by expressing ligands, for example, PD-L1, that interact with PD-1 on the surface of T-cells to reduce T-cell activity. Many anti-PD-1 antibodies have been developed for use in immuno-oncology therapies. For example, nivolumab has been approved in the United States for use in the treatment of, for example, certain melanomas, non-small cell lung cancers (NSCLC), small cell lung cancers (SCLC), mesotheliomas, renal cell carcinomas (RCC), Hodgkin lymphomas, squamos cell carcinomas of the head and neck, urothelial carcinomas, colorectal cancers, hepatocellular carcinomas, and esophageal squamous cell carcinomas; and pembrolizumab has been approved in the United States for use in the treatment of, for example, certain melanomas, non-small cell lung cancers (NSCLC), small cell lung cancers (SCLC), head and neck squamous cell cancers, Hodgkin lymphomas, primary mediastinal large B-cell lymphomas, urothelial carcinomas, gastric cancers, esophageal cancers, cervical cancers, hepatocellular carcinomas, Merkel cell carcinomas, renal cell carcinomas (RCC), endometrial carcinomas, cutaneous squamous cell carcinomas, and triple negative breast cancers.

[0006] 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 cells.

SUMMARY

[0007] The disclosure is based, in part, upon the discovery that it is possible to produce fusion proteins containing a sialidase enzyme and an anti-PD-1 immunoglobulin or a portion thereof, *e.g.*, an antigen-binding domain and/or an immunoglobulin Fc domain, and/or antibody conjugates including a sialidase enzyme and an anti-PD-1 antibody or a portion thereof, *e.g.*, an antigen-binding domain and/or an immunoglobulin Fc domain. The sialidase enzyme portion of the fusion protein and/or antibody conjugate may comprise at least one mutation relative to a wild-type sialidase. The mutations, or combination of mutations, can improve the expression, activity or both the expression and activity of the sialidase to improve its use in cancer diagnosis and/or treatment. The fusion proteins and/or antibody conjugates have suitable substrate specificities and activities to be useful in removing sialic acid and/or sialic acid containing molecules from the surface of cells, *e.g.,* PD-1-expressing 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] In one aspect, the present disclosure provides a fusion protein comprising: (a) a recombinant mutant human sialidase enzyme; and (b) an anti-PD-1 immunoglobulin antigen-binding domain, wherein the sialidase comprises: (i) a substitution of a proline residue at a position corresponding to position 62 of wild-type human Neu2 (P62); (ii) a substitution of an alanine residue at a position corresponding to position 93 of wild-type human Neu2 (A93); (iii) a substitution of a glutamine residue at a position corresponding to position 126 of wild-type human Neu2 (Q126); (iv) a substitution of an alanine residue at a position corresponding to position 242 of wild-type human Neu2 (A242); (v) a substitution of a glutamine residue at a position corresponding to position 270 of wild-type human Neu2 (Q270); and/or (vi) a substitution of an alanine residue at a position corresponding to position 42 of wild-type human Neu2 (A42). Accordingly, in one example, the disclosure provides a fusion protein comprising (or consisting essentially of): (a) sialidase enzyme; and (b) an anti-PD-1 immunoglobulin antigen-binding domain.

[0009] In certain examples, the sialidase is a human sialidase, *e.g.*, a recombinant mutant human sialidase. In certain examples, the sialidase 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 a lysine residue at a position corresponding to position 9 of wild-type human Neu2 (K9); (d) a substitution of an alanine residue at a position corresponding to position 42 of wild-type human Neu2 (A42); (e) a substitution of a proline residue at a position corresponding to position 62 of wild-type human Neu2 (P62); (f) a substitution of an alanine residue at a position corresponding to position 93 of wild-type human Neu2 (A93); (g) a substitution of a glutamine residue at a position corresponding to position 126 of wild-type human Neu2 (Q126); (h) a substitution of an isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 (I187); (i) a substitution of an alanine residue at a position corresponding to position 242 of wild-type human Neu2 (A242); (j) a substitution of a glutamine residue at a position corresponding to position 270 of wild-type human Neu2 (Q270); (k) a substitution of a serine residue at a position corresponding to position 301 of wild-type human Neu2 (S301); (l) a substitution of a tryptophan residue at a position corresponding to position 302 of wild-type human Neu2 (W302); (m) a substitution of a cysteine residue at a position corresponding to position 332 of wild-type human Neu2 (C332); (n) a substitution of a valine residue at a position corresponding to position 363 of wild-type human Neu2 (V363); or

(o) 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.

[0010] In certain examples, in the sialidase: (a) the methionine residue at a position corresponding to position 1 of wild-type human Neu2 is deleted (ΔM1), 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 lysine residue at a position corresponding to position 9 of wild-type human Neu2 is substituted by aspartic acid (K9D); (d) the alanine residue at a position corresponding to position 42 of wild-type human Neu2 is substituted by arginine (A42R) or aspartic acid (A42D); (e) 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); (f) the alanine residue at a position corresponding to position 93 of wild-type human Neu2 is substituted by glutamic acid (A93E) or lysine (A93K); (g) 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); (h) the isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 is substituted by lysine (I187K); (i) 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); (j) 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); (k) 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); (l) 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); (m) the cysteine residue at a position corresponding to position 332 of wild-type human Neu2 is substituted by alanine (C332A); (n) the valine residue at a position corresponding to position 363 of wild-type human Neu2 is substituted by arginine (V363R); or (o) 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 modification selected from ΔM1, M1A, M1D, V6Y, K9D, A42R, P62G, P62N, P62S, P62T, A93E, Q126Y, I187K, A242F, A242W, A242Y, Q270A, Q270T, S301A, S301R, W302K, W302R, C332A, V363R, and L365I, or a combination of any of the foregoing modifications.

[0011] In certain examples, 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 mutations.

[0012] In certain examples, the sialidase is selected from Neu1, Neu2, Neu3, and Neu4, *e.g.*, the sialidase is Neu2.

[0013] In certain examples, the sialidase has a different substrate specificity than the corresponding wild-type sialidase. For example, in certain examples the sialidase can cleave α2,3, α2,6, and/or α2,8 linkages. In certain examples the sialidase can cleave α2,3 and α2,8 linkages.

[0014] In certain examples, the sialidase comprises any one of SEQ ID NOs: 48-63, 94, 97, 100, or 126, 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: 48-63, 94, 97, 100, or 126.

[0015] In certain examples, the sialidase comprises mutation or combination of mutations set forth in any one of Tables 1-9.

[0016] In certain examples, the fusion protein further comprises an immunoglobulin Fc domain. In certain 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.

[0017] In certain examples, the anti-PD-1 immunoglobulin antigen-binding domain is associated (for example, covalently or non-covalently associated) with a second anti-PD-1 immunoglobulin antigen-binding domain to produce an anti-PD-1 antigen-binding site.

[0018] In certain examples, the anti-PD-1 immunoglobulin antigen-binding domain is derived from an antibody selected from nivolumab, pembrolizumab, cemiplimab, spartalizumab

(PDR001), TX-4014, camrelizumab (SHR1210), sintilimab (IBI308), tislelizumab (BGB-A317), toripalimab (JS 001), dostarlimab (TSR-042, WBP-285), INCMGA00012 (MGA012), AMP-514, and pidilizumab, *e.g.*, the anti-PD-1 immunoglobulin antigen-binding domain is derived from nivolumab or pembrolizumab.

[0019] In certain examples, the sialidase and the immunoglobulin Fc domain and/or the anti-PD-1 immunoglobulin antigen-binding domain are linked by a peptide bond or an amino acid linker.

[0020] In certain examples, the fusion protein comprises any one of SEQ ID NOs: 67-73, 78, 81-87, 95, 96, 98, 99, 101, 102, 105, 106, 108, 111, 112, 115, 122, 123, 125, 127, 128, 130, 132, 134, or 145.

[0021] In another aspect, the disclosure provides an antibody conjugate comprising any of the foregoing fusion proteins. In certain examples, the antibody conjugate comprises a single sialidase. In other examples, the antibody conjugate comprises two sialidases, which can be the same or different. In certain examples the antibody conjugate comprises two identical sialidases. In certain examples, the antibody conjugate comprises a single anti-PD-1 antigen-binding site. In other examples, the antibody conjugate comprises two anti-PD-1 antigen-binding sites, which can be the same or different. In certain examples, the antibody conjugate comprises two identical anti-PD-1 antigen-binding sites.

[0022] In certain examples, 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.

[0023] In certain examples, 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 anti-PD-1 antigen-binding site. The third polypeptide may, for example, comprise the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation. The first polypeptide may, for example, comprise SEQ ID NO: 77, the second polypeptide may, for example, comprise SEQ ID NO: 105, and/or the third polypeptide may, for example, comprise any one of SEQ ID NOs: 67-73, 78, 81-87, 95, 96, 98, 99, 101, 102, 106, 108, 111, 112, 115, 122, 123, 125, 127, or 128.

[0024] In certain examples, 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 anti-PD-1 antigen-binding site, and the third polypeptide and the fourth polypeptide together define a second anti-PD-1 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. The first and fourth polypeptide may, for example, comprise SEQ ID NO: 77. The second and third polypeptide may, for example, comprise SEQ ID NO: 145.

[0025] In certain 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 the first scFv defines a first anti-PD-1 antigen-binding site, and the second scFv, when present, defines a second anti-PD-1 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 orientation.

[0026] In certain 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 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 anti-PD-1 antigen-binding site and the scFv defines a second anti-PD-1 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 example, comprise the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation.

[0027] In another aspect, the disclosure provides an isolated nucleic acid comprising a nucleotide sequence encoding any of the foregoing fusion proteins or at least a portion of any of the foregoing antibody conjugates. In another aspect, the disclosure provides an expression vector comprising any of the foregoing nucleic acids. In another aspect, the disclosure provides a host cell comprising any of the foregoing expression vectors.

[0028] In another aspect, the disclosure provides a pharmaceutical composition comprising any of the foregoing fusion proteins or any of the foregoing antibody conjugates.

[0029] In yet another aspect, the present disclosure provides a use of the fusion protein as described herein, the antibody conjugate as described herein, or the pharmaceutical composition as described herein in the preparation of a medicament for treating cancer in a subject in need thereof. In another example, the 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 fusion proteins, any of the foregoing antibody conjugates, or any of the foregoing pharmaceutical compositions.

[0030] In certain examples, the cancer is selected from melanoma, bladder cancer, breast cancer, cervical cancer, esophageal cancer, gastric cancer, kidney cancer, lung cancer (*e.g.*, NSCLC), ovarian cancer, metastatic Merkel cell carcinoma (MCC), metastatic urothelial carcinoma (UC), pancreatic cancer, colon cancer, stomach cancer, AML, and multiple myeloma. For example, in certain examples, the cancer is NSCLC or melanoma.

[0031] These and other examples and features of the disclosure are described in the following detailed description and claims.

DESCRIPTION OF THE DRAWINGS

[0032] The invention can be more completely understood with reference to the following drawings.

[0033] 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.

[0034] FIGURE 2 is a bar graph showing the enzymatic activity of recombinant human Neu1, Neu2, and Neu3.

[0035] 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.

WHAT IS CLAIMED IS:

1. A fusion protein comprising:

   (a) a recombinant mutant human sialidase enzyme; and

   (b) an anti-PD-1 immunoglobulin antigen-binding domain,

   wherein the sialidase comprises:
   (i) a substitution of a proline residue at a position corresponding to position 62 of wild-type human Neu2 (P62);
   (ii) a substitution of an alanine residue at a position corresponding to position 93 of wild-type human Neu2 (A93);
   (iii) a substitution of a glutamine residue at a position corresponding to position 126 of wild-type human Neu2 (Q126);
   (iv) a substitution of an alanine residue at a position corresponding to position 242 of wild-type human Neu2 (A242);
   (v) a substitution of a glutamine residue at a position corresponding to position 270 of wild-type human Neu2 (Q270); and/or
   (vi) a substitution of an alanine residue at a position corresponding to position 42 of wild-type human Neu2 (A42).

2. The fusion protein of claim 1, 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 a lysine residue at a position corresponding to position 9 of wild-type human Neu2 (K9);

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

   (e) a substitution of a serine residue at a position corresponding to position 301 of wild-type human Neu2 (S301);

   (f) a substitution of a tryptophan residue at a position corresponding to position 302 of

wild-type human Neu2 (W302);

(g) a substitution of a cysteine residue at a position corresponding to position 332 of wild-type human Neu2 (C332);

(h) a substitution of a valine residue at a position corresponding to position 363 of wild-type human Neu2 (V363); or

(i) 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.

3. The fusion protein of claim 1 or 2, wherein, in the sialidase:

(a) the methionine residue at a position corresponding to position 1 of wild-type human Neu2 is deleted (ΔM1), 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 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 9 of wild-type human Neu2 is substituted by aspartic acid (K9D);

(e) 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);

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

(g) 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);

(h) the isoleucine residue at a position corresponding to position 187 of wild-type human Neu2 is substituted by lysine (I187K);

(i) 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);

(j) 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);

(k) 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);

(l) 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);

(m) the cysteine residue at a position corresponding to position 332 of wild-type human Neu2 is substituted by alanine (C332A);

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

(o) 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.

4. The fusion protein of claim 3, wherein the sialidase comprises a substitution selected from ΔM1, M1A, M1D, V6Y, K9D, A42R, P62G, P62N, P62S, P62T, A93E, Q126Y, I187K, A242F, A242W, A242Y, Q270A, Q270T, S301A, S301R, W302K, W302R, C332A, V363R, and L365I, or a combination of any of the foregoing substitutions.

5. The fusion protein of claim 4, wherein 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 mutations.

6. The fusion protein of any one of claims 1-5, wherein the sialidase is selected from Neu1, Neu2, Neu3, and Neu4.

7. The fusion protein of any one of claims 1-6, wherein the sialidase comprises any one of SEQ ID NOs: 48-63, 94, 97, 100, or 126.

8. The fusion protein of any one of claims 1-7, wherein the fusion protein further comprises an immunoglobulin Fc domain.

9. The fusion protein of claim 8, wherein the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, or IgG4 Fc domain.

10. The fusion protein of any one of claims 1-9, wherein the anti-PD-1 immunoglobulin antigen-binding domain is associated with a second anti-PD-1 immunoglobulin antigen-binding domain to produce an anti-PD-1 antigen-binding site.

11. The fusion protein of any one of claims 1-10, wherein the anti-PD-1 immunoglobulin antigen-binding domain is derived from an antibody selected from nivolumab, pembrolizumab, cemiplimab, PDR001, and pidilizumab.

12. The fusion protein of claim 11, wherein the anti-PD-1 immunoglobulin antigen-binding

domain is derived from pembrolizumab.

13. The fusion protein of any one of claims 1-12, wherein the sialidase and the immunoglobulin Fc domain and/or the anti-PD-1 immunoglobulin antigen-binding domain are linked by a peptide bond or an amino acid linker.

14. The fusion protein of any one of claims 1-13, wherein the fusion protein comprises any one of SEQ ID NOs: 67-73, 78, 81-87, 95, 96, 98, 99, 101, 102, 105, 106, 108, 111, 112, 115, 122, 123, 125, 127, 128, 130, 132, or 134.

15. An antibody conjugate comprising the fusion protein of any one of claims 1-14.

16. The antibody conjugate of claim 15, wherein the antibody conjugate comprises a single sialidase and/or a single anti-PD-1 antigen-binding site.

17. The antibody conjugate of claim 15 or 16, 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 covalently linked together, and wherein the first polypeptide and the second polypeptide together define an anti-PD-1 antigen-binding site.

18. The antibody conjugate of claim 17, wherein the third polypeptide comprises the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation.

19. The antibody conjugate of claim 17 or 18, wherein the first polypeptide comprises SEQ ID NO: 77.

20. The antibody conjugate of any one of claims 17-19, wherein the second polypeptide comprises SEQ ID NO: 105.

21. The antibody conjugate of any one of claims 17-20, wherein the third polypeptide comprises any one of SEQ ID NOs: 67-73, 78, 81-87, 95, 96, 98, 99, 101, 102, 106, 108, 111, 112, 115, 122, 123, 125, 127, or 128.

22. An isolated nucleic acid comprising a nucleotide sequence encoding the fusion protein of any

one of claims 1-14, or at least a portion of the antibody conjugate of any one of claims 15-22.

23. An expression vector comprising the nucleic acid of claim 22.

24. A host cell comprising the expression vector of claim 23.

25. A pharmaceutical composition comprising the fusion protein of any one of claims 1-14 or the antibody conjugate of any one of claims 15-21.

26. Use of the fusion protein of any one of claims 1-14, the antibody conjugate of any one of claims 15-21, or the pharmaceutical composition of claim 25 in the preparation of a medicament for treating cancer in a subject in need thereof.