[0005] The gene encoding HER2 is located on chromosome 17 and is a member of the EGF/erbB growth factor receptor gene family, which also includes epidermal growth factor receptor (EGFR, or HER1), HER3/erbB3 and HER4/erbB4. All of these genes encode transmembrane growth factor receptors, which are tyrosine kinase type 1 receptors with growth stimulating potential. Activation of HER family members generally occurs when the ligand and a dimer of the same monomer or other member of the HER family are bound together. HER2 has no known ligand. Once activation has occurred, tyrosine autophoshorylation of cytoplasmic signal proteins transmit signals to the nucleus, thus regulating aspects of cell growth, division, differentiation and migration.

[0006] Overexpression of HER2 receptors results in receptors transmitting excessive signals for cell proliferation to the nucleus (Gutierrez *et al.* (2011) ARCH. PATHOL. LAB. MED. 135(1): 55–62). Data supports the hypothesis that, for certain cancers, overexpression of HER2 directly contributes to the pathogenesis and clinical aggressiveness of tumor cells, and is associated with poor prognosis, including reduced relapse-free and overall survival (Iqbal *et al.* (2014) MOL. BIOL. INT. 2014: 852748). Anti-HER2 antibodies include trastuzumab, which has been approved in the United States for use in the treatment of, for example, breast cancer and gastric or gastroesophageal junction adenocarcinomas, and pertuzumab, which has been approved in the United States for use in the treatment of, for example, breast cancer.

[0007] Cancer immunotherapies have improved the outcome of many cancer patients. However, despite advances that have been made to date, many patients do not respond or only partially respond to currently available therapies. Accordingly, there is still a need for effective interventions for treating cancers associated with hypersialylated cancer cells.

SUMMARY

[0008] The disclosure is based, in part, upon the discovery that it is possible to produce fusion proteins containing a sialidase enzyme and an anti-HER2 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-HER2 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.

[0009] 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 cancer cells, *e.g.,* HER2-expressing 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.

[0010] In one aspect, the present disclosure provides a fusion protein comprising: (a) a recombinant mutant human sialidase, wherein the sialidase comprises a substitution of an alanine residue at a position corresponding to position 42 of wild-type human Neu2 (A42); and (b) an anti-HER2 immunoglobulin antigen-binding domain. Accordingly, in one example, the disclosure provides a fusion protein comprising (or consisting essentially of): (a) sialidase enzyme; and (b) an anti-HER2 immunoglobulin antigen-binding domain.

[0011] 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 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 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 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 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 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 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 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 substitutions.

[0016] In certain examples, the sialidase is selected from Neu1, Neu2, Neu3, and Neu4, *e.g.*, the sialidase is Neu2.

[0017] 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.

[0018] In certain examples, 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.

[0019] In certain examples, the sialidase comprises mutation or combination of mutations set forth in any one of **TABLES 1-11**, hereinbelow.

[0020] 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.

[0021] In certain examples, the anti-HER2 immunoglobulin antigen-binding domain is associated (for example, covalently or non-covalently associated) with a second anti-HER2 immunoglobulin antigen-binding domain to produce an anti-HER2 antigen-binding site.

[0022] In certain examples, the anti-HER2 immunoglobulin antigen-binding domain is derived from an antibody selected from trastuzumab, pertuzumab, CT-P6, trastuzumab-dkst, MGAH22 (margetuximab), PF-05280014, ertumaxomab, gancotamab, timigutuzumab, Ontruzant, ABP-980, SB3, DS-8201, MYL-1410, BCD-022, and HD201, *e.g.,* the anti-HER2 immunoglobulin antigen-binding domain is derived from trastuzumab.

[0023] In certain examples, the sialidase and the immunoglobulin Fc domain and/or the anti-HER2 immunoglobulin antigen-binding domain are linked by a peptide bond or an amino acid linker.

[0024] In certain examples, the fusion protein comprises any one of SEQ ID NOs: 66-85, 98-142, 150-153, 155-158, 160-163, 166-178, 185, 187, 189, 192-197, 203-210, 218, 220, 222, 224, 226, 228, 230, 232, 234, 236, 238, 240, 242, 244, or 249.

[0025] 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-HER2 antigen-binding site. In other examples, the antibody conjugate comprises two anti-HER2 antigen-binding sites, which can be the same or different. In certain examples, the antibody conjugate comprises two identical anti-HER2 antigen-binding sites.

[0026] 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.

[0027] 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-HER2 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: 66, the second polypeptide may, for example, comprise any one of SEQ ID NOs: 67 or 189, and/or the third polypeptide may, for example, comprise any one of SEQ ID NOs: 68-74, 98-112, 150, 151, 155, 156, 160, 161, 185, 187, 192, 195, 203-208. In certain examples, the first polypeptide comprises SEQ ID NO: 66, the second polypeptide comprises SEQ ID NO: 189, and the third polypeptide comprises SEQ ID NO: 205.

[0028] 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-HER2 antigen-binding site, and the third polypeptide and the fourth polypeptide together define a second anti-HER2 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.

[0029] 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-HER2 antigen-binding site, and the second scFv, when present, defines a second anti-HER2 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. The first polypeptide may, for example, comprise any one of SEQ ID NOs: 77-83, 166-178, 194, 197, 244, or 249, and/or the second polypeptide may, for example, comprise any one of SEQ ID NOs: 77-83, 166-178, 194, 197, 244, or 249.

[0030] 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-HER2 antigen-binding site and the scFv defines a second anti-HER2 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.

[0031] 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.

[0032] In another aspect, the disclosure provides a pharmaceutical composition comprising any of the foregoing fusion proteins or any of the foregoing antibody conjugates.

[0033] In 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 the treatment of 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.

[0034] In certain examples, the cancer is selected from lung bronchioloalveolar carcinoma (BAC), bladder cancer, a female genital tract malignancy (*e.g.,* uterine serous carcinoma, endometrial carcinoma, vulvar squamous cell carcinoma, and uterine sarcoma), an ovarian surface epithelial carcinoma (*e.g.,* clear cell carcinoma of the ovary, epithelial ovarian cancer, fallopian tube cancer, and primary peritoneal cancer), breast carcinoma, non-small cell lung cancer (NSCLC), a male genital tract malignancy (*e.g.,* testicular cancer), retroperitoneal or peritoneal carcinoma, gastroesophageal adenocarcinoma, esophagogastric junction

carcinoma, liver hepatocellular carcinoma, esophageal and esophagogastric junction carcinoma, cervical cancer, cholangiocarcinoma, pancreatic adenocarcinoma, extrahepatic bile duct adenocarcinoma, a small intestinal malignancy, gastric adenocarcinoma, cancer of

unknown primary (CUP), colorectal adenocarcinoma, esophageal carcinoma, prostatic adenocarcinoma, kidney cancer, head and neck squamous carcinoma, thymic carcinoma, non-melanoma skin cancer, thyroid carcinoma (*e.g.,* papillary carcinoma), a head and neck cancer, anal carcinoma, non-epithelial ovarian cancer (non-EOC), uveal melanoma, malignant pleural mesothelioma, small cell lung cancer (SCLC), a central nervous system cancer, a neuroendocrine tumor, and a soft tissue tumor. For example, in certain examples, the cancer is breast cancer, non-small cell lung cancer, bladder cancer, kidney cancer, colon cancer, and melanoma.

[0035] 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 promoting infiltration of immune cells into a tumor in a subject in need thereof. In another example, the disclosure provides a method of promoting infiltration of immune cells into a tumor 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. In certain examples, the immune cells are T-cells, *e.g.,* CD4+ and/or CD8+ T-cells, *e.g.,* $CD69^+CD8^+$ and/or $GzmB^+CD8^+$ T-cells. In certain examples, the immune cells are natural killer (NK) cells.

[0036] 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 increasing the number of circulating natural killer (NK) cells in a subject in need thereof, so as to increase the number of circulating NK cells relative to prior to administration of the fusion protein, antibody conjugate or pharmaceutical composition. In another example, the disclosure provides a method of increasing the number of circulating natural killer (NK) cells 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, so as to increase the number of circulating NK cells relative to prior to administration of the fusion protein, antibody conjugate or pharmaceutical composition.

[0037] 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 increasing the

number of T-cells in the draining lymph node in a subject in need thereof, so as to increase the number of T-cells in the draining lymph node relative to prior to administration of the fusion protein, antibody conjugate or pharmaceutical composition. In another example, the disclosure provides a method of increasing the number of T-cells in the draining lymph node in 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, so as to increase the number of T-cells in the draining lymph node relative to prior to administration of the fusion protein, antibody conjugate or pharmaceutical composition. In certain examples, the immune cells are T-cells, *e.g.,* CD4+ and/or CD8+ T-cells.

[0038] 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 increasing expression of Cd3, Cd4, Cd8, Cd274, Ctla4, Icos, Pdcd1, Lag3, Il6, Il1b, Il2, Ifng, Ifna1, Mx1, Gzmb, Cxcl9, Cxcl12, and/or Ccl5 in a cell, tissue, or subject, so as to increase the expression of Cd3, Cd4, Cd8, Cd274, Ctla4, Icos, Pdcd1, Lag3, Il6, Il1b, Il2, Ifng, Ifna1, Mx1, Gzmb, Cxcl9, Cxcl12, and/or Ccl5 relative to the cell, tissue or subject prior to contact with the fusion protein, antibody conjugate or pharmaceutical composition. In another example, the disclosure provides a method of increasing expression of Cd3, Cd4, Cd8, Cd274, Ctla4, Icos, Pdcd1, Lag3, Il6, Il1b, Il2, Ifng, Ifna1, Mx1, Gzmb, Cxcl9, Cxcl12, and/or Ccl5 in a cell, tissue, or subject. The method comprises contacting the cell, tissue, or subject with an effective amount of any of the foregoing fusion proteins, any of the

foregoing antibody conjugates, or any of the foregoing pharmaceutical compositions, so as to increase the expression of Cd3, Cd4, Cd8, Cd274, Ctla4, Icos, Pdcd1, Lag3, Il6, Il1b, Il2, Ifng, Ifna1, Mx1, Gzmb, Cxcl9, Cxcl12, and/or Ccl5 relative to the cell, tissue or subject prior to contact with the fusion protein, antibody conjugate or pharmaceutical composition.

[0039] These and other examples and features of the disclosure are described in the following detailed description and claims.

DESCRIPTION OF THE DRAWINGS

[0040] The invention can be more completely understood with reference to the following drawings.

[0041] FIGURES 1A-1I depict schematic representations of certain antibody conjugate constructs containing a sialidase enzyme, *e.g.*, a human sialidase enzyme, and an anti-HER2 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) anti-HER2 antigen binding site, each anti-HER2 antigen binding site may be the same or different. For each antibody conjugate construct that contains an Fc domain, it is understood that the Fc domain can be a wild type Fc domain or can be an engineered Fc domain. For example, the Fc domain may be engineered to contain either a "knob" mutation, *e.g.,* T366Y, or a "hole" mutation, *e.g.,* Y407T, or both, to promote heterodimerization, or the Fc domain may be engineered to contain one or more modifications, *e.g.*, point mutations, to provide any other modified Fc domain functionality.

[0042] FIGURE 2 depicts schematic representations of certain antibody conjugate constructs containing a sialidase enzyme, *e.g.*, a human sialidase enzyme, and an anti-HER2 antigen binding site. For each antibody conjugate construct that contains more than one (*e.g.,* two) anti-HER2 antigen binding site, each anti-HER2 antigen binding site may be the same or different. For each antibody conjugate construct that contains an Fc domain, it is understood that the Fc domain can be a wild type Fc domain or can be an engineered Fc domain. For example, the Fc domain may be engineered to contain either a "knob" mutation, *e.g.,* T366Y, or a "hole" mutation, *e.g.,* Y407T, or both, to promote heterodimerization, or the Fc domain may be engineered to contain one or more modifications, *e.g.*, point mutations, to provide any other modified Fc domain functionality.

WHAT IS CLAIMED IS:

1. A fusion protein comprising:

(a) a recombinant mutant human sialidase, wherein the sialidase comprises a substitution of an alanine residue at a position corresponding to position 42 of wild-type human Neu2 (A42); and

(b) an anti-HER2 immunoglobulin antigen-binding domain.

2. The fusion protein of claim 1, wherein the alanine residue at a position corresponding to position 42 of wild-type human Neu2 is substituted by arginine (A42R).

3. The fusion protein of claim 1 or 2, 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 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);

(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);

(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);

(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);

(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);

(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);

(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);

(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);

(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);

(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.

4. The fusion protein of any one of claims 1-3, 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.

5. The fusion protein of claim 3 or 4, 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);

(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);

(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);

(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);

(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);

(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);

(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.

6. The fusion protein of claim 5, 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.

7. The fusion protein of any one of claims 1-6, wherein 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 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.

8. The fusion protein of claim 7, 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 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.

9. The fusion protein of claim 8, wherein the sialidase comprises the M1D, V6Y, A42R, P62G, A93E, Q126Y, I187K, A242F, Q270T, and C332A substitutions.

10. The fusion protein of any one of claims 1-9, wherein the sialidase is selected from Neu1, Neu2, Neu3, and Neu4.

11. The fusion protein of any one of claims 1-10, wherein the sialidase comprises SEQ ID NO: 198.

12. The fusion protein of any one of claims 1-11, wherein the fusion protein further comprises an immunoglobulin Fc domain.

13. The fusion protein of claim 12, wherein the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, or IgG4 Fc domain.

14. The fusion protein of any one of claims 1-13, wherein the anti-HER2 immunoglobulin antigen-binding domain is associated with a second anti-HER2 immunoglobulin antigen-binding domain to produce an anti-HER2 antigen-binding site.

15. The fusion protein of any one of claims 1-14, wherein the anti-HER2 immunoglobulin antigen-binding domain is derived from an antibody selected from trastuzumab, pertuzumab, CT-P6, trastuzumab-dkst, MGAH22 (margetuximab), PF-05280014, ertumaxomab, gancotamab, timigutuzumab, Ontruzant, ABP-980, SB3, DS-8201, MYL-1410, BCD-022, and HD201.

16. The fusion protein of claim 15, wherein the anti-HER2 immunoglobulin antigen-binding domain is derived from trastuzumab.

17. The fusion protein of any one of claims 1-16, wherein the sialidase and the immunoglobulin Fc domain and/or the anti-HER2 immunoglobulin antigen-binding domain are linked by a

peptide bond or an amino acid linker.

18. The fusion protein of any one of claims 1-17, wherein the fusion protein comprises any one of SEQ ID NOs: 203-208.

19. An antibody conjugate comprising the fusion protein of any one of claims 1-18.

20. The antibody conjugate of claim 19, wherein the antibody conjugate comprises a single sialidase and/or a single anti-HER2 antigen-binding site.

21. The antibody conjugate of claim 19 or 20, 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-HER2 antigen-binding site.

22. The antibody conjugate of claim 21, wherein the third polypeptide comprises the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation.

23. The antibody conjugate of claim 21 or 22, wherein the first polypeptide comprises SEQ ID NO: 66.

24. The antibody conjugate of any one of claims 21-23, wherein the second polypeptide comprises any one of SEQ ID NOs: 67 or 189.

25. The antibody conjugate of any one of claims 21-24, wherein the third polypeptide comprises any one of SEQ ID NOs: 203-208.

26. The antibody conjugate of any one of claims 21-25, wherein the first polypeptide comprises SEQ ID NO: 66, the second polypeptide comprises SEQ ID NO: 189, and the third polypeptide comprises SEQ ID NO: 205.

27. An isolated nucleic acid comprising a nucleotide sequence encoding the fusion protein of any one of claims 1-18, or at least a portion of the antibody conjugate of any one of claims 19-26.

28. An expression vector comprising the nucleic acid of claim 27.

29. A host cell comprising the expression vector of claim 28.

30. A pharmaceutical composition comprising the fusion protein of any one of claims 1-18 or the antibody conjugate of any one of claims 19-26.

31. Use of the fusion protein of any one of claims 1-18, the antibody conjugate of any one of claims 19-26, or the pharmaceutical composition of claim 30 in the preparation of a medicament for the treatment of cancer in a subject in need thereof.

32. Use of the fusion protein of any one of claims 1-18, the antibody conjugate of any one of claims 19-26, or the pharmaceutical composition of claim 30 in the preparation of a medicament for promoting infiltration of immune cells into a tumor in a subject in need thereof.

33. The use of claim 32, wherein the immune cells are T-cells or natural killer (NK) cells.

34. The use of claim 33, wherein the T-cells are CD4+ T-cells and/or CD8+ T-cells.

35. Use of the fusion protein of any one of claims 1-18, the antibody conjugate of any one of claims 19-26, or the pharmaceutical composition of claim 30 in the preparation of a medicament for increasing the number of circulating natural killer (NK) cells in a subject in need thereof, so as to increase the number of circulating NK cells relative to prior to administration of the fusion protein, antibody conjugate or pharmaceutical composition.

36. Use of the fusion protein of any one of claims 1-18, the antibody conjugate of any one of claims 19-26, or the pharmaceutical composition of claim 30 in the preparation of a medicament for increasing the number of T-cells in the draining lymph node in a subject in need thereof, so as to increase the number of T-cells in the draining lymph node relative to prior to administration of the fusion protein, antibody conjugate or pharmaceutical composition.

37. The use of claim 36, wherein the T-cells are CD4+ T-cells and/or CD8+ T-cells.

38. Use of the fusion protein of any one of claims 1-18, the antibody conjugate of any one of claims 19-26, or the pharmaceutical composition of claim 30 in the preparation of a medicament for increasing expression of Cd3, Cd4, Cd8, Cd274, Ctla4, Icos, Pdcd1, Lag3, Il6, Il1b, Il2, Ifng, Ifna1, Mx1, Gzmb, Cxcl9, Cxcl12, and/or Ccl5 in a cell, tissue, or subject, so as to increase the expression of Cd3, Cd4, Cd8, Cd274, Ctla4, Icos, Pdcd1, Lag3, Il6, Il1b, Il2, Ifng, Ifna1, Mx1, Gzmb, Cxcl9, Cxcl12, and/or Ccl5 relative to the cell, tissue or subject prior to contact with the fusion protein, antibody conjugate or pharmaceutical composition.