLLYTHPTHSWQRADLGAY
LNPRPPAPEAWSEPVLLAKGSCAYSDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQA
FPAEYLPQ

[00289] SEQ ID NO: 2:

ATGGCCAGCCTGCCTGTGCTGCAGAAAGAAAGCGTGTTCCAGTCTGGCGCCCACGCCTACAG
AATTCCCGCTCTGCTGTATCTGCCAGGCCAGCAGTCTCTGCTGGCTTTCGCTGAACAGCGGG
CCAGCAAGAAGGATGAGCACGCCGAACTGATCGTGCTGCGGAGAGGCGATTACGACGCCCCT
ACACATCAGGTGCAGTGGCAGGCTCAAGAGGTGGTGGCTCAGGCTAGACTGGACGGCCACAG
ATCTATGAACCCCTGTCCTCTGTACGATGCCCAGACCGGCACACTGTTTCTGTTCTTTATCG
CTATCCCCGGCCAAGTGACCGAGCAGCAGCAGCTGCAGACAAGAGCCAACGTGACCAGACTG
TGTCAAGTGACCTCCACCGACCACGGCAGAACCTGGTCTAGCCCTAGAGATCTGACCGACGC
CGCCATCGGACCTGCCTATAGAGAGTGGTCCACCTTCGCCGTTGGACCTGGACACTGTCTCC
AGCTGCACGACAGGGCTAGATCTCTGGTGGTGCCTGCCTACGCCTATAGAAAGCTGCACCCC
ATCCAGCGGCCTATTCCTAGCGCCTTCTGCTTTCTGAGCCACGATCACGGCAGGACATGGGC
CAGAGGACATTTCGTGGCCCAGGACACACTGGAATGCCAGGTGGCCGAAGTGGAAACCGGCG
AGCAGAGAGTCGTGACCCTGAACGCCAGATCTCACCTGAGAGCCAGAGTGCAGGCCCAGAGC
ACAAACGACGGCCTGGATTTCCAAGAGAGCCAGCTGGTCAAGAAACTGGTGGAACCTCCTCC
ACAGGGCTGTCAGGGAAGCGTGATCAGCTTTCCATCTCCTAGAAGCGGCCCTGGCTCTCCTG
CTCAGTGGCTGCTGTATACACACCCCACACACAGCTGGCAGAGAGCCGATCTGGGCGCCTAC
CTGAATCCTAGACCTCCTGCTCCTGAGGCTTGGAGCGAACCTGTTCTGCTGGCCAAGGGCAG
CTGTGCCTACAGCGATCTGCAGTCTATGGGCACAGGCCCTGATGGCAGCCCTCTGTTTGGCT
GTCTGTACGAGGCCAACGACTACGAAGAGATCGTGTTCCTGATGTTCACCCTGAAGCAGGCC
TTTCCAGCCGAGTACCTGCCTCAA

[00290] SEQ ID NO: 3:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAG
THQVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRL
CQVTSTDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHP
KQRPIPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRARVQAQS
TNDGLDFQESQLVKKLVEPPPQGCQGSVISFPSPRSGPGSPAQWLLYTHPTHSWQRADLGAY
LNPRPPAPEAWSEPVLLAKGSAAYSDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQA
FPAEYLPQ

[00291] SEQ ID NO: 4:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRRSKKDEHAELIVLRRGDYDAG
THQVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRL
CYVTSTDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHP
KQRPIPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRFRVQAQS

TNDGLDFQESQLVKKLVEPPPTGCQGSVISFPSPRSGPGSPAQWLLYTHPTHSWQRADLGAY
LNPRPPAPEAWSEPVLLAKGSAAYSDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQA
FPAEYLPQ

[00292] SEQ ID NO: 5:

MGWSCIILFLVATATGVHS

[00293] SEQ ID NO: 6:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRASKKDEHAELIVLRRGDYDAG
THQVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRL
CQVTSTDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHP
KQRPIPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRARVQAQS
TNDGLDFQESQLVKKLVEPPPQGCQGSVISFPSPRSGPGSPAQWLLYTHPTHSWQRADLGAY
LNPRPPAPEAWSEPVLLAKGSAAYSDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQA
FPAEYLPQEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00294] SEQ ID NO: 7:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRRSKKDEHAELIVLRRGDYDAG
THQVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRL
CYVTSTDHGRTWSSPRDLTDAAIGPAYREWSTFAVGPGHCLQLHDRARSLVVPAYAYRKLHP
KQRPIPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRFRVQAQS
TNDGLDFQESQLVKKLVEPPPTGCQGSVISFPSPRSGPGSPAQWLLYTHPTHSWQRADLGAY
LNPRPPAPEAWSEPVLLAKGSAAYSDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQA
FPAEYLPQEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00295] SEQ ID NO: 8:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRRSKKDEHAELIVLRRGDYDAG
THQVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRL
CYVTSTDHGRTWSSPRDLTDAAIGPAYREWCTFAVGPGHCLQLHDRARSLVVPAYAYRKLHP
EQRCIPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRFRVQAQS
TNDGLDFQESQLVKKLVEPPPTGCQGSVISFPSPRSGPGSPAQWLLYTHPTDSDQRADLGAY
LNPEPPAPEAWSEPVLLAKGSAAYSDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQA
FPAEYLPQ

[00296] SEQ ID NO: 9:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRRSKKDEHAELIVLRRGDYDAG
THQVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRL
CYVTSTDHGRTWSSPRDLTDAAIGPAYREWCTFAVGPGHCLQLHDRARSLVVPAYAYRKLHP
EQRCIPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRFRVQAQS

TNDGLDFQESQLVKKLVEPPPTGCQGSVISFPSPRSGPGSPAQWLLYTHPTDSEQRADLGAY
LNPEPPAPEAWSEPVLLAKGSAAYSDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQA
FPAEYLPQ

[00297] SEQ ID NO: 10:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRRSKKDEHAELIVLRRGDYDAG
THQVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRL
CYVTSTDHGRTWSSPRDLTDAAIGPAYREWCTFAVGPGHCLQLHDRARSLVVPAYAYRKLHP
EQRCIPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRFRVQAQS
TNDGLDFQESQLVKKLVEPPPTGCQGSVISFPSPRSGPGSPAQWLLYTHPTHSWQRADLGAY
LNPRPPAPEAWSEPVLLAKGSAAYSDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQA
FPAEYLPQ

[00298] SEQ ID NO: 11:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRRSKKDEHAELIVLRRGDYDAG
THQVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRL
CYVTSTDHGRTWSSPRDLTDAAIGPAYREWCTFAVGPGHCLQLHDRARSLVVPAYAYRKLHP
EQRCIPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRFRVQAQS
TNDGLDFQESQLVKKLVEPPPTGCQGSVISFPSPRSGPGSPAQWLLYTHPTHSWQRADLGAY
LNPEPPAPEAWSEPVLLAKGSAAYSDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQA
FPAEYLPQ

[00299] SEQ ID NO: 12:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRRSKKDEHAELIVLRRGDYDAG
THQVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRL
CYVTSTDHGRTWSSPRDLTDAAIGPAYREWCTFAVGPGHCLQLHDRARSLVVPAYAYRKLHP
EQRCIPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRFRVQAQS
TNDGLDFQESQLVKKLVEPPPTGCQGSLISFPSPRSGPGSPAQWLLYTHPTHSWQRADLGAY
LNPEPPAPEAWSEPVLLAKGSAAYSDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQA
FPAEYLPQ

[00300] SEQ ID NO: 13:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRRSKKDEHAELIVLRRGDYDAG
THQVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRL
CYVTSTDHGRTWSSPRDLTDAAIGPAYREWCTFAVGPGHCLQLHDRARSLVVPAYAYRKLHP
EQRCIPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRFRVQAQS
TNDGLDFQESQLVKKLVEPPPTGCQGSVISFPSPRSGPGSPAQWLLYTHPTDSDQRADLGAY
LNPEPPAPEAWSEPVLLAKGSAAYSDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQA
FPAEYLPQEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00301] SEQ ID NO: 14:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRRSKKDEHAELIVLRRGDYDAG
THQVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRL

CYVTSTDHGRTWSSPRDLTDAAIGPAYREWCTFAVGPGHCLQLHDRARSLVVPAYAYRKLHP
EQRCIPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRFRVQAQS
TNDGLDFQESQLVKKLVEPPPTGCQGSVISFPSPRSGPGSPAQWLLYTHPTDSEQRADLGAY
LNPEPPAPEAWSEPVLLAKGSAAYSDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQA
FPAEYLPQEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00302] SEQ ID NO: 15:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRRSKKDEHAELIVLRRGDYDAG
THQVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRL
CYVTSTDHGRTWSSPRDLTDAAIGPAYREWCTFAVGPGHCLQLHDRARSLVVPAYAYRKLHP
EQRCIPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRFRVQAQS
TNDGLDFQESQLVKKLVEPPPTGCQGSVISFPSPRSGPGSPAQWLLYTHPTHSWQRADLGAY
LNPRPPAPEAWSEPVLLAKGSAAYSDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQA
FPAEYLPQEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00303] SEQ ID NO: 16:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRRSKKDEHAELIVLRRGDYDAG
THQVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRL
CYVTSTDHGRTWSSPRDLTDAAIGPAYREWCTFAVGPGHCLQLHDRARSLVVPAYAYRKLHP
EQRCIPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRFRVQAQS
TNDGLDFQESQLVKKLVEPPPTGCQGSVISFPSPRSGPGSPAQWLLYTHPTHSWQRADLGAY
LNPEPPAPEAWSEPVLLAKGSAAYSDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQA
FPAEYLPQEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00304] SEQ ID NO: 17:

DASLPYLQKESVFQSGAHAYRIPALLYLPGQQSLLAFAEQRRSKKDEHAELIVLRRGDYDAG
THQVQWQAQEVVAQARLDGHRSMNPCPLYDEQTGTLFLFFIAIPGQVTEQQQLQTRANVTRL
CYVTSTDHGRTWSSPRDLTDAAIGPAYREWCTFAVGPGHCLQLHDRARSLVVPAYAYRKLHP
EQRCIPSAFCFLSHDHGRTWARGHFVAQDTLECQVAEVETGEQRVVTLNARSHLRFRVQAQS
TNDGLDFQESQLVKKLVEPPPTGCQGSLISFPSPRSGPGSPAQWLLYTHPTHSWQRADLGAY
LNPEPPAPEAWSEPVLLAKGSAAYSDLQSMGTGPDGSPLFGCLYEANDYEEIVFLMFTLKQA
FPAEYLPQEPKSSDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHE
DPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAP
IEKTISKAKGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKT
TPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00305] SEQ ID NO: 18:

MEDLRP

[00306] SEQ ID NO: 19:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR
EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00307] SEQ ID NO: 20:

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00308] SEQ ID NO: 21:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV
MHEALHNHYTQKSLSLSPGK

[00309] SEQ ID NO: 22:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR
EPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
TSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00310] SEQ ID NO: 23:

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSREEMTKNQVSLTCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLTSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00311] SEQ ID NO: 24:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLTSKLTVDKSRWQQGNVFSCSV
MHEALHNHYTQKSLSLSPGK

[00312] SEQ ID NO: 25:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLTSKLTVDKSRWQQGNVFSCSV
MHEALHNHYTQKSLSLSPGK

[00313] SEQ ID NO: 26:

EPKSCDKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNW
YVDGVEVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKA
KGQPREPQVYTLPPSREEMTKNQVSLYCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSD
GSFFLYSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00314] SEQ ID NO: 27:

DKTHTCPPCPAPELLGGPSVFLFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGV
EVHNAKTKPREEQYNSTYRVVSVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPR
EPQVYTLPPSREEMTKNQVSLYCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFL
YSKLTVDKSRWQQGNVFSCSVMHEALHNHYTQKSLSLSPGK

[00315] SEQ ID NO: 28:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYNSTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
YCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV
MHEALHNHYTQKSLSLSPGK

[00316] SEQ ID NO: 29:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
YCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV
MHEALHNHYTQKSLSLSPGK

[00317] SEQ ID NO: 30:

ASTKGPSVFPLAPSSKSTSGGTAALGCLVKDYFPEPVTVSWNSGALTSGVHTFPAVLQSSGL
YSLSSVVTVPSSSLGTQTYICNVNHKPSNTKVDKKVEPKSCDKTHTCPPCPAPELLGGPSVF
LFPPKPKDTLMISRTPEVTCVVVDVSHEDPEVKFNWYVDGVEVHNAKTKPREEQYASTYRVV
SVLTVLHQDWLNGKEYKCKVSNKALPAPIEKTISKAKGQPREPQVYTLPPSREEMTKNQVSL
TCLVKGFYPSDIAVEWESNGQPENNYKTTPPVLDSDGSFFLYSKLTVDKSRWQQGNVFSCSV
MHEALHNHYTQKSLSLSPGK

[00318] SEQ ID NO: 31:

GGGGS

[00319] SEQ ID NO: 32:

(GGGGS)n

[00320] SEQ ID NO: 33:

GGGGSGGGGS

[00321] SEQ ID NO: 34:

EPKSS

[00322] SEQ ID NO: 35:

MDMRVPAQLLGLLLLWLPGARC

[00323] SEQ ID NO: 36:

YGTL

[00324] SEQ ID NO: 37:

HHHHHHHHHH

WHAT IS CLAIMED IS:

1. A recombinant human Neu2 enzyme having reduced binding affinity to heparin, the recombinant human Neu2 enzyme comprising a heparin-binding site comprising at least one amino acid selected from the group consisting of:

(a) an isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 (I187),

(b) a histidine residue at a position corresponding to position 300 of wild-type human Neu2 (H300), and

(c) an arginine residue at a position corresponding to position 314 of wild-type human Neu2 (R314),

wherein the at least one amino acid is substituted with an amino acid having less positive charge character under biologically compatible conditions, and

wherein the reduced binding affinity is relative to an otherwise identical human Neu2 enzyme that does not comprise the substitution of the at least one amino acid.

2. A recombinant human Neu2 enzyme having an enhanced serum half-life, the recombinant human Neu2 enzyme comprising a substitution of at least one amino acid selected from the group consisting of:

(a) an isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 (I187),

(b) a histidine residue at a position corresponding to position 300 of wild-type human Neu2 (H300), and

(c) an arginine residue at a position corresponding to position 314 of wild-type human Neu2 (R314),

wherein the at least one amino acid is substituted with an amino acid with a less positive charge character under biologically compatible conditions, and

wherein the enhanced serum half-life is relative to an otherwise identical human Neu2 enzyme that does not comprise the substitution of the at least one amino acid.

3. The recombinant human Neu2 enzyme of claim 2, wherein the recombinant human Neu2 enzyme exhibits at least a two-fold increase in serum half-life relative to an otherwise

identical human Neu2 enzyme that does not comprise the substitution of the at least one amino acid.

4. A recombinant human Neu2 enzyme comprising at least one amino acid substitution selected from the group consisting of:

(a) a substitution of an isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 (I187) with a negatively charged amino acid residue;

(b) a substitution of a histidine residue at a position corresponding to position 300 of wild-type human Neu2 (H300) with a negatively charged amino acid residue; and

(c) a substitution of an arginine residue at a position corresponding to position 314 of wild-type human Neu2 (R314) with a negatively charged amino acid residue.

5. The recombinant human Neu2 enzyme of any one of claims 1-4, further comprising a substitution of a serine residue at a position corresponding to position 155 of wild-type human Neu2 with a cysteine (S155C), and a substitution of a proline residue at a position corresponding to position 190 of wild-type human Neu2 with a cysteine (P190C).

6. The recombinant human Neu2 enzyme of claim 5, further comprising a substitution of an isoleucine residue at a position corresponding to position 277 of wild-type human Neu2 with a cysteine (I277C), and a substitution of a leucine residue at a position corresponding to position 337 of wild-type human Neu2 with a cysteine (L337C).

7. The recombinant human Neu2 enzyme of any one of claims 1-6, wherein the substitution of the isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 (I187) is an aspartic acid (D) or a glutamic acid (E).

8. The recombinant human Neu2 enzyme of any one of claims 1-7, wherein the substitution of the histidine residue at a position corresponding to position 300 of wild-type human Neu2 (H300) is an aspartic acid (D) or a glutamic acid (E).

9. The recombinant human Neu2 enzyme of any one of claims 1-8, wherein the substitution of the arginine residue at a position corresponding to position 314 of wild-type human Neu2 (R314) is an aspartic acid (D) or a glutamic acid (E).

10. The recombinant human Neu2 enzyme of any one of claims 1-9, wherein, when the recombinant human Neu2 enzyme has a substitution of a histidine residue at a position corresponding to position 300 of wild-type human Neu2 (H300), the recombinant human Neu2 enzyme further comprises a substitution of a tryptophan at a position corresponding to 302 of wild-type human Neu2 (W302) with an aspartic acid (D) or a glutamic acid (E).

11. The recombinant human Neu2 enzyme of any one of claims 1-10, further comprising a substitution of an arginine residue at a position corresponding to position 241 of wild-type human Neu2 (R241) with an aspartic acid (D), glutamic acid (E), or a tyrosine (Y).

12. The recombinant human Neu2 enzyme of any one of claims 1-11, further comprising a substitution of a valine residue at a position corresponding to position 276 of wild-type human Neu2 (V276) with a hydrophobic amino acid residue.

13. The recombinant human Neu2 enzyme of claim 12, wherein the hydrophobic acid is a leucine (L).

14. The recombinant human Neu2 enzyme of any one of claims 1-13, wherein the recombinant human Neu2 enzyme comprises one or more of the following substitutions: S155C, P190C, and I187E.

15. The recombinant human Neu2 enzyme of claim 14, wherein the recombinant human Neu2 enzyme comprises the following substitutions: S155C, P190C, and I187E.

16. The recombinant human Neu2 enzyme of claim 14 or 15, wherein the recombinant human Neu2 enzyme further comprises the following substitutions: M1D, V6Y, A42R, P62G, A93E, Q126Y, A242F, Q270T, and C332A.

17. The recombinant human Neu2 enzyme of any one of claims 1-13, wherein the recombinant human Neu2 enzyme comprises one or more of the following substitutions: S155C, P190C, I187E, and R314E.

18 The recombinant human Neu2 enzyme of claim 17, wherein the recombinant human Neu2 enzyme comprises the following substitutions: S155C, P190C, I187E, and R314E.

19. The recombinant human Neu2 enzyme of claim 17 or 18 , wherein the recombinant human Neu2 enzyme further comprises the following substitutions: M1D, V6Y, A42R, P62G, A93E, Q126Y, A242F, Q270T, and C332A.

20. The recombinant human Neu2 enzyme of any one of claims 1-13, wherein the recombinant human Neu2 enzyme comprises one or more of the following substitutions: S155C, P190C, I187E, R314E, H300D, and W302D.

21. The recombinant human Neu2 enzyme of claim 20, wherein the recombinant human Neu2 enzyme comprises the following substitutions: S155C, P190C, I187E, R314E, H300D, and W302D.

22. The recombinant human Neu2 enzyme of claim 20 or claim 21, wherein the recombinant human Neu2 enzyme further comprises the following substitutions: M1D, V6Y, A42R, P62G, A93E, Q126Y, A242F, Q270T, and C332A.

23. The recombinant human Neu2 enzyme of any one of claims 1-13, wherein the recombinant human Neu2 enzyme comprises one or more of the following substitutions: S155C, P190C, I187E, R314E, H300D, and W302E.

24. The recombinant human Neu2 enzyme of claim 23, wherein the recombinant human Neu2 enzyme comprises the following substitutions: S155C, P190C, I187E, R314E, H300D, and W302E.

25. The recombinant human Neu2 enzyme of claim 23 or claim 24, wherein the recombinant human Neu2 enzyme further comprises the following substitutions: M1D, V6Y, A42R, P62G, A93E, Q126Y, A242F, Q270T, and C332A.

26. The recombinant human Neu2 enzyme of any one of claims 17-19, wherein the recombinant human Neu2 enzyme further comprises a substitution of a valine residue at a position corresponding to position 276 of wild-type human Neu2 with a leucine (V276L).

27. The recombinant human Neu2 enzyme of any one of claims 1-26, wherein the recombinant human Neu2 enzyme comprises an amino acid sequence having at least 90% sequence identity to an amino acid sequence selected from the group consisting of SEQ ID NOs: 8-12.

28. A recombinant human Neu2 enzyme comprising: a substitution of a serine residue at a position corresponding to position 155 of wild-type human Neu2 with a cysteine (S155C), and a substitution of a proline residue at a position corresponding to position 190 of wild-type human Neu2 with a cysteine (P190C).

29. The recombinant human Neu2 enzyme of claim 28, further comprising a substitution of an isoleucine residue at a position corresponding to position 277 of wild-type human Neu2 with a cysteine (I277C), and a substitution of a leucine residue at a position corresponding to position 337 of wild-type human Neu2 with a cysteine (L337C).

30. The recombinant human Neu2 enzyme of any one of claims 1-29, wherein the enzyme is enzymatically active to remove a terminal sialic acid residue from a glycan moiety.

31. The recombinant human Neu2 enzyme of claim 30, wherein the glycan moiety is a glycoprotein or glycolipid.

32. A fusion protein comprising:

(a) the recombinant human Neu2 enzyme of any one of claims 1-31; and

(b) an immunoglobulin Fc domain and/or an immunoglobulin antigen-binding domain.

33. The fusion protein of claim 32, wherein the fusion protein comprises an immunoglobulin Fc domain.

34. The fusion protein of claim 33, wherein the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM Fc domain.

35. The fusion protein of claim 34, wherein the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, or IgG4 Fc domain.

36. The fusion protein of claim 35, wherein the immunoglobulin Fc domain is derived from a human IgG1 Fc domain.

37. The fusion protein of any one of claims 32-36, wherein the fusion protein comprises an immunoglobulin antigen-binding domain.

38. The fusion protein of claim 37, wherein the immunoglobulin antigen-binding domain is associated with a second immunoglobulin antigen-binding domain to produce an antigen-binding site.

39. An antibody conjugate comprising the fusion protein of any one of claims 32-38.

40. The antibody conjugate of claim 39, wherein the antibody conjugate comprises a single recombinant human Neu2 enzyme.

41. The antibody conjugate of claim 39, wherein the antibody conjugate comprises two recombinant human Neu2 enzymes.

42. The antibody conjugate of claim 41, wherein the two recombinant human Neu2 enzymes are identical.

43. The antibody conjugate of any one of claims 39-42, wherein the antibody conjugate comprises a single antigen-binding site.

44. The antibody conjugate of any one of claims 39-42, wherein the antibody conjugate comprises two antigen-binding sites.

45. The antibody conjugate of claim 44, wherein the two antigen-binding sites are identical.

46. The antibody conjugate of claim 44, wherein the two antigen-binding sites are different.

47. The antibody conjugate of any one of claims 39-46, wherein the antibody conjugate comprises:

(a) a first polypeptide comprising an immunoglobulin light chain;

(b) a second polypeptide comprising an immunoglobulin heavy chain; and

(c) a third polypeptide comprising an immunoglobulin Fc domain and the recombinant human Neu2 enzyme;

wherein the first and second polypeptides are covalently linked together and the second and third polypeptides are covalently linked together, and wherein the first polypeptide and the second polypeptide together define an antigen-binding site.

48. The antibody conjugate of claim 47, wherein the third polypeptide comprises the human Neu2 enzyme and the immunoglobulin Fc domain in an N- to C-terminal orientation.

49. The antibody conjugate of any one of claims 41-46, wherein the fusion protein comprises:

(a) a first polypeptide comprising a first immunoglobulin light chain;

(b) a second polypeptide comprising a first immunoglobulin heavy chain and a first recombinant human Neu2 enzyme;

(c) a third polypeptide comprising a second immunoglobulin heavy chain and a second recombinant human Neu2 enzyme; and

(d) a fourth polypeptide comprising a second immunoglobulin light chain;

wherein the first and second polypeptides are covalently linked together, the third and fourth polypeptides are covalently linked together, and the second and third polypeptides are covalently linked together, and wherein the first polypeptide and the second polypeptide together define a first antigen-binding site, and the third polypeptide and the fourth polypeptide together define a second antigen-binding site.

50. The antibody conjugate of claim 49, wherein the second and third polypeptides comprise the first and second immunoglobulin heavy chain and the first and second recombinant human Neu2 enzyme, respectively, in an N- to C-terminal orientation.

51. The antibody conjugate of any one of claims 41-46, wherein the antibody conjugate comprises:

(a) a first polypeptide comprising a first recombinant human Neu2 enzyme, a first immunoglobulin Fc domain, and a first single chain variable fragment (scFv); and

(b) a second polypeptide comprising a second recombinant human Neu2 enzyme, a

second immunoglobulin Fc domain, and a second single chain variable fragment (scFv);

wherein the first and second polypeptides are covalently linked together, and wherein the first scFv defines a first antigen-binding site, and the second scFv defines a second antigen-binding site.

52. The antibody conjugate of claim 51, wherein the first polypeptide comprises the first recombinant human Neu2 enzyme, the first immunoglobulin Fc domain, and the first scFv in an N- to C-terminal orientation, and the second polypeptide comprises the second recombinant human Neu2 enzyme, the second immunoglobulin Fc domain, and the second scFv in an N- to C-terminal orientation.

53. The antibody conjugate of any one of claims 39-46, wherein the antibody conjugate comprises:

(a) a first polypeptide comprising an immunoglobulin light chain;

(b) a second polypeptide comprising an immunoglobulin heavy chain and a single chain variable fragment (scFv); and

(c) a third polypeptide comprising an immunoglobulin Fc domain and a recombinant human Neu2 enzyme;

wherein the first and second polypeptides are covalently linked together and the second and third polypeptides are covalently linked together, and wherein the immunoglobulin light chain and immunoglobulin heavy chain together define a first antigen-binding site and the scFv defines a second antigen-binding site.

54. The antibody conjugate of claim 53, wherein the second polypeptide comprises the immunoglobulin heavy chain and the scFv in an N- to C-terminal orientation, and the third polypeptide comprises the recombinant human Neu2 enzyme and the immunoglobulin Fc domain in an N- to C-terminal orientation.

55. An isolated nucleic acid comprising a nucleotide sequence encoding the recombinant human Neu2 enzyme of any one of claims 1-31, the fusion protein of any one of claims 32-38, or at least a portion of the antibody conjugate of any one of claims 39-54.

56. An expression vector comprising the nucleic acid of claim 55.

57. A host cell comprising the expression vector of claim 56.

58. A pharmaceutical composition comprising the recombinant enzyme of any one of claims 1-31, the fusion protein of any one of claims 32-38, or the antibody conjugate of any one of claims 39-54 and a pharmaceutically acceptable carrier.

59. A method of treating cancer in a subject in need thereof, the method comprising administering to the subject an effective amount of the recombinant enzyme of any one of claims 1-31, the fusion protein of any one of claims 32-38, or the antibody conjugate of any one of claims 39-54, or the pharmaceutical composition of claim 58.

60. The method of claim 59, wherein the cancer is a solid tumor, soft tissue tumor, hematopoietic tumor, or metastatic lesion.

61. The method of claim 60, wherein the solid tumor is a sarcoma, adenocarcinoma, or carcinoma.

62. The method of claim 60 or 61, wherein the solid tumor is a head and neck (*e.g.,* pharynx), thyroid, lung (*e.g.,* small cell or non-small cell lung carcinoma (NSCLC)), breast, lymphoid, gastrointestinal (*e.g.*, oral, esophageal, stomach, liver, pancreas, small intestine, colon and rectum, anal canal), genital or genitourinary tract (*e.g.*, renal, urothelial, bladder, ovarian, uterine, cervical, endometrial, prostate, testicular), CNS (*e.g.,* neural or glial cell, *e.g.*, neuroblastoma or glioma), or skin (*e.g.,* melanoma) tumor.

63. The method of claim 62, wherein the hematopoietic tumor is a leukemia, acute leukemia, acute lymphoblastic leukemia (ALL), B-cell, T-cell or FAB ALL, acute myeloid leukemia (AML), chronic myelocytic leukemia (CML), chronic lymphocytic leukemia (CLL), *e.g.*, transformed CLL, diffuse large B-cell lymphoma (DLBCL), follicular lymphoma, hairy cell leukemia, myelodysplastic syndrome (MDS), lymphoma, Hodgkin's disease, malignant lymphoma, non-Hodgkin's lymphoma, Burkitt's lymphoma, multiple myeloma, or Richter's Syndrome (Richter's Transformation).