

of an immune response. The receptor can also function in antigen capture and presentation. ILT3 is thought to control inflammatory responses and cytotoxicity to help focus the immune response and to limit auto-reactivity. Multiple transcript variants encoding different isoforms of ILT3 have been identified.

5 Patent publications that disclose use of an antibody for modulating ILT3 activity with applications for inhibiting transplant rejection or for use in treatments for cancer or infectious diseases include U.S. Pub. Nos. 20090202544, 20150110714, 20150139986, and 20170267759; and, Intl. Pub. Nos. WO2013043569, WO2013181438, WO2014116846, WO2016049641, WO2016127427, WO2018089300, and WO2018148494. Of interest is Intl.
10 Pub. No. WO2017015227, which discloses CD166, also known as lymphocyte cell adhesion molecule (ALCAM), as a ligand for ILT3 and provides methods for treating cancer comprising in some embodiments an antibody against CD166 or ALCAM. Also of interest are U.S. Patent Nos. 7,777,008 and 8,901,281, which disclose monoclonal antibody 9B11 for use in various treatments where it is desirable to upregulate the immune system for anti-cancer treatments and
15 to downregulate the immune system for inhibiting transplant rejection.

While the patent publications disclose anti-ILT3 antibodies, in some instances no specific antibody is disclosed or specific antibodies are disclosed, which in some cases are shown to be promiscuous and cross-react with one or more ILT3-related receptors such as LILRA6 and ILT8. Promiscuous anti-ILT3 antibodies may have off-target effects, which may
20 have undesirable effects that contraindicate its use for therapeutic applications. Therefore there is a need for antibodies and antigen binding fragments that specifically bind ILT3 and have no measurable promiscuity towards other related receptors.

BRIEF SUMMARY

25 The present disclosure provides monoclonal antibodies and antigen binding fragments that bind specifically to immunoglobulin-like transcript 3 (ILT3) with no measurable binding to closely related proteins (e.g., ILT5, ILT7, ILT8, or ILT11) as determined by (i) a cell ELISA using 10 µg/mL antibody or antigen binding fragment or (ii) Biacore using 10 µg/mL antibody or antigen binding fragment. In particular examples, the antibodies and antigen binding
30 fragments specifically bind to both human ILT3 and Rhesus monkey ILT3. These antibodies and antigen binding fragments are capable of antagonizing ILT3 activity thereby enhancing dendritic cell activation and T cell priming. Tolerized dendritic cells and myeloid-derived suppressor cells (MDSCs) are also responsive to these antibodies. Furthermore, *in vivo*

studies of these antibodies in humanized NSGTM mouse model systems (The Jackson Laboratories, Bar Harbor, Maine) show that these antibodies may have the ability to reduce tumor burden and shift cellular phenotypes to a more activated state.

In clinical trial samples, ILT3 expression, like PD-L1, LAG3, and the GEP signature, was found to be associated with responsiveness to the anti-PD-1 antibody, pembrolizumab. Soluble ILT3 in circulation is also increased in certain cancer types. Taken together, the anti-ILT3 antibodies of the present disclosure may be useful for treating particular cancers either as a monotherapy treatment or in combination with an anti-PD-1 and/or anti-PD-L1 antibody to enhance responsiveness to the anti-PD-1 or anti-PD-L1 antibody, particularly in cancer treatments in which the cancer is non-responsive to anti-PD-1 or anti-PD-L1 monotherapies. In particular examples, the present disclosure provides chimeric or humanized anti-ILT3 antibodies. In certain examples, the antibodies may be fully human antibodies that compete with the antibodies disclosed herein for binding to the ILT3 epitope disclosed herein.

The present disclosure provides an antibody or antigen binding fragment comprising one, two, or three complementarity determining regions (CDRs) of a heavy chain variable V_H domain having heavy chain complementarity determining region (HC-CDR) 1, 2, and 3 and one, two, or three CDRs of a light chain variable domain V_L having LC-CDR1, 2, and 3, wherein the antibody or antigen binding fragment is capable of specifically binding human ILT3 wherein the the binding of the antibody or antigen binding fragment may be determined by cell ELISA or Biacore.

In a further example, the antibody or antigen binding fragment binds to an epitope on the human ILT3 or competes with an antibody disclosed for binding to an epitope on the human ILT3, wherein the epitope comprises at least one amino acid within one or more of the amino acid sequences set forth in the group consisting of SEQ ID NOs:3, 4, 5, 6, 7, and 8. In further examples, the antibody or antigen binding fragment binds to an epitope on the human ILT3 or competes with an antibody disclosed for binding to an epitope on the human ILT3, wherein the epitope comprises one or more of the amino acid sequences set forth in the group consisting of SEQ ID NOs:3, 4, 5, 6, 7, and 8. In further examples, the antibody or antigen binding fragment binds to an epitope on the human ILT3 or competes with an antibody disclosed for binding to an epitope on the human ILT3, wherein the epitope comprises the amino acid sequences set forth in the group consisting of SEQ ID NOs:3, 4, 5, 6, 7, and 8. In particular

examples, the epitope is determined by hydrogen deuterium exchange mass spectrometry (HDX-MS) analysis.

The present disclosure further provides an antibody or antigen binding fragment that binds human ILT3 comprising a heavy chain (HC) wherein the heavy chain variable domain (V_H) comprises a heavy chain complementarity determining region (HC-CDR) 3 having an amino acid sequence selected from the group consisting of SEQ ID NO: 22, 49, 57, 65, 73, 81, 89, 97, and 105, or having an amino acid sequence that has 3, 2, or 1 differences with an amino acid sequence selected from the group consisting of SEQ ID NO: 22, 49, 57, 65, 73, 81, 89, 97, and 105. In some examples the amino acid sequence differences are conservative changes/substitutions. In particular examples, the antibody or antigen binding fragment that binds human ILT3 comprises a heavy chain (HC) wherein the heavy chain variable domain (V_H) comprises a heavy chain complementarity determining region (HC-CDR) 3 having an amino acid sequence selected from the group consisting of SEQ ID NO: 23, 49, 57, 65, 73, 81, 89, 97, and 105, or having an amino acid sequence that has 3, 2, or 1 differences with an amino acid sequence selected from the group consisting of SEQ ID NO: 23, 49, 57, 65, 73, 81, 89, 97, and 105. In particular examples the amino acid sequence differences are conservative changes/substitutions.

In a further example, the antibody or antigen binding fragment binds to an epitope on the human ILT3 or competes with an antibody disclosed for binding to an epitope on the human ILT3, wherein the epitope comprises at least one amino acid from one or more of the amino acid sequences set forth in the group consisting of SEQ ID NO: 3, 4, 5, 6, 7, and 8. In further examples, the antibody or antigen binding fragment binds to an epitope on the human ILT3 or competes with an antibody disclosed for binding to an epitope on the human ILT3, wherein the epitope comprises one or more of the amino acid sequences set forth in SEQ ID NOs: 3, 4, 5, 6, 7, and 8. In further examples, the antibody or antigen binding fragment binds to an epitope on the human ILT3 or competes with an antibody disclosed for binding to an epitope on the human ILT3, wherein the epitope comprises the amino acid sequences set forth in SEQ ID NOs: 3, 4, 5, 6, 7, and 8. In particular examples, the epitope is determined by hydrogen deuterium exchange mass spectrometry (HDX-MS) analysis.

The present disclosure, in one aspect, provides an antibody or antigen binding fragment that binds to human immunoglobulin-like transcript 3 (ILT3) comprising (a) an HC having a variable domain (V_H) comprising a variable domain complementarity determining region (HC-CDR) 1 having the amino acid sequence set forth in SEQ ID NO: 17, 47, 55, 63, 71, 79, 87, 95, or 103; an HC-CDR2 having the

amino acid sequence set forth in SEQ ID NO: 18, 48, 56, 64, 72, 80, 88, 96, or 104; and an HC-CDR3 having the amino acid sequence set forth in SEQ ID NO: 23, 49, 57, 65, 73, 81, 89, 97, or 105; and, variants thereof wherein one or more of the HC-CDRs has one, two, or three amino acid substitutions, additions, deletions, or combinations thereof; and (b) a light chain (LC) having variable domain (V_L) comprising a variable domain complementarity determining region (LC-CDR) 1 having the amino acid sequence set forth in SEQ ID NO: 27, 50, 58, 66, 74, 82, 90, 98, or 106; an LC-CDR2 having the amino acid sequence set forth in SEQ ID NO: 43, 51, 59, 67, 75, 83, 91, 99, or 107; and an LC-CDR3 having the amino acid sequence set forth in SEQ ID NO: 44, 60, 68, 76, 84, 92, 100, or 108; and, variants thereof wherein one or more of the LC-CDRs has one, two, or three amino acid substitutions, additions, deletions, or combinations thereof. In particular examples the amino acid sequence differences are conservative changes/substitutions.

In a further examples of the antibody or antigen binding fragment, HC-CDR1 has the amino acid sequence set forth in SEQ ID NO:17; HC-CDR2 has the amino acid sequence set forth in SEQ ID NO: 19, 20, or 21; HC-CDR3 has the amino acid sequence set forth in SEQ ID NO: 23; and LC-CDR1 has the amino acid sequence set forth in SEQ ID NO: 34, 35, 36, 37, 38, 39, 40, 41, or 42; LC-CDR2 has the amino acid sequence set forth in SEQ ID NO: 43; and, LC-CDR3 has the amino acid sequence set forth in SEQ ID NO:44; and, variants thereof wherein one or more of the HC-CDRs and LC-CDRs has one, two, or three amino acid substitutions, additions, deletions, or combinations thereof. In particular examples the amino acid sequence differences are conservative changes/substitutions.

In a further example of the antibody or antigen binding fragment, HC-CDR1 has the amino acid sequence set forth in SEQ ID NO: 17; HC-CDR2 has the amino acid sequence set forth in SEQ ID NO: 20; and HC-CDR3 has the amino acid sequence set forth in SEQ ID NO: 23; and LC-CDR1 having the amino acid sequence set forth in SEQ ID NO: 41; LC-CDR2 having the amino acid sequence set forth in SEQ ID NO: 43; and, LC-CDR3 having the amino acid sequence set forth in SEQ ID NO: 44; and, variants thereof wherein one or more of the HC-CDRs and LC-CDRs has one, two, or three amino acid substitutions, additions, deletions, or combinations thereof. In particular examples the amino acid sequence differences are conservative changes/substitutions.

In a further example of the antibody or antigen binding fragment, the antibody or antigen binding fragment comprises (a) a V_H having a framework selected from the group consisting of human V_H1 , V_H2 , V_H3 , V_H4 , V_H5 , and V_H6 family and variants thereof having

1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, additions, deletions, or combinations thereof; and, (b) a V_L having a framework selected from the group consisting of human V_K1, V_K2, V_K3, V_K4, V_K5, V_K6, V_λ1, V_λ2, V_λ3, V_λ4, V_λ5, V_λ6, V_λ7, V_λ8, V_λ9, and V_λ10 family and variants thereof having 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, additions, deletions, or combinations thereof. In particular examples the amino acid sequence differences are conservative changes/substitutions.

In particular examples, the antibody or antigen binding fragment comprises (a) a V_H having a human V_H1 family framework or variant thereof having 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, additions, deletions, or combinations thereof; and, (b) a V_L having a human V_K5 family framework or variant thereof having 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, additions, deletions, or combinations thereof. In particular examples the amino acid sequence differences are conservative changes/substitutions.

In a further example of the antibody, the antibody comprises a human IgG1, IgG2, IgG3, or IgG4 HC constant domain or variant thereof having 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, additions, deletions, or combinations thereof compared to the amino acid sequence of the native human IgG1, IgG2, IgG3, or IgG4 isotype HC constant domain. In particular examples, the constant domain may comprise a C-terminal lysine or may lack a C-terminal lysine or a C-terminal glycine-lysine dipeptide.

In particular examples, the heavy chain constant domain is of the human IgG1 isotype, which has been modified to have reduced or minimal effector function. In further examples, the minimal effector function results from an effector-less Fc mutation, which may comprise or consist of the mutation N297A or D265A/N297A as identified using Kabat numbering in which case the minimal effector function results from aglycosylation (see for example, the amino acid sequence shown in SEQ ID NO: 211 wherein the N297A mutation corresponds to amino acid position 180; a D265A mutation, if present, would correspond to amino acid position 148). In particular aspects, the IgG1 has been modified to comprise or consist of an L234A, an L235A, and a D265S mutation as identified using Kabat numbering to render the Fc effector-less (see for example the amino acid sequence shown in SEQ ID NO: 12 or 13 wherein the L234A, L235A, and D265S mutations correspond to amino acid positions 117, 118, and 148, respectively).

In a further example, the HC constant domain is of the human IgG4 isotype and which isotype further includes a substitution of the serine residue at position 228 (EU

numbering) with proline, which corresponds to position 108 of SEQ ID NO: 9 or 10 (Serine at position 108).

In a further example of the antibody or antigen binding fragment, the antibody comprises a human kappa or lambda LC constant domain or variant thereof comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, additions, deletions, or combinations thereof compared to the amino acid sequence of the native human kappa or lambda LC constant domain. In particular examples the amino acid sequence differences are conservative changes/substitutions.

In a further example of the antibody or antigen binding fragment, the antibody comprises (i) a V_H having a framework selected from the human V_H1 , V_H2 , V_H3 , V_H4 , V_H5 , and V_H6 family and a human IgG1 or IgG4 HC constant domain or variant thereof comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, additions, deletions, or combinations thereof compared to the amino acid sequence of the native human IgG1 or IgG4 isotype HC constant domain; and, (ii) and a V_L having a framework selected from the human $V_{\kappa}1$, $V_{\kappa}2$, $V_{\kappa}3$, $V_{\kappa}4$, $V_{\kappa}5$, $V_{\kappa}6$, $V_{\lambda}1$, $V_{\lambda}2$, $V_{\lambda}3$, $V_{\lambda}4$, $V_{\lambda}5$, $V_{\lambda}6$, $V_{\lambda}7$, $V_{\lambda}8$, $V_{\lambda}9$, and $V_{\lambda}10$ family and a human kappa or lambda LC constant domain or variant thereof comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, additions, deletions, or combinations thereof compared to the amino acid sequence of the native human kappa or lambda LC constant domain. In particular examples the amino acid sequence differences are conservative changes/substitutions.

In a further example of the antibody or antigen binding fragment, the antibody comprises (i) a V_H having a human V_H2 family framework and a V_L having a human $V_{\kappa}5$ family framework; (ii) a human IgG1 or IgG4 HC constant domain or variant thereof comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, additions, deletions, or combinations thereof compared to the amino acid sequence of the native human IgG1 or IgG4 isotype HC constant domain; and, (iii) a human kappa LC constant domain or variant thereof comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, additions, deletions, or combinations thereof compared to the amino acid sequence of the native human kappa LC constant domain. In particular examples the amino acid sequence differences are conservative changes/substitutions.

In a further example of the antibody or antigen binding fragment, the antibody comprises (i) a V_H having a human V_H1 family framework and a human V_L having a human $V_{\kappa}5$ family framework; (ii) a human IgG4 HC constant domain or variant thereof comprising 1,

2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, additions, deletions, or combinations thereof compared to the amino acid sequence of the native human IgG4 isotype HC constant domain; and, (iii) a human kappa LC constant domain or variant thereof comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, additions, deletions, or combinations thereof compared to the amino acid sequence of the native human kappa LC constant domain. In particular examples the amino acid sequence differences are conservative changes/substitutions.

In a further examples of the antibody or antigen binding fragment, the antibody or antigen binding fragment comprises a V_H and a V_L having the amino acid sequences set forth in SEQ ID NO: 15 and SEQ ID NO: 16, respectively; SEQ ID NO: 45 and SEQ ID NO: 46, respectively; SEQ ID NO: 53 and SEQ ID NO: 54, respectively; SEQ ID NO: 61 and SEQ ID NO: 62, respectively; SEQ ID NO: 69 and SEQ ID NO: 70, respectively; SEQ ID NO: 77 and SEQ ID NO: 78, respectively; SEQ ID NO: 85 and SEQ ID NO: 86, respectively; SEQ ID NO: 93 and SEQ ID NO: 94, respectively; or SEQ ID NO: 101 and SEQ ID NO: 102, respectively.

In a further example of the antibody or antigen binding fragment, the antibody or antigen binding fragment comprises a V_H having the amino acid sequence set forth in SEQ ID NO: 117, 118, 119, 123, 124, or 125 and a V_L having the amino acid sequence set forth in SEQ ID NO: 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, or 141.

In a further example of the antibody or antigen binding fragment, the antibody or antigen binding fragment comprises a V_H having the amino acid sequence set forth in SEQ ID NO: 118 and a V_L having the amino acid sequence set forth in SEQ ID NO: 140.

In a further example of the antibody, the antibody comprises an HC constant domain comprising the amino acid sequence set forth in SEQ ID NO: 9, 10, 11, 12, or 13. In particular examples, the HC constant domain comprising the amino acid sequence set forth in SEQ ID NOs: 9, 11, 12, or 13 may lack a C-terminal lysine or a C-terminal glycine-lysine dipeptide. In particular examples, the HC constant domain comprises the amino acid sequence set forth in SEQ ID NO: 10.

In a further example of the antibody, the antibody comprises an LC constant domain comprising the amino acid sequence set forth in SEQ ID NO: 14.

In a further example of the antibody, the antibody comprises an HC comprising the amino acid sequence of SEQ ID NO: 142, 143, 144, 148, 149, 150, 167, 168, 169, 170, 174, 175, 176, 177, 178, 182, 183, 184, 185, 186, 187, 191, 192, or 193. In particular examples, the HC comprising the amino acid sequence set forth in SEQ ID NOs: 142, 143, 144, 148, 149, 150, 167, 168, 169, 170, 174, or 175, may lack a C-terminal lysine or a C-terminal

glycine-lysine dipeptide. In particular examples, the HC comprises the amino acid sequence set forth in SEQ ID NO: 143 or 177. In particular examples, the HC set forth in SEQ ID NO: 177 further lacks a C-terminal glycine.

In a further example of the antibody, the antibody comprises an LC comprising the amino acid sequence set forth in SEQ ID NO: 151, 152, 153, 154, 155, 156, 157, 158, 159, 160, 161, 162, 163, 164, 165, or 166. In particular examples, the LC comprises the amino acid set forth in SEQ ID NO: 165.

In a further example of the antibody, the antibody comprises an HC having the amino acid sequence set forth in SEQ ID NO:143 and an LC comprising the amino acid sequence set forth in SEQ ID NO:165. In particular examples, the HC comprising the amino acid sequence set forth in SEQ ID NO: 143 lacks a C-terminal lysine or a C-terminal glycine-lysine dipeptide.

The present disclosure, in another aspect, provides a chimeric, humanized, or recombinant human antibody or antigen binding fragment that binds to an epitope on a human immunoglobulin-like transcript 3 (ILT3), wherein the epitope comprises at least one amino acid from one or more of the amino acid sequences set forth in the group consisting of SEQ ID NOs:3, 4, 5, 6, 7, and 8. In a further example, the chimeric, humanized, or recombinant human antibody or antigen binding fragment binds to an epitope on a human ILT3 comprising the amino acid sequences set forth in SEQ ID NOs: 3, 4, 5, 6, 7, and 8. In these examples, the epitope is determined by hydrogen deuterium exchange mass spectrometry (HDX-MS) analysis.

The present disclosure further provides a chimeric, humanized, or recombinant human antibody or antigen binding fragment that binds ILT3 wherein the binding cross-blocks or competes with the binding of an antibody comprising a heavy chain having the amino acid sequence set forth in SEQ ID NO: 15 and a light chain having the amino acid sequence shown in SEQ ID NO: 16. In a further example, the chimeric, humanized, or recombinant human antibody or antigen binding fragment that cross-blocks or competes with an antibody comprising a heavy chain having the amino acid sequence set forth in SEQ ID NO: 15 and a light chain having the amino acid sequence shown in SEQ ID NO: 16 binds an epitope on ILT3 that comprises the amino acid sequences set forth in SEQ ID NOS: 3, 4, 5, 6, 7, and 8.

The present disclosure, in another aspect, provides a chimeric, humanized, or recombinant human antibody or antigen binding fragment that cross-blocks or competes with the binding of an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 15 and a light chain comprising the amino acid sequence shown in SEQ ID NO: 16 from
5 binding to human immunoglobulin-like transcript 3 (ILT3).

The present disclosure, in another aspect, provides a composition comprising one or more of any one of the antibody or antigen binding fragment disclosed or claimed herein and a pharmaceutically acceptable carrier.

The present disclosure further provides a method for treating a cancer in a subject comprising administering to the subject an effective amount of an antibody or antigen binding fragment disclosed or claimed herein sufficient to treat the cancer in the subject.

In a further example, the cancer is pancreatic cancer, melanomas, breast cancer, lung cancer, head and neck cancer, bronchus cancer, colorectal cancer, prostate cancer, pancreatic cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, or cancer of hematological tissues.

The present disclosure further provides a method for treatment of a cancer in a subject comprising administering to the subject concurrently or consecutively an antibody or antigen binding fragment disclosed herein in combination with one or more inhibitors or antagonists of PD-1, PD-L1 and/or PD-L2. In one example, the antagonist of PD-1 is an antibody or antigen binding fragment thereof that binds to human PD-1 and blocks the binding of PD1 to human PD-L1 and PD-L2. In one example, the antagonist of PD-L1 or PD-L2 is an antibody or antigen binding fragment thereof that binds to human PD-L1 or PD-L2 and blocks the binding of human PD-L1 or PD-L2 PD1.

In a further example, the anti PD1 antagonist is an anti-PD-1 antibody is nivolumab, pembrolizumab, cemiplimab, or pidilizumab and the PD-L1 inhibitor is durvalumab, atezolizumab, avelumab, YW243.55.S70, MPDL3280A, MEDI-4736, MSB-0010718C, or MDX-1105.

The present disclosure further provides an antibody or antigen binding fragment disclosed or claimed herein for treatment of cancer in a subject. The present disclosure, in another aspect, provides an antibody or antigen binding fragment as disclosed or claimed herein for use in the treatment of a cancer in a subject.

In a further example, the cancer is pancreatic cancer, melanomas, breast cancer, lung cancer, head and neck cancer, bronchus cancer, colorectal cancer, prostate cancer, pancreatic cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, or cancer of hematological tissues.

The present disclosure further provides an antibody or antigen binding fragment disclosed or claimed herein for treatment of a cancer in a subject wherein the treatment further comprises one or more inhibitors or antagonists of PD-1, PD-L1 and/or PD-L2. The present disclosure, in another aspect, provides an antibody or antigen binding fragment disclosed or claimed herein for use in treatment of a cancer in a subject that includes one or more inhibitors or antagonists of PD-1, PD-L1 and/or PD-L2

In one example, the antagonist of PD-1 is an antibody or antigen binding fragment thereof that binds to human PD-1 and blocks the binding of PD1 to PD-L1 and PD-L2.

In one example, the antagonist of PD-L1 or PD-L2 is an antibody or antigen binding fragment thereof that binds to human PD-L1 or PD-L2 and blocks the binding of human PD-L1 or PD-L2 PD1.

In a further example, the anti-PD-1 antibody is nivolumab, pembrolizumab, cemiplimab, or pidilizumab and the PD-L1 inhibitor is durvalumab, atezolizumab, avelumab, YW243.55.S70, MPDL3280A, MEDI-4736, MSB-0010718C, or MDX-1105.

The present disclosure further provides for use of an antibody or antigen binding fragment disclosed or claimed herein for the treatment of a cancer.

The present disclosure further provides for use of an antibody or antigen binding fragment disclosed or claimed herein for the manufacture of a medicament for the treatment of a cancer. The present disclosure, in another aspect, provides use of an antibody or antigen binding fragment disclosed or claimed herein in the manufacture of a medicament for treatment of a cancer.

In a further example, the cancer is pancreatic cancer, melanomas, breast cancer, lung cancer, head and neck cancer, bronchus cancer, colorectal cancer, prostate cancer, pancreatic cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, or cancer of hematological tissues.

The present disclosure further provides a composition comprising any one of the aforementioned antibodies or antigen binding fragments and a pharmaceutically acceptable carrier. In particular examples, the composition comprises a mixture of antibodies comprising a heavy chain having a C-terminal lysine and antibodies comprising a heavy chain lacking a C-terminal lysine. In particular examples, the composition comprises an antibody disclosed herein wherein the predominant antibody form comprises a heavy chain having a C-terminal lysine. In particular examples, the composition comprises an antibody disclosed herein wherein the predominant antibody form comprises a heavy chain lacking a C-terminal lysine. In particular examples, the composition comprises an antibody disclosed herein

wherein about 100% of the antibodies in the composition comprise a heavy chain lacking a C-terminal lysine.

The present disclosure, in another aspect, provides a nucleic acid molecule encoding an anti-ILT3 antibody or antigen binding fragment as disclosed or claimed herein.

5 The present disclosure, in another aspect, provides a vector comprising the nucleic acid molecule as disclosed or claimed herein.

The present disclosure, in another aspect, provides a host cell comprising the vector as disclosed or claimed herein.

10 The present disclosure, in another aspect, provides a method for producing an antibody or antigen binding fragment as disclosed or claimed herein comprising:

- (a) providing a host cell comprising the vector as disclosed or claimed herein;
- (b) culturing the host cell in culture medium under conditions to enable expression of the antibody or antigen binding fragment encoded by the vector; and,
- (c) obtaining the antibody or antigen binding fragment from the culture medium to
15 provide the antibody or antigen binding fragment.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1A, Fig. 1B, Fig. 1C, Fig. 1D, Fig. 1E, and Fig. 1F show a comparison of the selectivity of several of the anti-ILT3 antibodies disclosed herein to monoclonal antibody
20 9B11 and mouse IgG1 (mIgG1) using a cell-based ELISA format. CHO-K1 cells expressing human ILT3 (**Fig. 1A**), Rhesus monkey ILT3 (**Fig. 1B**), human ILT5 (**Fig. 1C**), human ILT7 (**Fig. 1D**), human ILT8 (**Fig. 1E**), or human ILT11 (**Fig. 1F**) were each tested with monoclonal antibody p40B5 (LB179.40B5.1A1), p49C6 (LB181.49C6.1A1), and p52B8 lb181.52B8.1B1); antibody 9B11 (U.S. Pat. No. 7,777,008 as having the amino acid sequences of SEQ ID NO: 33
25 (light chain) and SEQ ID NO: 34 (heavy chain)), and mouse IgG1.

Fig. 2A shows data characteristics on binding affinity, isoelectric point, purity of monomer species, and thermal stability measurements for variants of mAb 10. Terms: “huILT3” refers to human ILT3; “rhILT3” refers to Rhesus monkey ILT3; “pI” refers to isoelectric point; “T_m” refers to temperature mid-point of a thermal unfolding curve; “Tagg” refers to mid-point
30 of a thermal aggregation curve; “SEC” refers size-exclusion ultra-high performance liquid chromatography).

Fig. 2B shows the relationship of SEC purity and melting temperature of humanized light chain variants of mAb 10 (M64V VH1 IgG4). VL1-VL8 refer to variants having the amino acid sequence set forth in SEQ ID NOs: 126-133, respectively.

Fig. 3A shows a deuterium labeling difference heatmap of the human ILT3 extracellular domain amino acid residues that are bound by Chimeric Anti-ILT3 52B8 mouse 52B8 VH parental/human IgG4 (S228P): mouse 52B8 parental VL / human Kappa antibody (“c58B2”; mAb 73). These six peptide domains, which comprise the epitope bound by the antibody(residues 18-23 (ISWGNS; SEQ ID NO: 3), residues 64-69 (IPSMTE; SEQ ID NO: 4), residues 96-101 (MTGAYS; SEQ ID NO: 5), residues 124- 131 (QSRSPMDT; SEQ ID NO: 6), residues 152- 159 (AQQHQAEF; SEQ ID NO: 7) and residues 184-187 (LLSH; SEQ ID NO: 8)), are located near the border of the D1 and D2 domains of the ILT3 extracellular domain. The amino acid sequence of human extracellular domain with C-terminal His Tag is set forth in SEQ ID NO: 1.

WHAT IS CLAIMED:

1. An antibody or antigen binding fragment that binds to human immunoglobulin-like transcript 3 (ILT3), comprising:

(a) a heavy chain (HC) having a variable domain (V_H) comprising a variable domain complementarity determining region (HC-CDR) 1 having the amino acid sequence set forth in SEQ ID NO: 17, 47, 55, 63, 71, 79, 87, 95, or 103; an HC-CDR2 having the amino acid sequence set forth in SEQ ID NO: 18, 48, 56, 64, 72, 80, 88, 96, or 104; and an HC-CDR3 having the amino acid sequence set forth in SEQ ID NO: 23, 49, 57, 65, 73, 81, 89, 97, or 105; and, variants thereof wherein one or more of the HC-CDRs has one, two, or three amino acid substitutions, additions, deletions, or combinations thereof; and

(b) a light chain (LC) having variable domain (V_L) comprising a variable domain complementarity determining region (LC-CDR) 1 having the amino acid sequence set forth in SEQ ID NO: 27, 50, 58, 66, 74, 82, 90, 98, or 106; an LC-CDR2 having the amino acid sequence set forth in SEQ ID NO: 43, 51, 59, 67, 75, 83, 91, 99, or 107; and an LC-CDR3 having the amino acid sequence set forth in SEQ ID NO: 44, 60, 68, 76, 84, 92, 100, or 108; and, variants thereof wherein one or more of the LC-CDRs has one, two, or three amino acid substitutions, additions, deletions, or combinations thereof.

2. The antibody or antigen binding fragment of claim 1, wherein

(a) the HC-CDR1 has the amino acid sequence set forth in SEQ ID NO: 17; the HC-CDR2 has the amino acid sequence set forth in SEQ ID NO: 19, 20, or 21; the HC-CDR3 has the amino acid sequence set forth in SEQ ID NO: 23; and

(b) the LC-CDR1 has the amino acid sequence set forth in SEQ ID NO: 34, 35, 36, 37, 38, 39, 40, 41, or 42; the LC-CDR2 has the amino acid sequence set forth in SEQ ID NO: 43; and, the LC-CDR3 has the amino acid sequence set forth in SEQ ID NO: 44.

3. The antibody or antigen binding fragment of claim 2, wherein

(a) the HC-CDR1 has the amino acid sequence set forth in SEQ ID NO: 17; the HC-CDR2 has the amino acid sequence set forth in SEQ ID NO: 20; and the HC-CDR3 has the amino acid sequence set forth in SEQ ID NO: 23; and

(b) the LC-CDR1 has the amino acid sequence set forth in SEQ ID NO: 41; the LC-CDR2 has the amino acid sequence set forth in SEQ ID NO: 43; and, the LC-CDR3 has the amino acid sequence set forth in SEQ ID NO: 44.

4. The antibody or antigen binding fragment of any one of claims 1, 2, or 3, wherein the V_H comprises a framework selected from the group consisting of human V_H1 , V_H2 , V_H3 , V_H4 , V_H5 , and V_H6 , and variants thereof having 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, additions, deletions, or combinations thereof; and, the V_L comprises a framework selected from the group consisting of human V_K1 , V_K2 , V_K3 , V_K4 , V_K5 , V_K6 , $V_\lambda1$, $V_\lambda2$, $V_\lambda3$, $V_\lambda4$, $V_\lambda5$, $V_\lambda6$, $V_\lambda7$, $V_\lambda8$, $V_\lambda9$, and $V_\lambda10$, and variants thereof having 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, additions, deletions, or combinations thereof.

5. The antibody or antigen binding fragment of any one of claims 1, 2, 3, wherein the antibody comprises an HC having a human IgG1, IgG2, IgG3, or IgG4 HC constant domain or variant thereof having 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, additions, deletions, or combinations thereof compared to the amino acid sequence of the native IgG1, IgG2, IgG3, or IgG4 isotype constant domain.

6. The antibody or antigen binding fragment of any one of claims 4 or 5, wherein the antibody comprises an LC having a human kappa or lambda LC constant domain or variant thereof comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, additions, deletions, or combinations thereof compared to the amino acid sequence of the native human kappa or lambda light chain constant domain.

7. The antibody or antigen binding fragment of claim 3, wherein the antibody comprises:

(i) a V_H having a framework selected from human V_H1 , V_H2 , V_H3 , V_H4 , V_H5 , and V_H6 and a human IgG1 or IgG4 HC constant domain or variant thereof comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino acid substitutions, additions, deletions, or combinations thereof compared to the amino acid sequence of the native IgG1 or IgG4 isotype HC constant domain; and,

(ii) a V_L having a framework selected from human V_K1 , V_K2 , V_K3 , V_K4 , V_K5 , V_K6 , $V_\lambda1$, $V_\lambda2$, $V_\lambda3$, $V_\lambda4$, $V_\lambda5$, $V_\lambda6$, $V_\lambda7$, $V_\lambda8$, $V_\lambda9$, and $V_\lambda10$ and a human kappa or lambda LC constant domain or variant thereof comprising 1, 2, 3, 4, 5, 6, 7, 8, 9, or 10 amino

acid substitutions, additions, deletions, or combinations thereof compared to the amino acid sequence of the native human kappa or lambda LC constant domain.

8. The antibody or antigen binding fragment of claim 4, wherein the
5 antibody or antigen binding fragment comprises a V_H and a V_L having the amino acid sequences set forth in SEQ ID NO: 15 and SEQ ID NO: 16, respectively; SEQ ID NO: 45 and SEQ ID NO: 46, respectively; SEQ ID NO: 53 and SEQ ID NO: 54, respectively; SEQ ID NO: 61 and SEQ ID NO: 62, respectively; SEQ ID NO: 69 and SEQ ID NO: 70, respectively; SEQ ID NO: 77 and SEQ ID NO: 78, respectively; SEQ ID NO: 85 and SEQ ID NO: 86,
10 respectively; SEQ ID NO: 93 and SEQ ID NO: 94, respectively; or SEQ ID NO: 101 and SEQ ID NO: 102, respectively.

9. The antibody or antigen binding fragment of claim 4, wherein the
antibody or antigen binding fragment comprises a V_H having the amino acid sequence set forth
15 in SEQ ID NO: 117, 118, 119, 123, 124, or 125 and a V_L having the amino acid sequence set forth in SEQ ID NO: 126, 127, 128, 129, 130, 131, 132, 133, 134, 135, 136, 137, 138, 139, 140, or 141.

10. The antibody or antigen binding fragment of claim 9, wherein the
20 antibody or antigen binding fragment comprises a V_H having the amino acid sequence set forth in SEQ ID NO: 118 and a V_L having the amino acid sequence set forth in SEQ ID NO: 140.

11. The antibody or antigen binding fragment of claim 7, wherein the
antibody comprises a heavy chain (HC) comprising the amino acid sequence set forth in SEQ ID
25 NO: 143 and a light chain (LC) comprising the amino acid sequence set forth in SEQ ID NO: 165, and variants thereof wherein the HC lacks a C-terminal Lysine residue or a C-terminal glycine-lysine.

12. A chimeric, humanized, or recombinant human antibody or antigen
30 binding fragment that binds to an epitope on a human immunoglobulin-like transcript 3 (ILT3), wherein the epitope comprises at least one amino acid from one or more of the amino acid sequences set forth in SEQ ID NOs: 3, 4, 5, 6, 7, and 8.

13. A chimeric, humanized, or recombinant human antibody or antigen binding fragment that cross-blocks or competes with the binding of an antibody comprising a heavy chain comprising the amino acid sequence set forth in SEQ ID NO: 15 and a light chain comprising the amino acid sequence shown in SEQ ID NO: 16 from binding to human immunoglobulin-like transcript 3 (ILT3).

14. A composition comprising:
an antibody or antigen binding fragment of any one of claims 1-13 and a pharmaceutically acceptable carrier.

15. An antibody or antigen binding fragment of any one of claims 1-13 for use in the treatment of a cancer in a subject.

16. An antibody or antigen binding fragment of claim 15 for use in treatment of a cancer in a subject that includes one or more inhibitors or antagonists of PD-1, PD-L1 and/or PD-L2.

17. The antibody or antigen binding fragment of claim 16, wherein the one or more inhibitors or antagonists of PD-1, PD-L1 and/or PD-L2 is an anti-PD-1 antibody selected from the group consisting of pembrolizumab, nivolumab, cemiplimab, and pidilizumab.

18. The antibody or antigen binding fragment of claim 16, wherein the one or more inhibitors or antagonists of PD-1, PD-L1 and/or PD-L2 is a PD-L1 inhibitor selected from the group consisting of durvalumab, atezolizumab, avelumab, YW243.55.S70, MPDL3280A, MEDI-4736, MSB-0010718C, and MDX-1105.

19. The antibody or antigen binding fragment of claim 15 or 16, wherein the cancer is pancreatic cancer, melanomas, breast cancer, lung cancer, head and neck cancer, bronchus cancer, colorectal cancer, prostate cancer, pancreatic cancer, stomach cancer, ovarian cancer, urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small

bowel or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer, osteosarcoma, chondrosarcoma, or cancer of hematological tissues.

20. Use of an antibody or antigen binding fragment of any one of claims 1-13
5 in the manufacture of a medicament for treatment of a cancer.

21. The use of an antibody or antigen binding fragment of any one of claims
1-13 in the manufacture of a medicament for treatment of a cancer, wherein the cancer is
pancreatic cancer, melanomas, breast cancer, lung cancer, head and neck cancer, bronchus
10 cancer, colorectal cancer, prostate cancer, pancreatic cancer, stomach cancer, ovarian cancer,
urinary bladder cancer, brain or central nervous system cancer, peripheral nervous system
cancer, esophageal cancer, cervical cancer, uterine or endometrial cancer, cancer of the oral
cavity or pharynx, liver cancer, kidney cancer, testicular cancer, biliary tract cancer, small bowel
or appendix cancer, salivary gland cancer, thyroid gland cancer, adrenal gland cancer,
15 osteosarcoma, chondrosarcoma, or cancer of hematological tissues.

22. A nucleic acid molecule encoding an anti-ILT3 antibody or antigen
binding fragment of any one of claims 1-13.

20 23. A vector comprising the nucleic acid molecule of claim 22.

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

25. A method for producing an antibody or antigen binding fragment of any
25 one of claims 1-13 comprising:

- (a) providing a host cell comprising the vector of claim 23;
- (b) culturing the host cell in culture medium under conditions to enable
expression of the antibody or antigen binding fragment encoded by the vector; and,
- (c) obtaining the antibody or antigen binding fragment from the culture
30 medium to provide the antibody or antigen binding fragment.