

resulting in the expression of sialylated tumor-associated carbohydrate antigens. The expression of sialylated glycans by tumor cells is often associated with increased aggressiveness and metastatic potential of a tumor (Julien S., Delannoy P. (2015) "Sialic Acid and Cancer", In: Taniguchi N., Endo T., Hart G., Seeberger P., Wong CH. (eds) Glycoscience: Biology and Medicine. Springer, Tokyo. https://doi.org/10.1007/978-4-431-54841-6_193).

[0005] It has recently become apparent that Siglecs (sialic acid-binding immunoglobulin-like lectins), a family of sialic acid binding lectins, play a role in cancer immune suppression by binding to hypersialylated cancer cells and mediating the suppression of signals from activating NK cell receptors, thereby inhibiting NK cell-mediated killing of tumor cells (Jandus *et al.* (2014) J. CLIN. INVEST. 124: 1810-1820; Läubli *et al.* (2014) PROC. NATL. ACAD. SCI. USA 111: 14211-14216; Hudak *et al.* (2014) NAT. CHEM. BIOL. 10: 69-75). Likewise, enzymatic removal of sialic acids by treatment with sialidase can enhance NK cell-mediated killing of tumor cells (Jandus, *supra*; Hudak, *supra*; Xiao *et al.* (2016) PROC. NATL. ACAD. SCI. USA 113(37): 10304-9).

[0006] Cancer immunotherapy with immune checkpoint inhibitors, including antibodies that block 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

[0007] The ~~invention~~disclosure is based, in part, upon the discovery of anti-PD-L1 antibodies that impact or otherwise down regulate signaling mediated by PD-1 or PD-L1. In the appropriate circumstances, the antibodies can remove the PD-1 or PD-L1-mediated repression of a subject's immune system to mediate the removal of non-natural cells, for example, cancerous cells.

[0008] The ~~invention~~disclosure is also based, in part, upon the discovery that it is possible to produce fusion proteins containing a sialidase enzyme and an anti-PD-L1 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-L1 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.*, PD-L1-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. In one aspect, the present disclosure provides an isolated antibody that binds human PD-L1 comprising: (i) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 161, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 162, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 163 (PAL769- VH, h769-VH); and/or an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 165, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 142, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 203 (h769.T-VL); (ii) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 161, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 162, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 163 (PAL769- VH, h769-VH); and/or an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 165, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 142, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 166 (PAL769-VL, h769-IF3-VL, h769-tm2-VL, h769-tm3-VL); (iii) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 129, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 130, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 131 (PAL752-VH); and/or an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 133, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 134, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 135 (PAL752-VL); (iv) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 137, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 138, and/or a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 139 (PAL759-VH); and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 141, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 142, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 143 (PAL759-VL); (v) an immunoglobulin heavy chain variable

region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 145, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 146, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 147 (**PAL760-VH**); and/or an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 149, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 150, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 151 (**PAL760-VL**); (vi) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 153, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 154, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 155 (**PAL767-VH**); and/or an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 157, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 158, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 159 (**PAL767-VL**); (vii) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 161, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 168, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 169 (**PAL771-VH**); and/or an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 171, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 172, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 173 (**PAL771-VL**); (viii) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 175, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 176, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 177 (**PAL785-VH**); and/or an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 179, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 180, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 181 (**PAL785-VL**); (ix) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 183, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 184, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 185 (**PAL787-VH**); and/or an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 187, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 188, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 189 (**PAL787-VL**); or (x) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 191, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 192, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 193 (**PAL788-VH**); and/or an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 195, a

CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 196, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 197 (PAL788-VL).

[0009a] In a further aspect, the present disclosure provides an isolated antibody that binds human PD-L1 comprising: (i) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 199 (h769-VH), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 204 (h769.T-VL); (ii) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 164 (PAL769-VH), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 167 (PAL769-VL); (iii) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 199 (h769 VH), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 200 (h769-IF3-VL); (iv) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 199 (h769-VH), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 201 (h769-tm2-VL); (v) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 199 (h769 VH), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 202 (h769-tm3-VL); (vi) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 132 (PAL752-VH), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 136 (PAL752-VL); (vii) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 140 (PAL759-VH), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 144 (PAL759-VL); (viii) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 148 (PAL760-VH), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 152 (PAL760-VL); (ix) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 156 (PAL767-VH), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 160 (PAL767-VL); (x) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 170 (PAL771-VH), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 174 (PAL771-VL); (xi) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 178 (PAL785-VH), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 182 (PAL785-VL); (xii) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 186 (PAL787-VH), and an

immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 190 (PAL787-VL); or (xiii) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 194 (PAL788-VH), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 198 (PAL788-VL).

5 [0010] Accordingly, in one aspectexample, the inventiondisclosure provides an isolated antibody that binds human PD-L1.

[0011] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 161, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 162, and a CDRH3 comprising
10 the amino acid sequence of SEQ ID NO: 163 (PAL769-VH, h769-VH); and/or an immunoglobulin light chain variable region comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 165, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 142, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 166 (PAL769-VL, h769-IF3-VL, h769-tm2-VL, h769-tm3-VL).

15 [0012] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 250, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 251, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 163 (PAL769-VH); and/or an immunoglobulin light chain variable region comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO:
20 253, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 254, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 166 (PAL769-VL).

[0013] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 250, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 252, and a CDRH3 comprising
25 the amino acid sequence of SEQ ID NO: 163 (h769-VH); and/or an immunoglobulin light chain variable region comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 255, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 254, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 166 (h769-IF3-VL, h769-tm2-VL, h769-tm3-VL).

[0014] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 161, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 162, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 163 (PAL769-VH, h769-VH); and/or an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 165, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 142, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 203 (h769.T-VL).

[0015] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 250, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 252, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 163 (h769-VH); and/or an immunoglobulin light chain variable region comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 255, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 254, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 203 (h769.T-VL).

[0016] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 129, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 130, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 131 (PAL752-VH); and/or an immunoglobulin light chain variable region comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 133, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 134, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 135 (PAL752-VL).

[0017] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 137, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 138, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 139 (PAL759-VH); and/or an immunoglobulin light chain variable region comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 141, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 142, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 143 (PAL759-VL).

[0018] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 145, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 146, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 147 (PAL760-VH); and/or an immunoglobulin light chain variable region comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO:

149, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 150, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 151 (PAL760-VL).

[0019] In certain embodiment examples, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 153, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 154, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 155 (PAL767-VH); and/or an immunoglobulin light chain variable region comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 157, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 158, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 159 (PAL767-VL).

[0020] In certain embodiment examples, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 161, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 168, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 169 (PAL771-VH); and/or an immunoglobulin light chain variable region comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 171, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 172, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 173 (PAL771-VL).

[0021] In certain embodiment examples, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 175, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 176, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 177 (PAL785-VH); and/or an immunoglobulin light chain variable region comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 179, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 180, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 181 (PAL785-VL).

[0022] In certain embodiment examples, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 183, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 184, and a CDRH3 comprising the amino acid sequence of SEQ ID NO: 185 (PAL787-VH); and/or an immunoglobulin light chain variable region comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 187, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 188, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 189 (PAL787-VL).

[0023] In certain embodiment examples, the antibody comprises an immunoglobulin heavy chain variable region comprising a CDRH1 comprising the amino acid sequence of SEQ ID NO: 191, a CDRH2 comprising the amino acid sequence of SEQ ID NO: 192, and a CDRH3 comprising

the amino acid sequence of SEQ ID NO: 193 (PAL788-VH); and/or an immunoglobulin light chain variable region comprising a CDRL1 comprising the amino acid sequence of SEQ ID NO: 195, a CDRL2 comprising the amino acid sequence of SEQ ID NO: 196, and a CDRL3 comprising the amino acid sequence of SEQ ID NO: 197 (PAL788-VL).

5 [0024] In certain embodimentexamples of any of the foregoing antibodies, the CDRs are interposed between human or humanized immunoglobulin framework regions.

[0025] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 164 (PAL769-VH), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 167 (PAL769-VL).

[0026] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 199 (h769-VH), and/or an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 200 (h769-IF3-VL).

15 [0027] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 199 (h769-VH), and/or an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 201 (h769-tm2-VL).

[0028] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 199 (h769-VH), and/or an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 202 (h769-tm3-VL).

[0029] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 199 (h769-VH), and/or an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 204 (h769.T-VL).

[0030] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 132 (PAL752-VH), and/or an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 136 (PAL752-VL).

[0031] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 140 (PAL759-VH), and/or

an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 144 (PAL759-VL).

[0032] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 148 (PAL760-VH), and/or an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 152 (PAL760-VL).

[0033] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 156 (PAL767-VH), and/or an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 160 (PAL767-VL).

[0034] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 170 (PAL771-VH), and/or an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 174 (PAL771-VL).

[0035] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 178 (PAL785-VH), and/or an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 182 (PAL785-VL).

[0036] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 186 (PAL787-VH), and/or an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 190 (PAL787-VL).

[0037] In certain embodimentexamples, the antibody comprises an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 194 (PAL788-VH), and/or an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 198 (PAL788-VL).

[0038] In certain embodimentexamples of any of the foregoing antibodies, the antibody further comprises a heavy chain constant region (e.g., an IgG1, IgG2, IgG3, and IgG4 heavy chain constant region) and/or light chain constant region.

[0039] In certain embodimentexamples of any of the foregoing antibodies, the antibody binds to human PD-L1 with a KD of 5 nM or lower, 3 nM or lower, 2.5 nM or lower, 2 nM or lower, 1 nM or lower, 0.75 nM or lower, 0.5 nM or lower, 0.1 nM, 0.075 nM, or 0.05 nM or lower, as measured

by surface plasmon resonance or bio-layer interferometry. In certain embodimentexamples, the antibody also binds to *Macaca fascicularis* (cynomolgus) PD-L1.

[0040] In another aspect, the inventiondisclosure provides an isolated antibody that competes with any of the foregoing antibodies for binding to human PD-L1 and/or binds to the same epitope on human PD-L1 as any of the foregoing antibodies.

[0041] In another aspect, the inventiondisclosure provides an isolated nucleic acid comprising a nucleotide sequence encoding an immunoglobulin heavy chain variable region of any of the foregoing antibodies and/or an immunoglobulin light chain variable region of any of the foregoing antibodies.

[0042] In another aspect, the inventiondisclosure provides an expression vector comprising: (i) a nucleic acid comprising a nucleotide sequence encoding an immunoglobulin heavy chain variable region of any of the foregoing antibodies; and/or (ii) a nucleic acid comprising a nucleotide sequence encoding an immunoglobulin light chain variable region of any of the foregoing antibodies.

[0043] In another aspectexample, the inventiondisclosure provides a host cell comprising any of the foregoing nucleic acids or expression vectors.

[0044] In another aspect, the inventiondisclosure provides a fusion protein comprising (or consisting essentially of): (a) sialidase enzyme; and (b) an anti-PD-L1 immunoglobulin antigen-binding domain derived from any of the foregoing antibodies.

[0045] In certain embodimentexamples, the sialidase is a human sialidase, *e.g.*, a recombinant mutant human sialidase. In certain embodimentexamples, 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.

[0046] In certain embodiment examples, 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 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 substitution selected from Δ M1, 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.

[0047] In certain [embodimentexamples](#), 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.

[0048] In certain [embodimentexamples](#), the sialidase is selected from Neu1, Neu2, Neu3, and Neu4, *e.g.*, the sialidase is Neu2.

[0049] In certain [embodimentexamples](#), 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.

[0050] In certain [embodimentexamples](#), the sialidase comprises any one of SEQ ID NOs: 48-62, 94, 97, 100, 126, or 234, 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: 48-62, 94, 97, 100, 126, or 234.

[0051] In certain [embodimentexamples](#), the sialidase comprises mutation or combination of mutations set forth in any one of Tables 1-9.

[0052] In certain [embodimentexamples](#), the fusion protein further comprises an immunoglobulin Fc domain. In certain [embodimentexamples](#), 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.

[0053] In certain [embodimentexamples](#), the anti-PD-L1 immunoglobulin antigen-binding domain is associated (for example, covalently or non-covalently associated) with a second anti-PD-L1 immunoglobulin antigen-binding domain to produce an anti-PD-L1 antigen-binding site. For example, in certain [embodimentexamples](#) the anti-PD-L1 immunoglobulin antigen-binding domain is an immunoglobulin heavy chain fragment that is associated with an immunoglobulin light chain fragment to produce an anti-PD-L1 antigen-binding site. In other [embodimentexamples](#) the anti-PD-L1 immunoglobulin antigen-binding domain is an immunoglobulin light chain fragment that is associated with an immunoglobulin heavy chain fragment to produce an anti-PD-L1 antigen-binding site.

[0054] In certain [embodimentexamples](#), the sialidase and the immunoglobulin Fc domain and/or the anti-PD-L1 immunoglobulin antigen-binding domain are linked by a peptide bond or an amino acid linker.

[0055] In certain [embodimentexamples](#), the fusion protein comprises any one of SEQ ID NOs: 205-207, 211, 213, 214, and 219.

[0056] 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 anti-PD-L1 antigen-binding site. In other [embodimentexamples](#), the antibody conjugate comprises two anti-PD-L1 antigen-binding sites, which can be the same or different. In certain [embodimentexamples](#), the antibody conjugate comprises two identical anti-PD-L1 antigen-binding sites.

[0057] 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.

[0058] In certain embodimentexamples, 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-L1 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: 205, the second polypeptide may, for example, comprise any one of SEQ ID NOs: 206 or 213, and/or the third polypeptide may, for example, comprise any one of SEQ ID NOs: 207, 211, 214, or 219.

[0059] 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 anti-PD-L1 antigen-binding site, and the third polypeptide and the fourth polypeptide together define a second anti-PD-L1 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.

[0060] In certain embodimentexamples, 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-L1 antigen-binding site, and the second scFv, when present, defines a second anti-PD-L1 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.

[0061] In certain embodimentexamples, 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-L1 antigen-binding site and the scFv defines a second anti-PD-L1 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.

[0062] In another aspect, the inventiondisclosure provides an isolated nucleic acid comprising a nucleotide sequence encoding at least a portion of any of the foregoing antibodies, any of the foregoing fusion proteins, or at least a portion of any of the foregoing antibody conjugates. In another aspect, the inventiondisclosure provides an expression vector comprising any of the foregoing nucleic acids. In another aspect, the inventiondisclosure provides a host cell comprising any of the foregoing expression vectors.

[0063] In another aspect, the inventiondisclosure provides a pharmaceutical composition comprising any of the foregoing antibodies, any of the foregoing fusion proteins, or any of the foregoing antibody conjugates.

[0064] In another aspect, the present disclosure provides use of the antibody as described herein, 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 aspectexample, the inventiondisclosure 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 antibodies, any of the foregoing fusion proteins, any of the foregoing antibody conjugates, or any of the foregoing pharmaceutical compositions.

[0065] In certain embodimentexamples, the cancer is selected from non-small cell lung cancer (NSCLC), melanoma, bladder, breast, cervical, esophageal, gastric, kidney, lung, ovary, metastatic Merkel cell carcinoma (MCC), metastatic urothelial carcinoma (UC), and pancreatic cancer.

[0066] These and other aspectexamples and features of the inventiondisclosure are described in the following detailed description and claims.

DESCRIPTION OF THE DRAWINGS

[0067] The invention can be more completely understood with reference to the following drawings.

WHAT IS CLAIMED IS:

1. An isolated antibody that binds human PD-L1 comprising:

(i) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 161, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 162, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 163 (**PAL769-VH, h769-VH**); and/or an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 165, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 142, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 203 (**h769.T-VL**);

____(ii) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 161, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 162, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 163 (**PAL769-VH, h769-VH**); and/or an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 165, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 142, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 166 (**PAL769-VL, h769-IF3-VL, h769-tm2-VL, h769-tm3-VL**);

~~(ii) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 161, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 162, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 163 (**PAL769-VH, h769-VH**); and/or an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 165, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 142, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 203 (**h769.T-VL**);~~

——(iii) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 129, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 130, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 131 (**PAL752-VH**); and/or an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 133, a CDR_{L2} comprising the amino acid sequence of SEQ

ID NO: 134, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 135 (**PAL752-VL**);

(iv) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 137, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 138, and/or a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 139 (**PAL759-VH**); and an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 141, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 142, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 143 (**PAL759-VL**);

(v) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 145, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 146, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 147 (**PAL760-VH**); and/or an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 149, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 150, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 151 (**PAL760-VL**);

(vi) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 153, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 154, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 155 (**PAL767-VH**); and/or an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 157, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 158, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 159 (**PAL767-VL**);

(vii) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 161, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 168, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 169 (**PAL771-VH**); and/or an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 171, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 172, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 173 (**PAL771-VL**);

(viii) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 175, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 176, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 177 (**PAL785-VH**); and/or an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 179, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 180, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 181 (**PAL785-VL**);

(ix) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 183, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 184, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 185 (**PAL787-VH**); and/or an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 187, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 188, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 189 (**PAL787-VL**); or

(x) an immunoglobulin heavy chain variable region comprising a CDR_{H1} comprising the amino acid sequence of SEQ ID NO: 191, a CDR_{H2} comprising the amino acid sequence of SEQ ID NO: 192, and a CDR_{H3} comprising the amino acid sequence of SEQ ID NO: 193 (**PAL788-VH**); and/or an immunoglobulin light chain variable region comprising a CDR_{L1} comprising the amino acid sequence of SEQ ID NO: 195, a CDR_{L2} comprising the amino acid sequence of SEQ ID NO: 196, and a CDR_{L3} comprising the amino acid sequence of SEQ ID NO: 197 (**PAL788-VL**).

2. The isolated antibody of claim 1, wherein the CDRs are interposed between human or humanized immunoglobulin framework regions.

3. An isolated antibody that binds human PD-L1 comprising:

(i) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 199 (**h769-VH**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 204 (**h769.T-VL**);

_____ (ii) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 164 (**PAL769-VH**), and an immunoglobulin light chain variable region

comprising the amino acid sequence of SEQ ID NO: 167 (**PAL769-VL**);

(iii) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 199 (**h769 VH**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 200 (**h769-IF3-VL**);

(iv) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 199 (**h769-VH**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 201 (**h769-tm2-VL**);

(v) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 199 (**h769 VH**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 202 (**h769-tm3-VL**);

~~(v) —an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 199 (**h769-VH**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 204 (**h769.T-VL**);~~

(vi) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 132 (**PAL752-VH**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 136 (**PAL752-VL**);

(vii) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 140 (**PAL759-VH**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 144 (**PAL759-VL**);

(viii) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 148 (**PAL760-VH**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 152 (**PAL760-VL**);

(ix) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 156 (**PAL767-VH**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 160 (**PAL767-VL**);

(x) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 170 (**PAL771-VH**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 174 (**PAL771-VL**);

(xi) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 178 (**PAL785-VH**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 182 (**PAL785-VL**);

(xii) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 186 (**PAL787-VH**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 190 (**PAL787-VL**); or

(xiii) an immunoglobulin heavy chain variable region comprising the amino acid sequence of SEQ ID NO: 194 (**PAL788-VH**), and an immunoglobulin light chain variable region comprising the amino acid sequence of SEQ ID NO: 198 (**PAL788-VL**).

4. The isolated antibody of any one of claims 1-3, further comprising a heavy chain and/or light chain constant region.

5. The isolated antibody of claim 4, wherein the heavy chain constant region is selected from an IgG1, IgG2, IgG3, and IgG4 heavy chain constant region.

6. The isolated antibody of any one of claims 1-5, wherein the antibody binds to human PD-L1 with a K_D of 5 nM or lower, 1 nM or lower, 0.75 nM or lower, 0.5 nM or lower, 0.1 nM, 0.075 nM, or 0.05 nM or lower, as measured by surface plasmon resonance or bio-layer interferometry.

7. The isolated antibody of any one of claims 1-6, wherein the antibody also binds to *Macaca fascicularis* (cynomolgus) PD-L1.

8. An isolated antibody that competes with the antibody of any one of claims 1-7 for binding to human PD-L1.

9. An isolated antibody that binds to the same epitope on human PD-L1 as the antibody of any one of claims 1-8.

10. An isolated nucleic acid comprising a nucleotide sequence encoding the immunoglobulin heavy chain variable region of any one of claims 1-7 and/or a nucleotide sequence encoding the immunoglobulin light chain variable region of any one of claims 1-7.

11. An expression vector comprising: (i) a nucleic acid comprising a nucleotide sequence encoding the immunoglobulin heavy chain variable region of any one of claims 1-7; and/or (ii) a nucleic acid comprising a nucleotide sequence encoding the immunoglobulin light chain variable

region of any one of claims 1-7.

12. A host cell comprising the expression vector of claim 11.

13. A fusion protein comprising:

- (a) a sialidase enzyme; and
- (b) an anti-PD-L1 immunoglobulin antigen-binding domain derived from the anti-PD-L1 antibody of any one of claims 1-9.

14. The fusion protein of claim 13, wherein the sialidase is a human sialidase.

15. The fusion protein of claim 13 or 14, wherein the sialidase is a recombinant mutant human sialidase.

16. The fusion protein of claim 15, wherein the sialidase comprises:

(a) a substitution of a proline residue at a position corresponding to position 62 of wild-type human Neu2 (P62);

(b) a substitution of an alanine residue at a position corresponding to position 93 of wild-type human Neu2 (A93);

~~(a)~~(c) a substitution or deletion of a methionine residue at a position corresponding to position 1 of wild-type human Neu2 (M1);

~~(b)~~(d) a substitution of a valine residue at a position corresponding to position 6 of wild-type human Neu2 (V6);

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

~~(d)~~(f) 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.

17. The fusion protein of claim 16, wherein, in the sialidase:

- (a) 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);
- (b) the alanine residue at a position corresponding to position 93 of wild-type human Neu2 is substituted by glutamic acid (A93E) or lysine (A93K);
- ~~(a)~~(c) 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)~~(d) the valine residue at a position corresponding to position 6 of wild-type human Neu2 is substituted by tyrosine (V6Y);

~~(c)~~(e) the alanine residue at a position corresponding to position 42 of wild-type human Neu2 is substituted by arginine (A42R)

~~(d)~~(f) 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.

18. The fusion protein of claim 17, wherein the sialidase comprises a substitution selected from Δ M1, 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.

19. The fusion protein of claim 18, 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.

20. The fusion protein of any one of claims 13-19, wherein the sialidase is selected from Neu1, Neu2, Neu3, and Neu4.

~~21. The fusion protein of claim 20, wherein the sialidase is Neu2.~~

~~22. The fusion protein of any one of claims 13-21, wherein the sialidase has a different substrate specificity than the corresponding wild-type sialidase.~~

~~23. The fusion protein of claim 22, wherein the sialidase can cleave α 2,3, α 2,6, and/or α 2,8 linkages.~~

~~24. The fusion protein of claim 23, wherein the sialidase can cleave α 2,3 and α 2,8 linkages.~~

~~25.~~21. The fusion protein of any one of claims 13-~~20~~24, wherein the sialidase comprises any one of SEQ ID NOs: 48-62, 94, 97, 100, 126, or 234.

~~26. The fusion protein of any one of claims 13-25, wherein the sialidase comprises a mutation set forth in any one of Tables 1-9.~~

~~27.~~22. The fusion protein of any one of claims 13-~~21~~26, wherein the fusion protein further comprises an immunoglobulin Fc domain.

~~28. The fusion protein of claim 27, wherein the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, IgG4, IgA1, IgA2, IgD, IgE, or IgM Fc domain.~~

~~29.~~23. The fusion protein of claim ~~22~~28, wherein the immunoglobulin Fc domain is derived from a human IgG1, IgG2, IgG3, or IgG4 Fc domain.

~~30. The fusion protein of claim 29, wherein the immunoglobulin Fc domain is derived from a human IgG1 Fc domain.~~

~~31.24.~~ The fusion protein of any one of claims 13-~~2330~~, wherein the anti-PD-L1 immunoglobulin antigen-binding domain is associated with a second anti-PD-L1 immunoglobulin antigen-binding domain derived from the anti-PD-L1 antibody of any one of claims 1-9 to produce an anti-PD-L1 antigen-binding site.

~~32.25.~~ The fusion protein of any one of claims 13-~~2431~~, wherein the sialidase and the immunoglobulin Fc domain and/or the anti-PD-L1 immunoglobulin antigen-binding domain are linked by a peptide bond or an amino acid linker.

~~33.26.~~ The fusion protein of any one of claims 13-~~2532~~, wherein the fusion protein comprises any one of SEQ ID NOs: 205-207, 211, 213, 214, and 219.

~~34.27.~~ An antibody conjugate comprising the fusion protein of any one of claims 13-~~2633~~.

~~35.28.~~ The antibody conjugate of claim ~~2734~~, wherein the antibody conjugate comprises a single sialidase and/or a single anti-PD-L1 antigen-binding site.

~~36. The antibody conjugate of claim 34, wherein the antibody conjugate comprises two sialidases.~~

~~37. The antibody conjugate of claim 36, wherein the two sialidases are identical.~~

~~38. The antibody conjugate of any one of claims 34-37, wherein the antibody conjugate comprises a single anti-PD-L1 antigen-binding site.~~

~~39. The antibody conjugate of any one of claims 34-37, wherein the antibody conjugate comprises two anti-PD-L1 antigen-binding sites.~~

~~40. The antibody conjugate of claim 39, wherein the two anti-PD-L1 antigen-binding sites are identical.~~

~~41. The antibody conjugate of any one of claims 34-40, wherein the antibody conjugate has a molecular weight from about 135 kDa to about 165 kDa.~~

~~42. The antibody conjugate of any one of claims 34-40, wherein the antibody conjugate has a molecular weight from about 215 kDa to about 245 kDa.~~

~~43.29.~~ The antibody conjugate of any one of claims ~~2734-2842~~, 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-L1 antigen-binding site.

44.30. The antibody conjugate of claim 2943, wherein the third polypeptide comprises the sialidase and the immunoglobulin Fc domain in an N- to C-terminal orientation.

45.31. The antibody conjugate of claim 2943 or 3044, wherein the first polypeptide comprises SEQ ID NO: 205.

46.32. The antibody conjugate of any one of claims 2943-3145, wherein the second polypeptide comprises SEQ ID NOs: 206 or 213.

47.33. The antibody conjugate of any one of claims 2943-3246, wherein the third polypeptide comprises SEQ ID NOs: 207, 211, 214, or 219.

~~48. The antibody conjugate of any one of claims 34-42, 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 anti-PD-L1 antigen-binding site, and the third polypeptide and the fourth polypeptide together define a second anti-PD-L1~~

~~antigen-binding site.~~

~~49. The antibody conjugate of claim 48, 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.~~

~~50. The antibody conjugate of any one of claims 34-42, wherein the fusion protein 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 anti-PD-L1 antigen-binding site, and the second scFv defines a second anti-PD-L1 antigen-binding site.~~

~~51. The antibody conjugate of claim 50, 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.~~

~~52. The antibody conjugate of any one of claims 34-42, 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 anti-PD-L1 antigen-binding site and the scFv defines a second anti-PD-L1 antigen-binding site.~~

~~53. The antibody conjugate of claim 52, 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.~~

~~54.34. ____~~ An isolated nucleic acid comprising a nucleotide sequence encoding the fusion protein of any one of claims 13-~~2633~~, or at least a portion of the antibody conjugate of any one of claims ~~2734-3353~~.

~~55.35. ____~~ An expression vector comprising the nucleic acid of claim ~~3454~~.

~~56.36. ____~~ A host cell comprising the expression vector of claim ~~3555~~.

~~57.37. ____~~ A pharmaceutical composition comprising the antibody of any one of claims 1-9, the fusion protein of any one of claims 13-~~2633~~ or the antibody conjugate of any one of claims ~~2734-3353~~.

~~58.38. ____~~ ~~A method of treating cancer in a subject in need thereof, the method comprising administering to the subject an effective amount of the~~Use of the antibody of any one of claims 1-9, the fusion protein of any one of claims 13-~~2633~~, the antibody conjugate of any one of claims ~~2734-3353~~, or the pharmaceutical composition of claim ~~3757~~ in the preparation of a medicament for the treatment of cancer in a subject in need thereof.

~~59. The method of claim 58, wherein the cancer is selected from NSCLC, melanoma, bladder, breast, cervical, esophageal, gastric, kidney, lung, ovary, metastatic Merkel cell carcinoma (MCC), metastatic urothelial carcinoma (UC), and pancreatic cancer.~~