

[0005] Cancer immunotherapy with immune checkpoint inhibitors, including antibodies blocking the PD-1/PD-L1 pathway, has improved the outcome of many cancer patients. However, despite advances that have been made to date, many patients do not respond to currently available immune checkpoint inhibitors. Accordingly, there is still a need for effective interventions that overcome the immune suppressive tumor microenvironment and for treating cancers associated with hypersialylated cancer cells.

SUMMARY ~~OF THE INVENTION~~

[0006] The ~~invention~~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 are less sensitive to protease degradation than a corresponding sialidase enzyme without the mutation or mutations that render the sialidase less sensitive to protease degradation. The sialidase enzymes can be used in fusion proteins and/or antibody conjugates containing such sialidase enzymes that have suitable substrate specificities and activities to be useful in 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.

[0006a] In one aspect, the present disclosure refers to a recombinant mutant human sialidase enzyme, wherein the sialidase comprises: (a) a substitution of an alanine residue at a position corresponding to position 42 of wild-type human Neu2 (A42); (b) a substitution of a proline residue at a position corresponding to position 5 of wild-type human Neu2 (P5); (c) a substitution of a lysine residue at a position corresponding to position 9 of wild-type human Neu2 (K9); (d) a substitution of a lysine residue at a position corresponding to position 44 of wild-type human Neu2 (K44); (e) a substitution of a lysine residue at a position corresponding to position 45 of wild-type human Neu2 (K45); (f) a substitution of a leucine residue at a position corresponding to position 54 of wild-type human Neu2 (L54); (g) a substitution of a proline residue at a position corresponding to position 62 of wild-type human Neu2 (P62); (h) a substitution of a glutamine residue at a position corresponding to position 69 of wild-type human Neu2 (Q69); (i) a substitution of an arginine residue at a position corresponding to position 78 of wild-type human Neu2 (R78); (j) a substitution of an aspartic acid residue at a position corresponding to position 80 of wild-type human Neu2 (D80); (k) a substitution of an alanine residue at a position corresponding to position 93 of wild-type human Neu2 (A93); (l) a substitution of a glycine residue at a position

corresponding to position 107 of wild-type human Neu2 (G107); (m) a substitution of a glutamine residue at a position corresponding to position 108 of wild-type human Neu2 (Q108); (n) a substitution of a glutamine residue at a position corresponding to position 112 of wild-type human Neu2 (Q112); (o) a substitution of a cysteine residue at a position corresponding to position 125 of wild-type human Neu2 (C125); (p) a substitution of a glutamine residue at a position corresponding to position 126 of wild-type human Neu2 (Q126); (q) a substitution of an alanine residue at a position corresponding to position 150 of wild-type human Neu2 (A150); (r) a substitution of a cysteine residue at a position corresponding to position 164 of wild-type human Neu2 (C164); (s) a substitution of an arginine residue at a position corresponding to position 170 of wild-type human Neu2 (R170); (t) a substitution of an alanine residue at a position corresponding to position 171 of wild-type human Neu2 (A171); (u) a substitution of a glutamine residue at a position corresponding to position 188 of wild-type human Neu2 (Q188); (v) a substitution of an arginine residue at a position corresponding to position 189 of wild-type human Neu2 (R189); (w) a substitution of an alanine residue at a position corresponding to position 213 of wild-type human Neu2 (A213); (x) a substitution of a leucine residue at a position corresponding to position 217 of wild-type human Neu2 (L217); (y) a substitution of a glutamic acid residue at a position corresponding to position 225 of wild-type human Neu2 (E225); (z) a substitution of a histidine residue at a position corresponding to position 239 of wild-type human Neu2 (H239); (aa) a substitution of a leucine residue at a position corresponding to position 240 of wild-type human Neu2 (L240); (bb) a substitution of an arginine residue at a position corresponding to position 241 of wild-type human Neu2 (R241); (cc) a substitution of an alanine residue at a position corresponding to position 242 of wild-type human Neu2 (A242); (dd) a substitution of a valine residue at a position corresponding to position 244 of wild-type human Neu2 (V244); (ee) a substitution of a threonine residue at a position corresponding to position 249 of wild-type human Neu2 (T249); (ff) a substitution of an aspartic acid residue at a position corresponding to position 251 of wild-type human Neu2 (D251); (gg) a substitution of a glutamic acid residue at a position corresponding to position 257 of wild-type human Neu2 (E257); (hh) a substitution of a serine residue at a position corresponding to position 258 of wild-type human Neu2 (S258); (ii) a substitution of a leucine residue at a position corresponding to position 260 of wild-type human Neu2 (L260); (jj) a substitution of a valine residue at a position corresponding to position 265 of wild-type human Neu2 (V265); (kk) a substitution of a glutamine residue at a position corresponding to position 270 of wild-type human Neu2

(Q270); (ll) a substitution of a tryptophan residue at a position corresponding to position 292 of wild-type human Neu2 (W292); (mm) a substitution of a serine residue at a position corresponding to position 301 of wild-type human Neu2 (S301); (nn) a substitution of a tryptophan residue at a position corresponding to position 302 of wild-type human Neu2 (W302); (oo) a substitution of a valine residue at a position corresponding to position 363 of wild-type human Neu2 (V363); or (pp) a substitution of a leucine residue at a position corresponding to position 365 of wild-type human Neu2 (L365); or a combination of any of the foregoing substitutions.

[0007] In another aspect, the present disclosure relates to a recombinant mutant sialidase enzyme, wherein the sialidase comprises a mutation that increases resistance to cleavage by a protease. Accordingly, in one aspectexample, the inventiondisclosure relates to a recombinant mutant sialidase enzyme, e.g., a human mutant sialidase enzyme, wherein the sialidase comprises a mutation that increases resistance (decreases sensitivity) to cleavage by a protease. In certain embodimentexamples, incubation of the recombinant mutant sialidase (e.g., human sialidase) with the protease results in less than 50% (e.g., less than 40%, less than 30%, less than 10%, less than 5%, less than 3%, less than 1%, or less than 0.5%) of the proteolytic cleavage of a corresponding wild-type sialidase (or modified wild-type sialidase lacking the mutations described herein) when incubated with the protease under the same conditions. In certain embodimentexamples, the protease is trypsin.

[0008] In certain embodimentexamples, the sialidase is a human sialidase that comprises a substitution of an alanine residue at a position corresponding to position 213 of wild-type human Neu2 (A213); a substitution of a leucine residue at a position corresponding to position 240 of wild-type human Neu2 (L240); a substitution of an arginine residue at a position corresponding to position 241 of wild-type human Neu2 (R241); a substitution of an alanine residue at a position corresponding to position 242 of wild-type human Neu2 (A242);

a substitution of an arginine residue at a position corresponding to position 243 of wild-type human Neu2 (R243); a substitution of a valine residue at a position corresponding to position 244 of wild-type human Neu2 (V244); a substitution of a serine residue at a position corresponding to position 258 of wild-type human Neu2 (S258); a substitution of a leucine residue at a position corresponding to position 260 of wild-type human Neu2 (L260); a substitution of a valine residue at a position corresponding to position 265 of wild-type human Neu2 (V265); or a combination of any of the foregoing substitutions.

[0009] In certain embodiment examples, in the sialidase, the alanine residue at a position corresponding to position 213 of wild-type human Neu2 is substituted by cysteine (A213C), asparagine (A213N), serine (A213S), or threonine (A213T); the leucine residue at a position corresponding to position 240 of wild-type human Neu2 (L240) is substituted by aspartic acid (L240D), asparagine (L240N), or tyrosine (L240Y); the arginine residue at a position corresponding to position 241 of wild-type human Neu2 (R241) is substituted by alanine (R241A), aspartic acid (R241D), leucine (R241L), glutamine (R241Q), or tyrosine (R241Y); the alanine residue at a position corresponding to position 242 of wild-type human Neu2 (A242) is substituted by cysteine (A242C), phenylalanine (A242F), glycine (A242G), histidine (A242H), isoleucine (A242I), lysine (A242K), leucine (A242L), methionine (A242M), asparagine (A242N), glutamine (A242Q), arginine (A242R), serine (A242S), valine (A242V), tryptophan (A242W), or tyrosine (A242Y); the arginine residue at a position corresponding to position 243 of wild-type human Neu2 (R243) is substituted by glutamic acid (R243E), histidine (R243H), asparagine (R243N), glutamine (A243Q), or lysine (A243K); the valine residue at a position corresponding to position 244 of wild-type human Neu2 (V244) is substituted by isoleucine (V244I), lysine (V244K), or proline (V244P); the serine residue at a position corresponding to position 258 of wild-type human Neu2 (S258) is substituted by cysteine (S258C); the leucine residue at a position corresponding to position 260 of wild-type human Neu2 (L260) is substituted by aspartic acid (L260D), phenylalanine (L260F), glutamine (L260Q), or threonine (L260T); the valine residue at a position corresponding to position 265 of wild-type human Neu2 (V265) is substituted by phenylalanine (V265F); or the sialidase comprises a combination of any of the foregoing substitutions.

[0010] In certain embodiment examples, the sialidase comprises a combination of substitutions as set forth in **TABLE 2**, hereinbelow.

[0011] In another ~~aspect~~example, the ~~invention~~disclosure provides a recombinant mutant human sialidase enzyme, wherein the sialidase comprises: (a) a substitution of a proline residue at a position corresponding to position 5 of wild-type human Neu2 (P5); (b) a substitution of a lysine residue at a position corresponding to position 9 of wild-type human Neu2 (K9); (c) a substitution of an alanine residue at a position corresponding to position 42 of wild-type human Neu2 (A42); (d) a substitution of a lysine residue at a position corresponding to position 44 of wild-type human Neu2 (K44); (e) a substitution of a lysine residue at a position corresponding to position 45 of wild-type human Neu2 (K45); (f) a substitution of a leucine residue at a position corresponding to position 54 of wild-type human Neu2 (L54); (g) a substitution of a proline residue at a position corresponding to position 62 of wild-type human Neu2 (P62); (h) a substitution of a glutamine residue at a position corresponding to position 69 of wild-type human Neu2 (Q69); (i) a substitution of an arginine residue at a position corresponding to position 78 of wild-type human Neu2 (R78); (j) a substitution of an aspartic acid residue at a position corresponding to position 80 of wild-type human Neu2 (D80); (k) a substitution of an alanine residue at a position corresponding to position 93 of wild-type human Neu2 (A93); (l) a substitution of a glycine residue at a position corresponding to position 107 of wild-type human Neu2 (G107); (m) a substitution of a glutamine residue at a position corresponding to position 108 of wild-type human Neu2 (Q108); (n) a substitution of a glutamine residue at a position corresponding to position 112 of wild-type human Neu2 (Q112); (o) a substitution of a cysteine residue at a position corresponding to position 125 of wild-type human Neu2 (C125); (p) a substitution of a glutamine residue at a position corresponding to position 126 of wild-type human Neu2 (Q126); (q) a substitution of an alanine residue at a position corresponding to position 150 of wild-type human Neu2 (A150); (r) a substitution of a cysteine residue at a position corresponding to position 164 of wild-type human Neu2 (C164); (s) a substitution of an arginine residue at a position corresponding to position 170 of wild-type human Neu2 (R170); (t) a substitution of an alanine residue at a position corresponding to position 171 of wild-type human Neu2 (A171); (u) a substitution of a glutamine residue at a position corresponding to position 188 of wild-type human Neu2 (Q188); (v) a substitution of an arginine residue at a position corresponding to position 189 of wild-type human Neu2 (R189); (w) a substitution of an alanine residue at a position corresponding to position 213 of wild-type human Neu2 (A213); (x) a substitution of a leucine residue at a position corresponding to position 217 of wild-type human Neu2 (L217); (y) a substitution of a glutamic acid residue at a position corresponding to position 225 of wild-type human Neu2

(E225); (z) a substitution of a histidine residue at a position corresponding to position 239 of wild-type human Neu2 (H239); (aa) a substitution of a leucine residue at a position corresponding to position 240 of wild-type human Neu2 (L240); (bb) a substitution of an arginine residue at a position corresponding to position 241 of wild-type human Neu2 (R241); (cc) a substitution of an alanine residue at a position corresponding to position 242 of wild-type human Neu2 (A242); (dd) a substitution of a valine residue at a position corresponding to position 244 of wild-type human Neu2 (V244); (ee) a substitution of a threonine residue at a position corresponding to position 249 of wild-type human Neu2 (T249); (ff) a substitution of an aspartic acid residue at a position corresponding to position 251 of wild-type human Neu2 (D251); (gg) a substitution of a glutamic acid residue at a position corresponding to position 257 of wild-type human Neu2 (E257); (hh) a substitution of a serine residue at a position corresponding to position 258 of wild-type human Neu2 (S258); (ii) a substitution of a leucine residue at a position corresponding to position 260 of wild-type human Neu2 (L260); (jj) a substitution of a valine residue at a position corresponding to position 265 of wild-type human Neu2 (V265); (kk) a substitution of a glutamine residue at a position corresponding to position 270 of wild-type human Neu2 (Q270); (ll) a substitution of a tryptophan residue at a position corresponding to position 292 of wild-type human Neu2 (W292); (mm) a substitution of a serine residue at a position corresponding to position 301 of wild-type human Neu2 (S301); (nn) a substitution of a tryptophan residue at a position corresponding to position 302 of wild-type human Neu2 (W302); (oo) a substitution of a valine residue at a position corresponding to position 363 of wild-type human Neu2 (V363); or (pp) a substitution of a leucine residue at a position corresponding to position 365 of wild-type human Neu2 (L365); or a combination of any of the foregoing substitutions. For example, the sialidase may comprise a substitution of K9, A42, P62, A93, Q216, A242, Q270, S301, W302, V363, or L365, or a combination of any of the foregoing substitutions.

[0012] In certain ~~embodiment~~examples, in the sialidase: (a) the proline residue at a position corresponding to position 5 of wild-type human Neu2 is substituted by histidine (P5H); (b) the lysine residue at a position corresponding to position 9 of wild-type human Neu2 is substituted by aspartic acid (K9D); (c) the alanine residue at a position corresponding to position 42 of wild-type human Neu2 is substituted by arginine (A42R) or aspartic acid (A42D); (d) the lysine residue at a position corresponding to position 44 of wild-type human Neu2 is substituted by arginine (K44R) or glutamic acid (K44E); (e) the lysine residue at a

position corresponding to position 45 of wild-type human Neu2 is substituted by alanine (K45A), arginine (K45R), or glutamic acid (K45E); (f) the leucine residue at a position corresponding to position 54 of wild-type human Neu2 is substituted by methionine (L54M); (g) the proline residue at a position corresponding to position 62 of wild-type human Neu2 is substituted by asparagine (P62N), aspartic acid (P62D), histidine (P62H), glutamic acid (P62E), glycine (P62G), serine (P62S), or threonine (P62T); (h) the glutamine residue at a position corresponding to position 69 of wild-type human Neu2 is substituted by histidine (Q69H); (i) the arginine residue at a position corresponding to position 78 of wild-type human Neu2 is substituted by lysine (R78K); (j) the aspartic acid residue at a position corresponding to position 80 of wild-type human Neu2 is substituted by proline (D80P); (k) the alanine residue at a position corresponding to position 93 of wild-type human Neu2 is substituted by glutamic acid (A93E) or lysine (A93K); (l) the glycine residue at a position corresponding to position 107 of wild-type human Neu2 is substituted by aspartic acid (G107D); (m) the glutamine residue at a position corresponding to position 108 of wild-type human Neu2 is substituted by histidine (Q108H); (n) the glutamine residue at a position corresponding to position 112 of wild-type human Neu2 is substituted by arginine (Q112R) or lysine (Q112K); (o) the cysteine residue at a position corresponding to position 125 of wild-type human Neu2 is substituted by leucine (C125L); (p) the glutamine residue at a position corresponding to position 126 of wild-type human Neu2 is substituted by leucine (Q126L), glutamic acid (Q126E), phenylalanine (Q126F), histidine (Q126H), isoleucine (Q126I), or tyrosine (Q126Y); (q) the alanine residue at a position corresponding to position 150 of wild-type human Neu2 is substituted by valine (A150V); (r) the cysteine residue at a position corresponding to position 164 of wild-type human Neu2 is substituted by glycine (C164G); (s) the arginine residue at a position corresponding to position 170 of wild-type human Neu2 is substituted by proline (R170P); (t) the alanine residue at a position corresponding to position 171 of wild-type human Neu2 is substituted by glycine (A171G); (u) the glutamine residue at a position corresponding to position 188 of wild-type human Neu2 is substituted by proline (Q188P); (v) the arginine residue at a position corresponding to position 189 of wild-type human Neu2 is substituted by proline (R189P); (w) the alanine residue at a position corresponding to position 213 of wild-type human Neu2 is substituted by cysteine (A213C), asparagine (A213N), serine (A213S), or threonine (A213T); (x) the leucine residue at a position corresponding to position 217 of wild-type human Neu2 is substituted by alanine (L217A) or valine (L217V); (y) the threonine residue at a position corresponding to position 249 of wild-type human Neu2 is substituted by alanine (T249A);

(z) the aspartic acid residue at a position corresponding to position 251 of wild-type human Neu2 is substituted by glycine (D251G); (aa) the glutamic acid residue at a position corresponding to position 225 of wild-type human Neu2 is substituted by proline (E225P); (bb) the histidine residue at a position corresponding to position 239 of wild-type human Neu2 is substituted by proline (H239P); (cc) the leucine residue at a position corresponding to position 240 of wild-type human Neu2 is substituted by aspartic acid (L240D), asparagine (L240N), or tyrosine (L240Y); (dd) the arginine residue at a position corresponding to position 241 of wild-type human Neu2 is substituted by alanine (R241A), aspartic acid (R241D), leucine (R241L), glutamine (R241Q), or tyrosine (R241Y); (ee) the alanine residue at a position corresponding to position 242 of wild-type human Neu2 is substituted by cysteine (A242C), phenylalanine (A242F), glycine (A242G), histidine (A242H), isoleucine (A242I), lysine (A242K), leucine (A242L), methionine (A242M), asparagine (A242N), glutamine (A242Q), arginine (A242R), serine (A242S), valine (A242V), tryptophan (A242W), or tyrosine (A242Y); (ff) the valine residue at a position corresponding to position 244 of wild-type human Neu2 is substituted by isoleucine (V244I), lysine (V244K), or proline (V244P); (gg) the glutamic acid residue at a position corresponding to position 257 of wild-type human Neu2 is substituted by proline (E257P); (hh) the serine residue at a position corresponding to position 258 is substituted by cysteine (S258C); (ii) the leucine residue at a position corresponding to position 260 of wild-type human Neu2 is substituted by aspartic acid (L260D), phenylalanine (L260F), glutamine (L260Q), or threonine (L260T); (jj) the valine residue at a position corresponding to position 265 of wild-type human Neu2 is substituted by phenylalanine (V265F); (kk) the glutamine residue at a position corresponding to position 270 of wild-type human Neu2 is substituted by alanine (Q270A), histidine (Q270H), phenylalanine (Q270F), proline (Q270P), serine (Q270S), or threonine (Q270T); (ll) the tryptophan residue at a position corresponding to position 292 of wild-type human Neu2 is substituted by arginine (W292R); (mm) the serine residue at a position corresponding to position 301 of wild-type human Neu2 is substituted by alanine (S301A), aspartic acid (S301D), glutamic acid (S301E), phenylalanine (S301F), histidine (S301H), lysine (S301K), leucine (S301L), methionine (S301M), asparagine (S301N), proline (S301P), glutamine (S301Q), arginine (S301R), threonine (S301T), valine (S301V), tryptophan (S301W), or tyrosine (S301Y); (nn) the tryptophan residue at a position corresponding to position 302 of wild-type human Neu2 is substituted by alanine (W302A), aspartic acid (W302D), phenylalanine (W302F), glycine (W302G), histidine (W302H), isoleucine (W302I), lysine (W302K), leucine (W302L), methionine (W302M), asparagine (W302N), proline (W302P),

glutamine (W302Q), arginine (W302R), serine (W302S), threonine (W302T), valine (W302V), or tyrosine (W302Y); (oo) the valine residue at a position corresponding to position 363 of wild-type human Neu2 is substituted by arginine (V363R); or (pp) the leucine residue at a position corresponding to position 365 of wild-type human Neu2 is substituted by glutamine (L365Q), histidine (L365H), isoleucine (L365I), lysine (L365K) or serine (L365S); or the sialidase comprises a combination of any of the foregoing substitutions. For example, the sialidase may comprise a substitution selected from K9D, A42R, P62G, P62N, P62S, P62T, A93E, Q126Y, A242F, A242W, A242Y, Q270A, Q270T, S301A, S301R, W302K, W302R, V363R, and L365I, or a combination of any of the foregoing substitutions.

[0013] In certain embodimentexamples of any of the foregoing sialidases, the sialidase further comprises: (a) a substitution or deletion of a methionine residue at a position corresponding to position 1 of wild-type human Neu2 (M1); (b) a substitution of a valine residue at a position corresponding to position 6 of wild-type human Neu2 (V6); (c) a substitution of an isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 (I187); or (d) a substitution of a cysteine residue at a position corresponding to position 332 of wild-type human Neu2 (C332); or a combination of any of the foregoing substitutions.

[0014] In certain embodimentexamples, in the sialidase: (a) the methionine residue at a position corresponding to position 1 of wild-type human Neu2 is deleted (Δ M1), is substituted by alanine (M1A), or is substituted by aspartic acid (M1D); (b) the valine residue at a position corresponding to position 6 of wild-type human Neu2 is substituted by tyrosine (V6Y); (c) the isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 is substituted by lysine (I187K); or (d) the cysteine residue at a position corresponding to position 332 of wild-type human Neu2 is substituted by alanine (C332A); or the sialidase comprises a combination of any of the foregoing substitutions.

[0015] In certain embodimentexamples of any of the foregoing sialidases, the sialidase comprises: (a) the M1D, V6Y, P62G, A93E, I187K, and C332A substitutions; (b) the M1D, V6Y, K9D, A93E, I187K, C332A, V363R, and L365I substitutions; (c) the M1D, V6Y, P62N, I187K, and C332A substitutions; (d) the M1D, V6Y, I187K, Q270A, S301R, W302K, and C332A substitutions; (e) the M1D, V6Y, P62S, I187K, Q270A, S301R, W302K, and C332A substitutions; (f) the M1D, V6Y, P62T, I187K, Q270A, S301R, W302K, and C332A substitutions; (g) the M1D, V6Y, P62N, I187K, Q270A, S301R, W302K, and C332A substitutions; (h) the M1D, V6Y, P62G, A93E, I187K, S301A, W302R, and C332A

substitutions; (i) the M1D, V6Y, P62G, A93E, Q126Y, I187K, Q270T, and C332A substitutions; (j) the M1D, V6Y, P62G, A93E, Q126Y, I187K, and C332A substitutions; (k) the M1D, V6Y, P62G, A93E, Q126Y, I187K, A242F, Q270T, and C332A substitutions; or (l) the M1D, V6Y, A42R, P62G, A93E, Q126Y, I187K, A242F, Q270T, and C332A substitutions.

[0016] In certain embodimentexamples of any of the foregoing sialidases, the sialidase has a different substrate specificity than the corresponding wild-type sialidase. For example, in certain embodimentexamples the sialidase can cleave α 2,3, α 2,6, and/or α 2,8 linkages. In certain embodimentexamples the sialidase can cleave α 2,3 and α 2,8 linkages.

[0017] In certain embodimentexamples of any of the foregoing sialidases, the sialidase comprises any one of SEQ ID NOs: 48-54, 149, 154, 159, 191, or 198, 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: 48-54, 149, 154, 159, 191, or 198.

[0018] In addition, the inventiondisclosure provides a recombinant mutant human sialidase comprising a mutation or combination of mutations set forth in any one of TABLES 1, 2, 7-9, 11-13, 15-30, 34, or 35, hereinbelow. In certain embodimentexamples, the sialidase further comprises a mutation or combination of mutations set forth in any one of TABLES 3-6, hereinbelow.

[0019] In another aspect, the present disclosure provides a fusion protein comprising: (a) the recombinant enzyme as described herein; and (b) an immunoglobulin Fc domain and/or an immunoglobulin antigen-binding domain; wherein the enzyme and the immunoglobulin Fc domain and/or the immunoglobulin antigen-binding domain are linked by a peptide bond or an amino acid linker. In addition, the inventiondisclosure provides a fusion protein comprising (or consisting essentially of): (a) a recombinant mutant human sialidase disclosed herein; and (b) an immunoglobulin Fc domain and/or an immunoglobulin antigen-binding domain; wherein the sialidase and the Fc domain and/or the antigen-binding domain are linked by a peptide bond or an amino acid linker. In certain embodimentexamples, the fusion protein further comprises a linker, for example, an amino acid linker, connecting the sialidase enzyme and the Fc domain and/or the antigen-binding domain. In certain embodimentexamples, the immunoglobulin antigen-binding domain is associated (for example, covalently or non-covalently associated) with a second immunoglobulin antigen-binding domain to produce an antigen-binding site.

[0020] In certain embodiment examples, the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM Fc domain, *e.g.*, the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, or IgG4 Fc domain, *e.g.*, the immunoglobulin Fc domain is derived from a human IgG1 Fc domain.

- 5 [0021] In certain embodiment examples, the immunoglobulin antigen-binding domain is derived from an antibody selected from trastuzumab, daratumumab, girentuximab, ofatumumab, avelumab and rituximab.

[0022] In certain embodimentexamples, the fusion protein comprises any one of SEQ ID NOs: 203-210, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, or 242, 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: 203-210, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, or 242.

[0023] In addition, in another aspect the inventiondisclosure provides an antibody conjugate comprising any of the foregoing fusion proteins. In certain embodimentexamples, the antibody conjugate comprises a single sialidase. In other embodimentexamples, the antibody conjugate comprises two sialidases, which can be the same or different. In certain embodimentexamples the antibody conjugate comprises two identical sialidases. In certain embodimentexamples, the antibody conjugate comprises a single antigen-binding site. In other embodimentexamples, the antibody conjugate comprises two antigen-binding sites, which can be the same or different. In certain embodimentexamples, the antibody conjugate comprises two identical antigen-binding sites.

[0024] In certain embodimentexamples, the antibody conjugate has a molecular weight from about 135 kDa to about 165 kDa, or the antibody conjugate has a molecular weight from about 215 kDa to about 245 kDa.

[0025] In certain embodimentexample, 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 a sialidase; 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. The third polypeptide may, for example, comprise the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation.

[0026] In certain embodimentexamples, the antibody conjugate comprises: (a) a first polypeptide comprising a first immunoglobulin light chain; (b) a second polypeptide comprising a first immunoglobulin heavy chain and a first sialidase; (c) a third polypeptide comprising a second immunoglobulin heavy chain and a second sialidase; 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. The second and third polypeptides may, for example, comprise the first and second immunoglobulin heavy chain and the first and second sialidase, respectively, in an N- to C-terminal orientation.

5 [0027] In certain ~~embodiment~~examples, the antibody conjugate comprises: (a) a first polypeptide comprising a first sialidase, a first immunoglobulin Fc domain, and a first single chain variable fragment (scFv); and (b) a second polypeptide comprising a second sialidase, a second immunoglobulin Fc domain, and an optional second single chain variable fragment (scFv); wherein the first and second polypeptides are covalently linked together, and wherein
10 the first scFv defines a first antigen-binding site, and the second scFv, when present, defines a second antigen-binding site. The first polypeptide may, for example comprise the first sialidase, the first immunoglobulin Fc domain, and the first scFv in an N- to C-terminal orientation. The second polypeptide may, for example, comprise the second sialidase, the second immunoglobulin Fc domain, and the optional second scFv in an N- to C-terminal
15 orientation.

[0028] In certain ~~embodiment~~examples, 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 sialidase, wherein the first and
20 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. The second polypeptide may, for example comprise the immunoglobulin heavy chain and the scFv in an N- to C-terminal orientation. The third polypeptide may, for
25 example, comprise the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation.

[0029] In another aspect, the present disclosure provides an isolated nucleic acid comprising a nucleotide sequence encoding the recombinant enzyme as described herein, the fusion protein as described herein, or at least a portion of the antibody conjugate as described herein.

30 In another ~~aspect~~example, the ~~invention~~disclosure provides an isolated nucleic acid comprising a nucleotide sequence encoding any of the foregoing recombinant mutant human sialidases, any of the foregoing fusion proteins, or at least a portion of any of the foregoing

antibody conjugates. In another aspect, the ~~invention~~disclosure provides an expression vector comprising any of the foregoing nucleic acids. In another aspect, the ~~invention~~disclosure provides a host cell comprising any of the foregoing expression vectors.

[0030] In a further aspect, the present disclosure provides a pharmaceutical composition comprising the recombinant enzyme as described herein, the fusion protein as described herein, or the antibody conjugate of as described herein. In another ~~aspect~~example, the ~~invention~~disclosure provides a pharmaceutical composition comprising any of the foregoing recombinant mutant human sialidases, any of the foregoing fusion proteins, or any of the foregoing antibody conjugates.

[0031] In a further aspect, the present disclosure provides a use of the recombinant enzyme as described herein, the fusion protein as described herein, or at least a portion of the antibody conjugate as described herein, or the pharmaceutical composition as described herein in the preparation of a medicament for the treatment of cancer in a subject. In another

~~aspect~~example, the ~~invention~~disclosure provides a method of treating cancer in a subject in need thereof. The method comprises administering to the subject an effective amount of any of the foregoing sialidases, any of the foregoing fusion proteins, any of the foregoing antibody conjugates, or any of the foregoing pharmaceutical compositions. In certain ~~embodiment~~examples, the cancer is an epithelial cancer. In certain ~~embodiment~~examples, the cancer is a solid tumor, soft tissue tumor, hematopoietic tumor or metastatic lesion. In certain ~~embodiment~~examples, the solid tumor is a sarcoma, adenocarcinoma, or carcinoma. In certain ~~embodiment~~examples, 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 ~~embodiment~~examples, 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). In certain ~~embodiment~~examples, the cancer is selected from an endometrial cancer, ovarian cancer, cervical cancer, vulvar cancer, uterine cancer, fallopian tube cancer, breast cancer, prostate cancer, lung cancer, pancreatic cancer, urinary cancer, bladder cancer, head and neck cancer, oral cancer and liver cancer.

[0032] In another ~~aspeet~~example, the ~~invention~~disclosure provides a method of increasing expression of HLA-DR, CD86, CD83, IFN γ , IL-1b, IL-6, TNF α , IL-17A, IL-2, or IL-6 in a cell, tissue, or subject. The method comprises contacting the cell, tissue, or subject with an effective amount of any of the foregoing sialidases, any of the foregoing fusion proteins, any of the foregoing antibody conjugates, or any of the foregoing pharmaceutical compositions. In certain ~~embodiment~~examples, the cell is selected from a dendritic cell and a peripheral blood mononuclear cell (PBMC).

[0033] These and other ~~aspect~~examples and features of the ~~invention~~disclosure are described in the following detailed description and claims.

DESCRIPTION OF THE DRAWINGS

[0034] The invention can be more completely understood with reference to the following drawings.

[0035] **FIGURE 1** depicts an SDS-PAGE gel showing recombinant human Neu1, Neu2, Neu3, and *Salmonella typhimurium* (St-sialidase) under non-reducing and reducing conditions. Monomer and dimer species are indicated.

[0036] **FIGURE 2** is a bar graph showing the enzymatic activity of recombinant human Neu1, Neu2, and Neu3.

[0037] **FIGURE 3** is a line graph showing enzymatic activity as a function of substrate concentration for recombinant human Neu2 and Neu3 at the indicated pH.

[0038] **FIGURE 4** depicts a schematic representation of an exemplary sialic acid biotinylated probe that can be used in phage display or yeast display screening for Neu2 variants.

[0039] **FIGURE 5** depicts an exemplary protocol that facilitates phage display screening of Neu2 variants.

[0040] **FIGURE 6** depicts an exemplary protocol that facilitates yeast display screening of Neu2 variants.

[0041] **FIGURE 7A** depicts an SDS-PAGE gel showing recombinant Neu2-Fc (wildtype) and Neu2-M106-Fc under non-reducing and reducing conditions. **FIGURE 7B** is an SEC-HPLC trace of Neu2-Fc (wildtype) and Neu2-M106-Fc. The monomer species has a retention time of 21 minutes.

[0042] **FIGURE 8** is a line graph depicting the enzymatic activity of Neu2 variant M106.

[0043] **FIGURES 9A-9I** 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

WHAT IS CLAIMED IS:

- ~~1. A recombinant mutant sialidase enzyme (e.g., human sialidase enzyme), wherein the sialidase comprises a mutation that increases resistance to cleavage by a protease.~~
- 5 ~~2. The enzyme of claim 1, wherein incubation of the sialidase with the protease results in less than 50% (e.g., less than 40%, less than 30%, less than 10%, less than 5%, less than 3%, less than 1%, or less than 0.5%) of the proteolytic cleavage of a corresponding wild-type sialidase when incubated with the protease under the same conditions.~~
- ~~3. The enzyme of claim 1 or claim 2, wherein the protease is trypsin.~~
- 10 ~~4. The enzyme of any one of claims 1-3, wherein the sialidase comprises:~~
 - ~~(a) — a substitution of an alanine residue at a position corresponding to position 213 of wild-type human Neu2 (A213);~~
 - ~~(b) — a substitution of a leucine residue at a position corresponding to position 240 of wild-type human Neu2 (L240);~~
 - 15 ~~(c) — a substitution of an arginine residue at a position corresponding to position 241 of wild-type human Neu2 (R241);~~
 - ~~(d) — a substitution of an alanine residue at a position corresponding to position 242 of wild-type human Neu2 (A242);~~
 - ~~(e) — a substitution of an arginine residue at a position corresponding to position 243 of wild-type human Neu2 (R243);~~
 - 20 ~~(f) — a substitution of a valine residue at a position corresponding to position 244 of wild-type human Neu2 (V244);~~
 - ~~(g) — a substitution of a serine residue at a position corresponding to position 258 of wild-type human Neu2 (S258);~~
 - 25 ~~(h) — a substitution of a leucine residue at a position corresponding to position 260 of wild-type human Neu2 (L260);~~
 - ~~(i) — a substitution of a valine residue at a position corresponding to position 265 of wild-type human Neu2 (V265);~~
~~or a combination of any of the foregoing substitutions.~~
- 30 ~~5. The enzyme of claim 4, wherein, in the sialidase:~~
 - ~~(a) — the alanine residue at a position corresponding to position 213 of wild-type human Neu2 is substituted by cysteine (A213C), asparagine (A213N), serine (A213S), or~~

threonine (A213T);

~~(b) the leucine residue at a position corresponding to position 240 of wild-type human Neu2 (L240) is substituted by aspartic acid (L240D), asparagine (L240N), or tyrosine (L240Y);~~

~~(c) the arginine residue at a position corresponding to position 241 of wild-type human Neu2 (R241) is substituted by alanine (R241A), aspartic acid (R241D), leucine (R241L), glutamine (R241Q), or tyrosine (R241Y);~~

~~(d) the alanine residue at a position corresponding to position 242 of wild-type human Neu2 (A242) is substituted by cysteine (A242C), phenylalanine (A242F), glycine (A242G), histidine (A242H), isoleucine (A242I), lysine (A242K), leucine (A242L), methionine (A242M), asparagine (A242N), glutamine (A242Q), arginine (A242R), serine (A242S), valine (A242V), tryptophan (A242W), or tyrosine (A242Y);~~

~~(e) the valine residue at a position corresponding to position 244 of wild-type human Neu2 (V244) is substituted by isoleucine (V244I), lysine (V244K), or proline (V244P);~~

~~(f) the serine residue at a position corresponding to position 258 of wild-type human Neu2 (S258) is substituted by cysteine (S258C);~~

~~(g) the leucine residue at a position corresponding to position 260 of wild-type human Neu2 (L260) is substituted by aspartic acid (L260D), phenylalanine (L260F), glutamine (L260Q), or threonine (L260T);~~

~~(h) the valine residue at a position corresponding to position 265 of wild-type human Neu2 (V265) is substituted by phenylalanine (V265F);~~
~~or a combination of any of the foregoing substitutions.~~

~~6. The enzyme of claim 5, wherein, the sialidase comprises a combination of substitutions as set forth in **TABLE 2**.~~

~~7.1.~~ A recombinant mutant human sialidase enzyme, wherein the sialidase comprises:

~~(a) a substitution of an alanine residue at a position corresponding to position 42 of wild-type human Neu2 (A42);~~

~~(a)(b) a substitution of a proline residue at a position corresponding to position 5 of wild-type human Neu2 (P5);~~

~~(b)(c) a substitution of a lysine residue at a position corresponding to position 9 of wild-type human Neu2 (K9);~~

- ~~(e)~~(d) a substitution of a lysine residue at a position corresponding to position 44 of wild-type human Neu2 (K44);
- ~~(d)~~(e) a substitution of a lysine residue at a position corresponding to position 45 of wild-type human Neu2 (K45);
- 5 ~~(e)~~(f) a substitution of a leucine residue at a position corresponding to position 54 of wild-type human Neu2 (L54);
- ~~(f)~~(g) a substitution of a proline residue at a position corresponding to position 62 of wild-type human Neu2 (P62);
- 10 ~~(g)~~(h) a substitution of a glutamine residue at a position corresponding to position 69 of wild-type human Neu2 (Q69);
- ~~(h)~~(i) a substitution of an arginine residue at a position corresponding to position 78 of wild-type human Neu2 (R78);
- ~~(i)~~(j) a substitution of an aspartic acid residue at a position corresponding to position 80 of wild-type human Neu2 (D80);
- 15 ~~(j)~~(k) a substitution of an alanine residue at a position corresponding to position 93 of wild-type human Neu2 (A93);
- ~~(k)~~(l) a substitution of a glycine residue at a position corresponding to position 107 of wild-type human Neu2 (G107);
- ~~(l)~~(m) a substitution of a glutamine residue at a position corresponding to position 108 of wild-type human Neu2 (Q108);
- 20 ~~(m)~~(n) a substitution of a glutamine residue at a position corresponding to position 112 of wild-type human Neu2 (Q112);
- ~~(n)~~(o) a substitution of a cysteine residue at a position corresponding to position 125 of wild-type human Neu2 (C125);
- 25 ~~(o)~~(p) a substitution of a glutamine residue at a position corresponding to position 126 of wild-type human Neu2 (Q126);
- ~~(p)~~(q) a substitution of an alanine residue at a position corresponding to position 150 of wild-type human Neu2 (A150);
- ~~(q)~~(r) a substitution of a cysteine residue at a position corresponding to position 164 of wild-type human Neu2 (C164);
- 30 ~~(r)~~(s) a substitution of an arginine residue at a position corresponding to position 170 of wild-type human Neu2 (R170);
- ~~(s)~~(t) a substitution of an alanine residue at a position corresponding to position 171 of wild-type human Neu2 (A171);

- ~~(t)~~(u) a substitution of a glutamine residue at a position corresponding to position 188 of wild-type human Neu2 (Q188);
- ~~(u)~~(v) a substitution of an arginine residue at a position corresponding to position 189 of wild-type human Neu2 (R189);
- 5 ~~(v)~~(w) a substitution of an alanine residue at a position corresponding to position 213 of wild-type human Neu2 (A213);
- ~~(w)~~(x) a substitution of a leucine residue at a position corresponding to position 217 of wild-type human Neu2 (L217);
- 10 ~~(x)~~(y) a substitution of a glutamic acid residue at a position corresponding to position 225 of wild-type human Neu2 (E225);
- ~~(y)~~(z) a substitution of a histidine residue at a position corresponding to position 239 of wild-type human Neu2 (H239);
- ~~(z)~~(aa) a substitution of a leucine residue at a position corresponding to position 240 of wild-type human Neu2 (L240);
- 15 ~~(aa)~~(bb) a substitution of an arginine residue at a position corresponding to position 241 of wild-type human Neu2 (R241);
- ~~(bb)~~(cc) a substitution of an alanine residue at a position corresponding to position 242 of wild-type human Neu2 (A242);
- 20 ~~(ee)~~(dd) a substitution of a valine residue at a position corresponding to position 244 of wild-type human Neu2 (V244);
- ~~(dd)~~(ee) a substitution of a threonine residue at a position corresponding to position 249 of wild-type human Neu2 (T249);
- ~~(ee)~~(ff) a substitution of an aspartic acid residue at a position corresponding to position 251 of wild-type human Neu2 (D251);
- 25 ~~(ff)~~(gg) a substitution of a glutamic acid residue at a position corresponding to position 257 of wild-type human Neu2 (E257);
- ~~(gg)~~(hh) a substitution of a serine residue at a position corresponding to position 258 of wild-type human Neu2 (S258);
- 30 ~~(hh)~~(ii) a substitution of a leucine residue at a position corresponding to position 260 of wild-type human Neu2 (L260);
- ~~(ii)~~(jj) a substitution of a valine residue at a position corresponding to position 265 of wild-type human Neu2 (V265);
- ~~(jj)~~(kk) a substitution of a glutamine residue at a position corresponding to position 270 of wild-type human Neu2 (Q270);

- (~~kk~~)(ll) _____ a substitution of a tryptophan residue at a position corresponding to position 292 of wild-type human Neu2 (W292);
- (~~ll~~)(mm) _____ a substitution of a serine residue at a position corresponding to position 301 of wild-type human Neu2 (S301);
- 5 (~~mm~~)(nn) _____ a substitution of a tryptophan residue at a position corresponding to position 302 of wild-type human Neu2 (W302);
- (~~nn~~)(oo) _____ a substitution of a valine residue at a position corresponding to position 363 of wild-type human Neu2 (V363); or
- (~~oo~~)(pp) _____ a substitution of a leucine residue at a position corresponding to position 365 of wild-type human Neu2 (L365);
- 10 or a combination of any of the foregoing substitutions.

~~8. A recombinant mutant human sialidase enzyme, wherein the sialidase comprises:~~

- (a) ~~— a substitution of a proline residue at a position corresponding to position 5 of wild-type human Neu2 (P5);~~
- 15 (b) ~~— a substitution of a lysine residue at a position corresponding to position 9 of wild-type human Neu2 (K9);~~
- (c) ~~— a substitution of a lysine residue at a position corresponding to position 44 of wild-type human Neu2 (K44);~~
- (d) ~~— a substitution of a lysine residue at a position corresponding to position 45 of wild-type human Neu2 (K45);~~
- 20 (e) ~~— a substitution of a leucine residue at a position corresponding to position 54 of wild-type human Neu2 (L54);~~
- (f) ~~— a substitution of a proline residue at a position corresponding to position 62 of wild-type human Neu2 (P62);~~
- 25 (g) ~~— a substitution of a glutamine residue at a position corresponding to position 69 of wild-type human Neu2 (Q69);~~
- (h) ~~— a substitution of an arginine residue at a position corresponding to position 78 of wild-type human Neu2 (R78);~~
- (i) ~~— a substitution of an aspartic acid residue at a position corresponding to position 80 of wild-type human Neu2 (D80);~~
- 30 (j) ~~— a substitution of an alanine residue at a position corresponding to position 93 of wild-type human Neu2 (A93);~~

- (k) — a substitution of a glycine residue at a position corresponding to position 107 of wild-type human Neu2 (G107);
- (l) — a substitution of a glutamine residue at a position corresponding to position 108 of wild-type human Neu2 (Q108);
- 5 (m) — a substitution of a glutamine residue at a position corresponding to position 112 of wild-type human Neu2 (Q112);
- (n) — a substitution of a cysteine residue at a position corresponding to position 125 of wild-type human Neu2 (C125);
- 10 (o) — a substitution of a glutamine residue at a position corresponding to position 126 of wild-type human Neu2 (Q126);
- (p) — a substitution of an alanine residue at a position corresponding to position 150 of wild-type human Neu2 (A150);
- (q) — a substitution of a cysteine residue at a position corresponding to position 164 of wild-type human Neu2 (C164);
- 15 (r) — a substitution of an arginine residue at a position corresponding to position 170 of wild-type human Neu2 (R170);
- (s) — a substitution of an alanine residue at a position corresponding to position 171 of wild-type human Neu2 (A171);
- 20 (t) — a substitution of a glutamine residue at a position corresponding to position 188 of wild-type human Neu2 (Q188);
- (u) — a substitution of an arginine residue at a position corresponding to position 189 of wild-type human Neu2 (R189);
- (v) — a substitution of an alanine residue at a position corresponding to position 213 of wild-type human Neu2 (A213);
- 25 (w) — a substitution of a leucine residue at a position corresponding to position 217 of wild-type human Neu2 (L217);
- (x) — a substitution of a glutamic acid residue at a position corresponding to position 225 of wild-type human Neu2 (E225);
- 30 (y) — a substitution of a histidine residue at a position corresponding to position 239 of wild-type human Neu2 (H239);
- (z) — a substitution of a leucine residue at a position corresponding to position 240 of wild-type human Neu2 (L240);
- (aa) — a substitution of an arginine residue at a position corresponding to position 241 of wild-type human Neu2 (R241);

- (bb) — a substitution of an alanine residue at a position corresponding to position 242 of wild-type human Neu2 (A242);
- (cc) — a substitution of a valine residue at a position corresponding to position 244 of wild-type human Neu2 (V244);
- (dd) — a substitution of a threonine residue at a position corresponding to position 249 of wild-type human Neu2 (T249);
- (ee) — a substitution of an aspartic acid residue at a position corresponding to position 251 of wild-type human Neu2 (D251);
- (ff) — a substitution of a glutamic acid residue at a position corresponding to position 257 of wild-type human Neu2 (E257);
- (gg) — a substitution of a serine residue at a position corresponding to position 258 of wild-type human Neu2 (S258);
- (hh) — a substitution of a leucine residue at a position corresponding to position 260 of wild-type human Neu2 (L260);
- (ii) — a substitution of a valine residue at a position corresponding to position 265 of wild-type human Neu2 (V265);
- (jj) — a substitution of a glutamine residue at a position corresponding to position 270 of wild-type human Neu2 (Q270);
- (kk) — a substitution of a tryptophan residue at a position corresponding to position 292 of wild-type human Neu2 (W292);
- (ll) — a substitution of a serine residue at a position corresponding to position 301 of wild-type human Neu2 (S301);
- (mm) — a substitution of a tryptophan residue at a position corresponding to position 302 of wild-type human Neu2 (W302);
- (nn) — a substitution of a valine residue at a position corresponding to position 363 of wild-type human Neu2 (V363); or
- (oo) — a substitution of a leucine residue at a position corresponding to position 365 of wild-type human Neu2 (L365);
or a combination of any of the foregoing substitutions.

9.2. The enzyme of claim ~~7-1~~8, wherein the sialidase comprises a substitution of A42, K9, ~~A42~~, P62, A93, Q126, A242, Q270, S301, W302, V363, or L365, or a combination of any of the foregoing substitutions.

~~10. The enzyme of claim 7 or 8, wherein the sialidase comprises a substitution of K9, P62, A93, Q126, A242, Q270, S301, W302, V363, or L365, or a combination of any of the foregoing substitutions.~~

~~11.3.~~ The enzyme of ~~any one of claims 7-10~~ or 2, wherein, in the sialidase:

- 5 (a) the alanine residue at a position corresponding to position 42 of wild-type human Neu2 is substituted by arginine (A42R);
- ~~(a)(b)~~ the proline residue at a position corresponding to position 5 of wild-type human Neu2 is substituted by histidine (P5H);
- 10 ~~(b)(c)~~ the lysine residue at a position corresponding to position 9 of wild-type human Neu2 is substituted by aspartic acid (K9D);
- ~~(c) — the alanine residue at a position corresponding to position 42 of wild-type human Neu2 is substituted by arginine (A42R);~~
- (d) the lysine residue at a position corresponding to position 44 of wild-type human Neu2 is substituted by arginine (K44R) or glutamic acid (K44E);
- 15 (e) the lysine residue at a position corresponding to position 45 of wild-type human Neu2 is substituted by alanine (K45A), arginine (K45R), or glutamic acid (K45E);
- (f) the leucine residue at a position corresponding to position 54 of wild-type human Neu2 is substituted by methionine (L54M);
- 20 (g) the proline residue at a position corresponding to position 62 of wild-type human Neu2 is substituted by asparagine (P62N), aspartic acid (P62D), histidine (P62H), glutamic acid (P62E), glycine (P62G), serine (P62S), or threonine (P62T);
- (h) the glutamine residue at a position corresponding to position 69 of wild-type human Neu2 is substituted by histidine (Q69H);
- 25 (i) the arginine residue at a position corresponding to position 78 of wild-type human Neu2 is substituted by lysine (R78K);
- (j) the aspartic acid residue at a position corresponding to position 80 of wild-type human Neu2 is substituted by proline (D80P);
- (k) the alanine residue at a position corresponding to position 93 of wild-type human Neu2 is substituted by glutamic acid (A93E) or lysine (A93K);
- 30 (l) the glycine residue at a position corresponding to position 107 of wild-type human Neu2 is substituted by aspartic acid (G107D);
- (m) the glutamine residue at a position corresponding to position 108 of wild-type human Neu2 is substituted by histidine (Q108H);

- (n) the glutamine residue at a position corresponding to position 112 of wild-type human Neu2 is substituted by arginine (Q112R) or lysine (Q112K);
- (o) the cysteine residue at a position corresponding to position 125 of wild-type human Neu2 is substituted by leucine (C125L);
- 5 (p) the glutamine residue at a position corresponding to position 126 of wild-type human Neu2 is substituted by leucine (Q126L), glutamic acid (Q126E), phenylalanine (Q126F), histidine (Q126H), isoleucine (Q126I), or tyrosine (Q126Y);
- (q) the alanine residue at a position corresponding to position 150 of wild-type human Neu2 is substituted by valine (A150V);
- 10 (r) the cysteine residue at a position corresponding to position 164 of wild-type human Neu2 is substituted by glycine (C164G);
- (s) the arginine residue at a position corresponding to position 170 of wild-type human Neu2 is substituted by proline (R170P);
- 15 (t) the alanine residue at a position corresponding to position 171 of wild-type human Neu2 is substituted by glycine (A171G);
- (u) the glutamine residue at a position corresponding to position 188 of wild-type human Neu2 is substituted by proline (Q188P);
- (v) the arginine residue at a position corresponding to position 189 of wild-type human Neu2 is substituted by proline (R189P);
- 20 (w) the alanine residue at a position corresponding to position 213 of wild-type human Neu2 is substituted by cysteine (A213C), asparagine (A213N), serine (A213S) or threonine (A213T);
- (x) the leucine residue at a position corresponding to position 217 of wild-type human Neu2 is substituted by alanine (L217A) or valine (L217V);
- 25 (y) the glutamic acid residue at a position corresponding to position 225 of wild-type human Neu2 is substituted by proline (E225P);
- (z) the histidine residue at a position corresponding to position 239 of wild-type human Neu2 is substituted by proline (H239P);
- 30 (aa) the leucine residue at a position corresponding to position 240 of wild-type human Neu2 is substituted by aspartic acid (L240D), asparagine (L240N), or tyrosine (L240Y);
- (bb) the arginine residue at a position corresponding to position 241 of wild-type human Neu2 is substituted by alanine (R241A), aspartic acid (R241D), leucine

- (R241L), glutamine (R241Q), or tyrosine (R241Y);
- (cc) the alanine residue at a position corresponding to position 242 of wild-type human Neu2 is substituted by cysteine (A242C), phenylalanine (A242F), glycine (A242G), histidine (A242H), isoleucine (A242I), lysine (A242K), leucine (A242L), methionine (A242M), asparagine (A242N), glutamine (A242Q), arginine (A242R), serine (A242S), valine (A242V), tryptophan (A242W), or tyrosine (A242Y);
- (dd) the valine residue at a position corresponding to position 244 of wild-type human Neu2 is substituted by isoleucine (V244I) or proline (V244P);
- (ee) the threonine residue at a position corresponding to position 249 of wild-type human Neu2 is substituted by alanine (T249A);
- (ff) the aspartic acid residue at a position corresponding to position 251 of wild-type human Neu2 is substituted by glycine (D251G);
- (gg) the glutamic acid residue at a position corresponding to position 257 of wild-type human Neu2 is substituted by proline (E257P);
- (hh) the serine residue at a position corresponding to position 258 is substituted by cysteine (S258C);
- (ii) the leucine residue at a position corresponding to position 260 of wild-type human Neu2 is substituted by aspartic acid (L260D), phenylalanine (L260F), glutamine (L260Q), or threonine (L260T);
- (jj) the valine residue at a position corresponding to position 265 of wild-type human Neu2 is substituted by phenylalanine (V265F);
- (kk) the glutamine residue at a position corresponding to position 270 of wild-type human Neu2 is substituted by alanine (Q270A), histidine (Q270H), phenylalanine (Q270F), proline (Q270P), serine (Q270S), or threonine (Q270T);
- (ll) the tryptophan residue at a position corresponding to position 292 of wild-type human Neu2 is substituted by arginine (W292R);
- (mm) the serine residue at a position corresponding to position 301 of wild-type human Neu2 is substituted by alanine (S301A), aspartic acid (S301D), glutamic acid (S301E), phenylalanine (S301F), histidine (S301H), lysine (S301K), leucine (S301L), methionine (S301M), asparagine (S301N), proline (S301P), glutamine (S301Q), arginine (S301R), threonine (S301T), valine (S301V), tryptophan (S301W), or tyrosine (S301Y);
- (nn) the tryptophan residue at a position corresponding to position 302 of wild-type

human Neu2 is substituted by alanine (W302A), aspartic acid (W302D), phenylalanine (W302F), glycine (W302G), histidine (W302H), isoleucine (W302I), lysine (W302K), leucine (W302L), methionine (W302M), asparagine (W302N), proline (W302P), glutamine (W302Q), arginine (W302R), serine (W302S), threonine (W302T), valine (W302V), or tyrosine (W302Y);

(oo) the valine residue at a position corresponding to position 363 of wild-type human Neu2 is substituted by arginine (V363R); or

(pp) the leucine residue at a position corresponding to position 365 of wild-type human Neu2 is substituted by glutamine (L365Q), histidine (L365H), isoleucine (L365I), lysine (L365K) or serine (L365S);

or the sialidase comprises a combination of any of the foregoing substitutions.

~~12. The enzyme of any one of claims 7-10, wherein, in the sialidase:~~

~~(a) — the proline residue at a position corresponding to position 5 of wild-type human Neu2 is substituted by histidine (P5H);~~

~~(b) — the lysine residue at a position corresponding to position 9 of wild-type human Neu2 is substituted by aspartic acid (K9D);~~

~~(c) — the lysine residue at a position corresponding to position 44 of wild-type human Neu2 is substituted by arginine (K44R) or glutamic acid (K44E);~~

~~(d) — the lysine residue at a position corresponding to position 45 of wild-type human Neu2 is substituted by alanine (K45A), arginine (K45R), or glutamic acid (K45E);~~

~~(e) — the leucine residue at a position corresponding to position 54 of wild-type human Neu2 is substituted by methionine (L54M);~~

~~(f) — the proline residue at a position corresponding to position 62 of wild-type human Neu2 is substituted by asparagine (P62N), aspartic acid (P62D), histidine (P62H), glutamic acid (P62E), glycine (P62G), serine (P62S), or threonine (P62T);~~

~~(g) — the glutamine residue at a position corresponding to position 69 of wild-type human Neu2 is substituted by histidine (Q69H);~~

~~(h) — the arginine residue at a position corresponding to position 78 of wild-type human Neu2 is substituted by lysine (R78K);~~

~~(i) — the aspartic acid residue at a position corresponding to position 80 of wild-type human Neu2 is substituted by proline (D80P);~~

~~(j) — the alanine residue at a position corresponding to position 93 of wild-type human Neu2 is substituted by glutamic acid (A93E) or lysine (A93K);~~

- (k) — the glycine residue at a position corresponding to position 107 of wild-type human Neu2 is substituted by aspartic acid (G107D);
- (l) — the glutamine residue at a position corresponding to position 108 of wild-type human Neu2 is substituted by histidine (Q108H);
- 5 (m) — the glutamine residue at a position corresponding to position 112 of wild-type human Neu2 is substituted by arginine (Q112R) or lysine (Q112K);
- (n) — the cysteine residue at a position corresponding to position 125 of wild-type human Neu2 is substituted by leucine (C125L);
- 10 (o) — the glutamine residue at a position corresponding to position 126 of wild-type human Neu2 is substituted by leucine (Q126L), glutamic acid (Q126E), phenylalanine (Q126F), histidine (Q126H), isoleucine (Q126I), or tyrosine (Q126Y);
- (p) — the alanine residue at a position corresponding to position 150 of wild-type human Neu2 is substituted by valine (A150V);
- 15 (q) — the cysteine residue at a position corresponding to position 164 of wild-type human Neu2 is substituted by glycine (C164G);
- (r) — the arginine residue at a position corresponding to position 170 of wild-type human Neu2 is substituted by proline (R170P);
- 20 (s) — the alanine residue at a position corresponding to position 171 of wild-type human Neu2 is substituted by glycine (A171G);
- (t) — the glutamine residue at a position corresponding to position 188 of wild-type human Neu2 is substituted by proline (Q188P);
- (u) — the arginine residue at a position corresponding to position 189 of wild-type human Neu2 is substituted by proline (R189P);
- 25 (v) — the alanine residue at a position corresponding to position 213 of wild-type human Neu2 is substituted by cysteine (A213C), asparagine (A213N), serine (A213S) or threonine (A213T);
- (w) — the leucine residue at a position corresponding to position 217 of wild-type human Neu2 is substituted by alanine (L217A) or valine (L217V);
- 30 (x) — the glutamic acid residue at a position corresponding to position 225 of wild-type human Neu2 is substituted by proline (E225P);
- (y) — the histidine residue at a position corresponding to position 239 of wild-type human Neu2 is substituted by proline (H239P);

- (z) — the leucine residue at a position corresponding to position 240 of wild-type human Neu2 is substituted by aspartic acid (L240D), asparagine (L240N), or tyrosine (L240Y);
- (aa) — the arginine residue at a position corresponding to position 241 of wild-type human Neu2 is substituted by alanine (R241A), aspartic acid (R241D), leucine (R241L), glutamine (R241Q), or tyrosine (R241Y);
- (bb) — the alanine residue at a position corresponding to position 242 of wild-type human Neu2 is substituted by cysteine (A242C), phenylalanine (A242F), glycine (A242G), histidine (A242H), isoleucine (A242I), lysine (A242K), leucine (A242L), methionine (A242M), asparagine (A242N), glutamine (A242Q), arginine (A242R), serine (A242S), valine (A242V), tryptophan (A242W), or tyrosine (A242Y);
- (cc) — the valine residue at a position corresponding to position 244 of wild-type human Neu2 is substituted by isoleucine (V244I) or proline (V244P);
- (dd) — the threonine residue at a position corresponding to position 249 of wild-type human Neu2 is substituted by alanine (T249A);
- (ee) — the aspartic acid residue at a position corresponding to position 251 of wild-type human Neu2 is substituted by glycine (D251G);
- (ff) — the glutamic acid residue at a position corresponding to position 257 of wild-type human Neu2 is substituted by proline (E257P);
- (gg) — the serine residue at a position corresponding to position 258 is substituted by cysteine (S258C);
- (hh) — the leucine residue at a position corresponding to position 260 of wild-type human Neu2 is substituted by aspartic acid (L260D), phenylalanine (L260F), glutamine (L260Q), or threonine (L260T);
- (ii) — the valine residue at a position corresponding to position 265 of wild-type human Neu2 is substituted by phenylalanine (V265F);
- (jj) — the glutamine residue at a position corresponding to position 270 of wild-type human Neu2 is substituted by alanine (Q270A), histidine (Q270H), phenylalanine (Q270F), proline (Q270P), serine (Q270S), or threonine (Q270T);
- (kk) — the tryptophan residue at a position corresponding to position 292 of wild-type human Neu2 is substituted by arginine (W292R);
- (ll) — the serine residue at a position corresponding to position 301 of wild-type human Neu2 is substituted by alanine (S301A), aspartic acid (S301D), glutamic acid

(S301E), phenylalanine (S301F), histidine (S301H), lysine (S301K), leucine (S301L), methionine (S301M), asparagine (S301N), proline (S301P), glutamine (S301Q), arginine (S301R), threonine (S301T), valine (S301V), tryptophan (S301W), or tyrosine (S301Y);

~~(mm) the tryptophan residue at a position corresponding to position 302 of wild-type human Neu2 is substituted by alanine (W302A), aspartic acid (W302D), phenylalanine (W302F), glycine (W302G), histidine (W302H), isoleucine (W302I), lysine (W302K), leucine (W302L), methionine (W302M), asparagine (W302N), proline (W302P), glutamine (W302Q), arginine (W302R), serine (W302S), threonine (W302T), valine (W302V), or tyrosine (W302Y);~~

~~(nn) the valine residue at a position corresponding to position 363 of wild-type human Neu2 is substituted by arginine (V363R); or~~

~~(oo) the leucine residue at a position corresponding to position 365 of wild-type human Neu2 is substituted by glutamine (L365Q), histidine (L365H), isoleucine (L365I), lysine (L365K) or serine (L365S);~~

~~or the sialidase comprises a combination of any of the foregoing substitutions.~~

~~13.4.~~ The enzyme of claim ~~11-3~~~~or 12~~, wherein the sialidase comprises a substitution selected from the ~~A42R~~, K9D, ~~A42R~~, P62G, P62N, P62S, P62T, A93E, Q126Y, A242F, A242W, A242Y, Q270A, Q270T, S301A, S301R, W302K, W302R, V363R, and L365I substitutions, or a combination of any of the foregoing substitutions.

5. A recombinant mutant sialidase enzyme, wherein the sialidase comprises a mutation that increases resistance to cleavage by a protease.

6. The enzyme of claim 5, wherein incubation of the sialidase with the protease results in less than 50% (e.g., less than 40%, less than 30%, less than 10%, less than 5%, less than 3%, less than 1%, or less than 0.5%) of the proteolytic cleavage of a corresponding wild-type sialidase when incubated with the protease under the same conditions.

7. The enzyme of claim 5 or claim 6, wherein the protease is trypsin.

8. The enzyme of any one of claims 5-7, wherein the sialidase comprises:

(a) a substitution of an alanine residue at a position corresponding to position 213 of wild-type human Neu2 (A213);

(b) a substitution of a leucine residue at a position corresponding to position 240 of wild-type human Neu2 (L240);

- (c) a substitution of an arginine residue at a position corresponding to position 241 of wild-type human Neu2 (R241);
- (d) a substitution of an alanine residue at a position corresponding to position 242 of wild-type human Neu2 (A242);
- 5 (e) a substitution of an arginine residue at a position corresponding to position 243 of wild-type human Neu2 (R243);
- (f) a substitution of a valine residue at a position corresponding to position 244 of wild-type human Neu2 (V244);
- 10 (g) a substitution of a serine residue at a position corresponding to position 258 of wild-type human Neu2 (S258);
- (h) a substitution of a leucine residue at a position corresponding to position 260 of wild-type human Neu2 (L260);
- (i) a substitution of a valine residue at a position corresponding to position 265 of wild-type human Neu2 (V265);
- 15 or a combination of any of the foregoing substitutions.

9. The enzyme of claim 8, wherein, in the sialidase:

- (a) the alanine residue at a position corresponding to position 213 of wild-type human Neu2 is substituted by cysteine (A213C), asparagine (A213N), serine (A213S), or threonine (A213T);
- 20 (b) the leucine residue at a position corresponding to position 240 of wild-type human Neu2 (L240) is substituted by aspartic acid (L240D), asparagine (L240N), or tyrosine (L240Y);
- (c) the arginine residue at a position corresponding to position 241 of wild-type human Neu2 (R241) is substituted by alanine (R241A), aspartic acid (R241D), leucine (R241L), glutamine (R241Q), or tyrosine (R241Y);
- 25 (d) the alanine residue at a position corresponding to position 242 of wild-type human Neu2 (A242) is substituted by cysteine (A242C), phenylalanine (A242F), glycine (A242G), histidine (A242H), isoleucine (A242I), lysine (A242K), leucine (A242L), methionine (A242M), asparagine (A242N), glutamine (A242Q), arginine (A242R), serine (A242S), valine (A242V), tryptophan (A242W), or tyrosine (A242Y);
- 30 (e) the valine residue at a position corresponding to position 244 of wild-type human Neu2 (V244) is substituted by isoleucine (V244I), lysine (V244K), or proline

(V244P);

(f) the serine residue at a position corresponding to position 258 of wild-type human Neu2 (S258) is substituted by cysteine (S258C);

(g) the leucine residue at a position corresponding to position 260 of wild-type human Neu2 (L260) is substituted by aspartic acid (L260D), phenylalanine (L260F), glutamine (L260Q), or threonine (L260T);

(h) the valine residue at a position corresponding to position 265 of wild-type human Neu2 (V265) is substituted by phenylalanine (V265F);

or a combination of any of the foregoing substitutions.

~~14. The enzyme of claim 11 or 12, wherein the sialidase comprises a substitution selected from the K9D, P62G, P62N, P62S, P62T, A93E, Q126Y, A242F, A242W, A242Y, Q270A, Q270T, S301A, S301R, W302K, W302R, V363R, and L365I substitutions, or a combination of any of the foregoing substitutions.~~

~~15.10.~~ The enzyme of any one of claims 1-~~149~~, wherein the sialidase further comprises:

- (a) a substitution or deletion of a methionine residue at a position corresponding to position 1 of wild-type human Neu2 (M1);
 - (b) a substitution of a valine residue at a position corresponding to position 6 of wild-type human Neu2 (V6);
 - (c) a substitution of an isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 (I187); or
 - (d) a substitution of a cysteine residue at a position corresponding to position 332 of wild-type human Neu2 (C332);
- or a combination of any of the foregoing substitutions.

~~16.11.~~ The enzyme of claim ~~15.10~~, wherein, in the sialidase:

- (a) the methionine residue at a position corresponding to position 1 of wild-type human Neu2 is deleted (Δ M1), is substituted by alanine (M1A), or is substituted by aspartic acid (M1D);
- (b) the valine residue at a position corresponding to position 6 of wild-type human Neu2 is substituted by tyrosine (V6Y);
- (c) the isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 is substituted by lysine (I187K); or
- (d) the cysteine residue at a position corresponding to position 332 of wild-type

human Neu2 is substituted by alanine (C332A);
or the sialidase comprises a combination of any of the foregoing substitutions.

~~17.12.~~ 17.12. The enzyme of claim ~~1611~~, wherein the sialidase comprises:

- (a) the M1D, V6Y, P62G, A93E, I187K, and C332A substitutions;
- 5 (b) the M1D, V6Y, K9D, A93E, I187K, C332A, V363R, and L365I substitutions;
- (c) the M1D, V6Y, P62N, I187K, and C332A substitutions;
- (d) the M1D, V6Y, I187K, Q270A, S301R, W302K, and C332A substitutions;
- (e) the M1D, V6Y, P62S, I187K, Q270A, S301R, W302K, and C332A substitutions;
- (f) the M1D, V6Y, P62T, I187K, Q270A, S301R, W302K, and C332A substitutions;
- 10 (g) the M1D, V6Y, P62N, I187K, Q270A, S301R, W302K, and C332A substitutions;
- (h) the M1D, V6Y, P62G, A93E, I187K, S301A, W302R, and C332A substitutions;
- (i) the M1D, V6Y, P62G, A93E, Q126Y, I187K, Q270T, and C332A substitutions;
- (j) the M1D, V6Y, P62G, A93E, Q126Y, I187K, and C332A substitutions;
- (k) the M1D, V6Y, P62G, A93E, Q126Y, I187K, A242F, Q270T, and C332A
15 substitutions; or
- (l) the M1D, V6Y, A42R, P62G, A93E, Q126Y, I187K, A242F, Q270T, and C332A
substitutions.

~~18.13.~~ 18.13. The enzyme of claim ~~1612~~, wherein the sialidase comprises:

- ~~(a) the M1D, V6Y, P62G, A93E, I187K, and C332A substitutions;~~
- 20 ~~(b) the M1D, V6Y, K9D, A93E, I187K, C332A, V363R, and L365I substitutions;~~
- ~~(c) the M1D, V6Y, P62N, I187K, and C332A substitutions;~~
- ~~(d) the M1D, V6Y, I187K, Q270A, S301R, W302K, and C332A substitutions;~~
- ~~(e) the M1D, V6Y, P62S, I187K, Q270A, S301R, W302K, and C332A substitutions;~~
- ~~(f) the M1D, V6Y, P62T, I187K, Q270A, S301R, W302K, and C332A substitutions;~~
- 25 ~~(g) the M1D, V6Y, P62N, I187K, Q270A, S301R, W302K, and C332A substitutions;~~
- ~~(h) the M1D, V6Y, P62G, A93E, I187K, S301A, W302R, and C332A substitutions;~~
- ~~(i) the M1D, V6Y, P62G, A93E, Q126Y, I187K, Q270T, and C332A substitutions; or~~
- ~~(j)~~ (j) the M1D, V6Y, A42R, P62G, A93E, Q126Y, I187K, A242F, Q270T, and C332A
substitutions.

~~19.14.~~ 19.14. The enzyme of any one of claims 1-~~1813~~, wherein the sialidase is a human

~~sialidase selected from Neu1, Neu2, Neu3, and Neu4.~~

~~20.15.~~ The enzyme of claim ~~19~~14, wherein the sialidase is Neu2.

~~21. The enzyme of any one of claims 1-20, wherein the sialidase has a different substrate specificity than the corresponding wild-type sialidase.~~

5 ~~22. The enzyme of claim 21, wherein the sialidase can cleave α 2,3, α 2,6, and/or α 2,8 linkages.~~

~~23. The enzyme of claim 22, wherein the sialidase can cleave α 2,3 and α 2,8 linkages.~~

~~24.16.~~ The enzyme of any one of claims 1-~~23~~15, wherein the sialidase comprises any one of SEQ ID NOs: 48-54, 149, 154, 159, 191, or 198.

10 ~~25. The enzyme of any one of claims 1-23, wherein the sialidase comprises any one of SEQ ID NOs: 48-54, 149, 154, or 159.~~

~~26. A recombinant mutant human sialidase comprising a mutation or combination of mutations set forth in any one of TABLES 1, 2, 7-9, 11-13, 15-30, 34, or 35, and optionally further comprising a mutation or combination of mutations set forth in any one of TABLES~~
15 ~~3-6.~~

~~27.17.~~ A fusion protein comprising:

(a) the recombinant enzyme of any one of claims 1-~~26~~16; and

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

20 wherein the enzyme and the immunoglobulin Fc domain and/or the immunoglobulin antigen-binding domain are linked by a peptide bond or an amino acid linker.

~~28.18.~~ The fusion protein of claim ~~27~~17, wherein the fusion protein comprises an immunoglobulin Fc domain.

~~29. The fusion protein of claim 28, wherein the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM Fc domain.~~

25 ~~30.19.~~ The fusion protein of claim ~~29~~18, wherein the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, or IgG4 Fc domain.

~~31.20.~~ The fusion protein of claim ~~30~~19, wherein the immunoglobulin Fc domain is derived from a human IgG1 Fc domain.

~~32.21.~~ The fusion protein of any one of claims ~~27~~17-~~31~~20, wherein the fusion protein

comprises an immunoglobulin antigen-binding domain.

~~33.22.~~ The fusion protein of claim ~~32.21~~, wherein the immunoglobulin antigen-binding domain is associated with a second immunoglobulin antigen-binding domain to produce an antigen-binding site.

5 ~~34.23.~~ The fusion protein of claim ~~32.21~~ or ~~33.22~~, wherein the immunoglobulin antigen-binding domain is derived from an antibody selected from trastuzumab, daratumumab, girentuximab, ofatumumab, avelumab, and rituximab.

~~35.24.~~ The fusion protein of any one of claims ~~27.17-34.23~~, wherein the fusion protein comprises any one of SEQ ID NOs: 203-210, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, or 242.

~~25.~~ The fusion protein of claim 24, wherein the fusion protein comprises SEQ ID NO: ~~210~~.

~~36.26.~~ An antibody conjugate comprising the fusion protein of any one of claims ~~27.17-35.25~~.

15 ~~37.~~ The antibody conjugate of claim 36, wherein the antibody conjugate comprises a single sialidase.

~~38.~~ The antibody conjugate of claim 36, wherein the antibody conjugate comprises two sialidases.

~~39.~~ The antibody conjugate of claim 37, wherein the two sialidases are identical.

20 ~~40.~~ The antibody conjugate of any one of claims 36-39, wherein the antibody conjugate comprises a single antigen-binding site.

~~41.~~ The antibody conjugate of any one of claims 36-39, wherein the antibody conjugate comprises two antigen-binding sites.

~~42.~~ The antibody conjugate of claim 41, wherein the two antigen-binding sites are identical.

25 ~~43.~~ The antibody conjugate of any one of claims 36-42, wherein the antibody conjugate has a molecular weight from about 135 kDa to about 165 kDa.

~~44.~~ The antibody conjugate of any one of claims 36-42, wherein the antibody conjugate has a molecular weight from about 215 kDa to about 245 kDa.

~~45.27.~~ The antibody conjugate of ~~any one of~~ claims ~~36.26-44~~, 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 a sialidase;
- wherein the first and second polypeptides are covalently linked together and the second and third polypeptides are linked together, and wherein the first polypeptide and the second polypeptide together define an antigen-binding site.

4628. The antibody conjugate of claim **4527**, wherein the third polypeptide comprises the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation.

~~47. The antibody conjugate of any one of claims 36-44, 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 sialidase;~~
 - ~~(c) a third polypeptide comprising a second immunoglobulin heavy chain and a second sialidase; 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.~~

~~48. The antibody conjugate of claim 47, wherein 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.~~

~~49. The antibody conjugate of any one of claims 36-44, wherein the antibody conjugate comprises:~~

- ~~(a) a first polypeptide comprising a first sialidase, a first immunoglobulin Fc domain, and a first single chain variable fragment (scFv); and~~
 - ~~(b) a second polypeptide comprising a second sialidase, 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.~~

50. ~~The antibody conjugate of claim 49, wherein the first polypeptide comprises the first sialidase, the first immunoglobulin Fc domain, and the first scFv in an N- to C-terminal orientation, and the second polypeptide comprises the second sialidase, the second immunoglobulin Fc domain, and the second scFv in an N- to C-terminal orientation.~~

35. ~~51. The antibody conjugate of any one of claims 36-44, 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 sialidase;~~

~~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.~~

52. ~~The antibody conjugate of claim 51, 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 sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation.~~

5329. An isolated nucleic acid comprising a nucleotide sequence encoding the recombinant enzyme of any one of claims 1-2616, the fusion protein of any one of claims 2717-3524, or at least a portion of the antibody conjugate of any one of claims 3626-5228.

5430. An expression vector comprising the nucleic acid of claim 5329.

5531. A host cell comprising the expression vector of claim 5430.

5632. A pharmaceutical composition comprising the recombinant enzyme of any one of claims 1-2616, the fusion protein of any one of claims 2717-3524, or the antibody conjugate of any one of claims 3625-5228.

5733. ~~A method of treating cancer in a subject in need thereof, the method comprising administering to the subject an effective amount~~Use of the recombinant enzyme of any one of claims 1-2616, the fusion protein of any one of claims 2717-3524, or at least a portion of the antibody conjugate of any one of claims 3625-5228, or the pharmaceutical composition of claim 5632 in the preparation of a medicament for the treatment of cancer in a subject.

~~58.—The method of claim 57, wherein the cancer is a solid tumor, soft tissue tumor, hematopoietic tumor or metastatic lesion.~~

~~59.—The method of claim 58, wherein the solid tumor is a sarcoma, adenocarcinoma, or carcinoma.~~

5 ~~60.—The method of claim 58 or 59, 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.~~

10 ~~61.—The method of claim 58, 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~~
15 ~~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).~~